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ABSTRACTS

American Dairy Science Association®

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Dairy Foods Symposium: Championing Women in Dairy Science—Remembering the Legacy of Susan Duncan

1000 Engaging today's undergraduate women with the field of dairy science. G. Lewis*, *University of Wisconsin–River Falls, River Falls, WI.*

Dr. Susan Duncan had an incredible ability to guide students, both undergraduate and graduate level, through a warm, supportive mentoring style. Dr. Duncan's approach is fundamental to retaining students and guiding them toward careers in dairy foods-related fields, especially for undergraduate student populations. With this, this presentation intends to highlight some approaches I have taken to mentor undergraduate students in my early career as an Assistant Professor. The accomplishments of undergraduate students at the University of Wisconsin–River Falls will be highlighted, including research conducted on whey protein films, lactose-free ice cream, and dairy product development. The goal of this session is to recognize the extraordinary capacity of undergraduate researchers when encouraged and supported through mentoring styles motivated by Dr. Susan Duncan's legacy.

Key Words: dairy foods, undergraduate, research

1001 Mentoring while still being mentored: Championing women in dairy science as an early career faculty. S. Mann*, *Cornell University, Ithaca, NY.*

Effective mentorship can have a profound impact on professionals at every step of their career. Even though the field has seen a shift to have more than half of the graduates in agriculture bachelor degrees be women, female professionals and those belonging to minority groups are traditionally underrepresented among dairy scientists, particularly in leadership positions. Early career mentorship provides the opportunity to help navigate challenges typical to this stage of a scientist's career and provide ways to retain and promote a diverse workforce during this critical phase. As opposed to mentorship provided by senior professionals in a typical one-way relationship of providing advice and solutions to the less experienced mentee, peer mentorship can be an effective strategy to enrich both the mentor and mentee's development and create camaraderie among like-minded individuals learning and progressing together. The roles of mentor and mentee can be interchangeable in these relationships. This presentation as part of the Championing Women in Dairy Science: Remembering the Legacy of Sue Duncan symposium will focus on the unique aspects of being a mentor while still being mentored as an early career faculty member.

Key Words: peer mentoring, women, dairy science

1002 Navigating industry careers as a woman in dairy science. L. L. Colby*, *Land O'Lakes, Inc., Arden Hills, MN.*

Working in industry presents unique challenges and opportunities for women pursuing a career in dairy science. This is demonstrated through recent data on industry gender disparities. While we have made significant progress over recent decades, there are additional approaches we can take, which will be described. One area of emphasis we can continue to improve is technical leadership development within industry technical career tracks, in addition to people leadership development programs and training.

Key Words: dairy science, women, industry

1003 Being a mom and doing research about moms: When life and work collide. L. L. Hernandez*, *University of Wisconsin–Madison, Madison, WI.*

I knew I always loved mammals; however, it was not until graduate school that I was introduced to a project focused on understanding how a mother, specifically a dairy cow, could control the composition and amount of milk being made while maintaining her own physiology. I was hooked, the birth of a lactation physiologist. It was the first time I truly understood the word homeorhesis. I went on to study how molecules in the mammary gland influenced maternal physiology in the mouse and the human as a postdoctoral fellow. I was 7 mo pregnant when I started my faculty position, and 2 mo later my daughter was born, and a passion was cemented upon becoming a mother that has informed every research question I have had and every mentoring approach I have taken. No one prepared me for that experience: for being discharged from the hospital and being told we would see the baby for a bunch of appointments, but not you for 6 weeks; for what it would be like lecturing in lactation physiology and desperately needing to pump; or for how being up all night feeding or consoling a child would send my stress level through the roof. Experiences I have had as a mom have made me determined to focus my work on improving maternal health during the peripartal period, whether that is in a cow, mouse, or human, as well as making me a better mentor due to the expansion of my empathy for humans that is not to be explained until you become a parent. This path has led me to a location where it is impossible for me to separate my work and passion for improving maternal health, where every research question and experiment somehow impacts my actual life as a mom. Today I continue to focus my research on various aspects of improving the physiology of mothers, whether that be a dairy cow, mouse, or a human. I would like to think that the collision of a lactation physiologist with motherhood has made my research that much better, and that being a mom has made me a better scientist and mentor. Without those women in animal science before me, though, these things would not have seemed possible to the young woman who showed up in a lab and had a love for mammals.

Key Words: lactation, mom, mammals

1004 Building a strong team. M. A. Drake*, *North Carolina State University, Raleigh, NC.*

Team leadership requires vision, passion, commitment, and communication. An engaging environment with clear expectations is a pathway for success. The role of team leadership on graduate education will be discussed.

Key Words: leadership, team building, graduate students

1005 The critical role of leadership positions in the dairy industry. K. Plaut*, *Purdue University, West Lafayette, IN.*

In any industry, it is important to hear the perspectives of a wide variety of people. There are many distinct types of leadership roles in the dairy industry, from farmer to academician to industry or co-op leader, but they all have some common attributes. I will highlight some of the leadership values Dr. Susan Duncan brought to the table as a leader at Virginia Tech and in the dairy industry. Dr. Duncan brought a spirit of service to the dairy industry coupled with innovation—finding new ways to improve research facilities and processes for the future. She had passion for science and for leadership because she cared about her university and the industry. Dr. Duncan had a communication style that created an inclusive environment and made everyone in the room feel valued. We will discuss these leadership values and more as we discuss the critical role for leadership in the dairy industry.

Key Words: Susan Duncan, leadership, dairy

ADSA-GSD Competition: Dairy Foods Oral Semifinalists

1100 Understanding utility of acid-base buffering curve in determining insoluble calcium content in various cheese types.

R. Lindstrom* and P. Sharma, *NDFS Department, Utah State University, Logan, UT.*

Quality defects in cheese can arise from various reasons, including pH variation, which causes significant loss in revenue. Variations in pH impact the flavor, moisture, and textural properties. Insoluble calcium (INSOL Ca) associated with protein matrix slowly solubilizes, releasing H^+ ions affecting the final pH of cheese. Therefore, it is important to accurately measure INSOL Ca in the cheese. For determining INSOL Ca, cheese is converted into slurry using water and is subjected to forward (cheese pH to 3) and backward (pH 3 to 9) titration to obtain acid-base buffering curves. To better understand the utility of acid-base titration method and understand the impact of INSOL Ca on cheese pH, it was decided to test cheeses with a wide range of pH 4.8–6.6. Cheddar, Feta, Juustoleipa, and Mozzarella were used in this study, having pH 5.3, 4.8, 6.6, 5.6; total calcium 0.86, 0.46, 0.76, 0.55%; and INSOL Ca content 0.67, 0.15, 0.67, 0.41% respectively. Positive correlations were obtained for pH ($r = 0.6$, $P < 0.05$) and protein ($r = 0.89$, $P < 0.05$) with INSOL Ca suggesting the concomitant release of INSOL Ca with pH decrease from the colloidal phase to the aqueous phase and more retention of INSOL Ca with higher protein content. Despite being pH close to Mozzarella, Cheddar contained more INSOL Ca owing to the highest amount of protein due to low moisture content. Feta had the lowest amount of INSOL Ca owing to a low pH (4.8), more moisture content, low protein content, and higher extent of proteolysis as observed through UREA-PAGE analysis. Juustoleipa had the highest percentage of INSOL Ca out of total calcium (88%) due to higher pH and more intact casein, followed by Cheddar (79%), and least being with Feta (33%). It was evident that higher pH and higher protein content were associated with a higher proportion of INSOL Ca linked with the cheese protein matrix. These findings will help understand and mitigate potential causes of the pH variation in the early stage of ripening of cheese so that consistent quality cheese can be produced throughout the year.

Key Words: acid-base buffering curve, cheese, insoluble calcium

1101 The link of casein variants to casein composition, micelle size, and protein content in Dutch goat milk.

S. Breunig*[1,2], R. González Prendes[2,3], H. Bovenhuis[3], R. P. M. A. Crooijmans[3], K. Hettinga[1], and E. Bijl[1], [1]*Food Quality and Design Group, Wageningen University & Research, Wageningen, the Netherlands,* [2]*Ausnutria B.V., Zwolle, the Netherlands,* [3]*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands.*

Objective: The casein micelle structure is important for various functional properties of dairy products. Differences in protein content, casein (CN) composition, and micelle structure can be influenced by external factors, as well as by casein polymorphisms. Goat milk exhibits a wide range of casein polymorphisms, but knowledge on the link between casein variants, micelle structure and composition is limited, and mainly focused on α_{S1}-CN. Therefore, we aim to investigate how variants of all 4 caseins impact micelle size, protein content, and casein composition. Methods: Milk of 234 Dutch goats was analyzed for protein content (FTIR) and micelle size (dynamic light scattering). The casein composition and the casein variants were identified using LC-MS. To estimate the influence of each casein fraction and their variants on selected traits (protein content, casein composition, micelle size), we used a mixed mode approach. In the models, parity, lactation stage, family relation, herd effect, and the animal age were taken into account. Additionally, the heritability of the traits was estimated. Results: The α_{S1}- and κ-CN fraction showed the largest number of casein variants; in α_{S1}-CN also 2 previously uncharacterized variants were detected. Differences in protein content, casein micelle size and relative casein composition were observed between variants of all caseins. Casein variants were not independent from each other, but by using a mixed model, we could identify the casein fractions and variants that mainly contributed to differences in the analyzed traits. Variants of α_{S1}-CN impacted protein content. In contrast to previous findings, micelle size was found to be significantly influenced by α_{S2}-CN variants, and not by α_{S1}-CN. Additionally, some traits were significantly affected by herd and lactation stage. High heritability was found for casein micelle size. Conclusion: Casein composition and the casein variants affecting micelle size differ in Dutch goats from other dairy goats. The results provide new insights on effects of casein variants on micelle structure. This may help to understand functional properties of goat milk.

Key Words: goat milk, casein, genetic variants

1102 A new method for separating the milk fat globule membrane and the main buttermilk proteins.

J. Iung*[1,2], A. Doyen[1,2], Y. Pouliot[1,3], G. Remondetto[4], and G. Brisson[2,3], [1]*Insitute of Nutrition and Functional Foods (INAF), Quebec, Quebec, Canada,* [2]*Department of Food Science, Laval University, Quebec, Quebec, Canada,* [3]*Dairy Science and Technology Research Centre (STELA), Quebec, Quebec, Canada,* [4]*Agropur Cooperative, St. Hubert, Quebec, Canada.*

In recent years, butter production has surged, increasing buttermilk output. Buttermilk contains interesting compounds such as a milk fat globule membrane (MFGM) fragment and casein micelles (CM). Despite its nutraceutical and health properties, the presence of these membrane fragments limits its use in coagulated dairy products by introducing structural modifications. Additionally, their extraction poses a challenge due to their similarity in size to CM. One solution proposed for separating the buttermilk proteins involves using an adsorbent agent such as hydroxyapatite (HA) and adjusting physicochemical parameters to modulate their affinity for it. Therefore, the influence of pH, ionic strength, and temperature on mixed proteins and MFGM (4 g/L each) model solutions against HA were assessed using a 3-level, 3-factor Box-Behnken design. It was observed that each physicochemical parameter influenced their adsorption through different mechanisms (ANOVA, $P <$ 0,05), predominantly favoring the adsorption of CM. First, the temperature significantly impacted the adsorption process for all components by increasing the particle movement in solution reaching. Changes in component diffusion in solution and their different affinity for HA (CN > β-lactoglobulin > α-lactalbumin > MFGM) led to minimal adsorption at 10°C and maximum at 30°C. Second, interactions are more favorable at low (<100 m*M*) than at high ionic strength (>100 m*M*). Finally, pH changed the charge of proteins and the surface charge of HA, resulting in a minimum adsorption at pH 5.0 for each component. These findings have helped determine the temperature, pH, and ionic strength ranges needed to bind CM instead of whey proteins and MFGM preferentially. Furthermore, desorption characterization of CM revealed that using pH or sodium citrate partially or completely desorbed CM on HA, respectively. The optimal physicochemical parameters determined with the statistical model were tested and validated directly in buttermilk, demonstrating the feasibility of separating the buttermilk's proteins with HA.

Key Words: hydroxyapatite, buttermilk proteins, separation

1103 Mechanisms and factors impacting the interaction of flavonoids with β-lactoglobulin.

T. Mao*[1] and M. S. Mohan[1,2], [1]*South Dakota State University, Brookings, SD,* [2]*Lincoln University, Lincoln, New Zealand.*

Introduction: β-lactoglobulin (β-LG) is an effective carrier for flavonoids. However, factors affecting the interaction between different flavonoids and β-LG remain unclear. This study aims to explore the effects of the structural, physical, and chemical properties of flavonoids on the interaction with β-LG. Methods: UV-vis absorption spectroscopy, fluorescence spectroscopy, and Fourier-transform infrared spectroscopy (FTIR) were utilized to evaluate the binding mechanisms, affinities, and conformational changes in the structure of β-LG upon binding with cyanidin 3-O-glucoside (CG), cyanidin-3-galactoside (CGA), quercetin 3-glucoside (QG), and delphinidin 3-rutinoside (DR). Correlation analysis was employed to investigate factors including hydroxyl groups, benzene rings, molecular weight, hydrophobicity, acidity strength, and charge of flavonoids, on the interaction with β-LG. Results: The UV-vis absorption spectra confirmed the formation of β-LG-flavonoid complexes. CGA demonstrated the highest fluorescence quenching efficiency on binding with β-LG, followed by CG, DR, and QG at 25°C. Our multi-spectroscopy studies indicated that CG, CGA, QG, and DR spontaneously interacted with β-LG through hydrophobic interactions, hydrogen bonding, and electrostatic interactions, leading to secondary structural change of β-LG. The quenching efficiency was highly positively related to the ratio of molecular weight to the number of hydroxyl groups, hydrophobicity, and charge of the flavonoids. The binding affinity and quenching constant were significantly positively correlated with temperature. CGA exhibited the highest binding affinity at 45°C. Conclusion: These findings elucidate the binding mechanisms between CG/CGA/DR/QG and β-LG, emphasizing the importance of the factors influencing polyphenol binding to β-LG. A better understanding of the influencing factors will enable the tailoring of the delivery of bioactive flavonoids by complexing them with milk proteins.

Key Words: hydroxyl groups, hydrophobicity, charge

1104 Metabolic capabilities of *Latilactobacillus curvatus*, and the potential use as an adjunct culture for food safety and quality control in yogurt.

C. Wahlstrom* and T. Oberg, *Utah State University, Logan, UT.*

Latilactobacillus curvatus is a promising candidate as a bioprotective adjunct culture in dairy fermentations. Genomic analysis of a dairy isolate, WSU-1, identified the propane-diol utilization pathway which produces 3-hydroxy propionate with reuterin as an intermediate or propionate depending on the substrate. Initially, GC-MS analysis was conducted on fermentate from a controlled fermentation and showed production of 3-hydroxy propionate and propionate as products. A colorimetric assay for the determination of reuterin showed a concentration of 1 m*M* after 24 h. An in vitro method was developed to test antifungal capabilities of WSU-1 when grown in 3 conditions: standard MRS agar, carbohydrate restricted (CR) MRS + 200 m*M* glycerol, and CR-MRS + 200 m*M* 1,2-propanediol, using a modified overlay method in 24-well plates. A 5-point hedonic grading scale qualitatively showed some degree of anti-fungal capability against all 8 yeast and 9 mold strains. This capability was tested in a yogurt system with three 100-gallon yogurt vats produced in triplicate containing either the addition of WSU-1 or addition of WSU-1 and 200 m*M* glycerol. A control vat was produced without the adjunct or glycerol. Yogurt was packaged into 8-oz cups and inoculated in triplicate with either a single mold or yeast strain at an inoculation rate of 10^3 cfu/g. Uninoculated cups were kept as a control. Samples were held at 8°C for 4 wk. Yeast growth was analyzed weekly using plate counts, and data showed no statistically significant inhibition in any of the yeast (Tukey HSD post hoc analysis with adjusted p-values less than 0.05). Mold growth was analyzed visually using a 5-point hedonic scale, and data show no noticeable difference in any of the molds across any of the replicates or time points. These data show that in a controlled in vitro study, WSU-1 showed inhibition in all the yeast and molds tested. However, these results were not observed in a yogurt system, indicating the need for validation tests in real-life food systems.

Key Words: biopreservation, yogurt, fermentation

1105 Predicting phase stability of UHT reconstituted skim milk using vibrational spectroscopy and physicochemical properties coupled with partial least squares method.

Y. Shao*[1], L. He[2], and H. Zheng[1], [1]*North Carolina State University, Raleigh, NC,* [2]*University of Massachusetts Amherst, Amherst, MA.*

The occurrence of phase separation (i.e., sedimentation and age gelation) arises as a notable quality issue in UHT-treated milk over storage. Predicting the product phase stability after long storage is challenging when relying on physicochemical parameters such as pH, particle size, mineral content, etc. In this work, we aimed to investigate whether using Raman and Fourier-transform infrared (FTIR) spectral information with the addition of physicochemical properties can effectively predict the phase separation of UHT reconstituted skim milk (RSM) over storage. Milk samples were reconstituted to 3.4% protein content using nonfat dry milk (NFDM) and skim milk powders (SMP), UHT treated in duplicate ($n = 38$) and stored in sterilized 100-mL bottles for analyses. After 1 (M1) and 3 (M3) months of ambient storage, UHT-RSM samples were measured for pH, particle size, z-potential, and ionic calcium content. Samples were also vacuum-concentrated and characterized using Raman spectroscopy and FTIR. Mineral contents (Ca, Mg, K, Cl, and P) were analyzed on samples that were stored in a freezer after processing. At 7.5 mo, the liquid fraction of each sample was decanted for total solids (TS) analysis. The percentage of TS depletion at 7.5 mo (%$TSD_{7.5\text{-mo}}$) was calculated against the TS of the freshly processed sample. Partial least squares (PLS) regression models were constructed using Raman spectra, FTIR spectra, and physicochemical properties as X variables individually and in combination, and %$TSD_{7.5\text{-mo}}$ as the Y response. Raman spectra collected on M3 UHT-RSM were found effective for establishing a PLS model to predict %$TSD_{7.5\text{-mo}}$ ($R^2 = 0.91$ and root average squared error [RASE] = 0.61). The PLS fusing Raman spectra and FTIR spectra collected on M3 UHT-RSM did not improve the model performance ($R^2 = 0.84$ and RASE = 0.79), whereas the one fusing Raman spectra and physicochemical properties enhanced the prediction ability ($R^2 = 0.93$ and RASE = 0.53). The devised approach is advantageous to dairy food manufacturers regarding assessing the functionality of NFDM/SMP for UHT-RSM application.

Key Words: UHT milk, phase stability, prediction model

1106 Design and mastication simulation of orally self-disintegrating milk protein-based puffs for infants and the elderly.

J. Uhrin* and S. Rizvi, *Cornell University, Ithaca, NY.*

The objective of this study was to utilize supercritical fluid extrusion (SCFX) to design milk protein-rich, orally self-disintegrating puffs to address the needs of both infants and the elderly, who require high-protein foods and suffer from swallowing difficulties and often lactose intolerance. To enhance the flavor and fiber profile of the SCFX puffs, 2.5%–10% (dry weight) fruit powders (raspberry and apple pomace powder) were added to the formulations of milk protein concentrate (MPC80) and lactose hydrolyzed skim milk powder (LHSMP). The physio-mechanical properties of the puffs must be accurately evaluated, as the physiological processes occurring in the mouth are quite complex. Generally, mastication is simulated through a compressive strength test that utilizes a constant contact area, which does not well simulate the oral environment. In this study, the compressive strength properties of the puffs were determined considering experimentally measured variable contact areas for water-soaked puffs. The results for Young's modulus showed variance between variable and constant contact area ($P < 0.05$) and Poisson's ratio values close to 1.00, indicating that these puffs deform more like a liquid than a solid. The disintegration properties of the puffs were further measured as structural collapse and evaluated as a measure of change in strain (up to 80%) at a constant force (10.0 N) using a texture analyzer. The SCFX puffs had disintegration times ranging from 22.2 to 39.0 s, which were comparable to the market baby puff (27.4 s). To evaluate consumer acceptability of the SCFX puffs' physio-mechanical properties, a sensory study was conducted (n = 91). The acceptance test showed that incorporation of fruit and dairy powders in puffs either improved or maintained just about right (JAR) scores in 4 of the 5 attributes studied (texture, hardness, flavor, and purchase intent), except sweetness, compared with the market baby puffs. The current study provides valuable insights on the mastication simulation of orally self-disintegrating puffs for infants and the elderly, 2 populations that are difficult to test in a university sensory center.

Key Words: supercritical fluid extrusion (SCFX), puffs, disintegration

1107 Impact of cheese age and composition on the properties of microwaved shelf-stable puff snacks made from Cheddar cheese.

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Microwave heating of certain foods (e.g., popcorn) can produce an expanded, puffed structure. During baking, some cheeses (e.g., Mozzarella) can form expanded structures (e.g., blisters on pizza). However, cheeses during microwave heating usually melt instead of expanding. By altering the fat, moisture, and protein (P) content in cheese by drying, perhaps we can achieve a composition/texture favorable to expansion. A licensed Wisconsin cheesemaker made 3 batches (n = 3) of Cheddar cheese (CC) over a 3-mo period. Cylinders (9 mm high × 13-mm diameter) were cut from CC at 1 wk, 2 wk, and 1, 3, and 6 mo of age. Cylinders were dried for various periods in a ripening room (15.5°C, 70% relative humidity, ~10 m^3/min air flow) to make partially dried cheeses (PDC) with P/moisture/fat compositions (wt/wt) of 31.4/20.4/42.1% (low P; LP), 33.2/15.9/44.3% (medium P; MP), and 34.5/12.1/46.3% (high P; HP). During heating, all PDC did not melt when measured with small-strain oscillatory rheology. The PDC were puffed in a 900-W microwave oven (40–80 s at 100% power) to attain ~1.4% moisture and ~0.2 a_w. A split-plot design was used to analyze the impacts of age, composition, and their interactions on the properties of the puffs. The HP and MP expanded more at 1 wk (to 3.7 and 3.1 mL, respectively) than LP (2.2 mL, $P < 0.05$) when measured with an amaranth seed displacement method. Puff expansion decreased as the age of CC increased. Puff texture was assessed by trained sensory panelists (n = 8–10) using a 15-point quantitative descriptive analysis scale and a texture analyzer equipped with an acoustic detector (TAD). Puff sensory hardness increased as P content of PDC increased, whereas the quantity of TAD force/sound peaks decreased. Puffs made from HP achieved higher AD peaks (~90 dB) than puffs made from LP (~80 dB, $P < 0.05$). Scanning electron micrographs of the puffed cheeses showed defined strands of P in puffs made from MP and HP. We showed a way of making snack puffs from CC. In our system, increasing the ripening time of CC and decreasing the moisture content (while subsequently increasing P/fat contents) of PDC decreased puff expansion.

Key Words: snack, microwave, shelf-stable

1108 Southeastern US consumers' willingness to pay for farmstead cheddar cheese.

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Southeastern US farmstead creameries that process cheese from their farms' milk have increased in size and number since 2019. For these farmstead creameries to be successful, they must understand what consumers will pay for their product. This study estimates southeastern US consumers' willingness to pay (WTP) for 0.45-kg blocks of farmstead cheddar cheese (FSC) using a discrete choice experiment. Processor source, aging time, packaging type, purchase location, production method, and price per pound were used to develop 432 attribute combinations that were condensed to 14 choice scenarios using a fractional factorial design in JMP Pro 17. These choice scenarios were used in a Qualtrics survey that was distributed from October to November 2023, with 605 completed responses. Respondents were ≥18 yr old, primary food shoppers in dairy product-consuming households, and residents of either Tennessee (TN; $n_{tn} = 202$), North Carolina (NC; $n_{nc} = 200$), or Kentucky (KY; $n_{ky} = 203$). Results were analyzed in Stata 18 using mixed logit models and nonlinear combinations to calculate WTP. State and farmstead logos generated premiums ($2.27 and $2.15, respectively; $P < 0.001$) relative to products without source attributes. Grass-fed and all-natural production methods also commanded premiums ($0.85 and $0.80, respectively; $P < 0.001$) when compared with products without a specialty production method. Mild cheddar was the least desired aging time, requiring a $0.72 ($P < 0.001$) discount to purchase, compared with medium cheddar. Southeastern consumers desired a plastic-wrapped FSC with longer aging times and their respective states' promotional program or farmstead logos. Specialty production methods, such as grass-fed and all-natural, and farm-direct purchase sources, such as a farmers' market, farm store, or stand, were also preferred. North Carolina consumers paid the highest attribute premiums compared with TN and KY ($P < 0.001$). Farmstead cheese processors can use this information to refine cheese production, packaging, and marketing to appeal to local consumers and increase sales.

Key Words: value-added, cheese, willingness to pay

1109 Impact of different enzymatic hydrolysis treatments on characteristics of processed cheese products.

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Milk proteins and fat are essential for functionality. However, protein and fat often need to be modified to extract functionality. This research utilized enzymatic hydrolysis as way to enhance the properties of processed cheese products (PCP). This study aimed to evaluate the impact

of lipase and alkaline protease enzymes on the textural and rheological characteristics of PCP using micellar casein concentrate (MCC) and milk protein concentrate (MPC) as base powders. The PCP formulation contained 18% fat, 18% protein, and 50% moisture. These PCP with MCC treatments were categorized as MCC1 without enzymes, MCC2 with 0.8 g/100 g fat of lipase, MCC3 with 0.3 g/100 g protein of protease, and MCC4 with a combination of lipase and protease at the same concentrations. Similarly, PCP with MPC treatments included MPC1 without enzymes, MPC2 with lipase, MPC3 with protease, and MPC4 with both enzymes. Each batch of 700 g of PCP mixture was cooked in a thermomixer for 10 min at 95°C at 2 stirring speeds during the first 2 min and 5 for the last min. The PCP samples were evaluated for the modified Schreiber test (MST), tube melting test (TMT), texture characteristics, and rheological properties (storage modulus G′, loss modulus G″). The data were statistically analyzed using ANOVA, mean comparison was done using Tukey, and the experiment was replicated thrice. The MST results ranged from 50.86 mm^2 for MCC1 to 1917.76 mm^2 for MPC4. Meanwhile, TMT results ranged from 12.20 mm for MCC2 to 104.21 mm for MPC4; however, non-enzymatic treatments (MCC1 and MPC1) did not show any meltability and flow. Enzymatic treatments significantly ($P < 0.05$) increased the melting properties and reduced the hardness, elastic modulus (G′), and viscous modulus (G″) of PCP, particularly in MPC treatments, with the impact differing based on the enzyme type. Protease exhibited a greater influence on decreasing the hardness and increasing the meltability of PCP than lipase. However, combining both enzymes in MCC4 and MPC4 treatments led to more pronounced improvements in the PCP melting properties and texture than using each enzyme separately.

Key Words: enzymatic hydrolysis, processed cheese products, rheological properties

ADSA-USD Competition: Dairy Production Oral Presentations

1110 Oroesophageal tube versus bottle feeding colostrum impacts on immunoglobulin absorption. M. Brady* and E. Eckelkamp, *University of Tennessee, Knoxville, TN.*

During gestation in cattle, immunoglobulins (IgG) are not transferred via the placenta from dam to offspring. This results in calves born with naive immune systems offering little protection from the environment. Calves begin immune system development through the passive transfer of IgG in colostrum. The calf's gut is considered "open" during the first 24 h of life, allowing enterocytes to absorb molecules from the small intestine into the bloodstream. Producers feed colostrum via an oroesophageal tube (OET) or nipple bottle (NB) based on labor, delivery efficiency, and calf comfort. A 2012 study researched whether one method of feeding was superior for IgG absorption. Holstein bull calves (n = 26) were fed colostrum via an OET or NB, and serum levels were analyzed to determine the apparent efficiency of absorption (AEA) of IgG. The NB calves had a mean AEA of 45.2%, whereas OET calves had a mean AEA of 46.8%, with a difference of 1.6%. Feeding method did not significantly affect AEA. The factors that did impact successful passive transfer were IgG concentration and volume. In a 2017 study, Holstein bull calves (n = 20) were fed colostrum replacer through an OET or NB. Feeding method did not significantly affect IgG serum concentrations, and IgG absorption was not impacted. The maximum concentration of serum IgG for NB calves was 24.2 mg/mL and was 24.7 mg/mL for OET calves, with a difference of 0.05 mg/mL. The AEA of IgG was 52.7% for NB calves and 53.2% for OET calves, with a difference of 0.05%. The OET calves had an increase of 0.49 L of whole milk for their first post-colostrum meal compared with NB calves. Feeding method did not significantly affect IgG absorption. However, OET feeding could offer a slight increase of milk intake for the first 3 feedings post-colostrum due to the initial decreased feeding time. The management decision to feed calves colostrum via OET or NB can be determined based on what is most suitable for the individual producer and operation. Both methods are acceptable, and neither impacted long-term calf performance.

Key Words: immunity, calf performance, passive transfer

1111 Aspirin intervention strategies for multiparous fresh cows. K. C. Coffman* and D. O. Olver, *Pennsylvania State University, University Park, PA.*

Many management factors influence the success of a cow's transition period. These include diet formulation, housing strategies, diagnostic accuracy, and drug intervention (Nydamn et al., 2017). Recently more attention and emphasis have been placed on pain management and inflammation. Physiological changes around the time of parturition increase cytokine production by the liver, thus amplifying inflammatory pathways across the body and hindering performance (Trevisi, 2008). Other factors, such as calving ease and age, impact the severity and length of inflammation across the body. These pose different treatment opportunities for producers (Barragan, 2020). Acetylsalicylic acid (ASA) is a common nonsteroidal anti-inflammatory drug (NSAID) used to mitigate pain and inflammation. Although ASA is not routinely given to all multiparous cows after partition, recent studies suggest that the drug's anti-inflammatory properties can enhance animal comfort and performance. Multiparous cows given 2 consecutive oral doses of ASA post-calving produced 1.64 kg/d more milk than untreated multiparous cows (Barragan, 2020). In a separate study, Barragan found that 4 consecutive oral doses of aspirin reduced levels of haptoglobin in the blood, thus reducing localized inflammation and ketosis (Barragan, 2020). Acetylsalicylic acid is an effective agent at reducing inflammatory response in multiparous dairy cattle. However, veterinarian oversight and drug regulation may limit its widespread commercial use (Fritz, 2022). Therefore, a greater emphasis must be placed on future treatment strategies that adhere to FDA guidelines.

Key Words: aspirin, nonsteroidal anti-inflammatory drug (NSAID), inflammation

1112 Pros and cons of feeding transition milk to dairy calves after colostrum feeding. J. Hake*, K. Daniels, and D. Winston, *Virginia Tech, Blacksburg, VA.*

Transition milk (TM) is produced from the second to sixth milkings after calving. TM contains more energy, protein, immunoglobins, and other bioactive compounds than mature milk. Recently, renewed interest has been placed on potential gut function, growth, and health benefits of feeding TM to young (2- to 9-d-old) calves. Researchers at Shiraz University found that when compared with calves fed 4.3 L of milk replacer (MR) per day, calves fed 4.3 L of TM per day for 3 d had increased growth of 0.3 kg/d; this was attributed to TM calves digesting their feed more efficiently. Additionally, the TM calves increased body weight gain, heart girth, and hip height compared with those fed MR. Also found in the study were improvements of calf health. The TM calves had improved eye, ear, and nasal health scores. In controlled research settings, feeding TM to young calves appears to have clear benefits over MR in terms of ability to support health, growth, and gut function. These potential benefits should, however, be weighed against increased efforts related to preservation of the perishable product, variable daily supply of TM, and labor when compared with MR, especially in non-controlled settings. In conclusion, the use of TM can benefit the growth, gut function, and health in calves.

Key Words: transition milk, milk replacer, calves

1113 Liver abscesses in the beef on dairy market. M. Jones* and J. Bohlen, *University of Georgia, Athens, GA.*

The beef on dairy concept has increased in popularity as replacement selection has become more precise and efficient through tools such as genomics and sexed semen. At its onset, mating beef bulls to dairy animals was done with a focus on dairy outcomes more so than beef results. Often sire selection was focused on reduced input costs, which resulted in inconsistent genetic growth and carcass performance. Since that time, sire lines have developed to meet these needs, with carcass profiles improving and becoming known for excellent marbling and high-quality grades. However, the profitability of how these carcasses perform is countered by the costs associated with a major issue in the animals they are sourcing from. This major issue is liver abscesses. Liver abscesses are described as bacterial infection in the liver, which can occur in as many as 70% of beef on dairy crosses. These abscesses represent feedlot costs in poor gains and increased antibiotics usage as well as financial losses to the packer in the form of decreased yields and down time on the production line. Although many research programs are currently investigating the higher incidence risk, there is no singular, clear-cut explanation for the issue. However, there are 2 general lines of thinking that center on the contribution of early, high planes of nutrition and genetic components that increase susceptibility. Within nutrition, the early onset of high grain feeding in the beef on dairy crosses in combination with increased days on feed may predispose these animals to acidosis and ultimately liver abscesses. Variability in feeding practices and liver abscess results suggests that there are additional contributing factors. While there is research into the genetic predisposition of beef cattle populations to liver abscesses, the same work has yet to be completed in dairy cattle. In many cases, packers are no longer willing to take the financial risk that beef on dairy crosses represent. With the dairy industry keen on capturing this value-added component to marketing calves, this topic should be at the forefront of research and management considerations.

Key Words: liver abscess, beef on dairy, replacements

1114 The potential of artificial intelligence for enhanced cow care. S. Lawji*, E. Lindner, and A. De Vries, *University of Florida, Gainesville, FL.*

The world is continuously evolving with advancing technology, modern machinery, increased education, and now artificial intelligence. The dairy industry is constantly in pursuit of strategies to increase efficiency to improve farms not only for economic benefit but also for the welfare of the cows. Recently, farms that have implemented artificial intelligence have been able to better meet nutritional demands, predict potential milk production, and recognize diseases. As part of artificial intelligence, machine learning focuses on algorithms that can learn relationships in data collected by computer vision technology such as 2D and 3D cameras, which can help identity nutritional, reproductive, or overall health needs within the herd. Artificial intelligence allows for preventative action and provides possible solutions to ensure that these animals maintain good health and welfare. Additionally, caretakers can intervene earlier to prevent illnesses, make changes to an animal's routine, or evaluate reproductivity capabilities. Although these technologies can be costly and the proper training to assist with these predictions can be complex, the ability of these algorithms to recognize behavioral changes among individuals in a herd can save time and costs on specialized labor. The implementation of artificial intelligence within the dairy industry has the potential to conserve resources and more precisely meet the needs of the herd to provide more economic success for farms.

Key Words: dairy production, artificial intelligence, animal welfare

Animal Health 1

1115 Effects of breed and colostrum quantity on health and lymphocyte populations in blood of Holstein and crossbred calves. M. Kovacs*[1], H. McCarthy[1], T. Chapelain[1], L. R. Cangiano[2], D. L. Renaud[3], and M. A. Steele[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

The objective of this study was to explore the effects of breed and colostrum quantity on overall health and lymphocyte profiles in the blood of dairy calves during rearing. Male Holstein and Holstein × Angus calves (n = 40; 10/breed/treatment) were enrolled at birth and randomly assigned to either a low (LOW) or a high (HI) quantity of colostrum replacer (CR), which was tube fed at 0 and 12 h. The LOW treatment provided 2.5 g of IgG/kg of body weight (BW), while HI provided 5.0 g of IgG/kg of BW, and both were mixed at 27.5% solids. Calves were then fed milk replacer twice a day at 12% of birth BW for the first 2 weeks and 15% thereafter until weaning, which occurred from d 49 to d 70. Fresh water and a common starter feed were provided ad libitum throughout the experiment. Fecal consistency and respiratory health scores were recorded twice daily to estimate the incidence of disease. Blood was collected from a subset of calves (n = 20) on d 14 and 35 to perform complete blood cell counts, and on d 14, 35, and 84 for lymphocyte isolation and staining with monoclonal antibodies against several γδ T and B cell subsets and analyzed by flow cytometry. Data were analyzed using the GLIMMIX procedures in SAS 9.4, considering the fixed effects of breed, treatment, day, interactions, and the random effect of calf. No breed or treatment differences were detected ($P > 0.10$) for the proportion of days with diarrhea or respiratory disease. The proportion of IgM+ B cells expressing CD21 and CD32 tended to be greater ($P = 0.08$ and 0.05, respectively) in the HI calves; however, there was no impact ($P > 0.10$) of breed for either. The LOW calves tended to have a higher proportion ($P = 0.08$) of γδ T cells expressing WC1.1 than HI; however, WC1.1 was not impacted by breed ($P = 0.25$). The results suggest that the level of colostrum provided in the first 12 h of life impacts the lymphocyte profiles in blood of calves during the rearing phase. However, in this study, breed did not affect overall health and lymphocyte population in calves. A larger population size may be required to further characterize differences in health between breeds.

Key Words: dairy-beef, health, lymphocyte profile

1116 Investigating the IgM and IgG B cell repertoires and expression of ultralong complementarity determining region 3 in colostrum and blood from Holstein cows at calving. T. Altvater-Hughes*[1], H. Hodgins[2], D. Hodgins[1], and B. Mallard[1], *[1]Department of Pathobiology, University of Guelph, Guelph, Ontario, Canada, [2]Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.*

In cattle, maternal antibodies are not transferred across the placenta to the fetus. Instead, important immunological components are transferred through colostrum. Colostral maternal antibodies transfer across the neonatal calf's intestinal epithelium and provide immunological protection. Maternal lymphocytes also cross the intestinal epithelium, but their contributions to neonatal defenses and immune repertoire (IR) development have not been investigated intensely. A subset of bovine B cells in blood expresses ultralong complementarity determining regions 3 (UL-CDR3), = 40 amino acids in length, which occurs in 0.04% to 17.0% of B cells. There is preferential use of variable (V), diversity (D), and joining (J) genes to produce UL-CDR3s (VH1–7, DH8–2, and JH2–4). The objectives of this study were to characterize colostral B cells, identify a subset with heavy chain UL-CDR3s, and compare colostral and blood B cell IR. Blood mononuclear cells and colostral cells were isolated from 8 Holstein dairy cows. RNA was extracted, cDNA was produced, and IgM and IgG transcripts were amplified using polymerase chain reactions. Amplicons were sequenced using the Nanopore MinION with R10.4 flow cells. Blood and colostral percentages of UL-CDR3s were compared using a nonparametric Wilcoxon signed-rank test. In the colostral productive protein B cell IR, the mean percentage of UL-CDR3s for IgM was 7.61% (±1.47 SEM) and 14.65% (±1.71) for IgG. In the blood productive protein B cell IR, the mean percentage of UL-CDR3s for IgM was 4.22% (±0.61) and 7.05% (±1.04) for IgG. In colostrum, there was a significantly greater percentage of IgM B cells with UL-CDR3s compared with blood ($P = 0.05$). There was a significantly greater percentage of IgG B cells in colostrum with UL-CDR3s compared with blood ($P = 0.05$). The usage of the VH1–7 segment reflected the percentages of UL-CDR3s in IgM (6.3% to 27.0%) and IgG colostral B cells (15.1% to 45.3%). In conclusion, there is a population of B cells in colostrum that have UL-CDR3s, with greater percentages of UL-CDR3s in colostrum than blood.

Key Words: B cells, colostrum, sequencing

1117 Investigating gut permeability in neonatal calves with diarrhea. L. S. Zakia*[1], D. E. Gomez[2], M. A. Steele[3], P. D. Constable[4], S. J. LeBlanc[1], and D. L. Renaud[1], *[1]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [2]Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada, [3]Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph, Guelph, Ontario, Canada, [4]College of Veterinary Medicine, University of Illinois, Urbana, IL.*

This case-control study evaluated gut permeability in healthy and diarrheic calves. The study was conducted at a commercial calf-rearing facility, with calves enrolled at 7–21 d old. Fecal consistency was scored twice daily; calves were defined as diarrheic if they had runny or watery feces, or a control if their fecal consistency and physical exam were normal. Cases and controls were matched on the day of arrival at the facility (±1 d). Blood bacterial culture and gut permeability assessment were performed 24 h after the onset of diarrhea. Calves were considered bacteremic if culture was positive. Chromium-EDTA (Cr-EDTA) was administered orally (0.1 g/kg of BW). Blood samples were collected before Cr-EDTA administration, and 2 h and 4 h later. Plasma concentration of Cr was determined by inductively coupled plasma mass spectrometry. Chromium concentration was compared among groups using Wilcoxon signed-rank test and Kruskal-Wallis test, followed by Dunn's post hoc test. Twelve healthy calves and 11 calves with diarrhea were included. Among diarrheic calves, 5 were bacteremic and 6 were non-bacteremic. Pathogens detected on culture were *Clostridium* spp., *Staphylococcus* spp., *Enterococcus* spp., *Pasteurella* spp., and *Streptococcus* spp. Diarrheic calves had higher median (range) plasma Cr concentrations than healthy calves 2 h (176 ppb [43 to 333] vs. 59 ppb [37 to 133]; $P = 0.007$) and 4 h (207 ppb [43 to 333] vs. 92 ppb [57 to 234]; $P = 0.02$) after Cr administration when compared with healthy calves. Plasma Cr concentration at 2 h was higher in both bacteremic (195 ppb [91 to 209]; $P = 0.004$) and non-bacteremic (141 ppb [43 to 333]; $P = 0.04$) calves when compared with healthy; no difference was detected between bacteremic and non-bacteremic calves ($P = 0.20$). Plasma Cr concentration at 4 h was higher in both bacteremic (200 ppb [97 to 209]; $P = 0.06$) and non-bacteremic (245 ppb [47 to 384]; $P = 0.01$) calves when compared with healthy; no difference was detected between bacteremic and non-bacteremic calves ($P = 0.32$). This study shows that gut permeability is increased in calves with diarrhea, but not differentially so whether bacteremia was detected.

Key Words: sepsis, neonatology, scours

1118 Transport duration affects gastrointestinal permeability in preweaned calves.

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The objective of this randomized controlled trial was to determine the effect of transport duration on gastrointestinal (GIT) permeability in preweaning calves. On the day of transportation, male and female Holstein and dairy-beef calves were randomly assigned to 6, 12, and 16 h of continuous transportation by road (n = 25; median 11 d of age, range 3 to 18 d). Upon unloading, calves were weighed and evaluated for signs of dehydration, diarrhea, respiratory disease, and navel inflammation. Chromium-EDTA (Cr-EDTA) was administered orally (0.1 g/kg body weight) followed immediately by collection of a blood sample. Blood samples were also collected at 2 h relative to administration. A mixed linear regression model with repeated measures was built to determine the association between transport duration, body weight, breed, sex, transfer of passive immunity status, and age with Cr-EDTA concentration in serum, accounting for source farm as a fixed effect. In the multivariable model, the interaction between transport duration and time relative to Cr-EDTA administration was significant ($P = 0.05$), where calves transported for 16 h had higher Cr-EDTA concentration 2 h following administration (84.26 ppb, $P = 0.004$, 26.29 to 142.23) compared with calves transported for 6 h. There was no statistical difference between calves transported for 12 h compared with 6 h at 2 h following Cr-EDTA administration ($P = 0.66$). Additionally, for every 1-d increase in age, Cr-EDTA increased by 5.09 ppb ($P = 0.03$, 95% CI = 0.49 to 9.70). The results from this study demonstrate that transport for 16 h is associated with increased gut permeability.

Key Words: dairy beef, surplus calf, intestinal integrity

1119 The effect of meloxicam administration on the health and growth of transported surplus dairy calves.

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The objective of this randomized control trial was to investigate the impacts of administering meloxicam, a non-steroidal anti-inflammatory drug (NSAID), on blood parameters, diarrhea, respiratory disease, and growth of transported calves. Male and female surplus dairy calves approximately 3–10 d of age (n = 160) were enrolled on arrival at a calf-raising facility in Ontario and randomly assigned to receive an injection of either meloxicam (0.5 mg/kg body weight SQ) or saline. A blood sample was collected after unloading to assess transfer of passive immunity (TPI) and at 24 h and 48 h after arrival to determine serum creatine kinase (CK), nonesterified fatty acid (NEFA), and haptoglobin (Hp). Calves were health scored after transport and daily for 13 d for signs of diarrhea and respiratory disease, with the presence of diarrhea determined via a 4-point scale. Calves were weighed after unloading, daily for the first 6 d and again at 14 d. Changes in blood parameters and body weight at each time point were assessed via mixed models with repeated measures. A mixed linear regression model was used to determine variables associated with average daily gain (ADG) in the 13 d after transport. The number of days with an abnormal fecal and respiratory score was evaluated via mixed Poisson regression models. There was no difference in CK, NEFA, Hp, body weight, or ADG between treatment groups. Calves that received saline had an increased incidence of abnormal fecal scores compared with meloxicam calves in the 14 d after transport (IRR 2.90, $P = 0.04$, 95% CI 1.08 to 7.83). There was also an interaction between arrival weight and treatment on the number of days with an abnormal fecal score as larger calves that received meloxicam had a reduced incidence of diarrhea compared with larger calves that received saline (IRR 0.98, $P = 0.03$, 95% CI 0.96 to 1.00). There was no difference in the incidence of respiratory disease between groups. These findings support the use of meloxicam as a deterrent for diarrhea but do not support the use of meloxicam alone as a factor in improving the health and growth of calves following transport.

Key Words: veal calf, meloxicam, transportation

1120 Herd-level prevalence and risk factors associated with respiratory and entero-pathogens from dairy calves in Ontario: A cross-sectional study.

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This cross-sectional herd-level study aimed to determine the occurrence of and risk factors for pathogens associated with neonatal calf diarrhea and bovine respiratory disease on Ontario dairy farms. From April to August 2022, a convenience sample of 100 dairy farms were visited once. At each farm visit, a questionnaire was administered, and 5 calves between 2 and 35 d old were randomly selected for fecal sampling. Also, 5 calves between 21 to 122 d were randomly selected for nasopharyngeal sampling. In total, 363 fecal samples (from 83 dairy farms) and 390 nasopharyngeal swab samples (from 80 dairy farms) were collected. Fecal samples were analyzed individually using a multiplex PCR, whereas nasopharyngeal swabs were analyzed as one pooled sample per farm using bacterial culture and real-time PCR. The most common entero-pathogens detected at herd level were *Cryptosporidium parvum* (67.4%) and *Escherichia coli* K99+ (13.2%), whereas *Pasteurella multocida* (62.5%), bovine coronavirus (42.5%), and *Mycoplasma bovis* (21.2%) were the most common respiratory pathogens. Multivariable logistic models were built to explore the associations between the most common pathogens and herd-level predictors selected from the questionnaire. Presence in the herd of *C. parvum* was positively associated with herds having more than 61 preweaned calves per year and feeding mainly whole milk to calves. Presence of *M. bovis* was positively associated with herds that combined manual and automatic milk-feeding systems, and presence of bovine coronavirus was positively associated with having more than 98 preweaned calves during the year. Univariable Poisson regression models were built to explore the association between the most common pathogens and preweaning calf mortality. Herds that were positive for *C. parvum*, *M. bovis*, or bovine coronavirus had a greater risk of preweaning calf mortality. These results establish the risk factors for high herd-level prevalence of both entero- and respiratory pathogens in Ontario dairy calves and their associations with increased preweaning calf mortality.

Key Words: diarrhea, pneumonia, dairy youngstock

1121 The association of lung consolidation with pathogen shedding, and performance effects in weaned calves.

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The objective of this case-control study was to evaluate the association of lung consolidation (LC) at weaning on the odds of bacterial pathogen shedding (PATHO), and the association of PATHO on the odds of achieving above the 50th percentile for growth performance for beef (Angus, Red Angus, Simmental, Charolais) × Holstein cattle. Blood samples were taken at arrival <5 d to assess for serum total protein status (STP). Calves were fed milk replacer (0.84 kg/d), had ad libitum access to starter and water in buckets, and were weaned at 57 ± 14 d. At 4 ± 2 d post-weaning, calves were moved to a separate rearing facility. The ADG for each calf was calculated between weights: arrival, weaning, 83 d, and 238 d. Then, the top 50th percentile ADG was calculated across the study (HIGHADG). Calves with HIGHADG grew >1.00 kg/d. Thoracic ultrasonography was performed on each calf at 4 d post-weaning to assess calves for LC+ status (0, or LC+ = 1 to 2 cm^2, or = 3 cm^2 in one lobe). Calves that were LC+ (n = 37 case-control pairs) were pair-matched to healthy controls by sex, cohort, and body weight. A triple-guarded nasopharyngeal swab sample was taken from each calf, placed on Amie's charcoal, and shipped for PATHO culture at UW Diagnostics (*P. multocida*, *B. trehalose*, *M. haemolytica*, *H. somni*). Logistic regression models were used to evaluate the odds of LC on PATHO, and the association of PATHO and STP on the odds of HIADG, with the fixed effect of age and sire breed. Calves had *P. multocida* (20 cases, 9 controls) and *B. trehalose* (1 case, 1 control). Healthy calves had lower odds of having PATHO than LC calves at 1 to 2 cm^2 (OR 0.22, 95% CI: 0.11–0.70, $P < 0.001$) or LC at 3 cm^2 (OR 0.11, 95% CI: 0.05–0.26, $P = 0.001$). However, calves with different LC had similar probabilities of PATHO (OR 0.41, 95% CI: 0.16–1.06, $P = 0.64$). There was no association of PATHO with HIADG (OR 0.98, 95% CI: 0.42–2.29, $P = 0.96$), or STP with HIADG (OR 0.60, 95% CI: 0.33–1.12, $P = 0.11$). Therefore, lung consolidation was associated with the probability of a positive bacterial pathology in weaned calves, but pathology was not associated with changes in growth performance to 238 d.

Key Words: health, disease, growth

1122 High-resolution characterization of dairy calves' gut microbiota and identification of diarrhea-associated bacterial markers.

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Gut microbial dysbiosis often leads to calf diarrhea, a major contributor to calf mortality. However, early gut microbiota contains abundant antimicrobial resistance, making treatment difficult. Most previous research relied on partial 16S rRNA gene amplicon sequencing, providing limited taxonomic resolution. The aim of our study is to obtain species-level characterization using long-read sequencing and identify diarrhea-associated bacterial markers using machine learning. We collected fecal samples on d 4, 7, 11, 14, and 30 from 25 Holstein × Angus-cross calves that were housed individually. The calves were fed colostrum on d 1 and milk replacer from d 2 to 14 and transitioned to whole milk from d 15. The gut microbiota was analyzed using full-length 16S rRNA gene amplicon sequencing on GridION. The absolute number of total bacteria and bacteria resistant to cefotaxime were determined by plating. The taxonomic data were analyzed using MicrobiomeAnalyst, LEfSe, and ANOVA. The results showed a significant increase in microbial diversity ($P < 0.001$) with age, but with d 11 and d 14 showing similar diversity, when diarrhea prevalence peaked. Among the fecal scores of those days, 5 calves were consistently healthy and 6 calves were consistently unhealthy. The gut microbiota tended to differ on d 14 ($P = 0.07$) between healthy and diarrheic calves. Diarrheic calves exhibited a higher abundance of the pathogenic bacterium *Fusobacterium mortiferum*, while the commensal bacterium *Megasphaera elsdenii* was enriched in healthy calves. The machine-learning model built using SVM algorithms achieved 80% accuracy, identifying 7 bacterial species for predicting diarrhea. Moreover, the α diversity was significantly greater in diarrheic calves on d 4 ($P = 0.04$). The antimicrobial-resistant bacteria were significantly greater in diarrheic calves on d 14 ($P = 0.01$), and the trend continued into d 30 ($P = 0.06$). Our study provides species-level insights into neonatal dairy calf gut microbiota, indicating early, immediate, and long-term impacts of diarrhea on microbial composition.

Key Words: gut microbiota, calf diarrhea, machine learning

1123 Bacteria colonization and gene expression related to immune function in colon mucosa is associated with growth in neonatal calves.

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As Holstein calves are susceptible to gastrointestinal disorders during the first week of life, understanding the shift of intestinal bacterial colonization and immune function in neonatal calves is important to promote better intestinal health. The objective of this study was to characterize the impact of growth on colon mucosa-attached bacteria and host immune function in the same individual. Colon tissue biopsies were taken from Holstein bull calves (n = 20) within 2 h of life (D0) before the first colostrum feeding and 3 h after the morning milk replacer feeding at d 5 of age (D5) to analyze mucosa-attached bacteria and colon transcriptome. Metagenome sequencing showed that there was no difference in α and β diversity of mucosa-attached bacteria between days, but bacteria related to diarrhea were more abundant in the colon mucosa on D0 compared with D5 (false discovery rate [FDR] <0.05). In addition, qPCR indicated that the absolute abundance of *Escherichia coli* (*E. coli*) decreased in the colon mucosa on D5 compared with D0; however, that of *Bifidobacterium*, *Lactobacillus*, and *Faecalibacterium prausnitzii*, which could competitively exclude *E. coli*, increased in the colon mucosa on D5 compared with D0 (FDR <0.05). Functional analysis of differential expressed genes between D0 and D5 obtained from RNA sequencing suggested that pathways related to viral infection, such as Interferon Signaling, were activated in the colon mucosa of D5 compared with D0 (FDR <0.05). Growth and milk replacer affected mucosa-attached bacteria and host immune function in the colon mucosa during the first 5 d of life in dairy calves. During early life, opportunistic pathogens may decrease due to intestinal environmental changes by beneficial bacteria and/or host immune function. Predicted activation of immune function-related pathways may be the result of host immune function development or suggest other antigens in the intestine during early life. Further studies focusing on the other antigens and host immune function in the colon mucosa are required to better understand intestinal immune function development.

Key Words: RNA sequencing, immunity, calf health

1124 Evaluating the impacts of *Salmonella* Dublin on health and growth factors in surplus dairy calves.

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The objective of this cohort study was to determine whether positivity for *Salmonella* Dublin (S. Dublin) on serum ELISA was associated with health and growth of surplus dairy calves. At a commercial calf-rearing facility in southwestern Ontario, 344 Holstein and 40 crossbred calves, aged 3 to 10 d, had blood collected at arrival to the facility and at 4 wk and 12 wk following arrival. Serum samples were sent to the Animal Health Laboratory at the University of Guelph to determine positivity for S. Dublin using an antibody ELISA. Calves were declared as positive if they had a percent positivity (PP) of >35%. Calves were health scored twice daily for fecal consistency and respiratory score using the UC Davis Respiratory Scoring chart. Calves were weighed at arrival and weekly until departure from the facility at wk 12. A mixed linear regression model was built to assess S. Dublin's association with average daily gain (ADG) from arrival to 84 d, and mixed Poisson models were used to evaluate the number of days spent with a cumulative respiratory score of >5 and abnormal fecal consistency. To assess mortality, a mixed logistic regression model was used. Arrival group was used as a random effect in all models. Overall, 44 (11.5%) calves tested positive for S. Dublin throughout the study, with only 1 calf testing positive at arrival and wk 4 and the remainder being positive at wk 12. Calves that were positive for S. Dublin gained 124.2 g/d ($P < 0.001$; 95% CI: 72.1–176.4) less than calves who did not test positive for S. Dublin. Furthermore, calves positive for S. Dublin had a greater number of days with a respiratory score >5 (incidence rate ratio = 1.76; $P < 0.001$; 95% CI: 1.44–2.15) than calves that were negative. There were no differences detected between S. Dublin positivity and the number of days with an abnormal fecal consistency score ($P = 0.22$) and mortality ($P = 0.99$). This observational study brings awareness of the association of S. Dublin positivity with health and growth of surplus dairy calves.

Key Words: calf health, infectious disease, performance

1125 Test agreement for serum versus milk antibody ELISA for *Salmonella* Dublin.

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Salmonella Dublin (S. Dublin) is an emerging pathogen that can be tested with a commercial ELISA using serum or milk samples, but it is unclear whether the results are comparable. Cut points of 35% or 15% positivity have been used to define S. Dublin positivity. The objective of this study was to measure agreement between milk and serum results from individual animals using a commercial antibody ELISA test (PrioCHECK$^{\text{TM}}$ S. Dublin Strip Kit; Thermo Fisher Scientific). A convenience sample of 4 farms in British Columbia that had 3 bulk tank milk samples test positive for S. Dublin over the last year was selected. Approximately 50% of the lactating herd or 75 cows, whichever was smaller, was selected using random sampling, stratified by parity. Composite milk and blood samples were taken <12 h apart and frozen until processing following manufacturer's instructions (with dilution for serum but not milk). A total of 274 cows were sampled, of which 9.5% and 33.9% were classified positive for S. Dublin on serum and milk tests, respectively, using a cut point of 35% positivity relative to positive control, per the kit protocol. The apparent prevalence increased to 19% (serum) and 50% (milk) when the cut point was lowered to 15%. Lin's correlation coefficient for milk and serum values was 0.67 (95% CI 0.62–0.72). Kappa statistics were calculated to determine the agreement between serum and milk using the recommended 35% or the 15% positivity cut points, and a combination of both. When a cut point of 35% or 15% positivity was used for both milk and serum, the agreement between the 2 tests were 75.6% and 68.3% with kappa of 0.34 (95% CI 0.24–0.44), and 0.37 (95% CI 0.28–0.45), respectively, indicating fair agreement beyond chance. When 35% was used to evaluate milk samples and 15% for serum, there was moderate agreement, kappa = 0.54 (95% CI 0.43–0.64). As performed, results from milk and serum samples should not be used interchangeably, and exploration of optimal dilution or interpretive cut points for serum and milk samples is needed.

Key Words: infectious disease, diagnostics, titers

1126 The efficacy of vaccination and improvements in cleaning in mitigating *Salmonella* Dublin consequences in a Northeastern US heifer-raiser: A mathematical modeling study.

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We developed a mathematical model representing *Salmonella* Dublin transmission dynamics in a heifer raiser to evaluate the efficacy of a theoretical 2-dose vaccine in weaned calves and improvements in cleaning in preventing and controlling *S.* Dublin, and the influence of these strategies on a heifer raiser's operating income. We designed an SIRS model representing the transmission dynamics of *S.* Dublin in a heifer raiser in the Northeastern United States. Stochasticity was incorporated into the model via Monte Carlo simulations. The validated model was used to determine the *S.* Dublin outbreak probability and death, carrier, and abortion rates (per 100 head per year) by the end of a 5-year simulation period in scenarios with and without vaccination in response to infection and increased barn scraping frequency from once weekly (1×/week) to 12×/day. We also conducted an economic assessment of the impacts of these strategies on a heifer raiser's operating income (USD/100 head). Improving cleaning practices decreased the outbreak probability from 99% to 90% when cleaning 12×/day. At this cleaning frequency, the baseline median death, carrier departure, and abortion rates per 100 head/year were reduced from 3.1 (interquartile range [IQR] = 2.4–4.2), 2.4 (IQR = 1.8–3.0), and 3.3 (IQR = 2.1–5.1) to 2.4 (IQR = 1.4–3.5), 2.0 (IQR = 1.3–2.5), and 2.6 (IQR = 1.4–4.3), respectively, while vaccination reduced deaths to 0.9 (IQR = 0.6–1.3) per 100 head/year and slightly increased other rates. Based on the economic assessment, *S.* Dublin infection reduced the median operating income of a heifer raiser from USD 66,871 to USD 56,932 (IQR = 54,517–58,947) per 100 head. Depending on costs of implementation, vaccination improved the median operating income to between USD 58,076 and USD 60,112 per 100 head, whereas improved cleaning had less impact. Vaccine administration mitigated *S.* Dublin's impact on a heifer raiser's operating income by reducing mortality; however, cleaning improvements are also needed to lower its spread within the raiser operations and from these operations to dairy farms.

Key Words: compartmental model, cattle, dairy

Joint CSAS and ADSA Breeding and Genetics Committee Symposium: Beefing Up Dairy—Exploring the Advantages of Beef-on-Dairy

1127 Nutritional management of crossbred calves: Can they be a sustainable source of beef? M. A. Steele*[1], T. Chapelain[1], M. Kovacs[1], H. McCarthy[1], and D. L. Renaud[2], [1]*Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, Ontario Canada.*

In the past decade there has been a dramatic increase in the use of beef sires on dairy cows, efficiently generating more cattle for the world beef sector. Crossbred calves from dairy farms undergo a dramatically different rearing system compared with native beef, especially with respect to nutrition in early life. Native beef is reared with their dams and fed ad libitum milk and weaned after 6 mo of life. Conversely, crossbred calves are separated from their dam at birth, fed restricted levels of milk replacer, weaned before 2 mo of age and offered high-concentrate diets until slaughter. These dramatic differences in early-life nutritional management may lead to large differences later in the life, which may impact meat production and quality and lead to health challenges. Since crossbred calves are reared artificially, there are several opportunities for management intervention throughout life to improve health, productivity, and carcass quality. For example, pregnant dairy cattle can be offered specific nutrients and diets to prenatally program calves for desirable phenotypes compared with the traditional beef cow-calf farm. Furthermore, the macro- and micronutrients of milk and concentrates can be altered to program desirable outcomes in early life and during the finishing period. The opportunities for management interventions with crossbred calves, especially nutritional interventions, provide more flexibility to quickly adapt to market demands, in both product quality and sustainability aspects.

Key Words: crossbred, calf, nutritional management

1128 Insemination trends and the rise in beef and sexed sire semen in North America. A. Fleming*[1], H. Sweett[1], B. Van Doormaal[1], and F. Miglior[1,2], [1]*Lactanet Canada, Guelph, ON, Canada,* [2]*CGIL, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The dairy industry is rich in innovation, influencing the decisions producers need to make to help accelerate their herd sustainability and profitability. The wide availability and improved conception success of sexed semen have contributed to its increased usage within the last decade. In Canada, for the Holstein breed, sexed semen represented approximately 18% of inseminations in 2023, up from 4% in 2016. Sexed semen permits greater control of replacement heifers and improved breeding strategies. Top females in the herd can be bred using sexed semen to greatly increase, to about 95%, the probability that progeny will be female and the best genetics contribute to the next generation of the milking herd. With fewer pregnancies needed to yield replacement dairy heifers, there has been an associated increasing trend in beef sire semen to produce dairy beef crossbreds, translating to higher profits generated through surplus calves. In over 25% of Holstein cow inseminations in 2023 the service sire was a non-dairy breed, with Angus emerging as the dominant beef sire breed used. Traits prioritized in beef sire selection by dairy producers include fertility and calving traits to ensure successful pregnancy and easy calvings. Of lesser importance may be the selection of high genetic merit bulls for growth and carcass traits if there is little motivation to do so. Dairy data collection and storage centers accumulate insemination, fertility, and calving records for beef-on-dairy matings alongside traditional data. These data can be leveraged to discern the performance of sires, existing trends, and the producer's decision-making when applying beef-on-dairy strategies. National dairy genetic evaluation centers typically lack pedigree information and genotypes on beef sires and performance data on the crossbred calves once they leave the dairy. To address this gap, existing systems for beef sire evaluations could be expanded through collaborative efforts, providing dairy producers with essential information to identify optimal beef sire genetics for mutual benefit of the 2 industries.

Key Words: beef-on-dairy, beef semen, sexed semen

1129 Beef-on-dairy? Ireland has been doing that since flip phones were cool. D. P. Berry*, *Teagasc, Fermoy, Co. Cork, Ireland.*

Reasons for the intensifying interest in beef-on-dairy include (1) improving reproductive performance of dairy females necessitating fewer replacements, (2) growing use of X-bearing dairy sexed semen resulting in fewer dairy females required to generate replacements, (3) dairy herd expansion being curtailed or reversed in some territories, (4) exploitation of possible heterosis from crossbred embryos, (5) maintaining resilience to volatility in milk price as well as providing a highly liquid asset, (6) exploiting the greater market opportunities relative to dairy × dairy calves, and (7) mitigating mounting consumer concerns of fate of male dairy calves. The traditional fragmentation of Irish dairy farms also favors the retention of a beef enterprise on farm. Successful beef-on-dairy systems are predicated on a successful national and herd breeding program. All bovine data in Ireland are stored in a centralized database managed by a nonprofit organization, the Irish Cattle Breeding Federation. This enables across-breed genetic evaluations but also provides a single source of the truth for all actors in the beef-on-dairy pipeline. A single, across-breed, national beef-on-dairy breeding index is available on all male beef animals in Ireland; the goal of this index is to help dairy farmers identify beef bulls suitable for mating to their dairy females but also beef seedstock breeders interested in breeding bulls for the dairy market. Through the national infrastructure, dairy farmers have access to a sire advice mating tool which suggests matings between beef bulls and dairy females. A commercial beef value index is generated for each genotyped calf, which is made publicly available at the time of auction. Currently the prediction of future profit of each calf represented by its commercial beef value is based on additive genetic merit. Importantly, though, a strong correlation exists between the beef-on-dairy breeding index used for mating and the commercial beef value of the resulting progeny; the data used to populate both indexes originate from the same reliable source—the difference between the indexes is that the commercial beef value does not contain calving performance.

Key Words: dairy-beef, breeding, genetics

1130 Beef-on-dairy, sexed semen, and IVF. A producer prospective. M. Bowers*, *L'Alliance Boviteq, Saint-Hyacinthe, Quebec, Canada.*

The dairy industry is continuously evolving to meet the growing demand for both milk and beef production. To optimize milk yield while improving the quality and productivity of beef progeny, advanced breeding

strategies play a pivotal role by facilitating the selection of desired traits and accelerating genetic progress within the herd. Sexed semen technology offers precise control over offspring sex, enabling producers to make strategic mating decisions that maximize the production of beef progeny from dairy dams. By using sexed semen, producers can tailor their breeding strategies to meet market demands and increase profitability. This technology has transformed the dairy industry by providing a new level of control over breeding outcomes, allowing producers to reduce the number of replacement heifers and increase the number of beef calves produced from the dairy herd. By examining the producer perspective, we will explore the most commonly used breeding strategies and the role of advanced reproductive technologies, such as sexed semen and IVF. In particular, we aim to provide insights into the opportunities and challenges associated with adopting these advanced breeding techniques in the context of beef-on-dairy systems.

Key Words: beef × dairy, genetics, producers

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1131 Genomic selection for feed-efficient US Holstein cows: Maximizing lifetime efficiency. P. Khanal*, J. Johnson, G. Gouveia, V. Ribeiro, P. Ross, and N. Deeb, *STgenetics, Navasota, TX.*

Residual feed intake (RFI) is an important trait being used in dairy cattle breeding programs. Research toward integration of heifer RFI and cow RFI for selection of animals based on their lifetime feed efficiency is scarce. Therefore, the objectives of this study were to estimate the genetic parameters of RFI and its component traits (dry matter intake [DMI], body weight [BW], and energy-corrected milk [ECM]) in Holstein cows, to develop a system for genomic evaluation for RFI in Holstein cows, and to estimate the genetic correlation between RFI of US Holstein heifers and cows. DMI, ECM, and BW data were collected between 2020 and 2023 from 1,764 first- (n = 1362) or second- (n = 402) lactation cows, from 100 to 240 d in milk, located at STgenetics research farm as a part of the Ecofeed® program. Linear mixed models were used to estimate genetic parameters using LMT software. Pedigree information and genomic information were used to specify genetic relationships to estimate the variance components and genomic estimated breeding values (GEBVs), respectively. Animals with phenotypes and genotypes were used as training population, and 5,000 animals were randomly selected from a pool of Holstein animals with only genotypes to form a testing population. Breeding values of the testing population were estimated using the 2-step approach. RFI ranged from −9.75 to 9.67 kg/d with SD of 1.85 kg of DM/d. Heritability estimates (mean ± SE) of RFI, DMI, ECM, and BW, were 0.43 ± 0.07, 0.44 ± 0.04, 0.40 ± 0.05, and 0.46 ± 0.04, respectively. Average reliability of breeding values for RFI from training and testing populations were 44% and 30%, respectively. There were 1,184 animals with RFI records as heifers collected at the same research station that had also RFI recorded as milking cows. Using this data, the genetic correlation between heifer and cow RFI was 0.30 ± 0.06. Our results showed that the genetic component of RFI is not fully carried over from heifers to cows. We thus recommend considering the RFI of growing heifers in addition to that of lactating cows in genetic evaluation systems aimed at improving lifelong feed efficiency.

Key Words: lifetime feed efficiency, genomic evaluation, dairy cow

1132 Global trends in dairy cattle selection indexes with a focus on feed intake and methane. D. W. Bjelland*, C. M. Richardson, C. D. Quinton, B. F. S. Santos, E. C. Ooi, K. Stachowicz, and P. R. Amer, *AbacusBio Ltd., Dunedin, New Zealand.*

Selection indexes for cattle continue to be updated as new traits are calculated, economic situations shift, and breeding objectives are modified to match the current industry needs. Changes in relation to liveweight, feed intake, and methane inclusion are notable and are a particular focus of this paper. In the New Zealand national index, there has been a penalty on mature cow weight since 1996. The rationale for this has recently been modified, whereby the previous constraint on farm system feed intake has been translated into an additional opportunity cost component of feed. This creates a simplified and more robust structure for the economic weights models. Feed efficiency traits have also recently been added to many national indexes, such as those in the United States, Australia, the Netherlands, and Nordic countries. Recent updates to the US NM$ index included the addition of the trait feed saved, and an increase in the negative selection pressure on cow body size, leading to an expected increase of 0.59 kg more saved feed per cow annually. In the Netherlands, the inclusion of their feed saved trait is expected to reduce maintenance feed costs by €17.70 per cow per generation. Work also continues to produce genetic tools to monitor and reduce methane emissions. So far, index accounting for methane has been in the form of modification of weightings for existing traits, as the lack of phenotypic data available to produce reliable genetic evaluations has been a limiting factor. There are still many unresolved questions around the challenge to define breeding objectives and understand the environmental and economic impacts of the many different alternative definitions of directly measured or predicted proxy methane traits. Overall selection indexes in cattle continue to progress with the inclusion and research around health, welfare, and sustainability traits and the addition of more traits that tend to be favored by specific market niches. This is increasing opportunities to provide more targeted selection though niche indexes and customized index approaches led by private breeding companies rather than national genetic evaluation centers.

Key Words: selection index, methane, feed intake

1133 Impact of Dairy Wellness Profit Dollars (DWP$) index on lactating cow feed efficiency, nitrogen efficiency, and manure excretions: Insights from a RuFaS case study. H. Hu*[1], K. R. Briggs[2], J. Q. Fouts[2], J. Adamchick[3], Y. Gong[4], K. F. Reed[1], B. Fessenden[5], D. J. Weigel[5], and F. A. Di Croce[5], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Dairy Management Inc., Rosemont, IL, [3]Department of Public and Ecosystem Health, Cornell University, Ithaca, NY, [4]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [5]Zoetis Inc., Parsippany, NJ.*

This study evaluates the impact of genetic selection driven by the Dairy Wellness Profit (DWP$) index on lactating cow feed and nitrogen (N) efficiency, and manure excretions through simulation of herds representing high and low DWP$, with the Ruminant Farm Systems (RuFaS) model's animal module. Simulated performance attributes of each herd were defined based on empirical data collected over 6 lactations from the top (high DWP$, n = 266) and bottom (low DWP$, n = 267) 25% of Holstein calves born and ranked via the DWP$ in 2015, including average milk yield (kg of ECM/cow per day, 45.3 vs. 40.7) and body weight (kg, 798.3 ± 68.0 vs. 798.8 ± 74.4). The RuFaS animal module simulates daily events for individual animals, covering feeding, manure excretion, and lactation, among others. The stochastic nature of RuFaS generates probability distributions for event outcomes at the herd level. Daily values per cow for each variable were averaged over the herd and last 365 d. We collected 10 repeated simulation observations for each herd and applied a Mann-Whitney U test. Our results predict increased daily lactating cow manure total solids (kg, $8.81 \pm 1.29 \times 10^{-2}$ vs. $8.46 \pm 2.58 \times 10^{-2}$; $P = 0.0002$), manure N (kg, $0.383 \pm 5.01 \times 10^{-4}$ vs. $0.370 \pm 9.98 \times 10^{-4}$; $P = 0.0002$), and manure phosphorus (P; g, $26.6 \pm 3.72 \times 10^{-2}$ vs. $25.7 \pm 7.29 \times 10^{-2}$; $P = 0.0002$) excretions from the high DWP$ herd. However, smaller relative differences in feed intake and larger relative differences in milk yield lead to higher expected feed ($1.75 \pm 6.66 \times 10^{-3}$ vs. $1.65 \pm 4.40 \times 10^{-3}$; $P = 0.0002$) and N efficiency ($0.427 \pm 1.70 \times 10^{-3}$ vs. $0.401 \pm 1.10 \times 10^{-3}$; $P = 0.0002$), and lower excreted manure N (g, $9.07 \pm 3.82 \times 10^{-2}$ vs. $9.68 \pm 2.76 \times 10^{-2}$; $P = 0.0002$) and P (g, $0.630 \pm 2.73 \times 10^{-3}$ vs. $0.672 \pm 1.91 \times 10^{-3}$; $P = 0.0002$) per kilogram of milk in the high DWP$ herd. Our findings highlight the positive implications of using DWP$ for sustainable and efficient dairy farming. Future work should consider the impact of DWP$ on the performance of growing animals.

Key Words: DWP$ index, RuFaS, N efficiency

1134 Enteric methane production and intensity associated with Dairy Wellness Profit Dollars (DWP$) index ranking.

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The potential for carbon footprint reduction through improved dairy herd genetics has never been quantified. Using the Ruminant Farm System Model (RuFaS), to be launched in 2024, for a novel analysis, our objective was to estimate the difference in enteric methane production (MT/year) and intensity (kg/kg ECM) associated with genetic improvement in a dairy herd. A cohort of Holstein calves born in 2015 were genomically tested and ranked based on the Dairy Wellness Profit (DWP$) index. The bottom (low DWP$, n = 267) and top (high DWP$, n = 266) 25% of animals were distinguished at calving. Cows were tracked through 6 lactations, including average ECM (low DWP$: 40.7 kg ECM/cow per day; high DWP$: 45.3 kg ECM/cow per day), cull rate (low DWP$: 0.35; high DWP$: 0.26), and conception risk (low DWP$: 0.42 cows, 0.56 heifers; high DWP$: 0.45 cows, 0.59 heifers). These records were used to simulate 2 hypothetical 1,000-cow herds and associated youngstock using the animal module of RuFaS, in which individual animals experience daily events consistent with dairy farm management. Event outcomes are stochastic, with probability distributions specified for each herd. The last year of outputs from each of ten 5-year simulations was used to aggregate and summarize results for each herd. Methane emissions from animals sold were allocated to beef production (biophysical method) and discounted from methane intensity values. The higher DWP$ herd averaged 44.4 kg ECM/cow per day (95% CI: 43.9, 44.9), with allocated enteric methane intensity of 0.0132 kg CH_4/kg ECM (0.0128, 0.0136) and total herd enteric methane production of 206.3 MT CH_4/yr (201.4, 211.2). The lower DWP$ herd averaged 40.1 kg ECM/cow per day (95% CI: 39.8, 40.5), with allocated enteric methane intensity of 0.0144 kg CH_4/kg ECM (0.0138, 0.0151) and total herd enteric methane production of 209.1 MT CH_4/yr (199.7, 218.6). These results describe a lower methane intensity in the higher DWP$ herd and, as the first attempt to use a herd simulation model such as RuFaS for this purpose, demonstrate the potential for quantifying the environmental footprint of genetic differences on a dairy farm.

Key Words: DWP$ index, RuFaS, enteric methane

1135 Preliminary analysis of methane emissions traits in US Holstein cows.

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Enteric methane (CH_4) is a major source of greenhouse gas emissions from dairy farming. In dairy cows, CH_4 also represents a loss of 6 to 12% of gross energy intake, energy that could otherwise be available for milk production. Selective breeding is a promising tool for mitigating enteric CH_4 emissions from dairy cattle. We aimed to assess the variability of alternative CH_4 traits and estimate their heritability and phenotypic correlations with feed efficiency. We measured individually CH_4 production (MeP, in g/d), net energy for lactation (NE_L), and body weight (BW) in 303 mid-lactation Holstein cows, alongside dry matter intake (DMI) and residual feed intake (RFI) in 243 of these cows. These measurements were obtained from 8 studies (7 to 10 wk) conducted between April 2023 and January 2024 at the University of Wisconsin–Madison. Methane emissions were measured using GreenFeed. We calculated 2 residual methane traits: (1) residual methane intensity (RMI), defined as MeP regressed on NE_L and metabolic BW, and (2) residual methane yield (RMY), defined as MeP regressed on DMI. On average, CH_4 data consisted of 40 ± 17 records/cow per study, with a minimum duration of 2 min. Interestingly, both RMI and RMY traits exhibited variability, where RMI ranged from −192 to 145 g/d, and RMY ranged from −201 to 152 g/d. The heritability estimates of MeP, RMI, and RMY were 0.47 ± 0.16, 0.35 ± 0.15, and 0.18 ± 0.15, respectively. Methane production was moderately correlated with DMI (r = 0.59), NE_L (r = 0.49), mBW (r = 0.40), and RFI (r = 0.20). We found RMI correlated with RFI (r = 0.29) and DMI (r = 0.12). Furthermore, MeP was correlated with RMI (r = 0.71) and RMY (r = 0.69), and residual methane traits were highly correlated among them (r = 0.94). Overall, our findings suggest that CH_4 traits have considerable variability, moderate heritability, and favorable correlation with feed efficiency. The use of RMI offers a great opportunity for widespread phenotyping of lactating cows, since RMI calculations do not require daily intakes and thus can be performed on commercial dairy farms. Results obtained here will be confirmed using a larger data set as the records available increase monthly.

Key Words: methane emissions, feed efficiency, heritability

1136 A novel scalable, cost-effective method to quantify enteric methane emissions using the cow saliva microbiome.

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Enteric methane emissions (EME) are globally relevant, with 53% of all dairy-related greenhouse gases originating from the rumen itself. Existing methods to quantify EME are time and labor intensive, requiring specialized low-throughput equipment. Rumen microbiome composition has previously been linked with host EME, and the saliva microbiome has been shown to correlate with the rumen microbiome due to rumination behavior. Here we present a novel, cost-effective, and non-invasive method for estimating EME that leverages the saliva microbiome to predict dairy EME. We investigated 16S rRNA-based next-generation sequencing and a mcrA qPCR methodology for their abilities to describe and predict host EME output. Approximately 2,500 rumen and saliva samples were collected from 7 academic herds, along with performance data. For 3 sites (approximately 1,400 samples) EME measurements using GreenFeed systems were also collected. Bacterial populations were sequenced using an Illumina-based 16S rRNA (V1–V3, 300 bp) sequencing, and processed using Qiime (v2.2023.7). A mcrA qPCR assay was also used to link archaeal methanogen abundance and host EME output. Large-scale Dynamical Variational AutoEncoder (DVAE) models were then built on the 16S data set to explore the relationship between microbiome composition and host EME output, from which the top 200 microbes most correlated with and the top 200 microbes most anti-correlated with host EME output were identified via the deep-learning technique GradCAM. An ordinary least squares regression model was then generated that uses the log-ratio of the GradCAM-identified microbes, the cow's energy-corrected milk production, and dry matter intake to predict EME output. Archaeal mcrA abundance was found to correlate with host EME output when accounting for the time between the EME measurement and microbiome sampling ($P < 0.01$). The predictive model's accuracy was validated for both rumen (r = 0.72, $P <$

0.01) and saliva (r = 0.63, $P < 0.01$) data sets, regardless of sampling time. Together, these results demonstrate the feasibility of using saliva microbiome sampling to estimate EME output at the herd level.

Key Words: microbiome, methane, model

1137 Genetic parameters for residual metabolizable energy intake in Holstein calves including maternal effects.

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For dairy farmers, the 2 largest expenses are the animals' feed and the rearing of heifers. Feed efficiency in lactating cows has already been integrated into the genetic evaluation in many countries; however, studies on feed efficiency in dairy calves are still scarce. Additionally, it is unknown whether feed efficiency is influenced by maternal effects in pre-weaning calves. The objective of this study was to estimate genetic parameters of feed efficiency in pre-weaning dairy calves and investigate the significance of maternal genetic effects. The measure of feed efficiency used for the study was residual metabolizable energy intake (RMEI) of 471 Canadian Holstein calves in 2 time periods: the first month of age and the second month of age. Statistical analysis using animal models including maternal effects was performed with ASReml 4.1. Heritabilities ± SE for RMEI were 0.19 ± 0.11 and 0.32 ± 0.12 for first and second months of age, respectively. The estimated genetic correlation, 0.77 ± 0.36, indicated that RMEI in the 2 periods may be considered different traits. Maternal effects significantly ($P = 0.04$) improved model fitting; however, potentially due to the poor structure of the data set, direct and maternal effects showed high negative genetic correlation (−0.88 ± 0.02). Further analysis with more animals and herds, as well as an investigation on the potential relationships with other important traits, should be performed to better understand whether and how this trait could be included in selection decisions. However, moderate heritability estimates show the possibility to select for more feed-efficient calves.

Key Words: feed efficiency, maternal effects, residual metabolizable energy

1138 A genome-wide association study of rumination time in dairy cattle.

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Rumination time (RT) has been reported to be related to health, production, reproduction, and greenhouse gas (GHG) emissions in dairy cattle. Recent studies have estimated moderate heritabilities for RT and suggested that further investigations on the genetic architecture of the trait are still necessary. Genome-wide association studies (GWAS) and quantitative trait loci (QTL) enrichment analyses can be considered options to explore the genetic architecture of complex traits, such as RT. A GWAS on the single-nucleotide polymorphisms (SNP) was performed, followed by enrichment analyses of genes and QTL for RT in 452 mid-first-lactation Canadian Holstein cows. Animals were genotyped with a medium-density SNP panel (50K) and the quality control removed markers located on non-autosomal chromosomes, with minor allele frequencies <5%, and SNP and animals with call rates lower than 90%. The SNP effects were estimated via single-step genomic BLUP (ssGBLUP). A chromosome-wise modified Bonferroni correction, based on the expected number of independent chromosome segments, was used to identify significant markers. A total of 35 SNP were significantly associated with RT and 34 genes mapped within a 50-kbp interval up- and downstream from the SNP, while 19 QTL were found enriched in these genomic regions. From the list of genes, *KCND3* and *DAAM1* related to actomyosin and contractile fiber synthesis. QTL were associated with multiple traits, including fat and protein synthesis and their deposition in milk and muscle tissues, body height, and the level of immunoglobulins in the blood. Investigation of Gene Ontology terms and KEGG pathways showed possible associations of muscle contraction, neural development, neuronal communication, cell structure, and osmotic regulation processes with RT. Our results provide novel information regarding the genetic architecture of RT and a deeper understanding of the complex physiology behind this trait.

Key Words: rumination time, GWAS, genes

1139 Impact of heat stress on dry matter intake in mid-lactation Holstein cows.

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Studying the effects of heat stress on feed consumption, and consequently milk yield, can provide valuable information to identify more efficient and thermotolerant dairy cows. The goal of this study was to estimate genetic parameters of dry matter intake considering heat stress in mid-lactation Holstein cows. Data consisted of 530,046 daily dry matter intake records from 6,710 cows collected on 8 research stations in Florida, Iowa, Maryland, Michigan, and Wisconsin between 2007 and 2023. Heat stress was assessed by calculating first the hourly temperature-humidity index (THI) and then assigning for each day the maximum and average THI. Multi-trait repeatability test day models with random regressions on functions of THI values were used to estimate variance components for dry matter intake, considering the first 3 lactations as different traits. The models included cohort (trial-treatment) and DIM effects as fixed, and general (intercept) and thermotolerance (slope) additive genetic and permanent environmental effects as random. Heritability estimates for dry matter intake at heat stress level (THI = 78) ranged from 0.19 to 0.30 when using average THI and from 0.20 to 0.26 when using maximum THI. For both maximum and average THI, heritability for dry matter intake decreased across parities. These results suggest an important genetic variability underlying feed intake under heat stress conditions. Estimated genetic correlations between thermoneutral (intercept) and thermotolerant (slope) additive effects ranged from −0.08 to −0.44 for average THI and from −0.12 to −0.34 for maximum THI. These results indicate an unfavorable relationship between cows' ability to consume feed under thermoneutral versus thermal stress conditions, suggesting that continued selection without consideration of genetic variation in thermotolerance may lead to greater susceptibility to heat stress.

Key Words: feed consumption, temperature-humidity index, thermotolerance

1140 Beef-on-dairy genomic evaluation for feed efficiency, growth, and carcass traits. V. Ribeiro*, J. Jonhson, Y. Utsunomya, G. Canabrava, P. Kanal, F. Campagnari, P. Ross, and N. Deeb, *STgenetics, Navasota, TX.*

Implementing beef-on-dairy (B×D) strategy to dairy operation farmers can optimize profits while still driving genetic progress. This is achieved by employing sexed-sorted semen on the high-genetics cows to produce all-female replacement heifers, followed by utilizing beef semen on the remaining low-gEBV cows to increase the profitability of the terminal calves. However, the lack of accurate cross-breed performance prediction of pure-bred beef bulls has been a challenge. To identify high-performing beef bulls for B×D programs, STgenetics has implemented a multibreed genomic evaluation using terminal F_1 B×D calves as training population (phenotype and genotype). Several traits were recorded on live animals during the testing period (aged 250 to 500 d, on average) at our Genetic Development Center (Texas), including: dry matter intake (DMI), residual feed intake (RFI), body weight at 150 d (BW150), and average daily gain (ADG). After finishing at a commercial facility (890 lbs body weight, on average), hot carcass weight (HCW), ribeye area (REA), fat thickness (FAT), and marbling score (MBL) were recorded at the processing plant. Numbers of animals ranged from 5,455 for live traits to 3,590 with carcass records. Variance components were estimated using a single-trait animal model, with additive and non-additive effects, while accounting for parental breed genetic variations. Heritability estimates (±SE) of DMI, RFI, BW150, ADG, HCW, REA, FAT, and MBL were 0.21 ± 0.04, 0.18 ± 0.03, 0.24 ± 0.03, 0.29 ± 0.04, 0.29 ± 0.04, 0.32 ± 0.04, 0.33 ± 0.04, and 0.45 ± 0.04, respectively, indicating the viability of these traits as selection criteria to identify the best sires to produce B×D progeny. The reliability average was 62% for bulls with over 10 progenies tested and varied mainly by the number of progeny in the training population; for bulls with no progeny the reliability average was 21%. Considering that beef-bull breeding programs primarily rely on purebred training populations, we will discuss the merits and advantages of using B×D phenotypes for training genomic evaluation models aimed at identifying bulls that will produce high-value B×D calves.

Key Words: beef bull, dairy cow, cross-bred

1141 Efficient implementation of random regression models for dairy cattle genomic evaluations. A. Alvarez Munera*[1], M. Bermann[1], I. Aguilar[2], J. Bauer[3], J. Šplíchal[3], I. Misztal[1], and D. Lourenco[1], *[1]Department of Animal and Dairy Science, University of Georgia, Athens, GA, [2]Instituto Nacional de Investigación Agropecuaria (INIA), Montevideo, Uruguay, [3]Czech Moravian Breeders' Corporation, Hradištko, Czechia.*

Random regression models (RRM) with ssGBLUP are used worldwide for dairy cattle genetic evaluations. However, given the complex structure, challenges include solver convergence, using external information, and approximating reliabilities. This study aimed to improve the efficiency of implementing RRM with ssGBLUP for national dairy evaluations using the BLUPF90 software suite and to test a method to approximate reliabilities with genomic information. We used dairy data from Czechia, including 30 million test-day records for milk yield across 3 lactations. The pedigree had 2.5M animals, of which 55K were genotyped. To improve the convergence of the model, we added a general mean, reduced the number of genetic groups by combining groups with few records, used random genetic groups, and adopted the Algorithm for Proven and Young (APY). Models were solved using the preconditioned conjugate gradient (PCG) algorithm. External information was included as pseudo-phenotypes in the first lactation, calculated as deregressed proofs (DRP) of the cumulative 305-d MACE breeding values. The residual variance for the animals with external proofs was weighted by effective record contributions (ERC) adjusted for double counting and divided by 305. Reliabilities of 305-d GEBV combined the reliability of the average of cumulative 305-d GEBV across the 3 lactations without genomic information with the reliability from a GBLUP model in terms of ERC. All models converged successfully after implementing the strategies. Computing time with APY was 4.76 h, which is 10-fold faster than without it, and the correlation between GEBV with and without APY was 0.99. Approximated reliabilities showed a correlation of 0.94 with those based on the inverse of the MME, and the slope and intercept were 0.03 and 0.95, respectively. The elapsed time to approximate the GEBV reliabilities was less than 20 min. Adding the general mean and redefining genetic groups reduced the number of iterations, whereas using APY reduced computing time. Overall, the proposed modifications make ssGBLUP suitable for dairy evaluations with RRM.

Key Words: longitudinal traits, GEBV reliability, external proofs

1142 Shifting paradigms in daily milk yield correction factors: The DeLorenzo-Wiggans method revisited. X. Wu*[1,2], M. J. Caputo[1], G. R. Wiggans[1], H. D. Norman[1], A. M. Miles[3], C. P. Van Tassell[3], R. L. Baldwin VI[3], J. Burchard[1], and J. Dürr[1], *[1]Council on Dairy Cattle Breeding, Bowie, MD, [2]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [3]USDA Animal Genomics and Improvement Laboratory, Beltsville, MD.*

Various methods have been proposed to derive discrete correction factors for estimating daily milk yields from partial yields. These methods assume constant correction factors within discretized milking interval classes (MIC) but vary between classes. The recent introduction of continuous yield factors promises more accurate yield estimates. This study integrated continuous correction factors into existing methods, demonstrated using the DeLorenzo and Wiggans (D-W) method. This method is a 2-step process. It calculates discrete multiplicative correction factors (MCF) for specific MIC, which are then finalized by a smoothing process. Such an approach often results in computational inefficiency and estimation biases. Our modifications involve replacing separate local regressions with a single global linear regression, circumventing the need for smoothing MCF. The modified D-W model equation is as follows: y_i = F_t × x_i + gamma × (d_i − d_0) + e_i. Here, F_t is an MCF pertaining to time t; y_i, x_i, t, and d_i denote daily milk yield, partial yield, milking interval time (MIT), and days in milk, respectively; gamma is the regression coefficient of days in milk; d_0 is a constant value; and e_i is an error term. For cows milked twice daily, F_(t = t_i) = b_0 + b_1 × t_i, where t_i is MIT for animal i. For more frequently milked cows, F_(t = t_i) = b_0 + b_1 × t_i + b_2 × [t_(t = t_i)]^2. We evaluated the original and modified D-W methods using 7,544 milking records from Holstein cows. The modified method showed, on average, a 1–2% improvement in accuracy over the original, with more significant gains when individual MIT deviated from the midpoint of each class and as the milking interval became more uneven. The modified method fully leverages the available data, provides more accurate estimates, and better captures the relationship between the variables. Further enhancements

are discussed. These findings suggest a shift from discrete to continuous correction factors for yield to improve the computational efficiency and the accuracy of daily yield estimates.

Key Words: yield correction factors, milking interval, polynomial regression

Dairy Foods Symposium: Novel Technologies to Improve the Texture and Flavor of Cheese

1143 Use of membrane concentrates in cheesemaking. S. Govindasamy-Lucey*, *Center for Dairy Research, University of Wisconsin–Madison, Madison, WI.*

Historically there has been lot of interest in using highly concentrated milks for cheesemaking, which would avoid any whey drainage (thus improving yield due to whey protein retention). Those efforts have been largely unsuccessful. Reverse osmosis of milk has been used to concentrate cheese milk but suffers from increasing lactose levels, which raises the risk of excess cheese acidity. Ultrafiltration (UF) has been the membrane concentration approach most widely applied to cheese manufacture, where 3 different forms of UF, categorized on the basis of concentration factor (CF) or degree of milk protein concentration, are used. The most common approach is low-CF UF and is used mainly for milk protein standardization followed by cheesemaking and whey removal using conventional equipment. This has been successfully used in many parts of the world for various cheeses as long as the casein (CN) content is <4%; then only minor modifications are required. Higher levels of CN require more substantial changes, especially in coagulation and cutting phases. Use of highly concentrated UF can result in elevated whey protein in cheese, which can negatively impact ripening. Low-CF microfiltration (MF) has become popular over the last 10 years. It allows the cheese milk to be standardized to a CN content while removing serum proteins as milk-derived whey (which has flavor and functional benefits over traditional cheese whey). As CN content increases, moisture of the cheese is often reduced, unless corrective steps are taken. Similarly, the cheese can be tougher unless preacidfication is employed. Using MF to deplete most whey proteins in cheese milk does not impact the nutritional profile, and more characteristic ripening is observed. The benefits of using low-CF UF or MF include more consistent coagulation, improved yield, and in some cases higher fat or CN recoveries, as well as more consistent products. While concerns remain regarding the impact of using low CF UF or MF for standardizing protein or CN content in cheese milk on cheese quality and manufacturing cost, these approaches are likely to become mainstream because of the greater demand for more consistent cheese composition and quality.

Key Words: ultrafiltration, microfiltration, cheese

1144 Use of lactose standardization: A sustainability tool. R. A. Ibáñez*, *Center for Dairy Research, University of Wisconsin–Madison, Madison, WI.*

In various cheese varieties, fermentation of lactose into lactic acid is needed to target a required pH and thus obtain a desirable appearance, flavor, texture, and functionality. However, the quality of cheese is negatively impacted when excessive acid development occurs. To address this issue, water can be directly added into the cheese vat during manufacture (i.e., curd washing/rinsing, CWR; whey dilution, WD) to remove undesirable lactose from the curd and control final levels of lactic acid. This approach may require a large volume of water (up to 45% of initial cheese milk) and typically leads to cheeses with variable acid development, as this step is influenced by several factors (initial milk composition, temperature of water added, curd particle size). As an alternative, the lactose content of milk can be adjusted prior to cheese manufacture using an approach called lactose standardization (LS), which consists of applying low-concentration factor ultrafiltration (UF) in milk to increase levels of total solids to ~18%, followed by recombining the UF milk retentate with permeate and water to reduce the natural lactose-to-casein ratio (L:CN) found in milk (~1.8:1.0) and effectively control acid development and pH of cheese. The amount of water used in this technique could be lower than in CWR or WD, but its direct application into milk can affect the content and solubilization of Ca, which can modify the texture and melting properties of cheeses. The LS technique can be applied in either regular or concentrated milks (i.e., increased casein content), although modification to cheese manufacture protocols are required to achieve desirable moisture content; can be used to replace CWR/WD in Gouda and Colby cheeses; and can potentially reduce the incidence of common defects, such as calcium lactate crystals (Cheddar) and browning (Mozzarella) due to accumulation of excess lactic acid and sugars, respectively. Standardization of milk to a defined L:CN would allow cheese manufacturers to tightly control acidity and pH of cheeses. From a sustainable perspective, LS could also help reduce the use of water during cheese manufacture.

Key Words: lactose-to-casein ratio, cheese acidity, membrane filtration

1145 Metagenomic insights into cheese ripening and spoilage. G. LaPointe*[1,2], [1]*Dairy at Guelph, Guelph, Ontario, Canada,* [2]*University of Guelph, Guelph, Ontario, Canada.*

Metagenomics is generally defined as the study of the structure and function of all the nucleotide sequences isolated from a community of microorganisms. These sequences can be derived from live or dead microbial cells, or come directly from the sample matrix as eDNA, depending on the DNA extraction procedure applied. On their own, DNA sequences provide a view into the cumulative history of the microbial community from starter dominance in early curd formation to the later development of the non-starter lactic acid bacteria community and extending to phage as well as yeast and fungal sequences. The challenge resides in the computational methods to extract meaning from these sequences and integrate this data with the phenotypic, chemical, or sensory dimensions of cheese ripening and spoilage. Ultimately, with metagenomics, we hope to surpass the limitations of taxonomy to reveal how we can use these microbial functions and sequences to predict the progression of cheese quality. Case studies using metagenomics approaches have been published over the past decade, covering a breadth of cheese types (feta cheese, surface ripened cheeses, artisanal cheeses, washed rind cheeses, Cheddar cheeses), including a longitudinal study of Cheddar cheese over 3 years of ripening. Insights have been generated on the variations among cheese types, aging, countries, geographical regions, and processing conditions and techniques, revealing new species that could be contributing to taste and color. As an example, slit detection has been correlated with low levels of thermoduric heterofermentative *Lactobacillus* by sequencing propidium monoazide-treated DNA. With metagenomics, we can finally achieve near strain-level diversity analysis of specific taxa such as *Lacticaseibacillus paracasei* to predict their individual contributions to sensory parameters and volatile compounds produced during cheese ripening. Through machine learning, the consolidation of multiple sources of data can contribute to computer models potentially able to support the prediction of the optimal investment in ripening time for use by the dairy industry.

Key Words: cheese typicity, defects, microbial ecology

1146 Use of high-casein powders in cheese. P. Salunke*, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Caseins (CN) are predominant milk proteins and play a significant role in cheese. Increasing CN in cheese milk is directly correlated with yield and quality. Additionally, many cheese manufacturers are interested in changing functional properties such as firmness, meltability, and other properties of cheeses. Hence, cheese manufacturers are interested in increasing CN in cheese milk through concentrated milk protein, including high-casein powders (HCP). Although there is no clear definition of HCP, ingredients with varying CN (18–92%) content are available, including whole or skim milk powder, nonfat dry milk, milk protein or micellar casein concentrate, milk protein isolate, rennet or acid casein powder, buttermilk powder, and other modified powders such as calcium or sodium caseinate. Each manufacturing technique yields HCP with unique constituents, including micellar CN, calcium, serum proteins, and lactose, which affect functionalities and may limit its usage in cheese production. Fresh or stored HCP and its functionality, especially solubility, play a significant role in cheese manufacturing. Depending on the type of cheese, the HCP can be added directly to the milk or curd during processing. For natural cheese manufacturing, the higher protein in cheese milk requires the processing and handling parameters to be modified or manipulated depending on the type of HCP used such as changes in pH, coagulation time, cutting time, moisture retention, curd handling, ripening, flavor development, and more. Increased protein content produces tough cheese curd and has higher fat loss in whey. However, incorporating HCP in cheeses such as soft cheese, direct acid and heat-coagulated cheeses, imitation, or processed cheese products is more manageable, and many cheeses can be manufactured using 100% HCP. Since the cheese curd can be tough, the curd handling equipment may need modification. Adding HCP may require labeling to be modified. Many HCP flavors may be transferred, impacting cheese sensory properties. The HCP may be preferred for cheese-type products in 3D-printed cheese applications. In conclusion, the proper selection of HCP and manipulation of cheese-making parameters can produce good-quality cheese.

Key Words: high-casein powder, cheese, quality

1147 New instrumental techniques and approaches in understanding machinability of cheese. P. Sharma*, *Nutrition, Dietetics and Food Science Department, Utah State University, Logan, UT.*

Millions of kilograms of Cheddar cheese are sold in the United States each year. The cheese industry in the United States is growing at a 16% compound annual growth rate. In many cases, Cheddar cheese is sliced or shredded to use in various applications such as restaurant services and culinary dishes. During the process of slicing or shredding, problems such as loss of material can arise from cheese sticking to the moving parts of machinery or crumbling during high-speed operations. This wearing phenomenon can incur significant loss to cheese manufacturing operations. Currently, there is no objective way to determine or predict attributes of the machinability of Cheddar cheese from which these problems may arise. Sliceability is the ability of cheese to cut cleanly into thin slices, to resist breakage or fracture at slice edges, and also to undergo a high level of bending before breaking. On the other hand, shreddability is a comparable property to sliceability that includes several physical attributes such as ease of machinability of cheese, shape and integrity of shreds, absence of shredding defects such as propensity of shreds to mat or clump, and excessive production of fines. Intuitively, sliceability depends on the chemistry, microstructural, and rheological properties of the casein network. Good shredding or slicing behavior is observed in a relatively narrow range of textural properties and is not fully understood, or controlled, by cheese manufacturers. Poor performance of cheese during shredding or slicing would incur significant revenue loss to the cheese manufacturers. Understanding material response in a variety of conditions has been a subject of research in the past. Tribological (wear) behavior is believed to be closely related to machinability of cheese and can be used as a tool to predict slicing/shredding performance of Cheddar cheese. Similarly, tack force and tack energy measurements can be used to predict the tendency of cheese to stick to a metal blade. This talk will cover novel instrumental techniques and approaches in determining machinability of cheese.

Key Words: instrumental techniques, cheese, machinability

Joint ARPAS and ADSA Growth and Development Committee Symposium: From Birth to Lactation

1148 Connecting the dots: Calving ease, age at first calving, and enhanced cow production. M. I. Marcondes*[1], J. C. C. Chagas[2], A. F. Kertz[3], T. Allen[4], and J. M. Bewley[5], *[1]Washington State University, Pullman, WA, [2]Swedish University of Agricultural Sciences, Umeå, Sweden, [3]Andhil LLC, St. Louis, MO, [4]Agritech Analytics, Visalia, CA, [5]Holstein Association USA, Brattleboro, VT.*

Calving events are critical moments for dairies, with complications such as calving ease (CE) posing risks to cow performance and calf growth and development. A modeling approach was used to investigate relationships among CE, age at first calving (AFC), and production. Data came from 687 dairy farms (USA), comprising 1,048,574 CE observations (2017 to 2022). The CE was scored from 1 to 5 (Djemali et al., 1987). Breeds other than Holstein, Jersey, and dairy crosses (XD) were excluded. Outliers were excluded at 1% tails of data distribution, resulting in 794,870 CE. Lactation number (LN) was categorized as primiparous or multiparous. The study followed 3 steps. First, we evaluated the effect of CE on 305-d milk production (MP), energy-corrected milk (ECM), fat and protein, and milk at lactation peak (MLP). We used CE, LN, calf sex (CS), and dam's breed as fixed effects, and calving year (CY), calving season (CSA), and herd as random effects. Second, an AFC database was used following the same criteria but keeping only primiparous cows (90,574 CE observations). For this step, AFC was adopted as linear and quadratic covariates, evaluating the same parameters as in the first step and considering the same fixed and random effects. In step 3, CE was studied as the response variable using the step 2 database, where AFC, CS, and breed were adopted as fixed effects, and CY, CSA, and herd as random effects. In step 1, the effect of CE and its interaction was observed with LN, breed, and CS for MP, ECM, fat and protein, and MLP ($P < 0.01$). Milk production and components were impaired by CE >2, and the lowest values were observed for cows scored CE = 4. The lowest MLP was observed for Holstein, Jersey, and XD scored CE = 4 (43.1, 35.8, 39.2 kg/d). In step 2, linear and quadratic components of AFC adopted as covariates were significant ($P < 0.01$). As a result, previously observed CE and their interactions were absorbed by the covariates. In step 3, CE was quadratically affected by AFC while also affected by breed and sex. Cows calving males showed higher CE, and the lowest CE was observed for Holstein at 27 and Jersey at 22 mo AFC.

Key Words: calving difficulty, milk production, modeling

1149 Exploring symbolic regression for body weight prediction in dairy calves. E. Casella*, G. J. M. Rosa, and J. R. R. Dorea, *Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Monitoring heifer growth development is an important aspect of decision making in dairy farms, as a tool for health assessments, feed management, and breeding selection. Monitoring growth requires frequent body weight (BW) measurements; however, weighing calves is costly and labor-intensive. Computer vision and machine learning (ML) have been proposed as a powerful tool to predict BW in livestock animals. However, most ML techniques involve tuning a large number of parameters that increase the risk of overfitting in small data sets. Besides, these models lack interpretability and do not reveal biological associations between predictors and response variables. Symbolic regression (SR) is a ML approach that leverages genetic algorithms to find analytical equations and their coefficients to describe the output based on the input variables. In this study, we evaluated the use of SR versus gradient boosting trees (GBT) to predict BW in 67 pre-weaning Holstein dairy calves from 2 to 8 weeks of age, and BW of 57.0 ± 14.7 kg. A total of 400 3D images were captured from the dorsal area (top-down view) during weighing on a digital scale, and we extracted 27 biometric features from depth images (area, volume, length, 11 heights and widths along the dorsal area, eccentricity, and extent). Both SR and GBT were evaluated against observed BW using a nested cross-validation (5-fold for hyperparameter tuning and leave-one-out for testing). GBT achieved root mean squared error of prediction (RMSEP) = 7.7 kg, mean absolute error (MAE) = 5.8 kg, $R^2 = 0.59$, and concordance correlation coefficient (CCC) = 0.78. Among the frequently high-ranked equations we found (1) $BW = a + b \times Volume$, (2) $BW = \sqrt{Area} + Width6$, and (3) $BW = (Width5 + c)^d$. The median values for a, b, c, and d were 31.53, 0.22, 12.14, and 1.16. SR presented better predictive performance than GBT, with (1) presenting RMSEP = 6.0, MAE = 4.9, $R^2 = 0.67$, and CCC = 0.86. While further investigations are needed on more heterogeneous data sets, SR shows the potential to predict BW using simple linear and nonlinear equations that may generalize well, with low computational cost, and with the benefit of interpretability.

Key Words: body weight prediction, computer vision, interpretability

1150 Factors influencing colostrum production in multiparous Holstein and Jersey cows at multiple locations. P. S. Erickson*, *University of New Hampshire, Durham, NH.*

Feeding adequate amounts of good-quality colostrum after parturition is essential to the health and production of the future dairy herd. Colostrum quality, amount fed, and feeding time are known factors that have far-reaching impacts on animal performance. However, recently it has come to light that some Holstein and Jersey cows underproduce colostrum. Therefore, the purpose of this review is to evaluate the current research regarding factors influencing colostrum yield, quality, and immunoglobulin G (IgG) yield. Regression models were developed using environmental factors and previous lactation data including previous lactation 305 ME milk yield, milk component content and yield, and somatic cell count. Management factors included were previous days open and days dry, ordinal day, time of colostrum harvest relative to calving, parity, diet characteristics, number of dry cow feedings per day, and amount of time grazing. Farm-specific factors included the latitude of the farm and herd size. Specific to Holstein cows the number of days above the thermoneutral zone increased colostrum yield ($P < 0.01$) but negatively affected IgG/L ($P < 0.01$). Results for Jersey cows indicated that previous days open and previous days dry were positively associated with colostrum and IgG yields ($P < 0.02$). While the latitude of the farm was negatively associated with colostrum and IgG yields ($P < 0.01$). The respective models' ability to predict their respective outputs in comparison to actual colostrum production values will be presented. The results of this review may direct researchers to areas of study that will help decrease this colostrum production challenge.

Key Words: colostrum, Jersey, Holstein

1151 Unlocking the "potential" of bovine fetal mammary stem cells. T. Podles, H. Burchard, and A. Schiffmacher*, *University of Maryland, College Park, MD.*

Bovine mammary stem cell technologies can facilitate new methods to advance dairy animal health, growth, and performance. As early fetal mammary epithelial cells exhibit multipotency and give rise to all adult mammary gland tissues, we hypothesize that understanding fetal mammary development is critical for advancing bovine mammary stem cell research. Our objectives are to (1) identify gene regulatory networks governing fetal bovine mammary stem cell potential and (2) develop mammary stem cell tools that will benefit animal health and production. To ascertain when the stem cell niche forms, fetal bovine mammary tissues purchased from Applied Reproductive Technology (Madison, WI) were microdissected and partitioned into experimental groups by stage and crown-rump length (CRL, n = 5 fetal tissues per stage: hillock/bud, 21–88 mm; bud/teat, 81–120 mm; primary sprout, 120–160 mm; and secondary sprout, 161–230 mm). TRP63 and cytokeratin (14, 5, and 8) immunolabeling indicate that luminal/basal lineage segregation occurs before sprouting when P63+ cells segregate to the outer layer and inside luminal cells exhibit reduced TRP63 and CK14 immunolabeling relative to outer cells (no difference vs. differential inner/outer layer immunolabeling, chi square analysis, PROC FREQ, SAS). Multiplex fluorescent RNA in situ hybridization experiments reveal that early bovine mammary bud cells co-express specification (LEF1, SOX11), luminal (SOX9, ELF5), and basal (TRP63, LMO4) factors, indicating that they remain uncommitted yet transcriptionally primed for differentiation. Cell lines derived from fetal mammary epithelia (9 primary lines, 50 mm to 170 mm CRL) were generated via conditional reprogramming culture (irradiated 3T3J2 cells and 10 μM Y-27632 ROCK inhibitor). Lines have been propagated over 5 passages (5- to 7-d passage frequency). Together, this work will allow us to characterize fetal mammary stem cell stemness and provide new models for understanding mammary reprogramming due to gestational influences (heat stress, malnutrition).

Key Words: mammary stem cell, fetal development, multipotency

1152 The weaning transition in dairy calves: Why so traumatic?

J. K. Drackley*, *University of Illinois Urbana-Champaign, Urbana, IL.*

The weaning transition imposes many stressors on dairy calves, often compromising welfare and performance. These stressors include nutritional inadequacy, along with environmental and social stresses. According to the National Animal Health Monitoring System (2014), producers are feeding more milk, and death loss has decreased in pre-weaning calves, but growth post-weaning is often sub-optimal. Diseases such as respiratory disease and coccidiosis are common in newly weaned calves. Why is this period so often traumatic for the calf? The proportion of empty BW to BW decreases during the weaning transition, so that gut fill increase may contribute over 25% of measured BW gain. The gastrointestinal tract mass increases allometrically during the weaning transition, requiring large quantities of energy and amino acids. Starter intake before weaning is the largest determinant of growth and health post-weaning. Intake of starter drives rumen microbial and papillae development. Typical guidelines for adequate starter intake to allow weaning may only cover maintenance requirements. With larger volumes of milk fed before weaning, starter intake, and hence rumen development, is slower to develop than in situations in which less milk is fed. Key factors to help avoid poor weaning outcomes by having adequate rumen development include not weaning too early, weaning gradually, not allowing free access to alfalfa forage, feeding a high-quality starter, good water management, and not stacking stressors at weaning.

Key Words: weaning, starter feed, rumen development

Production, Management, and the Environment Symposium: Revisiting Progress in Feed Efficiency—Its Role in Advancing Dairy Sustainability

1153 Phenotypic and genomic relationships between energy efficiency and protein efficiency among dairy cows. M. J. VandeHaar* and E. Liu, *Michigan State University, East Lansing, MI.*

Feed efficiency is a function of level of production, differences in digestion and metabolism among cows, diet composition, and management. As dairy cows produce more milk on the same diet, milk energy per unit feed energy and milk protein per unit feed protein also increase because feed needs for maintenance are diluted by feed needs for production. Within a level of production, some cows use feed more efficiently than others; they need less feed than expected and have a negative residual feed intake (RFI). We recently showed that cows with negative RFI also use protein more efficiently. In animal breeding, both level of production and RFI are used in genetic selection, and selecting for energy efficiency will increase protein efficiency. In animal feeding, however, to maximize energy efficiency, dietary protein must not be limiting, and to maximize protein efficiency, no protein fraction should be in excess and instead metabolizable protein must be limiting. When cows are fed excess energy, they store the excess, but when cows are fed excess protein, they waste the excess, and this waste decreases not only protein efficiency but also energy efficiency. In the future, to improve dairy sustainability, we should continue to select for feed efficiency in breeding programs, feed to maximize milk production and health, and continue to refine protein requirements. We should also feed cows according to their needs by grouping or computerized supplementation and perhaps select cows that have lower protein requirements. By selecting for greater feed efficiency and production, methane emissions per unit milk will continue to decrease, although not dramatically. However, even if feed additives cause dramatic reductions in methane, cows will continue to produce methane. One justification for a continued thriving dairy sector is that cows can convert ecological leftovers into high-quality food for humans, and thus increase net food production and sustainability. The earth can feed more people with a thriving and efficient dairy sector than without one. We should do more to communicate this positive message to consumers.

Key Words: efficiency, genetics, nutrition

1154 The Resilient Dairy Genome Project synthesis—Overview of feed efficiency and methane emissions. C. Baes*[1,2], D. Hailemariam[3], G. Kistemaker[4], F. Miglior[1,4], F. Schenkel[1], A. Butty[5], E. Abdala[6], J. Lassen[7], O. Gonzáles-Recio[8], K. Parker-Gaddis[9], J. Koltes[10], F. Peñagaricano[11], M. VandeHaar[12], K. Weigel[11], H. White[11], *[1]University of Guelph, Guelph, Ontario, Canada, [2]University of Bern, Bern, Bern, Switzerland, [3]University of Alberta, Edmonton, Alberta, Canada, [4]Lactanet, Guelph, Ontario, Canada, [5]Qualitas AG, Zug, Zug, Switzerland, [6]VIT, Verden, Hessen, Germany, [7]Aarhus University, Aarhus, Denmark, [8]INIA, Madrid, Spain, [9]CDCB, Bowie, MD, [10]Iowa State University, Ames, IA, [11]University of Wisconsin–Madison, Madison, WI, [12]Michigan State University, East Lansing, MI.*

The demand for dairy products is increasing; however, the dairy industry is under growing scrutiny with regard to potential issues related to human and animal health, environmental impacts, sustainability, and overall social acceptability. The Resilient Dairy Genome Project (RDGP) is a significant international effort involving researcher and industry partners. The primary objective of the RDGP is to leverage genomic techniques to bolster the resilience of dairy cattle. To achieve this goal, the project focuses on collecting and analyzing phenotypic data in key areas such as fertility, disease resistance, and environmental efficiency. Within this project, resilience is defined as the capacity of an animal to adapt rapidly to changing environmental conditions, without compromising its productivity, health or fertility, while becoming more resource-efficient and reducing its environmental burden. The collection and analysis of phenotypes in the key areas of "closer-to-biology" fertility (e.g., estrous expression and embryo survival), enhanced disease resistance (e.g., fertility disorders, Johne's disease, leukosis, and calf health), and environmental efficiency (e.g., feed efficiency and methane emissions) contribute to this definition of resilience. Here we present a synthesis of this large-scale project, including an overview of various analyses involving interactions between genes and environment in the context of dairy cattle resiliency.

Key Words: genomics, methane, feed efficiency

1155 Interplay of rumen microbiome and host modulating feed efficiency and carbon emission in cattle. T. A. McAllister*[1,2], R. J. Gruninger[1], S. A. Terry[1], and L. L. Guan[2], *[1]Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, Canada, [2]University of British Columbia, Faculty of Land and Food Systems, Vancouver, BC, Canada.*

As the majority of energy and protein supplied to cattle arises as a result of ruminal fermentation, the rumen microbiome plays an integral role in determining host feed efficiency. Counterintuitively, current evidence suggests that a less diverse rumen microbiome is associated with improved feed efficiency, possibly as a result of greater metabolic precision and avoidance of energy spilling fermentative pathways. The composition of the rumen microbiome is mainly determined by diet, but host traits such as rumen volume, rate of passage, rumination, and immunity also have influence. Although less microbial diversity may improve feed efficiency in cattle fed a specific diet, reduced diversity may impair the ability of cattle to adapt to frequent changes in diet and the environment. Hydrogen exchange and capture is the energetic foundation of the rumen microbiome, and considerable capital has been invested to develop additives that redirect hydrogen flow away from the reduction of CO_2 to CH_4 and toward alternative sinks. These additives have been shown to reduce enteric CH_4 emissions by 30–80%, but improvements in feed efficiency have been less than the stoichiometric predictions. Approaches to improve the feed efficiency of cattle need to be multifaceted with consideration for host genetics, functional efficiency of the rumen microbiome, and the structure and composition of feed. Likewise, reductions in carbon emissions need to be broader than just CH_4, with an appreciation of how cattle can play a role within a circular bioeconomy to promote upcycling of nutrients and reductions in emissions from a farming systems perspective. Strategies to improve the efficiency of cattle production are a prerequisite for the sustainable intensification needed to ensure that the social license for milk and meat production from cattle is retained.

Key Words: efficiency, microbiome, systems

1156 The role of production systems in feed efficiency and carbon emissions. A. F. Brito*, *University of New Hampshire, Durham, NH.*

Evaluation of feed efficiency (calculated as ECM yield/DMI) and milk N efficiency (calculated as milk N yield/N intake) is needed to help dairy producers making decisions about the economic and environmental sustainability of their farms. It is well known that feed efficiency and milk N efficiency are both affected by production level and management practices, with high-output confinement systems yielding better efficiency metrics compared with their pasture-based counterparts. Furthermore, CH_4 yield (g/kg of DMI) and CH_4 intensity (g/kg of ECM) generally decrease in dairy cows fed TMR compared with pasture despite these responses being heavily influenced by the type of the grazing system and pasture nutritive value. Note that pasture-based systems perform multiple ecosystem services including carbon sequestration and improved biodiversity, which is not routinely quantified in typical life cycle analyses (LCA). The overarching objective of this symposium paper is to present results of feed efficiency, milk N efficiency, and enteric CH_4 emissions of dairy cows under different production systems (i.e., confinement, pasture-based, mixed). A second objective is to present whole-farm greenhouse gas emissions data from LCA studies published in the literature, highlighting the range in carbon footprint across various production and management systems. Specifically, greenhouse gas emissions and climate change mitigation potential of emerging production systems such as organic grass-fed dairy and silvopasture will be assessed. Factors including farm size, herd size, region, and animal breed relative to efficiency and carbon emissions will be covered as well.

Key Words: feed efficiency, methane, sustainability

Joint Ruminant Nutrition, Physiology and Endocrinology, and Lactation Biology Symposium: Update on the Arterial-Venous Approach and Application to Current Unanswered Questions

1157 What mammary flux has taught us about amino acid and protein metabolism in dairy cows. H. Lapierre*[1], R. Martineau[1], S. Lemosquet[2], G. E. Lobley[3], and D. R. Ouellet[1], [1]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [2]*PEGASE, INRAE, Institut Agro, Saint Gilles, France,* [3]*Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK.*

In dairy cows, milk protein yield (MPY) is the major net user of absorbed AA, but fates of individual AA vary across the mammary gland (MG). Mammary net fluxes have been used to classify EAA transfers into MPY. The MG net uptake of Group 1 EAA (His, Met, Phe+Tyr, Trp) is approximately equal to MPY, whereas net uptake of Group 2 EAA (Ile, Leu, Val, Lys) exceeds MPY, with the excess being variable. This reflects the site of EAA catabolism, from liver catabolism (Group 1) to oxidation in peripheral tissues, including MG (Group 2). For example, post-liver supplies of Lys and Met averaged 97 and 66% of their net portal absorption, whereas their MG uptake:output ratios (U:O) averaged, respectively, 1.15 and 0.98, indicating extensive Lys MG catabolism (n = 43, 14 studies). Mammary uptake of EAA can also increase with additional energy supply, but the U:O of Group 1 EAA is maintained at unity whereas the U:O of Group 2 EAA decreases, yet remains above unity. Increased MPY can be supported by greater arterio-venous difference (increased EAA supply) or by increased blood flow to the MG (extra energy provision). In contrast, for most NEAA, except for Ala, Gln, and Tyr, the U:O is usually less than 0.75, indicating important NEAA MG synthesis to support the MPY. To understand the dynamic mechanisms involved requires use of nutrients labeled with stable isotopes. For example, ^{13}C-Leu used for MG protein synthesis (PS) averages 40% of whole body (WB) PS, with approximately 75% of this PS appearing as MPY. The excess of Leu taken up is oxidized within the MG, and contributes 40 to 50% of WB Leu oxidation. There is no mammary oxidation of Phe. The ^{15}N of Lys taken up in excess of MPY is transferred to various NEAA: these products contribute to PS, including MPY, or are released into MG venous blood. Overall, the MG demand drives much of EAA metabolism but is very flexible and can switch between substrates to maintain MPY. What are the limitations to such flexibility? Resolution would require more studies combining measurements of MG fluxes using isotopes and of expression of genes related to AA metabolism and PS.

Key Words: uptake, milk, protein synthesis

1158 *ADSA-EAAP Speaker Exchange Presentation:* What have measurements of splanchnic flux taught us about ruminant gastrointestinal physiology? C. K. Reynolds*[1], L. A. Crompton[1], and M. Larsen[2], [1]*University of Reading, Reading, United Kingdom,* [2]*Aarhus University, Foulum, Denmark.*

There is a long history of research using measurements of venous-arterial concentration differences (VA) to describe ruminant splanchnic (portal-drained viscera [PDV] and liver) physiology and metabolism, from pioneering research with anaesthetized sheep, to long-term studies with high-yielding dairy cows. High rates of metabolism of PDV (gastrointestinal tract [GIT], spleen, pancreas, and associated adipose) and liver tissues are mirrored by high blood flow and O_2 consumption and thus substantial impacts on diet energy use. Measurements have quantified amounts of nutrients absorbed and available for productive purposes, as well as challenging dogma, in some cases regarding interpretation of results. The VA alone tell little about rates of metabolism unless coupled with blood flow to calculate flux; however, net flux reflects combined rates of a metabolite's uptake and release. Greater insight is gained when VA and blood flow are coupled with isotopic labeling to measure unidirectional flux, which has challenged the concept of substantial "first-pass" absorptive metabolism of acetate, glucose, and individual amino acids (AA). Changes in liver uptake of AA relative to glucose release in early lactation has also challenged the dogma of essential AA being required to meet glucose demands of high milk yields, as only alanine, with lactate, have greater relative contributions to glucose precursor supply. The integration of splanchnic and mammary gland fluxes is a powerful approach. Linking splanchnic flux with digestion and body energy and N balance, through mathematical models, also provides invaluable insights and a framework for revisions to nutrient requirement systems. Splanchnic tissues are a rich source of regulatory peptides, with neural connections integrating metabolism of the GIT, pancreas, liver, and other body tissues. Future studies should consider less invasive surgical procedures, as well as novel analytical approaches for measuring blood components and regulatory peptides integrating nutrient metabolism of the splanchnic tissues with requirements for production and maintenance.

Key Words: absorption, metabolism, flux

1159 Modeling arteriovenous difference data: Benefits and opportunities. M. D. Hanigan*, *Virginia Tech, Blacksburg, VA.*

For tissues with discrete arterial or venous blood supplies, the arterio-venous (AV) difference technique can be used to assess mass balance of individual metabolites across the tissue. When combined with mass balance data from the animal, one can determine the fraction of a nutrient supplied by the diet that is utilized by the tissue. When combined with observations from other tissues, a picture of metabolite flow through the post-absorptive system under varying circumstances can be assembled. However, AV is a net difference observation, and it does not provide unidirectional information for fluxes that have bi-directional flows, nor does it directly identify the pathways used to metabolize the metabolite. If one combines the technique with stoichiometry-based, mass balance models, the set of balanced equations greatly constrains the solution space allowing derivation of net flux rates for a range of pathways. Selective use of isotopic tracers with steady-state or nonsteady-state sampling allows the identification of additional pathway fluxes, and, if collected over time, bi-directional fluxes often can be parsed into unidirectional rates. Where the isotope is infused until steady-state, the models can often be solved algebraically. More complicated models or those applied to nonsteady-state conditions can be solved numerically. The functional flux data derived from these efforts can be combined with molecular observations of gene expression, RNA translation, post-translational modifications, and systemic observations of physiological, hormonal, and neural states to generate a complete picture of tissue metabolism and regulation under a range of feeding and management conditions. Challenges that must be weighed against the power of these approaches include surgical preparation of the animals, the need to sample over time to minimize temporal variation, the cost of labor to conduct the animal experiments, and the large number of samples generated, particularly when utilizing nonsteady-state isotope methods.

Key Words: arteriovenous difference, model, isotope

1160 Opportunities for understanding energy and protein efficiencies of dairy cows using arteriovenous difference methodology.

J. P. Cant*[1], A. J. Fischer-Tlustos[1], and K. Nichols[2], [1]*University of Guelph, Guelph, Ontario, Canada,* [2]*University of California at Davis, Davis, CA.*

The efficiency of capture of digestible energy and protein in milk depends on their partitioning between milk components, body components, and catabolism. Alternatively, one can view partitioning of nutrients between the main organs of gut, liver, mammary glands, adipose, and muscle/remainder. Within each organ, the input-output relationships obtained from arteriovenous difference measurements can be used to formulate the same maintenance/production model of nutrient utilization that is commonly applied to the whole animal. We use data from the mammary glands to illustrate how the 2 levels of organization—organ and whole animal—are reconcilable, to point out how efficiency of energy retention is an outcome of laws of thermodynamics whereas efficiency of amino acid retention is not, and to highlight some potential areas of interest needing further exploration, such as mammary nutrient utilization during the dry period and at calving, tissue retention of protein and fat, uptake of long-chain fatty acids, and non-invasive blood flow measurement.

Key Words: mammary glands, arteriovenous differences, lactation

Ruminant Nutrition 1: Gut Physiology, Fermentation, and Digestion

1161 Withdrawn.

1162 Effect of early-life supply of milk secretory immunoglobulin A on the performance of dairy calves. D. de Oliveira*, B. Schrag, T. Jackson, F. A. C. Mendonça, K. C. Jeong, and F. Batistel, *University of Florida, Gainesville, FL.*

The microbial composition and activity within the rumen ecosystem exert influence on nutrient metabolism, consequently affecting the efficiency of ruminant production. In non-ruminant models, recent research suggests that milk secretory immunoglobulin A (SIgA) promotes the colonization of commensal bacteria in the gut and influences the microbial composition and activity. The findings also imply that milk SIgA contributes to enduring beneficial changes in gut microbial composition. However, the effects of SIgA on ruminants remain unclear. We hypothesize that milk SIgA supply during early life will improve calf performance. This study aimed to identify how the early-life supply of milk SIgA influences animal performance during and after the intervention. Eighty-two female Holstein calves were blocked based on their body weight at birth and then assigned to the following treatments: (1) milk replacer (CON), (2) milk replacer with 7.2 mg of milk SIgA per day (SIgA), or (3) milk replacer with 7.2 mg of milk SIgA per day plus fresh rumen fluid from adult dairy cows (Rumen_SIgA). Treatments were administered from 2 to 28 d of age. Grain intake was measured daily from d 2 to 28, BW and BCS were measured weekly for 56 d, and rumen pH was recorded on d 4, 14, 28, and 56. The data were analyzed using a mixed model, with treatment, time, and their interaction considered as fixed effects and block as random effect. Grain intake was lower for SIgA (36.08 g/d) and Rumen_SIgA (39.70 g/d) compared with CON (45.41 g/d; $P < 0.01$). No differences were observed for ADG ($P = 0.36$), BCS ($P = 0.17$), or rumen pH ($P = 0.93$). Our preliminary results indicate that early-life supply of milk SIgA may improve calf feed efficiency.

Key Words: antibodies, calf, rumen

1163 Effects of microbial inoculum on the transcriptome and meta-transcriptome of rumen, reticulum, omasum, and abomasum epithelium in calves. P. Fregulia*[1,2], W. Li[2], and G. Zanton[2], [1]*Oak Ridge Institute for Science and Education, Oak Ridge, TN,* [2]*USDA-Agricultural Research Service, U. S. Dairy Forage Research Center, Madison, WI.*

Early life microbial inoculation may affect the rumen environment, but the effects in other stomach chambers (SC) are largely unexplored. We intraruminally dosed 3 types of microbial inoculum in dairy calves and evaluated the effects in the rumen (Rum), reticulum (Ret), omasum (Oma), and abomasum (Abo) epithelial tissue transcriptome (SC-T) and the associated meta-transcriptome (SC-M). Fifteen Holstein bull calves were enrolled at birth and randomly assigned to 1 of the 3 treatments dosed orally once weekly from 3 to 6 wk of age. Rumen fluid was collected from 4 adult cows and processed as autoclaved (control; C), bacteria-enriched (BE), or protozoa-enriched (PE) inoculum. After weaning at 7 wk of age, calves were euthanized at 9 wk, and stomach chamber tissues were collected for SC-T and SC-M analysis. We used Cufflinks for Differentially expressed gene (DEG) analysis ($P < 0.05$, fold-change >1.5), DeSeq2 for differential microbial abundance (DMA), and sPLS-DA for microbial community structure and taxonomic signature analysis. For the SC-T, comparing the SCs, 9,628 genes were differentially expressed (DE) on the Rum vs. Abo, 4,821 Rum vs. Oma, 2,212 Rum vs. Ret, 9,219 Abo vs. Ret, 8,728 Abo vs. Oma, and 3,557 Ret vs. Oma. For the DMA, 204 microbial taxa were differentially abundant (DA) on the Rum vs. Abo, 204 Rum vs. Oma, 126 Rum vs. Ret, 190 Abo vs. Ret, 174 Abo vs. Oma, and 159 Ret vs Oma. On the sPLS-DA analysis, C clustered differently from the other treatments, showing that animals that received BE and PE presented changes in the microbial structure community in almost all the stomach chambers. However, the Abo clustered differently from the all the other stomach chambers, regardless of treatment. We also found a strong microbial signature on the abomasum of the BE and PE, with 6 microbial taxa related to the abomasum of BE and 6 related to PE. Our study provides evidence that microbial inoculation affects SC-T and SC-M of all the stomach chambers. However, the microbiota on the Abo differs from the other chambers, regardless of the treatment received.

Key Words: stomach chambers, transcriptome, meta-transcriptome

1164 Meta-transcriptome analysis reveals temporal dynamics on the active rumen microbiome in transition Holstein cows. P. Fregulia*[1,2], G. Zanton[2], and W. Li[2], [1]*Oak Ridge Institute for Science and Education, Oak Ridge, TN,* [2]*USDA-Agricultural Research Service, U. S. Dairy Forage Research Center, Madison, WI.*

Dairy cows experience dramatic changes in metabolism during the transition period, during which the cows calve and switch from a high-forage prepartum to a high-concentrate postpartum diet. Understanding the changes in active rumen microbiome (ARM) community structure over the transition period may provide insights for improving animal health and production. We evaluated the combined effects of calving and diet changes on the ARM from 8 cannulated Holstein cows at 3 time points: 14 d before calving (−14), 2 d (±1 d) postpartum (PP), and 14 d after calving (+14). The cows were fed with a high-forage close-up diet before calving (containing 35.92% corn silage, 37.10% wheat straw, and 26.98% concentrate) and with a common high-cow diet after calving (BMR, 31.67% corn silage, 28.00% alfalfa silage, and 40.33% concentrate). Rumen liquid (RL) was collected by filtering rumen content obtained via cannula through 4 layers of cheesecloth. Total RNAs extracted from RL were used for meta-transcriptomic analysis by whole transcriptome sequencing. We used Kraken2 for quantifying microbial taxa-level abundance, DESeq2 for identifying differentially abundant genera (DAG) between time points, and sPLS-DA for analyzing microbial community profiles. Correlation between microbial taxa and time points was done using clustered image maps (CIM). The biggest changes in microbial genus abundance were observed between −14 and +14, where 361 DAG were identified, which agrees with the sPLS-DA analysis, where the microbial community profiles of +14 and −14 showed complete cluster separation and distinct microbial community structures. On the CIM plot, specific microbial taxa showed a strong correlation with the different time points. We found no difference on the α-diversity between the 3 time points (Simpson's index and Shannon's diversity, $P < 0.05$). Our results indicate that the combined effects of calving and diet changes result in shifts in the ARM structure in the rumen of transition dairy cows, with distinct ruminal microbial profiles pre- and postpartum.

Key Words: meta-transcriptome, microbial community, transition cows

1165 Interaction of supplemental branched-chain volatile fatty acids (BCVFA) and dietary RDP on nutrient degradation and

microbial protein synthesis in continuous culture. A. F. White*[1], A. Sanders[1], K. Mitchell[2], D. Kleinschmit[3], M. Socha[3], and J. Firkins[1], [1]*The Ohio State University, Columbus, OH,* [2]*Elanco Animal Health, Greenfield, IN,* [3]*Zinpro Corporation, Eden Prarie, MN.*

Dosed branched-chain volatile fatty acids (BCVFA: 2-methylbutyrate [2MB], isobutyrate [Ibu], and isovalerate [Ival]) increased NDF digestibility (NDFD) in previous work but microbial protein synthesis (MPS) only in one. We tested the effects of BCVFA and RDP on nutrient degradation and microbial protein. We hypothesized that BCVFA would improve NDFD more with low RDP and the optimal dose of BCVFA would be lower for high RDP. Treatments with high or low (10.0 or 8.5% NASEM formulated) RDP and 0, 1.18, or 2.15 mmol/d of each BCVFA were arranged in 2 × 3 factorial in a 6 × 6 Latin square design. Periods had 7 d of adaptation and 4 d of sampling. Total liquid and solids dilution rates were 10 and 5%/h. ^{15}N was used as a microbial marker, and urea was infused for adequate ammonia concentration. Data were analyzed in SAS v.9.4 PROC MIXED with fixed effect of treatment and random effects of period and fermenter. Contrasts were linear and quadratic doses of BCVFA with low and high RDP, respectively, and the main effect of RDP. Differences in RDP were verified, but calculated grams of degraded CP increased linearly ($P = 0.10$) with BCVFA in low-RDP diets. Dosing BCVFA linearly decreased ($P < 0.04$) net production (subtracting dose) of Ibu and Ival for both RDP treatments but 2MB only with high RDP, suggesting that rumen bacteria regulate 2MB more than Ibu and Ival. High RDP increased ($P < 0.09$) OM truly degraded compared with Low RDP, but NDFD increased linearly ($P = 0.06$) 5.9 percentage units only with high RDP. Microbial N flow was not different and increased linearly with BCVFA dosing ($P = 0.09$) for low RDP but quadratically ($P = 0.10$) for high RDP (both by about >5%). Microbial N derived from ammonia-N was lower ($P < 0.01$) with high RDP, suggesting increased preformed amino acid incorporation. Dosed BCVFA increased NDFD only with higher RDP, consistent with cow studies, even though ammonia was never limiting, and microbial N was increased by BCVFA with low RDP. The BCVFA dose appears to influence bacterial proteolysis and may interact with concentration of RDP to influence the rumen microbial community.

Key Words: isoacids, microbial protein, continuous culture

1166 RDP/RUP ratio affects ruminal fermentation and concentration of branched-chain VFA. M. H. De Oliveira*[1,2], T. Fernandes[1], A. Hruby-Weston[1], A. Beckman[1], J. Saunders[1], and M. D. Hanigan[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*State University of São Paulo, Botucatu, SP, Brazil.*

Dynamics between RDP and RUP, and the subsequent impacts on ruminal fermentation, are complex. Thus, we aimed to evaluate how proportions of RUP related to crude protein can affect rumen fermentation patterns in terms of VFA content, urea, and ammonia concentration. Six rumen-cannulated Holstein heifers (398 ± 28 kg BW on experiment onset) with ear monitoring tags were allocated in 2 sets of 3 × 3 Latin square design. Treatments were 3 dietary RUP proportion related to a fixed total dietary protein (15.25% CP): (1) 29% of RUP (29RUP; 1.72 NE$_L$/kg; 9.77% MP), (2) 39% of RUP (39RUP; 1.70 NE$_L$/kg; 10.72% of MP), and (3) 50% of RUP (50RUP; 1.69 NE$_L$/kg; 11.70% of MP), across 6 periods (3 for each square). For the first 10 d of each 14-d period, heifers were fed 1×/d; for the last 4 d of each period, heifers were fed 12×/d at 95% to establish a steady state. Rumen fluid samples were collected every 6 h on the last 4 d of each period for urea, ammonia, and VFA contents. Data were analyzed using the "lme4" package in R, with treatment, time (h), treatment × h, and period as fixed effects and either heifer or heifer nested in treatment sequence as random effects. Treatment did not affect DMI (10.9 kg/d; $P = 0.76$), time spent ruminating (474 min/d; $P = 0.39$), or ruminal pH (6.5; $P = 0.91$). Heifers on 50RUP had reduced rumen urea (0.68 mg/dL; $P = 0.02$) and rumen ammonia (4.5 mg/dL; $P < 0.01$) compared with 39RUP and 29RUP. Total VFA for 50RUP animals was lower; however, greater acetate (64.3%; $P < 0.01$) was observed, leading to a greater ($P < 0.01$) acetate:propionate ratio. Moreover, iso-valerate ($P < 0.01$), iso-butyrate ($P < 0.01$), and valerate ($P = 0.03$) were greater in 29RUP diets than in 39RUP and 50RUP. Because RUP is not hydrolyzed upon entering the rumen, it reduces AA availability into the rumen, potentially impairing microbial growth by limiting the N source and, consequently, affecting fermentation. Overall, our results indicate that while 29RUP heifers presented greater concentrations of branched-chain VFA, 50RUP heifers faced modulated ruminal fermentation due to lower available soluble proteins.

Key Words: fermentation, microbial growth, VFA

1168 Effects of branched-chain volatile fatty acids with different levels of rumen-degraded protein on rumen fermentation, plasma amino acids, and milk fatty acids in lactating cows. K. Park*[1], K. L. Clark[1], J. L. Firkins[2], D. H. Kleinschmit[3], M. T. Socha[3], and C. Lee[1], [1]*Department of Animal Sciences, The Ohio State University, Wooster, OH,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [3]*Zinpro Corporation, Eden Prarie, MN.*

The objective of the study was to determine the effects of branched-chain volatile fatty acids (BCVFA) under different levels of rumen-degraded protein (RDP) on rumen fermentation, plasma amino acids (AA), and milk fatty acid (FA) profiles. Eight ruminally cannulated Holstein cows were used in a replicated 4 × 4 Latin square design with 2 × 2 factorial arrangement: 9 or 11% RDP (dietary DM; NASEM, 2021) with or without BCVFA supplementation (IA, 0.09% isobutyrate and 0.06% 2-methylbutyrate in dietary DM). All samples were collected on the last week of each 4-week period. Data were analyzed using the PROC MIXED of SAS with RDP, BCVFA, RDP × BCVFA, and period as fixed effects, and square and cow within square as random effects. The model for rumen fermentation data included time as fixed effect (0, 3, and 6 h after feeding). Ruminal NH$_3$-N concentration was greater for high RDP (7.66 vs. 4.85 mg/dL; $P = 0.02$) compared with low RDP. Ruminal isobutyrate concentration was greater for IA ($P = 0.03$) compared with no IA. Interactions were observed between IA and time ($P < 0.05$) for ruminal concentrations of 2-methylbutyrate and total BCVFA. Those were not affected by IA within time. Within treatment, however, 2-methylbutyrate and total BCVFA decreased at 6 h for IA compared with 0 and 3 h, but those were maintained until 6 h for no IA. The concentrations of plasma AA were not affected by RDP and IA. The IA treatment did not affect de novo, mixed, and preformed FA concentration in milk fat. While C18:1 *trans*-11 increased ($P = 0.03$) for IA compared with no IA, an interaction between RDP and IA was observed ($P = 0.02$) for C18:1 *trans*-10. The IA treatment decreased C18:1 *trans*-10 only with high RDP. This interaction pattern for C18:1 *trans*-10 was opposite to that of milk fat concentration (interaction $P = 0.01$). In conclusion, IA seems to influence rumen microbes participating in biohydrogenation. However, the IA effect on biohydrogenation differed by RDP levels and IA likely required sufficient RDP to promote normal biohydrogenation in the rumen.

Key Words: isoacids, isobutyrate, 2-methylbutyrate

1169 Fecal metabolome analysis from Holstein cows with divergent methane emissions. A. H. Peres Assumpção*, G. Martinez Boggio, C. Chatman, E. Manjumder, H. White, K. Weigel, F.

Peñagaricano, and H. Mantovani, *University of Wisconsin–Madison, Madison, WI.*

Methane (CH_4) significantly contributes to greenhouse gas emissions in dairy farming. Its impact extends beyond environmental concerns and affects the energy efficiency of the host. This study aimed to evaluate differences in the fecal metabolome of dairy cows exhibiting divergent CH_4 emissions, employing a non-invasive approach. We used a GreenFeed system to measure CH_4 emissions from 96 mid-lactation Holstein cows in 3 different studies [HW1] (7 to 10 weeks). Parameters measured included CH_4 production (MeP, in g/d), net energy for lactation (NE_L), and body weight (BW). Residual methane intensity (RMI) was calculated by regressing MeP on NE_L and metabolic BW. Within each study, we identified the cows with 10% high and low RMI (n = 26) and collected fecal samples via rectal collection on the last day of each study. Metabolite extraction was performed using 0.5 mL of each sample, followed by untargeted metabolomics analysis on an ultra high-performance liquid chromatography mass spectrometer (UHPLC-MS). We performed statistical and functional analyses in MetaboAnalyst 5.0, with metabolite annotation and putative identification performed using Compound Discover (version 3.3). Of the 4,659 metabolites analyzed, 123 exhibited significant (*t*-test, $P < 0.05$) differences between high and low CH_4 emitter groups. Among the significant metabolites, 99 were upregulated in high-emitter cows, while 24 were upregulated in low-emitter cows. Future functional analysis is needed to elucidate pathway-level changes within the metabolome.

Key Words: metabolomics, microbiota, methane emissions

1650 Associative effects of buffers and tannins on modulation subacute ruminal acidosis and ruminal fermentation. A. Fernandez-Lehmann*, I. Fernandez-Marenchino, A. Maderal, F. Podversich, F. Tarnonsky, C. Gomez-Lopez, W. Cuervo, J. J. Vargas, and N. DiLorenzo, *University of Florida, Gainesville, FL.*

Global population growth intensifies nutrient demand. High-performance diets bolster productivity but can increase harmful outputs from fermentation, negatively impacting ruminants and the environment. External buffers and tannins offer promise in enhancing nutrient utilization and mitigating adverse effects. Our study aimed to evaluate different buffers and their interaction with tannins on ruminal fermentation and pH modulation, using a 8 ruminally cannulated steers in a duplicated 4 × 4 Latin square design. We used 4 treatments: BICARB (basal diet + 1.2% DM sodium bicarbonate), BRIC-050 (basal diet + 1.2% DM multi-element buffer), BRIC-150 (basal diet + 1.2% DM BRIC + 0.15% DM mixture of tannins), and TAN (basal diet + 0.15% DM mixture of tannins). The rumen sampling took place before and after feeding. The experimental period was 16 d total: 3 d of covariates, 7 d of treatment adaptation, 1 d of 50% feed restriction, 1 d of SARA challenge, and 4 d of recovery. Statistical analysis used the mixed procedure of SAS, with fixed effects of treatment, square, and covariates, and random effects of period and animal within square. On the challenge day, data were analyzed using repeated measures. The TAN treatment resulted in lower ruminal pH during adaptation 4 h after feeding (5.76 ± 0.09; $P = 0.01$) and feed restriction at 4 h after feeding (5.90 ± 0.09; $P = 0.01$). At 0 h feed restriction, TAN had lower NH_3-N (4.83 ± 1.18; $P = 0.04$), propionate had greater molar proportions for TAN (24.6 ± 1.37; $P = 0.02$), butyrate molar proportions significantly increased for BRIC-050 (11.89 ± 0.15; $P = 0.03$), branched-chain VFA (BCVFA) molar proportions decreased for TAN (2.06 ± 0.15; $P < 0.01$), and A:P decreased for TAN (2.69 ± 0.27; $P = 0.04$). At 4 h feed restriction, propionate molar proportions increased for TAN (26.1 ± 1.74; $P = 0.03$), BCVFA decreased for TAN (1.99 ± 0.13; $P < 0.01$), and A:P decreased for TAN (2.48 ± 0.13; $P = 0.02$). During the challenge with repeated measures, BRIC-150 increased butyrate molar proportions (11.53 ± 0.20; $P < 0.01$). At 0 h recovery, acetate decreased for TAN (62.9 ± 1.34; $P = 0.03$) and A:P decreased (3.29 ± 0.14; $P = 0.01$) for TAN.

Key Words: ruminal pH, ruminal fermentation, productivity

1171 Characterizing rumen fermentation profiles associated with early-lactation feed efficiency at different levels of milk production in transition dairy cows. G. G. Begalli*[1], L. D. P. Maldonato[2], V. A. de Oliveira[3], L. Buraschi[4], A. F. Souza Lima[1], J. Halfen[1], T. Fernandes[1], and J. S. Osorio[1], [1]*School of Animal Science, Virginia Tech, Blacksburg, VA,* [2]*Universidad de La Salle, Bogotá, DC, Colombia,* [3]*Universidade Federal de Lavras, Lavras, MG, Brazil,* [4]*Universidad Nacional de Mar Del Plata, Buenos Aires, Argentina.*

The objective of this study was to evaluate the effects of feed efficiency at 2 levels of milk production during early lactation on rumen fermentation profile. Thirty-five multiparous Holstein cows were monitored from −35 to 60 d in milk (DIM). From −35 to −22 DIM, cows were fed a far-off diet (1.52 Mcal/kg DM and 14.1% CP); from −21 d until calving, cows received a close-up diet (1.52 Mcal/kg DM and 14.5% CP). From calving to 30 DIM, cows received a fresh cow diet (1.62 Mcal/kg DM and 16.6% CP), and from 31 to 60 DIM, cows were fed a post-fresh diet (1.65 Mcal/kg DM and 16.8% CP). Cows were retrospectively classified into 4 groups based on their milk yield (MY) and fed efficiency (FE): High MY and high FE (HH, n = 11), high MY and low FE (HL, n = 7), low MY and high FE (LH, n = 7), and low MY and low FE (LL, n = 10). Fed efficiency was calculated based on averages of individual postpartal DMI and MY. Rumen fluid samples were collected once a day during the transition period (TP) at −35, −28, 7, and 28 DIM. Diurnal variations in rumen parameters were assessed through spot rumen fluid samples (0000, 0300, 0600, 0900, 1200, 1500, 1800, and 2100 h) taken at −14 and 14 DIM. From 0 to 11 DIM, blood BHB was measured every other day. The PROC MIXED of SAS was used for statistical analysis, and significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. Neither ruminal pH through the TP nor diurnal rumen pH at −14 DIM were affected ($P \geq 0.29$) by group. However, a treatment effect ($P = 0.04$) was observed for diurnal rumen pH at 14 DIM, where HH cows had greater ($P \leq 0.03$) rumen pH compared with HL, LH, and LL (6.8, 6.4, 6.4, and 6.4, respectively). A treatment effect ($P = 0.02$) was observed in blood BHB concentrations, where greater ($P \leq 0.01$) BHB was observed in HH cows compared with HL and LL, and tended ($P = 0.08$) to be greater than LH (1.78, 1.22, 1.14, and 1.35 mmol/L, respectively. Our results suggest that HH cows have a better ruminal adaptation to a high-energy diet after calving. Even though high blood BHB levels were predominant in HH cows, this did not impair milk production or feed efficiency.

Key Words: dairy cow, efficiency, rumen

1172 Effects of dietary betaine supplementation and partial rumen content transplantation on milk fatty acid profile and rumen microbial population in heat-stressed Holstein dairy cows. A. Ruiz-González[1,2], A. Javaid[3], C. Perdomo[2], D. Rico[1,2], and J. W. McFadden*[3], [1]*Université Laval, Québec, QC, Canada,* [2]*CRSAD, Deschambault, QC, Canada,* [3]*Cornell University, Ithaca, NY.*

Heat stress alters the rumen fermentation in cows, which may be modified by dietary betaine supplementation. Twelve rumen-cannulated multiparous Holstein cows (39 ± 6.4 kg milk/d; 82 ± 27 DIM) were used in a split-plot design testing the effects of betaine and partial ruminal content transplantation (RT) on cow performance during heat stress. The main plot was the level of dietary betaine supplementation (CON: unsupplemented; or BET: 100 g/d betaine hydrochloride 95%). Within each plot, cows were randomly assigned to (1) heat stress (HS), (2) thermoneutral pair-feeding (TNPF), or (3) HS with RT (HSRT; 25% replacement of rumen contents from 4 donor cows fed ad libitum under thermoneutrality; d 8–14) in a replicated 3 × 3 Latin square design with 14-d periods. The statistical model included the random effects of cow and period, and the fixed effects of plot, treatment, day, and their interactions. No plot effect was observed with the supplementation of betaine. Milk concentrations and yields of preformed fatty acids (FA), MUFA, PUFA, n-6, and C18 were decreased by more than 7% in HS, relative to TNPF ($P < 0.01$). The concentrations and yields of vaccenic acid, oleic acid, CLA (18:2 c9,t11) were lower by more than 20% in HS, relative to TNPF ($P < 0.01$). On d 13, the yields of total n-3 FA decreased by 22% in HSRT, relative to HS ($P = 0.02$). The relative abundance (RA) of phylum *Fibrobacterota* (~2%) decreased by 67% while *Proteobacteria* (~6%) increased by 58% in HS relative to TNPF ($P < 0.05$), and on d 13 RA of *Elusimicrobiota* (~0.01%) increased by 1.3% in HSRT, relative to HS ($P < 0.05$). Similarly, RA of genus *Saccharofermentans*, *Christensenellaceae*, and *Fibrobacteria* decreased, while *Oribacterium* and *Succinivibrionaceae* increased in HS, relative to TNPF ($P < 0.05$). On d 13, RA of *Ruminococcus* (~2%) increased by 40% in HSRT, relative to HS ($P = 0.01$). In conclusion, betaine had no impact on milk FA or rumen microbiota, while heat stress reduced FA production, whereas rumen transplantation and heat stress altered ruminal microbiota. Supported by FFAR.

Key Words: betaine, heat stress, ruminal microbiota

ADSA-USD Competition: Dairy Foods Oral Presentations

1173 The rise of reduced-lactose and lactose-free dairy products. M. Benfer* and D. Olver, *Pennsylvania State University, University Park, PA.*

In recent years, there has been an impressive increase in demand for reduced-lactose and lactose-free dairy products. According to Future Market Insights, the lactose-free dairy market grew 5.4% globally in the first half of 2021, and they predict that the demand for lactose-free products is going to continue to rise by 5.5% from 2022 to 2032. In earlier years, lactose-free milk served the purpose of consumers with lactose intolerance. Now consumers are finding that there are other benefits. With the absence of lactose, products have a reduced calorie count. Along with reduced calories, lactose-free dairy products benefit consumers by reducing their sugar consumption. One ongoing challenge that the lactose-free dairy market faces is competing with plant-based alternatives. Lactose-free milk excels over plant-based alternatives by containing more nutrients. According to the 2020–2025 US Dietary Guidelines, dairy products exceed plant-based beverages in calcium, potassium, and vitamin D content. Dairy products also excel in protein quality compared with plant-based alternatives. Lactose content in dairy products can be reduced by fermentation, filtration, and hydrolysis of lactose. Companies are now designing lactose-free products to become more appealing to consumers. Example products include lactose-free yogurts and various fermented milk products. There are also a variety of cheeses that have reduced lactose content or are lactose-free due to their production process. As the number of people that are lactose intolerant increases, the lactose-free food market is anticipated to rise across the world. For example, according to a 2023 article by Li et al., 25% of consumers in India are motivated to purchase or have purchased lactose-free drinks. Li also cited studies that showed almost 70% of the global population suffers from lactose malabsorption. As consumers across the world continue to demand healthier food and beverage options, the market for lactose-free dairy products should continue to expand.

Key Words: reduced lactose, lactose-free, dairy products

1174 Proposed WIC changes: How decreased availability to dairy products could contribute to a growing vitamin D issue. S. Dupont* and J. Bohlen, *University of Georgia, Athens, GA.*

WIC (Special Supplemental Nutrition Program for Women, Infants, and Children) is a federal program through the USDA that provides monthly nutritious food vouchers to low-income women and children who are at nutritional risk. WIC serves over 6 million women and children, including 43% of all infants in the US. This past November, the USDA proposed to reduce dairy voucher availability through WIC by as much as 25% each month. This "science-based" change reduces milk amounts provided from 128% of the daily recommended dairy to between 71 and 96%. Consequently, these new guidelines make the WIC allotment lower than the recommended daily dairy value for children. The USDA claims that this change is "consistent with the purpose" of WIC, as it is meant to be used as supplemental nutrition rather than the main food supply. However, this proposal ignores the USDA federal nutrition guidelines and discounts the food insecurity many families face. Moreover, this change potentially could have serious implications for the vulnerable groups it is meant to benefit, but none more important than the implications regarding vitamin D. Recent research shows that while 90% of people in the US do not consume enough dairy, which is rich in vitamin D, deficiencies in vitamin D are highest in low-income populations. Vitamin D is an essential nutrient that is vital for calcium absorption and bone health, and plays an active role in the immune system, nervous system, and cardiovascular system. Childhood deficiencies in vitamin D can lead to bone diseases such as rickets or osteoporosis. For pregnant women, a deficiency in vitamin D can cause spontaneous abortions, increased risk of pre-eclampsia, gestational diabetes mellitus, preterm birth, low birth weight, abnormal bone growth, and seizures. These medical issues that children and women could face from a simple nutrient deficiency, compounded with reduced or no medical resources as is common with WIC participants, exemplifies the serious impacts of this proposal. If the USDA is successful in passing this proposal, it will have economic and nutritional consequences for the exact people who are at greatest risk.

Key Words: vitamin D, WIC, deficiency

1175 Inclusion of insect proteins for clean labeling in ice cream. A. Trent* and E. Eckelkamp, *University of Tennessee, Knoxville, TN.*

Clean labeling in food focuses on informing consumers of food ingredients in a way that is easily readable and understandable. Clean labeling has been defined as a product positioned to be "organic," "natural," and/or "free from" artificial ingredients and additives. In food, clean labeling refers more to inclusion or removal of ingredients, such as additives and preservatives. Consumers perceive some additives and ingredients as harmful if names are unfamiliar or difficult to pronounce, such as carrageenan or guar gum. Stabilizers and thickening agents in particular had a lower mean naturalness rating in a 2021 study. To provide clean labels, new studies have implemented uncommon ingredients to replace ingredients with negative consumer perceptions. A 2022 Israeli study examined the addition of silkworm pupa flour (SPF) into ice cream in place of other stabilizers at 1.0, 2.5, 4.0, and 7.0% wt/wt formulation. Silkworm pupa flour was used as a stabilizing agent in the foaming of ice cream and improved product shelf life. Silkworm pupa flour, in addition to being a stabilizing agent, is a rich source of protein and an antioxidant, and could have anti-inflammatory affects. The SPF significantly improved viscosity and foaming at all inclusion levels. However, SPF of 4.0 and 7.0% significantly reduced ice cream overrun and added a brownish hue to the color. A tasting panel evaluated the ice cream made with 1.0, 2.5, 4.0, and 7.0% wt/wt formulation of SPF. Inclusion levels >1% wt/wt SPF decreased palatability and likelihood to repurchase. Ice cream containing 1% SPF was ranked from "like moderately" to "like very much" by respondents. This study indicated that low levels of SPF could improve viscosity and foam stability in ice cream without impacting consumer palatability, overrun, or color. By labeling the benefits of the item as well as how it is a natural ingredient to use, ingredients such as insect protein may become more common in the diet of the general public.

Key Words: clean label, silkworm pupa, ice cream

1176 Composition of fluid milk versus alternative plant-based beverages and impacts of processing. D. Welbaum* and D. Winston, *Virginia Tech, Blacksburg, VA.*

In the United States, fluid milk consumption is decreasing due to ever-changing consumer patterns and competition. A 61% increase in sales of non-dairy alternatives has been observed since 2013 whereas dairy beverage milks have decreased by 21% over the same time. Plant-based beverages had $2.3 billion in retail sales in 2018. These plant-based products continue to be labeled as milks, which is misleading to consumers. In January 2023, the US Food and Drug Administration proposed a new guidance that would allow alternative beverages with nut, soy, oat, and other non-dairy products to use labeling of "dairy." However, the bipartisan Dairy Pride Act has been proposed to define milk as a lacteal secretion by mammals. Nutritionally, whole milk has all 9 of the necessary amino acids in proteins required for healthy growth and internal repairs; plant-based beverages can only infuse 3 of these proteins into their drinks—lysine, glycine, and proline. Alternative beverages are becoming more popular to consumers because of their lower caloric content; however, to gain the 8 g of protein from drinking 240 mL of whole milk, a person would need to drink 7 times that amount of almond beverage. This would increase the caloric content to 400 kcal for the same amount of protein. Although these beverages may seem like a healthy option for consumers, they are actually highly processed, with an average of 14 steps in the cycle to get the products on the shelves. Comparatively, cow's milk is minimally processed. The dairy industry has reduced global greenhouse gas emissions by 11% since 2017. Informing consumers of nutrition and processing impacts could positively influence future dairy sales. Current regulations regarding milk labeling should be adjusted to keep consumers informed about what they are actually consuming, and how it may affect sustainability.

Key Words: milk consumption, plant-based beverages, nutritional composition

ADSA-USD Competition: Original Research Oral Presentations

1177 Evaluating the use of infrared thermal imaging of the eye as a non-invasive, rapid diagnostic tool to assess body temperature in calves. K. Glassman*, J. Crea, E. Arp, S. Sledge, and J. Bohlen, *University of Georgia, Athens, GA.*

Detecting sudden changes in a calf's body temperature provides necessary and vital insight into their health and allows for immediate disease intervention strategies. Though considered a gold standard, rectal temperatures can be an inefficient, ineffective, and invasive method to identify illness. Therefore, this study's objective was to assess the use of thermal imaging of the eye as a non-invasive and rapid technique to assess core body temperature of calves. Calves (n = 12) at 4 ± 3 d of age were enrolled and assessed weekly until 39 ± 3 d of age. For thermal imaging assessments, calves were moved to an enclosed barn with wood siding to reduce environmental variation with a HOBO data logger (Onset, Cape Cod, MA) used to assess temperature and humidity at the time of imaging. All images were collected using a FLIR T540 thermal imaging camera (Teledyne FLIR, Wilsonville, OR) with an emissivity setting of 0.95. Calves were positioned 2 feet from the camera, and images were collected perpendicular to each eye at an approximate 90-degree angle. Rectal temperature was collected at the time of thermal imaging. Images were analyzed using the FLIR Studio Pro software with the right (RE) and left eyes (LE) analyzed by a single operator for subjective identification of the coolest (pupil) and warmest (sclera) parts of the eye followed by autonomous identification of these points by the software program. All data were analyzed using PROC MIXED in SAS 9.4. Both warm and cool points identified by the operator differed in values from those autonomously identified ($P < 0.05$). The LE autonomous identifications of warm and cool points were related to the rectal temperatures ($P < 0.05$); however, RE indicators were not ($P > 0.05$). LE and RE differences may be related to lighting differences with calf rotation. Neither rectal nor thermal temperatures were influenced by environmental conditions ($P > 0.05$), but rectal temperatures were lower in older calves ($P < 0.05$). Additional data and modifications to the thermal imaging protocol are needed in the current study to utilize thermal imaging as an accurate core body temperature predictor.

Key Words: thermal imaging, calf, temperature

1178 Evaluating sire fertility and calving traits in lost Holstein Y-chromosome lineages. K. J. Jenkins*[1], H. D. Blackburn[2], W. S. Liu[1], and C. D. Dechow[1], [1]*Penn State University, University Park, PA,* [2]*USDA-ARS National Animal Germplasm Program, Fort Collins, CO.*

The modern Holstein (HO) breed has been reduced to 2 Y-chromosome lineages since the introduction of artificial insemination. This study aimed to contrast sire fertility, calving ease (CE), stillbirths (SB), and birth weight of modern HO sires, Angus sires, and sires from lost Y-chromosome lineages (lost-Y). The lost-Y lineages were recently reconstituted by mating historic bulls with semen available from the USDA National Animal Germplasm Program (NAGP) repository to modern HO dams. Breeding and calving records from one California herd, one New Mexico herd, one Pennsylvania herd, and the Penn State dairy herd were retrieved from herd management records. Records were retained from all cows bred during the time period the lost-Y semen was used on the respective farms, with 11,132 observations (8,140 modern HO, 2,435 Angus, and 557 lost-Y) for conception rate. Calving records included 2,941 SB observations, 2,921 CE observations, and 1,433 birth weight observations. Conception rate was evaluated with the HPMIXED procedure of SAS with fixed effects of lineage (modern HO, Angus, lost-Y), insemination number, and herd-year. Random effects were cow, sire, and herd-year-month of insemination. Models for calving traits included fixed herd-year, lineage (modern HO, Angus, lost-Y), and sex. The conception rate least squares means (LSM) for lost-Y (42.30% ± 4.14) were not different ($P > 0.05$) from Angus (36.06% ± 2.56) or modern HO (40.22% ± 1.45). Lost-Y SB (4.89% ± 1.51) was not significantly different from Angus (4.153% ± 1.29) or modern HO (4.28% ± 1.09). LSM for lost-Y CE (1.38 ± 0.05) was higher ($P < 0.01$) than Angus (1.20 ± 0.04) and modern HO (1.23 ± 0.02), likely due to higher ($P < 0.01$) lost-Y birth weight (44.67 kg ± 1.04) compared with Angus (41.40 kg ± 1.04) and modern HO (41.52 kg ± 0.42). This research suggests that the loss of Y-chromosome lineages since the introduction of artificial insemination was not due to repressed sire fertility, and that calves from these lost-Y lines weighed more at birth. Results suggest that semen from older sires in the germplasm collection of NAGP can be utilized with few or no detrimental effects.

Key Words: genetic conservation, sire fertility, calving

1179 Effects of adding *Bacillus* strains, bioactive clay, and yeast cell wall to milk replacer and starter grain on calf performance. E. N. Plunkett*[1], N. B. Litherland[2], E. Schwab[2], S. G. Onetti[2], B. Dado-Senn[2], A. Hoskins[2], M. A. Abeyta[2], W. Myers[3], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*Vita Plus Corporation, Madison, WI,* [3]*Phileo-Lesaffre, Milwaukee, WI.*

A multi-strain *Bacillus* direct-fed microbial (DFM; MicroSaf4C40; Phileo-Lesaffre, Milwaukee, WI) delivering 1 billion cfu/hd/d of *Bacillus licheniformis*, *B. amyloliquefaceins*, *B. subtilis*, and *B. pumilus*, bioactive clay (CLAY; MILPRO® TX; Brownsville, TX), and yeast cell wall (YCW; Safmannan; Phileo- Lesaffre, Milwaukee, WI) were fed to Holstein × Angus calves (n = 21/treatment) in a randomized design with 3 treatments during a 70-d nursery study: milk additive (MA), milk and starter additive (MSA), or control (CON). Objectives were to determine whether the MA and/or MSA improved calf growth. The control CMR (22:22; 5.5 g/hd/d, Safmannan) was mixed to 13% solids and fed at 5.0 L/d (d 1–10), 7.0 L/d (d 11–42), and 3.5 L/d (d 43–49), and discontinued on d 50. The milk additive of 25 mg/hd/d of DFM and 3 g/hd/d CLAY was added to CMR at mixing. Calves were offered a texturized starter with whole corn and a control pellet or pellet with DFM (25 mg/hd/d), CLAY (3 g/hd/d), and YCW (5.5 g/hd/d) pellet. Data were analyzed using the Mixed procedure of SAS using autoregressive covariance structure. Model effects included body weight and date of enrollment, calf sex, serum total protein (STP), treatment, week, and treatment × week interactions. Significance was declared at $P < 0.05$. Calves were 2–3 d of age at enrollment and weighed 48.5, 48.3, vs. 48.4 ± 0.82 kg (CON, MA, vs. MSA, respectively), and STP averaged 5.61 mg/dL. Starter intake at 70 d was similar (2.65, 2.72, vs. 2.64 ± 0.12 kg/d; $P = 0.49$) among treatments. Average daily gain (830, 876, vs. 839 ± 27.2 g/d; $P = 0.39$) and body weight at 70 d (102.5, 107.8, vs. 104.4 ± 2.0 kg; $P = 0.83$) did not differ among treatments. Hip height gain was also not different among treatments (15.3. 15.5, vs. 14.3 ± 0.58 cm; $P = 0.23$). While MA and MSA did not significantly affect calf growth, the numerical increase of 5.3 kg gain for MA vs. CON has economic implications and may justify further investigation.

Key Words: direct-fed microbial, yeast cell wall, bioactive clay

Joint American Society of Nutrition (ASN) and ADSA Symposium: Dairy and Dairy Products on Human Health

1180 Whole-milk dairy and cardiometabolic health in humans. R. Bruno*, *The Ohio State University, Columbus, OH.*

Bovine dairy milk is a nutrient-rich matrix, but consumption of its full-fat variety has historically been discouraged due to its saturated fat and higher energy content that may provoke poor cardiometabolic health. However, continued investigation has provided evidence suggesting that whole-milk dairy consumption does not elicit the predicted detriments on cardiometabolic health. The objective of this presentation is to discuss whole-milk dairy and its implications on cardiometabolic health, considering evidence from observational studies, randomized controlled trials, and preclinical models. It will emphasize the health effects of dairy foods, as well as milk polar lipids, which are concentrated in the milk fat globule fraction and reported to alleviate metabolic dysfunction and promote gut barrier function. Understanding the totality of evidence surrounding the bioactivities and health benefits of whole-milk dairy is expected to support a framework for evidence-based dietary recommendations aimed at reducing the risk of cardiometabolic disorders and/or reversing their progression toward premature mortality.

Key Words: full-fat dairy, cardiometabolic health, diet

1181 The dairy matrix and health benefits of dairy. C. Weaver*, *San Diego State University, San Diego, CA.*

Dairy products are nutrient-dense foods that are rich sources of bioavailable minerals, vitamins, and essential amino acids that support growth and development and decrease risk of chronic disease. On a global scale, milk consumption is ranked very high compared with other food sources for bone-building nutrients: first for calcium, second for phosphorus and potassium, third for protein, and fourth for magnesium. Milk is also ranked among the top 3 foods contributing to the global intake of essential amino acids such as lysine, threonine, methionine, and tryptophan. There is growing current interest in the dairy matrix, the physiochemical structure, and its interaction with chemical constituents including nutrients. Emerging evidence suggests that the consumption of whole food matrix in dairy foods has health effects distinct from that of isolated nutrients. Dairy foods are sustainable as well as nutrient dense and are more affordable than plant-based beverage alternatives to milk.

Key Words: bioavailable minerals, vitamins, essential amino acids

1182 Milk: The Rosetta stone for health innovation. J. B. German*[1,2], [1]*Department of Food Science & Technology, University of California, Davis, Davis, CA,* [2]*Foods for Health Institute, University of California, Davis, Davis, CA.*

The entire agriculture and food system is going through a revolution driven by the urgent need to meet the challenges of increasing sustainability of agricultural production and the challenges of improving human health through diets. These challenges are particularly daunting when one considers that the entire system may have to change without diminishing even briefly the massive productivity demands of feeding 8 billion people. Science itself is also challenged as it moves from a guiding a chemistry-based enterprise to a biotechnology-based system. Fortunately there is a magnificent biological model that combines the principles of sustainability and integrated diet: mammalian lactation. If we could understand scientifically how lactation manages to produce a comprehensive and sublimely nourishing diet for mammalian infants without compromising the health of mothers, we could transfer those same biological principles to the entire agriculture and food system of the future. Thus, scientific research and its translation needs to "decode" the language of lactation. Capturing the composition of milk will need to identify and quantify all of the dimensions of milk so that the functions can be assigned to molecules, structures, and mechanisms of action as an ensemble. Milk does not simply contain protein; it is a complex ensemble in which specific proteins, proteases, and anti-proteases act as a concerted, temporal, and spatial delivery system of proteins, catalysts, peptides, and amino acids. Similarly milk is not a simple source of fats but a profusion of globules, structurally complex and chemically diverse, with again an ensemble of catalytic activities that guide the disassembly of globules and the spontaneous assembly of complex structures within the infant. Milk is an extensively glycosylated biological fluid whose glycan structures and functions are only recently emerging. Just one of the roles of milk's glycans is to feed and fuel a unique microbiological community within the lower intestine of the infant, as an astonishing example of evolutionary genius of structure to function. Milk is alive, with cells, enzymes, immune factors, and microorganisms. As life scientists, we owe it to lactation, the central and most defended gene set of mammals, to give its understanding the priority it deserves.

Key Words: lactation, evolution, bioactivity

Animal Health 2

1183 Dynamics of individual quarter dry-off in organic certified dairies. C. Ibarguren*[1], J. Velez[2], and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Aurora Organic Farms, Platteville, CO.*

Drying off individual quarters represents an alternative for the treatment of severe or chronic clinical mastitis in US organic certified dairies, where antibiotic use is prohibited. The objective was to describe the dynamics of individual quarter dry-off (QD) in organic-certified dairies. Data from 2018 to 2023 from 4 organic dairies located in Colorado (USA) were collected and included 2,886 cows with at least one dried quarter (DQ). From this population, 769 cows (26%) had a second dried quarter (DQ2). Variables considered in the analyses were parity (primiparous [PP] and multiparous [MP]), DIM at the time of QD, and survival after the first QD (QD1). Dry quarters were identified by the date of the procedure and by location (left front [LF], left rear [LR], right front [RF], and right rear [RR]). Data were analyzed using the chi-squared test of goodness of fit, logistic regression, and ANOVA. During the study period, 16.9% of mastitis clinical cases resulted in DQ. The distribution of DQ1 by quarter in PP was 28.0% (LF), 20.2% (LR), 31.0% (RF), and 20.8% (RR). In MP the distribution was 29.8% (LF), 19.6% (LR), 31.7% (RF), and 18.9% (RR). In both groups, DQ1 was more frequent in the RF quarter (PP, $P < 0.01$; MP, $P < 0.01$), and no association was identified between quarter location and parity category. Frequency of DQ1 in PP was greater in summer (30.4%), followed by winter, (28.1%), spring (22.8%), and fall, (18.7%; $P < 0.01$). In MP cows DQ1 frequency was greater in summer (34.9%), followed by spring (24.8%), winter, (23.8%), and fall, (16.5%; $P < 0.01$). Mean ± SE DIM at DQ1 was smaller in PP (92.4 ± 2.82 d) than in MP (159.0 ± 2.71 d; $P < 0.01$), and the average times from DQ1 to DQ2 were 325 ± 21 d and 291.9 ± 19 d for PP and MP cows, respectively ($P = 0.22$). No effect on the occurrence of DQ2 was identified for parity category, and the odds (95% CI) of DQ2 for MP vs. PP were 0.95 (0.80–1.12). Days to culling after the date of DQ1 were not different between PP (347.0 ± 84.6 d) and MP (364.9 ± 18.4 d). These results contribute to the understanding of the dynamics of quarter dry-off, providing novel information for the control of mastitis in organic certified dairies.

Key Words: organic, mastitis, dry-off.

1184 Pathogen-specific clinical mastitis and its association with daily body condition score in dairy cows. P. Munoz-Boettcher*, C. Hernandez-Gotelli, and P. Pinedo, *Department of Animal Sciences, Colorado State University, Fort Collins, CO.*

The objective was to analyze the dynamics of automated body condition scores (BCS) in cows developing clinical mastitis (CM), considering the causal agent group. The study included data from 4,163 Holstein cows calving in 2022 and 2023 in a commercial dairy in Windsor, Colorado (USA). Milk samples were collected from quarters with CM at the time of diagnosis (d 0) to identify pathogen groups using a commercial on-farm plate system (Accumast, Ithaca, NY). Daily BCS were generated by video cameras (DeLaval, Tumba, Sweden). Data were analyzed by multivariable linear regression considering ΔBCS from d 0 to d 7 and d 14 post-diagnosis as the main outcome of interest. Potential predictors included the pathogen category (gram +, gram −, no growth, healthy), parity, other disease within ± 14 d from d 0, and BCS at d 0 as covariables. CM cases were categorized as occurring before or after nadir BCS (lowest BCS during the lactation) and analyzed separately. Overall, 25.7% of the cows had a CM case during the study period. Furthermore, 35.6% of the cultures indicated gram +, 36.3% gram −, and 29.1% had no growth. Multiparous cows affected with CM during the post-nadir period had greater loss of BCS (0–7 d and 0–14 d) compared with healthy cows (Table 1). The association between ΔBCS and the pathogen group depended on parity and time post-diagnosis. The results highlight the importance of preventing CM to avoid losses in BCS in dairy cows.

Key Words: BCS, mastitis, cow

Table 1 (Abstr. 1184). Least squares means (SE) for ΔBCS post-CM diagnosis, by pre-nadir or post-nadir category

	Pre-nadir (0–7 d)				Pre-nadir (0–14 d)			
	Primiparous		Multiparous		Primiparous		Multiparous	
Pathogen	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Gram +	0.045	0.033	0.012	0.015	0.097	0.034	0.061	0.017
Gram −	−0.014	0.036	0.043	0.014	0.068	0.038	0.072	0.015
No growth	0.032	0.031	0.038	0.014	0.053	0.033	0.081	0.016
No CM	0.019	0.011	0.035	0.007	0.051	0.012	0.051	0.008
	Post-nadir (0–7 d)				Post-nadir (0–14 d)			
	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Gram +	−0.007	0.024	-0.017^{a}	0.009	−0.052	0.03	-0.022^{a}	0.01
Gram −	−0.02	0.02	-0.012^{ab}	0.01	−0.035	0.024	-0.029^{a}	0.011
No growth	−0.0003	0.023	-0.022^{a}	0.011	−0.032	0.027	-0.031^{a}	0.012
No CM	−0.007	0.009	0.005^{b}	0.005	−0.007	0.011	0.012^{b}	0.006

[a,b]Different letters within columns indicate significant differences ($P < 0.05$).

1185 Relationship of differential somatic cell count with intramammary antibiotic success following subclinical mastitis diagnosis. C. Burner* and V. Ryman, *University of Georgia, Athens, GA.*

Subclinical mastitis (SCM) is diagnosed by a milk somatic cell count (SCC) ≥200,000 cells/mL and confirmed by microbial growth. Differential SCC (DSCC) considers the milk leukocyte distribution and could serve as a better diagnostic for SCM according to previous literature. In humans, elevated percentages of neutrophils (% NEU) have been correlated with negative medical outcomes. Our hypothesis is that elevated

% NEU at time of SCM diagnosis is associated with failure to cure after intramammary (IMM) antibiotic therapy. Quarter milk samples were collected from cows with SCC ≥200,000 cells/mL according to monthly SCC testing, plated on blood agar, and observed 48 h later for microbial growth. Infected quarters were enrolled if bacterial growth was detected. At enrollment, milk samples were aseptically collected and DSCC determined via QScout® Farm Lab. Enrolled quarters were treated with 1 tube of Spectramast LC IMM antibiotic for 2 d. Post-antibiotic milk samples were collected and cultured once a week for 5 weeks. A bacteriological cure was defined as no growth in any post-treatment samples. Differences in total leukocyte count (TLC), % NEU, percent macrophages, and percent lymphocytes between quarters that cured (CURE) and failed to cure (FTC) were analyzed in GraphPad 10 using nonparametric *t*-tests. Also, Pearson correlations were tested between parameters and outcome in GraphPad 10. The overall cure rate was 48.8%, with major pathogens (e.g., *Staphylococcus aureus*) having a cure rate of 25% and minor pathogens (e.g., non-*aureus* staphylococci) at 82.4%. The TLC was numerically lower, but trending, in CURE quarters (1,835.8 ± 933.6 cells/mL) compared with FTC (2,073.5 ± 374.8 cells/mL; $P = 0.066$). Importantly, there was a tendency for lower % NEU in CURE (54.8 ± 3.3%) compared with FTC (63.4 ± 3.0%; $P = 0.052$). Further, a successful outcome (CURE) tended to correlate with % NEU at $r = -0.29$, supporting earlier findings ($P = 0.061$). These preliminary data suggest that % NEU at time of SCM diagnosis could be associated with success following IMM antibiotic administration, although additional data are needed.

Key Words: subclinical mastitis, differential somatic cell count, antibiotic

1186 Evaluating effectiveness of two non-antibiotic options for management of mastitis in organic dairy cattle.

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The objective was to evaluate the effectiveness of 2 nonantibiotic mastitis treatment strategies: acoustic pulse therapy (APT) and cold laser therapy (CLT). A total of 129 multiparous lactating Holstein cows at a dairy farm in central Texas were enrolled in the study within 90 d in milk and with SCC >250,000/mL. Cows were randomly assigned to 1 of 3 treatment groups: APT (n = 47), CLT (n = 40), and control (CON; n = 42). The APT group was treated with the acoustic pulses generated using a commercial device (Armenta®) over 2 quarter regions (200 pulses/each region). Cows in the CLT group were treated using a cold laser device (Avant®) for 3 min, and treatments were repeated on d 3 and d 5, whereas CON did not receive either treatment. Quarter-level milk samples were collected before milking for the evaluation of SCC, fat percentage, protein percentage, lactose percentage, solid not fat (SNF), beta hydroxy butyric acid (BHBA), acetone, and milk urea nitrogen, and for the bacterial culture, identification, and cfu counts of the identified bacteria. All analyses were performed using repeated measures in the MIXED procedure of SAS (9.3). No significant effect of treatments was identified in SCC; however, both treatment cows (CLT and APT) had reduced ($P < 0.05$) cfu counts compared with CON on d 25 post-treatment. Cows in the CLT group had 0.17 more protein percentage ($P < 0.05$) than CON and APT groups on d 10 post-treatment. The CLT cows also had a lower proportion ($P < 0.05$) of bacterial growth among collected samples on d 10 (CLT 29%, CON 32%, APT 38%) and d 25 (CLT 29%, CON 33%, APT 35%) compared with the CON and APT groups. Milk BHBA concentration tended to be lower ($P = 0.06$) in the CLT group compared with the APT group by an average of 0.16 mmol/L, and MUN was greater ($P < 0.01$) in the CLT group compared with the CON and APT group by an average of 1.7 and 2.2 mg/dL, respectively. We conclude that the treatment effects varied between treatment groups and days; however, further research to identify appropriate doses and duration of treatment is warranted for optimal results.

Key Words: mastitis, laser, acoustic pulse

1187 The impact of maternal BLV status on daughters' status in Michigan dairy youngstock.

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Bovine leukemia virus (BLV) targets the immune system of animals. Research has shown that animals infected with BLV have decreased longevity, profitability, and welfare. Although the effects of BLV infection are well researched, the origin of infections in youngstock and the role that the dam's BLV status plays on her daughter(s) are not sufficiently understood. The objective of the study was to assess association between dams' BLV status and daughters' odds of being infected as youngstock. To do so, daughters born from previously tested and identified BLV-infected and uninfected dams were longitudinally sampled throughout the youngstock time frame, ranging from neonates to first-bred heifers. Whole blood samples were collected from each daughter at 3 time points: (1) neonates, (2) before being bred, and (3) 60 d following first breeding. DNA extracted from collected blood samples was screened via a BLV qPCR. Then, BLV infection status of dams and their daughters was statistically analyzed to perform a risk ratio analysis. A total of 254 daughters were sampled across 5 Michigan dairy farms, with 60% being born from a BLV-infected dam and 40% from an uninfected dam. The average ages at which animals were sampled were 5 ± 2.2 d, 370 ± 34.4 d, and 490 ± 43.2 d, respectively. The prevalence of BLV in daughters continually increased and reached 14% by the post-breeding time point. Of the 14% of infected daughters, 80% were born from an infected dam. Initial chi-squared analysis revealed a significant association between the BLV status of dams and the probability of their daughters being infected ($P = 0.0001$). Furthermore, the risk ratio (RR) analysis revealed that daughters born to an infected dam have a significantly higher risk of testing positive for BLV as youngstock compared with daughters born from uninfected dams (RR = 2.9; 95% CI: 1.6, 5.11). These findings indicate daughters born from an infected dam have an increased probability of testing positive before reaching the milking herd. Therefore, BLV status of dams may be a prominent aspect to consider when contemplating BLV management strategies in dairy herds.

Key Words: epidemiology, disease, management

1188 Evaluating the impact of mid-lactation hoof trimming on lesion risk in first-lactation dairy cows.

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We aimed to determine the effects of hoof trimming (HT) first-lactation dairy cows at 114–120 DIM on lesion occurrence at the subsequent HT and time to next lesion occurrence. First-lactation cows were enrolled from a single farm in a system in the Midwestern United States between

December 2022 and December 2023 and randomly allocated at calving to a TRIM (n = 4,774) or NOTRIM (n = 4,795) treatment. TRIM cows were automatically placed on the herd's trim list via the herd's management software for HT at 114 DIM (mean ± SD: 116 ± 1.5). NOTRIM cows were not trimmed during this period and considered enrolled at 117 DIM. Cows trimmed before 110 DIM were excluded. All enrolled cows received a scheduled HT at dry-off. All HT were done by farm personnel trained in the functional HT method with, at minimum, annual training by either local system trim leads or external consultants. Hoof trimmers were blinded to treatment allocation and monitored cows weekly for signs of lameness and hoof overgrowth in the pens and milking parlor. The HT records and cow-level information were extracted from the herd's management software. Time to next trim was calculated from the time of HT in TRIM cows and the 117 DIM enrollment time for NOTRIM cows. The lesions recorded by hoof trimmers were digital dermatitis (DD), foot rot (FR), sole ulcer (SU), white line disease (WLD), and toe ulcer (TOE). Cox proportional hazard models, with the fixed effect of treatment, were used to analyze time to next lameness event and lesion risk. The difference in median time to next trim of NOTRIM (169 d) and TRIM (177 d) was −5.6 (95%CI: −8.4, −2.9). Lesions at the next trim were reported in 5.5% of TRIM and 6.8% of NOTRIM cows, with a risk difference of 1.3 (95%CI: −0.23, 2.9). Similar proportions of infectious (DD, FR) and non-infectious (SU, WLD, TOE) lesions were reported in each group. The hazard ratio of cows getting a lesion was 1.01 (0.76–1.35) for NOTRIM vs. TRIM cows. The data show that mid-lactation HT of first-lactation cows did not affect lesion risk or time to lesion. Not doing mid-lactation hoof trims in first lactation can reduce cost and HT-associated behavior changes.

Key Words: dairy management, hoof health, lameness prevention

1189 Automated cattle lameness detection prevents development of severe lameness and reduces chronic cases: Preliminary results of a randomized clinical trial.

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Our aim was to assess the effectiveness of using an automated intelligent 2D imaging system (CattleEye Ltd., Belfast, UK) to detect lame cows and control lameness. Multiparous cows from a large dairy farm (2,100 Holstein cows) were enrolled weekly and randomly allocated to control (C, n = 208) or treatment (T, n = 211) groups. Enrolled cows were comingled with non-study cows in 3 pens numbering in total 780 cows. Only cows that were ≤30 d in milk (DIM) and not trimmed in the last 30 d before enrollment were eligible. Cows in both groups were managed under the farm's existing protocol, consisting of routine trims at 80 DIM and 180 DIM, and examination and treatment of any cow identified as lame by farm staff while in the parlor or while moving cows. There was no additional intervention for C cows. The weekly average automated mobility score (CE) was calculated only for T cows, and those with CE ≥50 or trending up (≥20-point increase during the last 2 weeks) with >28 d since last hoof check were examined and trimmed weekly. Farm staff and the trimmer were blind to which cows were enrolled. A veterinarian performed 5 monthly mobility scoring (MS) sessions using a 0-to-3 four-grade scoring system (scores 2 and 3 were considered as lame). Lameness prevalence (MS ≥2) and prevalence of severely lame cows (MS = 3) were calculated on each session. Chi-squared tests were used for comparisons. Lameness prevalence was numerically lower in the T group during the first 3 sessions by 5.8%, 2.5%, and 7.2%, respectively. Cumulative incidence of MS 3 was 6.7% and 0% for C and T groups, with the prevalence of MS = 3 cows being greater in the C group on the second session (9/163 vs. 0/164, $P = 0.002$). Moreover, the proportion of chronic cases (2 consecutive MS ≥2) was greater in the C group compared with the T group (24/169 vs. 11/168, $P = 0.031$). Preliminary results from this ongoing study indicate that using automated mobility scores for early detection of lameness, which is then treated, prevents development of severe lameness and reduces chronic cases.

Key Words: artificial intelligence, foot trimming, mobility

1190 Pilot implementation of a targeted bovine digital dermatitis control program on Swiss dairy farms.

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This non-randomized clinical intervention study was designed as a prospective, multicenter group comparison to evaluate the efficacy of a targeted control program for bovine digital dermatitis (BDD). This sanitation program was implemented over a 1-yr period on 10 pilot farms (intervention group) and 10 control farms (control group without intervention). Mainstays of this program derived from results of a previous BDD risk factor analysis. All farms were visited monthly to assess within-herd BDD prevalences and to perform risk assessments. Risk-associated management practices were identified on each farm, and management changes expected to prevent introduction or spread of BDD within farms were suggested and agreed upon with farmers of the intervention group. Diagnosis of BDD was based on visual inspection of the feet. Moreover, skin biopsies were taken from a subset of cows of the intervention group before and 2 mo after treatment for histopathological examination to confirm clinical and histological cure. The median number of dairy cattle was 48 (range 21–91) on intervention farms and 46 (range 25–94) on control farms. Initial prevalence averaged 32.5% (95% CI 28.2–36.6) and 38.8% (35.1–41.4). Preliminary findings suggest that, after 6 mo, BDD within-herd prevalence was 21.6% (17.5–25.9) on intervention farms and 37.1% (33.8–40.2) on control farms. After 1 yr, BDD prevalences were at 13.2% (11.4–15.8) on intervention farms and 42.3% (36.2–49.1) on control farms. Final data will be analyzed using mixed logistic regression models. Two months after successful clinical cure, biopsy samples revealed incomplete histological cure in 6/20 cows of the intervention group. Current descriptive results indicate that implementation of targeted BDD control measures could reduce within-herd prevalences. The suggested measures might provide the basis for a potential nationwide BDD control program that could be of importance also beyond the Swiss borders.

Key Words: cattle, lameness, treponemes

Breeding and Genetics Platform Session: Deriving Novel Traits from Sensors and Other Technologies for Breeding Purposes

1191 Deriving novel traits to predict feed intake using data from sensors and other novel technologies. J. E. Koltes*[1], L. M. James[1], M. S. Mayes[1], C. J. Cooper[1], K. L. Gaddis[2], R. L. Baldwin[3], J. E. Santos[4], F. Peñagaricano[5], H. M. White[5], K. A. Weigel[5], R. J. Tempelman[6], and M. J. Vandehaar[6], *[1]Iowa State University, Ames, IA, [2]Council for Dairy Cattle Breeding, Bowie, MD, [3]United States Department of Agriculture Animal Genomics and Improvement Laboratory, Beltsville, MD, [4]University of Florida, Gainesville, FL, [5]University of Wisconsin, Madison, WI, [6]Michigan State University, East Lansing, MI.*

Feed efficiency is an economically and environmentally important trait in dairy cattle. Estimating feed efficiency requires individual animal feed intake, milk yield and components, and ideally information about body weight and body condition score. Unfortunately, apart from milk traits, these data are rarely available on commercial farms as they are cost prohibitive. Thus, there is a need to identify new indicator traits for feed efficiency. Milk testing and sensor data represent existing information streams on dairy farms that may be able to serve as proxies for traits composing feed efficiency. Multiple experiments were conducted to evaluate the utility of these data. Five different types of sensors measuring activity, rumen traits, and temperature were evaluated for their phenotypic association with feed efficiency traits. Heritabilities were estimated for 3 sensor-based traits from repeated records for eartag and milking collar-based sensors, respectively. Estimated heritabilities were 0.21 (eartag activity), 0.14 (eartag rumination), and 0.17 (collar activity). Milk metabolites were also investigated to identify metabolites associated with or predictive of feed intake. Extreme cows for residual feed intake (RFI; top and bottom 15%) were selected. Three assays were performed: gas chromatography mass spectrometry, liquid chromatography mass spectrometry, and fatty acids inferred from Fourier-transformed infrared spectrometry. A total of 33 metabolites and 10 pathways were identified as statistically associated with feed intake. Milk fatty acid data were predictive of feed intake with an R^2 of 0.86 and a concordance correlation coefficient of 0.92 based on an across cow cross-validation approach using a random 25% of cows, independent of the training set, as the testing set. Although further validation is needed, these studies indicate that sensor data could be useful as indicator traits for feed intake and milk-based data may be predictive of feed intake.

Key Words: dairy cattle, feed efficiency, precision livestock technologies

1192 Genetic characterization of daily feeding pattern in lactating Holstein cows and its association with feed efficiency. L. Cavani*[1], K. L. Parker Gaddis[2], R. L. Baldwin[3], J. E. P. Santos[4], J. E. Koltes[5], R. J. Tempelman[6], M. J. VandeHaar[6], H. M. White[1], F. Peñagaricano[1], and K. A. Weigel[1], *[1]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, [2]Council on Dairy Cattle Breeding, Bowie, MD, [3]Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD, [4]Department of Animal Sciences, University of Florida, Gainesville, FL, [5]Department of Animal Science, Iowa State University, Ames, IA, [6]Department of Animal Science, Michigan State University, East Lansing, MI.*

Feeding behavior traits, such as number, duration, or intake per feeder visit, have been studied and associated with feed efficiency in dairy cattle. Those traits, however, do not fully capture a cow's feeding pattern throughout the day. The goal of this study was to propose a new phenotype for characterizing within-day feeding pattern and estimate its heritability and its genetic correlations with dry matter intake (DMI), secreted milk energy (MilkE), metabolic body weight (mBW), and residual feed intake (RFI). Feeding patterns were evaluated using 4.8 million bunk visits from 1,684 mid-lactation Holstein cows collected from 2009 to 2023 with an Insentec system. Daily bunk visits were ordered, with time zero designated as the time of the first feed delivery. Intake proportions per cow per day were calculated by dividing feed intake per visit by the total intake of the cow for that day. Feeding pattern was characterized by the area under the curve (AUC) of cumulative feed intake proportions for each cow each day. The feeding pattern phenotype per cow was defined as the average AUC across days, whereas consistency of feeding pattern was calculated as the log variance of daily AUC values (log-Var-AUC). Genetic parameters were estimated using an animal model, with lactation, days in milk, and cohort as fixed effects and animal as a random effect. Heritability estimates for AUC and log-Var-AUC were 0.35 ± 0.07 and 0.16 ± 0.06, respectively. Genetic correlations between AUC and DMI, MilkE, mBW, and RFI were −0.18 ± 0.12, −0.30 ± 0.14, 0.16 ± 0.10, and −0.18 ± 0.14, respectively. Log-Var-AUC was genetically correlated with DMI (0.47 ± 0.15), MilkE (0.40 ± 0.17), mBW (0.28 ± 0.13), and RFI (0.24 ± 0.14). These results suggest that cows that consume most of their total daily intake in the first few hours after feed delivery (larger AUC) and cows that have consistent daily feeding patterns tend to be more feed efficient. Overall, feeding pattern is heritable and genetically associated with feed efficiency.

Key Words: area under the curve, heritability, residual feed intake (RFI)

1193 Development and genetic evaluation of novel resilience traits in Holstein cattle utilizing precision calf-feeding technologies. J. Graham*[1], M. Taghipoor[2], L. Gloria[1], J. Boerman[1], A. Rocha[1], and L. Brito[1], *[1]Purdue University, West Lafayette, IN, [2]Université Paris-Saclay, Palaiseau, Île-de-France, France.*

Precision livestock farming (PLF) holds promise for assessing and enhancing resilience in dairy cattle. This study employs random regression models to analyze calf milk consumption, aiming to derive novel traits and estimate genetic parameters related to resilience in Holstein calves. We utilized the BLUPF90+ suite of programs to integrate genomic and pedigree data from 10,076 calves genotyped with a 50k SNP panel. Variance components were estimated using Bayesian sampling methodology. Longitudinal data, collected from 2015 to 2021, encompassed Förster-Technik calf feeding records and family and health status data from Dairy Comp software. We developed a data-driven model based on functional data analysis, using penalized quantile regression to quantify calves' responses to environmental perturbations. Various resilience indicators were assessed, including amplitude of deviation, perturbation time, recovery time, velocity parameters, and disease-related traits. Systematic effects such as feeding day, birth weight, pen density, dam parity, and contemporary groups (defined based on pen and management characteristics) were considered. Model selection for genetic parameters was based on Bayesian information criterion scores. Significant heritability estimates were obtained for several resilience indicators, ranging from 0.16 to 0.52, underscoring the genetic component of resilience traits in dairy calves. Our findings contribute to a deeper understanding of resilience mechanisms and inform breeding strategies for more resilient dairy cattle better capable of withstanding environmental and health challenges. This study highlights the potential of PLF technologies, combined with advanced genetic analyses, to enhance the resilience and sustainability of dairy farming operations, ultimately contributing to improved animal welfare and farm productivity. Further research is warranted to validate these findings and refine breeding programs targeting resilience traits in dairy cattle.

Key Words: resilience, precision livestock farming, genetics

1194 Calculating resilience indicators in US Holstein cows using pen-level data.

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Resilience is defined as an animal's capacity to bounce back to normal functioning after a perturbation or maintain specific functions in the face of change or stress. Dairy herds in the United States typically group cows into pens based on factors such as parity, lactation stage, reproductive status, and milk production. By coupling daily milk yield data with day-to-day pen location information, we can more precisely model management conditions and environmental stressors affecting individual cows for each day of the lactation. It also allows us to detect management and environmental perturbations that may occur each day at the pen level and subsequently measure the responses of individual cows to these stressors. Our data set included daily milk weights and pen locations of 4,183 primiparous cows in 10 US herds from 2018 to 2023. Individual lactation curves were fitted using polynomial quantile regression with a 0.5 quantile to derive expected lactation curves. Our resilience phenotype was defined as the percentage change in daily milk production for a cow on a given day relative to her preceding 7-d average. Perturbed days were identified based on residuals between expected and observed daily milk yields, and such days had 70% or more negative residuals for at least 5 consecutive days in a given pen. The statistical model included age at first calving and herd-pen-milking-date as fixed effects and additive genetic and permanent environmental as random effects. Estimated heritability of relative change in milk production on perturbed days was 0.08 (0.02), while estimated repeatability was 0.41 (0.01). Our findings suggest that animals differ in their response to perturbations at the pen level, and this measure of resilience in daily milk yield is heritable and repeatable. Identifying perturbations at the pen level on specific days can more effectively capture the management and environmental conditions affecting an individual cow at a given time, and resilience can be measured by comparing how her response differs from that of her contemporaries, which enables producers to make more informed decisions through selection and management strategies.

Key Words: resilience, pen-level perturbations, daily milk weights

1195 Novel phenotypes for selecting for increased heat tolerance.

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Cows are negatively impacted by heat stress, which results in reduced milk yield, decreased fertility, and diminished animal welfare. These effects will continue to grow as more dairy producing regions are increasingly impacted by climate change. Australia is the first country to introduce a genetic evaluation for heat tolerance, by evaluating the decline in milk production as temperature and humidity increase as a phenotype. Moreover, there are other opportunities to expand the selection criteria by exploring additional predictors as phenotypes for evaluating heat tolerance in dairy cattle, which can be incorporated into the current breeding value. This can enable us to identify cows that are more tolerant to heat. It has been shown that heat loss is related to the surface area per unit of body weight; therefore, cows with a larger ratio of surface area to mass (SA:M) could be more heat tolerant. Considering the practical difficulty to obtain a large amount of SA:M data from many commercial dairies, body depth is considered to be a proxy for SA:M. It was hypothesized that cows with less body depth, therefore higher SA:M, will have greater heat tolerance. For this analysis, 191,205 first-lactation Australian Holstein cows with test-day milk records and official classification scores from 1999 to 2021 were used. The test-day milk yield data were merged with temperature-humidity index (THI) from the nearest weather station. Cows were classified as shallow, average, or deep bodied based on classification score (1–9). Statistical analysis was performed using a linear mixed model that included fixed effects of year-season, age at calving, days in milk at test-day (DIM), THI, and the interaction between body depth and THI, and the random effect of herd-test-date. The result shows a significant effect ($P < 0.001$) of body depth-THI interaction on milk yield. Cows with a deep body depth (score ≥7) saw a greater reduction in milk yield as THI increased. The initial findings suggest that classification measurements can be used to predict heat-tolerant cows and warrant further investigation.

Key Words: heat tolerance, conformation, body depth

1196 Estimation of variance components for multiple electrical conductivity traits derived from automatic milking system data in Holstein cows.

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Mastitis is the most common and economically costly disease in dairy production. Subsequently, dairy producers stand to benefit from the development of novel traits that can improve resistance to mastitis. Therefore, traits derived from measures of electrical conductivity (EC) in milk may be key auxiliary traits for increasing resistance to mastitis in dairy populations. The primary objective of this study was to investigate the genetic background of multiple EC traits, incorporating data collected from automatic milking systems (AMS) and genomic information. Data were collected from 36 Lely Astronaut AMS for a period of 3 years on 3,868 Holstein cows. The AMS provided records of EC for each of the 4 quarters of the mammary gland (left-front_EC, left-rear_EC, right-front_EC, and right-rear_EC) for every milking visit. Two additional traits were derived from the data collected from the 4 quarters: the average of EC records per visit (averageEC) and the maximum EC of records per visit (maximumEC). To create daily estimates, records for each trait were averaged by day for all traits except for maximumEC, which was recorded as the maximum EC record within a day. All individuals were genotyped, and a total of 57,282 SNPs were utilized to calculate genomic relationships. Variance components were estimated for all daily traits using a single trait repeatability model utilizing (Average Information) REML in the BLUPF90 suite of programs. Heritability estimates ± SE for EC traits ranged from 0.12 ± 0.01 for maximumEC to 0.43 ± 0.02 for averageEC, while repeatability estimates ranged from 0.41 ± 0.006 for maximumEC to 0.64 ± 0.007 for averageEC. These preliminary results show that traits utilizing measures of EC have potential for genetic selection and future research needs to be done to fully evaluate the potential of EC traits as indictors of mastitis resistance.

Key Words: electrical conductivity, automatic milking system, Holstein

1197 Genetic and genomic evaluations of milking speed and duration from automated milk recording. J. R. O'Connell*[1], A. M. Miles[2], J. L. Hutchison[2], S. Toghiani[2], R. H. Fourdraine[3], and P. M. VanRaden[2], [1]*University of Maryland School of Medicine, Baltimore, MD,* [2]*USDA Animal Genomics and Improvement Programs Laboratory, Beltsville, MD,* [3]*Dairy Records Management Systems, Raleigh, NC.*

Many milking systems now record milk produced and the duration (DUR) of each milking for every cow. Suitability of milking speed (MSPD) or DUR traits for selection and phenotype data required to produce a reliable evaluation were investigated. Records from January 2022 to December 2023 were retrieved by Dairy Records Management Systems, comprising data from 305 herds, 9 different equipment manufacturers (OEM), and 23,201 complete lactations of 23,180 cows, including 4,246 genotyped cows. Milking speed (MSPD) was defined as milk yield divided by DUR for each individual milking. Eight traits were compared: (1) average of total lactation on data for all parities, (2) average of DHI test days (TD) for all parities, (3) average of total lactation data for first parity (P1) only, (4) average of TD for P1, for each MSPD and DUR. Breed, milking frequency, parity, and OEM were included in the genetic model along with genetic groups and permanent environment. The pedigree relationship matrix included 219,703 animals with records or descendants with records, plus 96 million other animals. Variances were estimated by both Gibbs sampling and REML and were very similar. For MSPD, residual variance was 51% higher for TD traits compared with total lactation. MSPD TD heritability was 28% vs. 37% for total lactation data; genetic correlation between them was 0.97. MSPD was less stable in P1 compared with other parities, but genetic correlation was 0.92, suggesting they are measuring the same trait. MSPD had a small favorable genetic correlation with milk yield but unfavorable with somatic cell score based on 756 Holstein bulls with reliability (REL) for MSPD >50%. Heritability for DUR was higher than MSPD at 44% across all parities and 48% for P1. Genetic correlation of DUR with MSPD was −0.81, and phenotypic correlation was −0.52. Genomic predictions for MSPD for young animals averaged 37% REL compared with ~70% REL for several other traits or 27% REL for genomic predictions of residual feed intake. We conclude that evaluations for MSPD or DUR are not only feasible but would have significant economic impact for producers using various milking systems.

Key Words: milking speed, heritability, genomic prediction

1198 Dairy calf health and growth monitoring through camera phenotyping techniques. M. Liao*, J. Duncan, Y. Bi, G. Morota, and R. Cockrum, *Virginia Tech, Blacksburg, VA.*

Effective monitoring of dairy calf health and growth is crucial for ensuring their well-being and productivity within the dairy industry. The objectives of this research were to (1) predict the body weight (BW) of dairy Holstein calves using an automatic recording system and (2) identify dairy Holstein calf diarrhea through predictive modeling. Heifer calves (n = 20) BW was collected manually via an electronic scale. Depth cameras captured videos of calves, and image processing techniques were employed to extract video body metrics, including width, length, height, and volume. A correlation matrix was computed using the Pearson correlation coefficient to determine the relationships between BW and body metrics using the ggcorrplot package and a linear regression model was used to predict BW, with 80% of the data set reserved for training using sklearn.linear_model package in R. A security camera was also used to collect videos of pre-weaning calves' (n = 54) posterior images that were manually labeled into 4 health levels (healthy, mild, moderate, severe) using the annotation tool *labelImg*. Using 80% of the data for training and 20% for testing, a customized YOLOv8 model was developed in a Linux environment. There were strong and positive correlations between body volume (0.83), height (0.75), width (0.71), and length (0.74) with BW. The predictive model from camera images achieved an accuracy of 0.8. Additionally, the automatic recording system was designed to activate once an object was detected within the region of interest. Moreover, the diarrhea prediction model demonstrated strong prediction accuracies: 0.94 for healthy, 0.97 for mild, 0.95 for moderate, and 1.0 for severe cases. This research demonstrates the application of camera technologies in monitoring dairy calf health effectively and non-invasively. By accurately predicting BW and detecting diarrhea at an early stage, these systems could assist producers in managing calf growth and health more efficiently.

Key Words: automated camera, body weight prediction, diarrhea detection

1199 Precision breeding opportunities using robotic milking data. J. P. Nani*, S. Arens, A. Sewalem, B. Shonka-Martin, L. Chang, and M. McClure, *ABS-Global, DeForest, WI.*

The increased use of robotic milking machines has revolutionized dairy farming practices by introducing advanced tools for milk production and herd management. These robotic systems also serve as data collection platforms, generating valuable information regarding milk production, cow health, and behavior that can be later used in breeding programs. Data were obtained from 136 Lely automatic milking systems (AMS) farms from 8 different countries from the Americas and Europe. In total 34,552 lactating cows from lactation 1 to 5 contributed with >60 million datapoints. Box time was calculated as the time in minutes spent in the milk pen for every visit. Dead time was calculated by subtracting the milk time to the total box time in the pen and averaged (Table 1). Although box time increased with lactation, dead time decreased from lactation 1 to 3, to start increasing again in lactation 4 and 5, indicating that there is an adaptation time to the system during early lactations. As expected, milk time was highly correlated with box time, and milk yield was moderately correlated with milk time (Table 2). Milk yield and milk time had lower correlation with dead time, indicating that cows with lower dead time would be desirable, as they seem to better adapt to AMS without compromising production. In summary, collecting and analyzing information from robotic milking machines is a powerful tool

Table 1 (Abstr. 1199). Average box time and dead time by lactation

Lactation	Avg. box time (min)	Avg. dead time (min)
1	6.86	2.35
2	6.83	2.30
3	7.04	2.29
4	7.30	2.32
5	7.51	2.35

Table 2 (Abstr. 1199). Correlations between box time (BT), milk yield (MY), milk time (MT), and dead time (DT)

Lactation	Cor BT/MT	Cor MY/MT	Cor MY/DT	Cor MT/DT
1	0.92	0.51	−0.15	−0.04
2	0.93	0.52	−0.12	−0.09
3	0.94	0.52	−0.12	−0.10
4	0.94	0.53	−0.11	−0.11
5	0.95	0.55	−0.10	−0.11

for the dairy industry to identify animals that will perform better in these systems, and that will in turn enhance productivity, improve animal welfare, optimize resource use, and drive continuous improvement for the industry through precision breeding.

Key Words: robot milking, precision breeding, animal welfare

1200 Exploring Siamese neural networks for few-shot individual recognition of dairy cows in open-set scenarios.

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Computer vision systems (CVS) for individual animal recognition are commonly developed to handle closed-set scenarios, limiting inferences to a fixed group of animals. These CVS require additional data annotation and model retraining when dealing with individuals not included in the initial training set. In this study, we explored Siamese neural networks (SNN) for few-shot individual recognition of dairy cows that were not included in the original data set (open-set scenarios). Top-down view images from 92 lactating cows were captured twice a day for about 3 mo, generating a total of 19,339 frames. This data set was split chronologically into training and validation sets, containing 15,659 and 3,680 images, respectively. Training images from 77 cows were used to implement different SNN models to classify image pairs as similar or dissimilar. We considered different architectures for the network backbone (VGG19, Xception, and ResNet50) as well as 2 different loss functions (binary cross-entropy and triplet loss). The trained networks were used to classify 15 cows that remained unknown during the training process. A limited number of training images (up to 11) for each of those 15 animals was randomly selected to compose the support data set. The SNN models were used to identify the animals in the validation data set based on their similarity with the support data set. The classification accuracy (mean ± SD) for the 15 cows ranged between 0.79 ± 0.05 and 0.91 ± 0.05 when using only one support image per animal. Additionally, average values for precision, recall, and F1-score were very similar to the obtained accuracies. Regression analysis showed accuracy plateauing after 5 or 7 support images per animal. The best overall performance was obtained with the ResNet50 architecture, achieving a classification accuracy of 0.96, considering 5 support images per animal. In conclusion, the results indicate the suitability of using SNN for recognizing new animals without need of extensive model retraining or data annotation, facilitating technology transfer to support data-driven decisions across farms.

Key Words: animal identification, computer vision, few-shot learning

1201 Genomic predictions for greenhouse gas emission traits in Holstein cows.

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Concerns about greenhouse gas emissions from dairy farms have been growing in recent years. Reducing methane emissions via genetic selection can be vital for the long-term sustainability of the dairy industry. The objective of this project was to estimate genetic parameters for methane production (MetP; g/d) and residual methane production (RMet; g/d) using data from commercial Holstein herds. A total of 32,615 weekly averages of daily MetP measured on 4,163 Holstein cows from June 2022 to November 2023 were used in this study. These data were collected in 4 commercial herds using the *GreenFeed* equipment. RMet was defined as the residual of a linear regression of MetP on energy-corrected milk, metabolic body weight, and other environmental effects. Genetic parameters were estimated using a univariate animal model with repeated records using the AIREMLF90 software. This statistical model included year-season of calving, week of lactation, lactation number, and herd-year of calving as fixed effects. The predicted variance components were then used to estimate breeding values (EBV) including genomic data of over 2 million animals in a single-step genomic BLUP (ssGBLUP). Reliabilities of EBV were estimated using accf90GS v2.54 program. The phenotypic mean (SD) of MetP and RMet was 364.28 (72.99) and 0.00 (59.94) g/d, respectively. Heritability estimates (SD) for MetP and RMet were 0.25 (0.04) and 0.30 (0.05), respectively. The EBV of phenotyped animals had mean (SD) of 1.69 (20.13) and 3.17 (29.62) g/d for MetP and RMet, respectively. The reliability of EBV for MetP had mean (SD) of 0.43 (0.04) for phenotyped animals, 0.35 (0.06) when considering only animals with genotype information, and 0.23 (0.16) when accounting for all animals. Likewise, the reliability for RMet's EBV had mean (SD) of 0.69 (0.04) for phenotyped animals, 0.55 (0.05) when including only animals with genotype data, and 0.35 (0.26) across all animals. The results of this study show that there is considerable genetic variation among the animals regarding methane emission and demonstrate that a large-scale genomic selection for reduced methane emission in dairy herds is feasible.

Key Words: genetics, dairy cattle, methane

Dairy Foods Platform Session: 44th Discover® Conference—Why Cheese from Milk?

1202 Strategies to control blistering and browning in low-moisture part-skim mozzarella cheese. K. Cook*[1], S. Govindasamy-Lucey[2], J. Jaeggi[2], R. Ibáñez[2], M. Johnson[2], and J. Lucey[1,2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

Blistering and browning is the phenomenon of bubble formation and browning during the baking of low-moisture part-skim (LMPS) mozzarella cheese on pizza, especially in Impinger-type ovens. Excessive browning is undesirable to pizza retailers and consumers. This study evaluated the effects of cheesemaking methods, preacidification (PA), and storage time (ST) on the browning coverage (BC) of melted cheese on pizza. Four types of LMPS mozzarella were manufactured using standardized 2.5% casein milk (n = 5). Treatments included a control vat (culture added, no PA), direct acid (DA) vat (no culture, PA to pH 5.6), pH 6.0 PA vat (culture added), and pH 5.6 PA vat (culture added). Cheeses were tested for composition, pH, insoluble calcium (ICa) content, proteolysis (pH 4.6 soluble nitrogen formed), and sensory descriptive analysis of melted pizzas (8–10 trained panelists). Cheeses were stored at 4°C for 3 mo. A split plot design was used to analyze the impacts of PA and ST, and their interactions ($\alpha = 0.05$, Tukey test). Composition did not vary between cheeses ($P > 0.05$). DA cheese had the highest pH during storage (5.67 compared with 5.06, 5.14, 5.17; $P < 0.05$). ICa levels in cheese decreased with increasing PA ($P < 0.05$). Control, DA, pH 6, and pH 5.6 had 15.5, 12.6, 13.0, 8.1 mg ICa/g protein at 2 wk, respectively. For total Ca in cheese, control, DA, pH 6, and pH 5.6 had 593, 468, 540, 430 mg/100 g cheese, respectively. Proteolysis did not vary at 2 wk ($P > 0.05$). Despite large differences in Ca and ICa contents, BC on pizza did not vary between cheeses at 2 wk ($P > 0.05$). BC on pizza increased over time for control, pH 6.0 PA, and pH 5.6 PA treatments, correlating well with the significant increase in proteolysis over time in these cheeses ($P < 0.05$). Proteolysis did vary significantly between treatments at 3 mo ($P < 0.05$). At 3 mo, control cheese had higher BC than pH 6.0 and pH 5.6 cheeses due to higher proteolysis ($P < 0.05$). DA cheese did not follow the same trends due to the impact of pH. Overall, pizza BC was most affected by proteolysis because it increases the free amines available for Maillard browning during blistering.

Key Words: blistering, browning, low-moisture part-skim (LMPS) mozzarella

1203 Development of feta and cheddar cheese flavor profiles with the substitution of animal lipase with microbial lipase. E. Caswell*, R. Larsen, and E. Ahlborn, *Brigham Young University, Provo, UT.*

Cheeses possess distinct flavors, aromas, and textures that are partially influenced by starter culture lipolytic activity and, in some varieties, added lipases. Our objective is to assess the validity of substituting exogenous animal lipase with microbial lipase and to see the impact on objective and subjective characteristics. Commercial microbial lipases were selected for their ease of production, availability, and appeal to current consumer trends. The experiment used conventional methods to create batches of Cheddar and feta cheese while keeping general production parameters constant. A Cheddar model was utilized as a shorter aging representation for Italian hard cheeses. After extensive testing, 3 microbial lipase treatments were compared against kid goat lipase and a control. The best 2 (*Mucor javanicus* and *Candida cylindracea*) were then compared against kid goat lipase and a control in a feta model. Measured parameters include microstructure, total fat content, volatiles, texture, moisture, water activity, and FFA composition. Quantitative descriptive analysis was used to observe sensory qualities. In the Cheddar model, no significant texture and minimal moisture changes ($\rho \leq 0.05$) were observed. However, in feta cheese, the use of *M. javanicus* lipase resulted in higher moisture content and a less firm structure ($\rho \leq 0.05$). *Candida cylindracea* had a firmer texture and a lower moisture content ($\rho \leq 0.05$). It was observed that microbial lipases liberated more medium and long-chain fatty acids while kid-goat lipase liberated more short-chain fatty acids. Exogenous lipases significantly influenced volatiles ($\rho \leq 0.05$), with notable changes in odd-numbered FFA and acetic acid levels. Sensory analysis showed that lipase-treated cheeses all had a general increase in FFA flavor notes and pungency. The results of this current study show that if kid goat lipase were to be replaced in feta cheese, *M. javanicus* would produce the closest flavor profile, but a softer texture. *Candida cylindracea* would not be a suitable replacement, due to significant differences in hardness and flavor volatiles.

Key Words: feta cheese, Cheddar cheese, exogenous lipase

Dairy Foods Symposium: Precision Components from Grass to Glass

1204 Nutritional strategies to regulate the synthesis of milk components. S. I. Arriola Apelo*, *Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Milk is the only single food that provides all the nutrients required to sustain the rapid growth and development of the newborn mammal. After closing of tight junctions, mammary epithelial cells are responsible for the uptake or synthesis of the more than 2,000 chemical compounds found in bovine milk. Nutritional, hormonal, and neuronal signals coordinate the supply, transport, synthesis, and secretion of each of those compounds to guarantee narrow enough concentrations of the required nutrients to balance the nutritional needs of the newborn. Cellular transduction pathways further transfer systemic signals to the metabolic machinery in charge of transport, synthesis, packaging, and secretion. Technological advances in the measurement of mRNA, proteins, post-translational modifications, and metabolites have allowed a deeper understanding of the roles of systemic and cellular signals on milk component synthesis. We now understand that mammary cells do not simply transform circulating metabolites into milk components at a concentration-dependent rate, but rather communicate their demands systemically to modify organism behavior, growth, and metabolic capacity of specific systems and organs, and ultimately mammary substrate supply. In turn, mammary cells also respond to systemic signals adjusting their metabolic activity to systemic capacity. Insulin is an example of a systemic signal that communicates nutritional status to peripheral tissues. Through cellular membrane receptors and a cascade of cellular signals, insulin regulates fatty acid, carbohydrate, and protein synthesis, including in milk. However, insulin generates an unfair competition for mammary cells. Unlike in mammary cells, in adipocytes and myocytes, insulin also stimulates glucose uptake, reducing glucose supply for milk synthesis. In turn, milk-secreting cells synthesize serotonin, which inhibits insulin secretion from pancreatic β-cells, increasing nutrient availability for milk production. We will discuss nutritional strategies to alter the balance between systemic nutrient supply and mammary demands with the overall goal of increasing nutrient efficiency for milk production.

Key Words: milk synthesis, insulin, serotonin

1205 Towards the future of next-generation dairy foods: A processing perspective. G. Ozturk*, *University of Wisconsin–Madison, Madison, WI.*

Milk contains an abundance of bioactive compounds and essential nutrients, including the milk fat globule membrane (MFGM). The MFGM is a complex structure comprising approximately 40% lipids (e.g., phospholipids) and 60% proteins (e.g., glycoproteins). Each of these compounds has unique functionalities, such as promoting brain development, modulating the immune system, exhibiting antimicrobial properties, improving the growth of desirable gut bacteria, and reducing inflammatory and metabolic diseases. The dairy industry produces abundant bioactive compound streams that can be examined for their valuable functionalities. Whey protein phospholipid concentrate (WPPC) is a by-product of whey protein isolate production and reveals a source of many bioactive compounds that can benefit the immune system and gut health. We recently demonstrated that WPPC contained relatively higher levels of MFGM proteins, representing 23% of the total protein, and phospholipids accounting for 20% of the total fatty acid pool. These bioactive compounds derived from WPPC have the potential to be utilized as cutting-edge ingredients. Therefore, it is crucial to harness these streams through isolation and characterize these bioactive compounds, and more importantly, to develop methodologies to isolate these components in purity to enable detailed mechanistic investigations. However, our earlier research indicates that the production of WPPC might involve heat-induced formations of complexes between whey proteins and MFGM. Therefore, investigating changes that occur in the WPPC matrix during thermal treatment becomes essential. Specifically, understanding the alterations that occur in whey proteins due to different thermal treatments enhances the efficiency of isolating compounds such as whey proteins, milk fat globule membrane proteins, and phospholipids, all of which hold substantial nutritional and economic value.

Key Words: whey protein phospholipid concentrate, bioactive compounds, isolation

1206 Effect of milk and its bioactive compounds on human health. M. Bionaz*, *Oregon State University, Corvallis, OR.*

Milk is a very complex product. Besides the main components that are typically measured in milk, many bioactive compounds, both nutritive and non-nutritive, are present in milk. Some of those compounds have been known for a long time, such as fatty acids, but others have been recently discovered, such as exosomes that cargo proteins and oligonucleotides (e.g., miRNA), peptides, naturally occurring bacteria, oligosaccharides, phytochemicals, and a large number of signaling molecules. Studies both in vitro, in animal models, and in humans overall indicate a positive effect of milk to human health; however, the effect of each bioactive compound present in milk on human health is difficult to dissect, as the effective dose of each bioactive compound is seldom known, and the effect of one bioactive compound cannot be studied in isolation, as there is a complex interaction between the various bioactive compounds in milk as well as with bioactive compounds in other feed. For instance, despite the assumption that milk positively affects bone health, an obvious positive effect of milk consumption on bone growth is observed only when calcium is deficient in the diet. The effect of milk might not be seen immediately, as the effect may be long-term; this is suggested by the discovery that milk consumption can affect the transcriptome of mesenchymal stem cells, which in turn can affect obesity and bone health. The studies of bioactive compounds in milk have important repercussions for producers, processors, and consumers, as they can help improve the health quality of milk. Some bioactive compounds can be enriched in milk via dietary intervention on the lactating animals; for instance, the still poorly understood phytochemicals can be easily enriched in milk by grazing lactating animals in specialized pastures containing such phytochemicals. Some bioactive compounds can positively affect the health of lactating animals and be enriched in milk, such as polyphenols and unsaturated fatty acids. Reality always surprises us. Milk, this beautiful and complex reality, is still full of surprises and, thus, opportunities, that need to be unpacked.

Key Words: milk, bioactive compounds, human health

1207 Role of MFGM in intestinal health. R. Jimenez-Flores*, *The Ohio State University, Columbus, OH.*

The milk fat globule membrane (MFGM) is a complex component of milk, and, in relation to relevance on dairy foods and human health, it is being defined as a biologically active part of milk. There is ample evidence and current research on the areas of cognitive development and gut-brain benefit with intervention of MFGM and the microbiome in the intestine. However, due to its complexity, there has not been a reductionist study that can pinpoint a single component as being responsible for this biological activity, nor a specific mechanism of action. There may not be an "only and single" component in MFGM that could be identified as a "silver bullet" for each health benefit ascribed to it. This is likely due to its origin and processing, having its structure and function dictated by lactation biology of the mammary epithelial cell, the physicochemical aspects involved in its natural function of engulfing every drop of milkfat, its tri-layer structure, and its natural evolutionary transformation to deliver nutrients and development signals to the mammalian neonate. Furthermore, this complexity is augmented by the way we process milk and the different methods for enriching this fraction in different dairy ingredients. In this presentation we will define the MFGM as a biological entity due to its origin, and we will summarize some of the methods used to enrich ingredients in MFGM. Then we will revise the latest information the scientific community has gathered in relation to the role that MFGM plays in maintaining and sometimes repairing intestinal health. However, this presentation will focus on evidence that the MFGM acts in part by regulating mucin production of goblet cells to ultimately enhance gut barrier function.

Key Words: dairy food biochemistry, milk lipids, dairy bioactive components

Extension Education Platform Session: Training Dairy Workforce—Industry Needs and Ongoing Initiatives

1208 Needs assessments to identify educational and outreach opportunities for dairy producers and employees. J. Heguy*[1] and R. Branco Lopes[2], [1]*University of California Cooperative Extension, Modesto, CA,* [2]*University of California Cooperative Extension, Tulare, CA.*

The US dairy industry has undergone substantial change in the last decade. Fewer dairies house a larger number of cows that produce more milk. Industry consolidation, increased technical services from allied industry specialization, and the widespread availability of web-based information have changed the way dairy producers obtain information. Delivery of unbiased, university research information is critical, but has become increasingly difficult in some dairy producing regions. A key challenge for extension professionals is to develop relevant programs that meet the needs of a changing industry. Conducting needs assessments offers a viable strategy to identify the changing needs of the dairy industry. Information gained can be utilized to better serve clientele through evolving extension programs. In this symposium, we will discuss the importance of needs assessments to guide research and extension education programs. In California, for example, the regulatory climate necessitates periodic assessment updates to identify emerging producer priorities. These key issues and concerns can be identified through direct or indirect assessment methods. This symposium will explore both direct methods, such as surveys and focus groups, and indirect methods that engage clientele through discussions to identify needs and priorities. Results from published literature and assessment methodology case-studies will be presented. We will highlight recent efforts by the University of California Cooperative Extension dairy team to build cooperative, effective working relationships with dairy producers and employees based on a needs assessment survey that prioritized research topics, preferred audience for extension programs, and method of information delivery. The presentation will include personal experiences highlighting the significant impact of needs assessments on refining and enhancing dairy research and extension outreach programs in California to be more aligned with, and responsive to, the dairy industry's needs.

Key Words: needs assessment, survey, California

1209 Framing extension strategies for facilitating the adoption of dairy farming practices. L. da Costa*[1], M. Rovai[2], and N. Silva-Del-Río[3], [1]*The Ohio State University, Columbus, OH,* [2]*South Dakota State University, Brookings, SD,* [3]*University of California, Davis, Davis, CA.*

Extension programs seek to enhance collaboration among farmers, researchers, and industry experts to share knowledge, conduct research, and advocate for best practices within the sector. Effective extension strategies are essential to bridge the gap between research and on-farm implementation. In a collaborative effort, scientists and extension practitioners should assist farmers by combining various extension methods (stacking) to provide practical guidance and mitigate challenges. These strategies encompass technologies, the adoption of practices, and the application of new knowledge from research. Over the years, attention has focused on understanding the drivers and barriers to the adoption of these methods, and it is progressively recognized as a dynamic process involving learning and gradual transformation rather than the implementation from a singular event. Different farmers follow diverse adoption pathways, influenced by social and behavioral changes. Despite progress, the adoption of practices within the dairy sector remains a challenge. Several aspects are crucial when designing extension programs to achieve success. These programs should be customized based on local context, considering available resources, socioeconomic conditions, and cultural nuances. It is crucial to avoid a "one size fits all" approach by tailoring messages based on farmers' existing knowledge, appropriate language, and delivery strategies, as well as considering farm size and management systems. Examples of technology used in extension programs include E-extension methods (utilizing digital tools and online platforms, such as webinars, to disseminate information and provide guidance), software data-driven decision-making (improving milk quality, production data, feed management, and breeding), technology development (creating mobile apps for herd monitoring). Framing extension strategies is complex and involves a holistic approach and the combination of methods (technology, field days, farm schools) strategically used to enhance their effectiveness and promote the adoption of practices that empower dairy farmers with relevant knowledge and skills.

Key Words: deliver strategies, extension communication, dairy

1210 Online dairy employee training with standardized SOPs: Assessing applicability and behavior change. W. Heuwieser*[1], M. Zurakowski[1], S. Neukirchner[1], and P. Virkler[1], [1]*Quality Milk Production Services, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [2]*Clinic of Animal Reproduction, Freie Universität Berlin, Berlin, Berlin, Germany.*

It is well known that poor implementation of critical steps can impact calf and udder health outcomes. Standard operating procedures (SOPs) are step-by-step instructions to ensure quality in task execution. We conducted 2 studies with ready-made SOPs (accessible through a website) to evaluate their perception in calf care and to assess the impact of SOPs on employees' behavior while performing dry-off procedures. In study 1 (S1), SOPs for 8 tasks in calf care were provided online to dairy farmers and employees in Germany (n = 301). With 5 questionnaires we collected data on demographics, use, and feasibility of SOPs. The effect of training on dairy employees' behavior has not yet been studied. Therefore, the objective of study 2 (S2) was to determine the effect of online training on employee behavior. Five training modules on udder health were developed. Participants from the US navigated the modules at their own pace or watched a narrated video. Employees (n = 60) were assessed performing 2 tasks related to dry cow treatment (antibiotic, teat sealant) with an objective structured clinical examination (OSCE). A discrepancy existed between availability (13.1%) and wish for SOPs (69.4%) in S1. Most participants rated ready-made SOPs as feasible for their farm (66.5%). This result provides evidence that the notion of calf rearing being too diverse to use ready-made SOPs is not justified. 85% fully agreed or agreed that SOPs are a suitable tool for training new employees. Interestingly, 64.2% of employees were interested in getting involved in the creation of SOPs. The courses increased the confidence in performing tasks in calf care, especially for tasks that were less often performed (tube feeding, testing colostrum). In S2 most participants (72%) watched the video, and 28% browsed the modules at their own discretion. In total, 29 and 19 employees were scored pre- and post-training for treating a cow at dry-off and for administering internal teat sealants, respectively. The OSCE score (max. 20 points) improved for both modules from 12.4 and 11.4 pre-training to 15.3 and 13.7 post-training. After training 83% and 90% of the participants had improved.

Key Words: extension, training, udder health

1211 From research to on-farm practices: An evidence-based approach to knowledge mobilization. E. Wynands*[1], J. Saraceni[1], C. Wright[1], D. Renaud[1,2], A. L'Espérance[3], É. Désilets[3], and S. Roche[1], *[1]ACER Consulting, Guelph, ON, Canada, [2]University of Guelph, Guelph, ON, Canada, [3]Dairy Farmers of Canada, Ottawa, ON, Canada.*

Mobilizing research knowledge is an important part of supporting on-farm decision-making and change. Best practices for knowledge mobilization (KM) include information exchange, dissemination, and collaboration among researchers and research users. The objective of this work was to use a collaborative approach to share results from 15 national, multidisciplinary, 5-year research projects funded in-part by Dairy Farmers of Canada. The project topics included dairy production, animal health, and sustainability. Knowledge mobilization outputs and events were planned and executed to meet the needs of the target audiences, including dairy farmers (primary audience), as well as farm advisors and dairy industry professionals (secondary audience). The KM outputs developed included podcasts (n = 22), trade publications (n = 15), infographics (n = 14), webinars (n = 12), animated videos (n = 8), in-person events (n = 7), live-action videos (n = 2), and a research highlights magazine (n = 1). A central hub website was used to host all materials, and a communication plan was created to share outputs with stakeholders. Communication efforts included a notification email to the 10 provincial milk marketing boards and industry organizations to request direct support in sharing with their members, and 4 unique emails sent to a mailing list of approximately 4,000 people (a combination of researchers, farmers, and industry stakeholders). Select individual outputs were promoted by research teams and shared via LinkedIn and newsletters. The collaborative development process highlighted the importance of early engagement and relationship building with the research teams. Research teams have different willingness and capacity to engage with developing KM outputs; therefore, having a flexible strategy to accommodate different interests and motivations was critical. When developing outputs, it was key to build consensus on the purpose of sharing the information and the needs of the target audience. This end-of-project KM strategy was a successful approach for developing and sharing research outputs with farmers and dairy industry professionals.

Key Words: knowledge mobilization, scientific communication, extension

1212 Building value in sustainability assessments for dairy farmers and advisors: A qualitative analysis. M. G. Erickson*[1], M. Rovai[2], P. Villamediana[2], A. M. Schmidt[3], R. R. Stowell[3], and E. L. Cortus[1], *[1]University of Minnesota, Minneapolis, MN, [2]South Dakota State University, Brookings, SD, [3]University of Nebraska–Lincoln, Lincoln, NE.*

Major industry-led efforts aim at reducing the cradle-to-farmgate environmental impacts of milk production (e.g., the US Dairy Net Zero Initiative). Sustainability metrics are increasingly available to producers, through assessment processes often mediated by processors. Our qualitative, exploratory work aimed to characterize farmer and advisor perceptions of an environmental sustainability assessment program (the FARM Environmental Stewardship Module) in the Upper Midwest. In autumn of 2023, we held a series of 2–3 focus groups for each of 5 regions (n = 14 meetings total) including farmers (n = 22), advisors (n = 13), and processor representatives (n = 1). For each region, we used an initial meeting centered on region-specific fictional assessment reports to investigate participant perceptions of the value in sustainability assessments. Facilitators guided participants through semi-structured prompts to generate qualitative data. We first coded transcripts to identify quotes related to the value of assessments and individually assigned open codes. Then, we organized results into themes and sub-themes through collaborative axial coding. Results suggested that participants valued the simplicity of the assessment, including its low requirements for input data and the availability of regional comparisons. Conversely, participants reported skepticism about accuracy and fairness, drawing attention to the model's over-simplicity and its potential to be leveraged in power dynamics among farmers and processors. Participants stated that the assessment supported baseline data numeration, creating value for processors and retailers. However, they expressed that the report alone had questionable value as a decision support tool or catalyst to action without other implementation support structures such as peer discussion, expert advising, and financial incentives. Our results illustrated that farmers and advisors identified tradeoffs between simplicity and accuracy, and a need to support dairies not only in completing baseline assessments but also in implementing environmental sustainability improvements.

Key Words: sustainability, environmental, assessment

1213 Identifying the antimicrobial stewardship competencies necessary for dairy farm workers. D. Wilson[1], E. Parker[2], R. Portillo-Gonzalez[2], P. Ruegg[3], and G. Habing*[2], *[1]Dr. Devon Wilson, DVM, Chilliwack, BC, Canada, [2]The Ohio State University, Columbus, OH, [3]Michigan State University, East Lansing, MI.*

Dairy farm workers have responsibilities for disease prevention, diagnosis, and treatment, and need to employ good antimicrobial use prac-

Table 1 (Abstr. 1213). Antimicrobial stewardship competencies necessary for dairy farm workers

Item	Disease diagnosis	Disease prevention	Disease treatment	General or personal
Knowledge	Etiology and pathology	Prevention strategies Routes of transmission Zoonoses	Implications of veterinary product use Veterinary product indications	Farm assurance programs
Skills	Observation Physical examination Use of diagnostic tools	Cleanliness Maintenance of farm operations	Following protocols Veterinary product administration	Animal handling Consistency Responsibility and initiative Record keeping Synthesizing data to guide decisions Willingness to learn Ability to ask for help

tices. The objective of this research was to determine the competencies needed by farm workers to ensure prudent antimicrobial use on dairy farms. Qualitative methods were used to explore this topic, with 7 focus groups conducted, first with dairy veterinarians at 3 different veterinary conferences in the United States (n = 6) and then 1 focus group of Ohio dairy producers via video link. Focus groups followed a semi-structured question guide, had 4–9 participants and 2 to 4 facilitators each, and explored which professional and technical competencies are needed by dairy farmers who work with lactating cows and youngstock. These discussions were audio recorded, transcribed, and evaluated using thematic analysis, where a theme captured an important competency and was discussed in all groups. Preliminary results indicated that farm workers required knowledge and skills as listed in Table 1. Stakeholders also noted the importance of farm managers being competent in delegation, evaluation and monitoring, teaching, and having knowledge of dairy farm regulations (e.g., milk withhold times). Shared competencies between managers and farm workers included having a common language and using open and ongoing communication. The evidence provided through these focus groups can be used to provide structured education efforts to dairy farm workers to improve antimicrobial stewardship on dairy farms.

Key Words: qualitative, extension, education

1214 Breakfast on the Dairy Farm: Dairy farmer experiences in delivering tours to members of the public. B. A. Ventura[1], S. M. Roche[2], A. Schaafsma[3], H. W. Barkema[4], W. Ruiter[3], and A. M. C. Smid*[4], [1]*University of Lincoln, Lincoln, Lincolnshire, UK,* [2]*ACER Consulting, Guelph, Ontario, Canada,* [3]*Utrecht University, Utrecht, Utrecht, the Netherlands,* [4]*University of Calgary, Calgary, Alberta, Canada.*

Events such as “Breakfast on the Dairy Farm” (BOTDF) are frequently used to educate the public about dairy farming. Despite work exploring the impacts of these public outreach efforts on the public, little is known about the farmers’ experiences regarding these efforts. Therefore, we investigated how Canadian dairy farmers (n = 26) experienced being a tour guide on a “Breakfast on the Dairy Farm” event. A secondary aim was to describe whether communication training of farmer tour guides using 1 of 2 styles (one-way education [A] versus dialog approach focused on identifying shared values [B]) influenced their experiences of the BOTDF events. A total of 4 focus groups (~90 min, 4–8 farmers/group) were conducted on Zoom and analyzed using reflexive thematic analysis. Here we focus on 2 themes: (1) farmers’ experiences with the event, and (2) farmers’ views toward the public. Overall, farmers described positive feelings toward the event, sharing that the tours went well, and that they enjoyed the chance to connect with and educate the BOTDF visitors. In addition, participants also shared that they enjoyed seeing their community coming together and collectively sharing their pride in their field. Participants shared both positive (e.g., public as curious and engaged), as well as critical (e.g., public as misinformed) views and mentioned that the most frequently mentioned visitor concerns revolved around cow welfare, antibiotic and hormone usage, and environmental impact of dairy farming. Group A farmers emphasized the importance of public education and maintained beliefs that this would reduce public concerns about dairy farming. Although group B farmers also shared beliefs around the importance of education, they also emphasized the importance of learning about public concerns as well. A mutual desire to understand others’ perspectives may present an important opportunity to align industry and public interests toward the shared goal of creating a sustainable dairy industry.

Key Words: questionnaire, social sustainability, farmer experiences

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1215 Impact of pre-training on dairy cattle automatic milking system acclimation.

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Automatic milking systems (AMS) can reduce labor and increase long-term milk production. However, the transition from conventional milking to AMS introduces stressors that negatively impact milk production and quality in the short term. Pre-training before first milking could reduce these impacts. We used 97 robot-naïve Holstein cows that were grouped into treatment (TRT; n = 48) and control (CON; n = 49) pens balanced for lactation and days in milk (DIM). Each pen was assigned to a separate AMS. Treatment cows underwent a pre-training protocol before first AMS milking: on d −5 to −1 relative to first AMS milking, they were routed through an open AMS pathway during 2× milking in the conventional parlor. On d −2 and −1, they were brought to the AMS between milkings for a pre-training mode involving air, AMS milking arm movement, and water sounds, mimicking typical milking. The CON cows were not acclimated before first AMS milking and were blocked from observing TRT pre-training processes. On d 0, TRT and CON began milking and were trained to the AMS across 3 milkings. Milk production and quality, milking activity, and pellet intake data were collected from the AMS on d 0 to 60 post-training. Data were analyzed in SAS 9.4 with the GLIMMIX procedure with significance $P < 0.05$ for effects of pre-training on milk production, quality, and pellet intake. The FREQUENCY procedure with chi-squared was used to evaluate milking activity: successful, failed, and refused milkings (visits outside of the approved milking range). No significant differences were found for pellet intake (10.1 ± 0.1 kg/d for CON and TRT; $P = 0.93$). Milk yield, fat, and lactose percentages were higher in TRT compared with CON (38.1 vs. 37.1 ± 0.2 kg/d; 4.70 vs. 4.47 ± 0.06%; 4.81 vs. 4.80 ± 0.00; $P < 0.01$, < 0.01, and 0.03, respectively). Control cows had greater somatic cell count and percent protein (331,020 vs. 205,400 ± 34,000 cells/mL; 3.11 vs. 3.02 ± 0.02%; $P < 0.001$, respectively) compared with TRT. Successful and refused milkings were different between TRT and CON ($P < 0.01$) with no difference in failed milkings ($P = 0.15$). Overall, pre-training may have some benefits for milk quality and components.

Key Words: precision technology, robotic training, production

1216 Characterizing failed milking events in a robotic milking system.

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Robotic milking systems are beneficial to productivity and cattle welfare; however, in some instances milking is not successful. Failed milking (FM) events during the robotic milking process increase total robot idle time and limit the opportunity for other cows to access the robot. The objective of this retrospective study was to characterize factors associated with failed milking attempts in a robotic milking system. Robotic milking information was obtained between January 2021 and March 2022 from the farm management system (Lely T4C) at a dairy farm located in central Texas, milking 1,200 cows with 18 robots (Lely(R)) utilizing a free-flow cow traffic system. The statistical analyses were conducted using ANOVA and associated post hoc tests in Python. Out of the 4,990 total failed milking (FM) events, 32.4% were connection time (CT) errors, 23.6% failed connection (FC) attempts on teats, 18.3% dead milk (DM) time, 15.16% automatic robot stop (RS), 9.2% stopped by the user (US), 16.5% other reasons (teats not found [NF], robot arm stopped [RS]). The frequency of FM was highest for the right rear teat (17.9%.), followed by the left rear (12.3%) and left front (7.4%), and lowest for the right front (4.7%). The total time (min) per milking attempt that was spent during a FM was longer ($P = 0.01$) for the right front (5.03 ± 1.8) than the left rear quarter (4.96 ± 0.76). The average time (min) spent in these failed attempts was longest ($P < 0.05$) during NF (18.79 ± 2.22), followed by CT (6.81 ± 0.63), RS (1.79 ± 2.88), and US (1.35 ± 1.78). The time for FM milking attempts was greater ($P < 0.01$) for late-lactation cows (5.14 ± 2.4) compared with those in early (4.67 ± 2.96) and mid (4.88 ± 2.61) lactation. The time spent during FM was greater ($P < 0.01$) for first-parity cows (5.02 ± 3.09) compared with second (4.75 ± 2.5), third (3.26 ± 2.3), and fourth (2.96 ± 2.8) parity. Failed milking attempts have the potential to negatively impact productivity and efficiency. These results present an overview of why these FM events may be occurring and illustrate that cow and quarter-level variables may be impacted differently.

Key Words: efficiency, failed milking, robotic

1217 Digitization of dairy farm operations using laser scanning and modeling.

S. Mirhassani, J. Louis, M. Bionaz*, and A. Fern, *Oregon State University, Corvallis, OR.*

Improving food security for growing populations in the midst of growing supply-chain crises and climate change mitigation efforts requires the optimal management of farm operations to lower costs, maximize production, and minimize greenhouse gas emissions. Opportunities for such operations optimizations exist at various decision points relating to farm layout design, resource allocation, automation of farm activities, feed generation, and harvest planning, but can be hindered by the lack of a systematic means of collecting, synthesizing, and analyzing available data for decision-making. Thus, our objective was to build a digital twin of the Oregon State University (OSU) dairy farm operations that can serve as a foundational platform for enabling the synthesis of multi-modal farm data, and operations analysis for decision-making. Specifically, this research developed a methodology to systematically digitize existing dairy farms through the collection of point-cloud data using an unmanned aerial vehicle (DJI Matrice 300 RTK), ground lidar (RTC360, BLK360), and a georeferencing system (Leica GS14 GNSS), which enabled the capture of approximately 1.8 billion points. These point-cloud data were then processed using Registe360 for scan registration, Agisoft Metashape for photogrammetry, and integration via Cyclone to eventually create a 3-dimensional building information model (BIM) of the OSU dairy farm using Autodesk Revit. The developed workflow resulted in a root mean square error of 0.017 m for the scanned point cloud and 0.099 m when integrated into the final point cloud. Next, discrete event simulation was used to simulate operations on the dairy farm and visualized them using the previously scanned digital model through the use of the Unity game engine. The created digital model of the dairy farm and its associated operations will be used to serve as a simulator platform for artificial intelligence-based optimization methods, and to generate virtual scenes for education and workforce training.

Key Words: dairy farm management, digital twin, laser scanning

1218 Applying model hybridization techniques to improve dry matter intake predictions.

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Empirical models, including those developed by the National Research Council (NRC, 2001), the Agriculture Research Council (ARC, 1980), and the Cornell Net Carbohydrate and Protein System (CNCPS, 2004) play a crucial role in estimating dry matter intake (DMI). However, their effectiveness relies on periodic refitting to accommodate for the evolution of research findings. Therefore, the current study aims to hybridize 3 empirical models (NRC, ARC, and CNCPS) with 8 machine learning (ML) models (linear regression, ridge, LASSO, decision tree, K-nearest neighbors, random forest, AdaBoost, and voting ensemble) to improve DMI predictions for lactating dairy cows. Data acquired from 2009 to 2020 at the Trouw Nutrition Global R&D farm in the Netherlands, representing 591 Holstein dairy cows, with 51 features that include variables such as body weight, milk composition, and week of lactation, were pre-processed and divided into training and testing sets balanced for parity. A 10-fold cross-validation and parameter optimization was performed on the training set to assess model performance and overfitting before evaluation on the testing set. In the first hybridization approach, each empirical model's DMI predictions served as additional feature inputs for the ML models (hybrid). Each empirical model was reparametrized by being fitted to the data set, and the DMI predictions were used as additional feature inputs for the ML models (fitted+hybrid). For comparison, ML models were also fit to the data that excludes the empirical predictions. Evaluation using the concordance correlation coefficient (CCC) revealed that all ML, hybrid, and fitted+hybrid models outperformed the 3 original empirical models in DMI prediction. The average CCC improved when the fitted empirical models (CCC = 0.51) were hybridized with the ML models (CCC = 0.72). Additionally, the NRC and CNCPS fitted+hybrid models (CCC = 0.74) improved the average CCC when compared with the average ML models (CCC = 0.71), whereas the ARC fitted+hybrid model showed no improvement. These results demonstrate that DMI predictions for dairy cows can be improved using a combination of modeling approaches.

Key Words: modeling, machine learning, lactating dairy cows

1219 Comparing alternative methods of body condition scoring to traditional live scoring.

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We aimed to assess the intra- and inter-observer agreement of on-farm body condition scores (BCS), image-based scores, and scores from an automated BCS system (AUTO). The BCS was assessed by 3 observers (BCS-O) in 2 farms. Additionally, 2 photos of each cow (side and rear views) were taken to create a photo record (PR). Each PR was duplicated, labeled as BCS-Image1 and BCS-Image2, and randomly combined and scored by 4 observers. To assess agreement between AUTO and PR, BCS-Mode values were created from the BCS observer scores of each PR set (BCS-Mode1 and BCS-Mode2). For PR sets where there were 2 modes, a high (BCS-High1 and BCS-High2) and low mode (BCS-Low1 and BCS-Low2) were created. For each cow with a BCS-O or PR corresponding to BCS data collected from AUTO on the same day (BCS-Auto), data were split into Farm A (BCS-AutoA) and Farm B (BCS-AutoB). Quadratic weighted kappa was used for all intra- and inter-observer agreement assessments. Observer 1 vs. 2 (n = 52) and observer 1 vs. 3 (n = 63) for BCS-O had agreements of 0.73 and 0.64, respectively. Observer 1 had the highest agreement between BCS-O vs. BCS-Image1 and BCS-Image2 (n = 430: 0.62 and 0.63), followed by observer 3 (n = 62: 0.56 and 0.41) and observer 2 (n = 105: 0.41 and 0.41). All observers had moderate to substantial intra-observer agreement for BCS-Image1 vs. BCS-Image2 (n = 493: 0.58–0.81). For BCS-Auto vs. BCS-Mode1(n = 269), BCS-High1 (n = 340), and BCS-Low1 (n = 340), agreement was poor (0.14–0.16). Low agreement was also found between BCS-Auto vs. BCS-Mode2 (n = 287), BCS-High2 (n = 339) and BCS-Low2 (n = 339), with agreements ranging from 0.05 to 0.11. There was moderate agreement for BCS-AutoA vs. BCS-Mode1 (n = 118: 0.66) and BCS-Mode2 (n = 133: 0.61), but poor agreement for BCS-AutoB vs. BCS-Mode1 (n = 151: 0.06) and BCS-Mode2 (n = 154: 0.05). For observer 1, BCS-O vs. BCS-AutoA (n = 210) was 0.73. Observers 1 (n = 116), 2 (n = 95), and 3 (n = 48) vs. BCS-AutoB had low agreements (0.09–0.25). Image-based BCS has the potential for evaluating BCS across multiple observers. The agreement between BCS-Auto vs. BCS from PR and our observer was impacted by farm.

Key Words: automated BCS system, inter-observer agreement, dairy

1220 Optimizing agricultural sustainability: A mixed integer linear programming approach for multi-year crop rotation and animal diet optimization under environmental policies.

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Crop rotation is widely recognized for its positive impacts on soil health, crop yield, and pest and weed control. However, its multi-year planning complexity poses significant challenges, especially for dairy farmers, who must also ensure that their animals' dietary needs are met while procuring reduced methane emissions. In this study, we developed a mixed integer linear programming model to facilitate proactive planning in crop rotation and diet formulation concurrently. Our model was applied to a case study of a North Italian dairy farm (300 ha, 45 fields, and 1,007 animals) to assess the implications of 3 environmental policy scenarios aimed at mitigating animal enteric methane emissions: (1) a multi-objective optimization that balances between economic gains and methane emission; (2) applying variable tax rates on methane emissions; and (3) setting explicit methane reduction targets. Results revealed intrinsic trade-offs between economic returns and methane emissions across all scenarios. Adjusting the environmental weighting factor from 0 (maximum possible net return) to 1 (minimum possible emissions) resulted in a decrease in net revenue from $3,392 to $1,738/cow per year, alongside the reduction in methane emissions from 131 to 117 kg/cow per year. Notably, the relationship between revenue loss and methane reduction is not linear. The cost for reducing 1 kg of methane increased dramatically as the reduction goal approached the maximum. Methane mitigation prompted dietary adjustments, leading to higher dry matter percentage, reduced neutral detergent fiber percentage, and a more diverse ingredient composition, in contrast to a predominant reliance on corn silage in diets when methane consideration is absent. This study highlights the potential of our model in optimizing multi-year, field-specific crop plans and simultaneously formulating nutritionally adequate animal diets. By considering both economic outcomes and environmental policies, this study offers valuable insights for dairy farmers, policymakers, and the broader agricultural community.

Key Words: crop rotation planning, diet formulation, enteric methane emission

1659 Effects of rumen-native microbial feed supplementation on production performance and methane emissions in lactating Jersey cows.

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The objective of this study was to evaluate the effects of a rumen-native microbial feed supplement (MFS) on production performance (milk production, milk composition and feed efficiency) and enteric methane emissions. Forty-six multiparous Jersey cows at 60 ± 8 DIM were blocked by milk yield and randomly assigned to: control (no MFS) or MFS (TMR) with 5 g cow per day of a MFS containing a minimum of *Clostridium beijerinckii* at 2×10^6 cfu/g, and *Pichia kudriavzevii*, *Ruminococcus bovis* and *Butyrivibrio fibrisolvens* at 2×10^7 cfu/g). Cows were housed in the same pen and fed the study diets ad libitum for 16 wk. Individual milk yield was recorded using AfiMilk electronic milk meters, and milk fat and protein were measured using optical in-line analyzers at each of 2 daily milkings. Individual cow TMR intake was recorded through the Biocontrol CRFI feed intake control and measurement system, and enteric methane emissions were measured using the GreenFeed system. Treatment effects claimed at $P < 0.05$ were assessed by multiple linear regression. Overall treatment effects were observed for ECM, milk and fat yields; yields were 1.94 ± 0.87, 1.40 ± 0.71 and 0.09 ± 0.04 kg/d higher for MFS compared with control cows, respectively. No overall treatment effects were observed for milk protein yield, milk fat and protein concentrations, as well as DMI. Feed efficiency tended to be 0.08 ± 0.04 units higher for MFS compared with control cows. Methane production and yield were similar for MFS and control cows (328.67 ± 8.15 vs. 324.20 ± 8.52 g/d and 14.22 ± 0.34 vs. 14.19 ± 0.35 g/kg of DMI). Methane intensity was lower for MFS compared with control cows when the model only included treatment and baseline effects (8.36 ± 0.22 vs. 8.84 ± 0.23 g/kg ECM). In conclusion, the supplementation of a rumen-native MFS effectively improved outcomes such as ECM, milk and fat yields, and feed efficiency. The potential of MFS to reduce methane intensity warrants further study.

Key Words: dairy cow, enteric emission, microbe

1221 Innovative practices dealing with the environment.

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The EU has high ambitions: improve quality of water (related to P and N leaching) and Nature 2000 areas (related to ammonia emissions); overall 90% GHG emission reduction in 2040 compared with 1990 (Green Deal); more biodiversity (4% of each farm area set aside); pesticide use reduced by 50% in 2030. Especially in intensive livestock regions, emissions have become a hot topic. The choice is between extensification (is stimulated) and technical innovation. Extensification can be achieved by transforming to organic or regenerative forms of agriculture. Examples of the latter will be presented, although the business model is under question. Regarding technical innovation, we search for integrated solutions: practices suitable to combat GHG emissions sometimes increase ammonia emission, and vice versa. We show the potential of reducing ammonia emissions with practices evaluated in case control studies: floor types to separate faces and urine, such as permeable plate on slatted floor (also improves walkability); quick removal of slurry from barn; cow-toilet (collects 35% of urine); dilution of manure with water (60 l./cow per day) on slatted floors. Reductions of 20% up to 35% can be achieved by such practices. Acidification of slurry or urine reduces both ammonia (−35%) and methane emissions (−90%) from manure, and reduces emissions when applying to the field. A freewalk housing system with woodchip bedding has an animal welfare and an ecological component, and affects the emissions. Filtering ammonia from the slurry pit is a proven technique to reduce ammonia emission (−70%), but asks a considerable investment. We study the possibility of simultaneously filtering methane and ammonia from the air in housing facilities. The low levels of methane concentrations in open barns (60 farms spread over Europe from 5 to 80 ppm at 2-m height) complicates removal by air filtering. Experiments with cubicle hood samplers are done to collect and concentrate methane from close to the mouth of the animal. Experience teaches that results achieved in these experiments were not automatically copied in the field, and that the effect of a technique or practice needs to withstand juridical complaints.

Key Words: environment, techniques, practices

1222 Near-real-time feed bunk monitoring for dairy cattle using stereo vision.

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The objectives of this study were to determine the capability of depth image analysis to measure feeding behavior and intake over a 12-h period. This study was conducted utilizing an OAK-D PoE stereo vision camera wire-routed to a computer configured with the Linux Ubuntu operating system. The camera was mounted directly above a single tiestall feed bunk at a height of 3.12 m. Python and open-source software libraries (DepthAI and Open3D) were utilized in tandem with the camera sensor's output to perceive objects including cows and feed present in the bunk and measure relative depth in near-real-time. The floor and surrounding area of the tiestall were manually excluded post-collection. Open3D was then employed to generate a point cloud using Delaunay triangulation to estimate volume. The diet was a total mixed ration with a bulk density of 0.406 kg/L. Mass, volume, and feeding behavior (n = 9 meal bouts) were recorded as manually measured data to be compared with the system's estimates. Volume was computed at a rate of ~50 estimations/min to comprise a cumulative data set of 36,750 frames for analysis. Volume estimates were compared with the manually measured volume of the diet present at 2-h intervals (n = 6). The cow was removed from the viewing area for 5 min to generate unobstructed frames of the bunk at each time point. Volume estimates were regressed with the manually measured volume present. Analysis involved overlay plotting of the volume estimates with manual volume and feeding behavior data. The system was able to detect the presence of cows eating at each meal bout, as evidenced by the larger volume estimate when cows were eating (103.5 vs. 33.9 L, $P < 0.0001$). The system detected changes in volume at each meal bout as well as at each 2-h sampling point. Initial and final feed volume estimates, compared with manually measured values were similar (initial 113.5 vs. 113.0 L; final 19.3 vs. 17.0 L). Estimates were consistent within individual meal bouts and 2-h sampling points (RMSE; 0.01 L and 0.06 L, respectively). Results indicate that stereo vision camera systems can be used to estimate feeding behavior and feed intake.

Key Words: precision livestock farming, feeding behavior, computer vision

1224 Qualitative research on actors in and around dairy farming: A narrative scoping review.

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Dairy science research has largely used natural science approaches to understand farm efficiency and production, disease control, and animal welfare. There is an increasing use of qualitative research to understand the human and social aspects of dairy farming. Our aim was to review qualitative social science research related to humans, dairy cows, and farming, and to explore how different views of the dairy industry are described, exposed, and discussed within disciplines such as anthropology, sociology, and animal geography. We conducted a broad search of journals (n = 14); 312 documents were returned, and after screening, 171 articles remained. The research applied a range of methodologies, such as case studies, narratives, discourse analysis, interviews, timeline analysis, document analysis, the Delphi method, and review articles. A broad range of topics was explored, including working conditions, inequity, technology, animal welfare, culture, and environmental sustainability. The reviewed articles included conceptual or philosophical frameworks not traditionally seen in literature focused on dairy cattle production and efficiency. For example, research that examined individual animals and their agency in using on-farm technology built upon concepts of freedom, identity, and relationships between humans, animals, and technology. Our review showed a clear difference between the focus of traditional dairy science literature and the focus of qualitative social and human science literature. Articles exposed more critical views and raised important ethical questions around dairy cattle, humans, and the dairy industry, and how to respond to contemporary issues in society, as well as in ecological and food systems. This review highlights an opportunity to build bridges between dairy scientists and social scientists, as well as the importance of creating a dialog between disciplines to produce high-quality research that is both accessible to the entire dairy value chain and socially informed.

Key Words: qualitative, social science, ethics

1225 Impact of heat stress abatement on growing cattle's response to respiratory disease. C. G. Savegnago*[1], A. M. Roper[1], T. N. N. Marins[1], J. Gao[1], G. G. Cunha[1], N. L. P. Kant[1], C. R. Czaykowsky[2], B. Credille[2], M. M. Woldemariam[3], and S. Tao[1], [1]*Department of Animal and Dairy Science, University of Georgia, Athens, GA,* [2]*College of Veterinary Medicine, University of Georgia, Athens, GA,* [3]*Veterinary Diagnostic and Investigational Laboratory, University of Georgia, Tifton, GA.*

Bovine respiratory disease (BRD) is one leading cause of morbidity in preweaning dairy calves. Our objective was to investigate the impacts of providing heat abatement during summer on immunity and inflammatory responses to experimentally induced bacterial bronchopneumonia in preweaning Holstein bull calves. Calves (n = 17, days of age = 21) were randomly assigned to 2 treatments based on body weight (BW) and serum Brix refractometry: (1) non-cooled (NC, n = 9) or (2) cooled (CL, n = 8). CL calves were housed under a barn and cooled with fans, and NC calves were housed adjacent to the barn without shade or fans. Milk replacer was offered twice a day, and calf starter (CS) was provided ad libitum. Health score, respiration rate, and rectal temperature were recorded daily, and BW was measured weekly. Plasma samples were collected at 1, 2, 3, 7, 10, 14, and 21 d of the experiment (DOE) to measure haptoglobin (HP). Additionally, blood was drawn at 3 and 17 DOE to assess neutrophil chemotaxis and peripheral blood mononuclear cells (PBMC) proliferation. On 22 DOE, all calves were inoculated with 10^7 cfu *Mannheimia haemolytica* at the tracheal bifurcation. Plasma samples were collected at 0, 6, 12, 24, 48, 72, and 96 h following inoculation, calves were sacrificed at 240 h, and lung lesions were scored. Data were analyzed using the MIXED procedure of SAS. Providing cooling reduced ($P \leq 0.01$) rectal temperature and respiration rate and tended ($P \leq 0.09$) to increase CS intake and reduce fecal score. CL calves had similar ($P = 0.40$) BW relative to NC. Compared with NC, CL calves had similar ($P \geq 0.14$) PBMC proliferation to concanavalin A and lipopolysaccharide and neutrophil chemotaxis in response to interleukin 8. Treatments did not ($P = 0.56$) affect plasma HP concentration before inoculation, but CL calves had greater HP concentration ($P < 0.05$) at 48 and 72 h following BRD than NC. Following inoculation, CL calves had lower ($P = 0.02$) nasal score and lower ($P = 0.02$) lung lesion score after euthanasia than NC. In conclusion, providing heat abatement did not improve immunity but lowered the lung lesion following BRD in preweaning dairy calves.

Key Words: bovine respiratory disease, dairy calf, heat abatement

1226 Assessing total mixed ration uniformity in a mixer wagon using image analysis. I. G. M. A. Santos*[1], A. R. Reibman[2], D. R. Buckmaster[3], L. J. Pfeiffer[4], and J. P. Boerman[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN,* [3]*Department of Agricultural & Biological Engineering, Purdue University, West Lafayette, IN,* [4]*Agricultural Science Education and Communication, Purdue University, West Lafayette, IN.*

Diet uniformity is essential to ensure that all cattle receive a balanced diet that meets their nutritional requirements. Diet uniformity of total mixed rations (TMR) can be assessed by repeated sampling of particle size and nutrient composition across the feedbunk; however, this is time consuming and provides information only after feed is delivered. Adopting precision agriculture technologies, such as image analysis, could offer a near-real-time way to measure TMR uniformity. Therefore, our objective was to determine whether image analytics could be used to assess TMR uniformity while feed is being mixed in a mixer wagon. To achieve this, 2 Ubiquiti UniFi G3 Bullet cameras were installed at fixed positions above the mixer wagon during the mixing process. The video was recorded and automatically segmented to contain only the times when the mixer wagon was present. A light bar was installed above the mixer wagon to ensure constant illumination during video recording. Initial and final frames were selected in each video, and 5 regions of interest (ROI) were identified in each image. Subsequently we analyzed each ROI, obtaining their intensity histogram, and the earth mover's distance (EMD) was calculated to measure the difference between each ROI within each frame. All computations were performed in Python using the OpenCV, NumPy, Matplotlib, and SciPy libraries. This process was applied to 2 videos of different TMR, with no ground truth data about diet uniformity collected. The first video displayed an EMD of 54.519 ± 27.041 (mean ± SD) in the initial frame, which significantly decreased to 17.161 ± 10.129 in the final frame ($P < 0.001$). In the second video, the initial frame showed a mean EMD of 28.047 ± 18.023, and the final frame exhibited a mean EMD of 14.780 ± 7.488, demonstrating a more uniform TMR post-mixing ($P = 0.03$). The reduction of EMD values between initial and final frame in both videos suggests increased uniformity over time. These findings illustrate the ability of image analysis to be used to determine TMR uniformity.

Key Words: feed consistency, precision livestock farming, video analytics

Reproduction 1

1227 Distinguishing open from pregnant beef cows and heifers based on detection of interferon-tau.

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Managing open cows so that they become pregnant early in the breeding season is critical for optimal reproductive efficiency and production. Pregnancy diagnosis and re-breeding open females following first artificial insemination is not typically completed until after d 42. Because interferon-tau (IFNT) is specific to the conceptus, it was hypothesized that open cows could be identified and managed based on lack of detection of IFNT much earlier compared with waiting for ultrasound results. A very sensitive (limit of detection, LOD ~60 to 80 pg/mL), antigen-specific (does not cross-react with other type I IFN or type II IFN), and rapid (5 h) ELISA was developed to detect IFNT. Previously, in dairy cow studies, we detected IFNT in external cervical os fluid using a swab device as early as d 15 of pregnancy. In the present study, the IFNT ELISA was used to qualitatively identify open beef females based on cervical swab values below the IFNT LOD, with focus on the false negative rate: incorrectly identifying open females, when in fact they were pregnant based on subsequent d-32 diagnosis by ultrasound. False negative rates are reported for 4 groups of beef females: (1) Heifers (n = 6) not artificially inseminated (AI'd) followed by external cervical os swab samples on d 19 to serve as negative (open) controls. The false negative rate was 0% (0/6) when incorrectly identifying open heifers. The assay was 100% (6/6) accurate when classifying these heifers as open. (2) Heifers (n = 101) AI'd following detection of estrus and then swabbed on d 18, 19, or 20. The false negative rate was 9% (9/101). (3) Beef cows (n = 135) AI'd and swabbed on d 18. The false negative rate was 5.9% (8 /135). (4) Beef cows (n = 126) that received a d-7 frozen/thawed embryo following embryo transfer on d 7 and swabbed on d 18. The false negative rate was 1.6% (2/126). It was concluded that open beef females can be identified by d 18 to 19 following AI so that they can be resynchronized and AI'd with reasonable accuracy based on lack of detection of IFNT. Funded by USDA-NIFA 2019-67015-29411, W4112 Regional Project, Colorado OEDIT and STRATA grants 010077-00002 and 010079-00002.

Key Words: open, pregnancy, diagnostic

1228 Effects of omega-3 fatty acids supplementation on reproductive biology in dairy cows.

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Our objective was to evaluate the effects of short- and long-term supplementation of omega-3 fatty acids (n3 FA) on ovarian and uterine biology. Healthy Holstein cows (n = 104) were assigned to 1 of 4 dietary treatments from 2 to 90 DIM: (1) UNSUP: unsupplemented control; (2) CaPO: calcium salts of palm oil (EnerGII; Virtus Nutrition) through 90 DIM; (3) CaFO35: calcium salts of fish oil (Strata; Virtus Nutrition) through 35 DIM; or (4) CaFO90: CaFO through 90 DIM. Fat was supplemented at 1% of DM. Estrous cycles were synchronized and cows were timed AI at 70 DIM (study d 0). Ovarian dynamics and progesterone concentrations were evaluated on d 0, 7, and 15. On d 15, uterine flushing (UF) and biopsy (UB) were collected for analyses of FA composition. Pregnancy status was determined based on concentration of interferon-tau (IFN-τ) in UF, using a cutoff of 336 pg/mL established on a ROC curve for conceptus recovery. Linear models included the fixed effects of treatment, parity, time, and their interaction, and the random effect of cow. No differences were observed in the size of the dominant follicle on d 0 and luteal volume on d 7 and 15. Progesterone concentration was higher in CaFO35 and CaPO than in CaFO90 (UNSUP: 3.6 ± 0.2^{ab}; CaPO: 4.0 ± 0.2^{a}; CaFO35: 4.0 ± 0.2^{a}; CaFO90: 3.3 ± 0.2^{b} ng/mL; $P = 0.05$). CaFO90 had greater proportions of EPA+DHA in UB (UNSUP: 2.0 ± 0.1^{b}; CaPO: 1.9 ± 0.1^{b}; CaFO35: 2.1 ± 0.2^{b}; CaFO90: 3.5 ± 0.1^{a}%; $P < 0.01$) and in UF (UNSUP: 3.5 ± 0.4^{b}; CaPO: 3.3 ± 0.4^{b}; CaFO35: 2.9 ± 0.4^{b}; CaFO90: 5.1 ± 0.4^{a}%; $P < 0.01$). Pregnancy in CaFO35 was higher ($P = 0.02$) than CaFO90 and tended ($P = 0.1$) to be higher than in CaPO and UNSUP (UNSUP: 37.5 ± 0.1; CaPO: 37.5 ± 0.1; CaFO35: 61.1 ± 0.1; CaFO90: 22.7 ± 0.1%). Within pregnant cows, IFN-τ concentrations in CaFO35 were higher than in UNSUP and CaFO90, and tended to be higher than CaPO (UNSUP: 1,847 ± 1662^{b}; CaPO: 3,938 ± 1,499^{ab}; CaFO35: 7,453 ± 1,458^{a}; CaFO90: 2,213 ± 2,061^{b} pg/mL; $P = 0.05$). Different superscript letters (a,b) denote statistical difference ($P < 0.05$). In conclusion, supplementation of n3 FA in the early postpartum period benefited subsequent ovarian and uterine function, but these benefits were lost when supplementation was extended until 90 DIM.

Key Words: pregnancy, interferon-tau, embryo

1229 Genetic merit for fertility and postpartum resumption of estrus behavior and ovulation in lactating dairy cows.

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Objectives were to characterize resumption of ovulation and estrus alerts (EA) using collar-mounted activity and rumination monitoring devices (AMD) according to genomic values for daughter pregnancy rate (GDPR) and heifer conception rate (GHCR). Lactating Holstein cows (n = 2,025) fitted with AMD were inspected for presence of corpus luteum (CL) by transrectal ultrasonography at 29 ± 3 and 43 ± 3 DIM. Cows were classified in quartiles for GDPR (average: Q1 = −1.24, Q2 = −0.12, Q3 = 0.64, Q4 = 1.83) and GHCR (average: Q1 = −1.25, Q2 = 0.02, Q3 = 0.80, Q4 = 2.03). Overall, 69.9% (n = 1,416/2,025) of cows had a CL ≥ 20 mm and were classified as cyclic by 43 ± 3 DIM. Estrus alerts and estrus intensity (heat index; HI) were recorded until 43 ± 3 DIM. Percentage of cyclic cows, cows with an EA, and cows with both (Cyc+EA) were analyzed using logistic regression and models included the fixed effects of parity (P), genomic quartile (Q), and its interaction. Orthogonal contrasts were built to evaluate linear (LN), quadratic (QU), and cubic (CB) effects. Proportion of cyclic cows and cows with EA increased linearly ($P < 0.01$) for both parities with increasing GDPR. Proportion of Cyc+EA cows increased linearly ($P <$

Table 1 (Abstr. 1229). Phenotypes per quartile (LSM ± SE)

Item	Q1	Q2	Q3	Q4	P	Q	P × Q	LN	QU	CB
QDPR										
Cyclic	63.2 ± 2.5	65.5 ± 2.5	70.9 ± 2.5	71.3 ± 2.2	0.16	0.05	0.77	<0.01	0.69	0.37
EA	41.9 ± 2.7	50.7 ± 2.6	56.5 ± 2.8	60.0 ± 2.5	0.03	<0.001	0.06	<0.001	0.37	0.80
Cyc+EA	32.3 ± 2.6	37.5 ± 2.6	47.9 ± 2.8	46.5 ± 2.5	0.08	<0.001	0.10	<0.001	0.17	0.10
QHCR										
Cyclic	64.4 ± 3.3	70.7 ± 2.6	62.4 ± 2.5	70.8 ± 2.1	0.12	0.03	0.08	0.25	0.69	<0.01
EA	48.8 ± 3.5	50.6 ± 2.8	50.3 ± 2.6	56.9 ± 2.3	0.07	0.13	0.50	0.07	0.39	0.57
Cyc+EA	37.8 ± 3.5	42.2 ± 2.8	35.8 ± 2.5	45.1 ± 2.3	0.13	0.05	0.32	0.19	0.39	0.04

0.01) with increasing GDPR for primiparous, whereas the relationship was cubic for multiparous. Proportion of cows with EA and HI ≥ 70 increased linearly ($P < 0.01$) with increasing GDPR (Q1 = 36.3 ± 2.6, Q2 = 44.2 ± 2.6, Q3 = 50.2 ± 2.8, Q4 = 50.1 ± 2.5%), but not GHCR ($P = 0.61$). Linear effect of GHCR quartile on cyclicity was observed only in primiparous ($P = 0.01$). In conclusion, larger proportion of cows with high GDPR resumed cyclicity and had EA compared with lower GDPR. Similar phenotypical patterns were not observed for GHCR.

Key Words: anovular, estrus detection, fertility trait

1230 Association of potential indicator traits correlating with fertility in Holstein heifers. M. A. Ajmal*[1], A. F. Machado[1,2], S. A. Paez Hurtado[1], J. V. Chaves[1,2], S. Duarte[1], J. D. Guimarães[2], J. S. Stevenson[1], S. E. Facioni Guimarães[2], and V. E. Gome-Leon[1], [1]*Kansas State University, Manhattan, KS,* [2]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*

Traits associated with fertility that are simple to collect, of low cost, and highly repeatable, and that result in continuous data, would help advance genetic progress for fertility. Our aim was to associate the fertility of Holstein heifers with (1) phenotypic traits that can be manipulated (body condition score [BCS] and body weight [BW]); (2) intrinsic ovarian reserve-related traits (Anti-Müllerian hormone [AMH], antral follicle count [AFC]); and (3) intrinsic traits (anogenital distance [AGD], vulva length). At 367 ± 3 d of age, heifers were weighed using an on-farm scale and moved to the breeding pen. Heifers were eligible for insemination for 200 d or 5 services. At 377 ± 3 d of age, BCS, AMH, AFC, AGD, and vulva length measurements were assessed in 698 Holstein heifers and associated with pregnancy diagnoses conducted at 36 ± 3 d post-AI. Data were divided into tertiles using the Rank procedure and compared with the Glimmix procedure. Tertiles of BCS were associated with AGD and vulva length ($P < 0.01$) and with pregnancy per AI (P/AI) at first service, and with P/AI in all services (bottom BCS [3.2 ± 0.01]: 52.6 ± 2.8%; middle BCS [3.5 ± 0.01]: 53.4 ± 2.5%; top BCS [3.8 ± 0.01]: 63.6 ± 3.0%; $P = 0.02$). In contrast, BW tertiles were not associated with any of the measured variables. Tertiles of AMH were associated with BCS, AMH, and P/AI at first service, and with P/AI in all services (bottom AMH [17.0 ± 0.7ng/mL]: 56.6 ± 2.9%; middle AMH [32.0 ± 0.6 ng/mL]: 62.2 ± 2.6%; top AMH [64.4 ± 0.7ng/mL]: 63.6 ± 3.0%; $P = 0.02$). In contrast, tertiles of AFC, tertiles of AGD, and tertiles of vulva length were not associated with P/AI at first service or P/AI in all services. Additionally, AGD and vulva length were strongly correlated according to a Pearson correlation (r = 0.66; $P < 0.01$). In conclusion, our results support that greater fertility can be achieved by manipulating BCS and selecting for ovarian reserve-related traits such as AMH. Our data, however, failed to support the hypothesis that intrinsic traits such as AGD or vulva length are associated with fertility.

Key Words: body condition score, anogenital distance, ovarian reserve

1231 Effect of delaying $PGF_{2\alpha}$ treatment and timing of AI on fertility during a 6-d timed AI protocol using sex-sorted semen in Holstein heifers. E. Karakaya Bilen*[1], F. Eski[1], J. P. N. Andrade[2], A. Gumen[3], and M. C. Wiltbank[2], [1]*Department of Veterinary Obstetrics and Gynecology, University of Cukurova, Adana, Turkey,* [2]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [3]*Department of Veterinary Obstetrics and Gynecology, University of Bursa Uludag, Bursa, Turkey.*

This study evaluated the effect of delaying $PGF_{2\alpha}$ (PG) treatment and time of AI (TAI) in a 6-d Co-Synch protocol. Nulliparous Holstein heifers (BW = 373.6 ± 0.92 kg, age = 14.4 ± 0.07 mo) were assigned randomly to 1 of 4 treatments (Trt). Heifers were given PG (150 µg of cloprostenol sodium) on d −2, GnRH (100 µg of gonadorelin acetate) and a progesterone releasing intravaginal device (PRID) on d 0, PG and PRID removal on d 6, and GnRH on d 8 (P-Synch protocol). Modifications evaluated were (Mod1) additional PG either 1d before (P-Synch5/6) or 1 d after (P-Synch6/7) PRID removal and (Mod2) timing of AI at either 48 h or 56 h after PRID removal. Thus the final treatments were P-Synch5/6-48 (n = 155), P-Synch5/6-56 (n = 154), P-Synch6/7-48 (n = 153), and P-Synch6/7-56 (n = 151). Heifers were inseminated with female sex-sorted semen from 3 Holstein sires. Pregnancy per AI (P/AI) was determined on d 33 and d 63 after TAI. Data were analyzed using a generalized linear model including heifer age, sire, AI technician, Mod1, Mod2, and their interaction as fixed effects. If the interaction between Mod1 and Mod2 was significant, pairwise comparisons were performed using Tukey's test. No interaction was forced using age, sire, or technician with modification. From the parameters included to enrich the model (age, sire, and AI technician), only age affected P/AI at d 33 ($P < 0.01$) and P/AI at d 63 ($P < 0.01$). The effects of treatment modifications on pregnancy outcomes are shown in Table 1. In conclusion, delaying the time of AI with sex-sorted semen increased P/AI in a modified 6-d Co-Synch protocol when PG was administered on d 5 and d 6, but not when administered on d 6 and d 7.

Key Words: heifer, timed AI (TAI), fertility

Table 1 (Abstr. 1231). Pregnancy outcomes from Holstein heifers inseminated with sex-sorted semen using different modifications to a 6-d Co-Synch protocol

Modification 1	Modification 2	P/AI d 33	P/AI d 63	Pregnancy loss
P-Synch5/6	AI-48h	36.8%^{b}	31.0%^{c}	15.8%
	AI-56h	59.1%^{a}	57.8%^{a}	2.2%
P-Synch6/7	AI-48h	45.8%^{ab}	42.5%^{b}	7.1%
	AI-56h	49.0%^{ab}	47.7%^{ab}	2.7%
P-value	Mod1	0.79	0.85	0.4
	Mod2	<0.01	<0.01	<0.01
	Interaction	<0.01	<0.01	0.34

$^{a–c}$Different lowercase letters indicate differences between treatments ($P < 0.05$).

1232 A reproductive management program aimed at maximizing insemination of cows at detected estrus after synchronization of ovulation improved first service outcomes.

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We evaluated the effects on first-service outcomes of a program aimed at increasing pregnancies per AI (P/AI) by increasing AI in estrus (AIE) after ovulation synchronization. Lactating Holsteins at the second $PGF_{2\alpha}$ (PGF) of the Breeding-Ovsynch (B-Ov) part of Double-Ovsynch (DO; GnRH [G]-7d-PGF [PG]-3d-G-7d-G-7d-P-1d-P) were blocked by parity and semen type for first service before random assignment to the G80 (n = 1,032) and DO+E+TAI (n = 1,030) treatments (Trt). Cows in G80 were eligible for AIE for 3 d post-first PG of B-OV and, if not AIE by 80 h, received G and TAI 16 h later. Cows in DO+E+TAI were eligible for AIE for 7 d post-first PG of B-Ov and, if not AIE, received Ovsynch plus progesterone (P4; G+P4in-7d-PG+P4out-1d-PG-32h-G-16h-TAI). All AIE were based on automated estrus alerts. Cows were grouped based on milk yield (MY; kg) to 100 DIM (primiparous: <3,446 low, medium 3,446–3,841, high >3,841; multiparous: low <4,703, medium 4,703–5,257; high >5,257). Binary data were analyzed by logistic regression with treatment, parity, milk yield, and their interactions as fixed effects. Based on the interaction of Trt and MY ($P = 0.02$) more cows were AIE in DO+E+TAI regardless of MY (low 87.7%, med 86.8%, high 89.9%), whereas for G80 fewer cows with high (40.1%) than medium (46.7%) and low (53.3%) MY were AIE. Overall, P/AI at 29–35 d post-AI were 48.9% and 56.7% for G80 and DO+E+TAI. Based on the interaction of Trt and parity ($P = 0.04$) primiparous cows in DO+E+TAI (64.7%) had more P/AI than the other groups (primiparous in G80, 52.1%; multiparous in G80, 45.6%; and DO+E+TAI, 48.3%) for which there was no difference. Based on the interaction between Trt and semen type ($P = 0.03$) pregnancy loss differed between G80 sexed (5.9%) and DO+E+TAI sexed (11.3%) but did not differ from G80 beef (10.0%) and DO+E+TAI beef (7.3%). In summary, a first-service program designed to increase P/AI by increasing expression of estrus after synchronization with DO was successful at increasing AIE and increased P/AI for primiparous compared with cows synchronized with DO with delayed induction of ovulation.

Key Words: targeted, fertility, timed AI

1233 The ovarian function and endocrine phenotypes of lactating dairy cows were associated with genomic merit for fertility.

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The objectives of this prospective cohort study were to evaluate associations among genomic merit for fertility and ovarian function, endocrine dynamics, and estrous behavior of lactating Holstein cows. Based on genomic predicted transmitting ability values for daughter pregnancy rate (gDPR) cows were classified into a high (Hi-Fert; gDPR >0.6 n = 36), medium (Med-Fert; gDPR −1.3 to 0.6 n = 45), and low fertility (Lo-Fert; gDPR <−1.3 n = 38) group. At 30 to 36 d after calving, cows were enrolled in a Presynch-Ovsynch protocol to synchronize ovulation and initiate a new estrous cycle. During an entire, non-hormonally manipulated estrous cycle, ovarian function was evaluated by ultrasonography, and concentrations of progesterone (P4), estradiol, and FSH were determined in plasma. Estrous behavior was monitored by an ear-attached sensor. Binary data were analyzed with logistic regression and continuous data by ANOVA with or without repeated measures. Mean plasma P4 concentrations (ng/mL) at 4 to 13 d after ovulation induction and at peak after ovulation were greater for the Hi-Fert (2.9 ± 0.1; 7.1 ± 0.4) and Med-Fert (2.7 ± 0.1; 6.9 ± 0.4) than the Lo-Fert (2.3 ± 0.1; 5.4 ± 0.4) group. From 3 to 1 d before the onset of luteolysis, P4 concentrations were greater ($P < 0.01$) for the Hi-Fert (3.1 ± 0.1) and Med-Fert (3.1 ± 0.1) than the Lo-Fert (2.7 ± 0.1) group. The frequencies of atypical estrous cycles were 24%, 20%, and 7% for the Lo-, Med-, and Hi-Fert groups, respectively, but did not differ ($P = 0.26$). The largest follicle of the ovulatory follicular wave was larger ($P = 0.04$) for the Lo- than the Med-Fert group 9 d before ovulation, and the Lo-Fert group (13.8%) had greater double ovulation rate than the Med-Fert (0.0%) and Hi-Fert (3.5%) groups. Plasma concentrations of FSH or estradiol and estrous behavior features evaluated with the ear-attached sensor did not differ during the estrous cycle ($P \geq 0.10$). In conclusion, cows of divergent genetic merit for fertility presented differences in concentrations of hormones and ovarian function during the estrous cycle, which might explain, at least in part, the differences observed in reproductive performance due to genetics.

Key Words: estrous cycle, genomics, fertility

1234 Association between serum pregnancy-specific protein B concentrations during conceptus attachment and parity in pregnant Holstein heifers and lactating cows.

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This observational study aimed to establish the association between serum pregnancy-specific protein B (PSPB) concentrations during conceptus attachment and parity in pregnant Holstein heifers and lactating cows. Ovulatory response between timed AI and 1 d later was evaluated in 614 lactating cows and heifers from the same herd that were submitted to timed-AI programs for first and subsequent services. Animals that ovulated were kept in the study (n = 514), and blood samples were collected from d 18 to 24 post-AI. On d 32 post-AI, pregnancy was diagnosed by ultrasound. On a sub-sample of pregnant animals on d 32 (n = 103), serum PSPB concentrations were measured by ELISA (bioPRYN assay). We categorized parity as heifers (n = 19), lactations 1 (Lact1; n = 25), 2 (Lact2; n = 15), and ≥3 (Lact3+; n = 44). Variables were analyzed by ANOVA, using the MIXED procedure with the REPETED statement in SAS. Table 1 shows daily mean serum PSPB concentrations from d 18 to 24 by parity category. We observed an interaction between parity and day post-AI with serum PSPB ($P < 0.01$). Heifers increased serum PSPB earlier than lactating cows, and Lact1 increased PSPB earlier than Lact2 and Lact3+. On d 24, heifers and Lact1 had similar serum PSPB, and both had greater PSPB than Lact2 and Lact3+. In summary, serum PSPB increase was negatively associated with parity class during conceptus attachment in d-32 pregnant Holstein heifers and lactating cows, based on our preliminary data.

Key Words: pregnancy-specific protein B, parity, pregnancy

Table 1 (Abstr. 1234). Serum PSPB concentrations from d 18 to 24 post-AI in heifers and lactating dairy cows by parity

	Parity				
Days post-AI	Heifers	1 (Lact1)	2 (Lact2)	3 (Lact3+)	*P*-value
18	0.11 ±0.05^{a}	0.18 ±0.05^{ab}	0.20 ±0.04^{ab}	0.23 ±0.03^{b}	0.04
19	0.26 ±0.09	0.18 ±0.05	0.18 ±0.05	0.21 ±0.02	0.57
20	0.86 ±0.18^{b}	0.48 ±0.09^{a}	0.35 ±0.13^{a}	0.35 ±0.04^{a}	<0.01
21	2.37 ±0.40^{b}	0.87 ±0.14^{a}	0.59 ±0.25^{a}	0.56 ±0.06^{a}	<0.01
22	4.23 ±0.67^{c}	2.30 ±0.29^{b}	1.36 ±0.43^{a}	1.36 ±0.16^{a}	<0.01
23	6.78 ±1.06^{b,B}	4.83 ±0.50^{b,A}	2.95 ±0.69^{a}	3.07 ±0.33^{a}	<0.01
24	10.09 ±1.60^{b}	8.99 ±0.83^{b}	6.24 ±1.02^{a}	5.61 ±0.47^{a}	<0.01

$^{a–c}$Means within a row with different superscripts differ at $P \le 0.05$.

^{A,B}Means within a row tend to differ at $0.05 < P \le 0.10$.

1235 Shifts in uterine microbiome associated with pregnancy outcomes at first insemination and clinical cure in dairy cows with metritis. C. C. Figueiredo*[1,2], H. F. Monteiro[3], F. Cunha[2], F. S. Lima[3], K. N. Galvão[2], and R. S. Bisinotto[2], [1]*Washington State University, Pullman, WA,* [2]*University of Florida, Gainesville, FL,* [3]*University of California, Davis, CA.*

The objectives were to assess differences in the uterine microbiome associated with clinical cure failure and subsequent pregnancy outcomes in dairy cows treated for metritis. Lactating Holstein cows from 2 herds in Florida were diagnosed with metritis (presence of fetid, watery, reddish-brownish vaginal discharge; VD) and paired with cows without metritis (metritis, n = 43; no metritis, n = 42). All cows with metritis were treated with antimicrobials. Uterine contents were collected through transcervical lavage with saline solution at the time of diagnosis (d 0), 5 d after diagnosis (d 5), and at 40 DIM. Cure failure was characterized by persistence of fetid, watery, reddish-brownish VD on d 5 (not cured, n = 25; cured, n = 18). Cows were artificially inseminated at 79 ± 1 DIM, and pregnancy outcomes (pregnant, n = 41; not pregnant, n = 44), metritis, and cure were used for statistical analysis. After sequencing the 16S rRNA gene and quality control procedures, amplicon sequence variants were created. Richness and diversity were analyzed using ANOVA, and β-diversity was analyzed using PERMANOVA and linear discriminant analysis. Although richness and diversity on d 0 and d 5 were similar between cows with and without metritis ($P \ge 0.36$), *Porphyromonas*, *Bacteroides*, and *Veillonella* were more prevalent in the uterus of cows with metritis, whereas *Streptococcus*, *Sphingomonas*, and *Ureaplasma* were more prevalent in the uterus of cows without metritis ($P < 0.001$). Cure was not associated with differences in uterine microbiome ($P \ge 0.15$) on d 0 or d 5. At 40 DIM, greater ($P < 0.04$) diversity of the uterine microbiome was associated with pregnancy success, and reduced ($P = 0.02$) richness of the uterine microbiome was associated with metritis. No differences ($P > 0.10$) in β-diversity were associated with pregnancy outcomes or metritis 40 DIM. No relationship between the uterine microbiome and pregnancy outcomes or clinical cure were observed, indicating other mechanisms associated with recovery of fertility and clinical cure, aside from intrauterine changes in bacterial community.

Key Words: metritis, pregnancy, cure

1236 Differences in lactational performance associated with antimicrobial therapy and clinical cure of metritis in dairy cows. A. Martelo Pereira*[1], P. R. Menta[2], E. B. de Oliveira[3], J. G. Prim[4], V. S. Machado[2], F. S. Lima[3], K. N. Galvão[4], and C. C. Figueiredo[1], [1]*Washington State University, Pullman, WA,* [2]*Texas Tech University, Lubbock, TX,* [3]*University of California, Davis, CA,* [4]*University of Florida, Gainesville, FL.*

The objective was to assess differences in lactational performance associated with antimicrobial therapy and clinical cure of metritis in dairy cows. Holstein cows from 5 farms in California, Florida, and Texas were diagnosed with metritis (fetid, watery, reddish-brownish vaginal discharge; VD) at 4, 7, and 10 DIM (d 0), and randomly assigned (regardless of fever) to receive ceftiofur (CEF) or to remain untreated (NT). At d 13 ± 1, clinical cure of metritis was assessed, and cows with cure failure had persistent fetid, watery, reddish-brownish VD (cure, C; no cure, N). Four groups composed this study (CEF-C = 381; CEF-N = 76; NT-C = 114; NT-N = 329) and used for statistical analysis. Risk of pregnancy and culling at 300 DIM were analyzed using logistic regression; time to pregnancy by 300 DIM was analyzed using Cox's proportional hazard; and milk production within 90 DIM was analyzed using ANOVA with repeated measures. Group was included as fixed effect and farm as random effect in all models. Orthogonal contrasts were set to evaluate the effects of cure (CEF-C+NT-C vs. CEF-N+NT-N), treatment (CEF-C+CEF-N vs. NT-C+ NT-N), and their interaction (CEF-C+NT-N vs. CEF-N+NT-C). Differences in risk of pregnancy and culling at 300 DIM ($P \le 0.06$) were associated with cure (CEF-C = 74.2 and 13.5; CEF-N = 63.7 and 21.9; NT-C = 74.1 and 12.7; NT-N = 64.5 and 15.1%, respectively), but not with treatment or interaction ($P \ge 0.38$). Reduced median time to pregnancy ($P = 0.03$) was associated with cure (CEF-C = 131; CEF-N = 150; NT-C = 145; NT-N = 162 d) but not with treatment or interaction ($P \ge 0.53$). Milk production was affected ($P < 0.001$) by the interaction between group, parity, and month. Whereas differences in milk production associated with cure were observed in multiparous cows (CEF-C = 43.4; CEF-N = 40.9; NT-C = 44.3; NT-N = 40.5 kg/d), minimal variations among primiparous cows were observed. No differences were associated with treatment or interaction ($P \ge 0.27$). The cure of metritis was positively associated with milk production and reproduction, regardless of antimicrobial therapy, warranting further investigation regarding selective therapy of metritis.

Key Words: antibiotic, cure, metritis

Ruminant Nutrition 2: Calves and Heifers and Ruminant Nutrition 3-Minute Poster Spotlights

1237 Unraveling the multifaceted mechanism of residual feed intake in Holstein female calves: A comprehensive multi-omics analysis across distinct digestive sites. T. Chen*[1], J. Xiao[1,2], S. Liu[1], S. Li[1], and Z. Cao[1], [1]*College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*Animal Nutrition Institute, Sichuan Agricultural University, Chengdu, Sichuan, China.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

This study investigated variations in rumen and hindgut microflora and metabolites in Holstein calves with high and low residual feed intake (RFI). Eighty female Holstein calves (41.7 ± 4.1 kg) were randomly selected and fed a consistent diet. Calves were weaned at 56 d, remaining in individual hutches until d 84, with daily records of starter and milk intake, and biweekly body weights. Rumen fluid was collected at d 84 before morning feeding, feces were taken from d 78 to 84 (twice a day: at 0800 h and 2000 h), and pooled for digestibility estimation. RFI for each calf was calculated based on body weight, average daily gain, and dry matter intake. Subsequently, 80 calves were divided into positive RFI value (HRFI; n = 40) and negative RFI value (LRFI; n = 40) groups. Data were analyzed by PROC MIXED and PROC REGRESSION within SAS 9.2. Wilcoxon rank sum test, student's *t*-test and linear discriminant analysis were employed for difference testing. False discovery rate correction was applied for multiple comparisons. Spearman correlation analysis was conducted. Random forest analysis identified markers. Results showed that *Erysipelotrichaceae_UCG-002*, as a marker in LRFI rumen fluid, was associated with carbohydrate metabolism, whereas *Rikenellaceae_RC9_gut_group*, as a marker in HRFI feces, showed associations with lipid metabolism. Metagenomic analysis revealed elevated levels of enzymes and genes related to carbon and nitrogen utilization pathways in LRFI calves, whereas HRFI exhibited superiority in fatty acid synthesis pathway. These findings align with the observation that protein and starch digestibility negatively correlate with RFI, whereas fat digestibility positively correlates with RFI. Integration of metagenomic and metabolomic analyses highlighted ribitol and taurine, enriched in the rumen and feces, as potential contributors to glutamate synthesis in serum. In summary, distinct RFI in calves led to variations in microbial composition and metabolomic profiles in both the rumen and hindgut, primarily associated with energy, protein, and fat metabolism.

Key Words: calves, residual feed intake (RFI), multiomics

1238 Lipid metabolism in calves fed milk replacer differing in fat composition. G. Berzoini Costa Leite*[1], J. N. Wilms[2], I. R. Rodrigues de Castro[3], M. I. Marcondes[1], and L. N. Leal[2], [1]*Washington State University, Pullman, WA,* [2]*Trouw Nutrition, North Brabant, the Netherlands,* [3]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

Fat composition in milk replacers (MR) for calves differs from milk fat due to the use of alternative fat sources, leading to differences in fatty acid profile and triglyceride (TG) structure. The aim of the study was to evaluate the effect of feeding calves MR with different fat compositions on lipid metabolism. Forty-five individually housed male Holstein calves were blocked by arrival day and age. Within each block of 3 calves, calves were randomly assigned to treatments (n = 15 each) including (1) a MR containing a fat blend with 60% rapeseed and 40% coconut fat (RC), (2) a MR containing 35% coconut and 65% palm fat (PC), and (3) a MR containing 65% lard and 35% dairy cream (LD). All MR were isoenergetic with 30% (DM basis) fat, 25% protein, and 36% lactose. All calves were fed 6 L/d from d 1 to 5, 7 L/d from d 6 to 9, and 8 L/d from d 10 to 35 (13.5% solids). Milk, water, and straw intakes were recorded daily, and body weight (BW) was measured weekly. Straw, milk, and water intakes and BW did not differ among treatments. Total plasma cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol of calves fed RC were greater than PC and LD, and greater in PC than LD (Table 1). Additionally, plasma TG was greater in PC than LD in wk 2, whereas RC did not differ. Inclusion of vegetable fats in MR, especially rapeseed, did not affect growth but increased plasma cholesterol in calves.

Key Words: cholesterol, triglycerides, fatty acid

1657 Liquefied cheese as a novel milk replacer ingredient for neonatal calves. D. P. Casper*[1,2], W. P. Hansen[3], and M. Scott[3], [1]*Casper's Calf Ranch, LLC, Freeport, IL,* [2]*College of Animal Sciences, North Carolina Agricultural and Technical State University, Greensboro, NC,* [3]*Milk Specialties Global, Eden Prairie, MN.*

By-product cheese (American, mozzarella, provolone) can be liquefied and used as an ingredient for the formulation and manufacture of

Table 1 (Abstr. 1238). Blood lipids of dairy calves in response to fat composition in milk replacer (MR)

	Treatment[1]			Pooled	*P*-value		
Blood lipids[2] (mmol/L)	RC	PC	LD	SEM	Treat	Week	Treat × Week
Triglycerides[3]	0.19	0.22	0.20	0.071	0.24	0.01	0.05
Cholesterol	3.41	2.67	2.03	0.178	0.01	0.01	0.01
HDL-cholesterol	2.22	1.80	1.38	0.104	0.01	0.01	0.03
LDL-cholesterol	0.98	0.74	0.53	0.110	0.01	0.01	0.05

[1]Treatments: RC = MR containing a blend of 60% rapeseed and 40% coconut fats; PC = MR containing 35% coconut and 65% palm fats; LD = MR containing 65% lard and 35% dairy cream (n = 15 per treatment).

[2]HDL = high-density lipoprotein; LDL = low-density lipoprotein.

[3]SEM is expressed as log; LSM are back-transformed log.

neonatal calf milk replacers. This study evaluated the optimal inclusion rate for maintaining or improving growth and health performance of neonatal calves. Eighty 2- to 5-d old neonatal Holstein bull calves were blocked by initial body weight and randomly allocated to 1 of 4 treatments for 56 d using a randomized complete block design with linear, quadratic, and cubic contrasts. Treatments comprised a 22:20 (CP:fat) AA balanced milk replacer (MR) with cheese (38:41; protein:fat) added at the rate of 0.00 (control; 0), 4.27%, 8.54%, and 17.08% of the MR. Calves received 0.283 kg MR in 1.9 L MR fed 2×/d for the first 14 d, then increased to 0.43 kg in 2.84 L fed 2x/d though d 35, followed by 0.43 kg MR in 2.84 L fed 1x/d through d 42, followed by weaning. The MR ash, Ca, P, and Na concentrations increased ($P < 0.05$)with increasing cheese inclusion rate. Regardless of treatments, all calves were similar ($P > 0.10$) in body weight (BW; 62.1, 63.3, 60.6, and 61.6 kg for 0.00%, 4.27%, 8.54%, and 17.08%, respectively), BW gains (37.4, 40.3, 36.7, and 35.7 kg), and average daily gains (ADG; 667.0, 720.0, 654.8 and 636.7 g/d). Calf starter intakes were similar ($P > 0.10$) for calves fed all MR treatments (0.79, 0.84, 0.78, and 0.74 kg/d), whereas, a cubic response ($P < 0.05$) for greater feed efficiency was demonstrated for calves fed 4.27% cheese compared with calves fed 8.54% cheese with other calves fed treatments being intermediate and similar (0.522, 0.555, 0.493, and 0.516 kg/kg). Body length, heart girth, withers height, hip height, and hip width gains were similar ($P > 0.10$) for calves fed all treatments. Fecal scores were similar ($P > 0.10$) for calves fed all treatments; however, fecal color was observed to vary with cheese inclusion rates. This study demonstrates that neonatal calves can be fed a MR containing up to 17.08% cheese resulting in similar ($P > 0.10$) growth performance and feed conversions. The use of cheese could lower feeding costs if economics are favorable for incorporating cheese into the MR.

Key Words: calves, cheese, growth

1240 Effects of branched-chain amino acids supplementation on plasma amino acids and growth of preweaning calves. M. Madureira Ferreira*[1], H. L. Thom[2], D. Wilson[2], E. A. Sells[2], C. C. Mills[2], R. A. Molano[1], A. F. Ortega[1], and F. A. Leal Yepes[3], *[1]Department of Animal Science, College of Agricultural and Life Sciences, Cornell University, Ithaca, NY, [2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Washington State University, Pullman, WA, [3]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Branched-chain AA (BCAA; Val, Leu, Ile) are essential amino acids (EAA) involved in muscle protein synthesis and growth. EAA deficiencies might disrupt calves' homeostasis and physiological functions. Our study aimed to evaluate the effects of BCAA supplementation on plasma AA concentration and growth of pre-weaning Holstein calves. We hypothesized that supplementing calves with BCAA during the preweaning period might change plasma AA concentration and enhance growth. Female Holstein calves (n = 12) were blocked by pairs, enrolled at 2 d of age until weaning, and randomly assigned to receive milk with no added BCAA (control) or supplemented with 15 g of L-Leu, 6 g of L-Ile, and 9 g of L-Val once daily (BCAA). Supplementation started at enrollment and lasted until weaning. Calves were fed 2.8 L of non-saleable milk 3 times a day from d 2 to 43, reduced to 2 meals until d 50, and one meal until weaning at d 56. Blood samples and BW were collected weekly. The statistical analysis was performed using repeated measurements ANOVA in SAS (SAS 9.4, SAS Institute Inc.). Compared with the control, BCAA calves had greater plasma concentrations of Val, Ile, and Leu (Table 1). The plasma concentrations of Lys and Met were higher in the control group compared with the BCAA group (Table 1). Body weight gain was affected by treatment ($P = 0.008$). The BCAA-supplemented calves grew at 0.73 kg/d (95% CI: 0.65, 0.81) whereas those in the control grew at 0.58 kg/d (95% CI: 0.50, 0.65). Supplementation of BCAA in non-saleable milk was effective to increase BCAA supply and promote growth of preweaned Holstein dairy heifers.

Key Words: branched-chain amino acid, average daily gain, nutrition

Table 1 (Abstr. 1240). Branched-chain AA (BCAA), Lys and Met plasma concentration of preweaned calves fed non-saleable milk with (BCAA) or without (control) BCAA

	Treatment		*P*-value		
Item	BCAA	Control	Treat	Time	Treat × Time
Val, μ*M*	717.1 (668.2, 769.5)	231 (216.3, 246.8)	0.0001	0.0001	0.0001
Ile, μ*M*	275.5 (259.4, 292.6)	100.6 (95.1, 106.3)	0.0001	0.0001	0.0001
Leu, μ*M*	575 (539, 613.3)	150.5 (142, 159.6)	0.0001	0.0001	0.0001
Lys, μ*M*	93.9 (86, 102.4)	124 (113.9, 135)	0.0001	0.0001	0.50
Met, μ*M*	18.3 (16.6, 20.2)	25.4 (23.3, 27.8)	0.0001	0.0001	0.002

1241 Effects of protected and unprotected butyrate supplementation on growth performance and fermentation profile in dairy calves. D. R. Martínez Mayorga*, K. R. Johnston, and A. H. Laarman, *University of Alberta, Edmonton, Alberta, Canada.*

The objective of this study was to evaluate rumen-protected and unprotected butyrate supplementation in calf starter on calf productivity and gastrointestinal fermentation profile. Calves (n = 31) were fed milk replacer at 900 g/d, and calf starter and water ad libitum. Animals were blocked by body weight, breed, and sex, and then assigned to 1 of 3 starter treatments: (1) 1% wt/wt palm fat as a placebo carrier (CON); (2) 1% wt/wt protected butyrate (2.5% of product; PRO); or (3) 1% wt/wt unprotected butyrate (1.5% of product) + 1% wt/wt palm fat (UNP). Calves were weaned with a 2-stage stepdown from d 49 to 63 of age and were slaughtered at 70 d of age. Feed intake was measured daily. Blood and weight were sampled weekly. Feces and rumen fluid were sampled at 28, 42, 56, and 70 d of age. Digesta were collected at slaughter. Data were analyzed as a generalized mixed model with fixed effects of treatment, block and treatment × time and random effects of breed and sex, with time as a repeated measure. At d 28, UNP had a higher ruminal pH than CON and PRO ($P = 0.05$). At d 42, ruminal propionate and butyrate concentrations were higher in UNP than CON and PRO ($P < 0.01$). At d 56, ruminal propionate concentrations were higher in PRO and UNP than CON ($P = 0.04$). At d 70, in the rumen, UNP ($P < 0.01$) and PRO ($P = 0.07$) had higher propionate concentration than CON calves. CON duodenal pH tended to be higher than PRO ($P = 0.07$), whereas propionate concentrations in the duodenum were lower in UNP than CON ($P = 0.05$). Starter intake and body weight were lower for UNP compared with CON and PRO ($P = 0.02$). These results suggest that both rumen-protected and rumen-unprotected butyrate supplementation similarly impact fermentation profile, but unprotected butyrate appears to compromise growth performance during weaning.

Key Words: calf, butyrate, nutrition

1242 Effects of calf starter, weaning, and butyrate supplementation on hindgut development in Holstein calves.

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The objectives of this study were to determine the effects of calf starter intake, weaning, and rumen-protected butyrate supplementation on hindgut enterocyte proliferation, barrier integrity, and immune responses of dairy calves. Thirty-six Holstein bull calves (age = 10.7 ± 4.1 d) were fed 1,200 g/d milk replacer and assigned to 1 of 4 treatments: (1) pre-weaning groups fed only milk replacer (PRE-M); (2) pre-weaning group fed milk replacer, hay, and calf starter (PRE-S); (3) post-weaning groups fed milk replacer, hay, and calf starter (POST-S); or (4) post-weaning group fed milk replacer, hay, and calf starter supplemented with 1% wt/wt butyrate during the weaning transition only (POST-B). PRE-M and PRE-S were harvested at 48 d of age. POST-S and POST-B groups were weaned over 14 d, with milk replacer provision being reduced to 75% on d 49, 50% on d 56, and 0% on d 63, and then harvested at 10 wk of age. Blood samples were collected on wk 3, 5, and 7; cecum, proximal colon, and distal colon tissue were sampled upon harvest. Gene targets were assessed via RT-qPCR, protein targets were assessed via ELISA, and data were analyzed with GLIMMIX in SAS. Expression of immune markers IL17A ($P < 0.1$), TLR4 ($P < 0.01$), and TLR10 ($P < 0.05$) were upregulated in PRE-M calves, independent of tissue type. In the cecum, IL17A ($P < 0.01$), TLR4 ($P < 0.0005$), TLR10 ($P < 0.005$), as well as enterocyte proliferation markers MKI67 ($P < 0.05$) and PCNA ($P < 0.1$), were upregulated; proliferation markers CAT ($P < 0.001$) and HSP70 protein ($P < 0.1$) were higher in the proximal colon than any other gut region. Barrier integrity marker CLDN1 ($P < 0.05$) was highest in the proximal colon, whereas OCLN ($P < 0.05$) was highest in the cecum. Serum amyloid A concentrations decreased from wk 3 to wk 5 ($P < 0.0001$). These data suggest that calf starter decreases inflammatory gene expression in the calf hindgut, but neither the weaning transition nor supplemental butyrate impact hindgut enterocyte proliferation, barrier integrity, or inflammation.

Key Words: pre-weaning calf, gut development, hindgut inflammation

1243 Effect of milk oligosaccharides on calf performance, gut development, and health.

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Colostrum and transition milk are rich in milk oligosaccharides (MO), which are known to be beneficial for health and development. Supplementation of MO in milk replacer (MR) could be beneficial to performance and health of calves. Three types of MO (2′-fucosyllactose [2′FL]; 3′-sialyllactose [3′SL]; and 6′-sialyllactose [6′SL]) were supplemented to MR and provided to calves until weaning at 63 d to evaluate the impact on performance and health. Two-week-old male surplus dairy calves (45.5 kg ± 2.9 kg SD) were randomly assigned to 4 groups of 100 calves: control (standard MR), B (0.005% 2′FL), C (0.025% 3′SL + 0.015% 6′SL), D (0.005% 2′FL + 0.025% 3′SL + 0.015% 6′SL). Calves were fed 3 L of MR twice daily at 150 g/L with a 4-week stepdown plan. Weight was taken at arrival and at 2, 6, 10, and 12 wk thereafter. Daily MR intake, solid feed intake, disease incidences, medicinal use, and daily fecal score for the first 10 d were recorded. Four calves per treatment were slaughtered at d 71, and a jejunal segment was excised for villi trophy and mucus measurements. Jejunal RNA was extracted, purified, and sequenced for transcriptomics. ANCOVA model, chi-squared test, and Kruskal-Wallis test with post-hoc Dunn were used to analyze growth, health, and histology data, respectively. MO-supplemented calves were heavier at wk 6 than the control group ($P < 0.001$). Post-weaning calves in B (+4.8 kg) and C (+3.4 kg) tended ($P < 0.10$) to be heavier than control, whereas D calves were heavier than control calves (+4.9 kg, $P = 0.021$). Feed intake, fecal consistency, villi height, crypt depth, and mucus thickness did not differ among groups, but villi height:crypt depth ($P = 0.055$) and feed conversion ratio (FCR; $P = 0.058$) of D tended to be better than the control. The cumulative disease incidence, specifically pneumonia, was lower in B and D compared with the control. MO treatment induced some changes in intestinal transcriptomes of calves, resulting in enhanced immune response. These results indicate that MR enriched with 2′FL, 3′SL and 6′SL, and especially their combination, can positively influence growth, FCR, gut development, immune response, and disease incidences in calves.

Key Words: calf, oligosaccharides, development

1244 Effects of yeast cell wall supplementation and/or vaccination on intestinal health, immunological status, and performance of calves.

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The aim of this trial was to evaluate the effects of yeast cell wall (YCW) and/or BRD vaccination on calves' health and performance. Female dairy calves (40) were randomly distributed to treatments: (1) control, no vaccine and no yeast (NoV/NoYCW); (2) no vaccine and yeast (NoV/YCW); (3) vaccine and no yeast (V/NoYCW); (4) vaccine and yeast (V/YCW). The trial started on d 4 (calves' age) and lasted until d 39. In yeast-supplemented calves, yeast cell wall (Immunowall®, 10 g/calf per day) was inserted in milk every day. The vaccine (Inforce3®)

Table 1 (Abstr. 1244).

	Non-vaccinated		Vaccinated			P-value[1]			
Item	No YCW	YCW	No YCW	YCW	SEM[2]	Vacc.	Supp. (YCW)	Vacc. × Supp.	Treatment × Day
Average fecal score[3]	1.49	1.34	1.48	1.39	0.047	0.673	0.019	0.510	0.336
IgA, mg/dL	477	515	568	737	51.3	0.003	0.045	0.190	0.121
IgG, mg/dL	2,413	2,678	2,769	3,021	170.0	0.042	0.128	0.965	0.371
ADG, g/day	727	891	708	831	32.8	0.231	<0.001	0.522	0.155

[1] Vacc. = vaccination; Supp. = supplementation with yeast cell wall (YCW).

[2] SEM for n = 9; 1 death in NoVacc./NoYCW and 1 in Vacc./YCW

[3] Score: 1 = normal; 2 = pasty; 3 = watery.

was administered in vaccinated calves on d 18. We measured fecal score daily, ADG weekly, and blood samples (IgA/IgG) on the following days: 4, 22, 25, and 39. Data were analyzed by using the MIXED procedure of SAS ($\alpha = 0.05$) to consider both treatments (vaccination, supplementation, and interactions) and the day of evaluation (repeated measures) as fixed effects. There was neither interaction effect between treatments nor treatments and day of evaluation (Table 1, $\alpha > 0.05$). The average fecal score improved (Table 1, $\alpha < 0.05$) in YCW-supplemented calves. IgA is a known mucosal protector; therefore, the increase (Table 1, $\alpha < 0.05$) of IgA may partly explain the better fecal score in YCW-supplemented calves. Vaccination also increased IgA (Table 1, $\alpha < 0.05$), but vaccination did not support better fecal scores. Therefore, mechanisms other than IgA protection may also be improving gut health in YCW-supplemented calves. The YCW calves probably benefited from the pathogen-binding capacity of YCW-mannans. In conclusion, whether calves are vaccinated or not, YCW supplementation enhances gut health and modulates the immune response, which explains the increased ADG of YCW-supplemented calves.

Key Words: β-1-3/1-6-glucans, immunonutrition, stool

1245 Withdrawn.

1649 Incorporation of butyric and caproic acids in milk replacer enhances feed intake, growth, and modulates the metabolic profile of calves on ad libitum feeding. J. N. Wilms*[1,2], S. Hendriks[1], M. A. Steele[2], T. Sugino[3], M. Ghaffari[4], H. Sauerwein[4], J. Martín-Tereso[1], and L. N. Leal[1], [1]*Trouw Nutrition R&D, Amersfoort, the Netherlands,* [2]*Department of Animal Bioscience, University of Guelph, Guelph, ON, Canada,* [3]*The Research Center for Animal Science, Hiroshima University, Higashi-Hiroshima, Japan,* [4]*Institute of Animal Science, University of Bonn, Bonn, Germany.*

Milk replacers for calves contain minimal levels of butyric (C4:0) and caproic acids (C6:0), which may negatively affect calf development. This study evaluated the effects of incorporating C4:0 and C6:0 as tributyrin (TB) and tricaproin (TC) in MR at the same levels as milk fat on growth, feeding intake, and metabolic profile in calves. A total of 48 male Holstein calves (46.9 ± 3.93 kg BW; 2.4 ± 0.73 d of age) were blocked by arrival day and age. Within each block of 2 calves, calves were randomly assigned to experimental treatments (24 calves each), including a MR containing a blend of vegetable fats (CON) or a MR containing the same fat blend to which TB and TC were incorporated (TRI). Both treatments were isoenergetic, with 23.7% (% DM) protein, 27.7% fat, and 35.6% lactose. Upon arrival, calves were group housed, and MR (13.5% solids), starter feed, straw, and water were provided ad libitum throughout the study. Weaning was gradual and induced between wk 7 and 10, after which the calves were fed only solid feeds in wk 11 and 12. Calves were weighed and blood was collected on arrival and weekly thereafter. Continuous variables were analyzed using a mixed-effects model in SAS, with the calf as the experimental unit. Treatment and time were treated as fixed effects, and the block was considered a random effect. Calves fed TRI had fewer days of therapeutic intervention ($P < 0.01$). Throughout the experimental period, MR ($P < 0.01$), starter feed ($P = 0.05$), and total metabolizable energy ($P < 0.01$) intakes were greater in calves fed TRI, leading to enhanced growth ($P < 0.01$). Serum NEFA ($P = 0.01$) and plasma cholesterol ($P = 0.05$) were lower in calves fed TRI. In addition, serum ghrelin concentrations ($P < 0.01$) were lower, whereas serum insulin-like growth factor I concentrations ($P < 0.01$) were higher in calves fed TRI. Incorporating TB and TC into MR is a suitable strategy to increase solid feed intake upon weaning and to maximize the overall growth performance and health of calves.

Key Words: milk replacer, tributyrin, tricaproin

1246 Response of Holstein neonatal bull-calves to a direct-fed microbials program. O. O. Adelusi*[1], D. P. Casper[1,2], and U. Y. Anele[1], [1]*North Carolina Agricultural and Technical State University, Greensboro, NC,* [2]*Casper's Calf Ranch, Freeport, IL.*

Neonatal calves are challenged by pathogens resulting in up to 9% death of the herd. Although antibiotics are known to lower mortality and scours, with higher feed consumption and gains, disease treatment costs exceed $250 million annually. Also, the FDA restricts their use due to antimicrobial resistance. Direct-fed microbials have been previously reported to help establish beneficial rumen flora, which leads to improved health and performance. The hypothesis was that feeding a culture mix (CM) of *Bifidobacterium animalis* and *Lactobacillus animalis* in milk replacer (MR) with calf starter (CS) containing *Lactobacillus plantarum* (LP) will support the transitioning of calves to solid feed by improving gut health and appetite. Eighty-six (86) 2- to 5-d-old Holstein bull calves were assigned to 1 of 4 treatments arranged in a 2 × 2 factorial using a randomized complete block design. Factors are MR (with [CM+] or without [CM0] culture mix) and CS (with [LP+] or without [LP0] *L. plantarum*). Calves were fed MR at 0.57 kg/d for 14 d via bucket, which was increased to 0.85 kg/d until d 35, and then fed once daily at 0.425 kg/d with weaning after d 42 of the 56-d experiment. Calves were fed at 0630 and 1800 h in equal allotments, with access to free-choice water and pelleted CS. Calves fed CS with LP+ recorded higher feed intake (49.03 vs. 44.17 kg; $P < 0.07$) and final body weight (85.19 vs. 82.41 kg; $P = 0.12$) compared with LP0. Average daily gain values were higher ($P < 0.07$) for calves fed CM+/LP+ and CM0/LP+ (0.73 and 0.72 kg) than those fed CM0/LP0 and CM+/LP0 (0.69 and 0.65 kg). These data show that including *L. plantarum* starter feed of neonatal calves improves feed intake and weight gain.

Key Words: direct-fed microbials, calves, *Lactobacillus plantarum*

1247 Micro-cooling interventions improved the resilience to heat stress of Italian Holstein heifers. G. Meli[1], A. Guerrini[2], D. Tedesco[2], G. Savoini[1], and G. Invernizzi*[1], [1]*Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy,*

Table 1 (Abstr. 1246). Effect of direct-fed microbials program on the growth performance of neonatal bull calves

	CM0		CM+			Significance		
Parameters	LP0	LP+	LP0	LP+	SEM	CM	LP	CM × LP
Average daily intake (kg/d)	0.89	0.91	0.78	0.94	0.025	0.40	0.07	0.15
Initial weight (kg)	45.01	44.83	44.82	44.59	0.30	0.73	0.74	0.97
Final weight (kg)	83.65	85.07	81.16	85.31	0.898	0.53	0.12	0.45
Weight gain (kg)	38.64	40.25	36.35	40.72	0.833	0.58	0.07	0.41
Average daily gain (kg/d)	0.69	0.72	0.65	0.73	0.015	0.58	0.07	0.41

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The trial was aimed to evaluate nutritional strategies and microstructural interventions to improve the resilience of Italian Holstein heifers under heat stress. The trial lasted 42 d and was performed during summer in Northern Italy. Thirty-six heifers (13.7 mo old; 374.96 ± 35.09 kg live body weight [LBW]) were allocated to 3 experimental groups (n = 12): F, with water-cooled mattresses and a micro-cooling system of feeders and waterers; N, fed twice a day at 0600 h and 2300 h with no coolers; C, fed once a day at 1200 h with no coolers. Temperature and relative humidity were continuously monitored, and temperature-humidity index (THI) calculated. Twice a day, rectal and skin temperature and respiration rate were measured. Feed, dry matter, and water intakes (FI, DMI, WI) were measured daily. LBW was recorded weekly, and average daily gain (ADG) and feed conversion ratio (FCR) were assessed. Data were analyzed by the Mixed procedure of SAS for repeated measurements. A significant treatment effect was observed in DMI and FI, but no difference was observed among the groups in WI and LBW. ADG was highest in group F in the third week of the trial (F: 0.76 ± 0.22; C: −0.66 ± 0.22; N: −0.43 ± 0.22 kg/d; $P < 0.05$), with no differences observed in FCR. Lower respiration rate (F:45.1 ± 1.6; C:54.9 ± 1.6; N:55.1 ± 1.6 breaths/min; $P < 0.05$), rectal temperature (F:38.7 ± 0.07; C: 38.99 ± 0.07; N: 39.09 ± 0.07°C; $P < 0.05$), and skin temperature (F: 34.07 ± 0.12; C: 34.7 ± 0.12; N: 34.68 ± 0.12°C; $P < 0.05$) were recorded when THI was above 75 in group F. Results suggest that heifers subjected to micro-cooling interventions were better able to withstand the summer heat compared with other groups.

Key Words: heat stress, nutritional strategies, micro-cooling interventions

Ruminant Nutrition Symposium: Integrating Statistics, Nutrition, and Economics—Recognizing the Contributions of ADSA Past President and Life Member Dr. Normand St-Pierre

1248 Connecting nutrition and statistics. J. L. Firkins*, *The Ohio State University, Columbus, OH.*

Dr. Normand R. St-Pierre developed his quantitative expertise during his PhD research with Dr. Harvey (statistician) and Dr. Conrad (dairy nutritionist) at The Ohio State University. Combining statistics and economics with dairy nutrition became a unique niche and expertise often sought by others. St-Pierre was focusing on how to improve statistics for on-farm nutrition trials in 1998, which culminated in his contribution to a 2016 paper by Bello et al. that is widely referenced today. Novel and improved software (Sesame) to estimate the economic value of feeds based on their nutrients has been used for years for lay outlets. Using all of the progression of thought and statistical processes used for a 1999 meta-analysis (Oldick et al.), St-Pierre published the archetype approach for mixed model meta-analyses in our field in his 2001 landmark paper. Although cited over 600 times so far, primarily to cite the random effect of study, arguably the best parts include weighting for variance, verifying lack of interaction with study, and combining class and continuous responses while minimizing overparameterization of a model. In 2003, St-Pierre extended this expertise to proper residuals evaluation of the NRC (2001) microbial N prediction equation. Further description and refinement of meta-analyses were included in his coauthored paper with Dr. Sauvant in 2008. St-Pierre finalized model evaluation approaches in 2015 (in a coauthored paper with Boerman et al.). Over the years, St-Pierre worked with many colleagues to improve screening of databases, properly assess random blocks and experimental units, and account for collinearity and inflation of variance when using predictions as observations. His papers demonstrated the value of composite experimental designs, consideration for Type II error, use of contrasts, and multiple regression. He always reflected on properly assessing biases and distribution of error when analyzing models, in the same way that nutritionists might analyze crude protein in feeds before explaining an experimental outcome. Normand St-Pierre's contributions to dairy science will be remembered for years to come.

Key Words: mixed model meta-analysis, nutrient value of feeds, statistical analysis

1249 Integrating quantitative data analyses: Application to the dairy sciences. R. J. Tempelman*, *Michigan State University, East Lansing, MI.*

Mixed effects modeling has become the primary statistical inferential engine for dairy science researchers. Originally harnessed by quantitative geneticists for estimating heritabilities and predicting breeding values of dairy cattle for economically important traits, the utility of mixed effects models has become more broadly appreciated in dairy science, particularly for the analyses of efficient experimental designs, such as Latin squares, crossovers, and repeated measures designs. The benefits of mixed models for meta-analyses in dairy science was first exposited by Dr. Normand St-Pierre in a landmark 2001 *Journal of Dairy Science* paper, inspiring many subsequent meta-analyses in the journal since then. With the emerging discipline of precision livestock science, dairy scientists are being increasingly drawn to various machine learning (ML) techniques for improving trait predictions. Unfortunately, many of these tools do not readily accommodate data structures that are typical of dairy science research, including environmental, temporal, or genetic relationships as well as experimental design constructs that involve effective blocking and different sizes of experimental units. Furthermore, data divisions into training and test sets for cross-validation are often purely random, thereby often leading to overoptimistic assessments of ML tools, particularly as they are applied to other typically heterogeneous environments for which these predictions are intended. The implications of different cross-validation scheme choices for assessing predictive performance of various ML tools are discussed, drawing examples from our own work and from others. Although generalized linear mixed models are not generally considered to be ML tools, their utility along with generalized additive model extensions make them an attractive option to predict responses across heterogeneous environments, particularly when, for example, genetic or environmental correlations can be specified between training and test sets. Bayesian hierarchical models will also warrant greater consideration for the analysis of discrete data, particularly when data structures are sparse.

Key Words: mixed models, cross-validation, meta-analysis

1250 Feeds vary: Should we care? W. P. Weiss* and N. R. St-Pierre, *Ohio Agricultural Research and Development Center (OARDC), Ohio State University, Wooster, OH.*

Based on the 2021 dairy NASEM, well-defined forages such as corn silage and mature alfalfa silage have coefficients of variation (CV) for CP and NDF ranging from 8% to 12%. Concentrates are usually less variable, but CV for CP and NDF are often >10%. For most feeds, mineral concentrations usually have CV >20% and often >40%. With this degree of variation, how can we formulate accurate diets? The SD in databases includes multiple sources of variation, many of which are not experienced by the cow. For forages, the major source of variation is farm to farm. This should have little consequence because nutritionists should sample forages for each farm. It does mean that table values for forages should not be used unless the inclusion rate is low (perhaps <5 of the TMR). Sampling and analytical variation (i.e., observer variation) contributes substantially to the SD in feed tables. For some feeds observer variation exceeds true variation, which means using a good table value is likely more accurate than sampling and analyzing the feed. Cows do not experience observer variation, but it can lead to poor diets if the observer (usually the nutritionist) assumes the data from the sample accurately reflects the feed being fed. Using good sampling protocols, taking duplicate independent samples, and using quality-certified labs will reduce observer variation. For most forages, within-farm true variation makes up much of the SD, and this variation is experienced by the cow. However, because the composition of feeds varies independently, a well-made TMR will be less variable than many of its ingredients. Even if best practices are applied to feed sourcing and TMR making, diets will still be variable. Based on the limited research that has been conducted evaluating the effect of diet variation on cows, substantial day-to-day variations in DM, CP, forage NDF, and fat have had little effect on production measures. Research is needed to evaluate effects of day-to-day variation in nutrients that rapidly affect rumen conditions (e.g., rumen fermentable starch). Arguably the greatest negative effect of variation in feed composition is reformulating what was a good diet by assuming observer variation is true variation.

Key Words: feed composition, variation, sampling

1251 A random view of the world: Because we are never absolutely sure of anything.

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Biological systems are by nature nonlinear, dynamic, and stochastic. However, we often characterize them as linear, static, and deterministic. In the process, we frequently reach improper conclusions. Examples follow. (1) Diets are often optimized using linear (LP) programming techniques, yet the exact composition of feed ingredients is not known, which is assumed by LP. Chance-constrained programming can be used to correctly optimize diets. (2) Nutrition models such as the NASEM and CNCPS contain nonlinear relationships. Thus, if they correctly predict the output of a group of animals using the average characteristics of the animals, they are inaccurate, but we conclude the opposite. Also, these models do not account for the uncertainty of the inputs. Hence, we do not know their precision, with the consequence that they cannot be properly validated. (3) Even if the concept of nutritional requirements is correct at the animal level, it does not apply to a group of heterogeneous animals that exhibits diminishing returns to scale. In these circumstances the economically optimal diet is not the one that maximizes output. (4) In research, we often measure things for which the interest is their rates of change (e.g., body weight gain) and not their absolute values (e.g., body weight). We do not know the true functional form underlying the process. Hence, we use a function that provides a good fit to the measurements. Approximate functions can produce unbiased predictions of the measurements, but they do produce biased estimates of the rates of change. This remains an unsolved problem in statistics. (5) Income risk on dairy farms arises from the uncertainty of future milk prices and the future amounts of milk sold. Futures and options markets can control price risks, but little has been done to forecast herd structure and production risk. A Markov-chain Monte Carlo approach can generate production forecasts including estimates of their uncertainty, a necessary condition to risk management. The integration of statistics, economics, and biology allows a better understanding of biological systems and proper decision-making under uncertainty.

Key Words: economics, randomness, uncertainty

Dairy Foods 1: Processing

1252 Efficient fat separation from raw milk using hydrophilic membranes—Effects of milk temperature and pore size. T. R. Dons*[1], V. M. Candelario[2], U. Andersen[3], and L. M. Ahrné[1], [1]*University of Copenhagen, Frederiksberg, Denmark,* [2]*University of Seville, Seville, Spain,* [3]*Arla Foods, Aarhus, Denmark.*

Traditionally fat has been separated from milk through centrifugation by a cream separator. This process induces mechanical stress, consequently disrupting the milk fat globule membrane, resulting in loss of nutritional components and decreasing the quality of milk. Ceramic silicon carbide (SiC) membranes are suitable for the separation of fat from raw milk due to their hydrophilicity. This work will present the potential of SiC membranes at varying temperature and pore sizes for separating fat from raw milk. The separation performance of milk fat globules (MFG) was investigated at different temperatures (15, 25, 35, and 50°C) [1] and varying pore sizes (SiC 1.4 µm, SiC 0.5 µm, and zirconia 0.06 µm). Furthermore, combinations of SiC 1.4 and 0.5 µm were used to fractionate MFG based on size. The process showed excellent yield in terms of fat retention ranging from 88% to 98% with filtration times varying from 12 to 106 min producing a fat rich retentate (cream), while shifting the protein profile and produce a skim milk-like permeate. At 50°C the separation performances showed an increased distribution of larger MFG in the permeate stream. Micrographs using confocal laser scanning microscopy depicted intact MFG in the retentate obtained, although the further fractionated 0.5-µm permeate stream showed coalescence and aggregation of MFG, probably induced by the local pore pressure in the membrane. Further, the 0.5-µm permeate showed a shift from 80:20 to 50:50 casein-to-whey ratio. Lastly scanning electron microscopy and elemental composition analysis by energy dispersive x-ray revealed no irreversible fouling. The findings provide valuable insights into the structural and compositional aspects of the separated components. [1] Dons, T., Candelario, V., Andersen, U., & Ahrné, L. M. (2023). Gentle milk fat separation using silicon carbide ceramic membranes. *Innovative Food Science & Emerging Technologies*, *84*, 103299. https://doi.org/10.1016/j.ifset.2023.103299.

Key Words: membrane filtration, raw milk separation, milk fat globule

1253 Microwave vacuum drying of fermented milks: Effect of processing on drying kinetics and powder quality. H. Chen* and C. Moraru, *Cornell University, Ithaca, NY.*

Microwave vacuum drying (MVD) offers opportunity for preserving dairy foods with active cultures. This study investigates the efficacy of MVD for producing dehydrated fermented dairy products and its effect on product quality. Commercial nonfat Greek yogurt, plain yogurt, and plain kefir with initial *Lactobacillus bulgaricus* and *Streptococcus thermophilus* levels of ~10^8 cfu/mL for were blended (3–6°C, 5,000 rpm) and dried using a pilot-scale MVD unit (Enwave NutraREV^{TM}, Canada). Product was spread in a uniform 2-mm thickness and dried (power: 0.8–2 W/g) using vacuum levels (2, 6, 10 kPa) as independent variables. The drying kinetics, microbial viability, moisture content, water activity, color, acidity, solubility, and dispersibility of the dehydrated products were assessed. The dehydrated products were pulverized (10,000 rpm, 10 s; Omni International, USA). Rehydration conditions were tested: high shear mixing (UltraTurrax, IKA, USA) at various temperatures (4, 21, 45°C), speeds (12,000, 18,000 rpm), and durations (2, 4, 6 min). Experiments were performed in triplicate, and statistical analysis was conducted by ANOVA. An inverse correlation between vacuum level and drying rates was found, with the highest efficiency observed at 2 kPa vacuum. MVD maintained product temperatures below 50°C, producing shelf-stable ($a_w < 0.4$) dehydrated powders within 120 min. An average reduction of 2.1 ± 0.5 log for lactic bacteria was noted; decrease in lightness and increase in yellowness were observed for all powders. Dehydrated kefir had highest dispersibility to spread in water, whereas dehydrated Greek yogurt had better solubility to dissolve in water. Rehydration time and speed had minimum effect on water absorption and solubility of powders ($P < 0.05$), while increasing rehydration temperatures adversely impacted these parameters ($P > 0.05$). Overall, mixing at 12,000 rpm for 2 min at 4°C was the optimum rehydration condition. MVD can produce shelf-stable, high-quality fermented dairy powders, providing convenience for consumers and facilitating long-term storage and transportation. This positions MVD as a key innovator in the dairy industry.

Key Words: microwave vacuum drying, fermented dairy, yogurt

Joint DCWC and ADSA Animal Behavior and Well-Being Symposium: Elevating Animal Comfort and Well-Being Using the Five Domains

1300 Explaining the Five Domains and using behavioral measures in commercial systems. T. Grandin*, *Colorado State University, Fort Collins, CO.*

The Five Domains Animal Welfare framework is now being used by many corporate buyers as the framework for animal welfare auditing programs. It is gradually replacing the Five Freedoms. Some commercial buyers have made this switch because it is not possible for an animal to be completely free from fear, distress, injury, or disease. For example, it is nearly impossible to have zero lame dairy cows. The percentage of severely lame dairy cows should be very low, and producers can work on continuous improvement to further reduce lameness. Another reason the Five Domains are being more widely adopted is that preventing distress or suffering is not always possible, nor is it sufficient. The animal should also have positive experiences and a life worth living. The Five Domains are (1) nutrition, (2) environment, (3) health, and (4) behavior, all of which will influence animals' (5) affective state. The first 3 domains are similar to Good Feeding, Good Housing, and Good Health from the European Welfare Quality System. The fifth domain (affective states) cannot be easily measured. In the Five Domains, the behavior domain has 3 parts. Each behavior category has both negative and positive behaviors that can be quantified. The 3 behavior components are as follows. Cow interactions with the environment: Such as quality of and access to resting spaces or access to a powered grooming brush. Cow interactions with people: Both positive and negative during handling and milking. Cow interactions with other animals: Such as fighting or allo-grooming. Some typical measures that would be used on the farm to assess nutrition, environment, and health are clean water, body condition, hygiene-scoring swollen hocks, falling on a slick floor, lameness, sickness, and lack of pain control for painful procedures. These are all key welfare indicators that can be assessed with ease to quantify outcome measures.

Key Words: animal welfare, animal health, behavior

1301 Measuring minds: Understanding the mental states of dairy animals in different management conditions. H. W. Neave*, *Purdue University, West Lafayette, IN.*

A key tenet of good animal welfare and a high quality of life is that animals experience positive emotional states, while negative emotional states are minimized. The assessment of emotional and mental states in animals remains difficult, but nonetheless is crucial for understanding how animals experience their life under farm management conditions. This presentation will provide some examples of behavioral, cognitive, and physiological methods for assessing emotional states in animals, with examples from dairy cattle and goats experiencing different management conditions. For example, cognitive bias tests and body language can reveal the emotional states of cows managed with or without their calves; anticipatory behavior demonstrates sensitivity to rewards when calves are housed in barren or enriched pens; and infrared thermography and decision-making tasks can inform about environmental conditions that goats like or avoid. Animal personality is also highly relevant to the discussion of mental states, as personality traits play a role in how individuals interact with and respond to challenges that are often stressful in their management environment. For instance, more exploratory dairy calves perform better around weaning; more fearful dairy goats are less likely to access the feed bunk during competition; and more curious dairy cows spend more time grazing and produce more milk. Together these examples demonstrate how different management conditions may positively or negatively affect the emotional states of animals, and how personality traits influence the way animals respond to stressful management practices. An understanding of and efforts to measure these mental states provide opportunities to tailor management practices to meet individual needs and improve animal welfare.

Key Words: emotional state, animal personality, positive animal welfare

1302 Importance of human-animal relationships and their influence on animal welfare and productivity. M. C. Ceballos*, *University of Calgary, Calgary, Alberta, Canada.*

Human-animal relationships (HAR) have existed since the Neolithic period, parallel to the domestication of many animal species. Establishing an HAR is a gradual process, strengthened by repeated interactions. Animals can develop relationships with humans with a long-lasting and integrative nature based on their ability to remember and predict future interactions. It is well documented that negative HAR can impair animal welfare, primarily through fear as an underlying mechanism, with negative consequences on animal health and productivity, including reductions in fertility and milk quantity and quality. Conversely, positive interactions with humans lead domestic animals to seek and engage with us, yielding intrinsic rewards for animals. However, our understanding of processes underlying positive perception of humans needs further research. In the recently updated Five Domains model, Domain 4, now called Behavioral Interactions, was extended to facilitate explicit and detailed assessment of humans' impact on welfare of animals in their care, reinforcing its importance to animal welfare. Some routine tasks on farms are aversive but are difficult or impossible to replace (e.g., vaccinations, transport); therefore, they should be done in the best possible and least aversive ways, minimizing unnecessary animal suffering. Furthermore, stockpeople may have misguided opinions and behaviors that are usually not due to intentional unkindness but rather from a lack of knowledge. On many farms, turnover rates for stockpeople are high, and there is limited investment in training. This creates a challenging cycle where insufficiently trained employees often have only short-term employment, reinforcing producers' reluctance to invest in their training. However, research in dairy and beef production systems has demonstrated the benefits of training programs incorporating behavioral and cognitive techniques to enhance stockpeople's attitudes and behavior toward animals and decrease animals' fear toward humans. Improving animal handling yields numerous benefits for both animals and workers, improving welfare and productivity for both.

Key Words: handling, training, human-animal relationships (HAR)

1303 Demonstrating U.S. Dairy's commitment to animal care. E. Yeiser Stepp*[1], C. DeCoite[2], B. Hampton Phifer[1], S. Roche[3], and J. Saraceni[3], [1]*National Milk Producers Federation, Arlington, VA,* [2]*Innovation Center for U.S. Dairy, Rosemont, IL,* [3]*ACER Consulting, Guelph, Ontario, Canada.*

The National Dairy Farmers Assuring Responsible Management (FARM) Program is the US dairy industry's national quality assurance program. The goal of FARM is to assist US dairy farmers, cooperatives, and processors in providing assurance to dairy buyers and consumers that dairy farmers care for their animals, workforce, and land in a responsible manner. FARM Animal Care encompasses voluntary participation from 99% of the US milk supply. The program sets industry standards with a few guiding principles: scientific research, facility and size neutrality, and outcomes-based standards. The focus of the program is to promote and ensure a strong veterinarian-client-patient relationship (VCPR), employee training and continuing education, care for high-risk animals, and animal observations. Noncompliance with standards that fall into these focus areas result in corrective actions. The program has seen strong compliance to standards related to animal and facility observations, and antibiotic stewardship. Opportunity areas exist related to documentation efforts (i.e., veterinary oversight, treatment records, cow care agreements, and employee continuing education). Management-related areas for improvement include the use of pain management for disbudding and injured tails. Nearly 85% of US dairy top-tier customers (brands, restaurants, retailers, and distributors) support the FARM Animal Care program through procurement requirements, global sourcing frameworks, and social responsibility commitments. This is a testament to the commitment of US dairy to have a program that is grounded in science, transparent in standards development, and maintaining relationships with key customers. Still, interest by these companies to differentiate themselves in the marketplace and address consumer concerns grows. Moving forward, animal welfare research, which is utilized to develop future FARM and global framework standards, must prioritize key areas of customer and consumer interest (i.e., painful procedures, freedom of movement, pharmaceutical use, and natural behaviors) with a lens on practical and economic feasibility for farmer implementation.

Key Words: animal welfare, supply chain, consumer perception

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1304 Impact of subclinical hypocalcemia on the behavior and productivity of dairy cows milked in robotic milking systems. S. Moore*, J. Marques, R. Conceicao, J. Denis-Robichaud, and R. Cerri, *University of British Columbia, Vancouver, BC, Canada.*

Limited research is available investigating subclinical hypocalcemia (SCH) within robotic herds, where cows are free to milk more often, and typically produce greater quantities of milk. Higher milk yields have been associated with a greater risk of SCH. This study aimed to explore how varying patterns and timing of SCH, impact production and behavior for cows in robotic herds. Multiparous Holstein cows (n = 279) were enrolled from a robotic milking herd in British Columbia, Canada, 1 week before dry off and followed until 60 d in milk (DIM). Blood samples were collected weekly in the dry period and every day following day of calving until 6 DIM. Blood was analyzed for total blood calcium (Ca, mmol/L) and used to classify SCH patterns as chronic SCH (Ca ≤1.77 at 1 DIM, and ≤2.2 at 4 DIM, n = 60), delayed SCH (Ca >1.77 at 1 DIM, and ≤2.2 at 4 DIM, n = 57), transient SCH (Ca ≤1.77 at 1 DIM, and >2.2 at 4 DIM, n = 44), and were compared with normal cows (Ca >1.77 at 1 DIM, and >2.2 at 4 DIM, n = 118). Calcium was analyzed at 6 DIM (SCH^+ for Ca ≤2.2) to test the effects of cows who remained hypocalcemic versus those who recovered. All data analyses were conducted using generalized linear mixed models. Cows classified as delayed and chronic SCH produced 3.6 kg/d and 2.9 kg/d less milk compared with normal-classified cows ($P = 0.03$, $P = 0.06$, respectively). No differences in milk production were found between transient SCH cows and normal-classified cows ($P = 0.9$). Chronic, delayed, and transient-classified cows spent 32.9 min/d, 29.6 mind/d, and 28.8 min/d less eating compared with normal cows ($P < 0.01$, $P < 0.01$, $P = 0.02$, respectively). Chronic cows were the only SCH group to reduce rumination time by 41.1 min/d compared with normal cows ($P < 0.01$). Delayed-classified cows who tested positive for SCH at 6 DIM produced 4.2 kg/d less milk compared with delayed cows who recovered by 6 DIM ($P = 0.04$). This research showcases the impacts of SCH patterns and timing on production and behavior outcomes and highlights the need for further SCH classification and threshold exploration within robotic milking herds.

Key Words: robotic milking systems, subclinical hypocalcemia, milk production

1305 Early postpartum calcium dysregulation is associated with acute phase responses in multiparous Holsteins. J. A. Seminara*, C. R. Seely, and J. A. A. McArt, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Calcium dysregulation at 4 DIM, or dyscalcemia, is common among multiparous Holstein cows and has been associated with increased risk of disease, decreased intake and production, and poor reproductive performance. Systemic inflammation early in lactation is well documented and has also been associated with suboptimal outcomes. The objective of this case-control study was to explore differences in the acute phase response, an important inflammatory process, between cows with and without dyscalcemia. We hypothesized that cows with dyscalcemia would experience more extreme inflammatory activation than eucalcemic cows. Blood was collected from multiparous Holstein cows on 2 farms in central New York, 1 to 3 d before parturition and once every 24 h postpartum through 4 DIM. Samples were analyzed at each time point for total calcium (tCa) and 3 acute phase proteins (APP): serum amyloid A (SAA), lipopolysaccharide binding protein (LBP), and haptoglobin (HP). Using 4 DIM serum samples, dyscalcemic cows (tCa <2.2 mmol/L; n = 20) were matched with eucalcemic cows (tCa ≥2.2 mmol/L; n = 36) such that the final proportion of dyscalcemic cows in the study population would be comparable to the dyscalcemia prevalence in US commercial herds (~35%). Patterns of APP over time were compared using linear mixed effects models including the fixed effects of calcium group, DIM, parity group (2, 3, or ≥4), farm, all relevant 2-way interactions, and the random effect of cow. Overall, the dynamics of APP showed that dyscalcemic cows experienced increased acute phase responses compared with eucalcemic cows. Dyscalcemic cows had elevated concentrations of HP, SAA, and LBP starting at 2 DIM (HP and SAA: $P < 0.001$; LBP: $P = 0.04$). This work, though preliminary in nature, strongly supports the hypothesis that dyscalcemia and excessive inflammation are associated in postpartum dairy cows. While the causal structure of this relationship remains unknown, improved understanding of inflammation and dyscalcemia may provide insight into mechanisms by which some cows become maladapted to the early postpartum period.

Key Words: dyscalcemia, inflammation, acute phase proteins

1306 Near-infrared spectroscopy analysis of blood plasma, urine, and feces during the prepartum period for predicting subclinical ketosis in dairy cows. L. R. Coelho, G. L. Menezes, T. Cunha, L. L. Hernandez, J. R. R. Dorea, and S. I. Arriola Apelo*, *University of Wisconsin–Madison, Madison, WI.*

During the transition period, dairy cows are often exposed to negative energy balance, leading to lipid mobilization and predisposing subclinical ketosis (SCK). Traditional diagnostic approaches for identifying subclinical ketosis involve measuring blood β-hydroxybutyrate, which only reflects the current state of the cow. However, predicting future events needs is crucial to optimal decision-making at the farm. In this context, spectral analysis can uncover subtle indicators in body fluids, potentially enabling the early detection of cows at risk of SCK. Therefore, this study aims to employ near-infrared (NIR) spectroscopy to analyze spectral data collected from different body fluids (blood, feces, and urine) over the dry period to predict SCK postpartum. Isolated plasma, urine, and dry, ground feces collected at 6, 5, 4, 3, 2, and 1 weeks before calving were analyzed using a FOSS DS2500 spectrometer. After calving, cows were sampled twice a week during the first 2 weeks. Cows that show BHB levels higher than or equal to 1.2 are considered to have SCK. BHB was measured by colorimetric assay in a chemical analyzer. The raw spectra data were pre-processed using spectral derivatives and scatter-correlation. The model was trained to detect events of SCK during the first 2 weeks postpartum using partial least squares discriminant analysis (PLS-DA), and predictive performance was assessed using leave-one-out cross-validation. The model predicting postpartum SCK from plasma collected at 6-weeks and 1-week prepartum achieved accuracies of 64.5% and 73.4%, respectively. For urine, the model presented a maximum accuracy of 60.0% to predict SCK at 4 weeks prepartum. When trained using spectral data of fecal samples, the model presented a maximum accuracy of 65.8% at 2 weeks prepartum. As a conclusion, spectral analysis of different body fluids could enable early management decisions to reduce the risk of SCK post-calving. This approach can improve animal health, welfare, and profitability in dairy farms by improving decision-making on optimal management strategies.

Key Words: near-infrared (NIR), transition period, subclinical ketosis

1307 Associations between hyperketonemia and rumen metagenome and metabolome in dairy cows during the first two weeks postpartum.

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Hyperketonemia, a pathological condition with elevated concentrations of blood ketone bodies, is a prevalent metabolic disorder that affects 20–40% of dairy cows during early lactation. The objective of this study is to investigate the potential associations of the rumen metagenome and metabolome with hyperketonemia status. In this study, blood and rumen fluid samples were collected from ruminally cannulated cows on 5, 10, and 14 d postpartum. Blood β-hydroxybutyrate (BHB) concentrations were used to classify cows as hyperketonemic (HYK; ≥1.2 mmol/L BHB) or control (CON). Five cows were identified as HYK and were matched based on calving date and lactation to 5 CON cows. Microbial DNA was extracted from rumen fluid and was sequenced using shotgun metagenomics with the Illumina platform. Kraken2 was used to map reads to microbial taxonomic groups, and Humann3.8 was used to predict potential functions. Metabolome profiling of rumen fluid was conducted using high-resolution liquid chromatography-mass spectroscopy. MetaboAnalyst 6.0 was used to identify potential changes in metabolic pathways. Comparison groups included ketonemic status and day (d 5, 10, 14). At the species level, there was no difference in the α (Shannon $P = 0.49$; Chao1 $P = 0.73$) or β (Bray-Curtis dissimilarity $P = 0.79$) diversity when comparing samples from HYK and CON cows. Abundance of *Corynebacterium variabile* ($P = 0.03$) and *Corynebacterium glyciniphilum* ($P = 0.03$) was greater in HYK. For bacteria functions, no differences were observed in β diversity ($P = 0.72$), and no functions were differentially abundant ($P > 0.05$) among groups. Although overall metabalome β diversity was not different ($P = 0.12$), HYK5 differed from HYK10 ($P = 0.01$), and HYK10 differed from CON10 ($P = 0.05$). No pathways differed between CON and HYK by day (false discovery rate >0.30). Overall, modest differences in rumen microbiome and metabolome were observed across different days based on ketonemic status.

Key Words: BHB, bacterial functions, pathways

1308 Associations of prepartum cytokine profile in peripheral blood with postpartum health in dairy cows.

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Our objectives were to investigate whether prepartum cytokine responses to ex vivo lipopolysaccharide (LPS) stimulation of whole blood is associated with incidence of postpartum clinical disease. Blood samples from 332 cows were collected 15 d before the expected calving and divided into 2 aliquots. One aliquot remained as an unstimulated control, and the other was stimulated with *Escherichia coli* O111:B4 LPS for a final concentration of 5 μg of LPS/mL of blood. Tubes were then incubated at 37°C for 3.5 h. After incubation, plasma was harvested for analysis of cytokine concentrations using the Bovine Cytokine 15-Plex Discovery Assay (EMD Millipore Corp.). Postpartum cows were examined for diagnosis of retained placenta, metritis, mastitis, lameness, and digestive and respiratory problems during the first 15 DIM, and then classified as healthy (NoClinD) or diseased (ClinD). Data on cytokine concentrations (IL-6, IL-8, IL-10, IL-36ra, IP-10, MCP-1, TNFα, and VEGF-A) were transformed ($\log_{10}$ or square root) and analyzed using mixed linear models, which included the fixed effects of postpartum health, LPS stimulation, parity, their interactions, season, and batch of samples, and the random effect of cow. Incubation with LPS increased the concentrations of all cytokines except for IL-8. Concentrations of prepartum IP-10 were higher ($P = 0.04$) in ClinD than in NoClinD regardless of LPS stimulation (434.7 [95% CI: 404.8–465.6] vs. 393.4 [95% CI: 367.9–419.8] pg/mL). In addition, the concentrations of prepartum IL-8 were lower ($P = 0.096$) in ClinD than in NoClinD regardless of LPS stimulation (252.5 [95% CI: 222.4–286.7] vs. 291.3 [95% CI: 260.0–326.6] pg/mL). An interaction between health group and LPS stimulation ($P = 0.08$) was observed for concentrations of VEGF-A (NoClinD-CON: 30.1 [27.2–33.2] vs. NoClinD-LPS: 39.9 [36.6–43.5] vs. ClinD-CON: 27.1 [24.3–30.2] vs. ClinD-LPS: 38.5 [34.9–42.4] pg/mL). For the remaining cytokines, no associations with postpartum disease were observed. In conclusion, prepartum concentrations of IP-10, IL-8, and VEGF-A in plasma were modestly associated with incidence of postpartum ClinD.

Key Words: immunity, transition, resilience

1309 The epidemiology of systemic inflammation: Associations of plasma haptoglobin in the first week postpartum with disease and milk yield in Holstein dairy cows.

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This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The objectives of this cohort study were to (1) establish thresholds for plasma haptoglobin (Hp) at 1, 3, 5, and 7 d in milk (DIM) associated with disease or removal (sold or died) within 60 DIM and (2) evaluate associations between Hp at each DIM with milk yield in the first 12 wk of lactation. Blood samples were collected from 513 cows from 2 herds at 1, 3, 5, and 7 DIM. Plasma Hp concentrations were analyzed using a bovine haptoglobin ELISA kit. Multivariable Poisson regression models were built to evaluate the associations of Hp at each DIM with the risks of metritis, clinical mastitis, clinical ketosis (CK) and/or displaced abomasum (DA), and herd removal. Critical thresholds were

Table 1 (Abstr. 1309). Risk ratios (RR) of health outcomes based on Hp thresholds derived from receiver operator characteristic curve analysis

Outcome	Critical threshold (g/L)	RR	95% CI	*P*-value
Metritis				
1 DIM	0.204	3.0	1.6–5.4	0.003
3 DIM	0.437	6.8	3.1–14.8	<0.0001
CK and/or DA				
3 DIM	0.517	9.4	3.1–28.7	0.005
Removal				
1 DIM	0.093	5.5	1.8–16.8	0.005
3 DIM	0.502	7.0	2.7–18.3	0.004
5 DIM	0.304	5.2	2.0–13.7	0.007
7 DIM	0.130	5.6	2.1–14.5	0.006

derived from receiver operating characteristic curve analysis for significant DIM. Linear mixed models were built to evaluate associations of Hp at each DIM with milk yield across the first 12 wk of lactation. Plasma Hp was associated with the risks of metritis, CK and/or DA, and removal (Table 1). In primiparous cows, Hp at 3, 5, and 7 DIM were associated with milk yield in the first 3 wk: 4.5 ± 1.5, 4.0 ± 1.4, and 5.0 ± 1.8 kg decrease in daily milk yield for every 1-g/L increase in Hp at 3 ($P = 0.003$), 5 ($P = 0.004$), and 7 ($P = 0.006$) DIM, respectively. In multiparous cows, Hp at 1, 3, 5, and 7 DIM were negatively associated with milk yield in the first 8 wk, with Hp × week interaction. Haptoglobin in the first week postpartum is associated with disease and decreased milk yield, and parity and DIM of measurement must be considered when evaluating these associations.

Key Words: systemic inflammation, haptoglobin, epidemiology

1310 Effects of postpartum acetylsalicylic acid administration and calcium supplementation on metabolism, inflammation, and health in Holstein dairy cows.

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This study is aimed to assess the effects of the combination of postpartum acetylsalicylic acid (ASA) administration and calcium supplementation on haptoglobin concentration (HP), calcium (Ca) concentration, β-hydroxybutyrate (BHB), and disease incidence in multiparous (≥2 lactation) cows. Within 12 h after calving, cows were randomly allocated to 1 of 4 treatment groups: (1) ASA (n = 155) = cows received 2 oral administrations with ASA 24 h apart (125 g/cow per day; 4× 480-grain aspirin boluses); (2) ASACAL (n = 164) = cows received 2 oral administrations with ASA (125 g/cow per day; 4× 480-grain aspirin boluses) and Ca (43 g/cow per day; 2 Ca boluses; Bovikalc®; Boehringer Ingelheim Animal Health) 24 h apart; (3) CAL (n = 171) = cows received 2 oral administrations with Ca (43 g/cow per day; 2 Ca boluses) 24 h apart; and (4) UNT (n = 156) = cows remained untreated. Blood samples were collected before each treatment and at 7 ± 3 DIM to assess Ca (total), HP, and BHB concentrations. On-farm records were collected to assess health events in the first 60 DIM. The data were analyzed using MIXED and GLIMMIX SAS procedures. There was no difference in BHB or HP concentrations between treatment groups. Regardless of treatment, cows that became sick in the first 60 DIM had lower concentrations of Ca and higher concentrations of BHB and HP in the first 24 h after calving compared with cows that remained healthy. Intriguingly, cows treated with ASA or ASACAL tended to have lower concentrations of Ca at the second treatment administration compared with UNT cows (ASA = 7.66 ± 0.07 mg/dL; ASACAL = 7.64 ± 0.06 mg/dL; UNT = 7.90 ± 0.07 mg/dL). Cows treated with CAL tended to have a lower incidence of subclinical ketosis compared with UNT cows (CAL = 36.96 ± 4.95%; UNT = 46.63 ± 5.19%). On the other hand, cows treated with ASA tended ($P = 0.1$) to have a lower incidence of clinical metritis compared with CAL cows (ASA = 15.92 ± 3.56%; UNT = 29.92 ± 4.40%). These findings suggest that postpartum ASA administration alone or in combination with Ca supplementation may alter Ca metabolism.

Key Words: postpartum period, calcium and acetylsalicylic acid, cow metabolism and health

1594 Evaluation of the plasma oxylipin and endocannabinoid profile and the platelet and plasma proteome in postpartum dairy cows experiencing elevated systemic inflammation.

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Unregulated, systemic inflammation can result in significant production losses and negative health outcomes in dairy cows. Soluble mediators and nonimmune cells (e.g., platelets) have been studied for their expansive role in mediating inflammation. Our objectives were to compare the plasma oxylipin and endocannabinoid (eCB) profiles and the platelet and plasma proteomic profiles of healthy cows against cows suffering from elevated systemic inflammation as indicated by plasma haptoglobin (Hp) concentrations. Postpartum cows at 3 DIM with plasma Hp concentrations ≥0.50 g/L and no clinical disease were enrolled into the high-inflammation group (n = 8). Cows with plasma Hp concentrations ≤0.1 g/L and no clinical disease were enrolled into the low-inflammation group (n = 8). Blood samples were collected for plasma lipidomics, proteomics, and platelet isolation. Lipidomics data were assessed using a mixed liner regression model where parity was included as a fixed effect. Targeted lipidomics revealed differences in the plasma oxylipin and eCB profile between high- and low-inflammation cows. Cows in the high-inflammation group had increased plasma concentrations of the oxylipins 9(S)-HpOTrE, 9(S)-HOTrE, 13(S)-HpOTrE, and 9,10-EpOME, and an increased concentration of the eCB, anandamide. Platelet proteomics identified 2,074 proteins at a 1% false discovery rate (FDR). One hundred fifty-five proteins were differentially abundant between the high- and low-inflammation groups, which included proteins involved in platelet granule release and cellular iron uptake. Three hundred thirty-one proteins were identified at a 1% FDR in plasma samples. Across high- and low-inflammation cows, 24 proteins were differentially abundant, including proteins involved in autophagy and immune mediation. These results suggest that cows suffering from an exacerbated systemic inflammatory response may have impaired disease resistance and platelets could contribute to their inflammatory state. More research is needed to elucidate the biological function of many of the proteins and lipid mediators identified in this study.

Key Words: lipid mediator, platelet, systemic inflammation

Joint EAAP and ADSA Breeding and Genetics Committee Symposium: Breeding for Environmental Sustainability

1312 The value of a national evaluation system in promoting dairy sustainability. A. M. Miles*[1], K. L. Parker Gaddis[2], and R. H. Fourdraine[3], [1]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD,* [2]*Council on Dairy Cattle Breeding, Bowie, MD,* [3]*Dairy Records Management Systems, Raleigh, NC.*

Sustainability is best defined as a balance of practices that promote economic vitality, protect the natural environment, and build healthy communities in the present without compromising the future. The dairy industry has seen tremendous gains in productive efficiency following decades of work with these goals in mind. A major tool contributing to this progress is the National Cooperator Database (NCD), which was developed in partnership with key industry groups. Involving a complex system of management and both domestic and international data sharing, the NCD now encompasses over 8.5 million genotypes, 90 million pedigrees, and 100 million lactation records. The primary outputs of this system are national genetic and genomic evaluations that are delivered to participants tri-annually. A comprehensive database with high participation from dairy herds can have additional impact. Greater genetic gains have resulted in a faster inbreeding rate, a well-established antagonist to cow health and performance. The marketability of sires with high genetic merit limits male genetic variation, so conserving female genetic diversity must be a top priority. With dairy genetics being traded all over the world, the NCD enables the monitoring of inbreeding at the global population level. Dairy systems are continually evolving thanks to technology advancements, changing consumer values, and resource availability. Frequent interrogation of the NCD can facilitate the early detection of changing industry trends such as the rise in embryo transfer or the popularity of beef-on-dairy to sustain farm profits. These changes impact the accuracy of existing evaluations (e.g., fertility), and also highlight the need for improvement tools that are just as dynamic as the dairy industry. An industry-wide effort is underway to develop a herd-level sustainability metrics platform that would support dairy producer ability to farm and track their progress in key health and production areas. This pre-competitive collaboration among dairy industry groups ensures the reliable flow of accurate data so we can continue to develop tools to support the dairy producer in the present and future.

Key Words: sustainability, genetics, breeding

1313 Breeding for improved heat tolerance: Methods, challenges, and progress. I. Misztal*, *University of Georgia, Athens, GA.*

Heat tolerance in dairy cattle likely deteriorates over time due to unfavorable correlations between production and heat tolerance, with stronger deterioration in later parities. Coupled with climate change, heat stress is felt in increasingly colder areas. The industry has responded to the challenge of heat stress by applying managemental modifications, such as better cooling devices, timed AI, and sexed semen. Cooling devices are justified in areas with a long hot season and high milk prices, and less so otherwise. Methodology exists to calculate GEBV for high production under heat stress, with high reliabilities under the genomic selection. However, cows that maintain production during the peak of the heat stress may be more likely to be disposed of, and detrimental effects of calving during the peak of the heat cycles can be permanent. One remedy would be selection for better fertility and survival under heat stress, but with low volume of data and low heritabilities, corresponding reliabilities may be too low for an efficient selection. In environments where intensive management is too expensive, an ideal cow would maintain production during a favorable weather, would reduce production during heat stress, and would restore production after the stress is over. Such a resilient cow could be identified by larger-than-expected fluctuations in production during the lactation. A special effort would be required to separate the desired fluctuations to cope with the heat stress, versus undesired fluctuations due to health issues (canalization).

Key Words: heat stress, genetics, management

1314 Strategies for breeding of feed-efficient dairy cows with lower methane emissions. B. Heringstad*[1] and K. A. Bakke[2], [1]*Norwegian University of Life Sciences, Ås, Norway,* [2]*Geno SA, Hamar, Norway.*

To meet future challenges, we should breed feed efficient cows that have lower methane emissions. The motivation for improving feed efficiency is high because feed costs are the largest cost in dairy production, reported to be more than 60% of the variable costs. At the same time the agricultural sector is expected or committed to reduce the environmental impacts of food production. By including new traits in the breeding goal we can save feed costs and at the same time reduce methane emissions from dairy production. Feed efficiency and methane emissions are challenging traits in dairy cattle, traits that are difficult, costly, and time- and labor-intensive to collect. However, reliable phenotypes are crucial for success. Strategies for genetic selection include direct selection and indirect selection using indicator traits or prediction equations based on, for example, mid-infrared spectra in milk. In any case, large-scale phenotyping of individual feed intake and methane emissions is needed. This is established in research herds and in commercial herds in many countries. These projects and initiatives will build reference populations for genomic evaluations, and the growing database of phenotypes of key parameters makes it possible to develop the tools needed for successful implementation. Results so far indicate that there is substantial genetic variation for methane emission, roughage intake, dry matter intake, and different measures of feed efficiency, and breeding for these traits is feasible. More knowledge is needed on the genetic associations between traits, and further research is needed to examine which trait definitions would be better for successful implementation in the breeding programs. The aim should be to balance feed efficiency, climate effects, production, health, and fertility in a sustainable breeding goal for the future.

Key Words: environmental impact, feed intake, genetic evaluation

1315 Genomic and epigenomic strategies for dairy breeding sustainability. H. Jammes*[1,2], G. Costa Monteiro Moreira[1,2], V. Costes[3], and H. Kiefer[1,2], [1]*INRAE, Jouy en Josas, France,* [2]*BREED, Maisons-Alfort, France,* [3]*ELIANCE, Paris, France.*

The livestock sector is a pillar of the global food system, supporting the food and nutrition security of nearly 1.3 billion people according to the FAO. Faced with global environmental changes, the health and fertility of farm animals are of paramount importance: they constitute the right way to maintain well-being and metabolic homeostasis while improving production in quality and quantity in variable environments. Genomic selection increasingly considers phenotype indicatives promoting robust and efficient animals. Nevertheless, environmental pressure on livestock is increasing and occurs throughout an individual's life; in this context, the full importance of epigenetic processes, such as DNA methylation, becomes clear, as they are needed for the development of the individual and serve as a memory of environmental events. Different examples will be presented to highlight the contribution of epigenetic process driving the phenotypes. First, to predict bull fertility, we studied epigenetic quality of semen variations in relationship with different intrinsic (age; breeding) and extrinsic (nutrition) factors. Using a unique collection of 100 sperm samples, it was demonstrated that the bull sperm methylome was a valuable source of male fertility biomarkers. DNA methylation changes in blood immune cells were also determined in response to various factors (age, nutrition, inflammation, heat stress) and used as biomarkers to diagnostic the individual health. A tool known as "epigenotyping," taking into account of all identified biomarkers combined with biomarkers selected from public data and genome annotation, is in progress (https://rumigen.eu) and will be presented, as well as the opportunities that this tool offers. Finally, the progress of annotation of functional genome elements in livestock animals (the FAANG initiative) and the utility of generated information (enhancers, promoters, insulators, small and large RNA transcripts) will be presented. All approaches taken together may improve the understanding of genome function as an adaptative response to environment.

Key Words: epigenetics, environment, adaptation

Dairy Foods Symposium: Next Generation of Scientific Leaders in the Field of Dairy Foods Science

1316 Harnessing research to boost innovation and develop future leaders: Fueled by dairy farmers. R. Kapoor*, *Dairy Management Inc., Rosemont, IL.*

The objective of this talk is to emphasize the importance of US dairy farmer-funded initiatives that fuel new product innovation and enable the development next generation of dairy leaders, consequently helping grow the US and global dairy industry. Dairy Management Inc. (DMI) was created in 1995 to help increase demand for US-produced dairy products on behalf of America's dairy farmers. DMI and its related organizations work to increase sales and demand for dairy through research, education, and innovation, and to maintain confidence in dairy foods, farms, and businesses. One of the key departments at DMI to help fulfill the above goals is the Dairy Products Research group. The DMI Dairy Products Research group strives to advance DMI's priorities through targeted research and development activities that address consumer needs and expand dairy choices in domestic and international markets. This includes funding research projects at various dairy research centers and universities in the United States. These programs lead to cutting-edge research findings, platform technologies, and quality and food safety solutions that enable the US dairy industry to generate high-quality, innovative products and ingredients. Furthermore, the group directs strategic resources to offer expert product and process development, technical support, and food safety and quality resources to the US dairy industry. Developing the current and future workforce through education and development through the National Dairy Foods Research Centers is also one of our primary goals.

Key Words: dairy farmers, research, future leaders

1317 Milk protein functionality: Colloidal and interfacial aspects. H. Zheng*, *North Carolina State University, Raleigh, NC.*

In general, fresh milk may be considered as a colloidal system containing dispersed fat globules and protein particles. Bovine milk, depending on the source dairy cattle breeds, may contain about 30 to 36 g/L of protein. There are 6 major types of milk protein. Four of them are α_{S1}-casein, α_{S2}-casein, β-casein, and κ-casein, assembled with calcium phosphate to form casein micelles; 2 of them are β-lactoglobulin and α-lactalbumin, presented in soluble form and known as whey proteins. Structure determines functionality. Milk proteins have been concentrated, fractionated, and even modified to make protein-based ingredients for constructing different mesoscopic and microscopic structures, including aggregates, gels, emulsions, and foams for improving texture and phase stability of many food products. The scope of this presentation focuses on (1) introduction of fundamentals of colloid and interface science and (2) investigation of how this knowledge guides research regarding modifying and charactering milk protein functionality using recently obtained results as examples. The presentation will discuss (a) approaches to assembling whey proteins into colloid particles as gelling agents and oil-water interface stabilizers; (b) significance of large deformation rheological properties on characterizing whey protein gel texture; (c) indicating phase stability of UHT reconstituted skim milk using information obtained from spectroscopy techniques and physicochemical properties.

Key Words: milk protein, whey protein, colloid and interface

1318 Dairy components in pharmaceutical and value-added applications. G. Lewis*, *University of Wisconsin–River Falls, River Falls, WI.*

The importance of adding value to dairy products has been an attractive research topic in recent years. The goal of the present work is to highlight 2 current research projects targeting value-added products, with applications in both the food and the pharmaceutical industries. First, a project looking to target milkfat as an intravenous lipid source for parenteral nutrition (PN). PN is a lifesaving strategy for patients (>300,000 patients per year in the United States) who cannot feed via the gastrointestinal tract due to trauma, surgery, premature birth, or other intestinal complications. Common PN lipid sources (e.g., olive oil, soybean oil, fish oil) cause a variety of complications, mostly due to their limited complexity. In this application, milkfat, with a more diverse fatty acid profile than typical PN lipids, must achieve emulsion particle sizes of 200–500 nm, and this was accomplished through high-pressure homogenization. Initial studies applying these emulsions in stimulated macrophage and mouse models have shown promising results, including reduced inflammatory response, anti-inflammatory response, reduced respiratory exchange ratio, and reduced pancreatic cell inflammation compared with soy-based intravenous lipid emulsions. Second, a project targeting optimized casein micelle dissociation for dairy product functionalization and nanoparticle applications. In this application, casein micelle dissociation was maximized through emulsifying salt addition and high-pressure homogenization, revealing more hydrophobic residues for target compound interactions. Together, these projects highlight novel, ongoing research with considerable economic potential for the dairy industry.

Key Words: milkfat, parenteral nutrition, casein

1319 Tailoring dairy-based ingredient functionality. P. Salunke*, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Milk is a heterogeneous mixture of soluble, colloidal, and suspended particles. Due to this heterogeneity, it is possible to separate or fractionate particles and develop new ingredients with altered fat-to-protein, casein-to-total protein, or protein-to-total solids ratios in the finished product compared with regular milk, which has an impact on microstructure and functionality. The functional and economic value of the ingredient becomes higher than the milk itself. Manufacturing dairy-based ingredients involves separation or fractionation, concentration, and drying. The developmental approaches for manufacturing milk can be broadly categorized into fat-only, lactose-only, and high-protein ingredients (normal ratio of milk constituents, whey or serum protein-rich, and casein-rich). These ingredients can be used as is, in concentrated or dried form. The fat- and protein-rich ingredients are used for flavor, structure, and texture development, and the molecular structure and surface properties play a significant role in functionality. These dairy-based ingredients can be used in various products, providing critical functional properties, including texture, water binding, viscosity, gelation, emulsifying, and foaming. However, some ingredients alone cannot deliver critical functional properties required in certain products, such as high heat-stable products, emulsions, cheese, and yogurt. Hence, modification of these ingredients is necessary for providing targeted and tailored functionality. In understanding factors that affect the functional properties, many technological, engineering, research, and development efforts have led to the production of dairy ingredients tailored for specific applications. Common approaches followed are processing modification, physical (e.g., membrane processing, heat) and chemical (e.g., pH, additives) modification, and biological (e.g., starter culture) or enzymatic (hydrolysis or crosslinking) modification, or a combination of 2 or more approaches. Various approaches for tailoring dairy-based ingredient functionality are available, but the selected approach should be controllable, efficient, safe, cost-effective, and eco-friendly.

Key Words: dairy-based ingredient, functionality, tailoring

1320 Generating added-value dairy products: A West Coast perspective.

C. Licon*, *Dairy Products Technology Center, California Polytechnic State University, San Luis Obispo, CA.*

In the dynamic landscape of California industries, innovation remains a pivotal part, and the dairy industry stands as a prime example, continually evolving to meet emerging trends and consumer preferences by adding value to its products. The purpose of this presentation is to outline my professional goals to establish robust infrastructure and educational capabilities within California higher education institutions, focusing on fostering innovation in dairy foods through extensive multidisciplinary research. With a commitment to promoting culturally diverse and artisanal dairy production, coupled with a fervent dedication to advancing innovation in dairy processing, my research endeavors are aimed at assisting industry businesses. This commitment extends to enhancing the overall quality of dairy products through various avenues: (1) the incorporation of regional products to increase value; (2) the identification of natural solutions to mitigate late blowing in cheese; (3) an exploration of the perceptions of cheese aromas; and (4) the upcycling of whey for beer production and algae cultivation. Recognizing the transformative potential of machine learning in recent years, I have observed a new perspective that can revolutionize the dairy industry and open avenues for applications that can significantly enhance innovation. A systematic review reveals that machine learning is successfully employed across diverse domains, covering composition analysis, quality control, optimization, authentication, processes, sensory evaluation, manufacturing, and detection of adulteration. Aligned with this paradigm shift, machine learning presents unprecedented opportunities to forge synergies and collaborations to support dairy producers in previously unexplored ways. The results of this research indicate that machine learning has the potential to not only transform but also deepen the impact on the dairy industry, creating a profound and lasting influence on dairy food innovation. In conclusion, innovation in dairy products is my approach to promoting collaboration and engagement, and to expand the offer of culturally diverse dairy products and educational opportunities.

Key Words: innovation, added-value, dairy products

1321 Understanding the structure-function relationship in dairy food matrices using material science approaches.

P. Sharma*, *Utah State University, Nutrition, Dietetics and Food Science Department, Logan, UT.*

Dairy foods are available in variety of forms, including liquid, semi-solids, solids, and powders. The desired functionality of these foods depends upon end user application. Any specific functionality in a food matrix is derived from the multiple layers of food structure, the way different molecules interact with each other at different length scales and form a basis for bulk material properties. Modifying food structure through changing formulation or processing conditions is an effective way of controlling functionality. This presentation will cover the dairy research being conducted at Utah State University by Assistant Professor Prateek Sharma in the area of functionality of dairy powders, cheese, and gels. His talk will focus more on how the use of novel material science approaches may not only be helpful in understanding structure-function relationship in various dairy food matrices but also could potentially improve profitability and sustainability of commercial dairy manufacturing operations.

Key Words: structure-function relationship, dairy foods, rheology

1322 Sustainable process and healthy dairy product solutions.

M. S. Mohan*, *South Dakota State University, Dairy and Food Science Department, Brookings, SD.*

The global dairy industry is one of the fastest-growing food industries, worth 893 billion US dollars in 2022, eliciting the most conversations of health and environmental impacts of dairy processing. Dairy products and ingredients are highly nutritious with versatile compositions, properties, functionality, and applications. The US fluid milk carbon footprint involves about 28% contribution of the dairy processing sector and the consumer supply chain. This footprint is associated with the high load of total solid waste and byproduct streams from dairy processing, elucidating the need for measures to evaluate the sustainability and efficiency of individual products lines. The US dairy Net Zero initiative 2050 plans to advance research and technology focusing on mitigating the large farm-to-fork carbon footprint of the dairy industries. There is awareness in the market and among consumers about the importance of sustainability and health impacts of dairy processing. Some novel trends have been identified that could impact the performance and future direction of the dairy industry, including novel processes and technologies, consumption and market patterns, and health and environmental impacts of dairy processing and products. We have investigated novel processing technologies, including nanobubble and ultrasound technology, that showcase huge potential as clean and sustainable technologies, as they do not require the use of processing aids and chemical additives. In addition, the diverse physicochemical and nutritional attributes of dairy products and ingredients can be utilized for the delivery of bioactive compounds and catering to consumers' healthy-eating demands such as high protein content. Themes that have emerged with the environmentally conscious consumer market include the importance of upcycling, reduction of waste, and sustainability in dairy processing. Overall, there is need for research in the areas of novel processes and scale-up, measurement of sustainability, innovative clean label products and ingredients, utilization of waste and byproduct streams, and overall effective use of consumer trend evaluations and artificial intelligence in the dairy industry.

Key Words: nanobubbles, protein, environment

Dairy Foods 2: Chemistry and Microbiology

1323 Relevance and future possibilities of utilizing the delivery properties of casein micelles. M. S. Mohan*, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Bovine caseins micelles are complex structures consisting of several thousands of caseins (αs_1, αs_2, β, and κ caseins), with sizes ranging between 50 and 500 nm. These micelles have open and loose structures with cavities (20–30 nm) and channels (5-nm diameter), and held together mostly by calcium phosphate bridges and protein-protein interactions (hydrophobic, hydrogen, electrostatic, and van der Waals interactions). Casein micelles act as carriers for vitamins, antioxidants, minerals, drugs, hormones, flavors, and fatty acids. However, there are limited studies elucidating the specific caseins interacting with the bound molecules, the binding mechanisms, the factors affecting the interactions, and the impact of the interactions on bioactivity and delivery of these molecules. Our research focuses on the interactions of casein micelles with different bioactive molecules or ligands, when mixed in aqueous solution, owing to noncovalent interactions. We studied factors including chemical properties of the ligands (molecular weight, number of hydroxyl groups, hydrophobicity, charge) and solution environment (pH, ionic strength, temperature). The hydrophobicity and the quantity of the hydroxyl groups in the ligands and the temperature played critical roles, especially when hydrophobic forces dominated the interactions of caseins with ligands. Studies on these interactions are limited about the effect of the solution environment and concentration of the ratio of caseins to ligands. Further understanding about the interactions of casein micelles with different bioactive compounds will enable possibilities for improving their delivery and bioavailability in the human body. Immense possibilities are available to utilize the delivery properties of the casein micelles for niche markets, in food products, as drug carriers for sensitive populations such as immune-compromised people, children, and geriatrics, and controlled-release delivery. The goal is to create new avenues in food, pharmaceutical, and health markets for milk proteins, and to enable industries to commercialize new processes and products, with the understanding developed by extensive research.

Key Words: hydrophobicity, bioavailability, drug

1324 Milk fat globule membrane structure with phytosterol replacement of cholesterol. R. Vaseghi Bakhshayesh[1,2], C. Thum[1,3], M. Golding[1,2], and D. W. Everett*[1,2], *[1]Riddet Institute, Palmerston North, New Zealand, [2]Massey University, Palmerston North, New Zealand, [3]AgResearch, Palmerston North, New Zealand.*

The milk fat globule membrane (MFGM) is a phospholipid-based trilayer structure enveloping fat globules. Cholesterol is found within the outer 2 layers in spherical, tightly packed liquid-ordered (L_o) domains bound to sphingomyelin. These domains modulate the kinetics of milk lipid digestion to ensure optimal absorption in the small intestine. Complexation with sphingomyelin delays the transition of fat globules into mixed micelles under digestive conditions and is a factor that may reduce cholesterol absorption. The recent trend toward hybrid plant- and animal-based food raises the question of how plant sterols may affect MFGM structure. Homogenized or pasteurized bovine milk was microfluidized (200 MPa, 32°C), mixed with β-cyclodextrin (β-CD; 0.4 to 1.0% wt/wt), held at 4°C for 16 h, and centrifuged (200 × *g*, 20 min), and the fraction containing cholesterol-reduced milk was retained. Plant β-sitosterol (β-S; 50 mg) was dissolved in 5 mL pH 7.4 phosphate-buffered saline with β-CD (300 mg), then vortexed and ultrasonicated to create a delivery structure to exchange cholesterol for β-S in a diffusion-driven process. Milk contained 2.4 mg β-S/100 mL as measured by gas chromatography equipped with flame ionization detection. Particle size and ζ-potential were measured in 20 m*M* imidazole, 50 m*M* NaCl, 5 m*M* $CaCl_2$, pH 7 buffer (n = 3), and the globule surfaces were examined by confocal laser scanning microscopy (CLSM) using the fluorescent dyes of Fast Green FCF to stain proteins, Nile Red and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) to stain lipids, and filipin to stain sterols. Cholesterol was reduced to the same extent (88–91%) by 0.6–1.0% β-CD ($P > 0.05$). The size of cholesterol-reduced globules (60 ± 4 nm) was the same as for β-S (60 ± 6 nm; $P > 0.05$). The ζ-potential for β-S globules was −9.88 ± 0.14 mV, lower in magnitude than cholesterol-reduced globules (−10.85 ± 0.24 mV; $P < 0.05$). L_o regions were not observed in cholesterol-reduced milk fat globules; however, β-S was observed on the membrane surface of β-S globules by CLSM. This study shows that cholesterol within the MFGM can be replaced with a plant sterol.

Key Words: milk fat globule membrane, phytosterol, cholesterol

1325 Fabrication and physicomechanical performance evaluation of k-carrageenan loaded casein nanofibers. D. Sharma*, G. R. Ziegler, and F. M. Harte, *Department of Food Science, The Pennsylvania State University, University Park, PA.*

Milk-based electrospun nanofibrous materials are gaining attention due to their unique biodegradability, biocompatibility, and nutritional properties. The low molecular weight and self-aggregation patterns of casein proteins (19–25 kDa) limit the structural stability of casein nanofiber-based electrospun mats (EMs). This study reports the influence of ethanol content (0–60%) and temperature (20–80°C) on the gelation of k-carrageenan homogeneous dispersions and its subsequent influence on the electrospinnability and physicomechanical attributes of casein-based EMs. Solid k-carrageenan gels were obtained for k-carrageenan dispersions of 40–50% ethanol-water mixtures at 80°C. Moreover, the influence of the temperature of casein and k-carrageenan solution preparation and temperature of mixing on the electrospinninability of casein/k-carrageenan dispersions was evaluated. Electrospinning samples prepared at a temperature of ~60°C resulted in the formation of EMs with minimum bead defects. Further, optimal conditions were analyzed, and homogeneous electrospinnable casein dispersions with varied k-carrageenan content varying from 0 to 2 wt.% with respect to casein content and a resultant solution concentration of 20 wt.%, were prepared using 50:50 vol/vol ethanol/water solvent mixtures at 60°C and pH 10. More uniform fibrous morphology with minimum bead defects was obtained for 1.5 wt.% k-carrageenan loaded casein-based EMs. An increase in the variation in enthalpy from 148 J/g to 192 J/g of the endothermic peak at ~90°C indicated an increase in the number of trapped water molecules in EMs with the increase in k-carrageenan content from 0.5 to 2 wt.%. A 3-fold increase in strain at break (ε_b) was observed with a maximum obtained for 2 wt.% k-carrageenan loaded casein EMs. Further, a 5-fold increase in strength (σ_{max}) was observed for 1 wt.% k-carrageenan loaded casein EMs. Therefore, the study demonstrates the influence of k-carrageenan loading on the electrospinnablility of casein proteins to fabricate novel biodegradable casein-based nanostructured mats with potential for food, cosmetic, packaging, and biomedical applications.

Key Words: electrospinning, casein, k-carrageenan

1326 Withdrawn.

1327 Mechanistic insights into the disruption of casein micelles by citrate and phosphate-based calcium sequestering salts.

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The aim of the present study was to understand how different types of calcium sequestering salts (CSS) disrupt the structure of casein micelles. The disruption of casein micelles through the addition of orthophosphate salt disodium phosphate (DSP), pyrophosphate salts disodium pyrophosphate (DSPP) and tetrasodium pyrophosphate (TSPP), polyphosphate salts sodium tripolyphosphate (STPP) and sodium hexametaphosphate (SHMP) and trisodium citrate (TSC) at 10, 15, 20, and 30 mEq/L levels was investigated in 5% micellar casein isolate (MCI) solution. Particle size of the MCI dispersion significantly ($P < 0.05$) decreased when 15–30 mEq/L of STPP, SHMP, and TSC was added at both pH 5.5 and 6.5, but for DSPP, significantly ($P < 0.05$) larger aggregates were formed. The addition of pyrophosphates significantly ($P < 0.05$) increased MCI viscosity with increasing concentration at pH 5.5 and 6.5. The viscosity and particle size findings of DSPP and TSPP suggest that after dissociation of casein fractions by CSS, protein, Ca, and phosphate can re-associate to form new complexes causing viscosity increase. The addition of 10–30 mEq/L of pyrophosphates, polyphosphates, and citrate to MCI significantly ($P < 0.05$) decreased the protein-bound sedimentable Ca and P at pH 6.5. The 10-kDa permeable Ca decreased significantly ($P < 0.05$) with increasing concentration of DSPP, TSPP, STPP, and SHMP, whereas it increased significantly ($P < 0.05$) for TSC at both pH 5.5 and 6.5. These results indicate that orthophosphates, pyrophosphates, and polyphosphates combined with Ca to form insoluble Ca phosphate complexes together with casein. The higher amount of 10-kDa permeable Ca for MCI samples with TSC confirms the formation of soluble Ca-citrate complexes. This study also demonstrated that phosphate-based CSS can cause peptization of calcium phosphate nanoclusters, citrate-based CSS can sequester micellar Ca, and hexametaphosphate can interact directly with caseins. The results of this study will help in elucidating the critical role of CSS in modulating the casein hydration and dispersion during processed cheese manufacture.

Key Words: micellar casein isolate, soluble calcium, processed cheese

1328 Impact of multispecies sward feeding and cow breed in a pasture-based seasonal dairy production system on the composition and quality of milk throughout lactation.

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Changes in cow feed, breed, and stage of lactation all cause variation in milk properties, resulting in challenges for processors to maintain or improve dairy product quality. The aim of this study was to evaluate the effects of 2 pasture feeding systems, the newly introduced, more sustainable, low chemical-nitrogen multispecies swards (MSS), and the typically used perennial ryegrass (PRG), on raw milk properties. Holstein Friesian (HF) and Jersey Holstein Friesian (JFX) cows were subjected to the 2 feeds and followed over an entire spring-calving lactation period. Bulk milk samples (n = 144) were collected from the 4 groups weekly (n = 36) and studied for yield, gross composition, fatty acid (FA) profile, and physicochemical properties, and then analyzed by mixed linear model ($\alpha = 0.05$) and redundancy analysis. Breed had significant impact on milk profile, with milk from HF cows having smaller milk fat globule (MFG) size, higher instability index values, higher milk yield, and lower total solids (TS) levels compared with that from JFX cows. Notably, when fed with MSS, HF cows had increased TS and fat levels in their milk, as opposed to the PRG-fed HF cows. The MSS groups showed increased milk creaming velocity compared with PRG groups, throughout lactation. In addition, MSS milk was associated with bigger MFG size in mid and late lactation. MFG size, creaming velocity, and instability index for the 4 groups decreased from early to late stage of lactation. Based on multivariate analysis, diet (76% contribution), followed by breed (17%), influenced the milk FA profile, and these differences increased as lactation progressed. For both breeds, the MSS diet increased levels of both beneficial omega-6 and omega-3 polyunsaturated FA in milk, as well as short-chain FA. JFX cows showed increased levels of short-chain and de novo FA in milk, compared with HF. Overall, these findings indicate potential to target desirable product characteristics and nutritive value within more sustainable, pasture-fed dairy production systems.

Key Words: multispecies swards, dairy cow breed, milk properties

1329 Electro-activation technology for integral valorization of whey.

M. Aider*, *Laval University, Quebec, QC, Canada.*

The objective of this work was to study the potential of electro-activation technology, a principle based on applied electrochemistry and process engineering, for value-adding of whey in a context of its whole valorization. Electro-activation was used for lactose conversion into lactulose and a formation of fructo-oligosaccharides (FOS), which are known as proven prebiotics. To achieve this objective, whey was electro-activated in a cathodic compartment of an electro-activation reactor modulated with anion and cation exchange membranes to create the required isomerization conditions and formation of FOS. Whey cathodic electro-activation was carried out at different total dry matter concentrations (7, 14, and 21%) and electric current intensities (500, 750, and 1000 mA), and treatment duration of 60 min. Electro-activation was conducted at ambient temperature of 23 ± 1°C. The measured dependent variables were the whey solution pH, total alkalinity, oxidoreduction potential, amount of the isomerized lactose into lactulose, GOS formation, formation of glucose, galactose, fructose, and residual lactose. Evolution of the solution color was also monitored. The obtained results showed that electro-activation allowed formation of adequate alkaline medium, which is necessary for the lactose isomerization reaction into lactulose and formation of FOS. A positive correlation was established between lactulose formation and pH alkalinity of the electro-activated whey. HPLC analyses of sugars with a refractive index detector showed that only galactose was present in the electro-activated whey as monosaccharide, while no glucose and fructose were found. Furthermore, the analyses revealed that lactose conversion into lactulose ranged between 30% and 45% and that FOS were formed following in situ isomerization of glucose into fructose. In conclusion, this study demonstrated that electro-activation technology is highly effective to convert whey into an added-value ingredient containing lactulose and FOS as prebiotics. This newly developed dairy ingredient can be used in the development of functional dairy products with high content of prebiotics.

Key Words: whey, electro-activation, lactulose

1330 Generation of a glycomacropeptide concentrate from industrial cheese whey and evaluation of its cryoprotective abilities. M. Fomich* and T. Wang, *University of Tennessee, Knoxville, TN.*

Cheese whey is a byproduct in the dairy industry and contains many underutilized molecules such as glycomacropeptide and α-lactalbumin. These proteins are naturally glycosylated and contain sugar residues and glycosidic bonds similar to those of known antifreeze glycoproteins. The objective of this study is to concentrate soluble glycosylated proteins from cheese whey and evaluate their ice recrystalization inhibition (IRI) and dynamic ice shaping (DIS) activities. The concentrate is made from whey by utilization of heat denaturation to remove precipitated proteins, dialysis to remove lactose, and preparative size exclusion chromatography to fractionate the proteins into distinct size fractions. These fractions were analyzed for IRI and DIS activity by the splat assay and a modified sucrose sandwiching assay under various salt concentrations and pH systems. This was performed using a cryo-cold stage and polarized microscope. The fractions demonstrated IRI activity in all solvents used, regardless of salt content, pH, and weight %. Fraction 1 was more effective in acidic conditions with the 1% 10 m*M* NaCl, pH 3.0 sample reducing the ice crystal size by 56.1 ± 1.2% and the same sample at the raised pH (9.0) reduced the ice crystal size by 45.8 ± 1.5%. The salt molarity also affected IRI activity. The previous 1% 10 m*M* NaCl, pH 3.0 sample reduced the ice crystal size by 56.2 ± 1.2%, and when the salt molarity was raised to 200 m*M*, the reduction in size was only 38.0 ± 2.1%. At 4% the samples exhibited DIS abilities by changing the morphology of the ice crystals to cubic and hexagonal shapes, which is consistent with naturally occurring antifreeze proteins. This was also a pH-dependent phenomenon. When the pH increased to 9.0 the rigidly shaped crystal became more relaxed and circular, showing a decline in DIS activity. The importance of pH and salt content indicates the importance of electrostatic interactions in these proteins' cryoprotective mechanisms. Further examination of this peptide's important functional characteristics is needed to help understand glycomacropeptide's cryoprotective mechanism.

Key Words: antifreeze peptide, glycomacropeptide, cryopreservation

Production, Management, and the Environment 2

1331 Effects of prenatal wildfire-$PM_{2.5}$ exposure on inflammatory markers and health of the postnatal dairy calf. A. Pace*[1], G. Ponce[1], C. Ehlers[1], N. Ellis[1], M. Larson[1], P. Villamediana[2], M. Rovai[2], P. Rezamand[1], and A. L. Skibiel[1], *[1]University of Idaho, Moscow, ID, [2]South Dakota State University, Brookings, SD.*

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Wildfire smoke contains hazardous emissions such as particulate matter ($PM_{2.5}$) that, when inhaled by pregnant women, causes lower birth weights, suggesting an impact on prenatal development. Wildfire-$PM_{2.5}$ also causes local and systemic inflammation in calves exposed to smoke during the pre-weaning period. However, the impact of prenatal wildfire-$PM_{2.5}$ exposure on the postnatal calf is unknown. Our study investigated impacts of prenatal wildfire-$PM_{2.5}$ exposure on calf health and inflammatory status. Holstein heifer calves were born to cows either exposed (WFS, n = 17) or not exposed to smoke (CON, n = 26) in mid-gestation. Management and nutrition between groups were matched. Passive transfer of immunity was confirmed using a Brix refractometer. Heart rate (HR), rectal temperature (RT), and health scores were assessed and blood samples were collected at birth and every 2 wk for 8 mo. Hematological parameters and acute phase proteins, serum amyloid A (SAA), and serum albumin (SA) were quantified. Thoracic ultrasounds (TUS) were conducted at birth and monthly for 8 mo. Data were analyzed in SAS using general or generalized linear mixed models with group (WFS or CON), time, and interactions as fixed effects, and calf within time as a random or repeated effect. Dams of WFS calves were exposed to $PM_{2.5}$ concentrations that reached daily averages of 113 µg/m^3 around 5 mo gestation. WFS calves had lower TUS and eye scores, but higher HR (140.14 ± 1.42 vs. 101.79 ± 1.16 beats/min), navel scores, and fecal scores relative to those for CON ($P \leq 0.05$). Lymphocytes and monocytes were lower in WFS compared with those for CON (5.21 ± 0.41 vs. 6.43 ± 0.35 × 10^3 cells/µL and 0.30 ± 0.15 vs. 0.72 ± 0.12 × 10^3 cells/µL, respectively; $P \leq 0.03$). SA was elevated in WFS (3.19 ± 0.07 vs. 2.92 ± 0.05 g/dL; $P < 0.01$) whereas SAA was reduced (107,858 ± 8,491 vs. 172,710 ± 6,854.29 ng/mL $P < 0.0001$) relative to CON. These results suggest that calves prenatally exposed to smoke have altered inflammatory status. Further investigation is warranted to evaluate whether this may affect calf responses to immune challenges and future productivity.

Key Words: wildfire, inflammation, air quality

1332 Comparison of group-scale emissions in the milking parlor and a tunnel-ventilated housing barn in a commercial dairy farm. D. Manriquez*, A. Houghton, and A. Jannat, *Colorado State University, Fort Collins, CO.*

This study aimed to compare the dynamics of group-scale emissions of ammonia (NH_3), carbon monoxide (CO), carbon dioxide (CO_2), methane (CH_4), particulate matter 2.5 (PM2.5), and volatile organic compounds (VOC) between a rotary milking parlor (MP) and a tunnel-ventilated barn (TV, freestalls bedded with sand). Two automated air quality sensor platforms (IEQMax, CO) were installed in a dairy farm located in Northern Colorado milking 6,000 Holstein cows. Sensors were situated in the middle of the MP and in the middle of the TV 10 feet from the ground. Data collection occurred between August and December 2023 at 1-minute intervals. Data were averaged by the hour and date and were analyzed using ANOVA (SAS 9.4, SAS Institute Inc., Cary, NC) considering sensor location (MP vs. TV), date, hour of day, and location and hour of day interaction as covariates. Table 1 depicts overall and maximum concentration (ppm) of NH_3, CO, CO_2, CH_4, and VOC, as well as PM2.5 (ug/m^3) in the MP and TV. Ammonia was not detected in the MP, whereas TV NH_3 reached maximum level at midnight. No differences in CH_4 were detected between the MP and the TV. The MP had greater emissions of CO and PM2.5 compared with the TV. The TV had greater emissions of CO_2 and VOC. A significant interaction between sensor location and hour of day was observed in all air components, indicating time-dependent dynamics of air quality that may vary dependent on specific farm operations. This research presents an opportunity to identify farm practices that might impact air quality.

Key Words: emissions, air quality, housing.

1333 Investigating the antimethanogenic effect of ethyl nitroacetate on methane production and methanogenic archaea in vitro. A. Castaneda*[1,2], K. Narayan[1], T. Webb[1], R. Anderson[3], and D. Pitta[1], *[1]University of Pennsylvania, Kennett Square, PA, [2]McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada, [3]United States Department of Agriculture, College Station, TX.*

Ethyl nitro-acetate (ENA) is a novel short-chain nitro-compound with a potent anti-methanogenic effect. Previous studies have shown its impact on methane (CH_4) production, total gas, and fermentation; however, its dose-by-hour effect on CH_4 production and methanogenic archaea is unknown. This study tested the dose-by-hour interaction of ENA on CH_4 production and methanogenic archaea in vitro. We conducted a preliminary experiment to determine doses and sampling hours using the ANKOM^{RF} Gas Production System. We tested ENA at 0, 8, and 16 m*M* doses and collected gas and liquid samples at 6, 12, and 24 h post-incubation under a 3 × 3 factorial design. Each dose-by-hour combination was tested

Table 1 (Abstr. 1332). Overall group-scale air emissions (LSM, SEM) in MP and TV and maximum average concentrations and their corresponding hour of day[1]

	Overall		Maximum		Hour of day	
Variable	MP	TV	MP	TV	MP	TV
NH_3	ND	6.3 (3.8)	ND	7.8 (0.2)	ND	11 pm
CO	1.1 (0.1)^a	0.4 (0.1)^b	1.8 (0.4)^a	2.5 (0.3)^b	3 pm	10 am
CO_2	609.9 (1.9)^a	655.5 (1.9)^b	642.6 (9.2)^a	729.9 (9.3)^b	0 am	1 am
CH_4	10.2 (0.2)^a	10.1 (0.2)^a	27.1 (0.8)^a	15.5 (0.8)^b	1 pm	7 am
PM2.5	5.2 (0.06)^a	3.1 (0.07)^b	7.4 (0.3)^a	5.3 (0.2)^b	5 am	3 pm
VOC	143.2 (1.4)^a	150.9 (1.3)^b	246.0 (6.5)^a	400.7 (6.5)^b	5 am	7 am

[1] ND = not detected. Different letters indicate significant differences at $P < 0.05$.

in triplicate. CH_4 production was analyzed by gas chromatography. The copy numbers of the 16S rRNA gene of methanogenic archaea were quantified using RT-PCR. We used a fixed effects model and analyzed the data using the PROC GLM procedure of the SAS® software. A post hoc analysis was performed for the dose-by-hour interaction using the lsmeans procedure and the Scheffé adjustment for pairwise comparisons. We detected a dose-by-hour interaction for CH_4 production ($P = 0.001$) and pH ($P = 0.03$), whereas for total gas, a main effect of hour ($P < 0.0001$) was found. The lowest CH_4 production (0.46 ± 5.38 mL) was found with the 16 m*M* dose at 6 h post-incubation, matching the lowest gas production (135 ± 7.47 mL) observed at the same dose and hour. We also observed that the copy numbers of methanogenic archaea were affected by hour ($P < 0.0001$) and dose ($P < 0.0001$), which were lowest (6.77 ± 0.15) with the 8 m*M* dose at 6 h post-incubation, suggesting a rapid suppressing effect. CH_4 production, total gas, and copy numbers of methanogenic archaea were at their lowest and highest points at 6 and 24 h post-incubation, respectively, suggesting that ENA was being metabolized as the incubation progressed. In summary, these findings indicate that the antimethanogenic effect of ENA is potentiated by dose and hour and that they act synergistically to reduce CH_4 production by suppressing the metabolism of methanogenic archaea.

Key Words: nitro-acetate, methane production, methanogenic archaea

1334 Monensin reduces enteric methane emissions and intensity in late-lactation Holstein cows consuming a high-concentrate diet. D. Onan-Martinez*[1], M. A. T. de Bari[1], H. Olmo[1], J. Lance[1], I. M. Toledo[1], J. M. Tricarico[2], and G. E. Dahl[1], *[1]Department of Animal Sciences, University of Florida, Gainesville, FL, [2]Dairy Management Inc., Rosemont, IL.*

Methane (CH_4) is a major greenhouse gas responsible for global warming. Multiple strategies have been tested to reduce enteric methane yield from cattle. Among them, supplementation with monensin has shown variable outcomes. However, we hypothesized that Rumensin (monensin) would reduce enteric CH_4 yield and intensity in late-lactation dairy cows on a high-concentrate diet (~207 DIM). Cows (n = 20) were fed a high-concentrate diet (51.3% grain mix and 48.7% forage on DM basis) during two 4-week periods in a crossover design with 2 treatments: control (CON) and monensin (MON). Statistical analysis by Proc Mixed used a model that included treatment, period, order, time, and the interactions as fixed effects. MON consisted of 300 mg/cow of monensin in 34 g/cow of dried distillers grains (DDG), whereas CON was DDG alone, both top-dressed daily on the TMR. All cows were milked 3 times daily and housed in a sand-bedded freestall barn equipped with Calan gates to collect individual dry matter intake (DMI). A GreenFeed system was used to measure enteric gas output. Daily milk yield and DMI were measured from each cow for the entire study. In addition, milk fat, protein, and lactose were measured weekly. Average DMI (MON = 25.4 ± 0.3, CON = 25.4 ± 0.3, kg/d ± SEM; $P > 0.1$) and energy-corrected milk (ECM) yield (MON = 35.8 ± 1.09, CON = 36.4 ± 1.09, kg/d ± SEM; $P > 0.1$) were similar between treatments. Conversely, daily methane emissions were significantly reduced ($P < 0.01$) with Rumensin (MON = 207.1 ± 13.1, CON = 257.2 ± 13.1 g/d). Methane yield also declined ($P < 0.05$) with treatment (MON = 8.1 ± 0.4, CON = 9.9 ± 0.4 g/kg DMI). Lastly, methane intensity relative to ECM tended to decline ($P = 0.08$) with Rumensin (TRT = 5.7 ± 0.3, CON = 6.5 ± 0.3 g/kg of ECM). The results suggest that Rumensin reduces enteric methane emissions and intensity in late-lactation dairy cattle fed high-concentrate diets, providing evidence to support the use of monensin to reduce CH_4 emissions from cattle.

Key Words: dairy cattle, GreenFeed, enteric gas output

1335 Feed intake regulation stabilized daily intake pattern but reduced lactational performance with little impact on rumen fermentation or efficacy of 3-nitrooxypropanol (3-NOP) in dairy cows. K. Wang*[1], S. E. Räisänen[1], L. Eggerschwiler[2], T. He[1], M. Z. Islam[1], Y. Li[1], X. Ma[1], R. Siegenthaler[2], Z. Zeng[1], F. Dohme-Meier[2], and M. Niu[1], *[1]Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH, Zürich, Switzerland, [2]Ruminant Nutrition and Emissions, Agroscope, Posieux, Switzerland.*

To study the effect of feed intake regulation (i.e., stabilizing intake fluctuations) on rumen fermentation and CH_4 emissions, 16 multiparous Holstein cows (126 ± 19.7 DIM, 34 ± 3.8 kg/d milk yield) were used in a 4 × 4 Latin Square design with four 21-d periods (14-d adaptation, 7-d sampling). Four treatments consisted of a 2 × 2 factorial arrangement of feed access levels (ad libitum [AL] vs. regulated [RE]) and 3-NOP levels (no vs. yes; 70 mg/kg DM). All cows were housed in a freestall barn, and TMR (14% CP and 42% NDF of DM) was delivered 2×/d at 0700 and 1700 h. The RE access to feed was programmed using the Insentec (Hokofarm Group B.V.) system as follows: a cow was allowed to consume up to 12% of the daily feed intake (based on the previous week's intake) in each meal; once the limit was reached, the cow was denied access to the feed trough for 1 h. Data were analyzed using linear mixed model in R. There was a feed access by time interaction ($P < 0.01$) on daily pattern of feed intake (FI): FI rate (% of DMI) was reduced ($P < 0.01$) for RE vs. AL at 2 conditional meals after feeding (5.7 vs. 9.0%/h and 6.1 vs. 12.7%/h, respectively) and was compensated for cows with RE access during night (4.2 vs. 3.1%/h from 1900 to 2400 h, $P < 0.01$). Cows with RE had a lower intraday variation (1.7 vs. 2.8%/h of SD) in FI compared with AL. There were no interactions between feed access and 3-NOP on production, rumen VFA, and CH_4 emission parameters. Cows with RE access had a lower ($P < 0.05$) DMI (−0.37 kg/d), milk yield (−1.04 kg/d), and ECM (−1.17 kg/d) but showed no difference in VFA and CH_4 production. The molar proportions (mol%) of acetate (−3.24) decreased, but propionate (0.93) and butyrate (2.56) increased in cows supplemented with 3-NOP ($P < 0.05$). Methane production (g/d), yield (g/kg DMI), and intensity (g/kg ECM) were reduced ($P < 0.05$) by 3-NOP by 33%, 32%, and 30%, respectively. In conclusion, the stabilized intake pattern did not further affect rumen VFA, CH_4 production, and mitigating efficacy of 3-NOP in the current study.

Key Words: intake regulation, rumen fermentation, methane emission

1336 Effect of different doses of 3-NOP combined with varying forage composition on methane yield, feed intake, and milk production in dairy cows. M. Maigaard*[1], M. R. Weisbjerg[1], C. Ohlsson[2], N. Walker[2], and P. Lund[1], *[1]Department of Animal and Veterinary Sciences, AU Viborg–Research Centre Foulum, Aarhus University, Tjele, Denmark, [2]DSM-Firmenich, Kaiseraugst, Switzerland.*

The objective of this study was to investigate the effect of different doses of 3-nitrooxypropanol (3-NOP) with varying forage composition on gas emission and production performance of dairy cows. Seventy-two lactating Danish Holstein cows were enrolled in a continuous randomized block design with 2 weeks of adaptation, after which treatments were applied for 12 consecutive weeks. Initial DMI and ECM yield were 24 ± 3.3 and 38 ± 7.1 kg, respectively. Cows were blocked according to parity and DIM. Treatments were organized in a 2 × 3 factorial arrangement. The first factor was diet type, reflecting 2 different forage compositions of the diet. Ratio of grass-clover silage to corn silage was 60:40% of total forage DM in grass-based diets and 40:60% in corn-based diets. Total forage constituted 56% of the DM in the partial mixed rations. The second factor reflected 3 doses of 3-NOP: 0, 60, or 80 mg 3-NOP/kg DM. Gas emission were measured using GreenFeeds and feed intake using Insentec. Weekly averages from the last 10 weeks were analyzed in a mixed model including fixed effects and interactions between diet type, 3-NOP dose, week, and parity, and included a covariate term and accounted for repeated measures. Methane yield (g/kg DM) was reduced by 34% and 31% at 60 and 80 mg 3-NOP/kg DM ($P < 0.01$), respectively, and there was no difference in CH_4 yield between the 2 doses ($P = 0.37$). The reduction in CH_4 yield was numerically greater in corn-based diets (−38%) compared with grass-based diets (−30%), although there was no interaction between dose of 3-NOP and diet type ($P = 0.27$). Total DMI was reduced by 9% in cows fed 80 mg 3-NOP/kg DM ($P < 0.01$) across diet types, whereas there was no effect at 60 mg 3-NOP/kg DM ($P = 0.41$). Similarly, ECM yield was reduced by 5% in cows fed 80 mg 3-NOP/kg DM compared with no 3-NOP supplementation ($P < 0.01$). In conclusion, 3-NOP at 60 mg/kg DM is efficient in reducing CH_4 without compromising production performance. Further, the CH_4-reducing effect of 3-NOP seemed numerically greater in corn-based diets compared with grass-based diets, despite similar NDF and starch levels.

Key Words: grass-based, maize-based, Bovaer

1337 Combined effects of 3-nitrooxypropanol (3-NOP; Bovaer®10) and tannin extract (*Acacia mearnsii*) on lactational performance, ruminal methane emissions, and nitrogen partitioning in Brown Swiss and Holstein Cows.

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This study aimed to investigate the combined effects of 3-nitrooxypropanol (3-NOP) and *Acacia mearnsii* extract (TAN), and their interactions with breed (Brown Swiss [BS] vs. Holstein Friesian [HF]), on lactational performance, enteric CH_4 emissions, and N partitioning. Sixteen cows, 8 HF (134 ± 31.3 DIM, 35.8 ± 6.04 kg MY) and 8 BS (161 ± 39.8 DIM, 27.0 ± 6.24 kg MY) were employed in a split-plot design, with breed as the main plot. Cows within each subplot were arranged in a replicated 4 × 4 Latin square with a 2 × 2 factorial design with four 24-d periods. The experimental diets were (1) CON (basal TMR), (2) 3-NOP (60 mg/kg DM), (3) TAN (3% DM), and (4) 3-NOP+TAN. Basal diets (47.1% DM, 39.3% NDF, and 15.9% CP) were fed 2×/d at 0830 h and 1730 h. Over three days, 8 spot samples of urine, feces, and gas emissions (GreenFeed) were collected at the end of each period. Data were analyzed via linear mixed model in R. No interaction was noted for 3-NOP × TAN for any of the variables. 3-NOP did not affect production performance. Cows fed TAN had 1 kg/d lower ($P < 0.01$) DMI, but ECM and feed efficiency were not affected. Further, TAN decreased ($P < 0.01$) MUN concentration (16.7 vs. 3.6 mg/dL) and increased ($P = 0.03$) milk true protein concentration and tended to ($P = 0.08$) increase milk true protein yield. 3-NOP decreased CH_4 production (g/d), yield (g/kg DMI), and intensity (g/kg ECM) by 18%, 16%, and 17%, respectively. A breed × 3-NOP interaction for CH_4 production (HF: 22% vs. BS: 13%; $P < 0.05$) was observed. Lastly, cows fed TAN had a lower (−23.5%; $P < 0.01$) urine N but greater (+28%; $P < 0.01$) fecal N (% daily N intake; total excretion estimated via creatinine and acid-insoluble ash). Overall, TAN reduced MUN and urinary N without affecting milk yield, whereas 3-NOP decreased ($P < 0.05$) CH_4 emissions by 16% to 17% on a high-fiber diet. Despite no observed interaction, combined supplementation of 3-NOP and TAN could offer benefits in CH_4 mitigation while minimizing excess N excretion. The varying effectiveness of 3-NOP across breeds warrants more research.

Key Words: enteric methane, N excretion, breed × 3-NOP interaction

1338 Grape pomace supplementation reduced methane emissions and improved milk quality in dairy cows.

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Grape pomace (GP), a viticulture byproduct, is rich in tannins and fat and may reduce enteric methane emission in dairy cattle. This study investigated the potential effects of GP on enteric methane emissions and milk quality in lactating dairy cows. Holstein dairy cows (n = 24) averaging 200 DIM were used in 3 × 3 Latin square design including a 2-week covariate period. Cows received 3 dietary treatments over 3 periods, each lasting 4 weeks: control diet (CON), 10% grape pomace (10% GP), and 15% grape pomace (15% GP) on DM basis. Methane emissions were measured using a GreenFeed system (C-Lock Inc.). Milk yield was recorded daily, and milk samples were collected on the last 3 d of each 28-d period. Data were analyzed using a mixed model ANOVA (Proc GLIMMIX in SAS 9.4) with LSM compared using Fisher's LSD at $\alpha < 0.05$. DMI (kg/d) was significantly higher in the CON group than in the 10% and 15% GP groups, and the 15% GP group had less DMI than 10% GP ($P < 0.0001$). Results showed that the CON group produced greater enteric methane (434.09 ± 23.4 g/d) compared with the 10% GP (386.11 ± 23.3 g/d) and 15% GP (389 ± 23.4 g/d) groups ($P = 0.01$). However, methane yield (g/kg DMI) did not differ among different treatments. Milk yield remained similar across treatments, although cows on 10% and 15% GP diets had numerically greater milk yield than the CON group. Milk fatty acids were affected by the GP dietary treatments. Linoleic acid, an omega-6 (n-6) PUFA, increased to 4.81 ± 0.76% in 10% GP and 4.99 ± 0.62% in 15% GP from 4.16 ± 0.50% in the CON group ($P < 0.0001$). Total PUFA increased to 6.34 ± 0.86% and 6.47 ± 0.72% in the 10% and 15% GP groups, respectively, from 5.39 ± 0.68% in CON group ($P < 0.0001$). PUFA has been shown to have beneficial effects on inflammatory and cardiovascular diseases. Both GP groups had lower palmitic acid compared with CON ($P < 0.0003$); palmitic acid has been linked to potential harmful effects on cardiovascular health. In conclusion, supplementing cows with GP reduced enteric methane emissions and improved milk quality without affecting milk production.

Key Words: grape pomace, methane emission, milk fatty acids

1339 A short-term intervention of a blend of quebracho and chestnut extracts did not impact lactating dairy cow methane emissions.

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Increased pressure has been placed by various stakeholders on the dairy industry to reduce its enteric methane (CH_4) emissions. The objective of this study was to evaluate the impact of a blend of quebracho and chestnut tannin extracts along with a carrier from cereals rich in saponins (TAN) on enteric CH_4 emissions. Twenty-four early- to mid-lactation cows (DIM = 89 ± 21.2) were blocked to treatment based on dry matter intake, parity, milk production, and days in milk. Cows were individually fed in a Calan feeding system, housed in a freestall pen, and milked twice daily at 0600 h and 1800 h. The 2 treatments were added as a top dress to the top of a standard ration that consisted of a control (50 g ground corn) or TAN fed at a rate of 0.15% dry matter. Tannins were weighed out before each feeding, and the difference from 50 g consisted of dry ground corn. Dairy cows were stagger-started to treatment to allow for same-day comparisons between blocks, and methane emissions were recorded via the use of a pair of ventilated head chambers. Emissions of CH_4 and CO_2 were measured in a discontinuous pattern from 0700 to 0900 h, 1300–1500 h, 1900–2100 h, and 0100–0300 h for a baseline

covariate (d 0) and at 16, 32, 48, and 64 d post-treatment initiation. Between measurements, cows were returned to a freestall pen. Methane emissions, dry matter intake, and milk yield were analyzed using R (Version 4.2.2). The TAN treatment was found to not impact gross CH_4 emission rate (14.9 vs. 15.1 ± 0.3 g CH_4/h), dairy cattle dry matter intake (28.5 vs. 28.5 ± 0.4 kg/d), or milk production (37.8 vs. 39.2 ± 1.7 kg/d; $P > 0.05$) on days of head chamber measurements. Similarly, no differences were identified between treatments for CH_4 intensity (g CH_4/h per kg milk) or CH_4 yield (g CH_4/h per kg dry matter intake). Previous research in essential oil and rumen modifiers has demonstrated treatment differences in CH_4 emissions under longer intervention periods. Long-term feeding studies using this TAN blend of quebracho and chestnut extracts may demonstrate efficacy in reducing CH_4 emissions in lactating dairy cows.

Key Words: methane, feed additive, sustainable agriculture

1658 Understanding the effect of halogenated compounds on methanogen isolates and methane production. K. Narayan*[1], A. Yelampalli[2], N. Indugu[1], A. Castaneda[1], A. Johnson[1], A. Post[1], T. Webb[1], and D. Pitta[1], *[1]University of Pennsylvania, Kennett Square, PA,* *[2]Avon Grove Charter School, West Grove, PA.*

Enteric methane (CH_4) is formed by methanogens in the rumen and is considered both an environmental problem and an energy loss (~12%) to the host. To overcome these adverse effects, several methanogenic inhibitors have been tested for their CH_4-reducing potential; however, their effect on individual methanogens and their relation with CH_4 production is largely unclear. The objective of this study was to examine the effect of 3 halogenated compounds (Bromoform, 65 µg; Iodoform, 65 µg; and Chloroform, 50 µg) and a seaweed (*Asparagopsis* taxiformis, 100 µg) on methanogenic populations and their effect on CH_4 production in vitro. Pure cultures of *M. ruminantium* M1 and *M. stadtmanae* DSMZ 3091 were cultured on H_2/CO_2 (80:20 ratio at 0.5 psi) and methanol (100 µL of 100%) respectively and inoculated with 5% (vol/vol). These strains were served as reference to observe their growth performance by colony forming unit (cfu) and CH_4 production potential by gas chromatography. Culture studies revealed that the growth of *M. stadtmanae* and methane emissions were 3 times more (10^{10} cfu/mL; 177 µg/mL) than *M. ruminantium* (10^{11} cfu/mL; 65 µg/mL). Halogenated compounds and seaweed had variable effects on CH_4 production from these 2 methanogenic isolates. Further, an in vitro assay was conducted using ANKOM^{RF} Gas Production System to evaluate the effect of these inhibitors on ruminal methanogen population and CH_4 production. A linear model was applied using R package (R Core Team, 2020) for statistical analysis. All inhibitors reduced ($P < 0.001$) CH_4 production which is consistent with the reduction ($P < 0.05$) in methanogenic population. RT-qPCR showed that bromoform reduced ($P < 0.05$) the copy number of *M. ruminantium*, *M. stadtmanae*, and *M. archaeon*, seaweed, and chloroform reduced ($P < 0.05$) *M. stadtmanae* and *M. archaeon*, and iodoform reduced ($P < 0.05$) *M. stadtmanae*. In conclusion, this study revealed that all inhibitors reduced CH_4 production and methanogens but had distinct inhibitory effects on individual methanogens. Future investigations are required to understand the effects of these inhibitors on more representatives of individual methanogens.

Key Words: in vitro, methanogenic inhibitor, methane production

Joint Reproduction and Physiology and Endocrinology Symposium: Physiology and Management of Previously Inseminated Cows—Approaches for Reinsemination

1340 Management strategies for reinsemination: An integrative approach. P. M. Fricke*, *University of Wisconsin–Madison, Madison, WI.*

In contrast to first artificial insemination (AI), submission of cows for second and greater AI requires coupling a nonpregnancy diagnosis with a management strategy to rapidly resubmit nonpregnant cows for reinsemination. Although an early nonpregnancy diagnosis can improve reproductive efficiency by decreasing the interval between AI services, the high rate of early pregnancy loss in lactating dairy cows limits the effectiveness of early resynchronization strategies based on an Ovsynch protocol (i.e., Resynch). One of the first Resynch studies aimed to identify the optimal interval from first timed AI (TAI) to initiation of a Resynch protocol to optimize fertility. Variation in cycle length among individual cows and the high frequency of early pregnancy loss, however, extends the interval between inseminations and increases variation in return to estrus after AI. Accurate identification and insemination of cows that return to estrus after AI is a practical and early method for nonpregnancy diagnosis in dairy herds that is facilitated by automated activity monitoring systems. A key factor affecting fertility to a Resynch protocol is the presence or absence of a corpus luteum (CL) and hence circulating progesterone concentrations at the onset of the Resynch protocol. Based on these collective findings, a common Resynch strategy is to identify and AI cows that return to estrus after AI and pre-treat all cows with GnRH 7 d before a pregnancy diagnosis conducted with transrectal ultrasonography at 25 or 32 d after TAI. Nonpregnant cows are identified 1 wk later at 32 or 39 d after TAI using transrectal ultrasonography and are segregated based on the presence or absence of a CL. Nonpregnant cows with a CL complete the Resynch protocol, whereas nonpregnant cows without a CL reinitiate a Resynch protocol that includes an intravaginal progesterone insert. This strategy has led to increased fertility to Resynch protocols used on commercial dairy farms. Future research should be aimed at developing practical and accurate methods for early nonpregnancy diagnosis and further refining Resynch strategies. *Supported by WI Experiment Station Hatch project WIS04012.*

Key Words: nonpregnancy diagnosis, pregnancy loss, Resynch

1341 Ovarian physiology of previously inseminated cows: Considerations for rebreeding. J. O. Giordano*, *Department of Animal Science, Cornell University, Ithaca, NY.*

Successful reproductive programs for dairy cows include a reinsemination strategy that minimizes the interbreeding interval (IBI) and maximizes pregnancies per artificial insemination (P/AI). Unique challenges of reinsemination programs (Re-AI) include the detrimental effects of early pregnancy loss (PL) on ovarian function and the need to determine pregnancy status before resynchronization protocols can be completed. The effects of PL on ovarian function have negative consequences not only on Re-AI at detected estrus (AIE) but also on hormonal interventions to resynchronize ovulation. Delayed corpus luteum (CL) regression has long been recognized as a major contributor to the variability in return to estrus in previously inseminated cows. Novel methods to monitor in more detail markers of early conceptus development (interferon stimulated genes, pregnancy-associated glycoproteins) and methods to characterize the CL and follicular wave dynamics enabled a more comprehensive characterization of the ovarian function of cows with and without PL. Through a better understanding of ovarian function and the development of more effective hormonal interventions regimens, novel resynchronization programs based on the ovarian status of cows at nonpregnancy diagnosis were developed and tested. Recent work demonstrated improved reproductive performance and management outcomes. However, these novel Re-AI programs have not yet been optimized. Preventing expression of estrus due to early resynchronization and suboptimal responses to key hormonal interventions continue to limit progress. Avenues for continued improvement include novel methods to identify cows affected by PL, predict occurrence of estrus, predict the stage of the estrous cycle, determine CL function and response to $PGF_{2\alpha}$, and predict the ability of follicles to continue growing or ovulate. Major gains in reproductive performance and management outcomes might be realized by focusing research efforts on incorporating novel technologies to predict and control ovarian function and thus enable further reduction of the IBI and enhancing fertility of reinseminations.

Key Words: ovarian function, resynchronization, detection of estrus

1342 In-line milk progesterone monitoring: Potential for identification of nonpregnant cows. T. C. Bruinjé*[1], S. J. LeBlanc[1], and D. J. Ambrose[2], *[1]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Department of Agricultural, Food, and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

In-line milk progesterone (P4) monitoring has become an increasingly available tool in Canada and Europe to support reproductive management. The in-line milk P4 analyzer (Herd Navigator, DeLaval) uses a built-in lateral flow immunoassay to measure real-time P4 to determine the onset of cyclicity, day of estrus, and pregnancy status after artificial insemination (AI). Sampling starts at 20 DIM and occurs on average every 2 d until pregnancy, with sampling frequency adjusted by a model according to the estimated stage of the estrous cycle. Sampling is more frequent between ~17 and 24 d after AI to identify return to estrus, characterized by a decline in P4 levels to below a threshold. Pregnancy alerts start ~27 d and stop ~55 d after AI in cows without a P4 decline. In a set of studies with 158,961 in-line milk P4 records from periods before and after 4,353 AI events in 1,821 lactations, we characterized predictors of pregnancy per AI and investigated P4 profiles in open cows that did not have timely return to estrus. Some of the predictors were prolonged luteal phase, suboptimal peak P4, elevated nadir P4 preceding AI, and suboptimal P4 following AI. Between 31 and 46% of cows had at least one metric of P4 profile unfavorable to pregnancy. However, these variables had low predictive abilities based on ROC cut points (AUC <0.60). Among open cows, 9%, 71%, and 20% returned to estrus ≤17, 19 to 23, or ≥25 d after AI, respectively. Among inseminated cows without a P4 decline, 14% had a false positive pregnancy alert at 30 ± 3 d after AI, and milk P4 declined ≤34, 37 to 44, or ≥48 d after AI in 22%, 59%, and 19% of those cows, respectively. Incorporating a confirmatory early pregnancy diagnosis may help determine if delayed return to estrus in 20% of cows without a pregnancy alert, or return to estrus shortly after a pregnancy alert in 14% of cows, are indications of abnormal estrous cycles or pregnancy losses. Monitoring in-line milk P4 is a valuable tool to identify predictors of pregnancy outcomes and opportunities for selective breeding decisions, as well as to identify nonpregnant cows for timely reinsemination.

Key Words: fertility, precision technology, reproduction

1343 Doppler ultrasonography versus on-farm milk progesterone (P4) for early pregnancy diagnosis: Opportunities and challenges for the development of rapid timed rebreeding programs.

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Daily detection of estrus in cows that were previously inseminated is the earliest and most common method for detecting nonpregnant cows, allowing an immediate reinsemination of these cows and enhanced reproductive efficiency. On the other hand, Resynch strategies that prioritize timed AI (TAI) boost reproductive performance by breeding all nonpregnants ~35 d after the previous TAI with a potential increase in pregnancy/AI optimizing hormonal milieu at AI. Nevertheless, most analyses indicate similar time to pregnancy in comparisons in rebreeding programs based on estrus detection versus Resynch programs. Thus, systems for rebreeding lead to substantial variation among farms based on management priorities (labor, facilities, goals, etc.). To develop an aggressive rebreeding program, we have evaluated programs that allow early detection of nonpregnant cows and immediate TAI, to increase the number of pregnancies before 130 DIM. Early pregnancy in lactating dairy cows is associated with more milk production, greater fertility, and fewer health disorders in the next lactation. On-farm methods to assess corpus luteum (CL) functionality such as color Doppler ultrasound (CDU) and milk P4 have been used for indirect detection of nonpregnancy. While CDU has been successfully incorporated into commercial beef cattle operations, it is still an area of research for high-producing lactating dairy cows. The problems with early rebreeding programs in lactating dairy cows are related to a later (~d 21) and more variable time to luteolysis in cows that do not become pregnant to previous AI. This leads to a greater false positive rate (active CL even though nonpregnant). Preliminary results indicate several factors can affect the accuracy of CDU compared with milk P4 as early pregnancy diagnosis methods. Furthermore, there are many challenges in developing early timed rebreeding programs, optimized for high-producing lactating dairy cows, particularly methods for synchronizing the time of luteolysis, without using $PGF_{2\alpha}$.

Key Words: Doppler, milk progesterone, dairy cow

Ruminant Nutrition 3: General

1344 Mycotoxin contamination trends in US corn grain: 2019–2023. P. N. Gott*, E. F. Schwandt, L. Zheng, and A. W. Levy, *dsm-firmenich, Animal Nutrition & Health, Plainsboro, NJ.*

Many molds can contaminate crops and feedstuffs, but few are known to produce secondary metabolites called mycotoxins that may affect animal health and performance. Mycotoxin occurrence and contamination levels in US corn grain were surveyed in this study. Sample pools differ each year but were submitted by feed mills, nutritionists, and producers mainly from Midwest and Southeast regions. Samples were analyzed via liquid chromatography with tandem mass spectrometry for 6 major mycotoxin groups: aflatoxins (Afla), type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A. Samples associated with clinical health or performance concerns on-farm were excluded. Results from samples that were above the limit of detection for each mycotoxin group were analyzed using the GLIMMIX procedure of SAS with harvest year as fixed effect and sample as the experimental unit (SAS 9.4, Cary, NC). Results are presented in Table 1. Initial results for 2023 corn grain (n = 189) identified FUM, ZEN, and B-Trich most frequently (86%, 76%, and 59%, respectively). Mean FUM concentration did not differ across the study period. The mean concentration of ZEN was highest in 2019 and lowest in 2023 (415 ppb and 117 ppb, respectively). Although occurrence of B-Trich was among the lowest observed across the study period, the highest mean concentration of B-Trich was detected in 2023 (1,562 ppb). Initial survey results indicate FUM, ZEN, and B-Trich continue to occur frequently in US corn grain.

Key Words: corn, fumonisin, mycotoxin

1652 Effects of emulsifying compounds blended in a fatty acid prill on milk production in lactating dairy cows. S. L. Burtnett*[1], J. J. Albrecht[2], O. R. Drehmel[2], P. Yoder[2], M. F. Scott[2], and K. J. Harvatine[1], [1]*Pennsylvania State University, University Park, PA,* [2]*Milk Specialties Global, Eden Prairie, MN.*

Fatty acid (FA) supplements are commonly fed to increase the energy content of the lactating dairy cow diets and improve milk and milk fat production and energy balance. However, increasing dietary fat commonly decreases FA digestibility. Our objective was to investigate the effects of reacting FA with magnesium and adding different emulsifiers on feed intake and milk yield and composition. We hypothesized that reacting FA with magnesium or the addition of an emulsifier would increase milk fat yield. Fifteen multiparous Holstein cows (>70 DIM) were randomly assigned to treatment sequence in a 5 × 5 Latin square design with 14 d periods. All cows received the same basal diet and treatments were prilled FA supplements with similar FA profile fed at 2% of diet DM. The treatments were (1) conventional FA prill (CON); (2) magnesium-reacted FA prill (MAG); (3) FA prill with lysophospolipids (LYSO), (4) FA prill with monoglyceride (MONO), and (5) FA prill with polysorbate 60 (POLY). Data were analyzed using a mixed model that included the random effects of cow and period and the fixed effect of treatment using PROC MIXED. Treatments had no effect on milk yield or milk fat and protein content or yield. As a group, cows produced 40.9 kg of milk per day with 4.26% milk fat and 3.13% milk protein. Intake averaged 30 kg/d and also did not differ with treatment. There was also no effect of treatment on milk palmitic acid or stearic acid concentration. The addition of lysolecithin did increase milk oleic acid compared with the monoglyceride treatment (19.0% vs 17.5%, $P = 0.03$). Additionally, the lysophospholipids treatment had higher total preformed FA in milk compared with monoglyceride treatment (40.3% vs 37.6%, $P = 0.05$). Overall, reacting a FA prill with magnesium or adding an emulsifier did not impact milk production however the inclusion of lysophospholipids did increase milk oleic acid concentration.

Key Words: lysophospholipids, polysorbate, monoglyceride

1346 Effects of a red macroalga on enteric methane emission and lactational performance of dairy cattle. D. E. Wasson*, C. V. Almeida, S. F. Cueva, L. F. Martins, A. Richards, N. Stepanchenko, and A. N. Hristov, *The Pennsylvania State University, University Park, PA.*

This study investigated the effects of a non-bromoform red macroalga (RM) supplementation on productive performance and enteric gas emissions in lactating dairy cows. Eight Holstein cows averaging (±SD) 79 ±

Table 1 (Abstr. 1344). Mycotoxin contamination in US corn grain

Parameter	2019	2020	2021	2022	2023	SEM[1]	*P*-value[1]
N	329	303	305	398	189		
Afla						79.1	0.294
Prevalence, %	3	5	7	11	11		
Mean, ppb	13	115	57	46	20		
B-Trich						124.9	0.007
Prevalence, %	81	58	69	68	59		
Mean, ppb	883^{b}	1,128^{ab}	899^{b}	1,054^{ab}	1,562^{a}		
FUM						399.9	0.376
Prevalence, %	78	64	64	79	86		
Mean, ppb	3,348	2,783	2,980	3,675	2,670		
ZEN						78.1	0.016
Prevalence, %	46	20	28	31	76		
Mean, ppb	415^{a}	353^{ab}	384^{ab}	329^{ab}	117^{b}		

^{a,b}Values across rows differ ($P < 0.05$).

[1] SEM and *P*-values generated using the GLIMMIX procedure of SAS to test the effect of harvest year on mean contamination level of each mycotoxin group. Other toxin groups are not presented due to limited number of positive samples.

11 DIM and 48 ± 5.6 kg/d milk yield (MY) were enrolled in a crossover design experiment with two 22-d periods with a 10-d washout period between the experimental periods. Treatments were control (basal diet, CON) or 1.5% RM (on a feed dry matter [DM] basis). The basal diet consisted of 46% (DM basis) corn silage, 13% alfalfa haylage, and 41% concentrates. Enteric CH_4 emission was measured using the GreenFeed system during the final week of each period. Data were analyzed using the MIXED procedure of SAS with treatment, parity, and period in the model, and square and cow within square as random effects. Dry matter intake (DMI) was decreased ($P = 0.006$) 12% by RM compared with control, whereas MY (42 ± 1.2 [mean ± SEM]) was unaffected, resulting in a trend for a 7% increase ($P = 0.10$) in feed efficiency by RM. Milk composition was largely unaffected, except trends for decreased ($P = 0.10$) true protein and lactose yields in cows fed RM by 8% for both. Daily CH_4 emission, yield (CH_4 g/kg DMI), and intensity (CH_4 g/kg MY) were decreased ($P \leq 0.05$) by RM compared with control 36%, 23%, and 33%, respectively. Conversely, daily hydrogen (H_2) emission was increased ($P = 0.002$) 2.75-fold by RM. If the negative impact on DMI can be managed, data suggest that RM can be an effective enteric CH_4 mitigation tool, at least during short-term supplementation. Long-term efficacy and impacts on animal health and performance need to be investigated. Further, the compound(s) within RM responsible for the CH_4 mitigation effect need to be identified and their modality of inhibition explained.

Key Words: macroalga, enteric methane, animal production

1347 Effects of concentrate allocation strategy on lactational performance and enteric methane emission in dairy cows. L. F. Martins*, S. F. Cueva, D. E. Wasson, C. V. Almeida, A. T. Richards, N. Stepanchenko, and A. N. Hristov, *The Pennsylvania State University, University Park, PA.*

The objective was to evaluate performance and CH_4 emission in dairy cows fed forages as a partial mixed ration (PMR) and concentrate premixes (CMix) delivered using an automatic feeding system (AFS). Forty-eight Holstein cows (30 primi- and 18 multiparous) averaging 91 ± 25 DIM, 25.4 ± 4.5 kg/d dry matter intake (DMI), and 43 ± 10 kg/d energy-corrected milk (ECM) yield, were enrolled in a randomized complete block design experiment. Cows were blocked based on parity, DIM, and ECM and, within blocks, were randomly assigned to 1 of 3 treatments: (1) basal diet fed as ad libitum (Ad) total mixed ration (TMR); (2) basal diet fed as separate feeds (SF) with Ad PMR and fixed amounts of CMix using AFS (SF^{Ad}); and (3) SF with Ad PMR and amounts of CMix calculated by an algorithm (Alg) and fed using AFS (SF^{Alg}). Concentrates used in TMR and SF^{Ad} were combined into 2 different CMix to be offered in the AFS as energy (13% CP and 45% starch) and protein (30% CP and 23% starch) at a ratio of 1:2.4, respectively. The CMix allowance for SF^{Alg} was calculated for individual cows based on predicted NE_L and MP balances, NE_L and MP allowable MY, and microbial protein synthesis (NASEM, 2021). Methane emission was measured using the GreenFeed system. Data were analyzed using PROC MIXED of SAS as repeated measures with the fixed effects of treatment, time, treatment × time, and a covariate measurement. Block and block × treatment were random effects. The PMR intake and overall DMI were increased ($P \leq 0.06$) by SF^{Ad} andSF^{Alg}, compared with TMR, whereas CMix intake was not affected by treatment. The ECM yield was decreased ($P \leq 0.10$) by 2.1 and 1.8 ± 0.73 kg/d with SF^{Alg}, compared with TMR and SF^{Ad}, respectively. The ECM feed efficiency (ECMfe) was decreased ($P < 0.01$) by SF^{Ad} and SF^{Alg}, compared with TMR. The SF^{Alg} increased ($P \leq 0.05$) CH_4 intensity (g CH_4/kg ECM), compared with TMR. Overall, allocation of CMix in AFS decreased ECMfe and increased CH_4 intensity in lactating dairy cows. Higher forage-to-concentrate ratio and competition for CMix in AFS by SF cows may have influenced feeding behavior and compromised lactational performance of the cows.

Key Words: methane, milk production, precision feeding

1348 On-farm supplementation of rumen-protected niacin: A randomized clinical trial. K. C. Krogstad*[1,3], R. Vlietstra[2], J. R. Pursley[1], and B. J. Bradford[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, [2]West Michigan Veterinary Service, Coopersville, MI, [3]Department of Animal Science, The Ohio State University, Wooster, OH.*

B-vitamins are synthesized by rumen microbes, but supplementation of B-vitamins may be beneficial for dairy cattle. Rumen-protected niacin (RPN) reduces lipolysis and inflammation and may improve health during lactation. Our objective was to determine if postpartum RPN supplementation may decrease disease, increase fertility, or increase milk yield. We enrolled 1,074 cows onto a randomized complete block experiment. At calving, cows were assigned to control (standard pellet; CON; n = 593) or to RPN (26 g/d of RPN in addition to standard pellet; n = 481). Treatments were administered to cows through an automated milking system for the first 21 d of lactation. Health records, reproductive records, and daily milk yield were collected from herd management software. Blood was collected from a subset of cows at 3 DIM (n = 99) and 10 DIM (n = 97) to assess plasma niacin concentrations and biomarkers of metabolism. Culling, pregnancy, and mastitis were analyzed using Cox proportional hazard models. Continuous variables, like milk yield, were analyzed with linear mixed models that included fixed effects of treatment, parity, time, and their interactions. Supplementing RPN increased plasma nicotinamide concentration by 1,740 ± 410 n*M* (difference ± SED; $P < 0.01$; 50% increase). The RPN reduced plasma insulin concentrations in all parities ($P = 0.01$). Free fatty acid concentrations were increased for cows receiving RPN ($P = 0.05$), while β-hydroxybutyrate increased in third+ parity cows supplemented with RPN ($P < 0.01$). Rumen-protected niacin increased milk yield for first- and second-parity cows by wk 15 and wk 19 of lactation ($P \leq 0.05$), respectively. Increased milk yield in those groups was sustained for the rest of lactation resulting in 639 ± 319.8 kg and 712 ± 364.4 kg more milk for RPN-supplemented first- and second-parity cows ($P \leq 0.05$). The hazard of leaving the herd, hazard of mastitis, and hazard of pregnancy were not affected by RPN ($P \geq 0.15$). Optimizing RPN supplementation may improve milk production and metabolism in dairy cows. Furthermore, increased milk yield in mid and late lactation demonstrates carryover benefits of early lactation RPN supplementation.

Key Words: vitamin B3, mastitis, fertility

1349 Associations between body temperature and feed efficiency traits in Holstein cows. L. C. Novo*[1,2], L. Cavani[1], F. S. Reyes[1], K. A. Weigel[1], and F. Peñagaricano[1], *[1]University of Wisconsin–Madison, Madison, WI, [2]Angus Genetics Inc., Saint Joseph, MO.*

Heat load and body temperature are influenced by several external and internal factors and directly impact dairy cow performance. The aim of this study was to investigate the associations between 3 body temperature traits, namely average body temperature, consistency of body temperature, and change in body temperature after the largest meal of the day, with 4 feed efficiency traits: DMI, metabolic body weight (mBW), secreted milk energy (MilkE), and residual feed intake (RFI) in lactating Holstein cows. Data were collected on 304 mid-lactation

Holstein cows enrolled in 11 different feed efficiency trials from 2020 to 2023 at the University of Wisconsin–Madison. Temperature records were obtained using an automatic temperature logger placed vaginally for 2 wk. Average body temperature was calculated as the individual mean during the trial, consistency of body temperature was calculated as the log-transformed variance of the deviations of individual records from the cow's mean, and change in body temperature was calculated as the difference in the temperature before and after the largest meal of the day. Data for DMI, mBW, MilkE, and RFI were collected from the same cows in the same feeding trials for 6 to 7 wk. The associations between body temperature traits and feed efficiency traits were assessed using univariate linear regression models including body temperature and cohort (trial-treatment) as independent variables. Average body temperature was positively associated ($P < 0.05$) with RFI, suggesting that higher body temperature is related to lower feed efficiency. In addition, consistency of body temperature was positively associated ($P < 0.05$) with MilkE, indicating that a greater variance in body temperature is linked to more MilkE. Finally, change in body temperature after the largest meal of the day was positively associated ($P < 0.01$) with both DMI and MilkE, suggesting that greater changes in body temperature after the largest meal is linked to greater DMI and MilkE. Overall, our results suggest that body temperature can be used as an indicator of feed efficiency measures in lactating Holstein cows.

Key Words: milk energy, residual feed intake, thermoregulation

1653 Effect of choline supplementation on milk production and milk lipids in post peak lactating Holstein cows.

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Choline supplementation is known to modify hepatic lipid metabolism in the dairy cow, but little is known about its effect on mammary lipid metabolism. The objective of this study was to characterize the effect of increasing doses of abomasally infused choline on milk production and milk lipids using untargeted lipomics. A total of 12 ruminally cannulated Holstein cows were used in a 4 × 4 Latin square design with 7-d experimental and 7-d rest periods. All cows were fed a common TMR diet formulated to have a low risk for milk fat depression. Treatments were continuous abomasal infusions of 0, 18, 36, or 54 g/d of choline. Infusion line patency and placements were checked daily. A 73% aqueous choline chloride stock solution was used as a source of choline and was infused in 5 L of water. Milk production was measured at the end of each period and milk samples were taken for analysis of milk component by FTIR and lipidomics analyzed using Thermo Orbitrap Exploris 120. Production data were analyzed with a mixed model that included the fixed effect of treatment and random effect of cow and period. Statistical analysis also included preplanned linear and quadratic contrasts. Lipidomics data were aligned, and reference matched with MS-Dial and analyzed using R-Studio. There was an effect of treatment ($P = 0.04$) on milk yield with a tendency for a quadratic contrast ($P = 0.10$) with maximum milk yield of 39.9 kg/d with 36 g/d of choline, compared with 37.3 kg/d for the control. There was no effect of treatment on milk fat or protein yield ($P \geq 0.13$). There was no visually apparent clustering by treatment when all the lipidomic data were visualized with a principal component analysis. Furthermore, there was no modification of the concentration of any lipid species at any choline dose compared with the control [Log_{10} (P-value) < 1.3 and −1 > Log_2 (Fold Change) < 1]. Overall, choline supplementation quadratically increased milk yield, but had no effect on milk component yield. The additional choline supply did not appear to influence mammary lipid metabolism, as there were no changes in the concentration of milk complex lipids including phospholipids.

Key Words: lipids, choline, phospholipids

1351 Effects of feeding a *Bacillus licheniformis* fermentation extract with or without monensin on milk production and feed efficiency of lactating dairy cows.

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Study objectives were to investigate the individual and interactive effects of monensin (Rumensin) and a *Bacillus licheniformis* fermentation extract (BLFE) on milk production, feed efficiency, ruminal volatile fatty acid (VFA) profile, and total-tract nutrient digestibility (TTD) of lactating dairy cows. Sixty Holstein cows (40 multiparous + 20 primiparous) at 108 ± 35 DIM were matched for parity and DIM and assigned to treatments in a 2 × 2 factorial arrangement (Rumensin: 0 and 14 g/t of DM and BLFE: 0 and 80 g/t of DM). After a 14 d baseline measurement period, cows were allowed 21 d to adapt to their respective dietary treatments, which were top-dressed daily. Daily DMI and milk yield (MY), weekly milk composition, and body weight were collected over the next 42 d during which cows continued to receive their respective treatments. The molar percentages of VFA in rumen liquid from a stomach tube (VFAp) and TTD with indigestible fecal NDF were determined in the last week. Individual and interactive treatment effects were analyzed by using the MIXED procedure of SAS with the REPEATED option. BLFE increased milk protein by 0.11 percentage units ($P = 0.01$) and tended to decrease DMI by 0.65 kg/d ($P = 0.07$) regardless of Rumensin. When fed alone, BLFE increased dietary net energy of lactation (NASEM, 2021) by 14% ($P = 0.04$), and tended to increase MY by 1.3 kg/d ($P = 0.06$) and milk protein efficiency (milk protein yield: crude protein intake) by 15% ($P = 0.054$). Those effects disappeared when BLFE and Rumensin were fed together. Rumensin increased DMI by 0.70 kg/d, milk fat yield by 0.08 kg/d, and fat-corrected MY by 2.08 kg/d ($P < 0.05$) independent of BLFE. Feeding BLFE alone or with Rumensin did not affect VFAp and TTD ($P > 0.15$). In summary, BLFE and Rumensin enhanced milk protein percentage and milk fat yield, respectively, independent of each other. BLFE seemed to improve dietary energy and protein utilization efficiencies of lactating dairy cows when fed in diets devoid of Rumensin.

Key Words: *Bacillus licheniformis*, feed efficiency, monensin

1352 Lactation performance of dairy cows in response to supplementing rumen-protected methionine and lysine, niacin, or both during the transition period.

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The objective of this study was to evaluate the effects of feeding rumen-protected lysine (RPL), rumen-protected methionine (RPM), and

rumen-protected niacin (RPN) separately or jointly on the performance and blood β-hydroxybutyrate (BHB) concentrations of transition cows. Two hundred multiparous Holstein cows were selected around 4 weeks before expected calving based on parity, days of pregnancy, body condition score (BCS), and previous milk yield; and randomly allocated to replicated pens (8 pens; 25 cows in each). The 8 pens were randomly assigned to 1 of 4 treatments arranged in a 2 × 2 factorial manner. The 4 treatments were (1) prepartum and postpartum basal diets (1.57 Mcal/kg DM and 1.81 Mcal/kg DM, respectively; CON); (2) CON diet supplemented with 0.61% RPL and 0.19% RPM prepartum and 0.31% RPL and 0.13% RPM postpartum DM (RPAA); (3) CON diet supplemented with 0.28% RPN prepartum and 0.19% RPN postpartum DM (RPN); and (4) CON diet supplemented with 0.61% RPL, 0.19% RPM, and 0.28% RPN prepartum and 0.31% RPL, 0.13% RPM, and 0.19% RPN postpartum DM (RPAA-N). Treatment diets were supplied from d 28 before expected calving until 28 DIM. Pen DMI and milk yield were measured daily. Milk samples were collected on d 7, 14, 21, and 28 DIM. Blood samples were collected on −28, −21, −14, and −7, 0, 7, 14, and 28 d relative to calving. Data were analyzed as a complete randomized design with repeated measures. Pre- and postpartum data were analyzed separately by ANOVA using PROC MIXED of SAS v9.4. Prepartum DMI did not differ among treatments; however, compared with CON, postpartum DMI was decreased for RPN and RPAA-N cows (18.20 and 18.58 vs. 20.15 kg/d; $P = 0.01$). Milk fat concentration (4.55% vs. 3.72, 4.30, and 4.14%; $P = 0.003$), yield (1.82 vs. 1.42, 1.80, and 1.68 kg/d; $P = 0.04$), and milk protein concentration (3.81% vs. 3.33, 3.57, and 3.59%; $P = 0.04$) were greater for RPAA-N cows compared with CON and other supplemented cows. In conclusion, RPAA-N supplementation improved milk protein content and milk fat yield and concentration.

Key Words: rumen-protected amino acid, rumen-protected B vitamin, transition cow

1353 Effects of dietary rumen-protected, ruminal-infused, or abomasal-infused choline chloride on milk, urine, and fecal choline and choline metabolite yields in lactating cows. M. You*[1], Y. Zang[1], A. T. Richards[1], P. Uzen[1,2], T. L. France[1], Y. Wei[1], N. D. Senevirathne[1], O. Malysheva[3], and J. W. McFadden[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Food Processing Department, Isparta University of Applied Sciences, Isparta, Turkey, [3]Division of Nutritional Sciences, Cornell University, Ithaca, NY.*

Current approaches to study choline bioavailability do not account for the ruminal or postruminal bacterial degradation of choline to trimethylamine (TMA) and TMA *N*-oxide (TMAO). To evaluate choline bioavailability, 8 multiparous Holstein cows (184 ± 109 d in milk) were enrolled in a study with a 4 × 4 Latin square design. Treatments were (1) 0 g/d of supplemental choline chloride (CTR), (2) 16.8 g/d of unprotected choline chloride (CC) delivered as a continuous ruminal infusion (12.5 g choline ion/d; UPC), (3) 27.9 g/d of rumen-protected CC (12.5 g choline ion/d; RPC), or (4) 16.8 g/d of unprotected CC delivered as a continuous abomasal infusion (ADC). Cows were fed every 4 h and milked twice per day. Choline and choline metabolites were analyzed using liquid chromatography and mass spectrometry. Each cow was administered 4.08 L of infusate daily for 5 consecutive days in each period, followed by a 2-d washout period. The statistical model included the fixed effects of treatment, square, period, treatment × square, and the random effect of cow. Mean comparisons of UPC, RPC, and ADC vs. CTR were conducted using the Dunnett test. Milk choline and betaine concentrations and yields (4% and 5% of infused choline for ADC), and plasma choline and betaine concentrations, were greatest for ADC (trt, $P < 0.01$). Urinary TMAO concentrations were greater for ADC vs. CTR (~4% of infused choline for ADC; $P = 0.03$). Urinary choline and betaine concentrations and yields were greatest for ADC (~3% and 9% of infused choline for ADC, respectively; trt, $P < 0.01$). Fecal TMA concentration and yields were highest for ADC ($P < 0.01$ and $P = 0.07$, respectively), while fecal betaine concentrations and yields were also greatest for ADC ($P = 0.07$ and $P = 0.03$, respectively), whereas fecal choline concentrations and yields were highest for RPC ($P = 0.07$ and $P = 0.02$, respectively). Our findings emphasize the importance of measuring choline and choline metabolite concentrations and yields when evaluating choline bioavailability.

Key Words: bioavailability, choline, trimethylamine N-oxide (TMAO)

1354 Effects of alternative protein sources in dairy cow diets on ruminal fermentation and nutrient utilization in a dual-flow continuous culture system. C. A. Hammond*, M. L. Johnson, E. Sarmikasoglou, M. U. Siregar, G. K. Salas-Solis, A. C. S. Vicente, K. S. Alves, R. R. Lobo, S. W. Ma, and A. P. Faciola, *University of Florida, Gainesville, FL.*

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Soybean meal (SBM), a staple in dairy diets, has highly degradable protein that limits N utilization. Alternative protein sources with lower protein degradability may improve N utilization. This study's objective was to evaluate the effects of partially replacing SBM with alternative protein sources on ruminal fermentation, nutrient utilization, microbial protein synthesis, and nitrogen utilization in dairy diets using a dual-flow continuous culture system. The experimental design was a replicated 4 × 4 Latin square with 8 fermenters. Treatments were control (CON, 18% SBM, in a DM basis), and approximately 50% replacement of SBM with feather meal (FM), blood meal (BM), or spray-dried blood cells (SDBC). Fermenters were provided with 106 g/d of diet DM divided into 2 meals per d at 0800 and 1800 h. Treatments were randomly assigned to fermenters within Latin square for each period. Four 10-d periods were conducted, and the last 3 d of each period were used for collection of samples for analyses of ruminal fermentation. Experimental samples were collected at 0, 1, 2, 4, 6 and 8 h after the morning feeding. Data were analyzed using the MIXED procedure of SAS and significance was declared when $P \leq 0.05$. Treatment and time effects were observed for pH and NH_3-N kinetics along with a treatment-by-time interaction for NH_3-N kinetics. Compared with CON (16.2 mg/dL), both FM (10.2 mg/dL) and BM (11.2 mg/dL) had lower NH_3-N pool concentrations, indicating lower microbial fermentation of these proteins. The SDBC NH_3-N concentration (15.2 mg/dL) was not different than CON. In addition, a treatment effect was observed for NDF degradability, where CON had the lowest fiber degradability among all treatments. Our results indicate that FM and BM have reduced ruminal protein degradation, and fiber degradability is improved when approximately 50% of the SBM is replaced by these alternative sources. Further analyses are needed to fully determine the impact of these alternative protein sources on nutrient digestibility, volatile fatty acid profile, and overall ruminal nitrogen metabolism.

Key Words: blood meal, feather meal, spray-dried blood cell

1355 Feeding high-byproduct diets to lactating cows as a strategy to improve net food production. M. Mills*, S. Naughton, E. Sarmikasoglou, and M. VandeHaar, *Michigan State University, East Lansing, MI.*

Dairy cattle produce methane but can also convert human-inedible byproducts, or leftovers, into nutritious food for humans. Diets higher in byproducts likely exacerbate the trade-off of environmental concerns versus food production. Our goal was to evaluate the effects of feeding a high-byproduct diet on production performance, methane emissions, and net food production. Multiparous Holstein cows (n = 31; 90 ± 23 DIM, 51 ± 8 kg milk/d, 730 ± 60 kg BW) were individually fed 1 of 2 diets in a crossover design. Diets were composed of 20% byproducts (CON) or 70% byproducts (BYP). BYP diet consisted of 25% corn silage, 18% corn gluten feed, 15% bakery waste, 10% whole cottonseed, 10% wet beet pulp, 8% wheat straw, and 13% supplements. The CON diet contained 36% corn silage, 13% haylage, and 25% corn grain. Cows were fed a 50:50 mix of both diets for 1 wk, then CON or BYP for 4 wk, mix for 1 wk, and then opposite diet for 4 wk. Data were analyzed using PROC GLIMMIX of SAS 9.4 with treatment, treatment sequence, and period as fixed effects and cow as random. Cows fed BYP consumed less DM (30.1 vs. 31.1 kg, $P = 0.02$) and produced less milk (46.9 vs. 49.1 kg, $P < 0.01$) with more fat (3.68% vs. 3.55%, $P < 0.01$), less protein (3.07% vs. 3.09%, $P = 0.01$), and less lactose (4.91% vs. 4.95%, $P < 0.01$) than cows fed CON. There was no change in BW or BCS. Corn grain, soybean meal, the grain portion of corn silage, and the leaves of alfalfa haylage were considered human edible. When evaluating efficiencies, cows fed BYP were considerably more efficient in converting human-edible feed energy into milk energy using Atwater energy values (171% vs. 54%, $P < 0.01$). The BYP cows were also more efficient in converting human-edible feed protein into milk protein (202% vs. 72%, $P < 0.01$). Preliminary data with GreenFeed suggest no difference in methane production. Adding more byproducts in dairy cattle diets presents tradeoffs for sustainability. Feeding more byproducts may decrease production and thus increase the number of cows needed and methane emissions; however, more byproducts result in less land needed to grow crops and a net increase in human-edible food.

Key Words: byproduct, sustainability, feed efficiency

Ruminant Nutrition Symposium: Functional Nutrients

1356 Fatty acids as bioactive nutrients in lactating dairy cows. A. L. Lock* and G. A. Contreras, *Michigan State University, East Lansing, MI.*

Our understanding of fatty acid (FA) digestion and metabolism in dairy cows has advanced significantly in the last few decades. We increasingly recognize that FA, both of dietary and rumen origin, can have different and specific effects on feed intake, rumen metabolism, small intestine digestibility, milk component synthesis in the mammary gland, energy partitioning between the mammary gland and other tissues, and inflammation. We will present recent research focusing on specific FA and how dairy cows respond differently to combinations of FA. Palmitic (C16:0), stearic (C18:0), and oleic (*cis*-9 C18:1) acids usually comprise the majority of FA present in milk fat and adipose tissue of dairy cows. While these FA have different functions in metabolism, they may also interact with each other by competitive or complementary mechanisms under different physiological conditions. In the mammary gland, lipid synthesis is dependent upon the simultaneous supply of short/medium-chain FA and long-chain FA. C16:0 has a higher preference as a substrate to start triglyceride synthesis than C18:0 or *cis*-9 C18:1. Also, if the amount of preformed FA surpasses the capacity of the mammary gland, these might be redirected to other tissues (e.g. adipose tissue) altering energy partitioning. In periparturient cows, *cis*-9 C18:1 limits lipolysis, improves adipose tissue insulin sensitivity, and may enhance mitochondrial biogenesis and function, which will minimize body weight loss and maximize lactation performance. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most bioactive FA in the omega-3 family, with known anti-inflammatory effects. Recent data demonstrate that DHA supplementation can increase the concentrations of DHA-derived anti-inflammatory and pro-resolving oxylipins with the potential to limit inflammatory responses driven by metabolic challenges. Research will undoubtedly continue to improve our understanding of the bioactive roles of FA rather than FA simply being considered as fuels and building blocks. The challenge and opportunity will be to effectively apply this knowledge in the feeding and management of today's high-producing dairy cows.

Key Words: milk fat, nutrient partitioning, adipose tissue

1357 The impact of fatty acids as bioactive nutrients on the development of offspring. A. Relling*, *The Ohio State University, Wooster, OH.*

Fatty acids (FA) are normally considered a source of energy; however, they are essential nutrients that can bind to membrane and nuclear receptors, modifying cell function and the expression of some transcripts. These effects will depend on the FA type and family FA (i.e., n-6 vs. n-3). The FA effect on developmental programs has been studied in cattle and sheep with some similitudes in the outcomes. Feeding n-3 FA during late gestation almost always improves offspring production performance (i.e., milk yield in dairy cows, growth in beef and sheep) compared with the offspring of dams supplemented with mono- and unsaturated FA or with offspring of dams with no FA supplementation. Opposite results were observed when n-3 FA were supplemented in late gestation in summer. Also, there is a sexual dimorphism in the outcomes of n-3 FA supplementation, where males have greater growth than females. Offspring born from an n-3 FA supplemented dam in early gestation had slower growth than the ones born from monounsaturated FA supplementation. No differences were observed when the FA supplementation was conducted in mid-gestation. There are multiple assumptions on how this physiological process occurs. The first unanswered question is if it is a direct effect of the FA on the fetus, if it is an effect of the FA manipulating nutrients' placenta passage, or both. The second unanswered question is which tissue is modified during developmental programming responsible for the changes in offsprings' growth. Research has demonstrated that the developmental effect is not due to changes in hypothalamic regulations of dry matter intake and energy expenditure or liver and adipose tissue functions. The changes in offspring growth can be attributed to changes in the gastrointestinal tract and the immune response. Feeding n-3 FA in late gestation to the pregnant dam increases the amino acid transporter expression (mRNA and protein) in the duodenum, changes associated with changes in DNA methylation. Regarding the immune function, the increase in offspring performance has been associated with decreased haptoglobin after weaning in calves or increases in Resolvin-D3 after lambing.

Key Words: DNA, n-3, programming

1358 Bioactive nutrients' role in gut development. A. H. Laarman*, *University of Alberta, Edmonton, AB, Canada.*

Ruminants are born without a functional rumen, thus requiring extensive gut development before weaning. In the dairy sector, especially, the severely shortened timeframe of calf rearing (i.e., weeks, instead of months) means enhancing gut development is a premium value add. A plethora of bioactive nutrients, primarily lipids (e.g., butyrate), carbohydrates (e.g., oligosaccharides), and protein (e.g., essential amino acids), have shown varied results, especially as calves age. Recently, efficacy of bioactive nutrients is determined to be dependent on the gut lumen environment in the calf. In early life, as calves develop from nonruminants to preruminants to ruminants, the rumen, microbiome, and immune system all undergo substantial development as well. Consequently, the gut luminal environment varies greatly from birth, preweaning, and postweaning, with resulting impacts on efficacy of bioactive nutrients. In early life, calves experiencing stressors are far more benefited by bioactive nutrients than are calves in an optimal, low-stress environment. As the early life stressors vary from health, management, and nutrition factors, so, too, do the bioactive nutrients best suited to boost gut development in the face of each of these stressors. An in-depth understanding of the optimal luminal environment for gut development is essential in optimizing the use of bioactive nutrients in young calves.

Key Words: bioactive nutrient, luminal environment, stress

1359 A case to be made for choline as a required nutrient for transition dairy cows. J. E. P. Santos*, U. Arshad, M. G. Zenobi, and J. M. Bollatti, *University of Florida, Gainesville, FL.*

The discovery of choline dates back to 1850s when French pharmacist, Théodore Gobley discovered a group of phospholipids that he named lecithin. Seventy years later, during the discovery of insulin by Frederick Banting, his student Charles Best noticed that depancreatized dogs developed hepatic lipidosis. Subsequently in the early 1930s, Best showed that supplementing lecithin reversed and prevented hepatic lipidosis in dogs and rats. It took another 67 years for dietary choline to be acknowledged as essential in human diets. Requirements have been established for several farm animal species; however, the specific amounts of dietary

choline to be fed to dairy cows have not been established. The development of rumen-protected choline (RPC) products has allowed extensive research to investigate the effects on dairy cows, in particular during the transition period. Dairy cows during transition accumulate lipid droplets into the hepatic tissue, which results in approximately 40% to 50% of them having hepatic triacylglycerol >5% in the first 2 to 3 weeks postpartum. Cows with hepatic lipidosis have increased risk of peripartum diseases. Experimental models using feed-restricted late-pregnant cows have unequivocally shown that supplementing RPC reduces the content of triacylglycerol in the liver. The mechanisms that support reduced hepatic lipidosis by RPC in feed-restricted cows are not completely understood, but involve enhanced export of triacylglycerol as nascent lipoproteins. Other mechanisms that facilitate the disposal of fatty acids from hepatocytes have been proposed and include increased cellular autophagy and lipophagy, and reduced endoplasmic reticulum stress response and hepatocyte inflammation. The effects of RPC on hepatic lipidosis are less clear in early postpartum cows, but extensive research shows an increase in production performance with some indication of benefits to peripartum health. Interestingly, the production response to supplementing RPC during the transition period extends well beyond the period of supplementation, thus suggesting effects on mammary cell metabolism that requires further investigation.

Key Words: choline, dairy cow, required nutrient

ADSA Interdisciplinary Symposium: Application of Artificial Intelligence to Dairy Systems

1360 Advancing precision dairy farming: Challenges and opportunities in data integration and methodological frameworks. M. Hostens*[1,2], [1]*Cornell University, Ithaca, NY,* [2]*Ghent University, Gent, Belgium.*

Precision dairy farming (PDF) encompasses advanced technologies for automatic, real-time monitoring of cow welfare, health, environmental impact, and production, prioritized globally to meet agricultural policy goals. Precision dairy farming facilitates the transition toward resilient, sustainable dairy farming, widely adopted in the industry. Technologies like milk robots, heat detection sensors, and new sensor technologies gather production and health data, while automated weight scales and imaging technologies are implemented. Real-time emission monitoring links farm management to emission rates, and wireless sensors and drones aid precision agriculture for crops and the environment. Integrating these data remains a challenge, yet offers numerous opportunities, including precision phenotyping, digital twin creation for simulation modeling, and genomic prediction of precision phenotypes. However, challenges such as data integration difficulties, data ownership, privacy, and security issues hinder full exploitation of dairy data by artificial intelligence. There is also a lack of a clear methodology in dairy science to analyze PDF data, with existing approaches scattered across research organizations. Considering the importance of sustainable food production and the scattered organizational data landscape, research should focus on developing methodological frameworks for heterogeneous dairy data. These frameworks aim to enable access to valuable agricultural data across different locations, addressing data heterogeneity through definitions and ontology mapping. These frameworks will encourage data reuse, stimulate the use of real-time integrated PDF data, and facilitate evidence-based decision-making in dairy farming.

Key Words: artificial intelligence, data-driven dairy, precision dairy

1361 Opportunities of artificial intelligence (AI) in enhancing quality control of the dairy food supply chain. C. Qian*, *Cornell University, Ithaca, NY.*

The ever-changing landscape of artificial intelligence (AI), particularly in computer vision and natural language processing, has lowered the barrier for developing and using AI tools. With increasingly transformative and accessible AI toolkits, more opportunities began to emerge throughout the dairy food supply chain to control the quality of dairy products more stringently and reliably, and therefore prevent food waste and loss. This presentation will cover 3 main areas where AI can benefit the quality control of dairy food supply chain, which are (1) diagnosis of critical factors and practices for decision-making in control strategies, (2) real-time prediction and monitoring of microbial contamination in the dairy product or dairy production environment, and (3) optimization of existing risk assessment tools with more data supply. Currently, we are at a phase where some of these technologies are developed for specific tasks in quality control, and yet, digitalizing the entire dairy food system would require much larger-scale effort and design thinking into investigating the connectivity between various tools at different stages of supply chain as well as addressing issues such as data privacy when sharing sensitive data across stakeholders or platforms. An ideal system will be envisioned and proposed to conclude this presentation, with highlights on currently available tools and emergent areas for future research needs.

Key Words: digital dairy, computer modeling, machine learning

1362 Generative artificial intelligence tools in journal article preparation: A preliminary catalog of ethical considerations, opportunities, and pitfalls. R. R. White*, *Virginia Tech, Blacksburg, VA.*

Public launch of large language models (LLM) such as ChatGPT has catalyzed considerable discourse about the role of generative artificial intelligence in scientific publishing. The goal of this work is to review viewpoints on LLM use in scientific writing and to explore strengths and weaknesses of contemporary LLM for journal article preparation. Scientific articles hold a unique place in humanity's knowledge structure as a primary information source. Primary information sources are trusted above secondary and tertiary information sources due to quality control practices to minimize biased, inaccurate, or misleading information. As such, protecting the fidelity of journal articles as a primary information source is critical for continuity of humanity's reliance on scientific findings as an underpinning for truth or reality. Most LLM are trained on a diversity of human-generated material, much of which is classified as secondary or tertiary information. Thus, use of existing LLM for unsupervised generation of scientific articles is unlikely to yield quality results, though evolution of LLM derived specifically for this purpose is likely inevitable. Careful use of current LLM in journal article preparation can serve as an initial exploration of this long-term possibility. Laboratory-specific procedures like careful prompt engineering can help support coupled human-LLM strategies wherein human attention is devoted primarily to the scientific content, while the LLM can support clarity, consistency, and completeness of language or presentation. Although debate exists surrounding the ethics of LLM use, the responsibility for the content of scientific articles, their interpretability, and accuracy lies with the authors. In complement to this responsibility, the peer-review process is intended to ensure quality control before information is broadly released. Maintenance of human insight and standards within the publication process is essential to maintain high ethical standards, though LLM can help alleviate communication challenges, allowing humans to focus more attention and energy to the science.

Key Words: artificial intelligence (AI), large language model (LLM), journal article

1363 Smart cows, smart farms: Unleashing the potential of artificial intelligence in the dairy sector. J. M. Bewley*, *Holstein Association USA Inc., Brattleboro, VT.*

Artificial intelligence, along with buzzwords like machine learning, deep learning, big data, is being applied in the dairy industry in multiple forms. This term generally refers to the use of computing technology to mimic and automate complex tasks normally accomplished by the human brain. These techniques are being applied within the dairy industry to process time-series data from wearable, vision-based, and in-line milk technologies. Results are often incorporated into decision

support systems to assist dairy producers make individual animal or group-based decisions. Artificial intelligence can be applied to process machine data (i.e., images or raw accelerometer data) into meaningful biometrics (i.e., objective scores or rumination time). Or, it can be applied to using biometric data to make specific intervention recommendations. Compared with traditional statistical techniques, artificial intelligence often employs more “black box” strategies without a statistical equation to help interpret how results were derived. Unfortunately, this provides an opportunity for overmarketing how artificial intelligence is being employed within an on-farm system. Further, for machine vision systems to work properly and continue to improve, new biological ground truth must be provided to the system. Ethical concerns exist centered around how much power machines should have in working with animals. Despite these concerns, what artificial intelligence is accomplishing on farms today already is groundbreaking and exciting. As dairy farms continue to grow and artificial intelligence techniques continue to advance in all industries, the impact of artificial intelligence on dairy farms will continue to grow.

Key Words: artificial intelligence, machine learning, technology

Animal Behavior and Well-Being 1

1364 Use of a calving blind that simulates natural features.

H. Olsen, K. Vogel, and K. Creutzinger*, *University of Wisconsin–River Falls, River Falls, WI.*

Dairy cows socially isolate themselves at calving. Many studies have investigated the use of solid blinds for isolation, but their designs do not reflect the elements of natural cover. Calving blind design may be important to cows' motivation to use them. The objectives of this study were to determine if cows preferred calving in a visibly separated (blind) or open area in group maternity pens, and if there was a preference for amount of visibility through a blind. Holstein cows and heifers (n = 79) were enrolled into a group calving pen 21 ± 3 d before calving and remained there until after calving. The study pen contained 2 side by side calving blinds (4.6 m width × 3.05 m depth × 3.05 m height) at the back of the pen. Cows could enter the blinds through the front, which was covered by hanging firehose, and the other 3 sides were solid. The degree of visibility through the blind was created from hoses hung from the top of the frame that lined the entire front of the blind (100%) or every other hose rolled up (50%). Cows had continuous access to both blinds. For cows who met the inclusion criteria (n = 65), video was used to determine calving location and the location of cows in the pen at each calving (open area, 50% blind, 100% blind). There was not evidence to support a difference in the number of cows that calved in or outside of a blind (28 vs. 37 calvings, respectively; $P = 0.26$). The odds of calving in a blind were greater during the day (OR: 3.4, 95% CI 1.10–10.50, $P = 0.03$) and as the number of cows in the pen increased (slope = 1.38, intercept = 0.18, $P = 0.01$). For cows that calved in a blind, there was no preference for calving in the 50% or 100% blind (10 vs. 18 calvings, respectively; $P = 0.13$). Environmental features likely important to cows' motivation to find a desirable place to calve. Further, providing a blind can improve welfare by allowing cows the opportunity to perform natural behaviors and choice over their environment.

Key Words: maternal behavior, parturition, enrichment

1365 Effect of cow personality and training method on adaptation of lactating primiparous cows to an automated milking system.

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The study objectives were to determine how cow personality affects their adaptation to an automated milking system (AMS) and whether certain personality types adapt better based on AMS training type. Primiparous Holstein cows (n = 48), with no previous AMS exposure, were enrolled at 85 ± 12.3 DIM (mean ± SD) and assessed for personality traits using a combined arena test including a novel arena, object, and human test. Cows were categorized based on latency to contact the novel object and human, and then randomly assigned within category to 1 of 4 AMS training treatments (n = 12/treatment): control (CON), AMS environment exposure only (ENV), environment and concentrate provision (PEL), and environment and AMS mechanics exposure (MEC). Cows in the ENV, PEL, and MEC treatments were subjected to training 4×/d for 4 d. The CON cows remained in their pen with no access to the AMS until first milking. All cows were milked in the AMS on the evening of training d 4. Milking activity was recorded for 14 d. Behavioral observations from personality testing were analyzed using principal component analysis (PCA) to determine personality traits and associated cow PCA factor scores for the identified traits. The PCA revealed 2 factors interpreted as personality traits: explore-active and bold. Factor scores ranged from −2.4 to +1.5 for explore-active and −1 to 2.6 for bold. The effect of personality trait (factor score) and treatment on AMS data were analyzed using mixed-effect linear regression models. The bold trait was not associated with any outcome. Across cows, more explore-active cows had greater milk yield (milk yield [kg/d] = 1.71 × explore-active score + 32.9; $P < 0.01$) compared with less explore-active cows. There were treatment × factor score interactions ($P \leq 0.08$) for milking outcomes, whereby ENV cows that scored low for explore-active had more visits ($P = 0.02$), rejections ($P < 0.01$), and voluntary milkings ($P < 0.01$) compared with CON cows that scored low for explore-active. To conclude, cow personality was associated with performance and adaptability of cows to the AMS and may influence cow performance differently depending on training.

Key Words: training, trait, robotic milking

1366 Associations of pre-milking waiting time with milking behavior and performance of cows in an automated batch milking system.

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Our objective was to examine associations between pre-milking waiting time (WT) and milking behavior and performance during the following visit to a milking robot in cows managed in a semi-voluntary batch milking system. Data considered 158,106 milking events in 2,209 cows from December 2023 to January 2024 in an organic grass-fed dairy in Texas. Cows were moved twice daily to the milking barn where they could select their milking visits among 22 AMS units (DeLaval, Sweden). Visit information was collected to calculate pre-milking WT, defined as the time elapsed between the entrance of the group to the holding pen and the entrance of each cow to the robot milking box. The WT were categorized into quartiles (Q1 <34 min; Q2 = 34 to 65 min; Q3 = 66 to 110 min; and Q4 >110 min). In addition, an extreme WT category was created considering the 90th percentile (P_{90} > 157 min). Data were examined using logistic regression and ANOVA. Results describing milking event data and performance by quartiles are presented in Table 1. The odds (95% CI) of incompletes and kick-offs in P_{90} cows were 1.16 (1.08–1.25) and 1.21 (1.28–1.31) times greater than the odds of incompletes and kick-off in Q1 cows, respectively. Greater WT was associated with lower milk yield on the next milking ($P < 0.0001$) and greater Mastitis DeLaval Index (MDi; $P < 0.0001$). The WT was associated with most of the milking variables in analysis and it should be considered when determining group sizes and cow movements during the milking.

Key Words: robot, waiting, time

1367 Modeling the effect of heat stress exposure on daily rumination time and milk yield in multiparous lactating cows.

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Table 1 (Abstr. 1366). Milking event data and performance presented as frequencies and averages by WT category

	WT category				
Item	Q1	Q2	Q3	Q4	P_{90}
Incompletes (%)	5.54^{b}	4.38^{a}	5.48^{b}	5.72^{b}	6.37^{c}
Kick-off milkings (%)	5.90^{b}	5.21^{a}	6.06^{c}	6.45^{d}	7.08^{e}
MDi	1.22^{a}	1.25^{b}	1.27^{c}	1.29^{d}	1.30^{e}
Milk duration (s)	363^{d}	348^{a}	355^{c}	352^{b}	350^{ab}
Milk flow duration (s)	265^{d}	254^{a}	260^{c}	256^{b}	254^{ab}
Mean flow (kg/min)	0.99^{c}	0.96^{a}	0.97^{b}	0.97^{b}	0.97^{b}
Peak flow (kg/min)	1.39^{c}	1.36^{a}	1.38^{b}	1.38^{b}	1.38^{b}
Milk yield: current milking (kg)	9.63^{d}	9.09^{a}	9.34^{c}	9.19^{b}	9.05^{a}
Milk yield: next milking (kg)	9.88^{e}	9.08^{c}	9.32^{d}	9.00^{b}	8.75^{a}
Milk yield: difference next − current (kg)	0.26^{d}	−0.01^{c}	−0.02^{c}	−0.19^{b}	−0.30^{a}
Average DIM	160	177	164	170	171

$^{a–e}$Different superscripts within rows indicate $P < 0.05$.

Husbandry Development Co., Ltd., Beijing, China, [4]CRSAD, Québec, QC, Canada.

Based on milk production losses, the onset of heat stress (HS) in dairy cows typically starts at a temperature-humidity index (THI) level of 68. However, this benchmark does not consider the duration of exposure to a high THI before changes in behavior or production are observed. The study aimed to determine HS thresholds of daily rumination time (RT) and milk yield (MY) in lactating cows based on HS exposure per day for various production levels or lactation stages. The HS exposure was defined as the length of day (h/d) when THI was above 68 (range: 0–24 h/d). Climatic data were collected using in-barn automatic stations, and RT data using neck collar sensors. Daily RT and MY were from 546 multiparous Chinese Holstein cows (524 ± 78.4 min/d and 40.3 ± 10.32 kg/d [mean ± SD], respectively). Two datasets were used to compare production levels (LOW or HIGH for cows averaging <44.1 and >48.0 kg MY/d, respectively) and lactation stages (EARLY, PEAK, MID, or LATE at 1–40, 41–60, 61–160, and 161–305 d in milk, respectively). The effect of HS exposure on RT or MY was analyzed using a generalized additive mixed model. The pointwise slopes of the fitted means were calculated, and the HS exposure threshold before the onset of the decrease in RT or MY ($P < 0.05$) was determined. The RT decreased when HS exposure exceeded 9.39 h/d for LOW, but decreased immediately for HIGH (i.e., any HS exposure above 0.00 h/d) by 2.58 and 2.71 min/d per hour more of HS exposure, respectively. Only HIGH showed a decrease in MY (−0.115 kg/d per hour more of HS exposure above 0.00 h/d). As for lactation stage, EARLY, PEAK, MID, and LATE decreased RT when HS exposure exceeded 4.80, 3.91, 0.00, and 8.45 h/d, respectively (−3.35, −3.17, −2.36, and −2.73 min/d per h more of HS exposure). Only MID decreased MY (−0.129 kg/d per h more of HS exposure above 11.2 h/d). In conclusion, HS exposure per day affects how dairy cows cope with HS, with a decrease in RT requiring a shorter duration of HS exposure compared with a decrease in MY. Further, high-producing cows in mid-lactation seem to be most susceptible to HS.

Key Words: dairy cow, heat stress, temperature-humidity index

1368 Development of a video-based method for identifying and tracking Holstein cows in a freestall barn environment. B. Zandona*, M. Wang, S. Li, X. Ma, S. E. Räisänen, A. M. Serviento, Z. Zeng, Y. Zhang, X. Zhao, and M. Niu, *ETH Zurich, Department of Environmental Systems Science, Institute of Agricultural Sciences, Zürich, Switzerland.*

In recent years, there has been an increasing interest in the development of contactless video-based approaches for dairy farming. However, latest studies involving video-based tracking of animals struggled with erroneous identifications (IDs) of reappearing animals. The objective of this study was to develop a video-based method to identify and track Holstein cows, with a specific focus on correctly re-identifying individuals as they come back into the field of view. The presented method consists of an object detector first recognizing and localizing cows in the frame. The detections are then sent to a classifier that predicts the classification probabilities of IDs for each individual. The association between detections and individual IDs is performed by maximizing the total probability through Hungarian matching. The method was trained and tested on video data that were collected from a squared pen containing 13 Holstein cows. Two separate trainings were performed (i.e., for object detection and classification) using YOLOv8 as the baseline model. The training set for detection comprised 5,250 annotated frames, whereas the classifier was trained on 2,831 frames (average of 218 ± 44 images per cow). Evaluated separately, the object detector showed a mean average precision @ intersection over union = 0.5 of 0.900, whereas the classifier had a top-1 accuracy of 0.936. Further, to test the tracking capability of our entire method (i.e., its capability to follow the trajectories of the different cows present in the frame), our approach was tested on 6 short video clips (length: 30 s each) obtaining 0.938 in multiple object tracking accuracy, 0.947 in ID F1 score, and 0.946 in higher order tracking accuracy. The ID F1 score provides a measure of the association accuracy. In other words, it gauges how well the IDs of the cows are preserved over the course of the video sequence. Based on those results, the presented method showed potential for video-based identification and tracking of Holstein cows. Further investigations will focus on longer videos used for the evaluation of long-term tracking performance.

Key Words: dairy cow, computer vision, tracking

1369 Evaluating the impact of sample-based pregnancy test procedures on milk production and daily cow time budgets of dairy cows. S. Paudyal*[1], J. M. Piñeiro[2,1], D. Duhatschek[2,1], A. Pilati[2,1], B. Shrestha[1], R. Neupane[1], and E. Kim[1], *[1]Department of Animal Science, Texas A&M University, College Station, TX, [2]Texas A&M AgriLife Research and Extension Center, Amarillo, TX.*

The objective was to evaluate the effects of milk sample-based pregnancy test (SBPT) procedures implemented at a commercial dairy farm on cow performance and behavior. Three open dry-lot pens (250 cows/pen) in a dairy farm in North Texas were enrolled under repeated crossover design as treatment (TRT) and control groups (CON). The pens went through crossover for 3 replications yielding a total of 6 individual treatment periods (n = 18 pens-period; 9 TRT and 9 CON pens). The TRT groups were tested for pregnancy using IDEXX milk sample-based pregnancy at a milking parlor and CON were tested using transrectal ultrasound at headlocks by a trained veterinarian. A leg-based accelerometer recorded daily cow-level activity behavior on the rear leg of a subgroup of cows. The statistical analysis was performed using the MIXED procedure in SAS 9.3. Overall, CON groups were restrained in headlocks for an average of 1.7 h per day whereas the TRT group was restrained for 0.6 h on the day of pregnancy detection. The average parlor turnaround time in the TRT was 14 min compared with 12 min in the CON group. Daily milk production was higher in TRT by 1.6 (±0.46) lbs on the day after test ($P < 0.05$). The weekly total milk yield was also greater for the TRT group (51.47 ± 0.49 vs. 50.57 ± 0.48; $P < 0.05$). The sampling procedure did not affect milk production on the day of the test (50.69 ± 0.8 vs. 50.33 ± 0.7 lbs; $P = 0.47$). On the day of test, TRT had a higher daily step count (1,839 ± 129 vs. 1,562 ± 150, $P = 0.02$), whereas the CON group tended to have fewer lying bouts (7.7 ± 0.5 vs. 8.3 ± 0.5, $P < 0.1$). Lying bout duration was greater in the CON group (56.64 ± 2.71 vs. 50.35 ± 2.87, $P = 0.04$) minutes observed among the treatment groups. The daily total lying time of cows was not different, indicating that cows lay down a similar amount of time mostly due to compensatory effect on bout frequency and duration. We conclude that SBPT procedures facilitate reducing the restraint time for cows managed in headlocks, leading to improved milk production and dairy cattle time budgets, thus minimizing the management-induced stress in dairy cows.

Key Words: headlock, restrain, sample-based pregnancy test

1370 Evaluating farm employees' knowledge gain after playing *Mooving Cows*™.

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Inappropriate cow handling negatively affects animal welfare, milk production, and public perception. Therefore, industry animal care programs require US dairy farm employees who work with animals to complete annual continuing education (CE) on appropriate animal handling. *Mooving Cows*™ is a digital educational game to simulate cow handling scenarios on a dairy farm. Upon completion, participants receive a CE certificate. Our objectives were to measure whether the game improves farm employees' knowledge of cow handling best practices and evaluate their impressions of the game. We recruited participants (n = 25; 15 male, 10 female) from 3 Wisconsin farms who had experience with adult cows, separated into groups by farm and language preference (16 Spanish, 9 English). Age (mean ± SD) was 30.2 ± 1.8 years old with 4.8 ± 1.4 years working on the current farm. Before and after playing the game on individual touchscreen tablets, participants answered 10 multiple choice knowledge questions (including an "unsure" option), with the order of questions and response choices scrambled. The postgame survey evaluated how useful they thought the game would be for those with little to no cow experience or those who already had experience (1 = not at all, 5 = very). A paired t-test was used to evaluate the Δ in correct, incorrect, and unsure responses before and after playing the game. Descriptive summary statistics are reported for the impressions of the game's usefulness. After playing the game, the number of correct responses increased by 0.68 (95% confidence interval [CI]: 0.08 to 1.28; $P = 0.029$), which was driven by a tendency for a decrease in the "unsure" responses by −0.48 (CI: −1.00 to 0.04; $P = 0.069$), but no change in incorrect responses (mean: −0.20, CI: −0.82 to 0.42; $P = 0.51$). Participants rated the game as very useful overall (median = 4 for both questions, interquartile range = 3 [moderately useful] to 4, and 2 [slightly useful] to 4 for inexperienced vs. experienced cow handlers). Based on the evaluation of the game with Wisconsin farm employees, our results suggest *Mooving Cows*™ can improve knowledge of best cow handling practices for both new and experienced staff.

Key Words: animal handling, stockmanship, employee training

1371 Impact of captive bolt placement on tissue dimensions of heads from Holstein cows greater than 30 months old.

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Penetrating captive bolt (PCB) is a cattle euthanasia and pre-slaughter stunning method. Industry guidelines include multiple PCB locations with minimal scientific justification. Objectives were to determine tissue depths, PCB position relative to anatomical features, and brain contact associated with FRONTAL, IDEAL, and POLL PCB locations for mature dairy cows. Cadaver heads were obtained from 10 Holstein cows >30 mo old. Heads were chilled before data collection. External head measurements were collected with a digital caliper. The FRONTAL location was at the intersection of 2 lines from the lateral canthus to the opposite poll; additional PCB applications were not made here. A Jarvis 0.25R Super Heavy Duty PCB and 6GR cartridges were used at the POLL: just below the external occipital protuberance at the midline, directed toward the central incisors. Heads were split at the midline after PCB application. Tissue depth was measured at the FRONTAL and POLL locations with a digital caliper. Images of each head were taken for measurement of tissue thickness at the IDEAL location in line with the thalamus. Mean separation was determined via Student's t-tests with the Bonferroni-Holm adjustment. Tissue depth was greater ($P < 0.05$) at the POLL (36.24 ± 1.66 mm) than the FRONTAL location (27.11 ± 1.66 mm); there was no evidence to support a difference ($P > 0.05$) in total tissue thickness between the FRONTAL and IDEAL (30.25 ± 1.85 mm) or IDEAL and POLL locations. There was no evidence to support a difference ($P = 0.48$) in nose to location distance (FRONTAL: 45.77 ± 0.71 cm; IDEAL: 45.00 ± 0.71 cm). Poll to location distance was greater ($P = 0.01$) for the IDEAL (11.51 ± 0.26 cm) than the FRONTAL location (10.47 ± 0.23 cm). Distance from location to the top of the eyes was greater ($P = 0.02$) for the FRONTAL (7.89 ± 0.20 cm) than the IDEAL location (7.20 ± 0.22 cm). More ($P = 0.001$) of the head was located above the PCB location for the IDEAL (20.36 ± 0.4%) than the FRONTAL location (18.52 ± 0.4%). These data suggest that a frontal shot placed slightly below the FRONTAL location and the alternative poll location may be possibilities for PCB use on dairy cows.

Key Words: captive bolt, euthanasia, welfare

Breeding and Genetics 2

1372 Single cell transcriptomics reveal immune changes related to selection practices in Holsteins. K. M. Sarlo Davila*[1], E. J. Putz[2], J. D. Lippolis[1], W. Weber[3], and B. A. Crooker[3], [1]*Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, Agricultural Research Service (ARS), USDA, Ames, IA,* [2]*Infectious Bacterial Diseases Research Unit, National Animal Disease Center, ARS, USDA, Ames, IA,* [3]*Department of Animal Science, University of Minnesota, St. Paul, MN.*

Selection practices since the mid-1960s have impacted a large number of immunity genes and reduced heterozygosity in the bovine major histocompatibility complex (BoLA) region. The University of Minnesota has maintained an unselected herd of Holsteins (UH) since the mid 1960s, which has a more effective immune system than contemporary Holsteins (CH). In this study, peripheral blood mononuclear cells (PBMC) from 3 CH and 3 UH heifers were challenged with lipopolysaccharide (LPS) or mock-challenged with PBS for 2 h. Mock- and LPS-challenged PBMC were processed for single-cell RNA-seq using the 10X Genomic Chromium Single Cell 3′ kits and were sequenced on an S2 lane of the Illumina NovaSeq 6000 to generate 100 bp reads. Reads were processed using the CellRanger 7.1.0 pipeline and secondary analysis was performed using the R package Seurat version 5.0.0. Cell types were annotated using the packages SingleR 2.4.1 and celldex 1.12.0 and the Database Immune Cell Expression (DICE) to assign specific cell types to the PBMC including B cell and monocytes. The FindMarkers feature of Seurat was used to identified differentially expressed genes (DEG) within the cell types. Significant DEG were analyzed for enriched pathways and upstream regulators using Ingenuity Pathway Analysis software. In the B cells cytokine genes crucial for immune response and bacterial clearance including *MYD88*, *TNF*, *ILA*, *ILB*, and *IL17A* were downregulated in LPS-challenged CH B cells compared with mock-infected cells but were upregulated in UH B cells compared with mock-infected cells. *XBP1* was identified as significant upstream regulator in both CH and UH monocytes. *XBP1* is a transcription factor that regulates MHC class II genes, including *BOLA-DQB*, *BOLA-DQA5*, and *BOLA-DQA2*, which were found to be significantly unregulated in UH monocytes compared with CH monocytes even in mock-infected cells. These results indicate that selection practices have induced genome changes in immune regions and have reduced immune function in contemporary Holsteins.

Key Words: cytokine, B cell, monocyte

1373 Informing dairy cattle precision breeding through nanopore adaptive sampling. E. K. Kiugu*[1], N. Flack[1], E. Kipp[1], L. Caixeta[2], B. A. Crooker[3], T. Sonstergard[4], and P. A. Larsen[1], [1]*Department of Veterinary Biomedical Sciences, University of Minnesota, St. Paul, MN,* [2]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN,* [3]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [4]*Acceligen, Eagan, MN.*

Recent advancements in genetic engineering have led to the integration of precision breeding in dairy production systems using novel techniques such as gene editing. The underlying principle of precision breeding is identifying DNA modifications associated with specific traits of interest and making informed breeding decisions to potentially pass these traits on to the next generation. The foundation for gene editing is the ability to identify and confirm target genes and gene variants using sequence data. Recent bioinformatic pipelines offered by Oxford Nanopore Technologies (ONT) allow for the selective sequencing of specific genomic targets across a given genome. Remarkably, this approach is purely bioinformatic and is conducted in real-time as sequencing is occurring, with DNA molecule “accept” or “reject” decisions made at the level of individual sequencing pores. Here we demonstrate how the ONT adaptive sampling technique can be leveraged to simultaneously target hundreds of loci across the *Bos taurus* genome, including specific regions critical to precision breeding efforts in cattle. We targeted 237 genes associated with thermoregulation and immune response in cattle using the latest *Bos taurus* genome assembly. The coordinates of these genes were compiled in a FASTA file and uploaded to the MinKnow software to inform selective sequencing. Genomic DNA was extracted from the buffy coat of a Holstein dairy cow using the MagAttract HMW DNA kit. The prepared libraries were sequenced using the ONT’s MinION sequencer for 48 h. Aligned reads were mapped to the reference genome using Geneious software. Our results indicate that adaptive sampling was successful as it enriched 98% of our 237 targeted regions at a median coverage depth of 11.5× (3.4-fold above background). Additionally, we present data on phased nucleotide variants and differential DNA methylation facilitated by ONT long reads and direct DNA modification detection, respectively. We conclude that the ONT adaptive sampling approach will serve as a valuable tool for the rapid and precise assessment of multiple loci of interest targeted for precision breeding in cattle.

Key Words: dairy, sequencing, gene editing

1374 Current challenges for the implementation of beef-on-dairy breeding programs: A beef industry perspective. L. C. Novo*[1], R. M. O. Silva[1], S. Miller[2], and K. J. Retallick[1], [1]*Angus Genetics Inc., Saint Joseph, MO,* [2]*AGBU, a joint venture of NSW Department of Primary Industries and University of New England, Armidale, NSW, Australia.*

The use of beef semen by the dairy industry has been increasing exponentially over the past years. While this represents a great business opportunity for beef and dairy, many challenges are still to be undertaken. We present here our perspective of the current state of the beef-on-dairy (BxD) fast-growing industry and the challenges and opportunities currently faced. The need for a combination in selection goals for 2 distinct end products set the demand for selection indexes focused on BxD. The ideal implementation of such a tool is limited by 2 main points of concern: poor record keeping and lack of standardization of identification numbers across industries. The common practice of the dairy sector is the early commercialization of crossbred calves, shortly after birth, with no data collection focused on the progeny. On the other hand, beef producers’ absence of data recording protocols at the commercial level is emphasized by the lack of records of animals’ early life events and no parent identification. The implementation of genetic selection is heavily dependent on phenotype and pedigree information, and they become more important on a commercial level where genotypes are usually not available. The diverse identification formats in cattle industry poses perhaps the biggest challenge for the necessary data exchange and consequently proper modeling of breeding schemes. While selection indexes focused on BxD such as $Angus-On-Holstein and $Angus-On-Jersey built by Angus Genetic Inc., a subsidiary of the American Angus Association, are available, the optimal solution is still dependent on the integration of dairy and beef industry organizations. Their collaboration and the consolidation of registration numbers, genotypes, identification codes, and phenotypes could be taken as the first step for the implementation of better data capture systems, genetic evaluations, and selection indexes for all parties involved. The joint effort of organizations will not only benefit the BxD chain, but also initiate partnerships in data and information that will advance the cattle industry regardless of where the animals come from.

Key Words: beef industry, carcass, dairy cross

1375 Genetic evaluation of fertility traits in US organic Holstein cows.

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Genetic evaluations of cow fertility in the United States are based primarily on performance in confinement herds and it is not clear how well such evaluations will correlate with fertility in organic systems that require grazing and no reproductive synchronization. The objectives of this study were to assess fertility in organic Holsteins and to determine the genetic relationships with fertility in conventional herds. The data contained 32,572 records from 18,565 cows and included the following fertility traits: days open (DOPN), pregnancy at first service (PG1ST), and number of inseminations (NSEM). A 3-trait single-step animal model implemented with Gibbs sampling and the BLUPF90 family of programs included 74,817 DNA markers for 1,339 genotyped cows, of which 898 were cows with records and the remaining were dams and siblings without records. We generated 250,000 iterations with 100,000 burn-ins; EBV and variance components were retained for every 100 samples. The EBV were averaged across the 1,500 retained samples and EBV with accuracy greater than 50% were merged with US genomic PTA (gPTA) for 355 bulls for daughter pregnancy rate (DPR), cow conception rate (CCR), and dairy form (DF). Heritability (±SD) for DOPN was 0.062 ± 0.008, PG1ST was 0.065 ± 0.012, and NSEM was 0.034 ± 0.012; the genetic correlation between DOPN and PG1ST was −0.508 ± 0.096, DOPN and NSEM was 0.717 ± 0.062, and PG1ST with NSEM was −0.775 ± 0.058. The correlation of EBV for DOPN with DPR was −0.480 (<0.0001), which corresponds to an approximate genetic correlation of −0.668 after adjustment for reliability. The EBV for PG1ST were positively and significantly correlated with CCR (0.337; $P < 0.0001$), whereas the EBV for NSEM were negatively correlated with CCR (−0.430; $P < 0.0001$). The gPTA for DF was positively correlated ($P < 0.01$) with DOPN (0.256) and NSEM (0.190) and negatively correlated with PG1ST (−0.156). In conclusion, heritabilities were very similar to expectations from conventional herds; gPTA from conventional herds will facilitate reproductive improvement in organic herds, but approximate genetic correlations less than unity suggested some level of genotype by environment interaction.

Key Words: fertility, Holstein, organic

1376 Genetic analysis of estrus expression traits in lactating dairy cows.

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Dairy cow estrus is marked by acute changes in behavior, such as increased activity, decreased feed intake, and decreased rumination. These behavioral changes can be measured on the farm using wearable sensors and other monitoring systems. The main goal of this study was to evaluate the genetic variability of alternative estrus expression traits in lactating dairy cows. Data consisted of bi-hourly activity and rumination records collected using automated monitoring devices in 12,000 lactating dairy cows during 2 years on a large commercial farm implementing timed-AI. We developed an algorithm that quantifies changes in activity and rumination to define estrus events and estimate its duration and strength. Four estrus expression traits were evaluated: estrus occurrence (yes/no), estrus duration, estrus strength based on activity, and estrus strength based on rumination. We assessed the heritability of these estrus expression traits using a sliding 21-d window between 21 and 100 DIM. We also evaluated the genetic variability in the number of detectable estruses between 11 and 70 DIM (voluntary waiting period). The statistical models included year-season and lactation as fixed effects and cow as a random effect. Between 21 and 50 DIM, heritability estimates for estrus occurrence, duration, activity strength, and rumination strength were 0.21 ± 0.02, 0.14 ± 0.02, 0.15 ± 0.02, and 0.16 ± 0.01, respectively. Between 50 and 100 DIM, the heritability for the 4 traits dropped to 0.09 ± 0.03, 0.08 ± 0.02, 0.09 ± 0.03, and 0.07 ± 0.02. The frequency of detectable estruses between 11 and 70 DIM showed a heritability estimate of 0.23 ± 0.02. Overall, estrus expression traits are heritable, and these heritability estimates are large compared with other female fertility traits. Interestingly, the initiation of a timed-AI protocol around 50 DIM was associated with a significant reduction in the genetic variability of all estrus expression traits, denoting how management interventions could mask genetic abilities.

Key Words: estrus behavior, genetic parameter, reproductive efficiency

1377 Exploring anogenital distance as an indicator of fertility in dairy cattle.

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Fertility is crucial to the productivity and profitability of the dairy industry. However, traditional fertility traits have shown slow genetic progress due to low heritability estimates and heavy environmental influences. Anogenital distance (AGD), a biologically relevant trait, has emerged as a promising indicator of fertility in dairy cattle. This study aimed to further explore the genetic basis of AGD and assess its potential as a selection criterion for improving fertility in dairy cattle breeding programs. The dataset consisted of 4,988 Canadian Holstein cows and heifers with AGD phenotypes with available pedigree. A single-trait animal model was used with fixed effects of recording technician, linear and quadratic regressions on age of animal in days, a linear regression on calving to measurement date in days, and herd-year-season of birth and random effects of animal and residual. The study estimated a moderate heritability for AGD (0.39 ± 0.04), significantly higher than traditional fertility traits, indicating its potential as a heritable trait for selective breeding. Reliability estimates of estimated breeding values (EBVs) for AGD ranged from 0.50 to 0.83, suggesting a strong potential for increased response to selection. These findings indicate AGD as a promising fertility indicator trait for selective breeding in dairy cattle, supporting the next steps of the investigation, which include estimation of genetic correlation with currently evaluated fertility traits. The potential incorporation of AGD into national breeding evaluations holds promise for enhancing overall fertility and accelerating genetic progress in the dairy industry.

Key Words: anogenital distance, fertility indicator, genetic parameter

1378 Six direct health traits for the United States: Updated variance component estimates and implementation of variance-adjusted weights.

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Six direct health evaluations for the resistance to milk fever (MFEV), displaced abomasum (DA), ketosis (KETO), mastitis (MAST), metritis (METR), and retained placenta (RETP) are provided to US dairy producers by the Council on Dairy Cattle Breeding (Bowie, MD). The evaluations were first incorporated into Merit Selection Indices as health dollars (HTH$) for Holstein in April 2018, Jersey in April 2020, and Brown Swiss in August 2022. The relative emphasis is 1.2% for Lifetime Net Merit, Fluid Merit, and Cheese Merit, and 1.4% for Lifetime Grazing Merit. The number of records have more than doubled since variance components were last estimated in 2018, currently including 5.8m (1.1%) MFEV, 5.8m (1.6%) DA, 4.3m (5.8%) KETO, 7.7m (11.7%) MAST, 6.3m (7.2%) METR, and 7.6m (3.4%) RETP records (incidence rate). Health evaluations use a single-trait linear animal repeatability model. The objective was to enhance the national genetic evaluation for health traits by applying an adjustment that gave more weight to parities with higher heritabilities. This is different from the previous adjustments for heterogeneous variance that standardized genetic variance across parities but gave all parities equal weight. Variance components were estimated resulting in new overall heritabilities (h^2), h^2-by-lactation, and repeatability. Except for MFEV, which maintained a 0.6% h^2, the h^2 increased from 1.1% to 3.1% for DA, 1.2% to 1.7% for KETO, 3.1% to 3.2% for MAST, 1.4% to 1.6% for METR, and 1.0% to 1.3% for RETP. Correlations between the official December 2023 evaluation and a test evaluation with the new updates were all ≥90% for genomic estimated breeding values (GEBV) and ≥97% for genomic reliability (GREL) for all traits except for DA. The DA correlations were ≥81% for GEBV and ≥84 for GREL due to the largest change in h^2, which also impacted the variance-adjusted weights. Some variation is expected in individual animals' GEBV. The impact on indices is expected to be small given the weighting in each index. Variance adjustments effectively capture the categorical trait of incidence in a linear model.

Key Words: categorical trait, health evaluation, heterogeneous variance

1379 Genetic parameters for lactation yields of milk, fat, protein, and lactose of New Zealand dairy goats undergoing standard and extended lactations.

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New Zealand dairy goat farmers are increasing the number of goats with extended lactations as an intentional management decision. Our objective was to estimate genetic parameters for lactation yields of milk, fat, protein, and lactose of dairy goats undergoing standard and extended lactations. The dataset comprised 3,941 standard and 1,981 extended lactation records from 3,282 Sannen-crossbred does kidding between 2014 and 2021 in a large (~1,250 milking goats) herd. A total of 149 sires and 130 maternal grandsires were represented. The raw average yields for standard lactations were 1,010 ± 325 L milk, 32.2 ± 10.3 kg fat, 31.9 ± 10.0 kg protein, and 46.4 ± 14.7 kg lactose from an average of 282 ± 29 d in milk. The raw average yields for extended lactations were 2,766 ± 899 L milk, 87.7 ± 29.1 kg fat, 88.8 ± 28.2 kg protein, and 124.1 ± 40.3 kg lactose from an average of 773 ± 174 d in milk. Estimates of (co)variances were obtained using the ASREML program with univariate and bivariate repeatability animal models. The model included the fixed effects of year and month of kidding, parity number, and proportion of Saanen as a covariate, and random additive genetic and permanent animal effects. From the univariate model heritabilities for standard lactation yields of milk, fat, protein, and lactose were 0.23 ± 0.04, 0.27 ± 0.04, 0.23 ± 0.04, and 0.23 ± 0.04, respectively. Corresponding heritabilities for extended lactations were 0.18 ± 0.05, 0.23 ± 0.05, 0.17 ± 0.05, and 0.20 ± 0.05. From bivariate modeles genetic correlations among milk, fat, protein, and lactose yields of standard lactations were from 0.72 to 0.97 and the genetic correlations among milk, fat, protein, and lactose yields of extended lactations were from 0.68 to 0.94. The genetic correlations of the same trait but expressed as standard and extended lactation were 0.88 ± 0.12, 0.91 ± 0.12, 0.88 ± 0.12, and 0.90 ± 0.12 for milk, fat, protein, and lactose, respectively. Estimates of (co)variances from this study are now used for the genetic evaluation for standard and extended lactations of animals from the Dairy Goat Co-Operative (NZ) Ltd.

Key Words: goat, extended lactation, genetic correlation

Dairy Foods Milk Protein and Enzymes Committee Symposium: Casein Structure and Chemistry

1380 Casein micelles structure and processing functionality. M. Corredig*, *Aarhus University, Aarhus, Denmark.*

Casein micelles are one of the most studied food colloids due to their major role in the stability and processing functionality of milk and milk ingredients. As novel ingredients become avaialable, obtained either by recombination of single caseins or by membrane filtration, our understanding of their structure and functionality relationship is put into question. Changes in environmental conditions, and ratios between the different components may modify the internal structure while maintaining similar size and shape, or modify their colloidal to soluble equilibrium. Furthermore, membrane concentration processes can cause in situ modifications of their colloidal properties, with consequences to their functionality, with new opportunities for innovation and the generation of new value-added ingredients. There is then a greater than ever need for understanding the details of the structure of casein micelles, to ensure their quality and consistency of functionality. This paper summarizes our current knowledge of the important factors affecting the colloidal structure of the casein micelles as well as the modifications leading to differences in their structuring properties.

Key Words: casein micelle, processing, structure

1381 Caseins: The structure-function relationship in cheese. J. J. Sheehan*, *Teagasc Food Research Centre, Moorepark, Moorepark, Co. Cork, Ireland.*

Caseins, their aggregation, properties, and interactions with other components are key to determining cheese structure function properties. During rennet coagulation, destabilized casein micelles aggregate into chains and clusters, leading to formation of a 3-dimensional gel concentration of casein. Other constituents (e.g. fat globules, water, minerals, bacteria, and dissolved solutes) are all interspersed within this casein matrix. Calcium bonding, electrostatic interactions, and hydrogen bonds (to a lesser degree) between caseins contribute to the formation and stability of the para-casein matrix (after the pressing stage) while interactions such as the localized balance of the attractive and repulsive forces between casein influence the functionality of cheese including the melting properties of heated cheese. Similarly, modulation of the levels of colloidal calcium associated with the casein present in the cheese matrix alters the texture and cooking properties of cheese, with decreased levels contributing to softening of the cheese texture (particularly early in ripening) while higher levels confer an elastic texture, important for sliceability in avoiding crumbliness or adhesion to cutting equipment. Cheese stretch properties have been linked to hydrophobic peptides, as products of casein hydrolysis, present in the pH 4.6-soluble nitrogen regardless of casein origin (i.e., whether derived from α_{S1}-CN, α_{S2}-CN, or β-CN) and are thought to interact with the protein matrix or with other large peptides via hydrophobic forces, possibly forming fibers in the cheese matrix. Cheese texture, rheological, and cooking properties are influenced by both varying pH and temperature. The former through alteration of casein-casein, mineral casein, and casein-water interactions while elevated temperature during heating of cheese is proposed to lead to reduction in the contact area between casein, leading to weakening of the cheese matrix and decreases in G' and G'' and an increase of loss tangent. Overall leverage of casein chemistry and interactions with other cheese matrix constituents are key to controlling cheese structure-function properties.

1382 Animal-free caseins: Challenges in recreating casein micelles and related functional properties. J. A. Lucey*[1,2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Center for Dairy Research, Madison, WI.*

Recently, various startups have stated that their goal is to create animal-free caseins that have identical functionality to those produced by the cow. Recombinant protein technology has been used to produce various enzymes like rennet. In this approach, genetically modified microorganisms like yeast/bacteria are engineered to express the gene for the target protein (e.g., a casein). This modified microorganism is then fermented to grow and produce the target protein, before isolation and concentration steps are applied. More recently, this approach has been described as precision fermentation. Other approaches include transgenic plants that can express the target casein, and lab-grown milk using cell culture lines of mammary cells. Caseins undergo significant post-translational modifications of the primary sequence, including phosphorylation (at primarily serine amino acids, which directly determines the extent of calcium binding) and glycosylation at a single site on kappa-casein. The bioassembly of casein micelles occurs in the Golgi apparatus of the mammary cell, under tightly controlled ionic conditions that promote the formation of insoluble calcium phosphate nanoclusters, which acts as a crosslinking material between casein molecules. Association of hydrophobic segments also promotes casein association and elongation. Incorporation of kappa-casein into the aggregating casein chains terminates the growth of the micelle since it cannot form further calcium phosphate crosslinks. At least 4 different types of caseins are involved in the structure of casein micelles produced by the cow. Artificial casein micelles were created in the 1970s by careful manipulation of the ionic environment, but the caseins used had already undergone post-translational modifications. Without a mammary cell, its unclear if the necessary and identical post-translational modifications will occur, and where the critical bioassembly process will occur, which is required for micelle formation. There have not been any published studies on the post-translational modifications or functionality of any animal-free caseins.

Key Words: precision fermentation, recombinant technology, animal-free

1383 Tearing down casein micelles: An alternative clean-label ingredient approach. F. Harte*, *Pennsylvania State University, University Park, PA.*

The casein micelles in bovine milk serve as the building blocks of a range of dairy foods, including yogurt and cheese. Despite outstanding progress in our capacity to extract functionality from casein, we still face roadblocks when it comes to leveraging the structure-function properties of the casein proteins and the casein micelles. Over recent years, members of the Harte laboratory have delved into exploring the physicochemical stimuli leading to the breakdown of native casein micelles, into monomeric casein proteins and smaller casein aggregates. This presentation will focus on 3 avenues conducive to the dissociation of the casein micelles: high-pressure technologies (e.g., hydrostatic, homogenization, jets), the modification of the ionic environment (e.g., by adding citrates, phosphates, tartrates), and co-solvation (e.g., by exposure to ethanol). The transformative effects on casein functionality resulting from "tearing down the casein micelles" will be stressed, highlighting

improvements in emulsifying properties, protein-polysaccharide stabilization, and the formation of casein-based nano-scale fibers, amorphous solid dispersions, and films. Throughout the presentation, attention will be drawn to 2 crucial aspects: (1) the current limitations in analytical tools that prevent us from better understanding structural features of the casein micelle, and (2) the significant potential for functionalizing casein toward clean-label applications in food and nonfood materials.

Key Words: casein, processing, clean-label

Dairy Foods Symposium: A Systems-Based Approach to Drive Innovation in Dairying

1384 An introduction to Dairy Management Inc.'s research priorities. K. Alexander*, S. Masiello Schuette, M. Pikosky, and C. Galer, *Dairy Management Inc., Rosemont, IL.*

Dairy Management Inc. (DMI) is focused on elevating US dairy through innovation, sustainability, and enhancing its public image, aiming to foster trust, drive sales, and ensure industry sustainability. The Strategic Intelligence team propels this vision with insights and analytics in areas like Foresight, Discovery and Trends, and Innovation and Growth, making informed decisions to navigate future challenges. The Environmental Research program champions sustainability, researching practices to reduce emissions and improve water efficiency, contributing to a greener dairy industry. Simultaneously, the Nutrition Research program explores dairy's health benefits, promoting its nutritional value and boosting consumer demand. The Dairy Products Research team furthers DMI's mission by innovating and expanding dairy choices through targeted R&D, addressing consumer preferences, and opening new market avenues. This coordinated effort across DMI's departments underscores a holistic strategy to not only rejuvenate US dairy's image but also solidify its market leadership and commitment to wellness and environmental responsibility. The objective of this talk is to illustrate how these diverse departments collaborate to achieve DMI's overarching goals, showcasing a unified front in redefining the future of the US dairy industry.

Key Words: Dairy Management Inc. (DMI), research, innovation

1385 Unlocking innovation opportunities for dairy in health and wellness. K. Alexander*, *Dairy Management Inc., Rosemont, IL.*

A growing number of US consumers are actively managing their health. In fact, health and wellness is the number 1 area of disposable income spending. As consumers look to functional foods and beverages for a variety of health and wellness reasons, dairy is in a prime position to be their solution. This session will dive deep into research that explores (1) who the health and wellness consumers are, (2) the top conditions they are looking to treat and prevent, (3) global health and wellness trends, and (4) dairy's opportunity to innovate within this space.

Key Words: innovation, trend, insight

1386 Innovative integrations: Genomics, milk spectrometry, and microbial manipulations to mitigate enteric methane emissions from dairy cattle. F. Peñagaricano*, *University of Wisconsin–Madison, Madison, WI.*

The overall goal of this multi-institutional, multi-disciplinary project is to mitigate enteric methane emissions from dairy cattle by combining selective breeding, milk mid-infrared spectra data, and rumen microbiome interventions. We will measure methane emissions, feed intake, energy-corrected milk, body weight, and milk mid-infrared spectra for 6 to 8 weeks in roughly 4,000 mid-lactation Holstein cows. To enable selective breeding for methane emissions, we will develop genomic evaluations for methane traits, including methane production, residual methane yield, and residual methane intensity, and incorporate these traits into US dairy cattle selection indices. To develop effective milk spectra-based solutions, we will determine the value of using milk mid-infrared spectra as a noninvasive approach to predict enteric methane emissions. To decipher how the rumen microbiome affects methane production, we will identify low and high methane-emitting individuals among the 4,000 reference cows and investigate the composition and activity of the rumen and oral microbiome using omics technologies. We will also exchange ruminal contents in a subset of low- and high-methane-emitting cows to decipher the relative contributions of hosts and microbes to methane production. Overall, we will deliver multiple solutions to mitigate methane emissions from dairy cattle, which will safeguard the economic, environmental, and social sustainability of the US dairy industry.

Key Words: greenhouse gas, selective breeding, sustainability

1387 Anabolic potential of dairy and dairy proteins in active youth. D. Moore*, *University of Toronto, Toronto, Ontario, Canada.*

Physical activity during childhood and adolescence is vital for the growth and development of lean tissues including muscle and bone. The net acquisition of lean tissue, which is constantly "turning over" through the breakdown of old/damaged proteins and the synthesis of new proteins in their place, must be underpinned by a positive net protein balance (NB = synthesis − breakdown). Provided energy needs are met, dietary protein provides the requisite amino acid building blocks to build body protein during growth. Dairy and dairy proteins have been shown to enhance skeletal muscle anabolism (i.e., growth) in adults after exercise primarily through the ability to support elevated rates of muscle protein synthesis. The anabolic potential of dairy and dairy proteins in adults may be related to the protein density of milk, the high essential amino acid content of dairy protein, and/or food matrix effects. This presentation will summarize the emerging research to date on the ability of dairy and dairy proteins to support a positive whole-body NB after physical activity in children and adolescents. Topics to be discussed include the quantity of dairy protein, its quality relative to plant-based proteins, and the anabolic potential of dairy vs. dairy-alternative beverages. Using whole-body stable-isotope methodology, our recent research has demonstrated that milk protein is able to induce a positive whole-body NB in children after exercise in a dose-dependent manner. As little as 5 g (~0.15 g protein/kg body mass) of MPC is able to induce a positive NB after exercise in children(2). Further, we have demonstrated that body protein balance is maximized and dietary amino acid oxidation (use as energy) is minimized at a MPC intake of ~0.3 g protein/kg(1, 2), which approximates ~1.5 servings of 250 mL of fluid milk for a 35-kg child. Thus, dairy protein represents an excellent source of dietary amino acids that would support efficient acute 'growth' after activity in children and adolescents. (1) Moore et al., J. Appl. Physiol. 117:1493, 2015. (2) Volterman et al., J. Nutr. 147:807, 2017.

Key Words: exercise, youth, growth

1388 Innovative pathways in scaling benchtop discoveries to health and wellness ingredients. S. Ropiak*[1], T. Lin[2], L. Yang[2], Y. Dadmohammadi[2], H. Meletharayil[1], R. Kapoor[1], E. Nashed[1], and A. Abbaspourrad[2], [1]*Dairy Management Inc., Rosemont, IL,* [2]*Cornell University, Ithaca, NY.*

Dairy Management Inc. (DMI) and its related organizations work to increase sales and demand for dairy through research, education, and innovation, and to maintain confidence in dairy foods, farms, and businesses. The DMI Dairy Products Research group strives to advance DMI's priorities through targeted research and development activities that address consumer needs and expand dairy choices in domestic and international markets. This includes funding research projects at various dairy research centers and universities in the United States. These programs lead to cutting-edge research findings, platform technologies, and quality and food safety solutions that enable the US dairy industry to generate high-quality, innovative products and ingredients. The objective of this talk is to demonstrate the powerful potential of collaboration, innovation, and consumer insights on shaping the future of the US dairy industry with a lens of health and wellness by showcasing a current project led by DMI Dairy Products Research group that has successfully progressed from conception to commercialization. One of the key focus areas for DMI is the development of novel dairy products and ingredients for health and wellness. In partnership with DMI's Strategic Intelligence team, a landscape study was completed to identify 2 dairy-forward bioactives, lactoferrin and tryptophan, to deliver toward consumer health and wellness needs related to immunity and calming, respectively. However, both ingredients face product development challenges when incorporated in foods and beverages, such as heat instability (lactoferrin) and off-flavors (tryptophan). Through a partnership with DMI and Cornell University, a novel encapsulation process was developed to protect these ingredients and have successfully scaled up these novel ingredients for their successful use in dairy and food products. This research model aims to serve as a template for future dairy innovation commercialization research.

Key Words: innovation, commercialization, encapsulation

1389 Existing and emerging microbial challenges in the dairy industry.

N. Martin*, *Cornell University, Ithaca, NY.*

The microbial quality and safety of dairy products are drivers of consumer acceptance and trust in the dairy industry and are critical to ensuring continued domestic and international demand. Addressing microbial contamination is critical throughout the grass-to-glass continuum, and requires a comprehensive approach that integrates monitoring dairy products for emergent microbial spoilage and safety risks, tracking and typing microbes for source detection, the development of digital tools to facilitate incremental improvements and aid in decision-making, and supporting the industry with guidance and training. In the Milk Quality Improvement Program at Cornell, our research is aimed at bridging the gap between practical strategies and innovative approaches to facilitate microbial quality and safety improvements in the dairy industry.

Key Words: microbial, spoilage, safety

Dairy Foods 3: Dairy Products

1390 Improving consumers' dairy knowledge and confidence in dairy through educational focus groups. C. Ozoh* and S. Clark, *Iowa State University, Ames, IA.*

Confusion around the meaning of code dates contributes to food waste at the consumer level. In the United States, dairy is the third highest food category wasted, accounting for 17% ($27 billion) annually. We conducted this study to (i) improve consumer understanding of fluid milk code dates, (ii) educate consumers about milk pasteurization and ultra-pasteurization, and (iii) improve consumers' knowledge on the sustainability practices of the dairy industry. Milk consumers over 18 (n = 233) participated in one of 20 focus groups, held between February and March 2023, in Ames, Iowa. Participants were led through the same scripted session by 2 to 4 facilitators, with 3 possible educational messages (EM): control (code date EM only), sustainability infographic (code date EM + sustainability infographic), or sustainability video (code date EM + sustainability video). The code date EM, along with a "decoding milk" infographic, was delivered to all participants. Participants completed a presurvey (before) and a postsurvey (after) their focus group intervention. All participants were invited, but only 177 completed the follow-up survey, conducted one month after their focus group. Analysis of variance and Wilcoxon rank tests were used to analyze differences in participants' survey responses. Panelists learned that pasteurization and ultra-pasteurization kill harmful bacteria but not all spoilage bacteria; after opening, milk should taste good for about 7 d; the dairy industry contributes only about 2% of total greenhouse gas (GHG) emitted in the United States. Knowledge gain was observed ($P < 0.05$) after intervention for code dates and milk processing. However, only participants in sustainability groups gained and retained knowledge about dairy GHG emissions. Confidence about sustainability efforts made by the dairy industry increased ($P < 0.05$) in both sustainability groups. Educating consumers with infographics, videos, and verbal interactions could help clarify and build confidence that code date is a quality indicator, milk is safe to consume (past its code date), and that the dairy industry is actively working toward reducing GHG emissions.

Key Words: code date, dairy sustainability, consumer education

1391 Impact of whey protein-to-casein ratio on the manufacture of ice cream. M. A. Alvi*[1] and S. I. Martinez-Monteagudo[1,2], [1]*Department of Chemical and Materials Engineering, New Mexico State University, Las Cruces, NM,* [2]*Family and Consumer Sciences, New Mexico State University, Las Cruces, NM,* [3]*Center of Excellence in Sustainable Food and Agricultural Systems, New Mexico State University, Las Cruces, NM.*

Milk used for ice cream manufacture is often fortified with protein powders (e.g., whey protein concentrate, milk protein concentrate, and micellar casein concentrate) as part of the milk solids-not-fat components. Altering the whey protein-to-casein ratio (WP/CN) can be an important determinant of the overall quality of ice cream. The objective of this work is to evaluate the impact of milk protein fortification, specifically altered WP/CN, on the manufacture of ice cream. Nonfat dried milk, whey protein concentrate, and micellar casein concentrate were used to formulate a basic ice cream formulation (42.15 ± 0.75% total solids, 13.13 ± 0.55% total fat, and 4.55 ± 0.25% total protein). Different concentrations of WP/CN were used, including 13/87, 20/80, 30/70, 50/50, and 70/30. Overall, ice cream mixes exhibited particle size distribution that spanned from 0.73 to 0.04 μm, regardless of the WP/CN. Additionally, the viscosity of all formulations decreased with increasing the shear rate, exhibiting a shear-thinning behavior. Increasing the amount of whey protein significantly reduced the viscosity of the mixes between 2- and 4-fold, depending on the shear rate and WP/CN. Ice creams formulated with a ratio of 50/50 exhibited relatively high values of hardness (76.53 ± 3.82 N) and onset of meltdown of 2,514 s, whereas a ratio of 20/80 exhibited moderate hardness (57.41 ± 2.87 N) and onset meltdown values of 2,376 s. The outcomes of the study provide relevant information for the manufacture of ice cream by changing the WP/CN.

Key Words: ice cream, whey protein, casein

1392 Impact of whey protein-to-casein ratio and hydrodynamic cavitation on the structural and rheological properties of Greek-style yogurt. A. Soler*, J. Agbawodike, M. Azeem Ur Rehman Alvi, and S. Martinez-Monteagudo, *New Mexico State University, Las Cruces, NM.*

The ratio of whey protein-to-casein (WP/CN) in the yogurt base is an important factor controlling the quality of set Greek-style yogurt. This study evaluated the feasibility of modifying the WP/CN ratio in combination with hydrodynamic cavitation (40°C, 48.26 MPa) during production, as an alternative to pasteurization (90°C for 10 min). The impact on acidification kinetics, syneresis, texture, microstructure, and rheological properties was evaluated. The milk base was fortified to a total protein content of 6% (wt/wt) by the incorporation of predetermined amounts of whey protein concentrate (80% whey protein) and micellar casein concentrate (88% casein) to obtain different WP/CN ratios (13/87, 20/80, 35/65, 50/50, and 65/35). A yogurt base fortified with nonfat dry milk was used as the control treatment. Texture profile analysis (TA.XT2i texture analyzer) and syneresis (via centrifugation) revealed significant statistical differences ($P < 0.05$) in the cavitated 65/35 ratio, which obtained the highest resilience (2.39 ± 0.17) and minimum syneresis (6.6 ± 1.54%). Dynamic rheology demonstrated an improved stability of the protein network in cavitated yogurts, with a longer linear viscoelastic region and the amplitude sweeps revealed an ability to recover deformation (weak gel behavior), with the storage modulus (G') consistently surpassing the loss modulus (G''). Scanning electron microscopy images displayed a finer network of caseins aggregates linked in clusters with small pores and dense crosslinks for the cavitated 65/35 ratio. The outcomes of this investigation suggest that quality characteristics of yogurt can be modulated by adjusting the whey protein content in combination of hydrodynamic cavitation.

Key Words: rheology, Greek-style yogurt, hydrodynamic cavitation

1642 Effect of ultrasound duration on the physicochemical, microstructural, and antioxidative properties of whey protein concentrate encapsulated 3,3′-diindolylmethane nanoparticle. A. Khan*[1] and M. Guo[2], [1]*University of Home Economics Lahore, Lahore, Pakistan,* [2]*University of Vermont, Burlington, VT.*

This study determined the impact of ultrasound duration on the encapsulation of 3,3′-diindolylmethane (DIM) using whey protein concentrate (WPC) nanoparticles. WPC-based DIM nanoparticles were prepared and treated with different ultrasound times (0–20 min) with 30% amplitude at 4°C. The results showed that ultrasound treatment significantly ($P < 0.05$) decreased (one-way ANOVA; post hoc Tukey's test by applying treatments in triplicates) the mean particle size from 265.96 ± 14.5 nm (Control; WPC-DIM 0 min) to 218.46 ± 9.37 nm (WPC-DIM: 20 min); and PDI value from 0.49 ± 0.02 to 0.43 ± 0.02; as well as zeta potential values were notably increased. The EE% increased with increasing sonication time (0–20 min) from 76 ± 0.05% to 77, 79, 81, and 88 ± 0.02%, respectively. The ultrasound treatment had a significant effect ($P < 0.05$) on the apparent viscosity, and a decrease in the viscosity as a function of shear rate was observed with increasing sonication time. The TEM micrographs demonstrated that all the formulations treated with different sonication times had a smooth and uniform spherical shape and ultrasound treatment led to the reduction of particle size, especially for 20 min of ultrasound. The thermal stability of the WPC–DIM nanoparticles was enhanced with increasing sonication time by increasing peak denaturation temperature and enthalpy. The FT-IR spectra analysis revealed that ultrasound treatment had a remarkable effect on the secondary structure of WPC–DIM nanoparticles; electrostatic interactions and hydrogen bonds between DIM and whey protein were strengthened. Moreover, the length of ultrasound treatment exhibited a significant effect on the DPPH scavenging activity (from 56% to 62%) and ABTS scavenging activity (from 47% to 68%). In conclusion, the ultrasound treatment successfully improved the physicochemical, microstructural, and anti-oxidative properties of WPC–DIM nanoparticles; therefore, it is considered an effective method to develop whey protein concentrate-based DIM nanoparticles for medical and nutritional applications.

Key Words: 3,3′-diindolylmethane, whey protein concentrate

1643 Concentration level of a novel culture starter on the quality of yogurt prepared from White Fulani raw milk.

D. O. Oshibanjo and G. Shallong*, *University of Jos, Jos, Plateau State, Nigeria.*

This study investigates the effect of *Paenibacillus polymyxa* as a culture starter on the quality of yogurt produced from raw milk obtained from lactating White Fulani cows. A freeze-dried starter culture with varying dosages (0.00%, 1.00%, 2.00%, 3.00%, 4.00%, and 5.00%) of *Paenibacillus polymyxa* was used, denoted as T1 (peptone water), T2, T3, T4, T5, T6, and T7, respectively. The yogurt was evaluated for gel formation, pH, water holding capacity, and syneresis using prism graph pad. Whey production after yogurt gel formation was significantly lower at the 1.00% inclusion level of *Paenibacillus polymyxa* (23.00%), whereas the highest whey values were recorded at the 3.00% inclusion level (59.00%). No gel formation occurred with peptone water and 0.0% inclusion of *Paenibacillus polymyxa*. The study revealed that the 1.00% inclusion level of *Paenibacillus polymyxa* resulted in the highest (P-value 0.0001) yogurt gel formation (77.23%), followed by the 2.00% inclusion level (73.53%), with the least gel formation observed at the 3.00% inclusion level (42.72%). Peptone water and no inclusion of *Paenibacillus polymyxa* showed no gel formation. An inverse relationship between *Paenibacillus polymyxa* inclusion levels and pH values was observed, except for peptone water and no *Paenibacillus polymyxa* treatment. Yogurt water holding capacity was significantly higher in the peptone water treatment, with the lowest value in the treatment with no *Paenibacillus polymyxa*. Syneresis was significantly higher in yogurt containing a 3.00% inclusion level of *Paenibacillus polymyxa*, followed by yogurt with no *Paenibacillus polymyxa*, while other yogurts showed no syneresis. In conclusion, based on the study findings, a 1.00% inclusion level of *Paenibacillus polymyxa* is recommended for improving yogurt quality.

Key Words: yogurt, *Paenibacillus polymyxa*, culture starter

1644 Dietary restriction in dairy goats does not affect free fatty acids levels in milk (lipolysis) in the same way as in cows or ewes.

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Spontaneous lipolysis results in the breakdown of milk fat by the lipoprotein lipase (LPL; EC 3.1.1.34), an enzyme present in milk. Subsequent release of free fatty acids (FFA) in milk can affect the organoleptic value of milk and dairy products (rancidity, off-flavor). Feed restriction was used as a model for studying milk lipolysis and its mechanisms in 3 species of dairy interest (cow, goat, and ewe) within the frame of the LIPOMEC project (ANR-19-CE21–0010). We have recently demonstrated that feed restriction in the cow increased both milk FFA levels and LPL activity (Hurtaud et al., 2023, Animal - Open Space 2, 100035), whereas a similar dietary restriction caused a decrease in milk FFA levels by more than 50% in the ewe species without affecting the LPL activity in milk (Bernard et al., *in revision*). We conducted a similar feed restriction study in the goat species. Two groups of 12 dairy goats (121 ± 7 DIM) balanced with regard to the αs1-casein (CSN1S1) genotype (7 A/A goats, strong CSN1S1 genotypes producing milk with high levels of αs1-casein, and 5 O/O goats, *null* CSN1S1 genotypes) received either a control diet (100% of the DMI ad libitum: unrestricted; NR) or the experimental diet (65% of the DMI ad libitum: restricted; R) according to a 2 × 2 crossover design. The duration of the restriction was 5 d. Statistical analyses, including effects of genotype, diet, and genotype × diet interactions, were performed using R software. Dry matter intake intake decreased by more than 25% in the R goats compared with the NR goats ($P = 0.031$). At the same time, milk yield decreased (−11% and −12% for A/A and O/O goats, respectively, $P < 0.001$) together with milk fat and protein yields ($P < 0.001$). However, dietary restriction in goats did not significantly affect FFA levels in milk nor milk fat globule size, whereas a sharp decrease in milk LPL activity was observed (−33% and −34% for A/A and O/O goats respectively, $P < 0.001$) without genotype × diet interaction. Overall, our results highlight a species-specific response to feed restriction with regard to FFA levels and milk LPL activity. Further studies are underway to better understand molecular mechanisms involved in the regulation of milk lipolysis in ruminant species of dairy interest.

Key Words: milk fat, free fatty acids, dairy species

1645 Effect of the availability of fresh pasture in the diet of dairy cows during autumn and spring seasons in Uruguay, on the nutritional and technological properties of butter models.

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There is significant potential for market capitalization of grass-fed dairy products. Thus, it is necessary to prove if pasture feeding systems under local conditions modify the technological and nutritional properties of high-fat products such as butter. The objective of this work was to evaluate the effects of different pasture feeding strategies on the technological and nutritional properties of butter models. The study was carried out from September to December 2022 (spring) and from April to July 2023 (autumn), collecting milk from Holstein North American cows from 10 dairy farms located in the northwest region of Uruguay. Farms were classified according to average percentage of fresh pasture supplied in each period into: high pasture (HP; >60%) and medium or low pasture (MLP; <60%). For every farm (HP and MLP) milk pool samples were taken twice a month during each season. For each sampling, laboratory-scale butter models were prepared in triplicate. The moisture and fat content were determined, and the fatty acid (FA) profile was analyzed. The atherogenic (AI), thrombogenic (TI), and spreadability (SI) indices were calculated. The firmness index (FI) was determined using a texturometer, and the color evaluation was carried out using a Konica Minolta CR-400 ChromaMeter. To determine the differences between the effects of the pasture feeding strategies, the sampling, and their interactions, ANOVA and Tukey tests were performed using a significance level of 0.05. Our response variables showed significant differences ($P < 0.05$) for both seasons. Butter models corresponding to HP samples showed: higher yellow index (46.06 vs. 37.52 on spring and 57.88 vs. 43.72 on autumn), higher CLA content (1.64% vs. 0.97% on spring and 1.75% vs. 0.79% on autumn), lower AI values (1.48 vs. 2.0 on spring and 1.58 vs. 1.92 on autumn) and lower TI values (1.78 vs. 2.13 on spring and 1.84 vs. 2.01 on autumn), which allows inferring about their better nutritional and technological quality.

Key Words: pasture intake, butter, technological property

Lactation Biology 1

1393 Fetal exposure to heat stress affects the mammary gland epigenome at birth. M. C. Guenther*[1], B. Dado-Senn[1], L. Liu[1], F. Peñagaricano[1], G. E. Dahl[2], and J. Laporta[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*University of Florida, Gainesville, FL.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Late-gestation heat stress in dairy cows negatively affects offspring mammary growth and development including decreased mammary size, reduced number of ductal structures, and altered cellular proliferation at birth. However, the molecular alterations underlying these changes are not well understood. Thus, this study aimed to characterize the epigenetic changes in mammary parenchyma (mPAR) triggered by *in utero* heat stress. In the last 54 ± 5 d of gestation pregnant cows were either heat-stressed (shade) or cooled (shade, fans, water soakers) during a subtropical summer, generating heifers that were *in utero* heat-stressed (IUHS) or *in utero* cooled (IUCL). A subset of heifers (n = 8/group) were euthanized at birth before colostrum feeding. The mammary glands of IUHS and IUCL calves were removed and mPAR was dissected and snap frozen. Total DNA was extracted from mPAR samples for whole-genome bisulfite sequencing. DNA fragments were sequenced with Illumina Hi-Seq 4000. Reads were mapped to the bovine genome (ARS-UCD1.2) using Bismark software. Differential methylation analysis was performed using the methylKit R Package. A total of 1.4 million CpG were evaluated across the entire bovine genome. A total of 1,449 of these cytosines were differentially methylated between treatments (DMCs; methylation difference ≥20%, q-value ≤0.10). These DMCs were assigned to 557 unique genes. Hypermethylated genes of interest in IUHS are related to mammary gland development (e.g., *CSMD1*), cell turnover (e.g., *XKR5*, *DDR2*), cell adhesion (e.g., *FAT3*, *CNTNAP5*, *CTNND2*, *NRXN3*), and immune response (e.g., *CADM1*, *IL1RAP*, *M-SAA3*), whereas hypomethylated genes of interest in IUHS are related to cell division (e.g., *CDC23*, *PTPRA*, *RBMS1*, *MAP3K4*) and cell differentiation (e.g., *FSTL5*, *TGFB1*). An over-representation analysis of methylated genes was performed using EnrichKit. Enriched pathways of interest were related to smooth muscle contraction, oxytocin signaling, cell-cell junction, steroidogenesis, and growth hormone signaling. Taken together, these epigenetic changes within the mPAR may in part explain the morphological and cellular alterations observed in IUHS heifers at birth.

Key Words: intrauterine, hyperthermia, methylation

1394 Carryover effects of maternal heat stress on growth, mammogenic hormones, and mammary microstructure through the daughter's first pregnancy. B. D. Davidson*[1], S. L. Field[1], K. A. Riesgraf[1], G. E. Dahl[2], and J. Laporta[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Maternal late-gestation heat stress negatively impacts calf stature and mammary growth from birth through puberty, decreasing milk production in the first lactation. However, the effects of in utero heat stress that persist through the first gestation, before onset of lactation, have not been investigated. Herein, cows were cooled (CL; shade, fans, soakers) or heat-stressed (HT; shade) for the last 56 d of pregnancy, generating in utero cooled (IUCL) or in utero heat-stressed (IUHT) heifers, respectively. All heifers were managed identically from birth until breeding. Once pregnant, body weight (BW), hip height (HH), chest girth (CG), and body length (BL) were measured monthly. Teat length, distances between teats, and mammary gland (MG) width were measured at 2, 4, 6, and 9 mo of gestation (n = 17–18/group). Plasma progesterone (P_4) was analyzed monthly, and estradiol (E_2) at 8 and 9 mo of gestation, via radioimmunoassay (n = 13–17/group). At 9 mo, MG biopsies were performed (n = 5/group) for histological evaluation. The number of mammary epithelial cells was counted, and the inner lumen and outer alveolar areas were quantified in ImageJ (4 images/animal, 20×). Data were analyzed using the MIXED procedure of SAS with treatment, month of gestation, and their interaction as fixed effects. The IUHT insult had no effect on BW, HH, CG, BL, teat lengths, distances between teats, or the concentration of plasma P_4 and E_2 through the first gestation ($P \geq 0.12$). There was a treatment by month interaction for MG width, whereby 9 mo IUCL heifers had wider glands, relative to IUHT ($P = 0.03$). Additionally, IUCL heifers tended to have more epithelial cells per alveoli ($P = 0.06$) and larger alveolar inner (lumen) and outer areas relative to IUHT at 9 mo of gestation ($P \leq 0.09$). Exposure to prenatal heat stress (2 years before the first parturition) has longstanding implications for alveolar size and mammary epithelial cell number, while sparing whole-body growth and hormonal profiles. These results contribute to understanding the reduced synthetic capacity of first-lactation heifers following in utero heat stress.

Key Words: parenchyma, estrogen, progesterone

1395 Characterization of milk vein blood flow via Doppler ultrasonography from dry-off to calving and its association with colostrum composition and yield in multiparous Holstein cows. A. J. Fischer-Tlustos*[1], K. Klein[1], J. Petrou[1], C. McQuaig[1], E. S. Ribeiro[1], J. Mergh Leao[2], C. A. Redifer[3], A. M. Meyer[3], J. P. Cant[1], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Saskatoon Colostrum Co. Ltd., Saskatoon, SK, Canada,* [3]*Division of Animal Sciences, University of Missouri, Columbia, MO.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Level of metabolic activity in the mammary gland is reflected in local blood flow (BF) during an established lactation; however, whether such a relationship exists during the dry period and its implications for colostrum production require further investigation. Thus, the aim of this study was to characterize milk vein BF (MVBF), as a proxy of mammary BF, from dry-off to calving and to determine its association with colostrum yield and composition. From d −58 ± 2.9 before calving, parity 2 (P2, n = 11) and 3 (P3, n = 10) Holstein cows were dried off and fed a controlled-energy diet (NE_L = 1.48 Mcal/kg DM) until calving. Milk vein BF was measured by Doppler ultrasound of both milk veins at d −49, −35, −21, and −7 relative to expected calving, then every 4 d until actual calving, and at 2 h after colostrum collection. Colostrum was collected within 1.4 ± 0.76 h of calving and composition was determined via mid-infrared spectroscopy and IgG by radial immunodiffusion. Time points were adjusted based on actual calving date and data were analyzed using PROC GLIMMIX of SAS considering the fixed effects of parity and time and the random effect of cow. Total MVBF ranged from 139.6 to 173.7 ± 20.8 L/h from wk −8 to −2 ($P > 0.52$), then increased ($P < 0.0001$) to 206.7 ± 18.4 L/h at wk −1 and to 415.0 ± 35.7 L/h at calving. Both left and right MV cross-sectional area increased ($P < 0.0001$)

from 1.5 ± 0.31 cm^2 at wk −8 to 2.0 ± 0.40 cm^2 at calving. On average, P3 cows had 24.9% and 29.5% greater left ($P = 0.06$) and right ($P = 0.02$) MVBF, respectively, compared with P2 cows. Colostrum IgG yield was correlated with MVBF at wk −1 ($r = 0.49$, $P = 0.05$) and d −2 ($r = 0.85$, $P = 0.03$). Colostrum yield was negatively correlated with wk −6 MVBF ($r = −0.51$; $P = 0.08$), but no further associations were observed. The results demonstrate that mammary BF during the dry period increases from P2 to P3 and suggests that BF—an indicator of mammary metabolic activity—in the week preceding calving may be an important determinant of colostrum IgG yield.

Key Words: dry period, mammary vein blood flow, colostrum

1396 Effects of *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 supplementation during late gestation on colostrum quality and components in primiparous and multiparous Holstein dairy cattle. M. Boerefyn*[1], S. Cartwright[1], S. Jantzi[1], K. Dekraker[1], A. Pineda[1], S. K. Kvidera[2], R. Sargent[3], C. Villot[4], and M. A. Steele[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Elanco Animal Health, Granger, IA, [3]Saskatoon Colostrum Company Ltd., Saskatoon, SK, Canada, [4]Lallemand SAS, Blagnac, France.*

Substantial evidence in monogastric and ruminant species suggests that prepartum nutrition has significant impacts on colostrum quality and composition. The objective of this study was to assess the effects of *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 supplementation (SCB; 1.0×10^{10} cfu/d; fed from d −28 to calving) and parity on Holstein cows' colostrum quality and composition. Fifty-two multiparous (MP) and 31 primiparous (PP) Holstein cows were blocked by previous milk yield, parity, body condition score, and body weight and were randomly assigned to either control (CON; n = 40) or SCB (n = 40) treatment groups. Colostrum (milking 1) samples were collected, and total yield was recorded. Colostrum was analyzed for IgG, fat, lactose, and protein and SCC. Data were analyzed using the MIXED procedure of SAS (v.9.4). The MP cows had greater colostrum yield ($P < 0.05$; 7.2 vs. 5.1 kg), IgG concentration ($P < 0.05$; 80.3 vs. 61.7 g/L), SCC ($P < 0.05$; 1,793.7 vs. 955.3 cells/mL) but lower fat content ($P < 0.01$; 4.9% vs. 7.2%) than PP cows, which supports the findings of previous literature. Colostrum yield, IgG concentration, and SCC did not differ ($P > 0.05$) between treatment groups and all cows had adequate IgG concentrations (SCB = 51.9 g/L; CON = 48.7 g/L) and colostrum yield (SCB = 5.9 kg; CON = 6.6 kg). However, the SCB cows had a greater ($P < 0.01$) fat percentage than CON cows (6.9% vs. 5.2%). Colostral protein and lactose concentrations were similar ($P > 0.05$) between treatment groups and parity. These data support previous literature findings that cows with a greater parity have a higher colostrum quality, yield, SCC, and lower fat concentration. These data also suggest that SCB supplementation during late gestation may be a feasible strategy to increase colostral fat content to support neonatal calf metabolism and development without impacting colostrum quality and yield.

Key Words: probiotic supplementation, colostrum composition, colostrogenesis

1397 Effects of repeated infusions of killed *Staphylococcus aureus* on milk yield and composition. B. D. Enger*, G. L. Hastings, C. S. Gammariello, M. X. S. Oliveira, and K. M. Enger, *The Ohio State University, Wooster, OH.*

Mastitis reduces milk yield of affected mammary glands but the mechanisms that reduce mammary gland productivity are unclear. We previously aimed to develop a reduced milk yield mastitis model via a single sterile intramammary infusion of oyster glycogen but failed to observe milk yield response even when milk SCC exceeded 3,000,000 cells/mL. The aim of this study was to assess the impact of repeatedly challenging mammary glands in a split udder model to determine if duration of immune response would affect milk yields. Four mid-lactation primiparous cows were used. Quarters of a randomly selected udder half were infused with saline (SAL) while the quarters of the opposite udder half were infused with 2 billion cfu of formalin-fixed *Staphylococcus aureus* (FX-STAPH). Intramammary infusions were repeated every 3 d over 12 d; milk yields and composition were measured at each milking (2×/d). Statistical analyses were conducted in SAS using PROC MIXED. Fixed effects included udder half treatment, time, and their interaction; cow was specified as a random effect. The interaction between treatment and cow was included as a repeated measure. Milk yield, composition, and somatic cell scores were similar between udder half treatments before challenge ($P \geq 0.28$). Somatic cell scores of FX-STAPH udder halves were greater than SAL udder halves at all postchallenge milkings ($P \leq 0.01$). Milk fat content was unaffected by udder half treatment ($P = 0.39$). Conversely, FX-STAPH udder halves had greater overall mean protein content and lesser lactose content than SAL udder halves ($P < 0.01$). FX-STAPH udder halves also had lower milk yields than SAL udder halves ($P < 0.01$). Generally, milk yield and component differences became more pronounced with repeated challenges. The results of this study indicate that the duration of somatic cell count elevation during subclinical or mild clinical mastitis significantly affects the degree of milk yield and compositional changes.

Key Words: mastitis, productivity, milk synthesis

1398 Impact of *Staphylococcus aureus* intramammary infections on mammary cell proliferation in pregnant heifers. M. X. S. Oliveira*, K. M. Enger, and B. D. Enger, *The Ohio State University, Wooster, OH.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Intramammary infection (IMI) is common in nonlactating pregnant heifers and can reduce future milk yields. The impact of IMI on mammary epithelial cell (MEC) proliferation may be negative, resulting in reduced mammary gland growth during first gestation. The objective of this study was to determine if IMI alters MEC and mammary stromal cell proliferation in dairy heifers. Twenty-one pregnant Holstein heifers divided across 3 different gestational ages (5.75, 6.75, and 7.75 mo pregnant) were utilized. One culture-negative quarter of each heifer was infused with saline (CTL) while the contralateral quarter was infused with 5,000 cfu of *Staph. aureus* (CHALL). Heifers were euthanized 21 d postchallenge and mammary tissue samples were collected from center parenchyma, located just above the gland cistern, and edge parenchyma, located near the mammary fat pad. At the time of sampling, animals were 6.5, 7.5, and 8.5 mo pregnant. Proliferating MEC and stromal cells were identified via Ki-67 staining. Total and Ki-67 positive MEC and stromal cells were quantified, and the data were analyzed using generalized linear mixed models, which included fixed effects of IMI status, gestational stage, mammary gland region, and all interactions. At 6.5 mo of pregnancy, greater MEC proliferation was observed in the edge region of CTL quarters compared with the central region ($P < 0.01$). In the central region, heifers at 8.5 mo of pregnancy exhibited increased MEC proliferation in their CTL quarters compared with those at 6.5 mo of pregnancy ($P = 0.04$). Interestingly, CTL glands at 8.5 mo of pregnancy displayed marginally greater MEC proliferation in the center region compared with the edge region ($P = 0.08$). The edge region of CHALL quarters in 6.5-mo pregnant animals showed reduced MEC proliferation compared with their CTL counterparts ($P =$

0.04). We were unable to identify any meaningful differences between CTL and CHALL glands on stromal proliferation. Our findings suggest that intramammary infections may have a greater impact on mammary epithelial cell proliferation at the sixth month of pregnancy.

Key Words: mastitis, mammary development, Ki-67

1399 Lactation stage-specific variations in health and lipid-associated milk fat globule membrane proteins in Holstein Friesian cow and Murrah buffalo.

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Bovine milk fat globule membrane (MFGM) proteins are crucial to calf health, animal physiology, and human nutrition. However, knowledge gaps exist regarding lactation stage-specific variations in MFGM protein profiles across cows and buffaloes. This study employed mass spectrometry (MS)-based proteomics to investigate (a) the inter- and intra-lactation stage/animal (Holstein Friesian cow [HF] and Murrah buffalo [Mu]) proteomics profiles and (b) the identification of functionally important MFGM proteins. Milk samples were collected from healthy HF cows (n = 9) and Mu buffalo (n = 9) during the winter season across lactation 1 (L1; 60–80 d), lactation 2 (L2; 120–140 d), and lactation 3 (L3; 220–240 d). The digested peptides were analyzed using MS and proteins identified against the *Bos taurus* database, followed by label-free quantitation (fold change with *P*-values = 0.05 and = 2). Protein functions were annotated and categorized using PANTHER Classification System, 13.1, and MetaboAnalyst was used to perform Multivariate analysis. A total of 571 proteins were identified across the 3 HF and Mu lactation stages, of which 250 in HF and 264 in Mu met stringent criteria. A lactation-specific comparison between HF L1 vs. Mu L1, HF L2 vs. Mu L2, and HF L3 vs. Mu L3. L3 showed the common and significant proteins between HF and Mu (n1 = 178, n2 = 93). In the L1, HF has more distinct proteins, whereas in the L2, Mu has more distinct proteins. Whereas L1 has the highest number of upregulated, L3 has the highest number of downregulated proteins. Further analysis within these cohorts yielded the identification of 48 upregulated and 16 downregulated proteins in HF L1 vs. Mu L1, 21 upregulated and 49 downregulated in HF L2 vs. Mu L2, and 38 upregulated and 55 downregulated in HF L3 vs. Mu L3. The functional significance of the MFGM proteins was investigated by categorizing them into health-associated proteins (47.1%) and lipid-associated proteins (44.1%). This analysis expands our understanding of lactation stage-specific MFGM proteins.

Key Words: milk fat globule membrane protein, lactation stage, mass spectrometry-based proteomics

1400 Establishment of physiologically more relevant bovine mammary epithelial cell lines.

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Bovine mammary epithelial cells (BMECs) serve as the fundamental secretory units of milk synthesis and are extensively used as in vitro models for investigating the mechanism of milk production. Although several BMEC lines such as MAC-T and BME-UV1 are available, they do not recapitulate in vivo function. This study aimed to establish an immortalized BMEC line that is physiologically relevant. Primary BMECs were isolated from mammary parenchymal tissue postmortem using enzyme digestion. Isolated cells were cultured and transduced with a lentivirus expressing the simian virus 40 T antigen (LVSV-40T). Immortalized cells were then cloned through limited dilution, resulting in 29 BMEC clones. These clones were subsequently characterized and evaluated using RT-qPCR for genes related to milk synthesis and secretion and epithelial cell identity (*CSN1S1*, *ESR1*, *LALBA*, *PRLR*, *OXTR*, *KRT14*, *PRGR*, *ITGA6*, *KRT18*, and *EPCAM*). Statistical analysis was carried out on R. Means were compared by one-way ANOVA followed by Tukey post hoc comparison. Five BMEC clones (clones #2, 13, 14, 17, and 24; Pass. 2) demonstrated a higher expression of genes *CSN1S1*, *ESR1*, *OXTR*, *LALBA*, and *PRGR* than the remaining clones ($P < 0.05$) and were selected for comparison with MAC-T (Pass. 8) and BME-UV1 cells (Pass. 7). Gene expression analyses revealed that clone 2 had a higher expression of milk protein genes *CSN1S1*, *CSN2*, *CSN3*, and *LALBA* ($P < 0.05$) compared with BME-UV1. Additionally, this clone exhibited a higher expression of EPCAM, a marker gene of epithelial cells, than MAC-T and BME-UV1 ($P < 0.01$). Interestingly, clone 17 showed an 800-fold higher expression of the estrogen receptor α gene, *ESR1* ($P < 0.01$) than BME-UV1, while the MAC-T cells showed no expression of *ESR1* and *PRGR*. The significantly higher expression of milk protein genes and hormone receptor genes in clones 2 and 17 than in MAC-T and BME-UV1 cells suggests that these BMEC clones could potentially serve as a physiologically more suitable model than the currently available cell lines for in vitro studies of the mechanisms of milk synthesis.

Key Words: bovine mammary epithelial cell, immortalized, in vitro study

1401 Mammary transcriptome response to palmitic acid and diet-induced milk fat depression in dairy cows.

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Milk fat yield can be increased by dietary supplementation with palmitic acid (PA) and decreased through diet-induced milk fat depression (MFD). Diet-induced MFD suppresses lipogenic transcription factors and key enzymes involved in milk fat synthesis, but the mechanisms underlying PA remain unclear. The objective was to characterize the mammary transcriptional response to PA and diet-induced MFD using RNA sequencing. Twelve multiparous mid-lactation cows were used in a 3 × 3 Latin square with 21-d periods; treatments were a low-fat control (CON), a high-PA acid supplement fed at 2% of diet dry matter (PALM), and MFD induced by a diet higher in starch and lower in fiber containing 2% soybean oil (MFD). Palmitic acid increased milk fat yield by 11%, while the MFD diet reduced milk fat by 37%. RNA from mammary biopsies collected at the end of each period were sequenced using Illumina NovaSeq. Differential gene expression MFD versus CON and PALM versus CON was analyzed using DESeq2 within R, and pathway analysis was conducted with Ingenuity Pathway Analysis (IPA). There was a distinct transcriptome response to MFD versus CON, with an upregulation of 705 genes and a downregulation of 780 (FDR <0.05). Key enzymes and transcription factors involved in fat synthesis were downregulated. Novel pathways related to interleukin-3,5,7, GM-CSF signaling, granzyme A signaling, and molecular mechanisms of cancer were downregulated. Pathways related to energy metabolism were upregulated, including those involved in electron transport, ATP synthesis, heat production by uncoupling proteins, and oxidative phosphorylation. Upstream analysis using IPA predicted potential transcriptional regulation by NFE2L2, XBP1, and HNF4A to be activated and TCF7L2, BACH1, and COPS5 to be inhibited. Comparing PALM to CON, only one gene, HBB, was identified as downregulated, and no genes were upregulated. This study highlights a complex mammary transcriptome response during diet-induced MFD, including possible ER stress, inflammatory signaling, epigenetic shifts, and energy metabolism alterations, and indicates that the response to PA is not transcriptionally regulated.

Key Words: milk fat, palmitic acid, diet-induced milk fat depression (MFD)

1402 Comparing the efficacy of serotonin and ethylene glycol tetraacetic acid (EGTA) on postpartum hypocalcemia prevention.

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Inducing a transient state of hypocalcemia prepartum mobilizes stored calcium (Ca) before the abrupt demand for Ca at parturition, which prevents postpartum hypocalcemia. Prepartum transient hypocalcemia can be achieved through intravenous infusions of either the precursor to serotonin, 5-hydroxy-tryptophan (5HTP), or a Ca chelating agent, ethylene glycol tetraacetic acid (EGTA). This study aimed to compare the efficacy of 5HTP and EGTA treatments in postpartum hypocalcemia prevention. We hypothesized that the 2 methods would be similarly effective compared with the control. Cows received 5HTP, EGTA, 5HTP+EGTA, or control saline (n = 6/treatment) starting 7 d before expected calving date through calving (range 4–13 d). 5HTP was administered daily as a single 1 L dose at 1.5 mg/kg BW. The EGTA and saline were infused for 6 h/d. Infusion rates of EGTA were manipulated to maintain blood ionized Ca (iCa) between 0.7 and 0.8 m*M* to induce subclinical hypocalcemia, which occurs when iCa is <0.9 m*M*. Mammary biopsies were taken from rear quarters at 6, 30, 54, and 78 h postpartum. 5HTP+EGTA cows required significantly less EGTA to maintain a low iCa during infusions, meaning 5HTP and EGTA had an additive effect in iCa reduction ($P < 0.05$). Control cows were subclinically hypocalcemic through 48 h postpartum, and 2 became clinically hypocalcemic. All other treatments were normocalcemic through 96 h postpartum and had significantly greater iCa than saline from 12 to 60 h postpartum ($P < 0.05$). Cows that received EGTA or 5HTP+EGTA had significantly lower mammary Ca compared with the control at 2 and 3 DIM ($P < 0.05$). Only EGTA tended to increase milk yield compared with the control with a mean difference of 4.19 kg/d through 7 DIM ($P < 0.09$), but there were no differences in milk Ca between treatments. qPCR and Western blot analysis showed a tendency for elevated gene expression and protein volume of secretory pathway calcium ATPase on the day of calving ($P < 0.10$). 5HTP and EGTA both prevented postpartum hypocalcemia, but EGTA may be more effective because it resulted in significantly greater iCa compared with 5HTP at 0, 6, 12, 24, and 48 h postcalving ($P < 0.05$).

Key Words: hypocalcemia, serotonin, calcium

Production, Management, and the Environment Platform Session | 45th Discover® Conference: Dairy Cattle Lifespan—New Perspectives

1403 Revisiting the 45th ADSA Discover Conference—Dairy Cattle Lifespan: New Perspectives. A. De Vries[1], M. Lucy*[2], C. Baes[7], N. Friggens[3], P. Kononoff[4], D. Nydam[5], and E. Vasseur[6], [1]*University of Florida, Gainesville, FL,* [2]*University of Missouri, Columbia, MO,* [3]*French National Institute for Agriculture, Food, and Environment (INRAE), Paris, France,* [4]*University of Nebraska, Lincoln, NE,* [5]*Cornell University, Ithaca, NY,* [6]*McGill University, Montréal, Quebec, Canada,* [7]*University of Guelph, Guelph, Ontario, Canada.*

The 45th ADSA Discover® Conference was held October 23 to 26, 2023, in Itasca, Illinois. Extended dairy cow lifespan is said to be good for sustainability and the image of the dairy sector. In practice, however, the topic is complex and simple perceptions do not capture the breadth of the underlying questions. The conference was designed, therefore, to take an in-depth look at dairy cow lifespan and explore new perspectives in the modern era of dairy production. Speakers and attendees explored the many facets of lifespan including definitions (productive life vs. longevity or lifespan), the underlying biology, genetics, and genomic selection, and important management factors including nutrition and housing. Importantly, economics were modeled across different countries and the environmental impact was assessed for herds under different production systems and with different age structures. Producer and industry panels provided diverse ideas about goals for lifespan in different countries and communities. The conference was successful in that there were topics that were generally agreed-upon (replacement of an individual cow should be based on her competitive economic merit; long lifespan should not be the goal unless it supports profitability). Disagreements existed across speakers and attendees on the importance of lifespan to consumer perspectives of dairying as well as its environmental impact. These disagreements identified important knowledge gaps and opportunities such as quantifying maturity and future cow performance, and the importance of early lactation culling. Rapidly changing genetics of cows, technological advances that affect heifer supply (embryo transfer, sexed semen, etc.), new lucrative markets for dairy farms (including meat from cull cows and the sale of beef-dairy crossbred calves), and impending legislative action on climate change will impact dairy farms and their decisions to keep or cull their older cows. Despite advances in husbandry, precision dairy farming technologies, and decision support tools, when cows should be replaced and what replacement rates should be remain areas for further investigation.

Key Words: longevity, productive life, culling

1223 Effect of dry period heat stress and rumen protected choline on productivity of Holstein cows. M. A. T. de Bari*[1], K. Estes[2], C. Zimmerman[2], H. Olmo[1], D. Onan-Martinez[1], J. Magalhaes[1], N. Jeronymo[1], J. Lance[1], M. Martin[1], L. C. Trevisan[1], I. M. Toledo[1], and G. E. Dahl[1], [1]*University of Florida, Gainesville, FL,* [2]*Balchem, Montvale, NJ.*

Dry period heat stress significantly reduces milk yield in the subsequent lactation in cows. Altered methylation patterns in mammary tissues is associated with the decline in milk yield. We hypothesized that as a methyl donor, choline (RPC) supplementation reverses similar patterns of methylation and thus productivity in heat-stressed dams. Late-gestation Holstein cows (n = 51) housed in freestall barns were enrolled to either heat stress (HT, n = 12), HT with RPC (HTC, n = 11), cooling (CL, n = 14), or CL with RPC (CLC, n = 14). A cooling system including shade, fans, and soakers was provided to CL and CLC cows, whereas HT and HTC cows only had shade. Choline treatment consisted of 30 g of RPC per day mixed with TMR. Dry matter intake (DMI), rectal temperature (RT), and respiration rate (RR) were assessed in the dry period (DP), and milk yield was tracked for 60 DIM. Statistical analyses were performed using the PROC MIXED procedure of SAS. HT+HTC increased RR and RT (78.7 ± 2.3 vs. 61.7 ± 1.9 bpm; 39.3 ± 0.07 vs. 38.8 ± 0.05°C; $P < 0.01$) relative to CL+CLC. HT exposure reduced DMI relative to CL (13.2 ± 0.4 vs. 14.4 ± 0.3 kg/d; $P = 0.01$). DMI of HTC cows was lower relative to HT (12.4 ± 0.4 vs. 13.8 ± 0.4 kg/d; $P = 0.02$). Gestation length (GL) was shorter in HT (HT+HTC; 271.2 ± 0.5) versus CL (CL+CLC; 274.9 ± 0.5 d; $P < 0.01$). HTC had a shorter GL and DP compared with HT (268.6 ± 0.9 vs. 273.6 ± 0.8 d; 38.6 ± 1 vs. 43.2 ± 0.9 d; $P < 0.01$), whereas CLC had a longer GL and DP than CL (275.8 ± 0.6 vs. 273.9 ± 0.6 d; 46.6 ± 0.6 vs. 43.9 ± 0.6 d; $P = 0.03$). Colostrum yield ($P < 0.01$) was lower in HT+HTC (7.4 ± 0.5 kg) relative to CL+CLC (11.5 ± 0.5 kg). Cooled calves (CL+CLC) were born heavier than heat-stressed (HT+HTC) calves (37.9 ± 0.4 vs. 33.3 ± 0.5; $P < 0.01$). HTC calves were born lighter than HS calves (31.7 ± 0.6 vs. 34.7 ± 0.6 kg; $P < 0.01$). In CL, ECM was increased compared with HT cows (54 ± 1.6 vs. 47.5 ± 1.7 kg/d; $P < 0.01$). An interaction of ECM yield was observed among treatments ($P = 0.06$): CL = 53, CLC = 54.9, HT = 47.9, and HTC = 47.1 kg/d. These results suggest that choline does not mitigate the negative impact of heat stress in prepartum cows but improves ECM yield when combined with cooling.

Key Words: dairy cattle, heat stress, choline

1239 Short- and long-term effects of pasteurized waste milk on calf growth, health, and subsequent performance and longevity. S. R. Li*, T. Y. Chen, S. Liu, S. L. Li, and Z. J. Cao, *China Agricultural University, Beijing, China.*

This study aimed to investigate the effects of pasteurized waste milk and milk replacer feeding on the growth and health of calves, as well as the long-term influence on milk production, health, and reproduction up to the fifth parity. A total of 240 newborn Holstein female calves (35.3 ± 5.7 kg) were randomly divided into 2 groups: pasteurized waste milk (PW, n = 120) or milk replacer (energy concentration adjusted to be consistent with PW based on the calculated energy content; MR, n = 120) feeding during the pre-weaning period. Upon birth, all calves received 4 L of colostrum within 1 h. From d 1 to d 7, calves were placed in individual hutches and fed 3 times a day. From d 8 to d 67, every 15 calves were transitioned sequentially according to age to a pen with automatic calf feeders, and gradually reduced feeding after d 40 and weaned on d 67. Data on feed intake, feces, cough scores, physiological parameters, and production up to fifth parity were collected. Data were analyzed using MIXED, ANOVA, GLM, and chi-squared test models of SAS 9.2. The results showed the PW group had a higher ADG (789.40 ± 195.31 g vs. 634.02 ± 192.29 g) but lower starter feed intake (251.60 ± 59.70 g vs. 359.80 ± 98.90 g) and diarrhea frequency (0.05 ± 0.13 vs. 0.14 ± 0.17) than the MR group during the pre-weaning period. Moreover, the MR group exhibited higher blood C3 (0.25 ± 0.11 vs. 0.37 ± 0.07) and cortisol (5.79 ± 5.00 vs. 12.63 ± 7.96) concentrations than the PW group 1 wk after weaning. The PW group's first-parity colostrum quality was lower (Brix, 24.50 ± 2.70 vs. 27.50 ± 3.30), whereas the 305-d milk yield was higher only in the second parity (4,928.63 ± 2,393.92 kg vs.

3,949.52 ± 2,424.16 kg). No significant differences were observed in reproductive performance, health, or culling rate up to the fifth parity. In summary, PW positively influenced pre-weaning calf health and growth, but long-term outcomes up to the fifth parity showed no disparities in health, performance, or longevity compared with MR-fed calves, despite some specific parameter differences.

Key Words: pasteurized waste milk, calf, long-term effects

1311 A health monitoring program based on automated health alerts identified more cows with health disorders and improved herd performance compared with exclusive use of visual observation to select cows for clinical examination. C. Rial*[1], M. L. Stangaferro[2], M. J. Thomas[2], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Dairy Health and Management Services, Lowville, NY.*

The objectives of this randomized trial were to compare the percentage of cows that underwent clinical examination and were diagnosed with clinical health disorders (HD), and the effects on milk yield (MY), the herd exit dynamics, and first insemination outcomes of a health monitoring program that relied only on automated health monitoring alerts or a program that relied only on visual observation to select cows for examination. Lactating Holstein cows fitted with a neck-attached rumination and physical activity monitoring sensor (SenseHub, Merck Animal Health) were randomly assigned to an automated health monitoring (AHM n = 607) or a visual observation (VO n = 597) group for monitoring health from 3 to 21 d in milk (DIM). Cows in the VO group were selected for clinical examination exclusively based on VO of clinical signs of disease, whereas cows in the AHM group were selected for examination based on health alerts (Health Index Score <86 arbitrary units, daily rumination <250 min, or a reduction of >20% in daily MY monitored 3×/d by parlor milk meters [MM32, DeLaval]). The clinical exam, conducted by the research team, was the same for both groups. Binary data were analyzed with logistic regression and continuous data by ANOVA with or without repeated measures. More cows underwent clinical examination ($P < 0.01$; AHM = 62%, VO = 29%) and were diagnosed with at least one HD ($P < 0.01$; AHM = 35%, VO = 21%) in the AHM than in the VO group. Cows in the AHM group had more accumulated milk than cows in the VO group from 2 to 21 DIM ($P = 0.01$; VO: 509 ± 14 kg, AHM: 542 ± 12 kg). Cows in the AHM group with HD produced more milk ($P < 0.01$) from 9 to 13 and 20 to 21 DIM than cows with HD in the VO group. No effects of treatments were observed on pregnancies per AI at first service ($P = 0.57$; VO = 37%, AHM = 39%) or the herd exit ($P = 0.13$; VO = 21%, AHM = 17%). Monitoring cow health based on automated monitoring systems data and alerts might be a more effective alternative for health monitoring than exclusive use of VO of clinical signs of disease.

Key Words: dairy cow, health monitoring, sensors

1535 Association of uterine health in the first lactation with transition cow health and reproductive performance in the second lactation of Holstein dairy cows. S. Borchardt[1], T. A. Burnett[2], M. Drillich[1], K. Wagener[3], and A. M. L. Madureira*[2], [1]*Clinic of Animal Reproduction, Freie Universität Berlin, Berlin, Germany,* [2]*University of Guelph, Ridgetown, ON, Canada,* [3]*University of Veterinary Medicine Vienna, Vienna, Austria.*

The objective of this study was to evaluate the effects of puerperal metritis (PM) on the first lactation (1st L) on transition cow health and reproduction of dairy cows in their second lactation (2nd L). Two farms were enrolled in this study (Farm A and B). In both farms, the following diseases were recorded during the first 30 DIM: clinical hypocalcemia, retained fetal membrane (RFM), PM, hyperketonemia, left displaced abomasum, and clinical mastitis. Cows were inseminated for first service using only timed AI after submission to a Double-Ovsynch protocol (Farm A) or inseminated after estrus detection using an automated activity sensor (Farm B). Based on the diagnosis of PM in the first and second lactation, cows were classified into 4 groups: (1) no PM in the 1st L and no PM in 2nd L (NoPM+NoPM), (2) no PM in the 1st L and PM in the 2nd L (NoPM+PM), (3) PM in the 1st L and no PM in the 2nd L (PM+NoPM), and (4) PM in the 1st L and PM in the 2nd L (PM+PM). A total of 4,834 cows (Farm A) and 4,238 cows (Farm B) in the 2nd L were considered for statistical analyses. Statistical analyses were performed using SPSS for Windows. On both farms, cows with PM in their 1st L had greater odds for RFM and PM in their 2nd L, while there was no association of PM in the 1st L with any other nonuterine diseases in the 2nd L. On Farm A, pregnancy per AI at first AI was 43.4 ± 1.3, 31.3 ± 2.7, 39.4 ± 2.3, and 44.3 ± 4.7% and on Farm B was 53.8 ± 3.4, 39.3 ± 4.9, 49.5 ± 4.3, and 23.7 ± 6.0% for 2nd L cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM, respectively. Pregnancy loss at first AI was 6.9 ± 0.7, 12.5 ± 3.0, 9.3 ± 1.8, and 18.8 ± 4.9% for 2nd L cows, on Farm A and on Farm B it was 8.6 ± 1.5, 19.6 ± 5.3, 11.8 ± 3.0, and 26.9 ± 11.6% for cows in NoPM+NoPM, NoPM+PM, PM+NoPM, and PM+PM, respectively. On both farms, there was an association with uterine health classification and pregnancy loss in second lactation at first AI. Puerperal metritis in the first lactation is associated with long-lasting negative effects for cows in their next lactation.

Key Words: puerperal metritis, pregnancy loss, pregnancy per artificial insemination (AI)

1542 Effect of dry cow vaccination and administration of a nonsteroidal anti-inflammatory drug on inflammation, immunity, and performance of dairy cows. J. Gao*, G. G. Cunha, N. L. P. Kant, C. G. Savegnago, T. N. Marins, A. M. Roper, and S. Tao, *Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

This study aimed to examine the effect of dry cow vaccination schedule and administration of a nonsteroidal anti-inflammatory drug on inflammation, immunity, and subsequent milk yield (MY) in a commercial dairy farm. Standard operating procedure (SOP) at dry-off (DO) includes blanket dry cow therapy and vaccination (5 mL Enviracor J-5 subcutaneously, 2 mL Scourguard 4KC intramuscularly, and 5 mL Ultrabac 7 subcutaneously, Zoetis) after last milking. Dry cows were housed in a barn until calving. Lactating cows were milked 3×/d with MY recorded. In late lactation, healthy cows were blocked by parity, days in milk, and MY and then randomly assigned to (1) SOP (n = 56); (2) vaccination at −14 d relative to DO (DRD) (EV, n = 55); (3) vaccination at −14 DRD and oral administration of meloxicam at 0 (the day of DO) and 3 DRD (EN, n = 56). SOP and EV cows received placebo orally at 0 and 3 DRD. All cows were managed similarly after 3 DRD. Plasma was collected at −16, −12, −10, −5, 0, 1, 2, 3, 4, 5, 6, 10 DRD to analyze haptoglobin (HP). Blood hematological profiles were assessed at −15, −5, 2, 6 DRD. Neutrophil chemotaxis to interleukin 8 was measured in a Transwell system at −16 and 2 DRD. Peripheral blood mononuclear cells (PBMC) proliferation stimulated by concanavalin A and lipopolysaccharide was assessed at −15 and 3 DRD. Data were analyzed using PROC MIXED of SAS with block as a random effect. Giving vaccines earlier reduced ($P \leq 0.02$) MY before DO but treatments did not ($P = 0.70$) affect MY up to 8 weeks in milk. EV and EN cows had greater circulating HP at −12 and −10 DRD while SOP cows had greater circulating HP at 1 to 6 DRD ($P \leq 0.01$). Vaccination at DO increased circulating neutrophil and lymphocyte counts at 2 DRD than EV and EN ($P \leq 0.01$). Treatments did not ($P > 0.70$) affect PBMC proliferation or neutrophil chemotaxis. In conclusion, giving vaccines 14 d before DO had no effects on MY in early lactation but reduced systemic inflammation and circulating immune cell number after DO. Administration of meloxicam at and following DO has no effects on systemic inflammation and immunity.

Key Words: dry-off, meloxicam, inflammation

1615 Exploring variables related to survival and production in dairy replacements. M. W. Overton* and A. K. McNeel, *Zoetis, Parsippany, NJ.*

The objective of the study was to examine relationships between variables available around first calving from the on-farm record system for production, reproduction, and survival through second lactation. Production and genomic data from the first 2 lactations from 53,333 cows first calving in 2019–2021 were extracted from 28 herds. First-lactation performance (projected 305-d milk, time to pregnancy through 250 DIM, time-to-removal and cumulative milk through 450 DIM), cumulative milk, and cumulative survival through 305 DIM of second lactation were evaluated using multivariable approaches. Milk production was evaluated using mixed models with herd, birth month, birth year, calving month, and calving year as random effects. Cox proportional hazards models were used for reproduction and survival. Logistic regression was used for cumulative survival through second lactation. For each model, fixed effects included age at first calving, age at first calving^2, Dairy Wellness Profit Index (DWP$®) either as a continuous or categorical outcome, body size composite (BDC) either as a continuous or categorical outcome, calf outcome (male, female, or twins), calf status (live or dead), previous gestation length, and previous gestation length^2. Herd, birth, and calving variables used as random effects within the mixed models were used as fixed effects in the other models. Age at first calving had varying effects depending upon lactation and outcome. In first lactation, older age at calving was associated with greater milk production, reduced reproductive performance, and a higher probability of removal. For cumulative results, age at first calving was not a significant predictor for either milk production or survival. Conversely, increasing DWP$ was consistently associated with greater milk production, improved reproduction, and improved survivability across both lactations. Increasing age at first calving is not a sound strategy to improve production and survivability through the first 2 lactations, but genetic improvement due to higher DWP$ ranking was associated with improved production, reproduction, and survival through these 2 lactations.

Key Words: replacement, genomic, lifetime

1618 Withdrawn.

1641 Extent of mobilization and histological changes of skeletal muscle tissue in transition dairy cattle. K. M. Gouveia*, H. Rehman, L. M. Beckett, J. F. Markworth, T. M. Casey, and J. P. Boerman, *Department of Animal Sciences, Purdue University, West Lafayette, IN.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

The objective of this study was to determine the effects of prepartum skeletal muscle reserves and branched-chain volatile fatty acid (BCVFA) supplementation on the extent of muscle mobilization through the transition period and to determine if muscle reserves and BCVFA affect *longissimus dorsi* (LD) muscle fiber type and size at −21 d pre- and 21 d postpartum. Muscle reserves were assessed by ultrasounds of LD depth and cows (n = 48) were assigned to either high muscle (HM; >4.6 cm) or low muscle (LM; ≤4.6 cm) groups based on LD depth 42 d before expected calving. Cows were then randomly assigned to either control (CON; soyhull pellets, 80 g/d) or BCVFA treatment (isobutyrate, isovalerate, and 2-methylbutryate at 40 g/d, 20g/d, and 20g/d, respectively) that were fed prepartum. Treatments resulted in 4 combinations of muscle group and treatment: HM-CON (n = 13), HM-BCVFA (n = 13), LM-CON (n = 11), LM-BCVFA (n = 11). The LD depth was measured on −42, −35, −21, −7, 0, 7, 14, 21, 28 d relative to calving to determine the extent and timing of muscle mobilization. Biopsies of the LD were performed on −21 and 21 d relative to calving and preserved for histological analysis. Blood samples were taken −42, −21, −14, 7, and 21 d relative to calving to determine plasma concentrations of 3-methylhistidine (3-MH) and creatinine. Prepartum, HM cows mobilized 0.4 cm of muscle, and LM cows accreted 0.2 cm of muscle ($P < 0.0001$). Postpartum, both HM and LM cows mobilized 0.9 cm of muscle. Compared with CON, 3-MH tended to be lower in BCVFA cows at 7 d postpartum ($P = 0.09$) with no differences in creatinine. Muscle histological analysis found a 10.5% reduction in mean muscle fiber cross sectional area (CSA) in all cows, pre- to postpartum ($P = 0.03$), with no change in the percent distribution of Type I and Type II muscle fiber types ($P = 0.97$). High muscle cows had greater myofiber CSA compared with the LM cows both pre and postpartum ($P = 0.003$). Branched-chain volatile fatty acids had no effect on muscle fiber CSA or percentage of fiber type. Results indicate that prepartum skeletal muscle reserves affect muscle mobilization timing, extent, and muscle fiber size.

Key Words: longissimus dorsi, branched-chain volatile fatty acids, muscle fiber

1648 Long-term effects of preweaning social housing on heifer performance and reproductive development. E. E. Lindner*[1], T. Martins[2], C. Burner[1], and E. K. Miller-Cushon[1], [1]*University of Florida, Gainesville, FL,* [2]*Mississippi State University, Raymond, MS.*

Evidence supports short-term performance and welfare benefits of dairy calf social housing, but longer-term implications for dairy heifer development remain unclear. The objective of this study was to assess the effects of social housing during the preweaning period on dairy heifer growth and reproductive success. Holstein heifers were housed either individually (IH; n = 55), in pairs (PH; n = 55; 1 focal heifer/pair), or in groups (GH; n = 53; focal animals selected from 9 groups of 10 calves/group) from birth until 9 weeks of age. Beginning at 6 mo of age (189.9 ± 11.8 d of age), body weight (BW) and hip height were recorded weekly for 24 wk. Heifers were also placed with an Estrotect breeding indicator, which was examined 1x/week for evidence of behavioral estrus. Ultrasounds were performed weekly until confirmation of estrus with the presence of a corpus luteum. Data were analyzed in general linear models including previous housing treatment, birthweight and preweaning health score (% of evaluations with temperature >38.9°C). Preweaning housing did not affect BW (263.9 kg; SE = 2.92; $P = 0.23$) but hip height was consistently greater for previously PH and IH heifers compared with GH (124.9 vs. 124.0 vs. 122.2 cm; PH vs. IH vs. GH; SE = 0.45, $P < 0.001$). Age at first expression of estrus behavior (298.8 d; SE = 7.2; $P = 0.33$) and first estrus cycle (326.6 d; SE = 8.0; $P = 0.59$) did not differ. Preliminary analysis of calving outcomes (n = 130; IH

= 46; PH = 50; GH = 34) suggested that treatment did not affect age at calving (695.7 d; SE = 6.2; *P* = 0.36), but previously PH heifers tended to weigh more at calving, compared with previously IH and GH heifers (657.3 vs. 631.7 vs. 636.2 kg; PH vs. IH. vs. GH; SE = 8.8; *P* = 0.06). These results indicate potential long-term effects of social housing on heifer growth, with similar reproductive outcomes across preweaning housing treatments.

Key Words: performance, social contact, welfare

Ruminant Nutrition 4: Protein and Amino Acids, and Ruminant Nutrition 3-Minute Poster Spotlights

1404 Effects of feeding controlled and high-energy diets with rumen-protected lysine and methionine prepartum on performance of Holstein cows. E. O'Meara*[1], D. del Olmo[2], J. Aguado[2], J. Drackley[1], and F. Cardoso[1], *[1]University of Illinois, Urbana, IL, [2]Kemin Industries Inc., Des Moines, IA.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

This study aimed to determine the effects of rumen-protected lysine (RPL) and methionine (RPM) fed in the same AA to metabolizable energy (ME) ratio in peripartum diets with different net energy of lactation (NE_L) concentrations on performance of Holstein cows. Sixty-two multiparous Holstein cows blocked by parity, previous 305-d mature-equivalent milk production, and BCS during the far-off dry period were assigned to 1 of 3 dietary treatments. Prepartum (−21 d to expected calving), animals were fed a controlled-energy diet (1.45 NE_L, Mcal/kg of DM) with rumen-protected lysine (RPL; Kemin Industries Inc., Des Moines, IA) and rumen-protected methionine (RPM; Kemin Industries Inc.) (CEAA; 0.15% RPL and 0.09% RPM of DMI), controlled-energy diet without RPL and RPM (control; CENAA), or high-energy diet (1.71 NE_L, Mcal/kg of DM) with RPL and RPM (HEAA; RPL 0.22% and RPM 0.12% of DMI). Postpartum, cows received the same lactation TMR (1.73 NE_L, Mcal/kg of DM) without RPL and RPM (CENAA, n = 19) or with RPL and RPM (CEAA, n = 21; and HEAA, n = 21; 0.38% RPL and 0.15% RPM of DMI) until 70 d relative to calving. Body weight and BCS were recorded weekly and DMI daily. Cows were milked twice daily. Milk samples were taken weekly and analyzed for components. Statistical analyses were performed using the MIXED procedure of SAS. Two treatment contrasts CENAA vs. CEAA and CEAA vs. HEAA were compared. The results of the study are found in Table 1. In conclusion, feeding RPL and RPM in a CE diet prepartum improved cows' performance.

Key Words: methionine, lysine, energy

Table 1 (Abstr. 1404). Effects of feeding RPL and RPM fed in the same AA:ME ratio on postpartum performance of Holstein cows

Period	Variable	Treatment			SEM	*P*-value	
		CENAA	CEAA	HEAA		CENAA vs. CEAA	HEAA vs. CEAA
Wk 1–4	Milk variable						
	Fat, kg/d	1.85	1.82	2.04	0.09	0.77	0.45
	ECM, kg/d	46.74	45.70	50.49	1.77	0.68	0.48
Wk 5–10	DMI, kg/d	22.94	25.12	24.55	0.65	0.02	0.53
	EBAL, Mcal/d[1]	−6.09	−3.18	−5.40	0.81	0.01	0.06
	BCS change	−0.51	−0.05	−0.24	0.12	<0.01	0.24
	Milk variable						
	Protein, %	2.72	2.89	2.85	0.05	0.02	0.53
	MUN, mg/dL	8.65	9.84	9.57	0.40	0.04	0.64

[1] Energy balance (EBAL) was calculated based on NASEM (2021).

1405 Development and characterization of a sustainable encapsulated methionine source. T. Danese[1], G. Mantovani[1], E. E. Gültepe*[2], D. Martinez Del Olmo[2], R. Spaepen[2], P. Mannina[3], M. Romero Huelva[4], I. Rivelli[4], D. Yáñez Ruiz[4], D. Van Wesemael[5], F. Righi[1], and F. Nuyens[2], *[1]Department of Veterinary Science, University of Parma, Parma, Italy, [2]Kemin Europa NV, Herentals, Belgium, [3]Kemin Cavriago, Cavriago (RE), Italy, [4]Estación Experimental del Zaidín, CSIC, Granada, Spain, [5]ILVO (Flanders Research Institute for Agriculture Fisheries and Food Research), Melle, Belgium.*

The study was conducted to characterize a sustainable encapsulated methionine (enMET) source that has been recently developed via the replacement of a synthetic polymer (2-vinyl pyridine/styrene; VP) with a chitosan-based biopolymer (CH; KESSENT® M*e*, Kemin Animal Nutrition and Health, Belgium). In experiment (Exp) 1, to evaluate the biodegradation of polymers, biological O_2 demand (BOD) was measured for 20 d in a chamber, and theoretical O_2 demand (ThOD) was estimated from chemical formula. Based on both values, biodegradation (BioDeg) was calculated. The BOD and ThOD (g O_2/L) were 66.0 and 1.2 for CH; 2.4 and 20 for VP. BioDeg for the CH vs. VP was calculated as 56.7 vs. 0.1. In Exp 2, rumen batch cultures were used to evaluate the effects of ruminal incubation on the physical structure of both polymers. Duplicate samples were obtained at 0, 2, 4, 6, 8, 10, and 12 h. Microscopy analysis showed structural integrity alterations of VP polymers after 4 h compared with the CH by 12 h. Euclidean distance algorithm was used to judge polymer integrity on the whole FTIR spectrum, $-COOH$, and $-NH_2$ groups. The VP showed greater spectral dissimilarity from the time-zero (1.0) compared with the CH (whole 0.75 vs. 0.91; $-NH_2$ 0.88 vs. 0.95; $-COOH$ 0.55 vs. 0.91). In Exp 3, in vitro ruminal and intestinal degradability of CH samples (n = 145) was evaluated. The means of ruminal degradability (%) of DM and CP were 2.2 ± 0.2 and 3.8 ± 0.5; 5.1 ± 0.2 and 10.3 ± 0.5; 9.9 ± 0.3 and 17.4 ± 0.7; and 20.7 ± 0.5 and 34.0 ± 1.0 after 4, 8, 12, and 24 h of incubation, respectively. Total-tract degradability (%) of DM and CP were 62.2 ± 0.5 and 91.0 ± 0.6 after 12 h rumen followed by 24 h enzymatic treatment. In Exp 4, 2 ruminal/duodenal fistulated cows were used. In situ rumen CP degradation (%) of CH were 0 and 11.4 ± 5.2 after 8 and 16 h incubation, respectively. After 8 h of rumen incubation, enzymatic treatment and intestinal incubation with mobile bags, CP of CH was completely digested after 9h:43m mean transit time. In conclusion, the biopolymer-based coating of enMET showed substantial biodegradation, while maintaining high ruminal stability and high intestinal availability.

Key Words: methionine, degradability, sustainability

1406 Evaluation of a rumen-protected methionine product to improve amino acid balance in lactating cows. T. Danese*[1], P. A. LaPierre[2], A. B. P. Fontoura[2], D. M. Barbano[3], O. R. Drehmel[4], J. J. Albrecht[4], P. S. Yoder[4], M. F. Scott[4], and M. E. Van Amburgh[2], *[1]Department of Veterinary Science, Parma University, Parma, Italy, [2]Department of Animal Science, Cornell University, Ithaca, NY, [3]Department of Food Science, Cornell University, Ithaca, NY, [4]Milk Specialties Global, Eden Prairie, MN.*

The study objective was to evaluate a rumen-protected methionine product (RP-Met, Milk Specialties Global, Eden Prairie, MN) designed to help meet the Met requirements for energy-corrected milk yield of dairy cattle. The study was conducted for 84 d with 144 lactating Holstein cows in replicated 16-cow pens, with 3 pens per treatment. Three diets were formulated, using the Cornell Net Carbohydrate and Protein System v6.5.5, to supply 0.96 g/Mcal ME (no RP-Met), 1.05 g/Mcal ME, and 1.19 g/Mcal ME, corresponding to 80%, 88%, and 100% of requirements, respectively. Cows were fed isocaloric diets, formulated for 5% sugar, 28.5% starch, 28.5% aNDFom, 4.3% EE (DM basis), and metabolizable Lys and His at 3.2 and 1.19 g/Mcal ME, respectively. Cattle were milked 3 times daily, where milk was sampled weekly in 3 contiguous milkings and analyzed for fatty acids (FA), true protein, MUN, and somatic cell count. Milk yield and pen dry matter intake were recorded daily by the farm software. Data were analyzed using a mixed model in SAS, with the fixed effect of diet and random effects of pen nested within diet and cow nested within pen and diet. Energy-corrected milk was greater in cattle fed diets 2 and 3 (50.1 and 50.4 kg/d, respectively) versus diet 1 (48.7 kg/d; SEM 0.31; $P = 0.02$). As supplemental Met increased, milk true protein percentage increased from 3.09% to 3.24% and 3.34% (SEM 0.01; $P < 0.01$), across diets 1, 2, and 3, respectively. Milk fat percentage was greater in diet 3 (4.36%; SEM 0.03; $P < 0.01$) compared with cattle fed diets 1 and 2 (4.21% and 4.25%, respectively). De novo FA concentration increased (SEM 0.01; $P < 0.01$) with increasing Met supply, consistent with the increase in milk protein, and mixed FA were greater in cattle fed diet 3 compared with diet 1 (1.70 vs. 1.65 g/100 g; SEM 0.015; $P < 0.01$). In summary, supplementation of this product improved the supply of metabolizable Met across diets, resulting in significant increases in milk protein percentage and yield and fat percentage, when metabolizable Lys and His were not predicted to be deficient.

Key Words: methionine, milk component, amino acid

1407 Sunflower meal as an alternative protein source for ruminants. C. Vande Berg*, S. McLeod, and V. Fellner, *North Carolina State University, Raleigh, NC.*

The effect of partially dehulled sunflower meal (SF) as a protein source in ruminant diets was evaluated using *in vitro* fermentation in batch cultures with mixed ruminal microorganisms. Sunflower meal is a byproduct of the sunflower oil industry (36.4% CP, 39.4% NDF, 0.74% Met, 1.32% Lys). Our objectives were (1) to compare the effect of SF to soybean meal (SB: 48.9% CP, 8.4% NDF, 0.64% Met, 2.80% Lys) and (2) to determine inclusion level of SF when included in corn silage (S) or orchard grass hay (G) diets. Six experimental diets were formulated on a dry-matter basis as follows: (1) 50% S + 25% SB (SSB: 18.4% CP, 78.6% TDN); (2) 50% G + 25% SB (GSB: 20.9% CP, 68.1% TDN); (3) 50% S + 25% SF (SSF: 14.3% CP, 69.9% TDN); (4) 25% S + 50% SF (SSFH: 21.2% CP, 68.8% TDN); (5) 50% G + 25% SF (GSF: 17.3% CP, 62.8% TDN); and (6) 25% G + 50% SF (GSFH: 22.4% CP, 62.7% TDN). All diets included 24% ground corn and 1% vitamin mineral premix. Diets were weighed in triplicate and inoculated for 24 h at 39°C in 2 independent runs (n = 2). Rumen inoculum was obtained from a cannulated heifer fed a predominantly forage diet. Data were analyzed using the Proc-Mixed procedure of SAS. Preplanned orthogonal contrasts were used to determine effect of SB vs. SF within forage (SSB vs. SSF and GSB vs. GSF) and level of SF inclusion (SSF vs. SSFH and GSF vs. GSFH). Soluble protein was greater in SF compared with SB (15.6% vs. 9.0%). Total VFA were not affected ($P > 0.10$) by treatment in either forage. GSF reduced ($P < 0.05$) propionate (P) when compared with GSB (30.7 vs. 38.6 m*M*, respectively, SE = 1.42 m*M*). SSF decreased ($P < 0.05$) methane when compared with SSF (5.0 vs. 5.5 mmol/L, respectively, SE = 0.17 mmol/L). Irrespective of forage source, SF included at 25% increased ($P < 0.05$) culture pH (5.7 vs. 5.6 in SSF vs. SSB [SE = 0.01], 5.6 vs. 5.5 in GSF vs. GSB [SE = 0.03]). Rumen NH_3-N increased ($P < 0.01$) with GSF compared with GSB (22.0 vs. 17.6 mg/100 mL, respectively, SE = 0.91 mg/100 mL). Data indicate that SF was comparable to SB as a protein source and better supported a fermentation consistent with enhanced fiber utilization. Increasing dietary energy may improve efficiency of N use in diets with greater SF inclusion.

Key Words: sunflower meal, soybean meal, rumen microbe

1408 Effects of a plant extract-based rumen modifier and rumen-degradable protein levels on nitrogen and energy utilization in lactating dairy cows. K. V. Almeida*[1], D. C. Reyes[1], R. A. Menezes[1], M. H. Khandakar[1], M. A. Rahman[1], M. M. Carvalho[1], P. Piantoni[2], G. F. Schroeder[2], K. Cowles[2], and A. F. Brito[1], *[1]Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH, [2]Cargill Animal Nutrition and Health, Innovation Campus, Elk River, MN.*

We aimed to investigate the effect of combining a rumen modifier (RM) containing condensed tannins and spice extracts with 2 levels of RDP on urinary excretion of purine derivatives (PD), CH_4 emissions, and N and energy utilization in dairy cows. Sixteen multiparous Holstein cows (81 ± 33 DIM and 750 ± 79 kg BW) were used in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments and 28-d periods. Data and sample collection was done during the last 7 d of each period. Treatments were (1) low RDP diet without RM (LRDP), (2) LRDP plus 15 g of RM top-dressed (Valido PRO®, Cargill Inc.), (3) high RDP diet without RM (HRDP), and (4) HRDP plus 15 g of RM top-dressed. Diets were formulated (DM basis) to yield 9.00% and 10.6% RDP and 15.0% and 16.8% CP for the LRDP and HRDP treatments, respectively. Urinary PD was measured using a spectrophotometer, and the gross energy of feed, feces, and urine samples was determined using a bomb calorimeter. Methane emissions were measured during 4 d of each period with a GreenFeed unit. Data were analyzed using the MIXED procedure of SAS. There were no interactions between RDP level and RM on the evaluated parameters. Treatments did not affect urinary PD excretion, as well as CH_4 yield and intensity. However, RM increased ($P = 0.02$) CH_4 production (470 vs. 449 ± 13.7 g/d) and tended to increase ($P = 0.09$) total urinary N excretion (285 vs. 273 ± 9.19 g/d). Retained N (% N intake) was greater ($P < 0.01$) in LRDP (7.29 vs. 4.70 ± 1.20%) compared with HRDP. Intakes of digestible energy, ME, and NE_L were not affected by RDP level and tended ($P = 0.08$) to increase with RM. Treatments did not affect fecal, CH_4, and tissue energy, but urinary energy (3.24 vs. 2.89 ± 0.20 Mcal/d) increased ($P = 0.01$) with HRDP vs. LRDP. Heat production and milk energy increased ($P = 0.01$) with RM (41.7 vs. 40.3 ± 0.79 Mcal/d and 32.2 vs. 31.4 ± 0.92 Mcal/d, respectively). In conclusion, RDP level and supplementation with a RM based on plant extracts affected N and energy utilization independently with no effect on CH_4 yield or CH_4 intensity in lactating dairy cows.

Key Words: rumen modifier, nitrogen balance, methane production

1409 Effect of close-up metabolizable protein supply on colostrum yield, composition, and immunoglobulin G concentration. T. A. Westhoff*[1], Á. Abuelo[2], T. R. Overton[1], M. E. Van Amburgh[1], and S. Mann[1], [1]*Cornell University, Ithaca, NY,* [2]*Michigan State University, East Lansing, MI.*

Colostrum yield and composition can be influenced by the prepartum diet. The objective was to examine the effect of increasing metabolizable protein (MP) supply on colostrum parameters. Multiparous Holstein cows (n = 96), blocked by calving date, were randomly assigned to 1 of 2 prepartum diets formulated to contain a control (CON; 85 g of MP/kg DM; 1,175 g of MP/d; 92% of MP requirement) or high (HI; 113 g of MP/kg DM; 1,606 g of MP/d; 129% of MP requirement) level of MP starting at 28 d before expected calving. Rumen-protected Met and Lys were used to formulate both diets to supply 1.24 and 3.84 g/Mcal of ME, respectively. Colostrum was harvested within 8 h of calving and yield (CY) was recorded. Immunoglobulin G (IgG) concentration was determined via radial immunodiffusion. Fat and protein were determined by Fourier transform infrared spectroscopy and lactose was determined by enzymatic colorimetric analysis. Data were analyzed in SAS v. 9.4 using chi-squared test or mixed-effects ANOVA with the fixed effects of trt, parity (2 vs. ≥ 3), and trt × parity with a random effect of enrollment block. Data are reported as LSM ± SEM. Treatment did not affect DMI (1.80 vs. 1.77 ± 0.03% of BW; $P = 0.29$). Feeding HI compared with CON tended to increase CY in cows entering parity 2 (9.4 vs. 7.2 ± 0.9 kg; $P = 0.08$), but trt did not affect CY from parity ≥ 3 cows (5.1 vs. 6.4 ± 1.0 kg; $P = 0.34$), respectively. The proportion of cows producing ≥ 6 kg of colostrum did not differ by trt (HI: 24 [52.2%] vs. CON: 22 [46.8%]; $P = 0.53$). Brix % (26.8 vs. 27.2 ± 0.6%; $P = 0.60$), and the concentrations of IgG (120.8 vs. 121.4 ± 5.8 g/L; $P = 0.94$), fat (5.0 vs. 4.3 ± 0.3%; $P = 0.11$), protein (16.7 vs. 16.9 ± 0.5%; $P = 0.79$), and lactose (2.00 vs. 1.96 ± 0.10%; $P = 0.70$) did not differ for HI and CON, respectively. Treatment did not affect the proportion of cows producing colostrum with ≥ 100 g of IgG/L (HI: 28 [60.9%]; CON: 30 [63.8%]; $P = 0.77$). In conclusion, increasing the prepartum MP supply tended to increase CY in cows entering parity 2 but did not affect the IgG concentration or composition of colostrum.

Key Words: metabolizable protein, colostrum, immunoglobulin G

1410 Metabolic and transcriptomic responses to dietary-induced milk fat depression and balanced amino acid supplementation in lactating dairy cows. K. E. Ruh*[1], M. Killerby[1], G. M. de Souza[1,2], A. D. Benn[1,3], E. M. Cohan[1], A. Krueger[1], L. A. Coelho Ribeiro[1], A. M. Larsen[1,4], A. Hoppmann[1], D. N. Sherlock[5], W. Li[4], and S. I. Arriola Apelo[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Universidade Federal de Lavras, Lavras, MG, Brazil,* [3]*Nutritional Sciences, University of California, Davis, Davis, CA,* [4]*USDA-Agricultural Research Service, Dairy Forage Research Center, Madison, WI,* [5]*Adisseo, Apharetta, GA.*

Conjugated linoleic acids and AA Met and Leu have opposite effects on milk fat synthesis. We aimed to determine the effect of AA supplementation on mammary gland (MG) and adipose tissue (AT) transcriptome and circulating concentrations of metabolites in cows fed control and milk fat depression (MFD) diets. Holstein cows (69 DIM+20 SD, n = 24) in tiestalls, fed and milked 2×/d, were subjected to 4 treatments over four 28-d periods in a 4 × 4 Latin square with a 2 × 2 factorial arrangement. The 2 factors were fat source (FS): soybean oil (SBO; 1.83% DM) or C16:0 (80%)–C18:1(10%) fat supplement (CTL; 1.89% DM); and metabolizable AA level (AAL), 5% deficient (AAD), or sufficient balanced for Leu and Met with corn gluten meal and rumen-protected Met (AAS). In each period, blood samples from 20 cows were collected d 27 and d 28, and 4 cows had MG and AT biopsies on d 26. Groups responded similarly for milk (SBO, AAS: increase) and fat (SBO: decrease, AAS: increase) yields. Plasma insulin, glucose, and AA were analyzed. Results were analyzed with a mixed model containing FS, AAL, FS × AAL, period, and square as fixed effects, cow as random. Extracted RNAs from MG and AT were sequenced, aligned to bovine reference genome (ARS-UCD1.2), and analyzed with edgeR to identify differentially expressed genes. Pathway enrichment analysis was implemented in Ingenuity Pathway Analysis with $|\text{Z-score}| \geq 2$ as cut-off. Glucose was not affected by either factor. Insulin was decreased by SBO but the effect was blocked by AAS (FS × AAL, $P = 0.007$). Leucine decreased in response to AAS ($P = 0.047$), whereas Met increased ($P = 0.002$). The FS affected expression of 523 genes in AT and 160 genes in the MG ($P < 0.05$), whereas AAL altered expression of 478 genes in AT and 318 genes in the MG. In AT, SBO was predicted to stimulate growth factor signaling and immune response while AAS was predicted to stimulate IGF1 and inhibit insulin signaling. In MG, AAL was predicted to alter phospholipase C and serotonin signaling. In conclusion, SBO seems to have stronger metabolic and transcriptomic effect in AT, while AAL altered relevant pathways in the MG.

Key Words: milk fat depression, amino acid (AA), RNA-seq

1411 Bioavailability of multiple rumen-protected amino acids by a stable-isotope technique in dairy cattle. T. Fernandes*[1], M. H. De Oliveira[1,2], A. Hruby-Weston[1], M. Morozyuk[1], B. Thelen[1], and M. D. Hanigan[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*State University of São Paulo, Botucatu, SP, Brazil.*

Multiple EAA drive milk protein production in dairy cows, including His, Ile, Leu, Lys, Met, and Thr. Therefore, determining bioavailability of rumen-protected AA (RPAA) is essential for optimal EAA supply. We aimed to evaluate bioavailability of lipid-encapsulated prototypes of His, Ile, Lys, Met, and Thr and an isopropyl ester of 2-hydroxy-4-(methylthio) butanoic acid (HMBi). Six Holstein heifers (518 ± 28 kg BW) were allocated in a 6 × 8 Youden square design, with 8 periods and 8 treatments. Treatments were (1) a base diet (BD, 1.72 Mcal of NE_L/kg and 15.8% of CP); (2) BD + RP-Lys1 + Met1; (3) BD + RP-His2 + RP-Lys2 + RP-Met2; (4) BD + RP-His3 + RP-Lys3 + RP-Met3; (5)

Table 1 (Abstr. 1411). Evaluation of rumen-protected prototypes

Prototype	RUP, %	Plasma entry, % AA fed[1]	Bioavailability, % fed AA[2]
RP-His 2	17.6	68.7	73.5
RP-His 3	54.3	44.7	47.9
RP-His 4	8.4	51.9	55.6
RP-Ile 5	16.6	21.6	23.1
RP-Lys 1	46.0	35.2	37.7
RP-Lys 2	20.6	41.8	44.8
RP-Lys 3	8.3	67.1	71.9
RP-Lys 4	20.8	44.0	47.1
RP-Lys 5	32.9	59.8	64.0
Met1 (HMBi)	—	58.6	62.7
RP-Met 2	93.9	89.2	95.5
RP-Met 3	86.9	43.0	46.0
RP-Met 4	24.7	28.9	30.9
RP-Met 5	22.4	71.0	76.0

[1] Derived from fits of the isotope dilution model to observed isotope enrichment.

[2] Calculated from total AA appearance and 6.6% gut use at first pass.

BD + RP-His4 + RP-Lys4 + RP-Met4 + RP-Thr4; (6) BD + RP-Ile5 + RP-Lys5 + RP-Met5; (7) and (8) extra treatments not presented here. In each 14-d period, heifers were fed 1×/d for 10 d and 12×/d at 95% for 4 d to establish a steady state. On d 14, a saline solution with stable-isotope U-[^{13}C]-labeled AA was infused into the jugular vein for 720 min, and blood samples were collected upstream. Isotopic ratio (IR) of plasma ^{13}C labeled AA was evaluated using an IR mass spectrometer coupled to gas chromatography by a combustion oven. Amino acid entry rates model was used to derive fractional availability values (g AA appearing in plasma/g AA consumed) for each RPAA by regression (Estes et al., 2018, J. Dairy Sci. 101:7040). Rumen-undegraded protein (RUP) was predicted by rumen kinetic degradation (0 to 96 h) using a passage rate of 7.11%/h. We could quantify EAA bioavailability for all RPAA except Thr (Table 1). The RUP values do not always represent the bioavailability of AA in RPAA.

Key Words: amino acid (AA) absorption, rumen undegradable protein (RUP), availability

1412 Previous level of protein nutrition effects on adaptive responses to a change in dietary protein in lactating Holstein cows. G. I. Zanton*, *USDA-Agricultural Research Service, US Dairy Forage Research Center, Madison, WI.*

Production responses to dietary changes in lactating dairy cows may depend on both the previous and current diet. The objective of this study was to characterize the implications of previous protein nutrition on the production responses to different levels of dietary CP. The hypothesis is that responses to level of CP will depend on the previous CP status in dairy cows in established lactation. To test this hypothesis, 60 lactating Holstein cows (DIM = 67.2 d ± 20.89 [SD], milk yield = 45.1 kg/d ± 5.45, and BW = 604 kg ± 77) were individually fed 2 diets of differing CP content (L: 14% CP, H: 18% CP) for 8 wk (P1). At the beginning of wk 9, cows were randomly assigned to 3 diets (L, H, and M: 16% CP) fed for an additional 8 wk (P2) resulting in 6 different nutritional histories in P2 (LL, LM, LH, HL, HM, and HH) fed according to a split-plot design. Diets were formulated to contain 37.5% DM BMR corn silage, 22.5% alfalfa silage, and 40% concentrates. Changes in CP were made by exchanging solvent soybean meal for ground corn and soybean hulls. Milk yield and DMI were measured daily and milk was collected for analysis of composition over 2 consecutive days each week. Production responses were evaluated by mixed modeling for the effect of diet during P1 and the direct and interaction effects of diet during P2; responses with $P < 0.05$ were considered significant. In P1, cows fed H had greater DMI (28.4 vs. 26.1 ± 0.29 kg/d [SEM]) and productivity (e.g., 31.0 vs. 29.5 ± 0.38 Mcal milk energy/d) than cows fed L. In P2, all yield responses interacted with P1 diet; DMI, components, and milk urea N concentrations were only affected by direct diet effects. Interaction in P2 resulted from a blunted production response in cows fed L during P1 compared with a pronounced linear response to diet in P2 when cows were fed H during P1. Cows fed the sequence HL had distinctly lower production during P2 (e.g., 26.2 ± 0.59 Mcal milk energy/d). In conclusion, previous level of CP affected subsequent production responses to diet, which has implications on interpreting responses to diet in nutrition experiments and on farms.

Key Words: nutritional history, dietary protein, production

Ruminant Nutrition Platform Session: Challenges and Opportunities for the Impactful Implementation of Greenhouse Gas Mitigation Strategies

1414 ***ADSA-EAAP Speaker Exchange Presentation:*** **Breeding strategies to reduce methane emission.** J. Lassen*[1,2], [1]*VikingGenetics, Randers, Denmark,* [2]*Aarhus University, Aarhus, Denmark.*

Selective breeding is a very effective way to improve traits that show quantitative genetic variance. The underlying strategy for selective breeding consists of several components. A prerequisite to do selective breeding is to have procedures that can be used to generate quality data. Preferably this is done in a continuous way at a low cost and with a minimum of labor per registration. This can be done using both indirect and direct measures of the trait that needs to be improved. Also, an effective selection procedure requires an economic incitement for the farmer to change the trait. Methane production is under some quantitative genetic control. Several studies worldwide, each based on more than 1,000 cows, have shown heritabilities higher than 20%. This means that methane production can be reduced through selective breeding. The trait to be improved will differ between countries. Some will choose to reduce methane production; others will reduce methane intensity. Methods have been developed that can be used to make large-scale registrations for the trait. Indirect approaches to reduce methane emission have mainly been based on milk sample data. This is an intriguing way to perform selection because such data are available through milk recording on each cow in many countries. It is important to continuously ensure that there is a high relationship between the trait that needs to be improved and information that is available in the milk samples. The effect of selecting for higher milk yield has a high effect on methane intensity in populations with low milk yield but limited effect in populations where the production level is higher than 6,000 L of milk per cow. The economic incitement to reduce methane emission from cattle will be visible worldwide soon. This will come both from higher income for milk and meat produced from cattle with documented lower methane emission as well as a cost for similar products if there is no documentation or a documented higher methane production. Selective breeding for reduced methane emission will be incorporated in many countries in the coming years.

Key Words: methane, cattle, breeding

1413 **Greenhouse gas emissions from dairy cows fed best-practice diets.** M. Managos*[1], C. Lindahl[2], S. Agenäs[1], U. Sonesson[3], and M. Lindberg[1], [1]*Department of Applied Animal Science and Welfare, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*Lantmännen Lantbruk foder, Stockholm, Sweden,* [3]*Research Institute of Sweden, Göteborg, Sweden.*

Dairy production faces the challenge of balancing conflicting goals: reducing greenhouse gas emissions, increasing food production, and achieving self-sufficiency within planetary boundaries. The disruptions caused by the global COVID-19 pandemic and armed conflicts have intensified these challenges, affecting the agricultural supply chain, prices, and resource availability. This study investigated milk production and methane (CH_4) emissions from high-yielding dairy cows fed grass/clover silage with 3 concentrate mixtures with varying degrees of carbon footprint in the production stage. The experimental diets included a control commercial concentrate (CON), a byproduct-based concentrate (BYP) addressing feed-food competition, and a homegrown concentrate mixture (HOM) based on domestically available resources. The experimental diets were formulated to have similar theoretical nutritional value as the control and the carbon footprint of the ingredients was used as the optimization parameter. Forty-eight Swedish Holstein cows, with a milk yield of 45 ± 6.4 kg/d (mean ± SD) at the start of the experiment, blocked by parity in 2 groups, were used in a randomized complete block design with a 2-week adaptation and 7 weeks of data collection. The cows were milked voluntarily in a single-station automatic milking system. Individual daily feed intake was recorded and the GreenFeed system was used for continuous measurements of CH_4. Dry matter intake (CON: 24.1, BYP: 24.6, HOM: 24.0 kg/d), energy-corrected milk yield (ECM) (CON: 47.3, BYP: 44.6, HOM: 47.1 kg/d), enteric CH_4 yield (CON: 391, BYP: 374, HOM: 390 g/d), and intensity did not differ between diets. Carbon dioxide equivalent (CO_{2e}) emissions from feed production were lower for BYP and HOM compared with CON, resulting in lower feed CO_{2e} emission intensity per unit ECM. Our results show that rations for high-producing dairy cows can be formulated using domestically available resources and by/products without compromising milk production or milk carbon footprint.

Key Words: enteric methane emission, high-yielding cow, sustainability

1170 **Associations between fecal methanogens and methane emission traits in Holstein cows.** N. Sheybani*, A. P. M. Assumpção, G. Martinez Boggio, H. M. White, K. A. Weigel, H. C. Mantovani, and F. Peñagaricano, *University of Wisconsin–Madison, Madison, WI.*

Enteric methane, produced by methanogenic archaea in the digestive tract of cattle as the by-product of anaerobic microbial digestion, contributes to 27% of all US methane emissions. Enteric methane not only has an adverse environmental impact, but it also represents an energy loss from feed utilization by the cow. The goal of this study was to quantify abundance of methanogens in fecal samples from dairy cows with divergent methane emissions. The data set comprises measurements of methane production (MeP), net energy for lactation (NE_L), and body weight (BW) from 128 mid-lactation Holstein cows in 3 different studies. We calculated residual methane intensity (RMI) by regressing MeP on NE_L and metabolic BW. Within each study, we identified the cows with 10% high and low RMI (n = 30) and collected fecal samples via rectum on the last day of each study. We quantified the genus *Methanobrevibacter* by targeting the 16S rRNA gene using ddPCR assay with the following primers: GAACTTGAGGTCGGGAGAGG and AGGTTCGTTCCAGTTAGCCG. The genus *Methanobrevibacter* is the most ubiquitous and predominant hydrogenotrophic methanogen found in the foregut and hindgut of herbivores. We evaluated the association between fecal *Methanobrevibacter*, measured as log-transform counts, and 2 methane traits, MeP and RMI, using linear regression. Notably, the amount of *Methanobrevibacter* in fecal samples was negatively associated ($P = 0.05$) with both methane production and residual methane intensity. Overall, our findings highlight the importance of targeted approaches to unveil the link between methanogen abundance and CH_4 emissions in dairy cattle.

Key Words: ddPCR, methane production, methanogens

1345 **Effects of enteric methane inhibitors on ruminal fermentation patterns in vitro using inoculum from cows with high and low methane yield phenotype.** N. Stepanchenko*[1], D. E. Wasson[1], S. F. Cueva[1], L. F. Martins[1], D. W. Pitta[2], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*School of Veterinary*

Medicine, University of Pennsylvania, New Bolton Center, Kennett Square, PA.

In vivo research has shown increased rumen butyrate concentration under inhibited methanogenesis in dairy cows. The objective of this experiment was to investigate the mechanism of butyrate concentration increase by evaluating the proportional changes of ^{13}C enrichment of rumen VFA using uniformly labeled D-glucose (GLU13) and sodium acetate (ACET13) and 2 potent CH_4 inhibitors (INH): *Asparagopsis taxiformis* (AT, at 0.25 and 0.5% of substrate DM), and 3-nitro-1-propionic acid (3NPA, at 0.5 and 1% of substrate DM). Four lactating Holstein cows were selected from a pool of 48 animals and 2 were designated as phenotypically (PHEN) high- (HM, 19.8 and 20.3 g CH_4/kg DMI) and 2 as low-CH_4-emitters (LM, 14.5 and 13.2 g CH_4/kg DMI). Repeated in vitro incubations were carried out for 24 h at 39°C with TMR as substrate (at 1%, wt/vol). Data were analyzed using a MIXED procedure of SAS with INH, PHEN, dose, and interactions in the model. Total gas production was decreased ($P < 0.01$) on average 32 ± 8.2% by INH. Methane production decreased ($P < 0.01$) 96 ± 5.8% by INH versus CON. Butyrate concentration increased ($P = 0.05$) by 3NPA, but not AT ($P = 0.61$). Concentrations of other VFA were not affected ($P \geq 0.27$) by INH. When GLU13 was used as a labeling substrate, ^{13}C-enrichment of all butyrate carbons was greater ($P < 0.04$) for HM by 20% to 37%, and ^{13}C-enrichment of second and third carbons of propionate tended to be lower ($P < 0.06$) by 9.5% and 16%, compared with LM. The INH increased ($P < 0.04$) ^{13}C-enrichment of the carboxylic carbon of propionate by 20%. When ACET13 was used, the INH increased ($P < 0.02$) ^{13}C-enrichment of carboxylic and second carbons of propionate by 27% and 25%, respectively. In conclusion, inhibition of methanogenesis resulted in increased ^{13}C enrichment of propionate, which serves as an alternative H_2 sync derived from pyruvate, while HM inoculum in combination with GLU13 reduced ^{13}C enrichment of butyrate through host-microbial interaction. Microbial analysis must be conducted to identify specific species contributing to these changes.

Key Words: methane, ruminal fermentation, phenotype

1350 Effects of dietary rumen-derived microbial product supplementation on lactation performance and enteric methane emission in lactating dairy cows.

M. Bulnes*[1], A. Celemin Sarmiento[1], M. R. A. Redoy[3], J. Lefler[2], C. Marotz[2], M. Embree[2], T. H. Swartz[1], and M. E. Uddin[3], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Native Microbials Inc., San Diego, CA,* [3]*Department of Animal Science, University of Connecticut, Storrs, CT.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The objective of this study was to evaluate the effects of a rumen-derived microbial product (MP; Galaxis Frontier, Native Microbials) on lactation performance and methane (CH_4) emissions in dairy cows. Thirty-four Holstein cows (10 primi- and 24 multiparous) averaging (±SD) 98 ± 37 DIM and 43 ± 13 kg/d of milk yield (MY) were enrolled in the study, which included a 2 wk covariate period and 16 wk of treatment supplementation. Cows were blocked based on parity, DIM, and MY, and randomly assigned to one of 2 treatments: control (CON, TMR + 150 g/d of ground corn; n = 17) or treatment group (MP, TMR + 150 g/d of ground corn + 5 g/d of MP; n = 17), top-dressed once a day. Milk yield, energy-corrected milk (ECM), milk components, somatic cell score (SCS), dry matter intake (DMI), and feed efficiency (FE; DMI/ECM) were monitored. Enteric CH_4 emission was measured using the GreenFeed system. Data were analyzed using linear mixed models (PROC GLIMMIX) as repeated measures with covariate, treatment, week, and their interaction as fixed effects. Block and cow were random effects. Compared with CON, MP cows tended to produce greater milk (38.3 vs. 36.8 ± 0.7 kg/d, $P = 0.06$), had greater DMI (26.9 vs. 25.9 ± 0.4 kg/d, $P = 0.04$), and had greater FE (0.67 vs. 0.64 ± 0.01, $P < 0.05$). The covariate ECM yield and fat % interacted with treatment ($P = 0.02$ and $P < 0.001$). When ECM covariate was <30th percentile, MP cows had greater ECM yield than CON. When fat % covariate was >40th percentile, MP cows had greater fat % than CON. Conversely, MP cows had lower SCS (2.87 vs. 3.32 ± 0.2, $P < 0.05$) and milk urea N (13.2 vs. 14.9 ± 0.3 mg/dL, $P = 0.03$). Treatment did not affect daily enteric CH_4 production (g/d per cow); however, MP supplementation decreased enteric CH_4 yield (CH_4/DMI) by 11.8% ($P < 0.001$) and intensity by 7.1% (CH_4/MY; $P < 0.05$) and 5.7% (CH_4/ECM; $P < 0.05$). In conclusion, supplementing MP may reduce CH_4 yield and intensity by improving feed efficiency and energy-corrected milk yield without increasing daily CH_4 production.

Key Words: direct-fed microbial, feed additive, sustainability

1553 Investigating rumination time, microbial populations, and volatile fatty acids to uncover predictive methane emission markers in lactating dairy cattle.

A. C. B. Johnson*, K. Narayan, A. Castaneda, T. Webb, J. Bender, L. Baker, S. Rassler, A. Post, R. Kashyap, N. Indugu, and D. Pitta, *University of Pennsylvania, Kennett Square, PA.*

Rumination time (RT) is a heritable trait in cattle that can be tracked on farm by precision technologies. We have previously shown that we could detect 2 distinct clusters of animals based on high RT (H-RT) and low RT (L-RT), and the current study expands upon that preliminary finding to determine if these RT phenotypes can be linked to methane (CH_4) emissions and thus serve as a marker for identifying dairy cows with high and low CH_4-yield phenotypes. We aim to gain a better understanding of the mechanistic connectivity between animal behavioral activities with rumen microbiota and CH_4 emissions. Over the course of one year, 159 animals were enrolled to the study including a 4-wk training/adaptation period, with 2 wk of RT (Aficollar, Afimilk Ag. Cooperative Ltd.) and CH_4 emissions sampling (GreenFeed, C-Lock Inc.), and 1 week of 16S bacterial and VFA surveying at 2 time points (2 and 8 h postfeeding). Animals with a minimum of 15 CH_4 readings covering a minimum of 19 h of the day were retained in the dataset (n = 87). All RT data were used to cluster the animals into 2 groups and an H-RT (n = 51) and L-RT (n = 36) phenotype were identified covering the spring, summer, and fall seasons. Over the entire year, RT was negatively correlated with CH_4 emissions, and particularly in summer and fall seasons ($P < 0.01$ and $P < 0.05$, respectively). Over all the seasons, *Bacteroidales*, 2 *Paraprevotellaceae*, and a *Christensenellaceae* genera and isobutyrate percent were negatively correlated ($P < 0.05$) to RT but valerate percent was positively correlated ($P < 0.05$). Bacterial richness was increased at 2 h postfeeding compared with 8 h postfeeding, but *Fibrobacter* increased at 8 h post feeding as expected. The L-RT phenotype differed significantly ($P \leq 0.01$) from H-RT in daily average RT (14.4 vs. 20.8 min/h), CH_4 emissions (284 vs. 255 g/d), acetate (58.1 vs. 60.1%), propionate (22.9 vs. 25.2%), and A:P ratio (2.75 vs. 2.41). The results of this study indicate that RT could be used to estimate CH_4 effectively in the summer and fall seasons.

Key Words: rumination, microbiome, methane

1633 Effects of dose, diet, and supplementation period on the methane-mitigating efficacy of feed additives: A meta-analysis. L. F. Martins*[1], S. F. Cueva[1], D. E. Wasson[1], C. V. Almeida[1], C. J. Eifert[1], M. B. de Ondarza[2], J. Tricarico[3], and A. N. Hristov[1], *[1]The Pennsylvania State University, University Park, PA, [2]Paradox Nutrition, LLC, West Chazy, NY, [3]Innovation Center for U.S. Dairy, Rosemont, IL.*

The objective was to quantify the CH$_4$-mitigating efficacy (MEff) of antimethanogenic feed additives (AMFA) in dairy cattle when accounting for the effects of dose, diet nutrient composition, and supplementation period (SP). Publications from 1988 through 2022 (n = 103) with 200 treatment and control mean comparisons were considered for analysis. Selected AMFA were macroalgae, 3-nitrooxypropanol (3-NOP), nitrate, lipids, and plant secondary compounds (PSC). Response variables were daily CH$_4$ emission (CH$_4^{D}$, g/d), yield (CH$_4^{Y}$, g/kg dry matter intake; DMI), and intensity (CH$_4^{ECM}$, g/kg energy-corrected milk). Dose (mg or g/kg of DMI), forage-to-concentrate ratio (F:C), diet nutrients (EE, NDF, ADF, and starch; as % of DMI), and SP (d) were used as continuous explanatory variables. Relative mean differences (treatment effect as % of control) were analyzed by robust variance estimation models. Data for macroalgae were limited and responses for studied species were contrasting but, overall, this class of AMFA did not affect CH$_4$emissions ($P \geq 0.15$). 3-NOP decreased CH$_4$ emissions by up to 32% ± 2.9% ($P < 0.01$; max response for CH$_4^{ECM}$). 3-NOP CH$_4^{D}$ and CH$_4^{Y}$ MEff increased by increasing starch and decreasing F:C or ADF ($P \leq 0.10$). Nitrate supplementation decreased ($P \leq 0.01$) CH$_4$ emissions by up to 18% ± 1.6% (max response for CH$_4^{D}$). Nitrate CH$_4^{D}$ MEff increased by increasing NDF, and SP ($P \leq 0.10$). Nitrate CH$_4^{Y}$ and CH$_4^{ECM}$ MEff increased by increasing its dose ($P \leq 0.06$). Lipids decreased CH$_4$ emissions by up to 14% ± 1.7% (max response for CH$_4^{ECM}$). Lipids CH$_4^{Y}$ MEff increased by increasing EE and starch but decreased with increasing ADF ($P \leq 0.09$). PSC decreased CH$_4^{Y}$ by up to 8%, and its CH$_4^{Y}$ MEff was increased by increasing diet fiber components, and SP ($P \leq 0.08$). Overall, increasing diet fiber and decreasing starch were positively associated with the MEff of nitrate, but negatively associated with the MEff of 3-NOP and lipids. SP was positively associated with the efficacy of nitrate, lipids (CH$_4^{ECM}$), and PSC. Variability in the MEff of AMFA can be explained by differences in dose, diet nutrients, and SP.

Key Words: methane, mitigation strategy, diet composition

1647 Evaluation of animal performance, energy requirements, and enteric methane emissions in lactating dairy cows ranked by residual methane production. V. Carneiro de Souza*[1], P. Niu[2], A. Schwarm[2], A. Guinguina[3,5], T. Yan[4], A. R. Bayat[5], M. Kreuzer[6], P. Lund[7], E. Kebreab[1], and P. Huhtanen[5], *[1]University of California–Davis, Davis, CA, [2]Norwegian University of Life Sciences, Ås, Norway, [3]Swedish University of Agricultural Sciences, Umeå, Sweden, [4]Agri-Food and Biosciences Institute, Hillsborough, UK, [5]Natural Resources Institute Finland, Jokioinen, Finland, [6]ETH Zurich, Zurich, Switzerland, [7]Aarhus University, Tjele, Denmark.*

The aim of this study was to compare animal performance, enteric methane (CH$_4$) emissions, metabolizable energy requirement for maintenance (MEm), and the efficiency of energy utilization (k_l) in dairy cows classified into low- (low-residual CH$_4$ [RCH$_4$] < − 14.2 g/d), medium- (medium-RCH$_4$; −14.2 to 14.5 g/d), and high-RCH$_4$ (high-RCH$_4$ > 14.5 g/d) groups. We compiled a database of 1,060 individual records of DMI, milk yield (MY) and composition, and enteric CH$_4$ emissions from 37 published and unpublished studies conducted in respiration chambers by research entities in Europe. Residual CH$_4$ was calculated as observed CH$_4$ − predicted, being CH$_4$ predicted from DMI (kg/d) and BW (kg). The lme4, lmerTest, and emmeans R packages were used for model development, and the experiment and diet within the experiment were included as random factors. Animal performance and enteric CH$_4$ emissions were compared among RCH$_4$ groups using the Tukey test. The MEm and k_l were derived from the linear regression of milk energy output corrected for zero energy balance (MJ/kg of BW$^{0.75}$) against metabolizable energy intake (MJ/kg of BW$^{0.75}$) and RCH$_4$ ranking. There were no significant differences between the low-RCH$_4$ and high-RCH$_4$ groups in animal performance (DMI, MY, ECM, and milk protein content), except for milk fat content, which was greater ($P < 0.05$) for the high-RCH$_4$ group. However, absolute CH$_4$ production, CH$_4$ yield, and CH$_4$ intensity were lower ($P < 0.05$) for the low-RCH$_4$ group compared with the medium- or high-RCH$_4$ groups. The MEm (MJ/kg of BW$^{0.75}$) was lower ($P < 0.01$) for the low-RCH$_4$ group (0.43 ± 0.021) compared with the high-RCH$_4$ group (0.48 ± 0.025) and tended ($P = 0.05$) to be lower than the medium-RCH$_4$ group (0.47 ± 0.023). However, k_l was not different ($P > 0.05$) among the RCH$_4$ groups (average = 0.65 ± 0.011). In conclusion, RCH$_4$ has the potential to be used as a phenotype for selecting cows with reduced enteric CH$_4$ emissions. Despite the energy savings due to the lower MEm and enteric CH$_4$ emissions in the low-RCH$_4$, no benefits in performance were observed compared with the high-RCH$_4$ group.

Key Words: greenhouse gas, energy requirement, model

Teaching Platform Session: The Scholarship of Teaching and Learning 101—Demystifying Using the Classroom for Research

1415 Modern publishing tools for modern science: An open-source web app of the 2021 Nutrient Requirements of Dairy Cattle. D. J. Innes*, B. G. Fieguth, and J. P. Cant, *Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Recent advances in free, open-source tools for publishing content from R and Python (e.g., Shiny and Quarto from Posit, PBC) provide us with an opportunity to modernize research and education materials. Shiny is a development framework for building web apps using syntax familiar to a typical R or Python user and is particularly useful for adding interactivity to otherwise static visual representations of data. Shiny also integrates with Quarto, which is an easy-to-use publishing framework for creating books, websites, slides, manuscripts, etc. It is a great option for developing modern, reproduceable training material, including features to showcase the underlying code. However, there might be some resistance to change; for example,we are still constrained to present at many conferences using only Microsoft PowerPoint, but our aim is to demonstrate what else is possible. For example, we recently converted the R code of the latest Nutrient Requirements of Dairy Cattle (NASEM, 2021) into a Python package to use in precision nutrition modeling, which also allowed us to write a simple “Shiny for Python” ration balancing app for teaching dairy nutrition. The main objectives for developing this web app were to simplify access to the software (e.g., NASEM 2021 software only available on Windows) and to allow for easy customization of the information that students interact with. We added buttons that would load scenarios based on different animal and diet inputs. These preloaded scenarios were useful for demonstrations and for loading assessment scenarios with a known dietary issue that the students were asked to re-balance using concepts taught in class. The app recalculates a “snapshot” of model outputs as the user modifies diet inputs, providing instant feedback. It also displays annotated outputs and generates a comprehensive report to download. By the end of semester, students were able to load custom feed libraries and evaluate commercial dairy rations. This interactive, scenario-based learning was appreciated by students. The app and its code are publicly available on GitHub, fostering a collaborative environment for ongoing development.

Key Words: Shiny, ration, teaching

Small Ruminant 1

1416 The effect of the inclusion of grape pomace in late gestation on colostrum composition of Sarda ewes. A. Nudda*[1], S. Carta[1], T. Stefaniak[2], J. Bajzert[2], M. F. Caratzu[1], A. Fenu[1], A. Mazza[1], and G. Pulina[1], [1]*Dipartimento di Agraria, University of Sassari, Sassari, Italy,* [2]*Department of Immunology, Pathophysiology and Veterinary Preventive Medicine, Wroclaw University of Environmental and Life Sciences, Wroclaw, Poland.*

The aim of this study was to evaluate the effect of the inclusion of a small amount of grape pomace in the diet of ewes in late gestation on colostrum composition. The trial was carried out using 22 ewes in late gestation (about 15 d before lambing) divided in 2 groups homogeneous for body condition score (BCS, 3.4 ± 0.1) and body weight (BW, 62.2 ± 07 kg, mean ± SD). The control group (12 animals) was fed with a basal diet composed by a total mixed ration (CON), whereas the second group, consisting of 10 animals, was supplemented by 50 g/d of grape pomace (GP). Animals were kept all together in a barn equipped with 10 individual automatic feeding systems (Biocontrol AS, Rakkestad, Norway), and the individual feed intake was recorded daily. The ewes received the supplementation of GP until the lambing. The colostrum was collected manually within 12 h after lambing and then analyzed for composition and fatty acid (FA) profile. The concentration of IgG in colostrum was determined using an indirect ELISA test. Data were analyzed with one-way ANOVA with diet as fixed effect. The IgG concentration was 54.1 ± 6.5 and 67.0 ± 7.2 g/L (mean ± SD) for CON and VIN, respectively ($P = 0.19$). The use of the GP did not influence the colostrum composition of the ewes. It was characterized by high fat and protein concentration with a mean of 10.4% and 14.7%, respectively, whereas the mean lactose concentration was 3%. A positive correlation was found between protein and IgG concentration in colostrum. Almost all FA were not affected by the inclusion of GP in the diet during the late gestation, except for some long-chain FA, which were increased. In conclusion, the quality of colostrum was not compromised using 50 g/d of grape pomace during the late gestation of dairy sheep. *Acknowledgments: We thank Rafal Baran for technical assistance in Ig analysis. This work was financially supported by NextGenerationEU; PNRR M4C2, CN00000022 AGRITECH.*

Key Words: colostrum composition, ewe, grape byproduct

1417 Weather conditions affect ewe milk production in mixed farms: Insights from a 21-year assessment. A. Mantino*[1,2], M. Milanesi[3], G. Conte[1,2], G. Chillemi[3], G. Foggi[1], and M. Mele[1,2], [1]*University of Pisa, Pisa, Italy,* [2]*Agro-environmental Research Center, Pisa, Italy,* [3]*University of Tuscia, Viterbo, Italy.*

The Mediterranean area is a climate-change hot spot. Climate models indicate warmer summers and a reduction up to 40% of local winter precipitation. This poses a significant threat to semi-extensive mixed farms, which, unlike intensive ones, are vulnerable to the increased frequency of dry spells, thus impairing pasture production and quality. The combination of elevated temperatures and intense solar radiation affects dry matter intake and animal welfare through heat stress. The aim of the present study was to evaluate the interaction between weather conditions and animal production in Tuscany (Italy). The study involved the analysis of milk production of about 400 farms with dairy ewes reared under semi-extensive systems. First, a 21-year (2002–2022) assessment of the spatial and temporal patterns of weather parameters was conducted on a monthly basis. Second, the monthly data of milk yield and quality were collected on farms over the same time span. Two crucial periods have been identified in determining milk production at farm level: April, the peak, and July, the trough. The assessment of the decline in production between April and July has been matched with meteorological variables. Principal component analysis has revealed a correlation between production decline and rising temperatures alongside dry spells, both in April and July. Employing conditional inference tree regression analysis has allowed us to highlight the critical role of April precipitation in sustaining high milk output throughout the spring-summer period, while elevated temperatures during this time exert a detrimental effect on production. Likewise, milk fat content tends to be higher when there are elevated levels of humidity and temperature in April. These findings underscore the crucial role of spring precipitation in ensuring good milk production levels for flocks, likely attributed to an optimal green biomass supply for grazing during the subsequent season. Notably, it is observed that high summer temperatures exert a lesser impact on reducing milk yields and fat content in sheep milk raised in Mediterranean regions.

Key Words: sheep, long-term, Mediterranean

1418 Exploring associations between production traits, adaptation, and resilience in sheep genomes through integration of selection sweeps. P. Augusto de Souza Fonseca*, A. Suárez-Vega, J. J. Arranz, and B. Gutiérrez-Gil, *Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Spain.*

Livestock animals face ongoing selective pressure for increased productivity across various purposes, leading to the emergence of individuals with distinct adaptive and production phenotypes. Investigating genomic regions linked to these phenotypes can enhance understanding of the adaptive process and its link to the maintenance of production levels even in undesirable environmental conditions, which is known as resilience. Here, data from 37 studies mapping selection sweeps across the sheep genome were synthesized to pinpoint confirmed selection sweeps (CSSs). A total of 518 CSSs across the sheep genome were identified. These CSSs were categorized as production (147 prod_CSS) and adaptation (219 adap_CSS) based on the prevalence of each trait in associated studies. The genes and previously reported QTLs (from Sheep QTLdb) mapping within the CSSs were annotated using the R software GALLO. Subsequently, enrichment analysis for Gene Ontology terms and QTLs was performed. Genes within adap_CSSs were implicated in regulating seasonality, circadian rhythm, and thermoregulation, while prod_CSS genes were associated with controlling feeding behavior, reproduction, and cellular differentiation. It is relevant to mention a larger number of milk fatty acid-related QTLs observed as exclusively enriched for adap_CSS, suggesting a putative effect of these regions over adaptability and milk composition simultaneously. Indeed, environmental factors such as heat and day length (represented by the circadian rhythm and thermoregulation associations) are described as associated with milk production in sheep. Furthermore, genes harboring prod_CSS and adap_CSS exhibited intriguing links to lipid metabolism. This study enhances comprehension of the genetic relationship between productivity and adaptability in sheep breeds, shedding light on potential mechanisms driving genetic correlations between these traits. These insights may facilitate a nuanced understanding of resilience, helping to minimize negative impacts on production levels while responding effectively to environmental stressors.

Key Words: selection sweep, productivity, adaptation

1419 Milk production and environmental impact of dairy ewes fed with a byproduct diet. G. Foggi*[1], B. Valenti[2], A. Silvi[1], V. Bolletta[2], A. Mantino[1,4], E. Lilli[2], M. Tognocchi[1], S. Tinagli[1], L. Turini[1,4], C. Viti[3], A. Buccioni[3], M. Pauselli[2], and M. Mele[1,4], *[1]Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy, [2]Dipartimento di Scienze Agrarie Alimentari Ambientali, University of Perugia, Perugia, Italy, [3]Department of Agriculture, Food, Environment and Forestry, University of Florence, Florence, Italy, [4]Centro di Ricerche Agro-Ambientali, Pisa, Italy.*

Public pressure on ruminant livestock systems is increasing due to concerns over GHG emissions and land use. However, ruminants can convert fibrous feed into energy, therefore valorizing feed sources undigestible for humans. This study aimed to assess the milk production and environmental impact of mid-lactation dairy ewes fed with conventional ingredients (barley, corn, and soybean meals; CTRL) or a mixture of sole byproducts (EXP), together with alfalfa hay and fresh pasture. The EXP concentrate feed included locally available byproducts: olive pomace, tomato peels, wheat bran, wheat distillers, and rice husk. Twenty-four ewes were distributed in 2 groups balanced for fat- and protein-corrected milk yield (FCPM) and days in milking. The trial lasted 6 weeks, during which FCPM, milk composition, milk fatty acid profile (FA), human-edible feed conversion efficiency (heFCE) of gross energy (GE), and crude protein (CP) were assessed weekly. Enteric CH_4 emissions were measured using a laser device during the first and last weeks. Data were analyzed using a mixed linear model considering fixed effects of treatment and period (excluding CH_4), their interaction, the relative covariate (only CH_4), and the random effect of the animal. Average FCPM yield did not differ between EXP and CTRL groups. Milk composition varied over time but remained consistent between treatments, except for lower casein and urea content in the EXP group compared with CTRL, likely due to differences in protein digestibility in EXP concentrate. Notably, the heFCE for CP of EXP group (1.4) was greater than CTRL (0.3), indicating that each unit of potentially edible CP from the EXP diet allowed production of 1.4 units of protein in milk. Likewise, the heFCE ratio for GE of the EXP group was almost 5 times that of CTRL. Milk FA originated by ruminal biohydrogenation of dietary PUFA were in a significantly greater concentration in milk from the EXP diet. Enteric CH_4 emissions did not differ between groups. In conclusion, the byproducts in dairy ewes feeding exhibit promising potential as substitutes for conventional feedstuffs, at least in the mid-lactation phase.

Key Words: feed-food competition, co-product, sheep

1420 Exploiting longitudinal phenotypic and transcriptomic data to evaluate the impact of feed efficiency on inflammatory response dynamics in dairy sheep. A. Suárez-Vega*, R. Pelayo, P. A. S. Fonseca, M. Alonso-García, B. Gutiérrez-Gil, and J. J. Arranz, *Dpto. Producción Animal, Facultad de Veterinaria, University of León, León, Castilla y León, Spain.*

The study aimed to assess longitudinal differences in the inflammatory response of divergent feed-efficient (FE) sheep subjected to an intramammary inflammatory challenge. Six high-feed efficiency (FE) and 6 low-FE primiparous Assaf ewes were selected from a group of 40 animals based on their residual feed intake (RFI) and feed conversion ratio (FCR) estimates. Approximately 150 d postlambing, the ewes underwent an intramammary infusion of *Escherichia coli* lipopolysaccharide (LPS). Plasma samples were collected and analyzed using a cytokine/chemokine multiplex immunoassay kit panel at 0, 2, 4, 6, 8, 12, 24, 48, 72, and 144 h following LPS inoculation. Concurrently, milk samples were obtained at 0, 6, and 24 h post-LPS inoculation for Poly(A) RNA-sequencing on a NovaSeq 6000 system (Illumina). A generalized linear model (GzLM) analysis was conducted to compare the systemic inflammatory markers between high-FE and low-FE groups, revealing 3 chemokines significantly reduced ($P < 0.001$) in the high-FE group. Furthermore, after the alignment and quantification of the RNA-seq data using STAR and RSEM software, differential gene expression trajectories were assessed using the TimeSeriesExperiment R package. We identified 23 genes exhibiting differences in longitudinal expression between divergent FE sheep. Among the genes identified, several were linked to cell-cell adhesion, a crucial biological process in mastitis that influences cell migration, proliferation, survival, and apoptosis in the lactating mammary gland. Consequently, these results suggest that variations in feed efficiency could potentially induce changes in the inflammatory response during lactation in dairy sheep.

Key Words: feed efficiency, mastitis, longitudinal data

1421 Complementary effects of breeds of goat and turmeric powder inclusion on milk yield for 12 weeks postkidding. O. A. Oderinwale*[1,2], B. O. Oluwatosin[2], S. D. Amosu[2], G. O. Sanusi[2], D. P. Toviesi[2], J. O. Alabi[1], J. O. Olalere[2], O. O. Adelusi[1], J. M. Enikunomehin[1], D. O. Okedoyin[1], M. Wuaku[1], L. K. Olagunju[1], and U. Y. Anele[1], *[1]North Carolina Agricultural and Technical State University, Greensboro, NC, [2]Federal University of Agriculture Abeokuta, Abeokuta, Ogun State, Nigeria.*

The study was conducted to investigate effects of goat breeds and varying levels of turmeric powder (TP) on milk yield for 84 DIM. Fresh TP rhizomes were sourced, processed, and added to a complete diet at the rate of 0, 2, and 5 g/kg as TP0, TP2, and TP5, respectively. The dietary treatments were fed to 45 lactating goats (15 Kalahari Red [KR], 15 KalaWAD [KW], and 15 West African Dwarf [WAD]), after weight balancing for the 3 groups. The goats were housed individually, fed twice a day with concentrate diets at 5% body weight at 8 a.m., and *Brachiaria ruziziensis* at 4 p.m. Kids were allowed to take colostrum for 7 d before the study commenced. The kids suckled directly from the dams overnight on all collection days. Draining was done each time after separating kids from the dams to empty the udders. Dams were milked manually after 4 h of draining. Milk collected was measured with a measuring cylinder, and the kids returned to the dams. Data obtained were arranged in a 3 × 3 factorial layout in a randomized block design. Analysis of variance, orthogonal contrast, and Duncan multiple range test for mean separation were done using SPSS (v29.0) at 5% probability. Results of the study indicated that KR and WAD fed TP0 had the highest and lowest ($P < 0.05$) milk yield of 1,100 and 373.3 mL/d, respectively, at the end of 7 DMI. In contrary, KR fed TP5 had the highest ($P < 0.05$) milk yield per day at 14 DMI (1,270), whereas the values (1,500, 1,560, and 1,020 mL/d) were highest for KR fed TP2 at 21, 24, and 42 DMI. Afterward, highest values are being shared among KR and KW fed TP2 and TP5 until 77 DMI. The KR goats fed TP5 and WAD fed TP0 had the highest and lowest ($P < 0.05$) yield of 701.3 and 206.7 mL/d, respectively, at 84 DIM. Overall, KR and KW fed TP2 had the highest ($P < 0.05$) values for mean yield (1,058.3 mL/d) and milk yield per body weight (29.11 mL/kg), respectively. The study concluded that inclusion of TP in goats' diets improved (linear, $P < 0.05$) milk yield throughout the study regardless of the breed. Inclusion of TP at 2 g/kg of concentrate is recommended to improve milk yield in goats.

Key Words: goat, turmeric, lactation

1422 Effects of chromium yeast supplementation on serum Hsp60 and 70 messenger RNA expression in heat-stressed lambs.

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The expression of heat shock genes hsp60 and 70 may be useful biomarkers of heat stress against current losses due to heat waves in small ruminant production, since they generate a mitigating effect. For its part, the use of organic chromium seems to be an alternative for stress, but there are few studies on its effect on Hsp gene expression. This study seeks to evaluate the effect of supplementation with organic chromium on the expression of serum Hsp 60 and 70 of sheep under heat stress. Forty-eight clinically healthy Katahdin sheep (1-yr-old and 20 ± 2.9 kg live weight) were used in a 2 × 4 factorial arrangement, with 2 temperatures (heat stress [hs] and thermoneutral [tn]) and 4 levels of supplementation (0, 0.2, 0.4, and 0.8 mg of organic chromium/kg of dry matter intake), and 6 experimental units were used per treatment, serum was sampled after 30 and 60 d of supplementation, RNA was extracted with the RNA solve protocol, and cDNA was synthesized, and quantitative PCR was carried out to perform a relative quantification of the transcripts. More hsp70 transcripts were observed in hs at a lower dose of chromium, and these were reduced with increasing dose, whereas in tn there were no significant differences, showing an interaction of dose of chromium and temperature effects with the level of transcripts; a similar trend was shown in Hsp60, reducing its concentration with increasing chromium dose and without significant effects in tn. Likewise, a resolution of gene expression was observed in the second sampling in the supplemented animals, which could be associated with a bio-accumulative effect in the supplemented animals. In conclusion, organic chromium supplementation during heat stress demonstrates promising results in stress mitigation. No changes were observed in thermoneutral conditions, so supplementation is not necessary.

Key Words: heat stress in sheep, heat shock protein, stress mitigation

ADSA Southern Branch Symposium: Seasonality in the Dairy Production System

1500 Seasonality of milk production and composition. M. A. C. Danes*[1], J. P. A. Rezende[1], C. R. Monteiro[1], V. A. Oliveira[1], L. H. Silva[1], and J. H. Carneiro[2], [1]*University of Lavras, Lavras, MG, Brazil,* [2]*Dairy Inside, Castro, PR, Brazil.*

The existence of an endogenous rhythm that affects biological phenotypes, such as milk production and composition throughout the year, is well known and reported. Quantifying the proportion of the observed variation in a trait that is due to endogenous rhythm allows for better data interpretation and management decisions. It is also important to understand the factors that can influence the endogenous rhythm so that it is possible to include these effects in a rhythm prediction model. For instance, one of the factors that appears to influence the seasonal rhythm is the photoperiod (both the absolute number of hours of light in the day and the day-to-day variation in this number). Data from the northern hemisphere have already demonstrated this effect by comparing regions at different latitudes. The evaluation of this behavior in different regions of the southern hemisphere can demonstrate more clearly the effect of photoperiod, as well as suggest other important factors affecting seasonality. Therefore, we explored different databases containing milk production and composition data from Brazil to conduct cosinor-based rhythmometry analyses. First, we analyzed milk composition data of the last 10 years from the national milk market database, grouped by region, to evaluate the effect of location (i.e., latitude) on seasonal rhythm. Next, we used individual cow data to evaluate not only the rhythm itself, but also the effects of breed, days in milk, location, and environmental temperature on the endogenous rhythm. For this analysis, we accessed data from Ideagri, a herd management software, from Instituto Clinica do Leite, a milk analysis laboratory, and from 2 breeders' associations, Holstein and Girolando (Gyr × Holstein crossbred). We then used data from Sebrae, a nonprofit private entity promoting sustainable and competitive development of small businesses, to evaluate the effect of herd structure and economic factors (milk price and production cost) using herd-level data from Minas Gerais state. Finally, we used individual cow data from 3 herds in Paraná state to evaluate the effects of genomics on seasonality. Results from these analyses are presented and discussed.

Key Words: Brazilian dairy, genomic, cosinor

1501 Seasonality of reproduction. P. J. Hansen*, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Depressed reproduction during warm periods of the year remains a limitation to optimal dairy production in most regions of the world. Using US records from DHIA, Guinn et al. (J. Dairy Sci. 101:11777) estimated that conception rate declines from 42.0% in winter to 32.4% in summer in the Southeast United States, from 41.1% to 37.5% in the Midwest United States, and from 42.0% to 37.5% in the Northeast United States. Several temporal trends are likely to interact to cause geographic-specific changes in the magnitude of seasonal variation in reproduction. Among those that will tend to exacerbate seasonal variation in reproduction are global climate change, which will increase the magnitude of heat stress, and increased milk yield, which will make cows more sensitive to heat stress. Trends that are anticipated to alleviate seasonal variation in reproduction include more widespread adoption of housing systems that provide effective cooling to cows during periods of heat stress as well as development of genetic strategies to make cows more resistant to the deleterious consequences of heat stress. There are also physiological strategies that can be used to enhance reproduction during heat stress including timed artificial insemination and embryo transfer. In addition, work continues to develop nutritional strategies to mimic cellular damage associated with hyperthermia during heat stress. It should be recognized that increasing reproductive function during heat stress could change seasonal calving patterns. A positive result could be a more consistent milk supply throughout the year. Negative consequences include more cows being in early and mid lactation during the hottest periods of the year and increased risk of adverse programming of fetal development. Thus, efforts to improve reproduction during heat stress should be carried out in tandem with efforts to reduce other effects of heat stress on cow function.

Key Words: heat stress, reproduction, seasonality

1502 The relationship between birth season and longevity of dairy cows. I. M. Toledo*[1], L. Cattaneo[2], J. E. P. Santos[1], and G. E. Dahl[1], [1]*University of Florida, Gainesville, FL,* [2]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Longevity of dairy cows is defined as the length of the animal's life or as the length of productive life, which is determined by either culling decisions made by the producer or death of the animal. Culling decisions are primarily driven by economic considerations. Therefore, dairy replacement management decisions largely determine the average productive life of dairy cows. In addition to economic consequences, dairy cow longevity is associated with the environmental footprint of the industry and welfare; thus, short productive life has environmental and social consequences, which are inversely associated with dairy sustainability. The ability of dairy farmers to keep their cows for longer could positively enhance the economic performance of the farms, by having a higher proportion of mature cows in the higher producing groups, reducing the environmental footprint of the milk production, and overall helping to justify the sustainable use of animals for food production. Multiple studies have documented that seasonal changes affect health, behavior, and performance of dairy cows throughout their life cycle. Management adjustments to decrease exposure to high temperatures during the hot months of the year give farmers the opportunity to decrease culling risk factors and possibly increase cow productive life. Recent analysis of seasonal records indicates that cows still on the farm in lactation 5 to 8 were more likely to be born in a cool season versus a hot season. Indeed, about 66% of longer-lived cows were born under cool conditions. This has implications for culling decisions even early in life. Birth in a cool season will increase the length of herd productive life and decrease the number of cows sold or dead during the first 4 lactations. A better understanding of the relationship between birth seasons and dairy cow longevity is an alternative to help farmers create opportunities to make management adjustments to possibly increase productive life in dairy herds. Increases in dairy cow productive life associated with birth season can possibly positively impact the dairy industry.

Key Words: season of birth, longevity, dairy cow

1503 Nutritional challenges and opportunities in seasonal grazing systems. M. Dineen*, *Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland.*

In pasture-based systems, the diet of the cow can comprise almost completely human-inedible ingredients resulting in a large contribution to net food production. High pasture inclusion levels can support resilient family-farm incomes and an animal welfare friendly image while also increasing concentrations of beneficial nutrients in milk including n-3 polyunsaturated fatty acids, vaccenic acid, and conjugated linoleic acid. Growing environmental challenges such as greenhouse gas emissions, nitrogenous losses to air and groundwater, and loss of natural ecological habitats will require pasture-based systems to adapt and innovate. Increasing animal productivity is an effective strategy to reduce the intensity of environmental emissions (i.e., per unit of milk). In the United States over the last 100 years, milk production per cow has increased 6-fold resulting in a 55% to 60% reduction in enteric methane emissions intensity. Experiments have demonstrated that robust cows bred for grazing systems possess the capability to increase milk production by 27% to 33% when fed a total mixed ration. This opportunity of achieving a step change in production, in tandem with continuous improvements in genetic merit for milk production, could lead to substantial reductions in the intensity of environmental emissions. However, improving milk production through increased purchased feeds typically results in unintended consequences, such as reduced family-farm income and increased intensity of environmental emissions in parts of the world with favorable characteristics for pasture-based systems. Therefore, other nutritional strategies must be examined, for example, improving pasture management, developing binary or multi-species pastures, selecting superior plant genetics, and optimizing concentrate supplementation. While improvements in performance have been achieved with such strategies, further research is required to understand the nutritional limitations of pastures across the grazing season and how pasture-based diets can be efficiently modified to overcome such limitations.

Key Words: pasture, milk production, environmental emission

Joint AAVI and ADSA Animal Health and Lactation Biology Committees Symposium: Colostrum—The Role It Plays in Calf Health, Development, and Future Productivity

1504 The regulation of colostrogenesis. C. Baumrucker*, *Penn State University, University Park, PA.*

Colostrogenesis is a unique phase of mammary epithelial cell differentiation occurring in the weeks before parturition and rapidly ending after birth in the bovine. What controls this process and how it accounts for the extreme variation of specific biologically active components that affect colostrum quality and neonatal health is the focus of new research. Among specific colostrum components whose concentrations exceed those in blood, immunoglobulin G1 (IgG1) is the only established marker of this mechanism. IgG1 is extremely variable in mass and concentration in cow colostrum. The Fc Receptor of the Neonate (FcRn; composed of FcGRT and B2M) is the major IgG and albumin binding protein that accounts for these proteins' long half-lives in the circulation. The high occurrence of only IgG1 in colostrum represents selective transcytosis by the FcRn. Fluid phase uptake of extracellular components into mammary cells and their merger with sorting endosomes containing membrane bound FcRn will be described. The FcRn means of binding IgG with increased endosome acidity and transcytotic delivery of IgG1 while recycling IgG2 and albumin will be explained. The low pH of colostrum (pH 6.1) presents a dilemma because the release of IgG1 from FcRn requires a pH > 6.5. We have proposed that this release occurs in the endosome and is related to mammary synthesized lactoferrin (Lf, pKa 8.7), which appears after FcRn-IgG1 binding and sorting and provides the pH needed for disassociation. Endosome cargo secretion into colostrum must be different than that of casein, and mechanisms will be proposed. Both IgG1 and Lf, while being highly variable in colostrum, are correlated in concentration and mass. Finally, regulation of lactoferrin and FcGRT synthesis during colostrogenesis represent potential regulatory mechanisms that account for the extreme variation in colostrum quality.

Key Words: colostrogenesis, Fc receptor of the neonate (FcRn), transcytosis

1505 Optimizing passive immunity with dam vaccination: When is too soon or too late? What is too much? A. R. Woolums*, *Mississippi State University, Mississippi State, MS.*

Dairy producers know that calves require high-quality colostrum given soon after birth to provide antibodies that protect calves from infection. Antibodies block infection by specific viruses or bacteria, and the cow's colostrum only contains antibodies to viruses or bacteria the cow has been exposed to in the past, through natural infection or vaccination. This is nature's way of helping the calf to be specifically protected from infections on the farm where they are born. It is possible to increase the concentration of antibodies to specific viruses or bacteria in newborn calves by giving cows a booster vaccination in the dry period. This dry period booster will increase the cow's blood antibodies, which then move into the colostrum. For dry cow vaccination to increase antibodies in colostrum, the cow must be boosted no sooner than 8 weeks before calving and no later than 3 weeks before calving. Booster vaccination in this window allows the cow's immune response time to make antibodies and to move them into her colostrum. Many studies have shown that calves nursing colostrum from cows given booster vaccines in the dry period have higher antibodies in their blood for the first 1 to 3 mo after birth, compared with calves getting colostrum from cows not boosted in the dry period. Moreover, experimental infection of calves with bacteria or viruses in the vaccine indicates that higher antibody concentrations in the calves can improve their resistance to infection. However, when the effect of dry cow vaccination on naturally occurring disease on farms is evaluated, the positive effects of vaccination do not always translate to less disease in the calves. This may be because other factors such as hygiene, nutrition, and crowding have a bigger effect on natural disease occurrence than specific antibodies in the blood of the calves. However, only a small number of research studies have evaluated the effect of dam vaccination on naturally occurring disease in calves, and the available studies are small. More research in large field trials could improve understanding of how much dam vaccination can truly prevent calf disease.

Key Words: vaccination, calf, colostrum

1506 The impact of prepartum nutrition on colostrum production and newborn calves. K. S. Hare*[1,2], K. M. Wood[2], G. B. Penner[1], and M. A. Steele[2], [1]*University of Saskatchewan, Saskatoon, SK, Canada,* [2]*University of Guelph, Guelph, ON, Canada.*

It is widely accepted that colostrum consumption is vital to newborn calves to enable their adaptation to the *ex utero* environment. Early consumption of high-quality colostrum supports the elevated metabolic rate of a newborn calf while promoting ontogenic development of their gastrointestinal tract and imparting the transfer of passive immunity. Colostrum quality is classically defined by its immunoglobulin G (IgG) concentration, but researchers are currently exploring the relative importance of other colostrum macronutrient and bioactive components to expand and provide a more nuanced definition of colostrum quality. Further, researchers aim to develop strategies for producers to modulate and improve colostrum composition to, ultimately, promote calf development. Manipulating feed intake before calving is a worthwhile approach since diet formulation can be readily adjusted. Early work demonstrated that both restricting or oversupplying nutrients relative to requirements reduced colostrum volume and IgG yield, importantly highlighting that prepartum nutrient intake can affect colostrogenesis. More recent research has focused on how the chemical composition of the prepartum diet can influence colostrum composition and yield. Substituting dietary starch in place of fiber, and altering the concentration and source of ether extract appear to have the most impact on colostrum composition and yield, while the effect of dietary protein content may be limited to the concentration and abundance of bioactive compounds in colostrum. Changes in the colostrum bioactive fraction due to prepartum nutritional management can occur independent of changes in the colostrum macronutrient composition and IgG concentration. The colostral bioactive fraction can affect the ontogenic development of newborn calves and, thus, should not be disregarded in instances where prepartum nutrient intake has marginal effects on other aspects of colostrum composition. By evolving our understanding of the relationship between prepartum nutrition and colostrogenesis in cattle, we may further support the health and development of newborn calves.

Key Words: colostrum, nutrition, calf

1507 Colostrum—More than immunoglobulin G (IgG): Colostrum components and effects on the calf.

S. Mann*, *Cornell University, Ithaca, NY.*

Colostrum has long been recognized as the critical source of immunoglobulins (Ig) to achieve adequate transfer of passive immunity in the agammaglobulinemic bovine neonate. Although the importance of colostral Ig, particularly IgG, is clearly documented, colostrum is also rich in low-abundant proteins such as growth factors, cytokines, hormones, complement, antimicrobial peptides, and enzymes, as well as other active components such as maternal immune cells and microRNA. Insulin and insulin-like growth factors are present in amounts that are substantially higher than those circulating in blood of the dam and play a role in gut maturation. Heat treatment of colostrum before feeding is a management approach on dairy operations to control bacterial contamination and the potential transmission of infectious agents from the dam to the newborn calf via milk. Heat treatment has been shown to preserve the important IgG fraction. Recent research shows that certain colostral components are affected by the heating process and are reduced in concentration or viability, such as IgA, insulin, IGF, complement proteins, enzymes, and live cells. The inherent antimicrobial activity of bovine colostrum is also greatly reduced due to heat treatment whereas it appears to be preserved in frozen colostrum. This presentation will review the non-IgG components of bovine colostrum and their significance in the physiology of the newborn calf, as well as take into consideration the effects of postharvest treatments on these components.

Key Words: colostrum, calf, non-IgG

1508 The role of colostrum in programming immune development of dairy calves.

M. Cid de la Paz and L. Rostoll-Cangiano*, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Neonatal calves are inherently susceptible to environmental pathogens as evidenced by the high incidence of mortality and morbidity during this phase of life. The placenta of the cow separates the maternal and fetal blood supplies, preventing in utero transmission of maternal leukocytes and immunoglobulins (Ig). As a result, calves are born immunologically naïve, and depend exclusively on colostrum for transfer of immunity to confer protection against environmental pathogens during the first weeks of life. Successful immune passive transfer via colostrum is one of the most important determinants of calf survival and can have long-lasting implications for health and productivity. While recent improvements have been made in colostrum management to minimize neonatal mortality and morbidity, studies and recommendations predominantly center on Ig transfer on calf health. However, while antibody-mediated protection is important, it is not the sole constituent of transferred immunity. In addition to Ig, colostrum is rich in growth factors, immune cells, and various bioactive molecules that collectively contribute to protecting against environmental pathogens while orchestrating the development of the nascent immune system of the calf. In this presentation we will discuss how colostrum can provide protection against both extra- and intracellular pathogens and how recent data suggest that it is instrumental in driving proper immune development, having long-term implications for animal health. Gaining a better understanding of the role of colostrum in shaping early life immune responses in calves can help us devise better management strategies to improve long-term health in dairy cattle.

Key Words: calf health, immune development, colostrum

1510 What can't colostrum do? Exploring the effects of supplementing colostrum after the first day of life.

D. L. Renaud* and M. A. Steele, *University of Guelph, Guelph, ON, Canada.*

Recently, there has been renewed interest in colostrum, specifically in the days following birth. It has been found that supplementing transition milk or small quantities of colostrum, in addition to milk, for 3 to 14 d following birth can result in improved growth performance and reduced levels of diarrhea and mortality. Although the mechanisms behind these outcomes are not completely clear, they are thought to be the result of colostrum's ability to stimulate intestinal development in dairy calves; however, addition work is needed to further elucidate the reasons for these effects. Furthermore, the optimal dose for supplementation is also unclear. Colostrum supplementation has also been explored as a therapy for disease. Specifically, when diarrheic calves were supplemented for 4 consecutive days with a colostrum and milk mixture, they had faster resolution of diarrhea and improved growth in the 56 d following the bout of diarrhea. Furthermore, when declines were noted in milk intake or drinking speed, measured by an automated calf feeder, colostrum supplementation for 3 d reduced the likelihood of developing respiratory disease, but not diarrhea, in the 7 d following intervention. Although few studies have been conducted exploring the therapeutic efficacy of colostrum, it shows promise as an antimicrobial alternative; however, more replication and further exploration of the mechanisms behind disease amelioration are needed. Finally, supplementation of colostrum has also been explored during stressful periods in the calf's life. During the weaning process, colostrum supplementation, in addition to milk, was associated with improved growth, although no other differences, including on intestinal permeability, were observed. Based on the literature available, supplementation of colostrum after the first day of life using various strategies can lead to improved health and growth of dairy calves. That said, more replication of studies, along with more mechanistic studies, are needed to fully understand the full potential of colostrum.

Key Words: calf, health, preventive

1509 The utility of whole colostrum and components in maintaining gut health and metabolism.

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Obesity is a major contributor to morbidity and mortality worldwide. Among contributing environmental and lifestyle causes, intestinal microbiome dysbiosis following antibiotics is linked with obesity through association and causality studies. In the United States, over 280 million antibiotics are prescribed annually, many to children. A common antibiotic class, macrolides, are necessary to treat common infections, but are linked to long-term risk of metabolic syndrome. In the sterile mammalian intestine at birth, colostrum serves as an initial food source, delivering nutritional components, but also concentrated bioactive metabolites (e.g., oligosaccharides, IgG) that shape optimal microbial colonization including *Lactobacillus* and *Bifidobacteria*, while limiting *Enterococcus* and pathogens. Given these bacterial genera are impacted by macrolide antibiotics, we hypothesized that therapeutic treatment with colostrum supplementation following antibiotic-induced microbial depletion would restore "eubiosis," leading to more normal gut community functions. Our initial study evaluated the impact of colostrum therapy immediately following brief early life exposure to antibiotics, and we tracked metabolic phenotypes in mice over the lifespan at multiple time points. Initially, colostrum appears to stimulate epithelial barrier function, leading to goblet cell enrichment and increased mucosal microbial colonizers. Metabolically, treated animals maintained adipose tissue compartments and glucose tolerance similar to controls and without altering total dietary intake, while antibiotic treatment alone leads to greater total adiposity and impaired glucose tolerance. These results suggest colostrum or milk derivatives may be developed and optimized to provide useful medical food approaches in preventing chronic disease driven by antibiotic use.

Key Words: colostrum, medical food, obesity

Breeding and Genetics 3

1511 Over-time genetic correlations based on accuracy in genomic estimated breeding values for 18 linear type traits in US Holsteins. S. Tsuruta*[1], D. Lourenco[1], T. Lawlor[2], and I. Misztal[1], *[1]University of Georgia, Athens, GA, [2]Holstein Association USA, Brattleboro, VT.*

Genetic parameters may change faster over time in populations under genomic selection. To investigate that, unbiased parameters can be estimated if using all available phenotypes, pedigree, and genotypes. However, the estimation process is challenging or even impossible with many genotypes and current methods. This study aimed to apply formulas based on predictive ability and accuracy of GEBV to compute genetic correlations and heritabilities with many genotyped animals. Heritability was computed as $h^2 = [c^2+\sqrt{(c^4+4c^2Me/N)}]/2$, where c is predictivity = $Corr(y\text{-}Xb\text{-}Zp,\hat{u})$, $y\text{-}Xb\text{-}Zp$ is the vector of adjusted phenotypes, $\hat{u}$ is the vector of predicted breeding values, Me is the number of independent chromosome segments (15,000), and N is the number of genotyped animals with phenotypes. Genetic correlations between traits i and j were computed as $r_{gij} = Corr(y_i\text{-}Xb_i\text{-}Zp_i,\hat{u}_j)/(h_i acc_j)$, where h is the square root of h^2 and acc is c/h. We used the 2023 national evaluation data from the Holstein Association USA, with phenotypes from 2001 to 2023. Longitudinal parameters were obtained using 6 validation datasets, one every 2 years from 2009 to 2020. Training datasets ended before the first validation year. Genotypes were available for 366K bulls and 326K phenotyped cows. A single-trait model was used to obtain GEBV for 18 traits. Correlations were compared with those from variance component estimation (VCE) via Gibbs sampler and Pearson correlations (r_p) among GEBV. Overall, genetic correlations and heritabilities with the formulas were lower compared with those with VCE but were like those from r_p. Some differences are because VCE reflects the base population, whereas the formulas and r_p reflect the last generation. Under certain conditions, estimates by GEBV become true parameters after reliability adjustment. Correlations of GEBV when using VCE and calculated genetic correlations with the formulas were high (0.99) over time, indicating good concordance. With the proposed formulas, computing time for genetic parameters in large populations is drastically reduced; however, fine-tuning is needed to obtain estimates on the proper scale.

Key Words: predictivity, genetic parameter, large population

1512 Comparison of genomic merit, pedigree completeness, inbreeding, and relationships of genotyped animals across continents. P. M. VanRaden*[1], T. M. McWhorter[2], and J. Carrillo[2], *[1]USDA Animal Genomics and Improvement Laboratory, Beltsville, MD, [2]Council on Dairy Cattle Breeding, Bowie, MD.*

Over a million foreign animals now have US genomic predictions. Recent females born from 2018 to 2023 including 4,259,017 Holsteins and 483,878 Jerseys were grouped by continental region. Those genotypes included 498,480 females from 13 countries in Asia, 378,650 from 17 countries in western Europe, 125,849 from 17 countries in eastern Europe, 153,362 from 12 countries in Latin America, 53,235 from 3 countries in Oceania, and 4,082 from 5 countries in Africa. Individual countries providing the most foreign genotypes were 320,350 from Canada, 186,499 from Saudi Arabia, 160,558 from China, 135,971 from Japan, 114,501 from Italy, and 102,000 from Brazil. For Holsteins, average pedigree completeness ranged from 64.2% for Latin America to 86.1% for western Europe. Average pedigree inbreeding ranged from 8.7% for Africa and Oceania to 9.6% for North America. Oceania also had the lowest expected future inbreeding (EFI) of 9.0% and genomic future inbreeding (GFI) of 9.4% compared with the highest EFI of 9.5% and GFI of 10.4% in North America. Average Net Merit (NM$) was $480 in North America, $335 in Latin America, $381 in Western Europe, $370 in Eastern Europe, $317 in Africa, $366 in Asia, and $211 in Oceania. For the recent genotyped Jerseys, pedigree completeness ranged from 57.3% in Latin America to 87.9% in North America. Western Europe had the lowest averages of 7.6% for pedigree inbreeding, 7.4% for EFI, and 5.7% for GFI compared with highest averages of 8.9% pedigree inbreeding, 9.0% GFI, and 7.7% EFI in North America. Those properties were compared with the first 200,000 foreign genotypes summarized previously in 2015. After 9 years of rapid growth in foreign genotyping since 2015, pedigree completeness decreased slightly on all continents except a small increase in Asia. Inbreeding levels increased quickly, but the foreign genotyped females are still almost as related to US animals as US animals are to each other (EFI and GFI). Average NM$ remains higher in North America than on the other continents.

Key Words: genomic prediction, global breeding, genetic merit

1513 Validation of genomic prediction for economic traits in heifers across 5 US dairy breeds. S. Toghiani*, P. M. VanRaden, D. J. Null, A. M. Miles, and C. P. Van Tassell, *USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD.*

While previous studies validating genomic predictions have primarily focused on bulls, most genotypes in the National Cooperator Database now originate from cows. This study paired official within-breed genomic predictions (GPTA) and parent averages (PA) for genotyped heifer calves born between 2019 and 2021 using August 2021 data with their corresponding yield deviations (YD) for 18 traits. The YD data became available after the heifers completed their first lactation and were extracted from August 2023 data. Using YD diminishes herd and other nuisance effects, providing a more unbiased assessment of prediction accuracy than validation using raw lactation performance records. The analysis included milk production YD for 340,578 Holsteins (HO), 56,405 Jerseys (JE), 1,436 Brown Swiss (BS), 437 Guernseys (GU), and 105 Ayrshires (AY). However, fewer YD records were available for other traits due to missingness. Reliability or squared correlations (R^2) were divided by corresponding trait heritability because only the heritable portion of cow records can be predicted and varied across different traits and breeds. In HO and JE, the predictive ability of GPTA outperformed PA in predicting cow YD for yield, productive life, somatic cell score, fertility, and health traits. The improvement ranged from 33% to 142% compared with PA's predictive ability. However, the results for AY, BS, and GU breeds were less consistent due to the smaller number of genotyped heifers. The gains of R^2 in those breeds were smaller and aligned with the published reliabilities of GPTA. The regression results for YD on GPTA and PA traits slightly exceeded the expected value of 2.00 when predicting the future trait YD using GPTA or PA. The larger number of observations and lower standard error for regression coefficient prediction in HO and JE contributed to more stable and consistent regression coefficients for all traits except Milk Fever and Heifer Livability. Our study suggests that herd owners may experience greater benefits from genomics than originally advertised.

Key Words: cow validation, genomic prediction, yield deviation

1514 Assessing the influence of heat stress on milk-based biomarkers using analysis of residuals.

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Research on milk mid-infrared (MIR) based biomarkers show not always clear results when studying their evolution across different levels of heat stress expressed as temperature-humidity index (THI). The objective of this study was to clarify the influence of heat stress on milk-based biomarkers using an innovative analysis of residuals approach. The rationale was that, through the application of an adapted random regression model, the evolution of generated residuals with THI would no longer be blurred by other systematic effects such as lactation stage and month of recording. Milk recording data (including MIR-based biomarker predictions) and THI (day of recording and the 3 previous days) from weather stations were available for 611,063 records from 2015 to 2022 observed on 97,042 Walloon (South Belgium) Holstein cows in first lactation. Single-trait models were used that included fixed effects for herd-year of recording, month of recording, age at calving, classes of days in milk, and random regression effects of lactation curves for herd-year of calving, additive genetic and permanent environment. Herd-year-test-day effects were excluded from the model as all animals in the same class had the same THI. This model was used for many MIR-based traits. Mean residuals were then computed per THI class. Among the results, observations on residuals showed clearer thresholds (e.g., protein), avoided unexpected fluctuation (e.g., C18:1 *cis*-9), disappearing THI effects (e.g., K), potentially dilution effects (i.e., effect comes from milk), and allowed many other findings. These results indicate that the proposed strategy was less subject to blurred heat stress effects because of the lactation stage and the general feeding strategy. Moreover, this 2-stage approach will also allow other studies on residuals exploring the potential improved detection and use of heat stress–sensitive biomarkers.

Key Words: mid-infrared spectra, confounding effect, random regression model

1515 Use of test-day model and principal component analysis to derive an index of heat tolerance in dairy sheep.

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Heat stress (HS) represents an issue also in temperate climates due to the consequences of climate change. A big constraint for the implementation of breeding programs aimed at improving HS tolerance is the identification of a suitable phenotype that could be recorded at population scale. In the present study, an index of HS tolerance was developed by combing mixed model and principal component analysis (PCA). Data were 2,009 test-day (TD) records for milk yield of 555 Sarda breed ewes. Data were analyzed with a mixed linear model that included the fixed effects of days in milk class, parity, production month, and THI max class (6 classes), and the random effects of farm and animal. The random effect of the animal was fitted with an Unstructured (UN) 6 × 6 covariance structure (corresponding to the 6 levels of THI_class). Principal component analysis was then carried out on the estimated UN covariance structure. The first 2 principal components represented the general level (PC1) and the slope of production across different THI levels (PC2), respectively. Scores of the 2 principal components for all the animals were calculated and then variance components were estimated using *gibbsf90+* software with 100,000 samples (first 10,000 discarded as burn-in) and saving one sample every 10 samplings. The estimated heritability values were 0.19 ± 0.12 and 0.15 ± 0.10 for PC1 and PC2, respectively. The 2 variables were analyzed also in a bivariate model and the estimated genetic correlation was not significantly different from zero. The nonsignificant genetic correlation would allow to select animals for one trait without negative consequences on the other trait. The PC2, even characterized by a low heritability, could represent a valid tool to improve heat resistance in sheep. *This work was funded by by the European Union–NextGenerationEU; PNRR M4C2, CN00000022 AGRITECH.*

Key Words: heat stress, sheep, genetics

1516 Multibreed single-step genomic best linear unbiased predictor evaluations for fertility traits in US dairy cattle.

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We tested ssGBLUP for fertility traits in realistic conditions, mimicking the CDCB December 2022 official runs. We included all historical phenotypes for daughter pregnancy rate (DPR), cow conception rate (CCR), heifer conception rate (HCR), and early first pregnancy (EFC), comprising 94 million records. The full pedigree consisted of 94 million individuals across the 6 official breeds and their crosses and 417 unknown parent groups (UPG) modeled as unrelated or metafounders. We used about 2 million genotyped animals with records or descendants with records in ssGBLUP with the algorithm for proven and young (APY). A total of 45K genotyped animals representing the 6 breeds and crosses were set as core. Results from the ssGBLUP run were compared with those from BLUP regarding dispersion bias and prediction reliability. For that, the whole dataset contained phenotypes up to December 2022, and the partial dataset was truncated in December 2018. Then, we compared (G)EBV of bulls with no daughters with records in the partial and at least 100 daughters with DPR records in the whole dataset, resulting in 1,891 bulls for Holstein and 303 for Jersey. The linear regression method (LR) was used for validation. Genetic trends were also investigated, which showed a steep decrease until about 2000–2010, followed by a fast increase after including fertility in the genetic evaluation and selection index and using genomic information. The regression coefficients (b_1) varied between 0.89 and 0.94 for ssGBLUP with metafounders and between 0.88 and 0.90 with UPG, resulting in a slightly biased evaluation. For BLUP, the values were from 0.76 to 0.93 and 0.72 to 0.92, respectively, which is more biased because BLUP cannot account for the genomic selection of young animals. The correlation between early (partial dataset) and late (whole dataset) proofs from BLUP varied from 0.49 to 0.65 for metafounders and 0.50 to 0.63 for UPG, whereas for genomic proofs, they varied between 0.80 and 0.91 with metafounders and 0.81 to 0.89 with UPG, showing good reliability of early genomic proofs. Overall, ssGBLUP seems viable for the genomic evaluation of fertility traits in US dairy cattle.

Key Words: daughter pregnancy rate (DPR), unknown parent group (UPG), metafounder

1517 Decreasing computing time of approximated reliabilities of genomic estimated breeding values in single-step genomic best linear unbiased predictor using different core sizes for the algorithm for proven and young.

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Animal and Dairy Science, University of Georgia, Athens, GA, [2]Zoetis Genetics, Kalamazoo, MI.

Single-step GBLUP (ssGBLUP) with the algorithm for proven and young (APY) is used to compute GEBV in livestock populations with extensive genomic data. Calculating GEBV reliabilities is computationally expensive with many genotyped animals because it requires inverting the left-hand side of the mixed model equations. The ACCF90GS2 software from the BLUPF90 suite approximates such reliabilities by considering the sparse structure of the APY inverse of the genomic relationship matrix. The bottleneck of the algorithm is a matrix multiplication that has a quadratic cost with the number of core animals. This study aimed to decrease the computing time for approximating GEBV reliabilities in ssGBLUP by reducing the size of the core set in APY without affecting the quality of the approximations. Reliabilities were approximated for 2 single-trait models with categorical traits. Tests involved commercial dairy cattle datasets comprising 4.5M animals in the pedigree, 1.6M genotypes, and 1.5M records for calf respiratory disease in Holsteins ($h^2 = 0.042$) and 427K animals in the pedigree, 107K genotypes, and 128K records for cystic ovaries in Jerseys ($h^2 = 0.054$). Core sets of varying sizes (25K, 20K, 15K, 10K, and 5K) were tested. Reliabilities obtained with a core set size of 25K were used as a comparison benchmark. Level and dispersion bias were evaluated based on intercept and slope of the regression of reliabilities with 25K core on the other core sizes. For Holsteins, correlations between approximated reliabilities obtained with different core sizes ranged from 0.96 to 0.99, intercepts ranged from 0.02 to 0.30, and slopes from 0.72 to 0.97. Computing times varied from 11.1 to 456.8 min for 5K and 25K core sizes, respectively. Results for Jerseys showed less variability among core sizes. Computing times ranged from 0.76 to 7.44 min. Decreasing the core size reduced computing time up to 40 times compared with the benchmark time. Having fewer genotyped animals in the APY core is a reasonable approach to obtain GEBV reliability faster; however, changes in the approximated reliabilities occur and are trait-dependent.

Key Words: accuracy, computing cost, genomics

1518 Developing genomic relationship values to monitor genetic diversity in the Canadian Holstein population. C. O. Obari*[1], B. O. Makanjuola[1], F. S. Schenkel[1], F. Miglior[1,2], C. Maltecca[3], and C. F. Baes[1,4], *[1]Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, Ontario, Canada, [2]Lactanet, Genetics Division, Guelph, Ontario, Canada, [3]Department of Animal Science, North Carolina State University, Raleigh, NC, [4]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

The increasing rate of inbreeding in the Canadian dairy cattle population has been attributed to the use of advanced reproductive technologies coupled with the adoption of genomic selection. Genomic selection increased genetic gain both by increasing the accuracy of selection and substantially reducing the generation interval, leading to an increased rate of inbreeding per year. Inbreeding could negatively impact economically important traits, such as production, health, and fertility. To mitigate the adverse effects of inbreeding and maintain genetic diversity, it is important to monitor inbreeding in breeding populations. Therefore, the objective of this study was to estimate the genomic relationship values (R-values) across the active Holstein cow and sire population and within herds in Canada. The genomic R-value represents the percentage of DNA an active sire shares with active cows of the same breed. The genotype dataset provided by Lactanet Canada consisted of 9,102 active sires and 84,854 active dams from 3,214 herds. The genomic R-values were estimated using the off-diagonal elements of the genomic relationship matrix. The results indicate that the average relationship of the active sires with the active dam population within herds ranged between 9.45% and 20.13%. Additionally, the across population estimated genomic R-values ranged between 0.00% and 94.79%. With this information, farmers can avoid mating closely related individuals on a population and herd level, since the R-value provides information on expected future inbreeding when closely related animals are mated.

Key Words: inbreeding, dairy cattle, genomic relationship value

1519 Leveraging representative learning to prioritize candidate genes for pleiotropic effects between somatic cell counts and milk technological traits in sheep. P. Augusto de Souza Fonseca*, A. Suárez-Vega, H. Marina, B. Gutiérrez-Gil, and J. J. Arranz, *Universidad de León, Departamento de Producción Animal, Facultad de Veterinaria, León, Spain.*

Achieving high productivity, sustainability, and welfare levels in production systems relies on breeding resilient animals. Understanding the genetic basis of correlations between traits, especially health- and production-related ones, is crucial for selecting resilient animals. Pleiotropy contributes to genetic correlations between traits, which can be better understood by pinpointing candidate genes of those effects. However, functional prioritization of genes remains challenging in genomic scan methodologies. We propose integrating genome-wide pleiotropic signals and weighted gene co-expression networks (WGCN) based on the transcriptome of Assaf sheep milk to enhance the detection of potential candidate genes. GWAS summary statistics were obtained for milk somatic cell counts, milk production/composition, and cheese-making-related traits in 1,039 Assaf ewes. Multitrait statistics for each SNP were calculated as follows: $\chi^2 = t'_i V^{-1} t_i$; where t_i is the vector of signed *t*-values of SNP_i for the traits, and t'_i is its transposed vector, V^{-1} is an inverse of the correlation matrix over the 12 traits. SNP *P*-values were combined with gene-level *P*-values using linkage disequilibrium-weighted Z-scores. Significant genes were identified using a 5% false discovery rate. Additionally, WGCN were estimated from the milk transcriptome of 28 Assaf ewes. Embedding vectors were obtained for genes in WGCN containing at least one significant gene through representative learning (RL). The REGENT pipeline integrated embedding vectors and gene-level *P*-values to create a posterior probability of association for each gene. The results indicate that WGCN harboring prioritized genes were enriched for relevant processes, including immune system activation, cellular proliferation, hormone release, and regulation of neuronal stimulus. These processes may be associated with improved immune response and higher productivity levels. The results obtained suggest that RL is a powerful tool to unravel candidate genes for complex traits by integrating different omics data.

Key Words: resilience, pleiotropy, representative learning

1520 Approximating reliabilities of indirect predictions based on SNP effects from large single-step genomic best linear unbiased prediction evaluations. D. Lourenco*[1], M. Bermann[1], J. M. Tabet[1], I. Aguilar[2], A. Legarra[3], S. Tsuruta[1], and I. Misztal[1], *[1]University of Georgia, Athens, GA, [2]Instituto Nacional de Investigación Agropecuaria (INIA), Montevideo, Uruguay, [3]Council on Dairy Cattle Breeding, Bowie, MD.*

With the algorithm for proven and young (APY), the computing cost of ssGBLUP is linear with the number of noncore animals and cubic with the number of core, which is less than 20K for single breed and 50k for multibreed evaluations. Based on that, ssGBLUP with APY can handle virtually any number of genotyped animals. However, from the almost 8.5M genotyped animals in the US dairy database, only about 2M may be informative. One way to reduce the computing costs due to the large number of genotyped animals in ssGBLUP is to include genotypes for informative animals and indirectly predict GEBV for the remaining genotyped animals that may not have their own or progeny phenotypes. Indirect predictions (IP) are highly correlated with GEBV and have the same scale, which makes them a practical option. However, IP need to be published together with their reliabilities. Reliability of IP are calculated based on prediction error covariance (PEC) of SNP effects, which relies on the inverse of the LHS of MME. If the official ssGBLUP evaluation has many genotyped animals, the inverse is not feasible. Therefore, in this study, we aimed to approximate the PEC of SNP based only on components associated with core animals. First, we approximated PEC for animals based on the block sparse inverse of the inverse of the genomic relationship matrix from APY (APYGi) after adding the contributions from phenotypes and pedigree as weights in the diagonal. This step was already implemented in an algorithm used to compute the reliabilities of GEBV from ssGBLUP in ACCF90GS2 from the BLUPF90 software suite. Changes were made in ACCF90GS2 to save PEC. Additional changes were made in POSTGSF90 to convert PEC for core animals into PEC for SNP. Finally, PREDF90 reads the output from POSTGSF90 and the SNP file to compute IP with their reliabilities. We tested this approximation with Holstein data with 570K genotyped animals. Correlations between the reliability of IP and GEBV for young animals were greater than 0.98. The reliability of IP can be successfully approximated using components from the official ssGBLUP run with only minor modifications.

Key Words: accuracy, genomic estimated breeding value (GEBV), interim evaluation

1521 Genetic correlations between heat tolerance for production traits and for somatic cell score in Canadian Holstein Cattle.

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Studies have shown the possibility of applying genetic selection for heat tolerance. Considering the detrimental impact already reported of heat stress on Canadian dairy production and the projected increase in temperatures during the summer, it has become worthy to consider including heat tolerance in the national genetic evaluation. However, it is crucial to determine the genetic correlation between the heat tolerance component of economically important traits. Therefore, the objective of the present study was to estimate the genetic correlations between the heat tolerance for milk production traits (i.e., milk, fat, and protein yields) and heat tolerance component for somatic cell score (SCS) in Canadian Holstein dairy cattle. Test-day records for milk, fat, and protein yields and SCS from 45,186 first-parity Holstein cows from 1,000 herds across Canada were provided by Lactanet Canada. Cows were required to have a minimum of 3 records within the interval from 5 to 305 DIM. Age at calving records were restricted to 18 to 40 mo. The temperature-humidity index (THI) was calculated for each farm location using weather data from the NASA POWER database and assigned to corresponding test-day records. The genetic correlations were estimated by a multiple trait repeatability animal model fitting a heat stress function. Thus, genetic parameters were estimated under thermal comfort and heat stress conditions. The genetic correlations between heat tolerance for SCS and heat tolerance for milk, fat, and protein were all negative, ranging from −0.51 to −0.65, while the correlations among the heat tolerance for production traits were all positive and moderate, ranging from 0.19 to 0.31. The estimated genetic correlations had large SE, nevertheless, these point estimates suggest that selecting for a production trait's heat tolerance would not have a detrimental effect on other production traits under heat stress. Moreover, selecting for production trait's heat tolerance would result in diminished increase in SCS as a result of heat stress.

Key Words: dairy cow, heat stress, Holstein

Forages and Pastures 1

1522 The effect of theoretical length of cut on leachate yield when chopping male-sterile sorghum with a self-propelled forage harvester equipped with kernel processor. D. Duhatschek*[1], A. Grando Pilati[1], D. Druetto[2], S. Paudyal[1], T. F. Kilcer[3], A. Mazzolari[4], R. Neupane[1], and J. M. Piñeiro[1], [1]*Department of Animal Science, Texas A&M University, College Station, TX,* [2]*Richardson Seeds Ltd., Vega, TX,* [3]*Advanced Ag Systems, Rutledge, TN,* [4]*Ferraroni Mangimi, Bonemerse, CR, Italy.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The objectives of this study were to assess the effect of theoretical length of cut (TLC) and the use of homolactic bacteria inoculant (INOC) on leachate yield (LY) when ensiling a male-sterile forage sorghum. We hypothesized that a longer TLC would reduce LY whereas INOC would not affect it. The experimental design was a randomized complete block design with a 2 × 2 factorial arrangement. Treatments were TLC of 6 mm (SHORT) or 20 mm (LONG), and INOC or distilled water (DW). Sorghum was seeded in 3 plots blocked by irrigation section in Texas, manually harvested at 23% DM, and fed to a self-propelled forage harvester equipped with kernel processors set at 2 mm. Two samples of 3.8 kg of wet matter (WM) from each plot and TLC were treated either with INOC or DW. Samples were packed and ensiled in 4.7 L buckets with 2 sandbags at the bottom. Leachate yield was calculated by subtracting the dry weight of the 2 sandbags from its wet weight after opening the silo. Particle size separation was assessed using a Penn State Particle Separator (PSPS). Mixed linear regression analyses and correlations were performed to assess LY with treatments and their interactions as predictor variables and plot as random effect. Compared with LONG, SHORT when sieved retained more particles at 4 mm ($P < 0.001$) and in the pan ($P = 0.03$), and tended to have more particles smaller than 8 mm ($P = 0.08$; Table 1). Reducing the TLC from 20 mm to 6 mm increased LY by 47% ($P < 0.01$) and LY for INOC was similar that of DW ($P = 0.31$). The LY was positively correlated with the proportion of particles retained on the 4 mm screen ($P < 0.01$; $R^2 = 0.52$). We concluded that decreasing the TLC increases leachate yield whereas the use of homolactic fermentative bacteria did not affect it.

Key Words: leachate yield, theoretical length of cut, male-sterile sorghum

Table 1 (Abstr. 1522). Leachate yield and particle size of male-sterile sorghum chopped with different theoretical length of cut

	Long		Short		
Item	LSM	SEM	LSM	SEM	*P*-value
Leachate yield, % of WM	2.6	0.21	3.83	0.22	<0.01
PSPS, % of WM					
19 mm	6.6	1.39	6.9	1.55	0.87
8 mm	59.3	2.23	56.0	2.47	0.17
4 mm	24.3	1.21	33.1	1.35	<0.001
Pan	9.9	1.87	4.0	2.15	0.03
Below 8 mm					0.08

1523 Comparison of on-farm techniques to assess kernel processing at corn silage harvest. R. C. C. Pinto[1], G. G. S. Salvati*[2], D. E. S. Farinha[1], J. M. Bragatto[1], A. F. Veríssimo[1], G. H. Zapponi[1], L. F. Ferraretto[3], H. A. R. Ramírez[4], W. P. Santos[2], and J. L. P. Daniel[1], [1]*State University of Maringa, Maringa, Paraná, Brazil,* [2]*Tracking Feed, Piracicaba, São Paulo, Brazil,* [3]*University of Wisconsin–Madison, Madison, WI,* [4]*Tecnología Forrajera, San Miguel de Allende, Guanajuato, Mexico.*

Corn silage processing score (CSPS) has been used as an indirect method to assess the degree of kernel breakage in whole-plant corn silage (WPCS). As a laboratorial method, however, CSPS does not allow for decision-making at WPCS harvest. Therefore, the objective of this study was to compare on-farm techniques to measure kernel processing in whole-plant corn forage. Fifty-one samples of whole-plant corn forage from various hybrids, maturities, forage harvesters, chop lengths, and processors were collected. Each sample was homogenized and used for measuring chemical composition, CSPS (% starch passing through a 4.75-mm sieve), intact kernels (n/250 g), and hydrodynamic separation of the grain fraction. A picture of the grain fraction was also captured by Silage Snap app. After samples were dried post separation, the grain fraction was sieved manually or using a Ro-Tap shaker to determine the proportion of grain passing through a 4.75-mm sieve (% DM). The geometric mean particle size (GMPS, μm) was also measured after Ro-Tap sieving and used for comparison with the other methods. Data were analyzed using the REG procedure of SAS. Whole-plant corn forage samples contained (mean ± SD) 37.4 ± 6.0% DM, 42.4 ± 6.4% aNDFom, and 29 ± 7.4% starch. Relationships between GMPS and other methods were significant ($P < 0.01$). Pearson correlation coefficients were −0.94, −0.93, −0.80, +0.68, +0.66, and −0.65, and root mean square errors were 198, 206, 348, 414, 433, and 444 μm for the relationships between GMPS and proportion of grain <4.75 mm determined using a Ro-Tap shaker, proportion of grain <4.75 mm determined using a 4.75 mm manual sieve, proportion of grain <4.75 mm determined using Silage Snap app, number of intact kernels, GMPS estimated using Silage Snap app, and CSPS, respectively. These findings support that on-farm hydrodynamic separation and manual sieving of grain fraction using a 4.75-mm sieve is a feasible tool to assess kernel breakage and allow for decision-making during WPCS harvest. Enumeration of intact kernels, Silage Snap app, and CSPS had the lowest relationships with grain GMPS in whole-plant corn forage.

Key Words: processing score, 4.75-mm sieve, whole-plant corn

1524 Climate-smart ruminant production through dietary manipulations in a climate-resilient environment. T. A. Arilekolasi*[1], O. B. Omotoso[2,3], and J. A. Alokan[2], [1]*Federal University Oye-Ekiti, Oye, Ekiti state, Nigeria,* [2]*Federal University of Technology, Akure, Ondo state, Nigeria,* [3]*Net Zero and Resilient Farming, Rothamsted, United Kingdom.*

Ruminants contribute significantly to global warming through emission of enteric CH_4. Farmers' friendly approach in ensuring climate-smart production is through dietary manipulations. This study was laid out in a 3 × 3 factorial arrangement to show the effect of 3 fertilizer types and 3 drying methods on the nutritive quality and CH_4 production of *Cajanus cajan* hay as feed for ruminant. *Cajanus cajan* pasture was artificially sown on a N_2 depleted tropical soil. Poultry manure (PM) was applied 2 weeks before planting (3 t/ha), while urea was applied to a portion 2 weeks after planting (WAP) at 30 kg/ha, a portion was established without any fertilizer application (control plot). Forages were harvested at 12 WAP and subjected to 3 drying methods (air-drying, sun-drying, and solar cabinet drying) for hay production, chemical analyses were carried out using standard procedures. Feed value of the hay was determined using in vitro gas production technique (IVGPT). Data collected were subjected to statistical ANOVA using GLM procedure of Minitab 18, and means were separated using Tukey's (HSD) test at 5% probability level. There were significant differences in the values obtained for the proximate components, fiber fractions, antinutritional factors composition (ANFs) (alkaloid, tannin, phytate), in vitro dry matter degradability (IVDMD), gas volume, and CH_4 production of the hay produced. Interactions of different combinations showed that unfertilized sundried hay had the highest (91.62%) dry matter (DM) content while solar cabinet dried urea fertilized hay had the highest crude protein (CP) content; however, the lowest CP was recorded in urea fertilized air-dried hay, PM X air-dried hay had the lowest crude fiber (CF) (22.68%), while PM air-dried hay had the highest energy content (15.34 kJ/100 g DM), which was similar to unfertilized sundried and PM sundried hay. Also, PM solar dried hay had the lowest CH_4 production (3.66 mL/200 mg) and the highest OMD (41.96%). Thus, PM is the most efficient fertilizer applied and the solar drying method improved nutrient and feeding value of the hay, and could be adopted for hay making for climate-smart feed.

Key Words: CH_4, feed, hay

1525 Evaluating corn silage nutritive value for dairy cattle: A comparison of MILK index models.

E. C. Diepersloot*, R. D. Shaver, and L. F. Ferraretto, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The objective of this study was to evaluate the performance of the recently released MILK2024 corn silage hybrid ranking index relative to the MILK2006 and MILK2000 indexes. A large dataset of corn silage nutrient composition was compiled from commercial laboratories. To exclude outliers, the normal range (average ± 1 SD) was calculated for each variable from each lab and any samples outside the normal range were removed. Thus, 101,025 samples remained in the final dataset. The final dataset was used to characterize the performance of MILK2024 compared with MILK2000 and MILK2006. To further understand how some nutrients in corn silage affect these models, the final dataset was ranked by starch, NDF corrected for residual ash (NDFom), and NDF digestibility corrected for residual ash (NDFDom). Low- and high-quality samples were designated as the 0 to 10th percentile or 90th to 100th percentile of samples for each nutrient. The overlap of predicted milk yield between high- and low-quality samples was calculated as the percentage of high-quality samples within the range of low-quality samples. The average predicted milk yield was greatest for MILK2000, followed by MILK2024, and MILK2006 (1,835, 1,547, and 1,493 kg milk/Mg, respectively). The range (549, 492, and 293 kg milk/Mg, respectively) and standard deviation (78, 58, and 45 kg milk/Mg, respectively) of predicted milk yield were greatest for MILK2000, followed by MILK2006 and MILK2024. For starch concentration, there was greater milk yield overlap between high- and low-quality samples for MILK2006 (98% of samples) than MILK2000 or MILK2024 (85% and 84% of samples, respectively). For NDFom, the milk yield overlap between high- and low-quality samples was 91% for MILK2000 and 95% for MILK2006, but only 39% for MILK2024. For NDFDom, the milk yield overlap between high- and low-quality samples was 90% for MILK2006, while the overlap for MILK2000 was 60% and MILK2024 was 76%. Based on a lower overlap for milk yield of high- and low-quality samples ranked by NDFom and starch concentration, a better separation between hybrids can be obtained by the MILK2024 than MILK2000 or MILK2006.

Key Words: MILK2024, MILK2006, energy prediction

1526 Spectral sensing for forage quality determination.

R. K. Wright*, R. K. Thompson, and R. R. White, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Forage quality determination is typically represented as an average of a sampled area, with comprehensive sampling being used to account for spatial or temporal variation. However, this approach may fail to capture variation in the quality of consumed forages in grazing settings. To precisely determine quality of grazed forage, there is a need for in-field, real-time quality sensing. The goal of this study was to determine the viability of a spectral sensing system in predicting pasture quality during the growing season. We hypothesized that the spectral sensed data would align with measurements from wet chemistry analyses of dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP). Each week between May and October, 12 fields were sampled, each in 2 randomly selected locations. A spectral reading was taken in-field, 15 cm above the tallest forage, before harvesting each sample. Samples were harvested by clipping an area 30 cm in diameter to a residual grazing height of 5 cm. After harvesting, samples were analyzed for DM, ADF, NDF, and CP. Spectral readings were aligned with wet chemistry results by date, field, and sample. These data were split into 3 parts before analysis such that 15% of observations were used for hyperparameter tuning, 55% for model training, and 30% for independent evaluation. A random forest regression was then fit using the 18 individual spectra and distance to the sample recorded by the sensor system to predict each nutrient. The root mean squared prediction error (RMSPE) was calculated using these models on the evaluation data to explore the ability of the spectral system to predict the forage quality. The RMSPE of DM, ADF, NDF, and CP were 23.0%, 9.5%, 11.4%, and 23.7%, respectively. Residuals were evaluated by both field and sample date and demonstrated minimal systematic variation in error. Given the success of this spectral sensing system when predicting forage quality, future work will focus on more comprehensive data collection to support refining prediction algorithms.

Key Words: sensing, forage quality, prediction

1527 Enhancing sustainability of forage production in Sri Lanka: The impact of vermicompost tea and effective microorganisms on chemical fertilizer reduction.

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This study addresses the growing concerns associated with the heavy reliance on chemical fertilizers (CF) in forage production in Sri Lanka. The experiment, conducted simultaneously within plot size 5 × 5 m across diverse soil and climate zones, aimed to assess the effectiveness of vermicompost tea (VT) and effective microorganisms (EM) in combination with a 50% reduction in recommended CF on the dry matter yield and nutritive value of 4 prominent fodder varieties: Super Napier (SN), Red Napier (RN), Fodder Sorghum (SO), and Fodder Maize (MA). The study spanned 6 mo and employed a 4 × 2 factorial design in a randomized complete block design (RCBD) with blocks as 3 replicates. It included 2 fertilizer treatments: 100% CF (45% N, 21% P, 50% K) and 50% CF combined with VT/EM (1.6% N, 0.3% P, 0.9% K). VT/EM was applied foliarly every 15 d. Fodder was harvested according to standard procedures: 60 d for SN, RN, and SO with 3 repeated harvests and 75 d for MA with a single harvest. Dry matter yield (DMY), CP, ADF, and NDF were evaluated. Data analyses were performed on statistical significance ($P < 0.05$) using the ANOVA and Tukey's LSD test. The results demonstrated significant differences in DMY across

the 4 fodder types, with SO exhibiting the highest yield (SN: 8,714 ± 975; RN: 8,019 ± 675; SO: 14,725 ± 975; MA: 12,699 ± 1,112 kg/ha). The utilization of VT/EM with a 50% CF reduction did not exhibit a significant decrease in DMY compared with the 100% CF treatment across sites and harvests. The application of VT/EM maintained nutritive quality comparable to 100% CF levels, as evidenced by similar values for crude protein (CP%: 9.32 ± 0.47–9.30 ± 0.32); ADF%: 41.65 ± 0.66–41.19 ± 0.74, and NDF%: 60.51 ± 1.31–60.14 ± 1.37). The study highlights that reducing CF by up to 50% did not significantly affect the biomass yield and nutritive quality of the selected fodder varieties. The utilization of VT/EM applications emerges as a robust strategy to reduce the heavy reliance on continuous CF applications, thus promoting sustainable forage production in Sri Lanka.

Key Words: biomass yield, effective microorganism, fodder

1528 Meta-analysis on the effects of physical characteristics of dairy rations on animal performance and feeding behavior in lactating dairy cows.

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A meta-analysis was performed with a dataset of 569 treatment means from 153 published peer-review journal reports from 1990 to 2021. The pooled standard error of the mean for response variables per study was included as a weighting factor for the data. Data were analyzed using PROC MIXED of SAS with fixed effects of treatment and random effect of trial. Dietary mean particle size (mm) tended to reduce intake of DM ($P = 0.08$) by 0.05% BW and milk yield ($P = 0.07$) by 0.37 kg/d for every 1-mm increase in mean particle size of the diet. Conversely, milk fat concentration increased ($P = 0.01$) 0.08% for every 1 mm-unit increase in diet mean particle size. Quadratic associations between feed efficiency calculations (kg milk/kg DMI; $P = 0.01$ and kg ECM/kg DMI; $P = 0.01$) and dietary mean particle size were detected. Similarly, dietary mean particle size had a quadratic relationship ($P = 0.01$) with milk fat and protein yields (kg/d). There was a quadratic relationship ($P = 0.03$) between eating time (min/d) and diet mean particle size. Increasing dietary mean particle size linearly decreased ($P = 0.01$) eating rate (kg/min). The proportion of particles above 19 mm (% DM) in the diet was or tended to be negatively related to DMI (% BW; $P = 0.001$), ECM (kg/d; $P = 0.09$), and milk fat (kg/d; $P = 0.06$) yields. However, protein concentration increased ($P = 0.01$) by 0.01% for every percentage-unit increase in the proportion of particles above 19 mm and tended to be positively related ($P = 0.06$) to feed efficiency (kg milk/kg DMI). There was a quadratic association ($P = 0.03$) between milk fat concentration and proportion of particles above 19 mm. Eating time increased ($P = 0.001$) by 2.2 min/d for every percentage-unit increase in the proportion of particles above 19 mm, whereas eating rate had a negative relationship ($P = 0.001$). Overall, feeding diets with coarser particles reduced intake and milk production by lactating dairy cows.

Key Words: particle size, total mixed ration (TMR), eating time

1529 Performance of pregnant dairy heifers on pasture relative to TMR-fed counterparts.

A. M. Grev*, J. W. Semler, and S. B. Potts, *University of Maryland, Keedysville, MD.*

The objectives of this study were to investigate the effects of improved grazing management on pregnant dairy heifer performance, along with its economic feasibility relative to a conventional management system. Holstein heifers at the University of Maryland Dairy were utilized for this study on a rolling basis, with heifers added following pregnancy confirmation and removed 3 weeks before calving. Heifers (n = 166; 2021–2023) were blocked by due date and randomly assigned to one of 2 treatment groups which varied in size throughout the study period (15 to 22 per group) but were kept consistent between treatments. Heifers in the control (CON) group received a daily total mixed ration and had access to one continuously managed 2-ha perennial pasture. Heifers in the grazing (ROT) group were rotationally grazed across 8 ha of both perennial and annual pastures subdivided into approximately 0.2-ha paddocks (1–2 d rotation); ROT heifers also received a daily corn/mineral mix (0.6 kg/head per d) to ensure dietary requirements were met. Both groups of heifers were measured (body weight [BW], body condition score [BCS], hip height [HH], fecal egg count [FEC]) on a bi-weekly basis throughout the grazing season (April–December). Average daily gain (ADG) was calculated by linear regression and data were analyzed using a mixed model analysis which included the fixed effect of treatment and random effect of block. Mean days on study was 144 and was similar for both groups. Initial BW (527 kg), BCS (3.7), and HH (147 cm) did not differ between ROT and CON heifers ($P > 0.05$). Final BCS (3.7) and HH (152 cm) did not differ ($P > 0.05$), but ADG (0.56 vs. 0.89 kg/d) and final BW (616 vs. 680 kg) tended to be lower for ROT heifers compared with CON heifers ($P = 0.09$). Both groups had very low FECs but ROT heifers were lower compared with CON heifers (9 vs. 16 eggs per gram; $P < 0.01$). These results demonstrate that bred heifers managed in an improved grazing system can achieve satisfactory growth relative to TMR-fed counterparts. Future work will evaluate the economic implications of this system and investigate potential carryover effects on first-lactation performance.

Key Words: pasture, replacement heifer, grazing

Physiology and Endocrinology Symposium: Peripheral Nervous System Regulation of Homeostasis

1530 Impact of postprandial butyrate production on hepatic glucose production via colonic and hepatic signaling pathways. R. M. Meyer*, H. R. Wachsmuth, A. I. Lane, S. N. Weninger, and F. A. Duca, *University of Arizona, Tucson, AZ.*

The liver is the predominant source of endogenous glucose synthesis in ruminants and is critical for many physiological states including fasting and lactation. Our lab focuses on understanding how the gut microbiome impacts hepatic glucose production (HGP), via interactions between ingested nutrients and bacteria that impact both intestinal-mediated and direct hepatic glucoregulatory pathways. For example, bacterial fermentation of nondigestible carbohydrates, either in the distal intestine of monogastrics or rumen of ruminants, leads to the production of volatile fatty acids (VFA), which are known to impact hepatic glucose production. Data from our laboratory show that butyrate increases temporally following a meal, and that intestinal and portal vein concentrations reflect butyrate production and absorption, respectively. However, butyrate levels are much lower in general circulation, indicating that the glucoregulatory impact of butyrate is due to intestinal and hepatic contributions. We have uncovered 2 distinct, tissue-dependent signaling mechanisms by which butyrate impacts HGP. The vagal pathway involves colonic butyrate activation of the SCFA receptor, FFAR2, to induce secretion of GLP-1. This GLP-1 activates a gut-brain vagal signaling axis that limits HGP. Direct signaling of butyrate at hepatocytes also reduces HGP. In fact, acute infusion of butyrate or HDAC inhibitors into the portal vein results in a reduction of HGP in mice. Furthermore, chronic portal vein administration during high-fat diet feeding, a state with excess HGP, results in reduced fasting blood glucose levels by limiting gluconeogenic flux. Our data suggest that hepatic butyrate acts as an HDAC inhibitor, decreasing expression of gluconeogenic enzymes to decrease HGP. Together these data support a strong role of colonic and hepatic butyrate signaling in affecting hepatic glucose production.

Key Words: short-chain fatty acid, gut microbiota, gut-brain axis

1531 Sympathetic circuits regulating hepatic glucose metabolism. A. Zsombok*, *School of Medicine, Department of Physiology, Tulane University, New Orleans, LA.*

The prevalence of metabolic disorders, including type 2 diabetes mellitus, continues to increase worldwide. Despite the availability of newer and more advanced therapies, current treatments are still inadequate and the search for solutions remains. The regulation of energy homeostasis including glucose metabolism, involves an exchange of information between the nervous systems and peripheral organs and tissues; therefore, altering central and/or peripheral neural pathways could be an alternative solution to modulate whole-body metabolism. Liver glucose production and storage are major mechanisms controlling glycemia, and the sympathetic nervous system plays an important role in the maintenance of hepatic glucose homeostasis. Pre-sympathetic neurons in the brainstem and hypothalamus govern the sympathetic output to the liver and change in neuronal activity is one of the underlying mechanisms of autonomic imbalance; therefore, modulation of the excitability of neurons involved in autonomic outflow governance has the potential to improve glycemic status. The talk will provide an overview of the brain-liver pathway and neuronal plasticity during diabetic and obese conditions.

Key Words: sympathetic neurocircuit, liver, viral tracer

1532 Fueling milk production carbon by carbon: Regulation of hepatic glucose production. H. M. White*[1], L. M. Beckett[2], S. J. Kendall[1], T. L. Chandler[3], T. M. Casey[2], and S. S. Donkin[4], *[1]University of Wisconsin–Madison, Madison, WI, [2]Purdue University, West Lafayette, IN, [3]Cornell University, Ithaca, NY, [4]Oregon State University, Corvallis, OR.*

Glucose metabolism in ruminant animals is nuanced and complex, yet is essential to support milk production. Understanding the regulation of hepatic glucose production (HGP) is key to supporting increased milk production, feed efficiency, and metabolic health. A combination of in vivo and in vitro research approaches have been used to better understand the regulation of HGP, including gene knockdown in cell culture systems, isotopic tracing, whole-animal nutritional interventions, and dry cow or feed restriction models. The collective knowledge gained from these studies underscores the complexity of glucose regulation and highlights several key themes that either regulate gluconeogenic pathways or shift nutrient partitioning. Precursor availability is essential for support of HGP, but precursors (e.g., propionate) can also serve to upregulate enzymes that support their own metabolism. Both traditional (e.g., high-starch diets) and nontraditional (e.g., ammoniated lactate, propionate supplements) feedstuffs can provide gluconeogenic precursors to support hepatic glucose production and subsequently mammary lactose synthesis. Although not HGP precursors, many fatty acids are gluconeogenic regulators and both the concentration and fatty acid profile influence the impact on gene expression and pathway flux. Beyond direct regulation of gene expression or protein abundance, some nutrients (e.g., fatty acids and choline) influence nutrient partitioning by shifting the use of specific precursors for HGP. Disrupting management and circadian rhythms can impact HGP by decreasing the use of propionate for glucose production and preferentially shift toward energy production, potentially overriding other regulatory mechanisms. Knockdown of pyruvate carboxylase in 2 different cell culture systems has highlighted the impact of disrupting the delicate regulation of these pathways and shifts nutrient use. Overall, HGP in dairy cows is complex and responsive to several signals requiring a broad suite of experimental tools and multi-omic approaches to examine gluconeogenesis directly, as well as the balance with other competing and supporting pathways.

Key Words: gluconeogenesis, propionate, fatty acid

Production, Management, and the Environment 3

1533 Impact of feeding calcareous marine algae to Holstein dairy cows during the transition period. L. Foley*, K. Mason, K. McLean, and E. Eckelkamp, *University of Tennessee, Knoxville, TN.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

Calcified red seaweed remains (*Lithothamnion calcareum*; calcareous marine algae, CMA) provide bioavailable essential macrominerals. This study evaluated feeding CMA compared with traditional ingredients on cows experiencing heat stress during the transition from late gestation to early lactation (−30 to 30 DIM). Thirty-one multiparous and 9 primiparous Holstein cows were studied to evaluate feeding CMA on dry matter intake (DMI), blood and urine metabolites, and milk yield and components. Data were collected from 21 treatment (TRT) and 19 control (CON) cows balanced for parity and projected calving date. Pre- and postpartum total mixed rations (TMR; 57.1% and 38.2% forage dry matter, respectively) partially replaced calcium carbonate ($CaCO_3$), magnesium oxide (MgO), and sodium bicarbonate ($NaHCO_3$) with CMA. The CON TMR was formulated to provide 1.8% dry matter (DM) as $CaCO_3$ and 0.07% DM as MgO in the prepartum diet, and 1.5% DM as $CaCO_3$, 0.3% DM as MgO, 1.5% DM as $NaHCO_3$ in the lactating diet. The TRT TMR was formulated with 0.8% DM CMA in the prepartum diet (0.7% $CaCO_3$ and 0.07% MgO DM replaced, respectively) and 0.4% DM CMA in the lactating diet (0.3% $CaCO_3$, 0.1% MgO, and 0.7% $NaHCO_3$ DM replaced, respectively). The GLIMMIX procedure of SAS 9.4 was used to determine the impact of treatment, DIM, temperature-humidity index, and projected 305 mature-equivalent milk yield and their 2-way interactions with significance set at $P \leq 0.05$. Stepwise backward elimination was used to remove nonsignificant interactions. No DMI differences were observed between the experimental diets prepartum (−30 to 0 DIM) or in early lactation (4 to 30 DIM). No differences between the experimental diets in early lactation were observed in colostrum quality or quantity, body weight, total milk, fat, or protein yield. Cows fed CMA maintained similar production efficiency values in early lactation compared with CON cows. There were no significant impacts on blood or urine metabolite status pre- or postpartum. Transition cows fed CMA performed as well as their counterparts fed traditional dietary sources of $CaCO_3$, MgO, and $NaHCO_3$.

Key Words: heat stress, transition period, feed additive

1534 Seasonal impact on colostrum quality and transfer of passive immunity in preweaned dairy calves in the Southeastern United States. A. M. Roper*, C. G. Savegnago, T. N. Marins, J. Gao, N. L. P. Kant, J. C. Castro, G. G. Cunha, and S. Tao, *Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

This abstract is part of the ADSA-Graduate Student Competition: Production (MS Oral Semifinalists).

Our aim was to evaluate the seasonal effects on colostrum quality and transfer of passive immunity (TPI) in the Southeastern United States. Holstein dairy heifer calves (n = 699) from a commercial dairy farm located in Central Georgia were enrolled at 2 to 4 d of age within a 12-mo span. Calves were fed maternal colostrum within 2 h of birth and then raised in individual hutches in a covered barn. Ambient temperature (AT) and relative humidity were measured at 15-min intervals during the entire experiment in close-up barn, calving area, and calf barn. At enrollment, serum was collected from all calves. Fresh colostrum samples (n = 1,151) from individual cows were collected. The immunoglobin G contents of colostrum and serum were estimated using Brix refractometry as proxy of colostrum quality and TPI, respectively. Segmental linear regression (R Studio) was used to analyze seasonal effect on TPI and colostrum quality by week of a year (January 1–December 31). Summer months had greater AT with the highest average daily AT observed in August (30.76°C). Winter months had the lower AT with the lowest average daily AT observed in December (−16.37°C). Colostrum quality maintained constant until April. Then colostrum quality started to decrease ($P = 0.02$) and reached nadir in June. From June to October, colostrum quality increased ($P < 0.01$) potentially due to a dilution effect by lower colostrum yield during heat stress. From October to December, colostrum quality decreased ($P = 0.01$). TPI increased ($P < 0.01$) from January to April, decreased ($P < 0.01$) from April to June, and then increased ($P = 0.01$) from June to July. These changes corresponded with changes in colostrum quality. However, we observed a decrease ($P < 0.01$) in TPI from July to October despite the increased colostrum quality, potentially indicative of the negative impact of maternal heat stress during the dry period on TPI. In conclusion, calf TPI is dependent upon colostrum quality during the first 7 mo of the year, but late-gestation heat stress decreases TPI regardless of colostrum quality during fall and winter months.

Key Words: passive immunity, season, heat stress

1656 Flawed relationships between sniffer-measured gas concentrations and daily methane emissions in dairy cattle. M. Harrison* and S. Zimmerman, *C-Lock Inc., Rapid City, SD.*

Successfully selecting and breeding low methane (CH_4) emitting animals requires accurate gas measurements, and for progress to be achieved at scale, individual gas emission phenotypes must be able to be easily collected on a large number of animals. In an effort to easily measure individual enteric emissions on-farm, sniffer gas concentration measurement systems have been used in various studies, but inconsistent methodologies, equations, and reporting standards makes systematic review and analysis challenging. The objective of this study was to quantify relationships between sniffer CH_4 concentrations and animal/production characteristics. A systematic review indicated 64 potential studies for inclusion, but the evaluation only included 16 group means from 9 published studies that reported mean (±SD) CH_4 concentration (547 ± 424 ppm) in addition to dry matter intake (DMI; 20.6 ± 1.9 kg/d), milk yield (MY; 26.2 ± 7.7 kg/d), or body weight (BW; 644 ± 61 kg), so methane concentrations could be compared with production parameters and methane predictions. Pearson correlation coefficients indicated poor relationships between CH_4 and DMI ($r = –0.88$), BW ($r = –0.16$), and MY ($r = 0.22$). A fixed regression model was used to evaluate mean emissions data (n = 12) from 4 direct comparison studies that measured CH_4 concentrations with sniffers and fluxes (g/d) using respiration chambers or GreenFeed. There was no relationship between CH_4 concentration and flux ($R^2 = 0.04$). Negative correlations between CH_4 ppm and production parameters and low phenotypic correlations suggest a systematic bias with sniffer measurements, indicating that sniffer data are not suitable for genetic selection and on-farm emissions monitoring.

Key Words: methane, sniffer, modeling

1536 Development of a Fresh Cow Index for first-lactation cows. A. Hanson*[1], R. Fourdraine[2], J. Clay[2], and A. De Vries[1], [1]*University of Florida, Gainesville, FL,* [2]*Dairy Records Management Systems, Raleigh, NC.*

The objective of this study was to develop a method to measure and benchmark the quality of management of heifers entering the milking herd. Heifer and first-lactation records between 2006 and 2024 were provided by Dairy Records Management Systems. The following variables were extracted for use: herd code, animal index, breed, birth date, breeding date, calving date, test date, DIM at first test, milk at first test, milking frequency, calving ease, parent average milk, and if the first lactation started from an abortion. The variables gestation length and age at first calving were calculated using the respective dates. The data were then filtered to exclude missing data, duplicates, outliers, multiple test dates for one individual, and lactations that started from an abortion. The resulting database consisted of 986,038 unique first-lactation cows from 5,290 farms across the United States. A mixed linear regression model for first test-day milk was fit using DIM, gestation length, parent average milk, age at first calving, calving month, breed, milking frequency, calving ease, and herd code (random). The coefficients from the model were then extracted to predict milk yield at the first test-day. The Fresh Cow Index (FCI) was calculated by dividing actual first test-day milk by the predicted first test-day milk and multiplied by 100. Farm average FCI were calculated by averaging FCI of all first-lactation cows. The mean ± SD of the FCI was 99.34 ± 13.36 and the mean FCI of all animals was 101.06 ± 21.64. Further work includes associating the FCI with future cow performance such as reproduction, risk of culling, and 305-d milk yield. In conclusion, the FCI allows dairy farms to benchmark the transition success of heifers into first-lactation cows, but more work needs to be done to further improve the index.

Key Words: heifer, transition, benchmark

1537 Deep learning and image processing for high-throughput udder phenotyping in dairy cows. M. E. Montes*[1], G. L. Menezes[1], D. Reinemann[2], L. L. Hernandez[1], and J. R. Dorea[1,2], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Biological Systems Engineering, University of Wisconsin–Madison, Madison, WI.*

Udder morphology is associated with performance and is important for genetic improvement in dairy cattle. However, systematic and large-scale udder phenotyping is labor-intensive. Therefore, this project aims (1) to automate the collection of udder phenotypes from depth images (img), (2) to generate phenotypes from the udder and quarter segments (e.g., teat geodesic distance, circularity, eccentricity, surface area, and volume), and (3) to investigate their association with voluntary milking system (VMS) records (e.g., milk flow, yield, and kick-offs). Videos of the udders of 150 Holstein cows (parity 2.2 ± 1.2, DIM 155 ± 103) were recorded at a VMS farm using a RealSense D455 depth camera at 30 fps. Images from 50 cows were incorporated into a larger dataset used to train (Tr) and test (Te) convolutional neural networks (CNN; YOLOV8) for classification, segmentation, and keypoint detection. The classifier (Tr = 14,164; Te = 4,778 img), trained to select images without occlusion, had an F1-Score of 0.91. The segmenter (Tr = 2,178; Te = 725 img) had an intersection over union of 0.93 ± 0.03 on the udder region. The keypoint model (Tr = 14,164; Te = 4,778 img) predicted teat locations with error (%; distance/udder diagonal) of 5.7 ± 3.8 left front, 4.6 ± 3.8 right front, 5.2 ± 4.1 left rear, and 6.1 ± 4.7 right rear. The Watershed algorithm was used to segment the udder into quarters, and a second classifier was trained to discard incorrect results (recall = 0.87 ± 0.12, precision = 0.56 ± 0.04, k = 5 folds, 300 img). The CNN models were used to get image class, udder region, and teat locations from images of the remaining 100 cows and unannotated images. Udder phenotypes were extracted from all nondiscarded images (170 ± 191 img/cow, 138 cows), and the median was used for correlation analysis. Udder surface area was correlated with session yield ($r = 0.53$, $P < 0.01$) and highest yield of current lactation ($r = 0.48$, $P < 0.01$), and front teat geodesic distance with session yield ($r = 0.41$, $P < 0.01$). Deep learning and image processing accurately produced large-scale udder morphology traits. Associations of the image-derived traits with performance and genetics should be investigated.

Key Words: udder phenotype, depth image, deep learning

1538 Identification of dam factors associated with immunoglobulin G mass in colostrum of dairy cows. H. M. Goetz*[1], M. A. Steele[2], O. N. Genther Schroeder[3], M. Gonzalez Cabrera[4], A. Pineda[2], T. Earleywine[3], and D. L. Renaud[1], [1]*Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada,* [3]*Research and Development, Land O'Lakes, St. Louis, MO,* [4]*IUSA-ONEHEALTH 4. Animal Production and Biotechnology, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, Arucas, Spain.*

The objective of this cohort study was to identify predictors of colostrum IgG mass in Holstein dairy cows. Colostrum and data were collected from 505 cows that calved from August 2017 to January 2019, enrolled across 3 trials with 4 negative DCAD prepartum diets, housed in a freestall barn and fed 1×/d using Calan gates at the Purina Animal Nutrition Center. Mixed linear models were used to assess variables associated with colostrum IgG mass (volume × concentration of IgG), including season, parity, number of days between the calving date relative to expected calving date, DMI, and metabolizable protein (MP) intake on the day of calving as well as −1, −2, −3, and −4 weeks relative to calving, with cow nested within diet as a random effect. The median (range) IgG mass produced was 621.64 g (20.41 to 2,488.41). In the final multivariable model, for every 1 d beyond expected calving date, IgG mass increased by 8.36 g ($P = 0.03$, 95% CI = 0.63 to 16.09) and multiparous cows produced more IgG than primiparous cows (310.70 g, $P < 0.001$, 95% CI = 214.51 to 406.90). At wk −2, DMI was negatively associated with IgG (−9.19 g, $P = 0.05$, 95% CI = −18.54 to 0.16), and at wk −3, MP intake was negatively associated with IgG (−94.20 g, $P = 0.04$, 95% CI = −185.67 to −2.73). The association of hematological variables measured in serum of the dam on the day of calving with IgG mass was also assessed in a subset of cows (n = 205). For every 1 g/dL increase in total protein on day of calving, IgG mass decreased by 66.95 g ($P < 0.01$, 95% CI = −114.81 to −19.09), whereas gamma-glutamyl transferase (GGT) was positively associated with IgG (446.47 g, $P < 0.01$, 95% CI = 147.76 to 745.17). A 1 g/dL increase in albumin concentration was associated with a 320.91 g increase in IgG mass ($P = 0.02$, 95% CI = 41.56 to 600.07) at wk −2 and an increase of 451.15 g ($P = 0.03$, 95% CI = 38.82 to 863.48; n = 117) at wk −3. Additionally, serum GGT was negatively associated with IgG mass at wk −3 (−16.99 g, $P = 0.02$, 95% CI = −30.97 to −3.01). These results suggest that IgG mass in colostrum can be predicted by dam-related factors before calving.

Key Words: immunoglobulin, transfer of passive immunity, colostrum quality

1539 Early detection of subclinical ketosis in Holstein dairy cows using computer vision and recurrent neural networks. R. E.

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In the transition period, the severity of NEB can increase the likelihood of peripartum disorders, resulting in significant financial impact on dairy operations. Computer vision (CV) emerges as a powerful tool for improving management decisions in a nonintrusive and scalable manner. Our objective was to develop a CV algorithm for the early detection of postpartum subclinical ketosis (SCK) in dairy cows using depth images collected between 3 and 1 week before calving. Videos from 115 cows were recorded weekly, tentatively between 3 weeks before the expected calving date and 8 weeks after the calving date, with body condition score (BCS) assessments performed during recording by 3 evaluators. A convolutional neural network (CNN) coupled with a recurrent neural network (RNN) was trained to predict the BCS of a cow in the subsequent week using sequences of images extracted from previous consecutive videos of that cow. Using CNN-RNN, features were extracted from sequences of 3 images obtained from the 3 prepartum videos. Of the 115 cows, 92 had exactly 3 videos collected before calving, and the features extracted from those were used to train machine learning (ML) algorithms for SCK prediction. As a comparison baseline, a CNN network for BCS prediction was used to extract features from individual images, and the concatenated features from images of the 3 prepartum videos were used to train ML algorithms for SCK prediction on the same 92 cows. Using nested 5-fold cross-validation, the best SCK prediction models using CNN-RNN and concatenated CNN features achieved average accuracies of 69.6% (±7.1%) and 63.0% (±4.5%), respectively. These results indicate that features extracted from a sequence of prepartum depth images are better postpartum SCK predictors than those extracted from individual images, taking advantage of the relationships between images of the same cow taken in different time points. These findings allow for the development of more accurate models for SCK prediction, potentially improving animal health and reducing economical losses caused by SCK in dairy farms.

Key Words: computer vision, ketosis, transition

1540 Liver function and its relationship with milk production and ovarian activity in dairy cows.

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The liver functionality index (LFI) is a marker that measures how well liver function is, and it could be a determinative tool during the transition period. Our study aimed to assess the LFI and its relationship with milk production and the onset of ovarian activity. The study was conducted in Bijagual, Chiriqui province, Panama. The experimental design consisted of repeated measurement using a mixed model in SAS. Thirty dairy cows were enrolled and randomly distributed into 2 experimental groups, CON (supplemented with concentrate) and MIX (supplemented with corn silage and soybean plant). Blood samples were taken on d 3 and 28 postpartum and analyzed for bilirubin (mg/dL), cholesterol (mg/dL), and albumin (g/L). Data were included in the LFI equation to determine the liver LFI (positive or negative). The variables studied were weight loss at 28 d, milk production at 7 (initial), 28, and 100 d, ovary and follicle size, and d to first heat. Diet CON or MIX did not affect any of the variables studied ($P > 0.05$). However, cows with positive LFI had higher initial milk production ($P = 0.07$) and 28 d ($P = 1.28$) but not at 100 d ($P = 1.67$). That means cows with positive LFI tend to improve milk yields at 7 d but not at 28 and 100 d. Weight loss was unrelated to LFI ($P = 0.605$). In contrast, positive LFI increased ovary ($P = 0.009$) and follicle ($P = 0.006$) size for both transverse (T) and longitudinal (L) compared with negative status. Cows with positive LFI had more prominent preovulatory follicles ($P = 0.006$) than negative, showing differences of 9.00 and 4.64 mm for T and L, respectively. Additionally, LFI was positively correlated with preovulatory follicle size T ($r = 0.28$) and L ($r = 0.52$). Within the first 90 DIM, 89% of cows were in heat, averaging 49 d to first estrus postcalving. However, LFI was not statistically different ($P = 4.43$) with d to the first heat. We conclude that LFI status was correlated with initial milk production but not at 28 and 100 d. However, the most significant implications of LFI were related to the preovulatory follicle, showing remarkable size improvement.

Key Words: biomarker, ovarian activity, transition period

1541 The economic impact of clinical endometritis in dairy cows.

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The objectives were to calculate the cost of clinical endometritis (CE) and to evaluate the individual and combined effects of metritis (MET) and CE on the profitability of dairy cows. The dataset was composed of 11,051 cows. The cost of CE was calculated by subtracting the gross profit of cows with CE from the gross profit of cows without CE. Continuous and dichotomous outcomes were analyzed using linear mixed-effects and generalized linear mixed-effects models, respectively. Models included the fixed effects of CE, MET, parity, morbidity in the first 60 DIM, their interactions, region, season of calving, and random effect of farm and the interaction between CE and farm. An additional model was used in which cows were classified as having no uterine disease (NUD), only MET (MET), only CE (CE), or both MET and CE (METCE). This model included the fixed effects of uterine disease (NUD vs. MET vs. CE vs. METCE), parity, morbidity, their interactions, region, season of calving, and random effect of farm and the interaction between uterine disease and farm. A nonorthogonal comparison was also performed to evaluate the additive effect between MET and CE. A stochastic analysis was performed using 10,000 iterations with varying relevant inputs. Cows with CE produced less milk, were less likely to be pregnant, and were more likely to be culled by 305 DIM compared with cows without CE. Consequently, milk sales and residual cow value were lesser for cows with CE. Replacement and reproductive management costs were higher for cows with CE. The mean cost of CE was $202 per case. The stochastic analysis also showed that the mean cost ranged from $152 to $265 per case. The combination of MET and CE led to additive negative effects, which resulted in a mean cost of $457 per case compared with NUD, whereas MET and CE resulted in a cost of $179 and $163, respectively. In conclusion, CE caused a large economic loss to dairy herds by being associated with decreased milk yield, reproductive efficiency, and the proportion of cows remaining in the herd. The negative effect of CE on profitability was independent but additive to the negative effect of MET.

Key Words: economics, endometritis, dairy cow

1654 Effects of dietary lipid supplements and feeding level on milk production and methane emissions in Holstein dairy cows.

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Lipid supplements are commonly fed to dairy cows; however, the effects of fatty acids (FA) on methane emissions requires clarity. Our objective was to investigate the effects of 2 FA supplements on milk production and methane (CH4) emissions. In a split-plot design, 36 multiparous Holstein cows (2.1 ± 1.0 lactations; 43 ± 6.4 kg milk/d; 138 ± 13 DIM) were enrolled. Cows were fed a corn silage-based diet (43% DM and 24% starch). The main plots were a palmitic acid-enriched supplement (HP) or calcium-salts of palm FA (PO). Within each main plot, cows were assigned to 3 supplementary levels (0%, 1.5%, or 3% total FA, DM basis) in a 3 × 3 Latin square design with three 21-d periods. Dietary FA predominantly replaced aNDFom. Ruminal greenhouse gas emissions were measured 3 times daily on d 19 to 21. Data were analyzed in a mixed model with fixed effects of plot, FA level, period, square, treatment × period, and the random effect of cow nested in the square. Preplanned contrasts compared HP 1.5% versus PO 1.5% and HP 3.0% versus PO 3.0%. Increasing FA level in the diets decreased DMI regardless of FA supplement ($P < 0.05$). Increasing FA level in the diets increased yields of milk and ECM for both supplements ($P < 0.05$). Cows fed PO at 3.0% FA had greater milk yields relative to HP (42.9 vs. 40.6 kg/d; $P = 0.03$). Cows fed HP at 1.5 or 3.0% FA had greater fat and true protein contents relative to PO (4.63 vs. 4.40% fat and 3.54 vs. 3.34% true protein for 3.0% FA level, respectively; $P < 0.05$). Fat, lactose, and TS yields increased as the FA level increased for both supplements ($P < 0.05$). Methane production (g/d), intensities (g/kg of milk or ECM), and yields (g/kg of DMI) decreased as FA level increased regardless of FA supplement ($P < 0.05$). Feed efficiencies (milk/DMI or ECM/DMI) increased as the FA level increased for both supplements ($P < 0.05$). Cows fed PO at 1.5% or 3.0% FA had greater feed efficiency (MY/DMI), relative to HP ($P < 0.05$). In conclusion, lipid supplementation type and level uniquely modify lactation performance and methane emissions

Key Words: fatty acid, methane, milk

Ruminant Nutrition 5: Gut Physiology, Fermentation, and Digestion

1543 The effect of undegradable fiber of alfalfa and grass hays on neutral detergent fiber passage rate and digestibility. H. Galyon* and G. Ferreira, *Virginia Tech, Blacksburg, VA.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The objective of this study was to determine how forage undegradable neutral detergent fiber (uNDF) influences rumen NDF passage rate (K_p) and potentially degradable NDF (pdNDF) digestibility. Twelve primiparous Holstein cows were randomly assigned to 1 of 2 diets containing either alfalfa hay (Alfalfa) or grass hay (Grass) with corn silage as a secondary forage in a crossover design with two 28-d periods. The alfalfa hay contained 38.0% NDF and 14.1% uNDF and the grass hay contained 63.2% NDF and 20.2% uNDF (DM basis). Diets were formulated for hays, silage, and concentrate to each contribute 33.3% of the dietary NDF. Six of the cows were rumen-cannulated to determine uNDF pool size (PS) and K_p. To determine PS, rumen evacuations were performed 2 h before feeding and 2 h after feeding 7 d apart each period. To determine K_p, a pulse dose of externally marked NDF was inserted into the rumen. The dilution of the marker in rumen contents determined at 0, 3, 6, 9, 12, 24, 36, 48, 60, and 72 h were fitted to an exponential model using PROC NLIN of SAS. Nutrient digestibility was determined using uNDF as an internal marker. All variables were evaluated using PROC MIXED of SAS and the model included the fixed effect of diet and the random effects of cow and period. Significant differences were declared when $P < 0.10$. Cows consuming Alfalfa consumed more DM (26.7 vs. 24.6 kg/d; $P < 0.01$) and uNDF (2.8 vs. 2.3 kg/d; $P < 0.01$) but consumed less NDF (8.2 vs. 8.7 kg/d; $P < 0.01$) than cows consuming Grass. The uNDF PS did not differ between Alfalfa and Grass (2.4 kg; $P = 0.99$). Cows consuming Alfalfa had a faster K_p (5.02 vs. 4.03%/h; $P = 0.06$), a shorter rumen retention time of NDF (21.0 vs. 26.2 h; $P = 0.09$), and greater pdNDF digestibility (58.1 vs. 52.3%; $P = 0.02$) than cows consuming Grass. The feces from cows on Alfalfa contained less NDF (46.1 vs. 52.1% DM; $P < 0.01$) and less pdNDF than cows on Grass, both on a NDF basis (47.7 vs. 60.9%; $P < 0.01$) and on a DM basis (22.0 vs. 31.8%; $P < 0.01$). In conclusion, although alfalfa hay contains more uNDF on an NDF basis than grass hay, the pdNDF is utilized more efficiently when included in the diet.

Key Words: uNDF, passage rate, rumen retention time

1544 Effect of a blend of essential oils on production performance and nutrient digestibility in Jersey cows transitioning from confinement to grazing. M. A. Arshad*, K. V. Almeida, D. C. Reyes, A. L. Konopoka, M. A. Rahman, M. M. H. Khandakar, R. A. Menezes, and A. F. Brito, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH.*

Essential oils (EO) have been used to improve milk yield and composition. However, we are not aware of any research to date that has investigated the effect of supplementing EO on DMI, milk yield and composition, and nutrient digestibility in dairy cows transitioning from winter (i.e., confinement) to the grazing season. Twenty-two (6 primiparous and 16 multiparous) organic certified Jersey cows (83 ± 39 DIM and 468 ± 51 kg of BW) were assigned to 1 of 2 treatments in a randomized complete block design with repeated measures over time: (1) 0 g/head/d of EO (control = CON) or (2) 1 g/head/d of EO (Agolin® NATURU). The experiment lasted 12 wk, with 2 wk for covariate measurements and 10 wk for treatments evaluation. Data and samples collected during wk 5 (confinement) and wk 10 (grazing) were used for statistical analyses. Cows in confinement received a diet with a 60:40 forage:concentrate ratio, while during grazing 35% (DM basis) of grazed herbage replaced the grass-legume baleage. Except for feed efficiency, no treatment by week interactions were observed for the remaining variables shown herein. Diets did not affect ($P \geq 0.12$) DMI and concentrations of milk fat, true protein, and MUN. Contrarily, cows fed EO had greater ($P < 0.01$) milk yield (31.3 vs. 30.1 kg/d), 4% FCM yield (34.5 vs. 31.9 kg/d), and ECM yield (34.4 vs. 32.1 kg/d) than CON. Feeding EO also increased ($P = 0.05$) milk fat yield (1.42 vs. 1.34 kg/d) and tended ($P \leq 0.06$) to increase that of milk true protein. Feed efficiency did not change during confinement, but it was greater ($P < 0.05$) with EO versus CON during grazing (1.41 vs. 1.33 kg/kg and 1.41 vs. 1.32 kg/kg for milk yield/DMI and ECM yield/DMI, respectively). Whereas the apparent total-tract digestibility of DM, OM, and CP were not affected ($P \geq 0.34$) by diets, that of aNDFom tended ($P = 0.06$) to increase in EO versus CON. Overall, yields of milk, 4% FCM, ECM, and milk fat increased with EO, and the lack of interactions for most variables evaluated in our study indicate that the effect of EO was independent of feeding system (i.e., confinement or grazing).

Key Words: digestibility, essential oil, milk yield

1545 Measures of rumen health and function in organic pasture-raised dairy heifers. C. Goodey*[1], S. Fonnesbeck[1], K. Hintze[1], B. Miller[1], J. Cuthbert[1], K. Thornton-Kurth[1], B. Waldron[2], M. Greenland[1], R. Stott[1], A. Sweat[1], E. Creech[1], and C. Isom[1], [1]*Utah State University, Logan, UT,* [2]*USDA-Agricultural Research Service, Forage and Range Research Laboratory, Logan, UT.*

We hypothesized that variations in the constitution and performance of the rumen microbiome are responsible for a substantial part of the unexplained variance noted in the growth of grazing dairy heifers. To test this hypothesis, 288 dairy heifers from 4 distinct genetic backgrounds (“breeds”; Holstein, Jersey, Holstein/Jersey crossbred, and ProCROSS) were grazed on one of 2 treatment types (monoculture grass [MONO], or grass interseeded with the legume birdsfoot trefoil [MIX]) over the course of three 105-d grazing seasons. Additionally, 48 heifers were fed a total mixed ration (TMR; forage:concentrate ratio of 77:23 on a dry matter basis) in confinement for reference. Rumen fluid was collected via esophageal tubing on the first day of the study each year, and at the end of each of three 35-d grazing rotations thereafter. Rumen fluid was analyzed for ammonia content via phenol-hypochlorite assay and for short-chain (volatile) fatty acid (VFA) content via gas chromatography. Rumen microbiome profiling was accomplished by ultra-deep 16S rRNA sequencing and associated bioinformatic analyses. Results indicate that there was a significant effect of treatment on rumen ammonia content (with MIX > MONO > TMR; $P < 0.05$), but no effect of breed or breed × treatment interactions on ammonia levels ($P > 0.05$). There were main effects of treatment ($P < 0.05$) on the acetate, propionate, and butyrate content in the rumen fluid. There was a main effect of breed on propionate and butyrate content ($P < 0.05$), but not acetate levels ($P > 0.05$). There was no significant breed × treatment interaction for any of the VFAs ($P > 0.05$). Preliminary analyses of the microbiome profiles suggest significant effects of calendar year (season), collection date within a calendar year, and treatment on α diversity and β diversity scores

(FDR adjusted $P \leq 0.05$). The influence of breed on α and β diversity scores was inconsistent between years. We anticipate that this research will provide clarification of the complementary relationships between animal genetic background and rumen health and function, and their effect on feed intake and performance in livestock animals.

Key Words: rumen microbiome, heifer development, birdsfoot trefoil

1546 An interplay of viruses, bacteria, and protozoa in the rumen of dairy cows may contribute to improved feed efficiency. H. F. Monteiro*[1], R. Profeta[1], M. Van Heule[1], B. C. Weimer[1], C. T. Brown[1], J. E. P. Santos[2], R. S. Bisinotto[3], E. S. Ribeiro[4], F. Penagaricano[5], and F. S. Lima[1], *[1]Department of Population Health and Reproduction, University of California–Davis, Davis, CA, [2]Department of Animal Sciences, University of Florida, Gainesville, FL, [3]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, [4]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [5]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI.*

Previous findings from our group indicate the rumen microbiome of dairy cows may play a role in feed efficiency through greater ruminal ornithine production. The aim of the study was to investigate mechanisms by which the rumen microbes can potentially modulate residual feed intake (RFI). It was hypothesized that predation of ruminal bacteria by protozoa was upregulating their protozoal urea cycle and, thus, ornithine production in highly feed-efficient cows. From a cohort of 454 lactating cows, 24 cows with only diverging RFI were chosen. Total rumen content RNA was extracted, ribo-depleted, and analyzed using ultra-deep dual-RNA sequencing. After quality control, sequencing reads were split into host and microbial reads using the bovine ARS-UCD1.2 reference genome. The microbiome composition was assessed using *Sourmash* against the GenBank database. De novo gene assembly was performed for the host and microbial reads in *Trinity*. Gene functional annotation was performed using several databases in *MEGAN* and *Prokka*. Differential gene expression was performed using *DESeq2*. Preliminary results indicate the presence of 478 bovine genes in the rumen content. One extra manually annotated mitochondrial gene potentially from bovine was upregulated in most feed-efficient cows. For the rumen microbiome, a *Crassvirales* sp. virus known to infect *Bacteroidetes* was upregulated in most efficient cows, which may have contributed to a shift in several bacteria and potentially protozoa. The ribosomal gene of the protozoa *Entodinium caudatum* was downregulated, while a similar one in *Ostracodinium dentatum* from the same family was upregulated. Certain genes linked to bacteria nitrogen metabolism showed an upregulation in most efficient cows, hinting at a potential cascade of microbial interactions leading to ornithine production. While it is still uncertain whether an RNA virus is the initiator of these changes, understanding these intricate mechanisms marks a significant milestone in harnessing improvements in feed efficiency toward more sustainable livestock production.

Key Words: gene expression, metabolomics, metatranscriptomics

1547 Effect of a biosynthetic bacterial 6-phytase on nutrient digestibility in lactating dairy cows. R. García-González*[1], S. Ped[2], G. Dusel[2], C. Koch[3], T. Christensen[4], L. Marchal[1], and Y. Dersjant-Li[1], *[1]Danisco Animal Nutrition and Health (IFF), Oegstgeest, the Netherlands, [2]University of Applied Sciences Bingen, Bingen am Rhein, Germany, [3]Educational and Research Centre for Animal Husbandry Hofgut Neumühle, Münchweiler an der Alsenz, Germany, [4]Danisco Animal Nutrition and Health (IFF), Brabrand, Denmark.*

The effective degradation of phytate in the rumen has been shown to be variable. Undegraded phytate leads to incomplete phytate-P utilization and possible antinutritional effects of phytate. In this study, we examined the effects of a novel biosynthetic bacterial 6-phytase (PhyG) on nutrient digestibility in lactating dairy cows. Forty-eight multiparous Holstein cows averaging 44.1 ± 6.0 kg milk/d and 124 ± 62 DIM were assigned to 3 treatments in a randomized block design. Diet was fed ad libitum as a TMR via electronically operated feeding throughs and was formulated to meet nutrient requirements, except for P which was fed at ca. 92% recommendation. TMR comprised corn silage, grass silage, ensiled beet pulp, and concentrates, and was supplemented with TiO_2 as external digesta marker and PhyG at 0 (CON), 2,000 (PhyG2000), or 5,000 (PhyG5000) phytase units (FTU)/kg DM. The experiment comprised 14 d of adaptation and 5 d of fecal collection and measurements. Fecal grab samples were collected twice daily in a scattered schedule including morning and evening hours; diets were sampled 4 times; DMI, milk production, and BW were monitored daily; and milk composition was analyzed from 2 consecutive milkings. Data were fitted to a mixed model with treatment as fixed effect and block as random, and linear and quadratic contrasts were tested. The apparent total-tract digestibility (ATTD) of total P (33.4%, 40.8%, and 47.1% for CON, PHyG2000, and PhyG5000, respectively), CP (57.1%, 57.7%, 61.2%) and ash (22.4%, 32.8%, 39.0%) linearly increased ($P < 0.05$) with PhyG dose while the ATTD of phytate-P (88.5%, 96.2%, 97.7%) and NDF (49.1%, 55.6%, 56.6%) increased ($P < 0.05$) both linearly and quadratically with increasing PhyG dose. DMI, BW, and milk yield did not differ across treatments, while milk protein yield increased linearly ($P = 0.05$) and tended to increase quadratically with PhyG dose (1.31, 1,40, 1.40 kg/d). The addition of PhyG phytase to the diet of lactating cows can be a tool to maximize nutrient efficiency and minimize costs and environmental impacts in dairy operations.

Key Words: phytase, phytate, digestibility

1548 Metabolomics analysis investigating the impacts of essential oil blends and fumaric acid on rumen fluid metabolites in dairy cows using a RUSITEC system. J. O. Alabi*, M. Wuaku, C. C. Anotaenwere, D. O. Okedoyin, O. O. Adelusi, O. A. Oderinwale, J. M. Enikuomehin, K. A. Ike, and U. Y. Anele, *North Carolina A&T State University, Greensboro, NC.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

The potential of essential oils and fumaric acid (FA) to modulate ruminal fermentation and mitigate greenhouse gas emissions in dairy cows has attracted significant attention. However, little is known about the specific metabolites produced as a result of their interaction. This study investigated the combined effects of essential oil blends (EOB) and FA on rumen metabolites using a rumen simulation technique system. Three rumen-cannulated, nonlactating Holstein Friesian cows served as inoculum donors. The total mixed ration (TMR) comprised corn silage (60%), alfalfa hay (20%), and a concentrate mix (20%). Three distinct EOB were formulated: EOB1 (Garlic, Lemongrass, Cumin, Lavender, and Nutmeg; 4:2:2:1:1), EOB2 (Anise, Clove, Oregano, Cedarwood, and Ginger; 4:2:2:1:1), and EOB3 (Clove, Anise, Peppermint, and Oregano; 4:3:2:1). Four treatments evaluated were control (CON), EOBFA1 (EOB1 + FA), EOBFA2 (EOB2 + FA), and EOBFA3 (EOB3 + FA). The EOBs and FA were included at 10 μL/g feed and 3% of TMR, respectively. Rumen effluents were collected over 5 d for metabolome analysis using a liquid chromatography-mass spectrometry (LC-MS). A total of 495 metabolites were detected and identified. Volcano plot analysis revealed no differentially abundant metabolites between CON and EOBFA groups ($P \leq 0.05$). Partial least squares-discriminant analysis (PLS-DA) showed no clear separation between CON and EOBFA groups, indicating no alteration in the metabolome. Metabolic pathways, including phosphonate, glyoxylate, pyrimidine, purine, amino acids, vitamins, nitrogen metabolism, primary bile acid, and aminoacyl-tRNA biosynthesis, remained unaltered by EOBFA treatments. Overall, our findings suggest that the inclusion of EOBFA had no adverse effects on rumen metabolites and metabolic pathways.

Key Words: dairy cow, essential oil, metabolome

1549 Exhalomics as a mirror to rumen fluid metabolomics in dairy cows. M. Z. Islam*[1], S. E. Räisänen[1], J. Lan[2], Y. Li[1], F. Wahl[3], E. C. Slack[2], R. Zenobi[4], S. Giannoukos[4], and M. Niu[1], *[1]Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland, [2]Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland, [3]Food Microbial Systems Research Division, Agroscope, Bern, Switzerland, [4]Department of Chemistry and Applied Biosciences, Analytical Chemistry, ETH Zürich, Zürich, Switzerland.*

Understanding rumen function is crucial for improving nutrient utilization efficiency. This study investigated the rumen metabolomic profile through noninvasive exhalomics (EX) vs. rumen fluid (RF) analysis. A switchback design of three 9-d periods, with 7 d for adaptation and 2 d for sampling, was utilized with 4 rumen-cannulated Swiss Brown (Braunvieh) cows. The 2 experimental diets were low-starch (LS, 6.31% of DM) and high-starch (HS, 16.2% of DM) in an ABA/BAB arrangement. Cows were fed 1×/d at 0830 h. Exhalome (all exhaled volatiles, with the GreenFeed system), and RF samples were collected 8× to represent a full day at 3 h intervals. Both EX and RF samples were analyzed using secondary electrospray ionization-mass spectrometry and liquid chromatography-mass spectrometry, respectively. Data were analyzed in R and MetaboAnalyst 6.0, with raw features normalized by Pareto scaling. Recursive feature elimination (RFE) with a linear kernel support vector machine (SVM) was used as a classifier. Pathway analysis was conducted using Mummichog (v2.0) with *Bos taurus* reference (KEGG). In total, 1,096 RF and 1,694 EX features were detected. Using SVM, both EX and RF profiles effectively differentiated between the HS vs. LS diets ($P < 0.01$; EX: 84, RF: 92% accuracy). The SVM-RFE model identified 66 RF and 48 EX metabolites that were potentially related to dietary alterations and were mapped to distinct metabolic pathways. Notably, the galactose metabolism ($P < 0.01$; enrichment factor [EF] >3) and starch and sucrose ($P = 0.03$; EF >3) metabolism were common to both matrices and identified as upregulated in the HS group (normalized enrichment score = 2.0). Additionally, tryptophan metabolism ($P = 0.04$; EF >4.5) was found to be significantly altered in RF, while compounds associated with sulfur ($P = 0.02$; EF >4.0) and pyrimidine metabolism ($P = 0.03$; EF >3) were enriched in EX. Overall, both RF and EX profiles captured similar diet effects on metabolism, particularly within shared enriched pathways, which underscores the high potential of exhalomics for noninvasive assessment of rumen metabolic status.

Key Words: bovine-breath, metabolomics, rumen function

1550 Rumen inoculum as a factor in studying methanogenesis in vitro. C. V. Almeida*, S. F. Cueva, L. F. Martins, A. Richards, N. Stepanchenko, and A. Hristov, *The Pennsylvania State University, University Park, PA.*

Source and activity of the rumen inoculum have a considerable impact on results obtained using in vitro gas production (IVGP) systems. The objective of this study was to investigate the effects of rumen inoculum collected from cows fed increasing levels of dietary starch on total gas (TG) and CH_4 production and fermentation variables in vitro. Eight cows enrolled in a separate, replicated 4 × 4 Latin square design experiment evaluating the effects of increasing dietary levels of starch on lactational performance and enteric CH_4 emissions were used as donors of rumen inoculum for the study. Cows were fed diets containing 10, 20, 30, or 40% starch on a dry matter basis (ground corn grain replacing soy hulls). The experiment consisted of 4 periods in which rumen inoculum was collected from the same 2 cows during each experimental period of the study at 4 h after feeding using the stomach tubing technique. In vitro incubation substrates were dried and ground alfalfa haylage (AH) and ground corn grain (CG) included at 1% (wt/vol) in the incubation medium. Replicated incubations were carried out for 24 h at 39°C using an automated IVGP system. Gas production was continuously monitored, and headspace samples were collected at 12 and 24 h and analyzed for CH_4 concentration. Data were analyzed using the MIXED procedure of SAS with fixed effects of substrate, inoculum, and substrate × inoculum interaction. Polynomial orthogonal contrasts were used to evaluate linear and quadratic effects of the ruminal inoculum. When substrate was CG, incubation end-point medium pH was lower ($P < 0.001$) and TG and CH_4 production were greater ($P < 0.001$), compared with AH. For both substrates, increasing the starch content in the diets fed to the donor cows linearly decreased ($P < 0.001$) pH, CH_4 production, and CH_4 concentration in TG, without affecting TG production. In conclusion, increasing starch content in the diet of cows used as donors for rumen inoculum linearly decreased CH_4 production in vitro, highlighting the importance of diet composition and rumen environment of donor animals for studying rumen methanogenesis in vitro.

Key Words: rumen inoculum, enteric methane, in vitro

1551 An anionic exchange resin can sequester perfluorooctane sulfonic acid (PFOS) under in vitro ruminal conditions. K. Nishimwe*[1], J. Poblete[1], D. Ayala[1], M. Cardoso[1], A. Jimenez[1], Y. Jiang[2], G. Pereira[1], and J. Romero[1], *[1]University of Maine, Orono, ME, [2]Kentucky State University, Frankfort, KY.*

The aim of this study was to evaluate the efficacy of potential binders in sequestering PFAS (per- and poly-fluoroalkyl substances) under simulated ruminal conditions using contaminated grass (PFOS: 10 ± 1.72 μg/kg; DM basis). Binders included clay binder 1 (CLY1) and 2 (CLY2), polysaccharide binder (PLS), carbonaceous binder (CRB), and anion exchange resin 1 (AER1) and 2 (AER2). PFAS-contaminated grass (3 g) was mixed with 0.05 g of each binder (except AER1, 0.01 g) before adding 100 mL of rumen media. ANKOM bottles were shaken at 60 rpm for 48 h at 39°C. The percentage of PFOS binding affinity of the respective binders was calculated in relation to the control (CON). A randomized complete block design (n = 4) was used to analyze the data using SAS v.9.4. Differences were declared at $P \leq 0.05$. Only AER2 was found to bind PFOS under ruminal conditions, with a relative binding of 52.5 ± 8.59%, which was higher than any other binder tested. The asymptotic maximum gas production ($\bar{x} = 132.4 \pm 10.9$ mL/g of DM), rate of gas production ($\bar{x} = 3.37 \pm 0.23$%/h), ammonia-N ($\bar{x} = 9.96 \pm 2.27$ mg/dL), and total volatile fatty acids ($\bar{x} = 121.34 \pm 5.6$ m*M*) were not affected by binder type. However, propionic acid was lower in AER1 vs. CON (27.6 vs. 28.3 ± 0.16%). In conclusion, AER2 was the only binder that effectively sequestered PFOS under in vitro ruminal conditions, and it did so without negatively affecting ruminal gas production kinetics and microbial fermentation.

Key Words: binder, per- and poly-fluoroalkyl substance (PFAS), rumen

1552 Hydroponic fodder as alternative feeds to reduce methane (CH_4) emission, and their effects on ruminal digestibility: An *in vitro* study. Y. Li*[1], R. Peng[1], C. Kunz[1], M. Terranova[2], Y. Zhang[1], M. Macsai[3], E. Frossard[3], and M. Niu[1], [1]*Group of Animal Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland,* [2]*AgroVet-Strickhof, ETH Zürich, Lindau, Switzerland,* [3]*Group of Plant Nutrition, Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, Zürich, Switzerland.*

Malate is an intermediate metabolite in the ruminal propionate-producing pathway, which mitigates enteric CH_4 production by hydrogen competition. When seeds germinate, mobilization of stored fat produces a high transient malate content via glyoxylate cycle. The objective of this study was to investigate the mitigating effect of malate-containing hydroponic fodder on ruminal CH_4 production in vitro. The chronological malate profiles of 7 feed fodders during germination (d 0–10) were established, including alfalfa (AF), forage pea (FP), Italian ryegrass (IR), rye (R), soybean (S), triticale (T), and wheat (W). The hydroponic fodders were grown under natural light with an average of 20.7°C, 60% relative humidity and freeze-dried after harvesting. Time points with the highest malate content (AFd10, FPd10, IRd9, Rd9, Sd10, Td10, and Wd7, with 15.7, 20.1, 1.1, 26.0, 1.0, 5.3, and 1.3 mg malate/g DM, respectively) were selected for the Hohenheim gas test to assess the CH_4 mitigating capability in vitro, with a basal diet consisting of 40% DM grass silage, 40% maize silage, 15% hay, and 5% concentrate. Hydroponic fodder replaced 20% of basal diet (20R) or complete silage replacement (CSR). Twelve m*M* malate was used as positive control. The gas production and composition, volatile fatty acid (VFA) profile, and ammonia (NH_3) production over the 24 h fermentation were recorded. Data were analyzed in mixed model in R. The most effective fodder at reducing CH_4 production was AF. The 20R by AFd10 reduced CH_4 production (mL/g DM per 24 h) by 6.6% ($P < 0.05$), CSR by AFd10 reduced CH_4 production by 17.7% ($P < 0.05$). The NH_3 of CSR AFd10 increased by 174% ($P < 0.05$), which suggests an over-abundant protein supply. Organic matter digestibility increased in absolute percentage with CSR rye (12.6%; $P < 0.05$). Marginal effects of dietary variables were analyzed in a generalized additive model. A negative relationship between dietary malate content and CH_4 production ($P < 0.05$) was observed, whereas dietary NDF and starch content were positively correlated with CH_4 production ($P < 0.05$).

Key Words: grass fodder, enteric CH_4, malate

1554 Effects of *Asparagopsis taxiformis* supplementation on enteric gas emission and expression of methyl coenzyme reductase in dairy cows. D. E. Wasson*[1], S. F. Cueva[1], L. F. Martins[1], N. Stepanchenko[1], K. Welter[1], N. Indugu[2], K. Narayan[2], D. Pitta[2], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of Pennsylvania, New Bolton Center, Kennett Square, PA.*

This study investigated the effects of long-term supplementation of *Asparagopsis taxiformis* (AT), a known enteric CH_4 mitigating feed additive, on the rumen microbiome and CH_4 emission in lactating dairy cattle. Following a 2-wk covariate period, 24 Holstein cows averaging 118 d in milk and 44 kg/d milk yield (MY) were assigned to one of 2 treatments in a 15-wk randomized complete block design experiment. Treatments were control (CON; basal diet) or basal diet supplemented with 0.25% AT (feed dry matter [DM] basis). Enteric CH_4 emission was measured using the GreenFeed system. All cows were sampled for rumen contents in wk 3 and wk 10 and processed for metagenomic analysis to investigate the effect of AT on methanogenesis pathways. Data were analyzed using the MIXED procedure of SAS with fixed effects of treatment, week, and treatment × week in the model. Block and block × treatment were random effects. Spearman correlation coefficients were calculated to evaluate relationships between microbial genes (copies per million) and enteric gas emission. The average reduction in CH_4 emission (g/d) by AT over the entire experiment was 32% ($P \leq 0.001$). The mitigation effect, however, diminished over time, being on average, 47% in wk 3, and 19% in wk 9, preceding the second rumen fluid sampling. Methane yield (g/kg of dry matter intake) was not affected by treatment, except it was decreased 27% by AT in wk 3. Overall, a positive correlation was observed between copy number of methyl coenzyme reductase (MCR) and CH_4 yield ($r = 0.59$; $P < 0.001$) and daily CH_4 emission ($r = 0.46$, $P = 0.002$). At wk 3, copy number of MCR was decreased ($P = 0.002$) by 47% in AT compared with control. Conversely, AT only tended ($P = 0.09$) to decrease MCR by 26% in the final stages of the experiment, when CH_4 yield was not different ($P = 0.83$) from CON. Hydrogen emission was negatively correlated ($r = -0.54$, $P < 0.001$) with the copy number of MCR. Interpretation of the data suggests that the mitigation effect of AT on enteric CH_4 emission is mediated by MCR but may be transient, possibly due to rumen adaptation.

Key Words: methane, seaweed, methyl coenzyme reductase

Ruminant Nutrition 6: Carbohydrates and Lipids, and Ruminant Nutrition 3-Minute Poster Spotlights

1555 Effects of raw and roasted high-oleic soybeans on milk production responses of high-producing dairy cows. A. M. Bales* and A. L. Lock, *Michigan State University, East Lansing, MI.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

We determined the effect of feeding roasted and raw, ground high-oleic acid soybeans (HOSB) on production responses of dairy cows. Thirty-six multiparous Holstein cows (45.6 ± 6.22 kg/d of milk; 110 ± 61 DIM) were randomly assigned to treatment sequences in a 4 × 2 truncated Latin square design with 35-d periods. Treatments were (1) control (CON), (2) 16% roasted HOSB (RST), (3) 16% raw HOSB (RAW-D), and (4) 16% raw HOSB + by-pass protein (RAW-U). HOSB replaced conventional soybean meal and hulls in CON and by-pass protein replaced soybean meal in RAW-U to maintain diet nutrient composition (% DM) of 21% forage NDF, 28% starch, and 17% CP. Total dietary fatty acid content was 2.8, 4.9, 5.1, and 5.1% DM, respectively. The statistical model included the random effect of cow within square and fixed effects of square, treatment, period, and week and their interactions. Pre-planned contrasts were the overall effect of HOSB {CON vs. SOY [1/3 (RST + RAW-D + RAW-U)]}, effect of roasted HOSB {RST vs. RAW [1/2 (RAW-D + RAW-U)]}, and effect of protein source (RAW-D vs. RAW-U). The order of results is CON, RST, RAW-D, and RAW-U. Interactions were observed between treatment and week; SOY increased milk yields compared with CON and RST increased milk yields compared with RAW, with only the magnitude of increase varying across weeks. Overall, SOY increased DMI (28.4, 29.0, 29.1, 29.1; $P = 0.04$) and yields of milk (42.3, 45.9, 42.2, 43.7; $P < 0.01$), 3.5% FCM (43.6, 49.4, 45.9, 46.6; $P < 0.001$), ECM (44.0, 49.0, 45.6, 46.4, $P < 0.001$), and milk fat (1.56, 1.83, 1.71, 1.70; $P < 0.001$), but did not affect milk protein yield (1.40, 1.47, 1.37, 1.41; $P = 0.21$). Compared with RAW, RST did not impact DMI ($P = 0.91$) but increased yields of milk, 3.5% FCM, ECM, milk fat, and milk protein (all $P < 0.001$). Compared with RAW-D, RAW-U did not impact DMI ($P = 0.90$), increased yields of milk ($P < 0.01$) and milk protein ($P = 0.01$), and tended to increase ECM ($P = 0.07$). Overall, HOSB inclusion at 16% DM increased production yields of high-producing dairy cows, with roasted HOSB having greater benefit than raw HOSB. The use of by-pass protein improved milk production for raw HOSB.

Key Words: oleic acid, soybean, milk fat

1556 Effects of increasing amounts of lipid from conventional or high-oleic soybeans on nutrient and energy balance in lactating dairy cows. T. L. Hagemann*, E. A. Petzel, E. A. Bailey, and D. W. Brake, *University of Missouri, Columbia, MO.*

Eight cannulated lactating Holstein cows were split into 2 groups and placed in a strip-plot design to evaluate effects of increasing amounts of conventional (CON) or high-oleic (HO) soybeans on nutrient and energy balance. Source of soybean (CON or HO) was the whole-plot factor and provided in a crossover arrangement. Cows were subsequently provided 0%, 4%, 8%, or 12% soybeans from CON or HO. Each cow was provided each combination of amount and source of soybeans in a manner balanced for carryover effects. Samples of feed were collected in the last 4 d of each 14-d period, and measures of respired air from indirect calorimetry on the last 2 d of each period. Data were analyzed with the MIXED procedures of SAS as a strip-plot design accounting for fixed effects of soybean source, amount, the interaction of source and amount, and the interaction of crossover square and source. Over all amounts of soybean source, DMI was 2.13 kg greater ($P \leq 0.01$) when cows were fed HO versus CON. Additionally, measures of N intake tended to increase linearly ($P = 0.07$) when cows were fed CON, but decreased linearly ($P = 0.04$) when fed HO. There was an interactive effect of soybean type and amount on measures of digestible energy (DE; $P \leq 0.01$) where increased inclusion of CON resulted in a linear increase in DE ($P \leq 0.01$), but increased inclusion of HO quadratically decreased DE ($P = 0.04$). Measures of DE corresponded to linear increases ($P \leq 0.04$) in measures of DM, OM, and NDF digestion to increased CON, but linear decreases ($P \leq 0.01$) in measures of DM and OM to increased amounts of HO. Increased amounts of soybeans in the diet resulted in a linear decrease in urine energy and N losses ($P \leq 0.01$). Measures of heat production were not influenced by soybean type or amount. Consequently, measures of metabolizable and retained energy reflected differences in DE. NE_L decreased quadratically ($P = 0.02$) when cows were fed greater amounts of HO, but not for increased amounts of CON. Overall, changes in energy balance in response to soybean lipid profile were driven largely by dynamic changes in intake and digestion.

Key Words: energy, digestion, soybean

1557 Effects of dietary palmitic acid and rumen protein degradability on milk production and composition in dairy cows. M. Landry*[1,2], P. Denis[1,2], F. Huot[1,2], C. Rheault[1,2], K. Robitaille[1,2], M. Breton-Bernier[1,2], M. Guimont-Martin[1,2], Y. Lebeuf[1,2], K. Nichols[3], J. Chamberland[1,2], G. Brisson[1,2], E. R. Paquet[1], D. E. Rico[4], P. Y. Chouinard[1,2], R. Gervais[1,2], *[1]Université Laval, Québec, QC, Canada, [2]Dairy Science and Technology Research Centre (STELA), Québec, QC, Canada, [3]Department of Animal Science, University of California–Davis, Davis, CA, [4]Centre de recherche en sciences animales de Deschambault, Québec, QC, Canada.*

This study aimed to determine the interaction between dietary palmitic acid (PA) and RUP level on milk production. In a double 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments, Holstein cows (95 ± 22 DIM) received a diet with 0% or 1.8% PA (DM basis) and one of 2 levels of RUP (4.5% or 6.1% of DM; LRUP and HRUP, respectively). Dietary RUP was increased by replacing soybean meal with heat-treated soybean meal. Diets were formulated to be isonitrogenous (mean ± SD; 16.5 ± 0.2% CP, DM basis) and isoenergetic (1.73 ± 0.03 Mcal/kg DM) by replacing corn with PA and soyhulls. Treatment periods lasted 21 d, with the last 7 d used for data collection. The statistical model included period, square, and level of PA and RUP and their interaction as fixed effects and cow as a random effect. Dry matter intake was not affected (28.8 ± 0.9 kg/d; $P \geq 0.22$). Inclusion of PA decreased milk yield only in combination with LRUP. Milk fat content was increased by PA, but fat yield was only increased by PA in combination with HRUP. Milk protein content decreased independently in response to PA and HRUP. Protein yield was decreased by PA only in combination with LRUP. There was a PA × RUP interaction for ECM, feed efficiency, and milk fat globule diameter ($D_{4,3}$). Free fatty acid (FFA) content after 24 h storage at 4°C and mean casein micelle size were increased by PA. This study demonstrates that dietary PA can be

Table 1 (Abstr. 1557). Impacts of PA and RUP on milk composition

	0% PA		1.8% PA			*P*-value		
Item	LRUP	HRUP	LRUP	HRUP	SEM	PA	RUP	PA × RUP
Milk yield, kg/d	33.8^{a}	34.0^{a}	29.7^{b}	34.6^{a}	1.92	0.01	<0.01	<0.01
ECM, kg/d	35.6^{b}	35.8^{ab}	32.6^{c}	38.3^{a}	1.44	0.67	<0.01	<0.01
Feed efficiency[1]	1.22^{c}	1.27^{b}	1.12^{d}	1.33^{a}	0.026	0.11	<0.01	<0.01
Fat, %	4.44	4.42	4.77	4.93	0.232	<0.01	0.32	0.20
Fat, kg/d	1.47^{b}	1.48^{b}	1.40^{b}	1.68^{a}	0.062	0.03	<0.01	<0.01
Protein, %	3.52	3.47	3.45	3.39	0.147	<0.01	0.01	0.96
Protein, kg/d	1.18^{a}	1.16^{a}	1.01^{b}	1.16^{a}	0.046	<0.01	0.01	<0.01
$D_{4,3}$, µm	4.17^{bc}	4.08^{c}	4.35^{ab}	4.52^{a}	0.106	<0.01	0.46	0.02
FFA, mEq/100 g fat	0.91	1.09	1.58	1.42	0.181	<0.01	0.97	0.31
Casein micelle size, nm	219	224	238	235	9.6	0.01	0.88	0.44

[1] ECM/DMI, kg/d.

more efficient combined with an increased RUP level and can affect physicochemical properties of milk.

Key Words: dairy cow, 16:0, protein degradability

1558 Fatty acid supplementation during heat stress improved milk production of mid-lactation dairy cows. S. R. Naughton*, M. N. Mills, J. M. dos Santos Neto, E. Sarmikasoglou, A. L. Lock, and M. J. VandeHaar, *Michigan State University, East Lansing, MI.*

Heat-stressed dairy cows typically have reduced DMI and milk production. Fatty acid (FA) supplementation is a potential way to mitigate heat stress. We hypothesized that feeding a FA supplement containing palmitic (PA) and oleic (OA) acids would improve production during warm weather. Holstein cows (50–150 DIM; 17 primi- and 24 multiparous) were blocked by parity, DIM, and energy-corrected milk (ECM)/metabolic body weight. Both control (CON) and high FA (HF) diets were 40% corn silage; CON was 19.4% forage NDF and 16.8% CP, and HF was 19.8% forage NDF and 17.2% CP. The fat supplement was a Ca-salt fed to provide 2.0% FA in diet DM with 59% PA and 29% OA. For a 2-wk preliminary period, cows were fed CON followed by 6 wk of treatment diets. Milk samples were collected for analysis 2 d/wk and body weights were recorded 3×/wk. Barn temperature and humidity were measured continuously. Average daily temperature-humidity index (THI) values ranged from 70 to 87, which exceeds the heat stress THI threshold of 68. We measured methane emissions every 9 h for 6 d. Data were analyzed using repeated measures with the random effect of cow nested within parity and the interactions between the fixed effects of treatment, time, and parity. Compared with CON, HF increased yields of ECM (43.6 vs. 41.5 kg/d, $P = 0.02$) and milk fat (1.59 vs. 1.47 kg/d, $P = 0.01$). We observed no treatment by parity interactions for yields of milk and milk components. Compared with CON, HF had greater yields of $C > 16$ milk FA (545 vs. 500 g/d, $P = 0.04$) and C16 milk FA (614 vs. 519 g/d, $P < 0.01$), but not $C < 16$ FA (363 vs. 385 g/d, $P = 0.25$). Compared with CON, HF increased ECM/DMI (1.74 vs. 1.66 kg/kg, $P < 0.01$) but not milk energy/feed net energy (67% on average). Treatment did not alter methane emissions ($P = 0.7$). In conclusion, dietary supplementation of a Ca-salt of PA and OA increased milk energy output and yield of milk fat through increases in C16 and $C > 16$ milk FA, regardless of parity, during warm weather and did not alter methane emissions.

Key Words: palmitic acid, heat stress, methane

1559 The effect of palmitic and stearic acid supplementation on plasma fatty acids and mammary arteriovenous fatty acid difference in Holstein cows. A. N. Staffin* and K. J. Harvatine, *Penn State University, University Park, PA.*

Palmitic acid (PA) and stearic acid (SA) are commonly fed to dairy cows but the effects of these fatty acids (FA) on circulating FA concentration and mammary FA uptake are not well described. The objective was to characterize these parameters in cows fed a supplement high in PA or SA. The hypothesis was that PA supplementation would have a greater increase in mammary FA arteriovenous (AV) difference and extraction efficiency compared with SA. Twelve Holstein cows (106 ± 31 DIM) were arranged in a replicated 3 × 3 Latin square design with a ≥10 d washout between periods. Treatments included a no-supplement control (CON) and 500 g of a FA supplement high in palmitic acid (HPA; >80% palmitic) or high in stearic acid (HSA; 80% stearic and 10% oleic). HPA was previously reported to increase milk fat yield by 144 g/d while HSA tended to increase milk fat by 61 g/d. Coccygeal vessel (representing arterial) and mammary vein samples were taken together at 4 time points over the last 48 h of each period to determine plasma FA and analyzed using GLC. Data were analyzed with repeated measures in a mixed model that included the random effects of cow and period and the fixed effects of treatment, time, and their interactions with preplanned contrasts testing CON versus HPA and CON versus HSA. HPA increased enrichment of PA and oleic acid (OA) as a percent of FA in both arterial and venous plasma ($P < 0.01$). HSA enriched SA ($P = 0.01$) and OA ($P < 0.001$) in arterial plasma and enriched OA in venous plasma ($P < 0.001$). HPA also increased total arterial plasma FA concentration by 360 mg/L ($P < 0.001$) while HSA tended to increase it by 111 mg/L ($P = 0.10$). HPA numerically increased mammary AV difference of total plasma FA by 27 mg/L while HSA did not affect total AV difference. HPA increased mammary AV difference of PA by 11 mg/L ($P < 0.001$), but HSA did not affect AV difference of SA. In conclusion, increasing dietary PA and SA increased total plasma FA and altered plasma FA profile while only PA increased mammary AV difference, which may mechanistically explain greater increases in milk fat yield with PA supplementation.

Key Words: nutrition, lipid, metabolism

1560 Abomasal infusion of docosahexaenoic acid to mid-lactation dairy cows alters cytokine production following whole-blood in vitro stimulation. H. L. Reisinger*[1], M. L. Miller[1], L. K. Mamedova[1], G. A. Contreras[2], A. L. Lock[1], and B. J. Bradford[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, [2]Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

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Cytokines are signaling molecules that elicit physiological responses, particularly in the immune system. Docosahexaenoic acid (DHA; 22:6 n-3) is known for its anti-inflammatory properties and may alter cytokine production. However, DHA delivery to the ruminant intestine for absorption is challenging due to rumen biohydrogenation. Our objective was to evaluate the effect of abomasally infused DHA to mid-lactation cows on cytokine production following in vitro whole-blood gram-positive bacterial stimulation. Ruminally cannulated multiparous Holstein cows (n = 8; 97 ± 37 DIM, 49 ± 3 kg/d milk) enrolled in a 4 × 4 Latin square design received 0, 2, 4, or 6 g/d DHA abomasally infused for 11 d (4 infusions/d) with a 10-d washout period between treatment periods. DHA was provided via an enriched algal oil (64.5% DHA) suspended in ethanol (~200 g/d). Blood collected on d 11 of each period was stimulated with heat-killed *Staphylococcus aureus* (HKSA; 10^7 HKSA/mL) or water (CON) for 6 h (39°C, 5% CO_2). Following stimulation, plasma was collected and cytokine concentrations were measured using a magnetic bead multiplex assay. Data were analyzed to assess the fixed effect of treatment and bovine leukemia virus status was tested in each model and retained if $P < 0.10$ with random effects of cow, block, and period. Compared with CON, HKSA increased IFN-γ, IL-1α, IL-1β, IL-6, IL-10, IL-17A, MIP-1α, MCP-1, TNF-α, and VEGF-A concentrations ($P < 0.01$), but not IL-4 or IL-36RA ($P > 0.10$). In HKSA normalized with CON, DHA treatment did not affect IFN-γ, IL-4, IL-6, IL-10, MIP-1α, MCP-1, or TNF-α concentrations ($P > 0.10$). The pro-inflammatory cytokine IL-17A decreased linearly with increasing dose ($P < 0.01$) and abundance of VEGF-A and the anti-inflammatory cytokine IL-36RA showed a quadratic effect with a nadir at 2 g/d ($P < 0.01$). A tendency for a linear decrease in IL-1α and IL-1β was observed with increasing dose ($P < 0.10$). In conclusion, DHA supply to the intestine may dampen cytokine production, which has the potential to alter immune function.

Key Words: n-3 (omega-3), inflammation, immune response

1561 The effects of the inclusion of sprouted barley in diets of high-yielding dairy cows on production, efficiency, rumen environment, and digestibility. U. Moallem*[1], J. Shpirer[1], T. Alon[1,2], L. Lifshitz[1], and H. Leibovich[3], *[1]Department of Ruminants Science, Agriculture Research Organization, Volcani Institute, Rishon LeZion, Israel, [2]Department of Animal Science, the Hebrew University of Jerusalem, Rehovot, Israel, [3]Israeli Dairy Board, Yehud, Israel.*

The sprouted fodder system is the process of growing grains in trays. Recently there is a growing interest in using sprouts as fodder for ruminants. The objectives were to test the effect of including sprouted barley in the diet of dairy cows on production, digestibility, and efficiency. Forty-two mid-lactation cows were assigned into 2 groups (n = 21) and were fed (1) control (CTL), a typical Israeli milking-cow diet; or (2) sprouted barley (SB), a similar diet that contained 11.5% (DM basis) of sprouted barley produced by Hoshen FoodTech Company Ltd., Israel. Milk yields and body weight were recorded daily, and milk samples were taken 9 times for milk content analysis. Four blood samples were taken for metabolite analysis, 4 rumen samples were collected for VFA and ammonia, and 8 fecal samples for digestibility measurements. Production and efficiency data were analyzed as repeated measurements with the MIXED Procedure of SAS. The milk yield was higher (45.7 and 44.1 kg/d, respectively; $P = 0.001$), and the milk fat content was lower (3.85 and 4.08%, respectively; $P = 0.004$) in the SB cows. Protein percentage tended to be higher in the SB cows ($P = 0.07$), with no differences in fat and protein yields. Milk urea nitrogen (MUN) was 17% lower in the SB cows ($P < 0.001$). The 4% FCM and ECM yields ($P > 0.75$) and DMI ($P = 0.90$) were not different between groups, and the energy balance was higher in the SB cows ($P = 0.04$). The milk-to-DMI ratio tended to be higher in the SB cows ($P = 0.11$). The rumen ammonia concentration was 21% lower in the SB cows ($P = 0.006$), and rumen acetate, propionate, and total VFA were higher in the CTL cows ($P = 0.02$). The apparent total-tract digestibility of DM, OM, protein, and fat were higher in the SB cows ($P = 0.001$), and tended to be higher for NDF ($P = 0.1$). No differences were observed in glucose and NEFA concentrations in blood, and lower urea and triglycerides were observed in the SB cows. In summary, the inclusion of sprouted barley increased the milk yield, reduced the milk fat, MUN, rumen ammonia, and blood urea, and improved the digestibility of nutrients.

Key Words: sprouted barley, dairy cow, nutrition

1562 Effects of dietary starch on methane production and lactational performance in dairy cows. S. F. Cueva*, L. F. Martins, C. V. Almeida, S. Issabekova, C. J. Eifert, D. E. Wasson, A. Richards, N. Stepanchenko, and A. N. Hristov, *The Pennsylvania State University, University Park, PA.*

The objective of this study was to evaluate the effects of increasing dietary starch concentration (ST) on lactational performance, rumen fermentation, and enteric gas emissions in dairy cows. Twenty-four (12 primi- and 12 multiparous) Holstein cows averaging 65 ± 14 DIM were used in a replicated 4 × 4 Latin square design experiment with 4 periods of 28 d each (with the last 10 d for data collection). Cows were grouped based on parity, DIM, and milk yield (MY) and were assigned to 4 diets formulated to contain 10% (control), 20%, 30%, or 40% ST (ST10, ST20, ST30, and ST40, respectively). Starch concentrations were achieved by substituting ground corn grain for soyhulls (ST20 and ST30) and molasses and alfalfa haylage (ST40). Enteric CH_4 emission was measured at 8 different time points over 3 d using the GreenFeed system. Three cows per treatment were equipped with electronic rumen boluses to monitor rumen pH. Data were analyzed using the MIXED procedure of SAS with the fixed effects of ST, period, and parity in the model. Group and cow within group were random effects. Contrasts were used to evaluate linear (L) and quadratic (Q) effects of ST. Treatment did not affect DM intake (DMI; 24.0 kg/d; SEM = 0.50). Energy-corrected MY (ECM; 36.7, 37.6, 39.5, 38.1 kg/d; SEM = 0.87) increased ($P = 0.02$; L) with increasing dietary ST. Feed and ECM feed efficiency also increased ($P < 0.01$ and $P = 0.03$, respectively; L), whereas milk fat content decreased ($P < 0.01$; L) and milk fat yield tended to decrease ($P = 0.06$; L) with increasing ST. Milk true protein (MTP) content and MTP and lactose yields increased ($P < 0.01$ for all variables; L) with increasing ST. Increasing ST decreased ($P \leq 0.03$; L) rumen pH between 5 and 15 h after feeding. Daily CH_4 production, yield (18.9, 18.0, 18.1, 16.4 g/kg DMI; SEM = 0.53), and intensity (12.4, 11.6, 11.2, 10.3 g/kg ECM; SEM = 0.35) were decreased ($P < 0.01$ for all variables; L) with increasing ST. Increasing ST from 10 to 40% did not affect DMI but increased ECM yield, despite a decrease in milk fat, and decreased daily CH_4 emission and CH_4 yield and intensity in dairy cows.

Key Words: enteric methane, dietary starch, dairy cow

1638 Effect of abomasal infusions of docosahexaenoic acid on production responses and plasma fatty acids of mid-lactation dairy cows. M. L. Miller*, H. R. Reisinger, G. A. Contreras, B. J. Bradford, and A. L. Lock, *Michigan State University, East Lansing, MI.*

Our objective was to evaluate the effect of abomasal infusions of increasing doses of docosahexaenoic acid (DHA; C22:6 n-3) on plasma and milk fatty acids and milk production of mid-lactation dairy cows. Eight multiparous ruminally cannulated Holstein cows (97 ± 37 DIM, 49.2 ± 3.3 kg/d milk) were used in a 4×4 Latin Square design. Treatments were abomasal infusions of 0, 2, 4, and 6 g/d of DHA with 11-d treatment periods and 10-d washout periods. DHA was provided via an enriched algal oil (64.5% DHA) and suspended in ethanol (~200 g/d). Samples were collected during the last 4 d of each infusion period. The statistical model included the random effect of cow nested within square and fixed effects of treatment, square, period, and their interactions. Preplanned contrasts tested the linear, quadratic, and cubic effects of increasing doses of DHA. Results are presented in the following order: 0, 2, 4, 6 g/d of DHA. There was no effect of treatment on yields of milk, FCM, ECM, milk fat, or milk protein. Increasing DHA dose linearly decreased SCC (13.3, 12.0, 10.4, 10.8 × 10^3/mL; $P = 0.05$). Increasing DHA dose linearly increased DHA in plasma phospholipids (0.30, 0.50, 0.64, 0.82 g/100g FA; $P < 0.01$), triacylglycerols (0.09, 0.25, 0.40, 0.51 g/100g FA; $P < 0.01$), and cholesterol esters (0.04, 0.04, 0.05, 0.06 g/100g FA; $P < 0.01$). Increasing dose of DHA linearly increased milk fat content of DHA (0.01, 0.04, 0.06, 0.08 g/100g FA; $P < 0.01$), total n-3 fatty acids (0.56, 0.57, 0.63, 0.63 g/100g FA; $P < 0.05$), and the yield of DHA in milk (0.19, 0.58, 0.91, 1.18 g/d; $P < 0.01$). Apparent transfer efficiency of DHA into milk was linearly decreased by DHA dose for 2, 4, 6 g/d, respectively (29, 23, 20%; $P < 0.01$). In conclusion, increasing abomasal infusions of DHA did not impact short-term production responses of mid-lactation cows, but increased DHA content of key plasma lipids and milk fat, and reduced milk SCC.

Key Words: omega-3 fatty acid, abomasal infusion, plasma lipid

1639 Interaction between dietary palmitic acid and genotypes on milk fat of dairy cows. J. M. dos Santos Neto*, A. L. Lock, and R. J. Tempelman, *Michigan State University, East Lansing, MI.*

Our objective was to perform a genome-wide association study (GWAS) to determine possible interactions between dietary palmitic acid (C16:0) and single nucleotide polymorphisms (SNP) for milk fat responses of dairy cows. Our data set included 21 studies conducted at MSU comparing diets with (n = 1,016) and without (n = 866) C16:0 supplementation. Dietary C16:0 averaged (±SD) 1.14 ± 0.66% and ranged from 0.75 to 2.50% DM. We had a total of 62,856 SNP markers on 365 cows with information on milk fat and milk fatty acid (FA) sources, categorized as de novo (<16 carbons), mixed (16 carbons), and preformed (>16 carbons). Our model included fixed effects of dietary C16:0, SNP, and their interaction, and random effects of genomics, study, period within study, and cow-specific linear and quadratic random regressions on DIM. Estimated heritabilities (h^2; ± SE) for milk fat yield and content, and the yields of de novo, mixed, and performed FA were 0.24 ± 0.09, 0.45 ± 0.09, 0.24 ± 0.08, 0.28 ± 0.07, and 0.08 ± 0.04. On a content basis (g/100 FA), h^2 for de novo, mixed, and preformed FA were 0.25 ± 0.07, 0.35 ± 0.07, and 0.22 ± 0.07. Our GWAS analysis inferred interactions (false discovery rate <0.20) between dietary C16:0 and SNP for several FA. Overall, we observed 2 for milk fat yield (chromosome [Chr] 5 and 6), 1 for mixed FA yield (Chr 24), and 8 for mixed FA content (one on each Chr 1, 2, 7 and 24; 4 on Chr 14). Interactions for milk fat yield showed an allelic substitution effect whose magnitude increased when increasing dietary C16:0. We found no interactions for de novo FA as a group, but observed interactions for C14:1, mostly on Chr 26 (n = 10), whose magnitude of the allelic substitution effect decreased as dietary C16:0 increased. Similarly, in most interactions for the yield and content of mixed FA, the magnitude of the allelic substitution effect decreased as dietary C16:0 increased. In conclusion, our study indicates that some SNP markers interact with dietary C16:0, affecting milk fat, with most interactions occurring for mixed FA on Chr 14 and C14:1 on Chr 26. Identifying candidate genes may further elucidate genetic mechanisms underlying these interactions.

Key Words: fatty acid, gene, nutrigenomics

1640 Whole cottonseed and fatty acid supplementation affect production responses during the immediate postpartum in multiparous dairy cows. J. E. Parales-Giron*, J. M. dos Santos Neto, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of whole cottonseed (WCS) and fatty acid (FA) supplementation on production responses of early-lactation cows. A total of 52 multiparous cows were used in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Treatment diets were fed from 1 to 24 DIM and contained 0% or 10% DM WCS (NC and CS) and 0% or 1.5% DM supplemental FA (LF and HF). The FA supplement was a Ca-salt containing 60% palmitic and 30% oleic acid. Treatment diets were formulated to contain 18% CP, 21% forage NDF, and 24% starch. The statistical model included the random effect of block, cow within block and treatment, Julian date, and the fixed effects of WCS, FA supplementation, time, and their interactions. Results are presented in the following order: NCLF, CSLF, NCHF and CSHF. We observed interactions between dietary WCS and FA supplementation for yields of milk fat (1.85, 2.15, 2.12, 2.12, kg/d; $P = 0.03$), and 3.5% FCM (47.9, 54.8, 53.1, 54.5, kg/d; $P = 0.09$), and a tendency for ECM (48.2, 54.3, 52.3, 53.6, kg/d; $P = 0.13$) because FA supplementation increased these variables only in a diet without WCS, and WCS only increased these variables in a diet without FA supplementation. We found that WCS increased DMI (22.8, 25.8, 23.3, 24.5, kg/d; $P < 0.01$), the yields of milk fat ($P = 0.03$), 3.5% FCM ($P = 0.02$) and ECM ($P = 0.03$), and tended to increase milk protein yield (1.47, 1.59, 1.50, 1.54, kg/d; $P = 0.10$). In addition, FA supplementation tended to increase milk fat yield ($P = 0.07$) and had no effect on DMI and the yields of 3.5% FCM and ECM ($P > 0.10$). There was no effect of treatments on body weight and body weight loss ($P > 0.15$). In conclusion, feeding WCS postpartum increased DMI during the immediate postpartum. The effect of FA supplementation on the yields of milk fat, 3.5% FCM, and ECM of early-lactation cows depended on dietary WCS level, with increases in ECM of 6.1, 4.2, and 5.4 kg/d for CSLF, NCHF, and CSHF compared with NCLF. Lastly, WCS and FA supplementation did not affect body weight loss during the immediate postpartum.

Key Words: whole cottonseed, early lactation, fatty acid

ADSA Dairy Foods Division International Partnership Program (IPP) Symposium: Dairy Research in Denmark—Focus on Sustainability and Functionality

1563 The dairy matrix and calcium bioavailability. W. He[1], Z. Xie[2], N. K. Wittig[1], L. F. Zachariassen[2], H. J. Andersen[2], H. Birkedal[1], D. S. Nielsen[2], A. K. Hansen[2], and H. C. Bertram*[1], [1]*Aarhus University, Aarhus N, Denmark,* [2]*University of Copenhagen, Frederiksberg, Copenhagen, Denmark,* [3]*Arla Foods amba, Aarhus N, Denmark.*

Objectives: Being rich in calcium, dairy products provide an excellent food matrix to prevent bone loss in postmenopausal women, but recent research supports that the inclusion of dietary fibers also exerts this capability. The milk matrix (e.g., casein micelles) is likely to benefit the controlled release of calcium into the intestinal lumen, thereby increasing calcium fractional absorption. Both yogurt and dietary fibers may improve bone homeostasis in postmenopausal women by modulating gut microbiota. However, the mechanisms underpinning these effects are scarce. This study aimed to compare the effects of milk, yogurt, and yogurt-inulin combination on gut-bone associations. Methods and results: A 6-week dietary intervention study was conducted in ovariectomized rats. Calcium content, inulin, and milk/yogurt addition were the main differences in composition among the diets. Milk supplementation did not influence bone mineral density (BMD) or bone mineral content, femur mechanical strength, or femoral microstructure as compared with the positive control receiving a similar calcium dose from a calcium carbonate source, whereas yogurt supplementation significantly increased spine BMD. The serum metabolome revealed that yogurt also modulated endogenous glycine-related pathways with reduced concentrations of serum glycine, serine, and threonine. No additive effects of yogurt and inulin were observed for the examined bone mineralization parameters. Microbiota analysis showed that increased lactobacilli and reduced *Clostridiaceae* members in the gut of the yogurt-supplemented group were linked with an increased spine BMD, whereas increases in some bacteria (*Bifidobacterium pseudolongum*, *Turicibacter*, *Blautia*, and *Allobaculum*) and gut short-chain fatty acids in the yogurt-inulin supplemented group were not reflected in measured bone parameters. In conclusion, the present study demonstrates that yogurt intake changed the gut microbiota composition and serum metabolites related to glycine-related pathways with a concomitant increased spine BMD, suggesting that yogurt as a vehicle is superior to milk in terms of securing optimal conditions for bioaccessibility of calcium to enhance bone mineralization.

Key Words: fermentation, prebiotic, bone health

1564 Sustainable dairy: Effect of feed additives on milk composition and functionality. G. M. S. Lokuge[1], M. Maigaard[2], N. I. Nielsen[3], L. B. Larsen[1], P. Lund[2], L. Wiking[1], and N. A. Poulsen*[1], [1]*Department of Food Science, Aarhus University, Aarhus N, Denmark,* [2]*Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark,* [3]*SEGES Innovation, Aarhus N, Denmark.*

Mitigating methane emissions through specific feed practices and feed additives are intensively studied, whereas the possible effects on milk quality are less examined. We explored the effect of antimethanogenic feeding strategies on milk quality and functionality with a focus on supplementation of 3-nitrooxypropanol (3-NOP), nitrate, and whole-cracked rapeseeds in several animal trials and farm experiments. In the first animal experiment, 48 lactating Danish Holstein cows were allocated to 8 different rations (2 levels of cracked rapeseed, +/− nitrate, +/− 3-NOP) over 6 periods of 21 d each, according to an incomplete Latin square design. In total, 288 milk samples were collected and analyzed for detailed milk quality traits. The trial confirmed the methane reducing effect of the tested feed additives. Furthermore, the individual and combined use of whole-cracked rapeseeds, nitrate, and 3-NOP affected the milk composition to varying degrees, and especially supplementation of whole-cracked rapeseeds altered milk fatty acid (FA) composition by decreasing the proportion of saturated FA and increasing the proportion of unsaturated FA. In contrast, no additive effects of fat, nitrate, and 3-NOP were observed on milk protein and mineral composition, whereas individual fat supplementation largely altered protein composition. Milk composition changes by nitrate and 3-NOP supplementation were relatively minor. However, feeding nitrate increased riboflavin and nitrate concentrations in milk, whereas 3-NOP increased vitamin B_{12} concentration. In addition, large-scale butter production was performed using milk from cows fed 3-NOP. 3-NOP butter had higher proportions of short- and medium-chain FAs, which slightly affected the physical properties of butter. Use of 3-NOP has also been tested across different breeds where differences in milk FA composition were observed, suggesting that breeds differ in their response to 3-NOP. Implementation of the different methane emission mitigating strategies is currently being tested on commercial farms and targeted milk analyses are conducted to ensure that no impairment of milk quality is at play.

Key Words: milk, 3-nitrooxypropanol, nitrate

1565 Innovative cheese products by extrusion. L. Ahrne*, *University of Copenhagen, Denmark.*

The development of dairy products with innovative structures, which can offer new experiences to consumers, is of great importance to the dairy industry. A unique characteristic of the world-popular mozzarella cheese is its fibrous structure, which is traditionally created during the cooking-stretching process. During this process, the shear forces applied to the cheese curd during kneading and extrusion play a key role in the final structure of the cheese. Extrusion technology has gained industrial interest to texturize plant proteins, whereas applications to dairy proteins to create cheese are still limited. New insights into extrusion of cheese curds and the relationship between extrusion parameters, extruded cheese microstructure and its functional properties will be presented. By combining confocal laser scanning microscopy and X-ray micro-tomography techniques, and low field nuclear magnetic resonance, a better understanding of the structure of the protein, fat, and serum phase, as well as salt and water distribution, and anisotropic characteristics of the structure can be obtained. Application of high-shear to the curds using a twin-screw extruder show that extrudates with a variety of characteristics can be produced by modifying the extrusion parameters and/or curd properties. Controllable extrusion parameters—heating and cooling temperatures—are crucial to create fibrous curd structures. Exit temperature, residence time, and specific mechanical energy were selected as promising indicators for a better understanding of the extrudate properties, since these can comprehensively summarize the influence of multiple controllable parameters and their interactions. Modifications of the protein matrix of curds shows significant impact on the extrusion process and consequently on meltability and oiling off of the extruded cheese. Overall, the results show the potential to create a variety of extruded cheese products by exploring the relationships between extrusion parameters and curd composition. The knowledge generated provides new knowledge to improve equipment design and to produce structured cheese products with customized behavior.

Key Words: extrusion, cheese, functional property

1566 Healthier milk fat fractions through cow feeding or by volatility-based enrichment process. L. Wiking*, S. S. Mathiesen, L. B. Larsen, and N. A. Poulsen, *Department of Food Science, Aarhus University, Aarhus N, Denmark.*

Milk fat contains several fatty acids (FA) with potential health benefits. This presentation will focus on ways to increase n-3 FA and medium-chain fatty acids (MCFA) in milk. Feeding dairy cows with grass in the field has great potential, as the dairy cow is supplied with its supply of energy and nutrients through grazing, either entirely or partly. This can contribute to sustainable and economically profitable milk production, while at the same time changing the milk's nutritional profile in a positive direction in relation to human health. In one project we compared fat composition from 2 herds, one practicing an exclusive grass feeding strategy and the other feeding high levels of grass, but not exclusive, providing bulk milk from an organic and a conventional milk line, respectively. Exclusive grass feeding resulted in the most favorable n-3/6 ratio compared with other grass feeding regimens, and this milk also contained significantly more vitamin E. Moreover, we explored the growing practice of feeding fresh grass within the barn and how this affects milk quality. MCFA have been shown to be linked with health benefits in humans, such as improving insulin sensitivity and reducing appetite, which may be useful in managing diabetes and obesity. The content of MCFA in milk can be elevated by feeding high-starch diets or choosing milk from Jersey cows. Our approach to significantly increase the MCFA in milk or milk fractions was to use a volatility-based enrichment process for short-chain fatty acids and MCFA conducted by short path distillation. Here, a 2-fold increase in C8–C12 proportions of distillates compared with native milk fat, corresponding to 21% of the total fat, was obtained. The highest increase was for C8:0. Vitamin E was also greatly enriched in the distillate, from 2.5 mg/100 g fat in native milk fat to 47 mg/100 g fat in the distillate. This novel distillate may hold significant metabolic advantages.

Key Words: milk fat, medium-chain fatty acid, n-3 (omega-3) fatty acid

1567 Lacprodan bLG-100: An enabler for advances in controlling taste, flavor, and functionality in ready-to-drink beverage applications. S. B. Nielsen*, *Arla Foods Ingredients Group P/S, Videbæk, Denmark.*

Lacprodan bLG-100 is a novel ingredient from Arla Foods Ingredients that represents a new era in providing large product volumes of a highly pure and previously expensive β-lactoglobulin source high in leucine and essential amino acids. Beyond nutritional value the emergence of β-lactoglobulin has enabled a cascade of further development in new process and application opportunities that build and extend on decades of academic and industrial research into whey protein stability, aggregation, and functionality. This talk showcases how some of the molecular insights obtained from studying β-lactoglobulin via continued research in both an industrial setting and in collaboration with university partners has enabled the development of new and emerging ingredients, processes, and applications ranging to molecular aspects of taste improvements in acid (astringency, acidity) and neutral (off-flavor) ready-to-drink beverage applications, as well as highly dense protein aggregates with superior heat stability and minimal viscosity contribution in both process and application, which make them particularly relevant in high-protein beverages.

Key Words: whey protein beverage, taste and flavor, protein functionality

1568 Exopolysaccharide-producing lactic acid bacteria and texture of acid dairy gels. V. Vojinovic*, *Chr. Hansen S/A, Hørsholm, Denmark.*

Many lactic acid bacteria (LAB) produce exocellular polysaccharides. These macromolecules are known to play an important role in the texture of the dairy products produced through use of these bacteria. Despite the ubiquity within bacteria species (and LAB in particular), and their relevance to the dairy industry, knowledge about the mechanisms through which bacterial exocellular polysaccharides modulate the texture of dairy products is limited. A common approach to leveraging the texturizing potential of exocellular polysaccharides produced by different strains of LAB is to screen for the texturizing strain candidates and the optimal starter culture blends. The world's largest LAB culture collection, together with internally developed robotic-based high-throughput screening techniques have enabled Novonesis (previously Chr. Hansen) to develop and market well-recognized highly performant texturizing starter cultures for fermented dairy applications. However, understanding the role of exocellular polysaccharides in the development of texture in acid-based dairy gels is expected to enable a shift from screening-based to rational, targeted development of starter cultures. This presentation will address the following topics: (i) current knowledge of the role of exocellular polysaccharides in the development of the texture of acid-based dairy gels; (ii) development and use of exocellular polysaccharide-producing cultures in fermented milk: current approaches and challenges; and (iii) future prospects.

Key Words: fermented milk, exocellular polysaccharide, texture

1569 The way we look at the membrane for protein fractionation. B. R. Parjikolaei*, *Arla Food Ingredients Group P/S, Viby J, Denmark.*

Membrane technology plays a crucial role in the dairy industry, enabling the separation of valuable components while preserving their nutritional integrity. However, the inherent limitations of membrane processes necessitate further research to address the challenges and optimize their performance. Our research focuses on membrane filtration and protein fractionation to enhance functionality and reduce the environmental impact of specialized dairy ingredients. One key aspect of our research has been to untangle the fundamental mechanisms that govern membrane performance. By identifying critical process parameters e.g., pH and concentration, and understanding the complex interplay between feed-membrane properties and separation efficiency we have gained valuable insights into optimizing membrane processes in a more predictable way. Recognizing that protein fractionation is a dynamic process, we have also explored the challenges of mimicking industrial-scale membrane processes in laboratory-scale systems and the feasibility of in-line measurement of separation mechanisms and fouling formation in real-time. Additionally, we have devoted significant attention to the Cleaning-in-Place (CIP) protocols and exploring the impact of CIP on membrane properties. In the interest of sharper selectivity in protein fractionation, we have also studied the feasibility of manipulating driving forces to modify the separation mechanisms, producing protein fractions with enhanced purity and functionality, paving the way for the development of innovative dairy ingredients. These efforts enable us to gain a comprehensive understanding of dynamic aspects of the protein fractionation, optimizing the CIP, membrane performance, and lifespan while producing specialized dairy ingredients that have improved functionality and lower environmental impact.

Key Words: mechanistic understanding, membrane, protein

1570 How research and innovation turn into dairy products in your home.

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The performance and nutritional value of butter and other butter-derived products is linked to the microstructural features of the fat. At Arla Foods, we always strive to develop innovative solutions to optimize and improve the structure and functionality of our dairy products such as butter. In this presentation, we will go over the research aimed to study the crystallization and shear-induced structure formation of fat blends composed of anhydrous milk fat (AMF) and rapeseed oil (RO). The investigations were centered around different shear rates applied to a 500 g mass of AMF and 20 wt/wt% RO blends. Structural build-up and breakdown were demonstrated by solid fat content determination and large deformation rheology. However, the effects of shear forces on structural formation were found to be greater in the 20% RO blends. The incorporation of liquid oil into the solid milk fat structure was found to lower the melting behavior depending on the shear force, which affects the internal oil binding. Furthermore, a procedure to investigate the crystallization behavior of milk fat globules (MFGs) was developed by confocal Raman microscopy (CRM). Results showed crystallization patterns where triacylglycerol crystals are positioned around the inner membrane surface of the milk fat globule membrane. CRM was also used to identify solid from liquid fat domains within MFGs, which eliminated the effects of interference patterns observed by polarized light at the spherical lipid membrane. In conclusion, we gained insight into how we can study the performance of milk fat and rapeseed oil blends, by determining structural parameters that can potentially be fine tuned by processing to obtain the best dairy products in our market.

Key Words: milk fat, innovation, functionality

Animal Behavior and Well-Being 2

1571 What does quality assurance look like at the international level? A review of animal care standards for dairy calves. J. Saraceni*[1], P. Lawlis[1], E. Yeiser Stepp[2], B. Hampton-Phifer[2], E. Wynands[1], D. Renaud[1,3], and S. Roche[1,3], *[1]ACER Consulting, ACER Consulting, Guelph, Ontario, Canada, [2]The National Dairy FARM Program, National Milk Producers Federation, The National Dairy FARM Program, National Milk Producers Federation, Arlington, VA, [3]The University of Guelph, Guelph, Ontario, Canada.*

Calves are a vulnerable population and of particular interest for dairy consumers; therefore, calf health and welfare has been a priority area for many dairy-producing nations. The primary objective of this work was to investigate differences in standards across dairy cattle quality assurance (QA) programs related to calf health and welfare. An environmental scan was conducted to investigate 10 established, industry-run QA programs across 8 nations (Canada, US, France, UK, Ireland, Denmark, Austria, the Netherlands). A representative from each program participated in an interview to share additional insights. The QA programs included in this review were evaluated for program-specific standards related to group/pair housing of calves, pain management for disbudding, calf nutrition, cow-calf contact, surplus calves, and calf transport. Group housing of calves is mandated by about half of the programs (60%), with age at group housing often aligning with weaning age. The vast majority (90%) of programs require pain management when disbudding calves, with many programs specifically requiring anesthetic and analgesic. Colostrum expectations are fairly consistent (10% BW) and higher planes of milk nutrition (8 L) tend to be a focus, although minimum volumes vary. Less than half (40%) of the programs investigated have specific standards for surplus calves, with the majority of programs making specific reference to equal treatment of male and female calves while on the dairy. Standards related to cow-calf contact are uncommon, though many program representatives are monitoring this topic closely. Programs often have specifications for transport of calves, adult cows, and animals intended for slaughter that tend to align with regulatory expectations for their jurisdiction. Ongoing discussions, research, and upcoming priority areas for QA programs reflect a continued focus on calf care, with expected elevations to calf health and welfare standards to come. This highlights the importance of focused and quality scientific evidence, which can be used to guide and inform the development of these standards in the future.

Key Words: calf, quality assurance, animal welfare

1572 Producer perceptions of dairy calf management, behavior, and welfare. S. B. Doyle[1], C. L. Wickens[1], J. M. C. Van Os[2], and E. K. Miller-Cushon*[1], *[1]University of Florida, Gainesville, FL, [2]University of Wisconsin, Madison, WI.*

We surveyed dairy producers and calf managers to assess perception of relationships between calf management, behavior, and welfare. Survey questions addressed how calf management practices (including social housing and milk allowance) and observed calf behaviors were related to aspects of welfare (termed "calf health/performance" and "calf comfort"). Responses (n = 93 dairy producers, managers, and personnel) were collected via digital surveys. Response rates between categories and equality of distributions were analyzed using nonparametric methods (Kolmogrov-Smirnov test, Fisher's exact test). Respondents generally viewed social housing positively, particularly for calf comfort versus health/performance ($P = 0.0056$). Respondents from farms using social housing (56%) had a more positive perception of how it affected calf comfort and health/performance ($P < 0.04$), viewed social play as being associated with both better calf comfort and health/performance ($P < 0.019$), and considered "freedom to express natural behavior" as more important for calves (64 vs. 8% selected "very important"; respondents from farms using social housing vs. not; $P < 0.001$). Providing greater milk allowances (>7.6 L/d) was generally perceived positively, although this practice was viewed as better for both calf comfort and health/performance ($P < 0.039$) by respondents on farms implementing it. Abnormal oral behaviors (cross-sucking, pen-directed sucking) were perceived negatively, although the perceived value of resources which may reduce these behaviors (e.g., a teat, forage) was more ambiguous and depended on current on-farm practice (e.g., respondents from farms providing forage viewed it as more highly preferred; $P = 0.0098$). We observed a positive relationship between how respondents perceived the human-animal bond (i.e., agreement with "calves enjoy contact with humans") and stated personal behavior related to calf contact (frequency of contacting calves to scratch or pet them; $P = 0.015$). Overall, these results suggest that most calf management personnel place a high value on calf welfare and individual perceptions of animal welfare may depend on practical experience.

Key Words: survey, calf, animal welfare

1573 Wound characteristics following hot-iron and 4 approaches to caustic paste disbudding in dairy calves. A. M. Drwencke*[1], S. J. J. Adcock[2], and C. B. Tucker[1], *[1]University of California–Davis, Davis, CA, [2]University of Wisconsin–Madison, Madison, WI.*

This abstract is part of the ADSA-Graduate Student Competition: Production (PhD Oral Semifinalists).

Disbudding is a common painful procedure. Farms vary in method (hot-iron or paste), if horn bud hair is shaved or not, or the quantity of paste applied, for example. Yet, little research compares the effects of these methods. Our objective was to describe wound characteristics following 5 disbudding methods, with an emphasis on how paste is used. Female Holstein calves were assigned to 1 of 6 treatments (n = 24–25/treatment): nondisbudded control, 1.25 mm hot-iron leaving the bud in, or paste applied in 1 of 4 ways: 0.2 or 0.3 mL on either shaved or unshaved horn buds, respectively. Before disbudding, calves received a local block and systemic pain relief. Wounds were scored 2×/wk until covered with new epithelium. Wound diameter and mechanical nociceptive threshold (MNT) measures to evaluate sensitivity were collected 4 d, 2 wk, and 4 wk after disbudding. Linear mixed models were used to evaluate the effects of treatment and observation (4 d, 2 or 4 wk) on outcome variables with calf as a random effect; pairwise comparisons were used among the treatments. Compared with controls, all disbudded calves exhibited lower MNT values in the first 4 wk (mean N of force ± SE, control: 2.1; disbudded: 1.7 ± 0.08; $P < 0.01$). Sensitivity was similar among disbudding treatments ($P > 0.4$). The size of hot-iron wounds did not consistently differ from the paste treatments over time. Among the paste treatments, wounds were larger when created with 0.3 versus 0.2 mL (21.9 and 19.2 ± 0.4 mm, $P < 0.01$) and when applied to shaved versus unshaved buds (22.2 and 18.9 ± 0.4 mm, $P < 0.01$). Paste wounds were slower to re-epithelialize compared with hot-iron (mean ± SE: hot-iron: 7 wk; 0.2 mL unshaved paste: 14 wk; 0.2 mL shaved paste: 16 wk; 0.3 mL unshaved paste: 16 wk; 0.3 mL shaved paste: 19 ± 1 wk, $P < 0.01$). All wounds were more sensitive than undamaged tissue for at least 4 wk, applying more paste and shaving created larger wounds, and paste wounds took at least twice as long to re-epithelize compared with those from hot-iron disbudding.

Key Words: pain, disbudding, burn

1574 Effects of a maternal bovine appeasing substance on heifer dairy calf stress response to disbudding.

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To examine the effects of applying maternal bovine appeasing substance (MBAS) before caustic paste disbudding on heifer calf stress, cortisol was measured via serum and hair concentrations. Blood was collected via jugular vein within 1 h before disbudding (d0BLOOD), as well as 4 h (4hBLOOD) after disbudding. Tail hair samples were collected upon arrival (d0HAIR) and d 14 (d14HAIR) after arrival to the rearing ranch. Calves were sourced from 3 dairies. One day after arrival, calves were disbudded according to 1 of 4 randomly assigned disbudding protocols: CON (n = 29): disbudding using caustic paste only; MBAS (n = 30): disbudding 24 h after topically administering 5 mL of MBAS (FerAppease®) above muzzle and 5 mL behind poll; AA-CON (n = 29): disbudding using caustic paste 10 min after administering pain mitigation (AA: analgesic (3.3 mg/kg BW transdermal flunixin meglumine) and anesthetic (2 mL per horn bud of 2% Lidocaine); or AA-MBAS (n = 29): administering MBAS and AA at respective time points relative to disbudding. The impact of disbudding protocol on 4hBLOOD and d14HAIR were analyzed using a Generalized Linear Mixed Model (PROC GLIMMIX) that included d0BLOOD and d0HAIR as covariates and the random effect of farm. Cortisol concentrations (μ/dL) increased after disbudding ($P = 0.004$); however, 4hBLOOD differences were not observed among disbudding treatments ($P = 0.33$; CON: 2.23 ± 0.18, MBAS: 1.82 ± 0.17, AA-CON: 1.99 ± 0.17, and AA-MBAS: 1.85 ± 0.17). Hair cortisol concentrations decreased over time ($P < 0.001$), and d14HAIR were also similar ($P = 0.29$) among treatment groups (CON: 29.48 ± 2.46, MBAS: 27.29 ± 2.21, AA-CON: 24.95 ± 2.37, AA-MBAS: 24.95 ± 2.22). In conclusion, the use of MBAS into the disbudding protocol did not significantly lower the 4hBLOOD and d14HAIR cortisol concentrations versus 0hBLOOD and d0HAIR, respectively. Research investigating the effects of MBAS during other stressful events may be worthy of investigation. *Funding was provided by FERA Diagnostics and Biologicals.*

Key Words: maternal bovine appeasing substance, disbudding, dairy calf

1575 Effects of individual versus pair housing on cortisol, growth, and health of dairy calves during the preweaning period.

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In the dairy industry, it remains standard practice to raise preweaning calves individually. There is growing scientific evidence that housing calves in pairs or small groups largely benefits behavior, cognition, and productivity, but relatively few studies have investigated the impact of social housing on stress. The study aim was to investigate the effects of individual versus pair housing on cortisol, growth, and health of preweaning dairy calves. Thirty Holstein calves (18 females, 12 males) were enrolled into 1 of 2 housing treatments: individual (INDV; n = 10 calves) or pair housing (PR; n = 10 pairs; 20 calves). Calves were fed 3 L of milk replacer via bottle 2×/d until 49 d of age, then 3 L 1×/d until 56 d of age. Data collection continued through 63 d of age. Hair samples were collected from the tail switch at birth, 35, and 63 d to assess hair cortisol concentration (HCC). All calves were weighed at birth and 56 d to calculate average daily gain. Calves were assessed daily for clinical signs of disease (dehydration, depression, diarrhea, fever, respiratory disease), and lung ultrasounds were performed weekly to identify respiratory disease. Data were analyzed in SAS using linear mixed-effects models with Gaussian or Poisson link functions; models included the fixed effects of treatment, sex, treatment × sex interaction, and the random effect of pen. HCC decreased significantly with age (birth: 2.5 ± 0.1 pg/mL; 35 d: 1.5 ± 0.1 pg/mL; 63 d: 0.4 ± 0.1 pg/mL), but we did not observe any evidence for differences among treatment groups in HCC. We found no evidence for a significant main effect of treatment on growth, the frequency of clinical signs of disease, or abnormal lung ultrasound scores. However, there was marginal evidence for a treatment × sex interaction for weaning weight; female PR calves were 5.8 kg heavier compared INDV female calves, and INDV male calves were 4.2 kg heavier than male PR calves. These findings indicate PR housing did not adversely affect calf HCC or health. More research is needed to gain a better understanding of the potential differential effect of housing on female and male calves.

Key Words: dairy calf, growth, health

1576 Impact of paired social housing on behavior and growth of organic dairy calves.

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The objective of this study was to determine the impact of paired social housing on behavior and growth of organic dairy calves. Sixty heifer calves (36 Jersey, 12 Holstein, and 12 crossbred) from a Northern California dairy were assigned to 3 housing treatments: individual housing (IH; 2.4 m^2 total interior space; n = 28; 2 Holstein, 10 Jersey, 4 crossbred), paired housing in a double-hutch system (PD; 4.8 m^2 total interior space; n = 16; 3 Holstein, 12 Jersey, 1 crossbred), or paired housing in a large single hutch (PS; 3.5 m^2 total interior space; n = 16; 7 Holstein, 14 Jersey, 7 crossbred) using rolling enrollment from March to August 2023. Following birth, calves were kept with cows and allowed free access to nursing until enrolled at 4.2 ± 2.3 d. Calves were fed 2 L of pasteurized bulk tank milk twice daily with the amount gradually increased to 8 L by 60 d until weaning (104.4 ± 10.5 d old). Calves had free-choice grain and water at enrollment and hay at 8 wk. Calf weight, hip height, and wither height were measured at enrollment and weaning. Data loggers were attached to the rear fetlock of each calf from enrollment to weaning to monitor changes in lying behavior. Data were analyzed using mixed linear models in SAS. Fixed effects included housing treatment, breed, and their interaction. Calf within housing treatment was considered random. Housing treatment did not affect average daily gain or average daily wither and hip height gain. Crossbreds grew at a faster rate (0.71 ± 0.05 kg/d) compared with Jersey calves (0.56 ± 0.03 kg/d; $P < 0.05$), but not compared with Holsteins (0.66 ± 0.05 kg/d; $P = 0.73$). Housing treatment and breed did not affect daily lying bout frequency or lying bout duration. IH calves tended to lie for longer (17.3 ± 0.1 h/d) compared with PS (16.8 ± 0.2 h/d; $P = 0.07$) but did not differ from PD (17.2 ± 0.2 h/d; $P = 0.23$). Paired calf housing may provide an environment for more activity depending on space while maintaining performance metrics. Identifying and quantifying calf behaviors may give insight into the benefits or risks that increased activity and social contact may provide.

Key Words: paired housing, calf behavior, calf growth

1577 Effects of modified wooden group hutches on dairy calves' growth and behavior.

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Previous research suggests welfare improvements with greater space per calf. To investigate group housing benefits without increased land demand, we modified existing California wooden hutch systems. A total of 42 female calves, either Jersey or Jersey–Holstein cross, were randomly and equally assigned to either individual wooden hutch (IH, n = 21) or group modified hutches (GH, n = 21). A modified wooden group hutch was created by removal of the 2 middle dividers of each wooden hutch. Calves were weighed on a digital scale and measured for height at birth, 1, 4, and 8 wk of age. A subset of 37 calves (19 in GH and 18 in IH) was fitted with triaxial accelerometers to the left hind leg to monitor calf activity including daily lying time, lying bouts, and motion index. Mixed-effects linear regression models were specified to estimate the effects of treatment group, week, breed, and their interactions on BW and calf activity, with calf as a random effect. The initial BW ($P = 0.84$), withers height ($P = 0.78$), hip height ($P = 0.84$), and chest girth ($P = 0.72$) of calves at the time of enrollment were not significantly different. There was no significant difference in final BW between the housing types, and interaction between housing type and week effects ($P = 0.77$). However, a significant interaction between breed and week was observed for BW ($P < 0.01$). The mean (±SE) BW for GH and IH calves were 61.24 ± 2.13, and 59.4 ± 1.53 kg/calf, respectively ($P = 0.49$). The mean (±SE) daily lying times of calves housed in GH and IH throughout the experiment were 1,075.40 ± 20.30, and 1,107.44 ± 16.57 min/d, respectively. No treatment ($P = 0.98$) or treatment × week ($P = 0.08$) effect was observed on daily lying daily lying time. Frequency of lying bouts (mean ± SE) did not differ between calves housed in IH (21.24 ± 1.77 bouts/d) and GH calves (21.66 ± 0.44 bouts/d; $P = 0.94$). Further, no treatment ($P = 0.99$) or treatment × week ($P = 0.06$) effect was observed for motion index. The current trial findings provide evidence that simplified group housing offers the benefits of GH without detrimental short-term impacts on calf growth.

Key Words: accelerometer, behavior, group housing

1579 Dairy calves weaned and separated after either 4 or 6 months of full cow-calf contact show signs of stress.

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Cow-calf contact (CCC) systems provide an alternative to early cow-calf separation. However, while calves raised with CCC have opportunities to suckle and form strong social bonds with their dams, they are still weaned and separated at a younger age than observed for semi-feral cattle. The aims of this study were to (1) evaluate the behavioral responses of dairy calves to weaning and separation at 4 or 6 mo of age, and (2) determine if dam-calf spatial proximity—a potential measure of attachment between dam and calf—before weaning influences these responses. Dam-calf pairs (Swedish Red: n = 16; Swedish Holstein: n = 9) were housed together in a freestall pen with 24-h CCC and automatic milking for 4 or 6 mo. During the end of the CCC period cows and calves also had access to pasture, and the calves were weaned outdoors via fenceline separation. Scan samples of proximity (≤4 m indoors and ≤8 m outdoors) within each dam-calf pair were performed during 3 d before weaning. Calf activity (lying time and step count) was recorded both 6 d before and for 11 d after weaning using triaxial accelerometers. Polynomial regression was performed to assess the fixed effects of treatment (4MO vs. 6MO), time after weaning (d 1–11), treatment × time and dam-calf proximity on the changes in daily lying time and step count, compared with levels before weaning. Directly after weaning, calves increased their step count from an average (SD) 2,457 (515) to 10,832 (3,324) steps/d and reduced their lying from 15(1) to 8(2) h/d. Step count returned to preweaning levels within 11 d while lying remained reduced. While treatment groups did not differ in step count ($P = 0.45$), there was a significant treatment-by-time interaction for change in daily lying time ($P < 0.001$), with more intense initial responses observed for 4MO calves. Dam-calf proximity before weaning influenced neither step count ($P = 0.36$) nor lying time ($P = 0.39$). The results indicate that weaning via fenceline causes strong behavioral signs of distress, even in calves nearing an age at which nutritional and social independence might be expected.

Key Words: dam-rearing, fenceline separation, welfare

1580 Development of abnormal oral behaviors in dairy cattle in the first 6 months of life.

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Dairy cattle perform oral abnormal repetitive behaviors (ARBs) including tongue rolling (TR) and non-nutritive oral manipulation of nonfeed items (NNOM). Research on ARBs has often focused on the milk-fed period or a single day during weaning or adulthood. Furthermore, there is some evidence that Jerseys perform ARBs more than Holsteins. We assessed breed differences and the ontogeny of ARBs (n = 24 Holsteins, n = 6 Jerseys) in the first 24 wk of life. We predicted that NNOM would be higher during the milk-fed period and peak around weaning while expression of TR would become prominent at weaning and persist into subsequent weeks. Calves were limit-fed milk and individually housed from birth until the end of step-down weaning (6–7 wk). Weaned animals were housed in similarly aged groups. Throughout these stages, there were different types of feeding: milk via a bottle, then a bucket, weaned from milk, TMR and starter, and then TMR only. Each individual was observed once every 2 wk until 24 wk, resulting in 12 d of data/animal. We used behavioral sampling to record TR and NNOM in 5-min intervals from 0800 to 2000 h and calculated the percent of observations each ARB occurred. A linear mixed model was used to assess the effect of breed and feeding method on ARBs with individual as a random effect. TR and NNOM occurred at the highest levels when calves were experiencing a reduction in milk during weaning (6.8 ± 1.2%, 47.9 ± 2% respectively) or being fed milk from a bucket (10.4 ± 1.3%; $P < 0.001$; 43.9 ± 1.8%; $P < 0.001$) compared with all other feeding types (4.0 ± 0.6%; $P < 0.05$; 27.5 ± 1.2%; $P < 0.001$). Among these other feeding types, NNOM performance was lower when fed from a bottle (31.3 ± 1.3%), TMR and starter (28.3 ± 1.3%), or when fed only TMR (22.9 ± 1%; $P < 0.001$) compared with during weaning or bucket feeding. Despite our limited sample size, Jersey cattle performed significantly more TR and NNOM compared with Holsteins (20.2 ± 1.1 vs. 27.2

± 2.2%; 39.1 ± 1.3 vs. 30.7 ± 0.7%; $P < 0.05$). Regardless of breed, NNOM and TR are expressed at the highest levels in the weeks around and before weaning, compared with other points in their first 6 mo.

Key Words: abnormal repetitive behavior (ARB), dairy cattle, breed

1581 Behavioral and hair cortisol responses of heifers raised in barn or on pasture.

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We assessed behavior and hair cortisol responses of heifers (Hfs) raised in barn and fed a total mixed ration (TrtB) or on pasture (TrtP) rotationally stocked on cool-season pastures and given grain with a vitamin and mineral mix. Holstein Hfs ($n = 64$) aged 4.8 ± 0.45 mo were assigned to 8 groups; 4 of TrtP and 4 of TrtB (8 Hfs/group). A TrtB animal died due to cause unrelated to the study. Hair samples were obtained in May before treatments (Trt) began (in late May 2023) and in June and August, 6 and 14 wk after imposing Trt. Hair cortisol was analyzed via ELISA. Behavioral observations were recorded every 10 min for 12 h during daylight on 1 d in June and 1 d in July by trained observers. Behavior data were converted to minutes or incidents per animal (Hf) by dividing observations by number of Hfs in a group. Data were analyzed as repeated measures in a model with Trt, sampling date, the interaction, and with group as a random variable and the experimental unit. For cortisol, May data were used as a covariate, and also analyzed separately with Trt and group in the model. Eating/grazing time was greater for TrtP than TrtB (403 and 243 min/Hf, SE = 6.6; $P < 0.0001$) as were incidents of vocalization (0.96 and 0.15 times/Hf, SE = 0.173, SE not back transformed; $P < 0.001$). Incidents per Hf of drinking, grooming, and stereotypy were greater for TrtB (TrtP and TrtB, SE, P, respectively: 3.92 and 5.75, 0.47, $P = 0.03$; 4.59 and 9.79, 0.73, $P < 0.01$; 0.17 and 2.15, 0.27, $P < 0.01$). Time spent standing (491 TrtP and 468 TrtB min/Hf, SE = 7.7; $P = 0.07$) or lying (229 TrtP and 252 TrtB min/Hf, SE = 7.8; $P = 0.08$) tended to differ between Trt. Hair cortisol pg/mg hair did not differ between Trt in May (7.2 TrtP and 6.8 TrtB, SE = 0.51; $P = 0.62$) but was higher for TrtP than TrtB in later months (June: 19.2 TrtP and 5.3 TrtB; August: 17.3 TrtP and 5.5 TrtB, SE = 0.48; Trt, $P < 0.001$, Trt × date, 0.08). Stereotypy and grooming in TrtB may be time budget–related responses. Based on hair cortisol, heifers in the barn were apparently less stressed than those on pasture, though the latter appeared to adapt.

Key Words: heifer, behavior, cortisol

1582 Behavior and stress of first-lactation horned and dehorned organic dairy cattle during milking.

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Most US dairy producers disbud cattle. Raising cattle that are horned may more closely align with organic values of natural living while removing a welfare concern in early life. However, many producers perceive horns as a safety concern. Therefore, the objective of this study was to compare the behavior and salivary cortisol concentrations of dehorned ($n = 37$) and nondehorned ($n = 14$) first-lactation dairy cows at the University of Minnesota West Central Research and Outreach Center organic dairy in Morris, Minnesota. Cattle were milked twice daily during the first 8 d of first lactation. Handlers provided scores for ease of milking parlor entry and behavior in the milking parlor and recorded occurrences of kicks and stomping in the milking parlor. Saliva samples were collected immediately after milking. Linear mixed models were used for analysis of salivary cortisol and logistic regression was used for analysis of behavior with the fixed effects of dehorning status, days in milk, time of milking (a.m. or p.m.), and the interaction of dehorning status and days in milk with cow as a random effect. Dehorned cattle (163.3 pg/mL, 95% CI = 152.4 to 174.9) had higher salivary cortisol ($P = 0.037$) than horned cattle (142.3 pg/mL, 95% CI = 128.1 to 158.0). Mean salivary cortisol was lower ($P < 0.001$) for increased days in milk from 203.2 pg/mL on d 1 to 140.4 pg/mL on d 8. Cortisol was higher ($P = 0.006$) during the morning (157.7 pg/mL, 95% CI = 147.6 to 168.3) than during the evening (147.4 pg/mL, 95% CI = 138.20 to 157.3). There was no effect of dehorning status, days in milk, or time for parlor entry or behavior scores or the occurrence of kicking or stomping in the milking parlor. The results indicate that dehorning may have long-term impacts on animal wellbeing. Dehorned dairy cattle had higher salivary cortisol than horned cattle and similar behavior during milking in the first 8 d of lactation.

Key Words: dairy cattle welfare, horned cattle, organic farming

Animal Health 4

1590 Single-nuclei RNA sequencing reveals macrophage heterogeneity in adipose tissue of hyperketonemic dairy cows. H. Ford*[1], T. Michelotti[2], and C. Strieder-Barboza[1], *[1]Texas Tech University, Lubbock, TX, [2]INRAE Clermont-Auvergne-Rhône-Alpes, Saint-Gènes-Champanelle, France.*

Our objective was to elucidate the transcriptional heterogeneity of macrophages in subcutaneous (SAT) and visceral adipose tissue (VAT) of dairy cows with hyperketonemia. Ten Holstein dairy cows (8 ± 2 DIM, parity 3.2 ± 1.4, BCS 3.6 ± 0.3) were blocked by parity, BCS, and serum BHB and assigned to 2 groups based on BHB: nonketotic (NK, n = 5, BHB ≤1.0 m*M*) and hyperketonemic (HK, n = 5, BHB >1.4 m*M*; range = 1.4 to 1.7 m*M*). Flank SAT and retroperitoneal VAT samples were collected via laparotomy and nuclei were isolated for library construction (10X Genomics), sequenced (Illumina NovaSeq 6000), aligned (CellRanger), and analyzed using the Seurat package in R (v1.4). The clusterProfiler package in R was utilized for functional analysis of differentially expressed genes. Identification of distinct cell types was performed based on the expression of signature genes. We identified populations of adipocytes (AD; *ADIPOQ*), adipose stem/progenitor cells (ASPC; *PDGFRA*), endothelial cells (EC; *PECAM1*), pericyte/smooth muscle cells (PE/SMC; *NOTCH3*), and immune cells (IMC; *PTPRC*) in both SAT and VAT. Sub-clustering analysis of the IMC revealed subpopulations of lipid-associated macrophages (LAM; *CD9*, *LPL*) in both SAT and VAT, perivascular macrophages (PVM; *LYVE1*) in SAT, and tissue-resident macrophages (TRM; *MRC1*) in VAT. In SAT, LAM from HK cows had higher expression of TGFB and VEGF signaling genes and lower expression of genes associated with NF-kB signaling and inflammatory pathways, suggesting that the SAT LAM adopt an anti-inflammatory phenotype in HK conditions. In VAT, LAM from HK and NK cows exhibited a more inflammatory gene profile when compared with LAM from SAT. HK was associated with a pro-inflammatory profile of SAT PVM with increased expression of genes associated with TNF, and IL-17 signaling pathways. There were fewer differences between VAT TRM from HK and NK, with both cell populations exhibiting a pro-inflammatory profile. Together, these data indicate that HK has depot-specific effects on macrophage transcriptomes and highlight the heterogeneity of macrophage populations in the adipose tissue of dairy cows.

Key Words: transcriptomics, adipose, macrophage

1583 Effect of bovine-derived *Bifidobacterium longum* ssp. *longum* and resistant potato starch on the gut bacterial colonization in colostrum-restricted calves. X. Wu*[1], P. Griebel[2], K. Rajamanickam[2], N. Malmuthuge[3], R. Nakandalage Don[3,4], and L. L. Guan[1], *[1]The University of British Columbia, Vancouver, British Columbia, Canada, [2]University of Saskatchewan, Saskatoon, Saskatchewan, Canada, [3]Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada, [4]University of Alberta, Edmonton, Alberta, Canada.*

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Probiotics are reported to improve gut health, but findings are controversial due to the lack of host-specific products and limited understanding of their mode of action in vivo. This study assessed if probiotics (bovine-derived *Bifidobacterium longum* ssp. *longum*, B), prebiotics (resistant potato starch, RS), and synbiotics (B-RS) could affect the colonization of *Bifidobacterium*, *Lactobacillus*, *Escherichia coli*, and *Clostridium perfringens* when calves were colostrum-restricted at birth. Forty-five newborn Holstein calves were randomly assigned to 2 groups: (1) optimal calves (OC, n = 9) receiving the recommended IgG (100 g of IgG/L of colostrum replacer, CR) + placebo; (2) suboptimal calves (SOC, n = 36) receiving one-fourth of the recommended IgG (25 g of IgG/L of CR). SOC calves were further allocated into 4 groups (n = 9/group) and received either placebo (negative control, NC), probiotics (10e9 cfu/d, B), prebiotics (80 g/d, RS), or synbiotics (B-RS). Calves were fed CR within 2 h after birth and treatments from d 2 to 14 postpartum. Fecal samples were collected at d 2, 7, 14, 21, 28, and 35 to estimate the density of total bacteria, *Bifidobacterium*, *Lactobacillus*, *E. coli*, and *C. perfringens* using quantitative real-time PCR. The use of Kruskal-Wallis test revealed that the density of total bacteria in NC tended to be higher than that in both RS and B-RS regardless of the time point ($P < 0.1$). The abundance of *Bifidobacterium* increased 6.4-fold in RS compared with NC at d 7 ($P < 0.05$). The B-RS treatment increased *Lactobacillus* abundance by 8.8-fold compared with NC at d 7 ($P < 0.05$). *Escherichia coli* had 5.1-fold colonization in B compared with NC at d 7 ($P < 0.05$). No significant difference in the abundance of *C. perfringens* was observed between the groups. In conclusion, supplementing calves with RS either alone or in combination with B may promote the colonization of beneficial bacteria in the gut and further investigation is needed to explore the impact of B and RS on the gut microbiota in colostrum-restricted calves.

Key Words: bovine-derived probiotic, colostrum-restricted calf, gut bacteria

1584 Effect of probiotic supplementation on fecal microbiota in preweaning Holstein dairy calves. Y. Lee*[2], H. A. Rossow[1,2], D. R. Williams[1], S. Cheong[2], and E. Okello[1,2], *[1]Veterinary Medicine Teaching and Research Center, University of California–Davis, Tulare, CA, [2]School of Veterinary Medicine, University of California–Davis, Davis, CA.*

Probiotics have been suggested as a possible alternative to reduce the use of antimicrobials for diarrhea, due to the concern of emerging antimicrobial resistance. Reported benefits of feeding probiotics include improved weight gain and immune function, but the specific effect of probiotics on the fecal microbiota of preweaning Holstein dairy calves is not elucidated. Our study objective was to compare fecal bacterial diversity and abundance between preweaning calves fed probiotic (PRO) and nonprobiotic (CON) diets and among diarrheic and nondiarrheic calves within each group. A randomized controlled trial was conducted from October to December 2021 on a California calf ranch. A total of 30 calves were enrolled and randomly assigned to PRO (n = 15) and CON (n = 15). Probiotic (*Bacillus subtilis*, *Bacillus lichenformis*, *Lactobacillus animalis*, and *Propionibacterium freudenreichii*, 1.1×10^{10} cfu/g for each species) was administered daily in milk from birth to weaning. Fecal samples were collected at 7, 14, 21, and 42 d of age, and fecal consistency scored on a 3-point scale. PRO and CON were subgrouped into diarrheic versus nondiarrheic calves to compare bacterial diversity and abundance between and within groups. 16S rRNA amplicon sequencing was used to determine the bacterial diversity and abundance. Alpha and β diversity were measured for bacterial diversity, and species abundance was analyzed using a negative binomial regression model. Diarrhea prevalence and bacterial diversity were not different between PRO and CON. PRO had decreased *Clostridium perfringens* (0.009 times abundance, $P < 0.01$) and *Fusobacterium varium* (0.045 times abundance,

$P < 0.01$) compared with CON at d 7. At d 7 in CON, *Fusobacterium varium* was more abundant in diarrheic compared with nondiarrheic calves (108.38 times abundance, $P < 0.01$). There were no significant differences in pathogenic bacteria after d 7. In conclusion, feeding probiotics decreased the abundance of *C. perfringens* and *F. varium* in calf feces at d 7. Administering probiotics during early life may mitigate fecal pathogen shedding in calves, thereby reducing disease spread.

Key Words: probiotic, microbiota, preweaned dairy calf

1585 Effects of feeding different microencapsulated sources of long- and very-long-chain unsaturated fatty acids on production performance and response to an immune challenge. V. Sáinz de la Maza-Escolà[1,2], A. Celemin-Sarmiento[1,3], M. da Silva[1], L. Boback[1], E. Grilli[2,4], and J. E. Rico*[1], [1]*University of Maryland, College Park, MD,* [2]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy,* [3]*Department of Dairy and Food Science, South Dakota State University, Brookings, SD,* [4]*Vetagro Inc., Chicago, IL.*

Omega-3 (n-3) fatty acids are known immunomodulators that can attenuate the inflammatory response. The objective of this study was to evaluate the effects of feeding microencapsulated fish oil, algae oil, and oleic acid on production performance and response to an immune challenge in dairy cows. Nine Holstein cows (mean ± SD: 82 ± 18 DIM; 3.7 ± 0.9 lactations) were randomly assigned to treatments in a replicated 3 × 3 Latin square design with 9-d periods and 7-d washouts. At d 9 of each period all cows received an i.v. lipopolysaccharide (LPS) challenge (30 ng/kg of BW of *Escherichia coli* O55:B5, Millipore Sigma). Cows were fed at 115% of nutrient recommendations and milked twice daily. Treatments were top-dressed daily and consisted of microencapsulated fish oil (FO; Prototype 6; 36% fish oil), algae oil (ALG; Prototype 39; 45% algae oil), and oleic acid (OA; Prototype 40; 20% oleic acid). The FO and ALG products delivered 25 g of the sum of n-3 (20:5 and 22:6), while OA provided 40 g of *cis*-9, 18:1. Treatments were balanced for lipid supply at 226 g/cow per d. Data were analyzed under a mixed model with fixed effects of treatment, parity, time, and their interactions. Although no overall treatment effect was detected for milk yield ($P = 0.54$), FO and ALG cows outperformed OA on d 6–8 (treatment × time, $P = 0.04$). Cows in OA tended to have higher DMI ($P = 0.12$) and lower feed efficiency (treatment × time, $P = 0.06$) relative to FO and ALG. Milk fat yield was higher in OA compared with FO and ALG ($P < 0.05$). Energy- and fat-corrected milk (3.5%) were greater in OA and FO than in ALG cows ($P < 0.05$). Relative to FO and OA, cows in ALG had lower milk SCS ($P < 0.05$). No changes were detected in milk protein and MUN. Rectal temperature area under the curve (AUC) tended to be lower in ALG relative to FO and OA during initial 6 h from LPS challenge ($P < 0.15$). Similarly, respiration rates as AUC at 24 h tended to be lower in ALG compared with FO and OA ($P = 0.15$). The effects of feeding unsaturated fat supplements on performance and health may depend upon chain length degree of saturation.

Key Words: n-3 (omega-3), oleic acid, immune challenge

1586 Integrating multi-omics and immune profiling reveals causal networks driving uterine dysbiosis in dairy cows that develop metritis. S. Casaro*[1], J. Prim[2], T. Gonzalez[1], F. Cunha[1], R. Bisinotto[1], R. Chebel[1], J. E. Santos[1], C. Nelson[1], S. Jeon[3], R. Bicalho[4], J. Driver[5], and K. Galvão[1], [1]*University of Florida, Gainesville, FL,* [2]*Auburn University, Auburn, AL,* [3]*Long Island University, Brookville, NY,* [4]*FERA Diagnostics and Biologicals, College Station, TX,* [5]*University of Missouri, Columbia, MO.*

The objective was to integrate uterine microbiome, uterine and plasma metabolome, and peripheral blood immune profiling to advance the understanding of metritis development. Holstein cows (n = 100) had blood collected at 14 d before calving (PP), and blood and uterine fluid collected at calving (D0) and the day of metritis diagnosis (Dx). Uterine fluid samples had the 16S rRNA gene sequenced and quantified. Untargeted gas chromatography time-of-flight mass spectrometry was performed for metabolome characterization. Flow cytometry was used for blood leukocyte counts, proportions, and activation. Total immune cells, live cells, single cells, monocytes, polymorphonuclears (PMN), B cells, T-helper, T-cytotoxic, and γδ T-cells were evaluated. CD62L was used as a marker for cell activation. Major pro- and anti-inflammatory cytokines were quantified. Prepartum body weight (ppBW) and body weight change (BWC) were evaluated. Sparse principal component analyses were conducted for microbiome and metabolome latent variables (LV) identification. Bayesian network modeling was used to integrate the datasets. Greater ppBW predicted lesser PP total cells and greater BW loss. These predicted greater PP PMN, lesser PP plasma amino acids (AA), lesser PP and D0 carbohydrates and antioxidants, lesser D0 monocyte activation, and greater PP, D0, and Dx B cell activation. Lesser PP plasma AA predicted lesser Dx plasma AA, which predicted lesser Dx uterine LV2 (galactose metabolism). Greater Dx B cell activation predicted lesser Dx PMN, which predicted greater Dx uterus LV3 (AA and energy metabolism). Lastly, PP plasma PC5 (antioxidants) predicted lesser D0 and Dx live cells. Lesser Dx live cells, together with lesser Dx uterine LV2 and greater Dx uterine LV3 predicted greater Dx opportunistic pathogenic bacteria (*Fusobacterium*, *Bacteroides*, and *Porphyromonas*). In summary, our integrative analysis revealed that ppBW and BW loss correlated with several immune and metabolic changes, promoting the overgrowth of opportunistic uterine pathogenic bacteria on the day of metritis diagnosis.

Key Words: metritis, multi-omics, immune dysregulation

1587 Metagenomics-guided isolation and genetic antibiogram of emerging pathogens associated with metritis in dairy cows. F. Cunha*, S. Casaro, K. L. Jones, M. Hernandez, K. C. Jeong, Y. Zhai, and K. N. Galvão, *University of Florida, Gainesville, FL.*

Metritis is a highly prevalent postpartum uterine disease caused by mixed bacterial infection in dairy cows, affecting productivity, survival, and welfare. Culture-independent studies have found that *Fusobacterium*, *Bacteroides*, *Porphyromonas*, and *Helcococcus* sp. are more abundant in the uterus of dairy cows with metritis, whereas there is no difference in the abundance of *Escherichia coli* and *Trueperella pyogenes*. The objective of this study is to use microbial culture to evaluate the association of these bacteria and metritis, and to investigate their genomic antimicrobial resistance. Our hypothesis is that *Fusobacteria* sp., *Bacteroides* sp., *Porphyromonas* sp., and *Helcococcus* sp. are associated with metritis, and that their genetic antimicrobial resistance profiles will warrant re-evaluation of treatment strategies. Uterine luminal content was collected transcervically from 20 healthy and 18 metritic cows by flushing 50 mL of sterile saline via a plastic pipette and a sterile syringe, avoiding contamination. Samples were inoculated onto selective media and incubated in various conditions. Isolates were identified by 16S Sanger sequencing and their genomes were sequenced on an Illumina NextSeq 2000. The association between bacterial prevalence and disease was analyzed using Fisher's exact test. We used Abricate, a bioinformatics tool designed for screening of contigs for acquired antimicrobial resistance genes, against comprehensive databases. *Fusobacterium necrophorum* ($P = 0.008$), *Bacteroides pyogenes* ($P < 0.001$), and *Hel-*

cococcus ovis ($P < 0.001$) were significantly associated with metritis. There was no association between *E. coli* ($P = 0.49$) or *Porphyromonas levii* ($P = 0.10$) and metritis. A wide range of antimicrobial resistance genes were found in metritis-associated isolates including tetracycline resistance genes in 97% (30/31) *H. ovis* strains. Our work confirms the importance of bacteria identified by culture-independent studies and challenges the importance of *E. coli* in the pathogenesis of metritis. The genetic antimicrobial resistance profile of these pathogens suggest that tetracyclines are not an effective treatment for the disease.

Key Words: metritis, WGS, metagenomics

1588 A microfluidic-based approach to investigate dynamic cytokine release of RAW 264.7 macrophage under lipopolysaccharide stimulation. J. Halfen*[1], V. A. Oliveira[1], J. McGill[2], and J. S. Osorio[1], [1]*School of Animal Science, Virginia Tech University, Blacksburg, VA,* [2]*College of Veterinary Medicine, Iowa State University, Ames, IA.*

Microfluidic cell culture is a method that, unlike most static culture protocols, aims to approach physiological conditions for in vitro experiments with dynamic nutrient supply and waste removal. Therefore, this study aimed to use microfluidic technology to characterize the inflammatory activation of RAW 264.7 mouse macrophage under 12 h of lipopolysaccharide (LPS; *Escherichia coli* O111:B4) challenge. Cells were harvested with 85% of confluency by scraping. Cells were counted and then transferred to 5 treated microfluidic slides (Ibidi u-Slide 0.2 μm) at 3×10^5 cells/slide and kept overnight in the incubator at 37°C, 5% CO_2. Each replicate consisted of cell-seeded slides randomly assigned to one of 5 treatments; CON (control; complete media without LPS), LPS (100 ng/mL of LPS diluted in complete media), DEXA (10 u*M* of dexamethasone + 100 ng/mL of LPS), siRNA (mouse TNFα silencer RNA + 100 ng/mL of LPS), and NC (Select Negative Control siRNA + 100ng/mL of LPS). The siRNA and NC were transfected with 50 pmol of TNFα siRNA or NC siRNA at 24 h before starting the microfluidic test. The microfluidic flow was set up to run at 50 μL/min range (0.2dyn. cm^2 shear stress) controlled by a flow sensor. Flow-through media was collected at −1, 0, 1, 3, 6, and 12 h relative to the LPS challenge and used to measure TNFα concentration via ELISA. Data was analyzed using the MIXED procedure of SAS, including a total of 4 replicates. A trend for Treatment × time (Trt × T, $P = 0.06$) reflected in greater TNFα (Trt × T, $P < 0.05$) in LPS group compared with CON, DEXA, and siRNA at 3 h and greater TNFα in LPS group compared with CON and DEXA at 6 h relative to the LPS challenge. The TNFα secretion from LPS and NC groups increased rapidly, reaching maximal levels at 3 h post-LPS challenge, followed by constant decline until 12 h. Overall, LPS and NC groups had greater TNFα (Trt, $P = 0.01$, 34.2 and 32.9 pg/mL) than CON, DEXA, and siRNA (24.6, 24.3, and 25.2 pg/mL, respectively). The innovative use of microfluidics to study macrophage activation and modulation under continuous LPS challenge provides a unique perspective on cytokine release dynamics.

Key Words: microfluidic, inflammation, siRNA

1589 Prepartal liver glutathione improves liver function, and it may be regulated by prepartal energy status in transition dairy cows. A. F. Souza Lima*[1], G. Goncalves Begalli[1], M. H. Oliveira[1], R. Chaves Barcellos Grazziotin[1], J. Halfen[1], E. Trevisi[2], and J. S. Osorio[1], [1]*School of Animal Science, Dairy Science, Virginia Tech, Blacksburg, VA,* [2]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Sciences, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Our objective was to investigate the impact of prepartal liver glutathione (GSH) concentration on blood and liver metabolites during the transition period. Sixty multiparous Holstein dairy cows were monitored from −21 to 30 DIM. Liver biopsy was performed in all cows at −10 DIM to measure total GSH and classify cows based on prepartal liver GSH content as high (HGSH; n = 15), medium-high (MHGSH; n = 14), medium-low (MLGSH; n = 16), and low (LGSH; n = 15). Blood and liver samples were collected at various time points from −21 to 21 DIM to evaluate biomarkers associated with metabolism and liver function. Target genes related to GSH metabolism were evaluated via RT-qPCR. Statistical analysis was performed using the PROC MIXED of SAS. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. A Group × Time (G × T) interaction ($P = 0.02$) was detected in cholesterol, where HGSH cows had greater concentrations ($P < 0.01$) than LGSH at −10 d and greater cholesterol in HGSH compared with all other groups at 21 d. A trend ($P = 0.09$) for G × T in albumin was explained by greater ($P = 0.02$) levels in HGSH compared with LGSH at 21 d. Additionally, lower albumin was observed in MLGSH compared with MHGSH at 7 d ($P = 0.02$) and LGSH at 14 d ($P = 0.02$). Greater glucose levels ($P = 0.02$) were observed in HGSH and MHGSL compared with MLGSH and LGSH. In contrast, greater insulin levels ($P = 0.03$) were observed in MLGSH compared with the MHGSH and HGSH. A positive correlation was found between liver GSH and glucose ($r = 0.36$; $P < 0.01$) and insulin ($r = 0.31$; $P < 0.01$) at −10 d. The mRNA expression ($P \leq 0.04$) of *GGCT*, *GCLC*, *GGT*, and *GSR* had a linear downregulation across HGSH to LGSH groups. Our findings suggest a link between prepartum liver GSH levels and postpartal liver function. The correlation of prepartal liver GSH with glucose and insulin suggests a possible role of metabolic energy in regulating liver GSH levels. Downregulation of GSH-related genes, as prepartal liver GSH decreases, indicates a potential molecular regulation of liver GSH synthesis. Prepartal liver GSH promotes better liver function and may be regulated by energy status.

Key Words: peripartum period, dairy cow, glutathione

1591 Lipolysis pathways modulate endocannabinoid production and release by dairy cows' adipocytes. M. N. Myers*[1], M. Chirivi[1], J. C. Gandy[1], J. Tam[2], M. Zachut[3], and G. A. Contreras[1], [1]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI,* [2]*Institute for Drug Research, School of Pharmacy, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel,* [3]*Department of Ruminant Sciences, Agriculture Research Organization, Volcani Institute, Rishon LeZion, Israel.*

In periparturient dairy cows, adipose tissue (AT) lipolysis is stimulated through canonical and inflammatory pathways. While necessary to meet near-calving energy demands, protracted and excessive lipolysis predisposes cows to metabolic and infectious disease. Endocannabinoids (eCB), including 2-arachidonoyl glycerol (2-AG) and anandamide (AEA), derived from free fatty acids (FFA), are potent modulators of AT metabolism and inflammation. Presently, the role of lipolysis pathways on eCB production in bovine adipocytes is unknown. Therefore, our study's goal was to define the capacity of lipolysis pathways to modulate eCB biosynthesis and release by adipocytes and reveal associated transcriptional changes. Preadipocytes from 5 nonlactating Holstein cows were induced to differentiate, exposed to untreated (BAS) or media treated with isoproterenol (ISO; 1 μ*M*) or lipopolysaccharide (LPS; 1 μg/mL). Lipolysis intensity, eCB production, and gene expression were assessed. Statistical methods included one-way ANOVA with Tukey's HSD for eCB/glycerol data and Student's *t*-tests for DESeq2R-normalized gene counts ($P < 0.05$ for differences unless otherwise stated).

ISO and LPS increased lipolysis and tended to enhance eCB release ($P < 0.09$). ISO promoted 2-AG release, while LPS enhanced AEA production. ISO upregulated transcription of the 2-AG biosynthesizing *GDPD5* and *INPP5F*, while LPS increased NAE-affiliated *PTPN22*. LPS promoted eCB-degrading *PTGS2*, *CYP27B1*, and *MGLL*. ISO and LPS heightened adipocytes' transcription of transporters *FABP3* and *FABP7*. *SCP2* and *HSP1A1* were elevated in LPS while ISO enhanced *CD36* abundance. *PPARG* was elevated in ISO and LPS increased the expression of *TRPV3*. Collectively, our results indicate that lipolysis enhances eCB production and release by cows' adipocytes in a pathway-dependent manner, with canonical lipolysis enhancing 2-AG and inflammatory lipolysis promoting AEA biosynthesis. These findings suggest FFA may be diverted toward 2-AG or AEA production based on the cow's health status and presence of pathogens in AT, holding potential implications for the health and management of periparturient cattle.

Key Words: adipose, endocannabinoid, lipolysis

1592 Cyclooxygenase activity is regulated by TLR4 signaling and is determinant for lipolytic responses in bovine adipocytes. M. Chirivi* and G. A. Contreras, *Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Endotoxin triggers lipolysis and reduces insulin sensitivity in adipose tissue (AT), evidencing a link between metabolic and infectious diseases. However, the mechanisms underlying endotoxin-induced adipocyte lipolysis remain unclear. We aimed to characterize the role of cyclooxygenase (COX) on bovine adipocyte lipolysis upon TLR4 activation. Adipocyte progenitors (AP) were obtained from subcutaneous AT from 6 nonlactating nongestating multiparous Holstein dairy cows by nonenzymatic isolation. AP were transfected with siRNA targeting *TLR4* (si*TLR4*) or a noncoding (ncRNA) control, and induced to differentiate into adipocytes using pro-adipogenic media for 7 d. Next, adipocytes were stimulated for 7 h with the lipolytic agent isoproterenol (ISO = 1 µ*M*, basal = 0 µ*M*) or lipopolysaccharide (LPS; O55:B5; 1 µg/mL). To quantify the contribution of COX on lipolysis, adipocytes were preincubated with a COX inhibitor, flunixin meglumine (FM). Lipolysis was determined by quantification of glycerol release and results are presented as units relative to basal (±SEM). Arachidonic acid (ArA)-derived oxylipids were quantified by LC/MS. Statistical analyses used a linear mixed model in JMP. Compared with basal, ISO and LPS increased glycerol release by 86.91 ± 16% and 72.66 ± 16%, respectively ($P < 0.01$). si*TLR4* drastically reduced LPS -induced lipolysis (+3.5 ± 4.5%) compared with ncRNA (+42.66 ± 5.1%, $P < 0.05$). *siTLR4* reduced the transcription of COX-2 gene (*PTGS2*) by 83.89% compared with ncRNA. Incubation with FM inhibited LPS-induced lipolysis, reduced *PTGS2* by 52.45%, and reduced the amount of LPS-induced ArA in the culture media by 63.51%. Adipocytes treated with FM showed lower concentrations of Ara-derived oxylipids during LPS incubation, including PGE2, PGD2, 6-Keto PGF1a, and 5-HETE. Collectively our results suggest a link between TLR4 and COX activity in bovine adipose tissue during lipolysis. The inhibition of COX activity may represent a new approach to reducing inflammation-induced lipolysis in dairy cows. Future studies will evaluate the role of prostaglandins in adipose tissue function.

Key Words: adipocyte, COX, lipolysis

1593 Prepartum acetylsalicylic acid in over-conditioned and nulliparous cows: Effects on metabolic stress and inflammatory status. E. Jimenez*[1,2], P. Zarei[1], J. Spring[1], M. Dailey[1], C. Zheng[1], J. Lection[1,3,4], R. Sorto[1,2,4], M. Martinez[1], E. Hovingh[1], and A. Barragan[1], *[1]Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA, [2]Veterinary Medical Center, Iowa State University, Ames, IA, [3]Intercollege Graduate Degree Program in Integrative and Biomedical Physiology, Penn State University, University Park, PA, [4]Department of Animal Science, Penn State University, University Park, PA.*

This study aimed to assess the effects of prepartum administration of acetylsalicylic acid on metabolic and inflammatory biomarkers in high-priority dairy cows (i.e., over-conditioned cows [OVERC = BCS ≥ 3.75 points] and nulliparous cows [NUL]). At −21 ± 3 d relative to calving (d.r.c), cows were blocked by parity (NUL, n = 75; parous [PAR, n = 130]) and body condition score (OVERC, n = 145; optimal conditioned [OPT] = BCS 3–3.5 points, n = 60), and randomly allocated to one of 2 treatment groups: (1) ASA (n = 96): Cows received one oral administration with 4 boluses of acetylsalicylic acid (200 mg/kg) at −14 ± 3 d.r.c.; or (2) PLC (n = 109): Cows received one oral treatment with 4 gelatin capsules filled with water at −14 ± 3 d.r.c. Blood samples were collected weekly, starting 1 week before treatment until 3 weeks after calving, to measure the serum concentration of nonesterified fatty acids (NEFA), interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α). The data were analyzed using linear regression models with the MIXED procedure of SAS as a complete randomized block design. Cows treated with ASA had lower prepartum NEFA concentrations compared with PLC cows (ASA = 273.5 ± 15.5 mEq/L; PLC = 303.7 ± 13.9 mEq/L; $P = 0.05$). ASA PAR OVERC cows had lower NEFA concentration during the postpartum period than PLC PAR OVERC cows. There was a statistical tendency ($P = 0.1$) for IL-1β, where cows treated with ASA had lower concentrations than PLC cows (ASA = 7.46 ± 1.50 pg/mL; PLC = 10.68 ± 1.37 pg/mL). Similarly ($P = 0.08$), over-conditioned cows that were treated with ASA had lower concentrations of TNF-α compared with PLC OVERC cows (ASA OVERC = 272.77 pg/mL, 95% CI = 208.09–346.19; PLC OVERC = 327.97 pg/mL, 95% CI = 59.12–404.94). OPT cows treated with ASA had higher concentrations of IL-6 than PLC OPT cows. These results suggest that prepartum ASA treatment may yield positive effects on the metabolic and inflammatory status of high-priority dairy cows throughout the transition period, while it may be nonbeneficial for nonpriority cow groups.

Key Words: prepartum anti-inflammatory therapy, metabolic and inflammatory status, high-priority cow group

Breeding and Genetics Symposium: Genomic Strategies for Alternative Dairy Production Systems

1595 Defining breeding goals for improved sustainability in organic production systems. I. W. Haagen*[1], L. C. Hardie[2], B. J. Heins[1], and C. D. Dechow[2], [1]*University of Minnesota, St. Paul, MN,* [2]*Pennsylvania State University, State College, PA.*

The objective is to describe breeding goals for organic production systems that achieve profitable, healthy, and sustainable cattle. Organic dairy operations account for approximately 3% of dairy cows in the United States. The organic market demands that products come from healthy animals and are produced sustainably, all while maintaining herd profit. However, in the United States, genetic evaluations are largely based upon conventional production systems from herds participating in Dairy Herd Improvement testing, and organic dairy producers are less likely to participate in milk recording than conventional producers. Currently there are approximately 50 genetic traits evaluated in the United States. Research suggests that current US national selection indexes, such as Net Merit, will produce positive changes related to production, health, efficiency, and sustainability in organic production systems. Further, several selection indexes have been developed for alternative production systems, such as Grazing Merit, which places greater emphasis on fertility traits. However, while genetic correlation estimates between conventional and organic production systems are generally favorable, many estimates are less than unity suggesting there is reranking of sires across systems. Additionally, there are considerations for organic producers that may not be fully accounted for in current national evaluation systems. For instance, our research suggests that increased selection emphasis should be put on youngstock stayability and disease resistance in organic production compared with conventional production systems. While current national selection decisions are driving improvements in dairy production efficiency and sustainability, organic dairy producers must consider if current national breeding goals fully meet the needs of their operations.

Key Words: organic, genetics, sustainability

1596 Implementation of genomic selection in French dairy sheep and goats and opportunities for sustainable animal breeding. H. Larroque*[1], J.-M. Astruc[2], M. Arnal[2], D. Buisson[2], M. Chassier[2], V. Clément[2], G. Lagriffoul[2], I. Palhière[1], R. Rupp[1], M. Teissier[1], A. Legarra[1], and C. Robert-Granié[1], [1]*GenPhySE, Université de Toulouse, INRAE, ENVT, Castanet-Tolosan, France,* [2]*Institut de l'élevage, Castanet-Tolosan, France.*

In France, the implementation of genomic selection in dairy sheep and goats seemed less obvious than in dairy cattle, due to higher relative cost of genotyping, small reference populations, and high genetic diversity. Our research work using the single-step GBLUP approach has, however, yielded genomic reliability similar to those obtained in some dairy cattle breeds, as well as higher theoretical accuracies of genomic estimated breeding values (GEBV) for genotyped young male candidates. This work led selection schemes to switch to genomic selection in the late 2010s. Studies then focused on ways of increasing reliability of genomic evaluations. In sheep, the over-dispersion of GEBV for young candidates can be reduced by taking into account missing pedigrees using the metafounders method. In goats, the genomic prediction quality can be improved by weighting SNPs (WSSGBLUP) to take account of major genes or QTLs, or by using sequence data. To assess the possibilities of building an international reference population, across-countries genomic correlations were estimated. These correlations make it possible to envisage collaborative projects between France and Spain for 2 sheep breeds, and France and Italy for goats. Genomic selection opens up the possibility of selecting new traits. Small ruminant farms are often located in areas with severe environmental constraints where they suffer the consequences of climate change. In this context, breeders are concerned about resilience and efficiency traits, key elements for sustainable animal breeding. Thus, a genomic evaluation of artificial insemination success of females has been developed for goats, and a genomic evaluation of rams for their resistance to gastrointestinal parasites is now operational in 3 breeds of sheep. Finally, research work has been carried out in both species on functional longevity, feed efficiency, milk persistency, and maturity, opening up new selection prospects. *Part of this work has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 772787 (SMARTER).*

Key Words: genomic selection, dairy sheep, dairy goat

1597 Angus genetics—Providing crossbreeding solutions for the dairy industry. K. J. Retallick*, *Angus Genetics Inc., Saint Joseph, MO.*

The utilization of sexed semen alongside robust genetic predictions tools in the dairy sector has led to a common practice of enhancing the value of dairy byproducts through crossbreeding dairy females with beef bulls. Although this practice of integrating dairy animals into the beef supply chain is not new, the emphasis on the carcass capabilities through the right genetic selection has transformed the value of these carcasses to the beef industry. Over time targets in the beef industry have shifted from a commodity-driven to a quality-driven marketplace. In 2008, 33% of the total fed cattle populations harvested graded USDA select to that number is less than 17% today. With that, the demand for premium beef from consumers has continued to grow demand for labeling programs like Certified Angus Beef. It is approximated that today Angus has greater than 60% market share in the beef-on-dairy space. In response to the trend, Angus Genetics Inc. (AGI) worked to create profitability indexes tailored for this specific sector, including Angus-On-Holstein and Angus-On-Jersey dollar values. These indexes include fitting traits not traditionally included in our terminal beef indexes, including calving ease and modeling growth all the way from birth to harvest date. In addition, traits addressing muscling and height requirements have been added to decrease the prevalence of known issues with these carcasses. While terminal indexes designed for both these segments have a strong correlation of approximately 0.75, it is known that not all Angus bulls are suitable for this purpose. In addition, the dairy industry has the luxury of focusing on a strictly terminal mating, if those females are getting bred and calving without assistance. This allows for rapid genetic progress to take place with this supply as well as a unique opportunity for beef industry groups to collaborate on data capture refining genetic tools to support the growth in this industry. Nonetheless, with the right tools in hand, this supply can be both a profitable opportunity for the dairy producer and an asset for the beef industry.

Key Words: beef, dairy, crossbreeding

1598 Crossbreeding dairy cattle for improved production and profitability. B. J. Heins*[1,2] and L. B. Hansen[1], [1]*University of*

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Interest in crossbreeding of dairy cattle continues to increase globally. The Holstein breed has been tremendously successful in selection for milk production over the past 50 years. However, the Holstein breed was also selected for larger body size and more angularity of cows on top of the selection for production. Therefore, the Holstein breed experienced a rapid decline in these functional traits. The average inbreeding coefficient of US Holstein females born in 2023 surpassed 10%, and the annual rate of increase in average inbreeding has approached +0.4%. Heterosis from crossbreeding is the most influential for traits related to fertility, health, and survival, and it comes on top of genetic improvement within breeds. The support for breeds with robust genetic improvement programs is critical for successful crossbreeding programs. Dairy producers should select 3 breeds of dairy cattle that are appropriate for their specific management systems and use the highest-ranking AI bulls from each of the 3 breeds. A 10-year study compared cows from a 3-breed rotational crossbreeding program using the Holstein, Viking Red, and Montbeliarde breeds with their pure Holstein herdmates. Health treatment cost was 23% lower for the 2-breed crossbreds and 17% lower for 3-breed crossbreds than their Holstein herdmates. Lifetime death loss was 4% lower for both the 2-breed crossbreds and the 3-breed crossbreds than their Holstein herdmates. Daily profit was 13% higher for the 2-breed crossbreds and 9% higher for the 3-breed crossbreds than their Holstein herdmates. An ongoing crossbreeding research study at the University of Minnesota West Central Research and Outreach Center, Morris, Minnesota, dairy will evaluate the production, health, and most importantly, the economic performance of crossbred dairy cattle compared with their Holstein herdmates. Future studies will evaluate methane emissions, fatty acid composition of milk, parasite resistance, as well as economic impacts of crossbreeding. The results will help improve the long-term profitability and social sustainability of the dairy industry by improving its public image.

Key Words: crossbreeding, organic, Holstein

Physiology and Endocrinology 1

1599 Polymerized octadecyltrichlorosilane constructed microfluidic field-effect transistor amplified with a negatively charged DNA nanostructure for sensing magnesium ions (Mg^{2+}). H. Wang*, L. Yang, and B. Xiong, *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Accurate detection of magnesium ion (Mg^{2+}) levels in the bloodstream is crucial for the diagnosis and treatment of hypomagnesemia. A paper-based microfluidic chip with low-cost, simple fabrication, customized, and specific structural production is suitable for point-of-care diagnostics, but breathability and water absorption properties limit its sensitivity, stability, and durability for biosensing applications. To address these challenges, we proposed a cellulose paper-based microfluidic field-effect biosensor (MFB) fabricated using a cutting and pasting method, along with a hydrophobic polymeric material and a designed RNA-cleaving DNAzyme to improve its performance for Mg^{2+} levels determination. Cellulose paper was first decorated with polymerized octadecyltrichlorosilane or not to produce hydrophilic/hydrophobic paper, which was cut into a special structure and pasted with PDMS to construct a MFB with stability and durability in the water environment. The sensitive area of the chip was then modified with semiconducting single-walled carbon nanotubes (SWNTs) and RNA-cleaving DNAzyme to build the MFB. The p-type nature of SWNTs enables further sensitivity amplification by hybridization with a negatively charged DNA nanostructure. Characterization and optimization of the proposed biosensor revealed that it combines sample pre-treatment and detection analysis with high selectivity and sensitivity. It has a linear range of 1 μ*M* to 10 m*M* with a detection limit of 0.65 μ*M*, demonstrating its promising potential for application in the diagnosis and treatment of animal diseases. This method effectively addresses the limitations of traditional paper-based biosensors with low cost, which can work even in real samples with satisfactory accuracy.

Key Words: hydrophilic/hydrophobic paper, magnesium ion, microfluidic chip

1600 Immune cell phenotypes and bioenergetic measures in $CD4^+$ T cells differ between high- and low-feed-efficient dairy cows. U. Arshad*, K. Kennedy, M. Cid de la Paz, S. Kendall, L. Cangiano, and H. White, *University of Wisconsin–Madison, Madison, Wisconsin.*

Identifying sources of variance that contribute to residual feed intake (RFI) can aid in improving feed efficiency. An activated immune system represents an energy sink, yet it has not been evaluated in high or low RFI cows. Objectives were to investigate immune cell phenotypes and bioenergetic measures only in $CD4^+$ T cells in low-efficient (LE) and high-efficient (HE) cows. Holstein cows (n = 64) were enrolled at 93 ± 22 DIM and monitored for 6 weeks to measure the RFI. Cows with the highest RFI (LE; n = 14) or lowest RFI (HE; n = 14) were selected to determine the immune cell phenotype using flow cytometry. Blood samples were collected at 234 ± 22 DIM to isolate the peripheral blood mononuclear cells followed by magnetic separation of $CD4^+$ T cells using bovine specific monoclonal antibodies. Isolated $CD4^+$ T cells from each cow were divided into 2 tubes and randomly assigned to be incubated in either control (resting state; RST) or phorbol myristate acetate and ionomycin media (activated state; ACT) to evaluate their metabolic function. Analyses of RST and ACT states were done separately. The statistical models included the fixed effects of feed efficiency, time, and interaction between feed efficiency and time, and the random effects of plate and cow nested within feed efficiency category. The HE cows had an increased ($P \leq 0.02$) median fluorescence intensity (MFI) of $CD62L^+$ cells within $CD8^+$ T cells (LE = 569 vs. HE = 608 ± 11.2 MFI) and $CD21^+$ B cells (LE = 637 vs. HE = 653 ± 4.5 MFI) compared with LE cows. The HE cows showed an increased ($P = 0.001$) mitochondrial respiration, coinciding with an increased ($P \leq 0.02$) cytoplasmic aerobic glycolysis during RST and ACT states compared with LE cows. The increased metabolic activity in $CD4^+$ T cells during RST or ACT states in HE cows specifically demonstrates their enhanced fitness, which may be linked to maintaining effective immune function. These preliminary data suggest that even in healthy cows, immune readiness may contribute to nutrient use and be a source of variance between cows of divergent RFI that warrants further investigation.

Key Words: dairy cow, feed efficiency, immune readiness

1601 Association of postpartum inflammation with feeding behavior, milk yield, and metabolic profile in Holstein dairy cows. L. Cattaneo*, M. M. Passamonti, L. Benedetti, A. Minuti, and E. Trevisi, *Department of Animal Sciences, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Inflammation is a key factor in determining the outcomes of the transition period. The aim of this study was to evaluate the associations of postpartum inflammation with feeding behavior, milk production, and blood biomarkers during the transition period in Holstein dairy cows. Data from 128 cows involved in previous trials were used. Plasma haptoglobin concentrations from 3 d postpartum were used to retrospectively classify cows into quartiles (means ± SD were 0.08 ± 0.05, 0.34 ± 0.10, 0.58 ± 0.06, 0.94 ± 0.19 g/L, respectively). Blood samples were collected at −21, −7, 3, 14, and 28 d relative to calving. Feeding behavior, rumination, and milk yield were automatically monitored daily and condensed into weekly averages. Data were analyzed with repeated measures mixed models, considering the fixed effects of quartile, distance from calving, their interaction, parity, dietary treatment, calving month, and the random effect of cow. By design, HP differed overall among groups ($P < 0.01$), with significant differences only at the 3 and 14 d samplings. Milk yield in the first 8 wk of lactation decreased with increasing postpartum inflammation ($P < 0.01$). Dry matter intake was smaller postpartum in the fourth quartile (i.e., high haptoglobin) compared with the others, but no differences were noted before calving. However, the fourth quartile had the shortest eating time before calving ($P = 0.01$). Rumination time had different trends after calving ($P < 0.01$), with the lowest value observed the first week postpartum in the fourth quartile. Plasma Ca concentration was greater in first quartile than in the other ones ($P < 0.01$). Albumin was greater 14 and 28 d after calving in the first and second quartiles compared with the third and fourth ($P \leq 0.01$). Myeloperoxidase and bilirubin concentrations overall increased with increasing postpartum inflammation, whereas paraoxonase had the opposite trend. Our results show that postpartum inflammatory conditions are associated with changes in milk yield and alterations in feeding behavior, mineral balance, oxidative stress, and liver function during the transition period.

Key Words: inflammation, transition period, postpartum

1602 Effects of feed restriction on basal and stimulated energetic metabolism in fat and thin lactating cows. T. A. Flemming*, G. J. Combs, S. Rodriguez-Jimenez, E. J. Mayorga, J. Opgenorth, B.

M. Buol, and L. H. Baumgard, *Department of Animal Science, Iowa State University, Ames, IA.*

Over-conditioned transition cows are notoriously poor performers and many blame excessive adipose mobilization on this phenomenon. Objectives were to evaluate the effects of feed restriction (FR) on basal and stimulated metabolism in fat and thin cows. Eleven multiparous Holstein cows (80 ± 8 DIM) were assigned to 1 of 2 treatments: (1) FAT (4.20 ± 0.25 BCS; 666 ± 48 kg BW; n = 6) and (2) THIN (2.85 ± 0.25 BCS; 578 ± 90 kg BW; n = 4). Cows were enrolled in 3 experimental periods (P): in P1 (3 d) baseline data were collected, in P2 (5 d) cows were FR to 60% based on P1 intake, and in P3 (3 d) cows were fed ad libitum to recover from FR. To evaluate whole-body nutrient partitioning, epinephrine challenges (EPI; 1.4 µg/kg BW) were performed on d 3 of P1, d 5 of P2, d 3 of P3. Data were analyzed using PROC MIXED of SAS. There were no treatment differences in DMI (27.7 kg/d) and milk yield (21.8 kg/d) during P1. DMI decreased during P2 and subsequently all cows similarly decreased milk yield ($P < 0.05$; 38% by d 5). Overall milk yield during P3 was not different during P2. There was an interaction ($P < 0.05$) on basal NEFA during FR as it acutely increased more in THIN cows (280 vs. 196%), but basal NEFA remained elevated in FAT cows while it slightly decreased as P2 progressed in THIN cows. NEFA acutely and similarly returned to basal levels in P3. Circulating insulin uniformly decreased during FR ($P < 0.05$; 89%). In P3 insulin acutely increased in FAT versus THIN cows (24.5- vs. 12.8-fold; $P < 0.01$) on d 1. Blood glucagon did not differ by period but was overall increased in THIN versus FAT cows (39%; $P < 0.05$). Blood BHB did not increase during P2 in either treatment but did similarly increase during P3 (19%; $P < 0.01$). In response to EPI, the glucose area under the curve (AUC) tended ($P = 0.10$) to decrease during P2 in THIN cows, but did not change in FAT cows. The EPI-induced NEFA AUC did not differ by BCS, but was increased (2.1-fold) during FR and it returned to basal levels in P3 ($P < 0.01$). Overweight cows do not appear to mobilize more adipose tissue during FR than THIN cows. Reasons why periparturient FAT cows are less successful are not likely related to energetics.

Key Words: fat, off-feed, lipid mobilization

1603 Methodology for breath profiling in dairy cows: A novel approach for metabolic assessment.

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Frequent eructation in ruminant animals results in a blend of exhaled ruminal and breath volatile organic compounds (VOC). This physiological distinction might limit the applicability of breathomics in describing the metabolic phenotype of cows. The primary focus of this study was to differentiate the origin of exhaled VOC, with the aim to utilize breathomics for the assessment of dairy cow metabolism. Eighteen multiparous lactating Holstein cows (203 ± 60 DIM; 32.7 ± 4.95 kg/d milk yield) were enrolled in the study. Cows were fed a similar diet. Exhaled VOC in breath (Br; blood-borne metabolites) and exhalome (Ex; a mixture of ruminal eructation and Br) were sampled separately using a head chamber (GreenFeed System®; GF) 8 × to represent every 3 h of a day. Methane (CH_4), originating solely from ruminal fermentation, was used as the marker to differentiate breath from eructation events. Using a previously established method by our laboratory, GF real-time readings were used to collect eructation CH_4 peak events as Ex samples. A threshold of <100 ppm CH_4 was set to sample Br. Both samples were analyzed using secondary electrospray ionization-high resolution mass spectrometry. Data were analyzed using mixed model with a random effect of cow and origin × time of day interactions. Logarithmic transformation on Br over Ex mean ratio and fold change (FC) were calculated to differentiate the origin of metabolites. Putative metabolite annotation and most enriched pathways were conducted using MetaboAnalyst. Identified as Ex ($P < 0.01$), ruminal VFA acetate, propionate, and butyrate were differentiated with FC >0.5. Energy metabolites such as pyruvate and TCA cycle precursors citrate, cis-aconitate, succinate, fumarate, and malate were detected in Br ($P < 0.01$), and differentiated with FC >0.2. While variations in VFA concentrations over time were linked to feeding events, TCA cycle precursors seemed to vary relative to milking time. Our study not only established a method to sample Br versus Ex but also provided compelling evidence of the potential to implement breathomics as a noninvasive tool for conducting metabolic assessment in ruminant research.

Key Words: dairy cow, metabolomics, breath

1604 Links between volatile organic compounds from exhaled breath and metabolites of blood and ruminal fluid of lactating dairy cows.

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Exhaled breath sampling is a low-invasive intervention and an interesting matrix as it contains information about the organism's metabolism in form of volatile organic compounds (VOC). However, the exact physiological information of exhaled VOC about a cow's metabolism is mainly unknown. Therefore, we performed an untargeted analysis of exhaled VOC and investigated their relationship with blood and ruminal fluid metabolites. Thirty-two lactating healthy Holstein cows (83.27 ± 31.40 d in milk) were fed either hay (n = 16) or silage (n = 16); all cows were supplemented with concentrate feed. The study lasted 12 weeks with sampling of exhaled breath, blood, and ruminal fluid every 4 weeks. The VOC were analyzed by dynamic headspace vacuum-in-tube extraction GC-MS. Concentrations of glucose, nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHB), cholesterol, and urea in blood were analyzed by a BT1500 autoanalyzer, short-chain fatty acids (SCFA) by HPLC, and ammonia in ruminal fluid colorimetrically. Pearson correlations between exhaled VOC and blood and ruminal fluid metabolites were calculated separately for each sampling time point. Significant ($P < 0.05$) diet-independent correlations with $r > 0.35$ were selected and the implicated VOC were identified (NIST 17, Gaithersburg, MD). Five exhaled VOC (4-hydroxy-4-methylpentan-2-one, tetradecanal, 3-penten-2-one, γ-hydroxybutyrate, dodecanal) correlated with blood metabolites (BHB and cholesterol, BHB and NEFA, NEFA, glucose and urea, glucose, respectively) and 6 (2-ethylhexanal, 4-hydroxy-4-methylpentan-2-one, γ-hydroxybutyrate, 3-penten-2-one, 2-ethylhexan-1-ol, p-cymene) correlated with ruminal fluid metabolites (acetate and butyrate, acetate, acetate and valerate, butyrate, butyrate, ammonia, respectively). The correlated VOC are e.g., part of the fatty alcohol cycle and the GABA shunt. This suggests that exhaled breath of cows contains information about the fatty acid, amino acid, and therefore energy metabolism. The usability of exhaled breath sampling as low-invasive intervention to assess other metabolic states needs further investigation.

Key Words: noninvasive, metabolism, volatilome

1605 Plasma biomarkers and pathways associated with heat stress resistance in lactating dairy cows.

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This study aimed to identify plasma biomarkers and pathways associated with heat stress resistance, to facilitate the future selection of heat-resistant (HR) dairy cows. Sixteen multiparous lactating Holstein dairy cows were enrolled and fed TMR once daily. The study consisted of a 10-d baseline and 49-d heat stress phase (by depriving the evaporative cooling). Vaginal temperature (VT) was recorded every 5 min from d −1 to 3. The 4 cows with the smallest increase in average VT (1.4%–2.7%) during d 1–3 of heat stress, compared with the baseline (d-1), were selected as HR, while the remaining 12 cows served as the control. In the first week of heat stress, the HR cows selected had a lower reduction in milk yield (8% vs. 15%) and DMI (3 vs. 15%) compared with the control. Blood samples were collected on d −3 (baseline) and d 1 (heat stress) before feeding. Metabolomic analysis of plasma was performed using liquid chromatography-mass spectrophotometry. Data were analyzed using MetaboAnalyst 6.0: KEGG pathway enrichment analysis was performed, and biomarkers were selected based on highest area under the curve (AUC) values, indicating strong predictive accuracy. On d 1 of heat stress, 2 pathways (alanine, aspartate, and glutamate metabolism, and lipoic acid metabolism) were enriched in the HR cows. Before heat stress (d −3), 3 metabolic pathways were enriched ($P \leq 0.05$) in HR cows, namely butanoate metabolism, cysteine and methionine metabolism, and lysine degradation pathways. The most promising biomarkers for predicting heat stress resistance in dairy cows we identified are 5-hydroxyindole-3-acetic acid, 4-hydroxyglutamate semialdehyde, and glutamyl-lysine. Glutamyl-lysine and 4-hydroxyglutamate semialdehyde are precursors of gluconeogenesis and may support energy production, contributing to HR by maintaining metabolic function. 5-Hydroxyindole-3-acetic acid was reported to be a breakdown product of serotonin, which was reported to be reduced during chronic heat stress. Future studies should validate the biomarkers we identified for selecting heat-resistant dairy cows.

Key Words: lactating cow, heat stress, plasma metabolome

1606 Effects of heat stress and dietary protein on milk fatty acid profile and blood metabolites in dairy cows.

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Milk fatty acid (FA) concentrations can serve as indicators of metabolic disorders and could potentially help identify risk of heat stress in dairy cows. Twelve Holstein cows (42.2 ± 10.6 kg milk/d; 83 ± 28 DIM) were used to evaluate the effect of balancing for Lys, Met, and His supply on milk FA profile and blood metabolites under heat stress. Using 4 replicated 3 × 3 Latin squares (14-d periods), 3 treatments were evaluated: (1) heat stress (HS; 17% CP, THI = 82; supply d 7–14 [g/d]: 107 Lys, 34 Met, 37 His, and 1,715 MP); (2) pair feeding in thermoneutrality (PF; 17% CP; THI = 64; same diet and AA and MP supply as HS); or (3) heat stress with a diet balanced to supply 178, 64, and 43 g/d of Lys, Met, His, and 1,730 MP, respectively; AA supply was adjusted based on DMI reductions (HS+AA; 17% CP; THI = 82; observed DMI −32%). Milk and blood samples from d 0, 7, and 14 were analyzed for FA profile and for plasma metabolites, respectively. Multivariate analyses were performed on normalized (sum method), generalized log-transformed, and Pareto-scaled data. Treatment differences were evaluated using a mixed model including cow and period as random effects, and treatment, time and their interactions as fixed effects. Discriminant analysis revealed distinct clustering of the HS and PF, where low plasma free FA and low milk fat >16C concentrations were predictive of heat stress (VIP scores >1). Iso 15:0 concentrations in milk fat were lower in HS+AA and PF relative to HS ($P < 0.03$), whereas total odd-chain FA and the odd- to branched-chain ratio were lower in HS relative to PF ($P < 0.05$). Plasma concentrations of 3-methyl His increased by over 50% in both heat-stressed groups compared with PF ($P < 0.001$). Additionally, a positive correlation was observed between plasma 3-methyl His levels and plasma lipopolysaccharide-binding protein ($r = 0.46$; $P < 0.001$). Changes in milk FA profile could be used to identify cows undergoing metabolic stress from hyperthermia; however, supply of individual AA may alter cow responses to heat stress and therefore impact predictions.

Key Words: amino acid, heat stress, fatty acid

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1607 Heat stress in late gestation disrupts placental gene expression in the dairy cow. L. T. Casarotto*[1], J. J. Bromfield[1], H. N. Jones[2], P. Chavatte-Palmer[3], and G. E. Dahl[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Florida College of Medicine, Gainesville, FL,* [3]*Université Paris-Saclay, Jouy-en-Josas, France.*

Late-gestation heat stress in dairy cows can adversely affect dam productivity and offspring health. This may be due to placental dysfunction caused by heat stress, which reduces cotyledon number and increases cotyledon size, leading to decreased calf weight at birth and increased morbidity and mortality of offspring. To better understand the impact of heat stress during late gestation we employed global transcriptomics of placental cotyledon tissue. Multiparous pregnant Holstein cows were randomly enrolled to receive either cooling (CL) with access to shade, forced ventilation, and water soakers; or heat stress (HT) with access to shade only for 54 ± 5 d before parturition. After parturition (4.0 ± 0.1 h), a subset of placentas (n = 5/treatment) were collected. Total RNA was extracted from cotyledon tissue and subjected to RNA sequencing using the Illumina NovaSeq platform. Differential expression analysis of 2 groups was performed using the DESeq2 R package. A total of 289 differentially expressed genes were identified in HT versus CL, with 179 transcripts increased and 110 transcripts decreased. Heat stress increased the expression of *NPSR1* (a vasopressin/oxytocin receptor gene) by 22.7 $\log_2$ fold change, while expression of *SPATC1L* (associated with actin polymerization/depolymerization) was decreased by 5.5 $\log_2$ fold change. Other transcripts impacted by HT related to placental regulation and function increased including *IL6*, *IGFPB2*, *MMP12*, *PAPPA2*, and *PAG14*. Also, the expression of *PGF*, *HSPB8*, *HBA/HBB*, and *SLC7A10* were decreased by HT. Dysregulated pathways in the HT placenta were associated with gas and oxygen transport, nutrient transport and uptake, inflammatory response, and cortisol biosynthesis. Our research demonstrates that late-gestation heat stress causes significant transcript alterations in the placenta. Notably, these alterations signify the initial steps toward comprehending the long-term consequences reported for several years on fetal development. This highlights the grave concern regarding the detrimental effects that late-gestation heat stress can have on fetal and neonatal health.

Key Words: hyperthermia, placenta, fetal development

1608 Supplementation of rumen-protected methionine to late-gestation heat-stressed cows: Impact on calf amino acid metabolism and liver gene expression. G. A. Larsen*[1], B. D. Davidson[1], A. Hoppmann[1], E. M. Tabor[1], D. N. Sherlock[2], D. Luchini[2], S. I. Arriola Apelo[1], and J. Laporta[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Adisseo USA Inc., Alpharetta, GA.*

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We evaluated postnatal growth, plasma AA profile, and liver transcriptomics of Holstein heifers gestated by cows under heat stress (HS, induced via electric-heat blankets) with or without rumen-protected Met supplementation (M, 2.6% CP) relative to heifers born to cows fed a control diet (C, 2.2% CP) under thermoneutrality (TN). Heifers were exposed to maternal treatments *in utero* (IU) during the last 4 weeks of gestation (IU-CTN, IU-CHS, IU-MHS, n = 17/group). Throughout the preweaning period, BW, hip height (HH), and withers height (WH) were measured weekly (0–49 d), DMI daily (28–49 d), and plasma AA quantified at d 1 and 49 (n = 10/group). A liver biopsy was performed on d 1 (n = 6/group). Growth and AA data were analyzed using PROC MIXED in SAS, gene expression data were analyzed using edgeR, and pathway enrichment was predicted with IPA Software. Pre-determined orthogonal contrasts (C_1: IU-CTN vs. IU-CHS+IU-MHS, and C_2: IU-CHS vs. IU-MHS) were used. Significance is set at $P < 0.05$. Growth and DMI were similar across the preweaning phase (all $P > 0.20$), except for d 49, where IU-CHS heifers were lighter than IU-CTN ($P_{Interaction} = 0.04$). Prenatal HS reduced circulating essential AA (EAA) on d 1 ($P_{C1} < 0.03$), particularly Met, Lys, and His ($P_{C1} < 0.03$), while nonessential AA (NEAA) and branched-chain AA (BCA) were not impacted ($P_{C1} > 0.14$). At d 49, there was no difference in EAA, NEAA, or BCA between groups ($P_{\mathrm{C1/C2}} > 0.23$). There were 286 differentially expressed genes in the liver (DEG, q-value ≤ 0.2; fold change ≥ 2) for C_1, whereas for $C_{2,}$ there was one DEG (SYT6, upregulated in IU-MHS) involved in calcium-dependent exocytosis. Enriched pathways for C_1 included growth hormone, IGF-1 signaling, ER signaling, and mitochondrial biogenesis. In summary, in utero heat stress reduced circulating concentrations of limiting AA and modulated hepatic expression of genes involved in biological functions important to neonatal heifers. Herein, maternal Met supplementation does not overcome these impairments, underlining the importance of HS abatement to unlock known benefits of maternal Met supplementation on the progeny.

Key Words: methionine, in utero, heat stress

1609 Navigating antimicrobial stewardship in dairy farming: A cross-contextual study of dairy veterinarians' perspectives in Alberta, Canada, and Northern Italy. J. A. Ida*[1,2], E. R. Russell[3], S. Allodi[4], P. Buniotto[4,5], P. Moroni[2,6], D. V. Nydam[2], and H. W. Barkema[1], [1]*Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada,* [2]*Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY,* [3]*Animal Welfare Program, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC, Canada,* [4]*Dairy veterinarian, Northern Italy,* [5]*Soluzione Latte, SRL, Italy,* [6]*Department of Veterinary Medicine and Animal Sciences–DIVAS, University of Milan, Milan, Italy.*

Antimicrobial use (AMU) in animals is an important component in emerging antimicrobial resistance (AMR). Veterinarians serve as the link connecting the pharmaceutical industry with the client-patient, making it critical to assess the context and perspectives of these actors. This study aims to provide a cross-contextual understanding of veterinarians' self-perceived role in antimicrobial stewardship (AMS) and their perspectives on current policy in dairy farming in South-Central Alberta, Canada, and Northern Italy. Ethnographic data, consisting of semi-structured interviews with 20 local dairy veterinarians (9 in Canada, 11 in Italy) in the context of immersive fieldwork, were collected. Interviews were audio-recorded and transcribed, and thematic analysis was used to analyze the interviews. This study revealed that dairy veterinarians in Alberta recognize a global responsibility for industry-wide AMS, but do not always see themselves as effectively promoting appropriate stewardship practices. Trade-offs in the use of critically important antimicrobials, and the role of the milk supply management system in antimicrobial prescribing practices were acknowledged by veterinarians in Alberta. The results demonstrate variability in defining AMS success, highlight concerns of blame placed on the dairy industry, and reservations about adopting a "European" model. Dairy veterinarians in Italy identified a variety of stewardship considerations. The focus was on upstream drivers of AMU, emphasizing the function of prevention, animal welfare, and nutrition in AMS. Veterinarians perceived themselves as mediators between governmental policy, individual farm contexts and constraints, and farmers' belief systems. Landscape variability, farm heterogeneity, and feasibility concerns regarding new policy implementation timelines were identified as key considerations. The results of the study underscore the importance of understanding the specific context and the overarching role of veterinarians before implementation of new antimicrobial policies and interventions.

Key Words: antimicrobial stewardship, dairy veterinarian, cross-contextual

1610 Assessing solutions for resilient dairy farming in Europe.

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The European dairy sector faces challenges within the context of climate change, market trends and societal demands, under a set of EU regulations concerning nutrient management, Nature 2000 areas, and recently, the Green Deal. Project Resilience for Dairy (R4D) deals with these challenges (https://resilience4dairy.eu) by developing a Thematic Network on 100 resilient dairy farms in 15 EU countries. Our research is based on expert opinions by assessment of solutions (practices, techniques) that contribute to future resilient farming. First, farmer needs were collected during 2021 by a survey filled in by 535 stakeholders (70% farmers and 30% stakeholders) in the participating countries. Next, outcomes were discussed in national dairy AKIS (NDA) focus groups and in European expert workshops, resulting in the formulation of 190 possible solutions in the resilience areas: socioeconomics, technical efficiency, and environment/animal welfare/health. The solutions were evaluated using a developed assessment scheme (Likert scale: scores 1 to 5). Sixty-six expert assessors selected by the participating organizations performed 3,329 assessments with a focus on resilience (no. of assessments per solution from 15 to 30). Additionally, the readiness and attractiveness of the solutions were evaluated in NDA focus groups in 2022/23. Each NDA group was asked to select the 20 solutions with highest attractiveness, resilience, and readiness. There was variation in focus over Europe, especially in the NDA groups, and also, expert and stakeholder opinions appeared to differ. Technical efficiency remains a leading strategy at farm level. Improved labor organization, strategic hoof trimming, early detection of diseases, hospital pens, technology for heat stress management, various aspects of calf rearing, and agroforestry were much mentioned topics of interest, while communication with society, renewable energy production, and use of farm economic indicators, among others, were specifically emphasized by farmers and stakeholders. It appeared that the choices of solutions were somewhat affected by facilitation, choice of farmer groups, and societal unrest during the study period.

Key Words: solution, resilient, dairy farming in Europe

1611 Effect of dry period length on energy balance and performance of dairy cows.

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The hypothesis was that short dry period improves energy balance (EB) and uterine health with little or no effect on production. Objectives were to investigate the effect of dry period length (DPL) on pre- and postpartum dry matter intake (DMI), EB, uterine health, and milk yield in cows. Holstein cows were blocked on 207 ± 3 d of gestation by parity and previous lactation milk yield, and within block, they were randomly assigned to either a traditional dry period of 60 d (TDP; n = 14) or a short dry period of 30 d (SDP; n = 14). Dailly DMI and weekly body weight (BW) and body condition scores (BCS) were measured from −21 to 105 d postpartum. Milk yield was recorded for 105 d postpartum. Milk components were assessed on a weekly basis. Vaginal discharge (VD) collected on 7, 14, and 21 d postpartum scored on a 1 to 5 scale

Table 1 (Abstr. 1611). Effect of short dry period on energy balance and performance in dairy cows

	Dry period length, d			*P*-value		
Item	30	60	SEM	DPL	Week	DPL × Week
Prepartum						
DMI, kg/d	12.7	11.0	0.8	0.20	0.02	0.01
BCS	2.99	3.03	0.12	0.81	0.04	0.07
BW, kg	604	643	20	0.19	<0.01	0.08
EB, Mcal	3.48	0.91	1.38	0.24	0.01	0.01
Postpartum						
DMI, kg/d	14.8	13.8	1.2	0.56	0.01	0.89
BCS	2.72	2.76	0.06	0.67	<0.01	0.19
BW, kg	521	557	16	0.14	<0.01	0.95
EB, Mcal	0.39	−1.59	1.15	0.26	0.02	0.77
Yield, kg/d						
Milk	18.5	21.8	1.3	0.09	<0.01	0.39
Fat	0.59	0.75	0.04	0.03	<0.01	0.90
Protein	0.64	0.76	0.05	0.08	<0.01	0.51
VD score	3.27	3.23	0.21	0.90	0.28	0.01
Metritis, %	6.1	20.8	7.0	0.16	—	—

based on color, consistency, and smell. Cows with fetid reddish brown watery discharge were declared with metritis. Data were analyzed with mixed-effects model in SAS. Results are shown in Table 1. The statistical model included fixed effects of DPL, week, and interaction between DPL and week, and random effect of block and cow nested within DPL. Prepartum DMI and EB were greater in SDP than TDP in last 2 weeks of gestation, but postpartum DMI and EB did not differ between treatments. Incidence of metritis did not differ between treatments, but VD score was higher on d 7 but lower on d 21 postpartum in SDP cows than TDP. Yields of milk, and components were less in SDP than TDP cows. A short dry period improved prepartum EB and uterine health and reduced milk yield by 3.3 kg/d in dairy cows.

Key Words: short dry period, energy balance, dairy cow

1612 Milk component-, farm-, and industry-level factors associated with elevated free fatty acids in bulk tank milk on conventional dairy farms. H. M. Woodhouse*, T. J. DeVries, S. J. LeBlanc, K. J. Hand, and D. F. Kelton, *University of Guelph, Guelph, ON, Canada.*

Elevated concentrations of free fatty acids (FFA) in bulk tank milk indicate excessive milk fat breakdown. Levels ≥1.20 mmol FFA/100 g of milk fat compromise milk taste and function and pose a quality threat to the dairy industry. Previous research found average FFA levels on Ontario, Canada, conventional (CON) dairy farms to be highest in the fall months, and this study aimed to identify the factors associated with elevated FFA. Using a subset of Ontario CON dairy farms, average monthly milk components, the presence of incentive days (a feature of the Canadian quota system that allows producers to ship more milk when the demand exists), and milking system data between August 2018 and December 2022 were considered in a mixed multivariable logistic regression model of monthly mean FFA ≥1.2 mmol FFA/100 g fat with herd as a random effect. The final dataset included 3,009 farms and 145,897 observations over 53 mo. The average monthly bulk tank FFA was 0.83 mmol/100 g of fat (SD = 0.32, range 0.04 to 7.68) and 7% (n = 10,016) of samples were elevated. Fifty-three percent of samples came from tiestall farms (n = 1,578), 29% from parlor-milked herds (n = 878), and 18% from farms with automated milking systems (AMS) (n = 553). The odds of elevated FFA were higher for tiestall farms (OR = 28.8, 95% CI [14.2, 58.4] $P < 0.001$) and AMS farms (OR = 11.2, 95% CI [5.3, 23.8], $P < 0.001$) when compared with parlor farms. Tiestall farms had higher odds of elevated FFA than AMS farms (OR = 2.6, 95% CI [1.9, 3.4], $P < 0.001$). The odds of elevated FFA increased with more incentive days (OR = 1.14 per extra incentive day, 95% CI [1.1, 1.2], $P < 0.001$) and increased milk fat (OR = 1.3 per weighted volume percentage of milk fat increase, 95% CI [1.1, 1.6], $P = 0.007$). A decrease in milk protein was associated with increased odds of elevated FFA (OR = 35.6 per weighted volume percentage of milk protein decrease, 95% CI [25.3, 50.1], $P < 0.001$). These results suggest that other milk components, production incentive days, and nonparlor milking systems are associated with elevated FFA on CON Ontario dairy farms.

Key Words: milk fat, lipolysis, milk quality

1613 Exploring new predictive approaches to selective dry-off criteria using machine learning and genomic data. T. J. Almand*[1], H. F. Monteiro[1], S. Mukku[2], D. Bruno[3], E. Okello[1], and F. S. Lima[1], [1]*Department of Population Health and Reproduction, University of California–Davis, Davis, CA,* [2]*Amazon Sciences, Banglore, India,* [3]*Cooperative Extension, University of California Agriculture and Natural Resources, Davis, CA.*

Selective dry cow therapy (SDCT) has emerged as an effective strategy to reduce antimicrobial use by treating only cows at risk of developing mastitis. However, other potential predictors, such as genomic prediction of mastitis (GMAS), have not been explored. The aim of the study was to evaluate the predictive performance of a model based on SCC <200,000 at dry-off incorporating genomic data and machine learning algorithms (ML). The hypothesis is that a model with multiple predictive variables more accurately detects cows that develop mastitis than a model based solely on SCC <200,000 at dry-off. The data used for analysis were derived from a dairy located in the north Central Valley, California. Data were collected from DairyComp305 and analyzed using JMP Pro 17 and the sci-kit-learn library in Python. A total of 3,454 cows with at least 150 d in milk and 2 lactations were selected. All cows in the study had production, health, and genomic data available from previous and current lactation and received antibiotics and teat sealant at dry-off. A one-way analysis of variance (ANOVA) was performed to evaluate the incidence of mastitis in the variables of interest. A random forest ML was applied with data from the previous lactation to predict the future incidence of mastitis. There were 2 models, one with only log-transformed SCC and a more complex one with production and genomic traits. Results were validated using a 10-fold cross-validation technique. The full model (all variables included) was more accurate at identifying cows who are less likely to develop mastitis at dry-off. Sensitivity and specificity for the model with SCC >200,000 were 6.1% and 94.7%, respectively, while sensitivity and specificity for the complex model were 35.0% and 97.9%, respectively. The accuracy and F1-Score for the SCC <200,000 model was 75.5% and 9.8%, respectively. Meanwhile, for the full model, the accuracy and F1-Score were 84.3% and 49.1%, respectively. Our data demonstrate that the ML model, with genomic data and cow factors associated with mastitis, enhances the predictability of mastitis and potentially improves the SDCT odds for success.

Key Words: mastitis, dry cow, antibiotics use

1614 Investigating the impact of diet and parity on the rumen microbiome: Insights from shotgun metagenomics. I. C. Hermisdorff*[1], H. F. Monteiro[2], B. Mion[1], J. F. W. Spricigo[1], M. G. S. Santos[1], R. E. Jahnel[1], F. S. Lima[2], E. S. Ribeiro[1], C. F. Baes[1,3], and F. S. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA,* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

The rumen microbiome of dairy cows is affected by parity and diet, especially dietary changes from pre- to postpartum periods. Despite this understanding, there is limited knowledge regarding the interaction between these factors and changes in the microbiome. Here, we investigated such interactions using a deep shotgun metagenomics approach. The rumen content of 72 dairy cows was collected at days −21, 23, and 65 relative to parturition. Total DNA was extracted from rumen samples and shotgun metagenomics was performed at 50 million reads. Sequences were quality controlled, host reads were removed, and taxonomy classification was performed using the *sourmash* software against the GenBank database, controlling for false discovery rate (FDR). Final microbial counts were normalized using a centered-log ratio. Microbial abundances were analyzed via a mixed model including the fixed effects of collection day, parity, 2 types of trace mineral (TM) supplementation (organic vs. inorganic) and all possible interactions using the *ASReml* software. A correction for potential host genetic influence was performed by including the random sire effect in the model. A post hoc correction for FDR (FDR < 0.05) was applied. A total of

3,616 microbial species were identified, out of which 116 species had significant day by parity interaction with no significant triple interaction with TM source. Most of the interaction effects were related to changes in primiparous cows from pre- to postpartum samples, especially for the abundance of *Oceanobacillus* sp. and *Butyrivibrio* sp. *XB500–5*, which increase and decrease by the onset of the lactation, respectively. Parity was the only significant factor for 27 species, with *Christensenella* sp. *UBA4379*, *Lachnospira* sp. *UBA4285*, and *Methanobrevibacter ruminantium* abundance notably lower in primiparous cows. Postpartum diet familiarity may help explain a more stable microbiome in multiparous versus primiparous cows. This sheds light on rumen microbiome changes during the challenging transition from prepartum to lactation, which seems to be parity dependent and more pronounced in primiparous cows.

Key Words: transition, primiparous, rumen fermentation

1655 Evaluating the effect of grazing intensity on biodiversity in US ecoregions: Indicators for life cycle assessment.

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The expansion of pastures across the United States has accompanied the intensification of agricultural production systems. This strategy is considered an alternative to mitigate the adverse effects on natural ecosystems, including resource overconsumption and biodiversity loss, resulting from land use changes. Our objective was to assess how biodiversity of United States has been affected by pasture lands for cattle grazing, and to compare how effects differed between intensive and extensive (light) grazing systems. We assessed the potential biodiversity damage caused by areas occupied by cattle pastures (intensive and light managements) in 23 American states, divided into 5 ecoregions. The indicators used are those from the Countryside SAR model, adapted by Chaudhary and Books (2018), for 5 taxa: mammals, birds, amphibians, reptiles, and plants. The functional unit and area, in m2, to raise 1 animal. Our findings indicate that intensive rotational grazing leads to greater production efficiencies and lower biodiversity effects compared with extensive grazing. This outcome is attributed to the optimization of yield and the alignment of forage quantity and quality with livestock nutritional requirements. Among the evaluated taxa, plant species were the most adversely affected by pasture establishment, except in the Southern Plains ecoregion, where the effect was similar across both grazing intensities. Following plants, the effect on biodiversity was ranked in descending order for amphibians, birds, mammals, and reptiles. The ecoregions experiencing the highest biodiversity loss were identified as follows: Southern Plains and Delta and Southeast States, each with a loss of 3.07E-07 species; Mountain and Pacific, with 9.22E-07 species; Northern Plains and Western Corn Belt, with 5.53E-07 species; and Appalachian, with 4.61E-07 species. The study concludes that intensive grazing systems can mitigate biodiversity loss effects if managed to reduce animal occupancy rates without compromising production levels.

Key Words: land use, pasture, habitat

1616 Evaluation of milk energy predictions based on inclusion of energy content of individual milk components and their constituents.

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The aim of this project was to evaluate the performance of selected milk energy equations (Eqn) from Hall (2022): (EqS: Sjaunja et al., 1990, Eqn. 12; EqTR: Tyrell and Reid, 1965, Eqn. 3; EqNA: NASEM, 2021, Eqn. 21) compared with a new prediction equation (EqNew) based on our analysis of gross energy (GE; bomb calorimeter) of pure milk components: lactose monohydrate, fat, and true protein (TP). Milk samples (n = 120) from 49 lactating Holstein, Swiss Brown, and Original Swiss (Braunvieh) cows were collected from 4 a.m. milkings. The samples were analyzed for lactose monohydrate, fat, crude protein (CP), and milk urea N (MUN) concentrations. Milk TP % was calculated as milk N − MUN × 6.38. The average milk yield (±SD) was 13.1 (±3.44) kg/milking. Lactose, fat, and TP concentrations (%) averaged 4.46 (±0.270), 4.49 (±0.700), and 3.67 (±0.330). The GE of the milk samples was 779 (±71.4) kcal/kg, whereas the GE of pure milk components were 3.65, 9.10, and 4.88 kcal/g for lactose monohydrate, fat, and TP, respectively, and were used as coefficients in EqNew. The predicted milk energy based on EqNew was 757 (±69.4) kcal/kg, which gave a ratio of 0.97 for predicted versus measured milk GE, and were 811 (±71.2), 803 (±70.2), and 815 (±72.3) kcal/kg, based on EqS, EqTR, and EqNA, respectively. These gave a ratio of 1.04, 1.03, and 1.05 between predicted and measured milk GE. Pearson correlation was similar among the Eqn (r = 0.87), and confidence interval varied from 0.798–0.981 for EqTR to 0.771–0.952 for EqNA. The lowest root means squared prediction error as a percentage of observed mean varied from 2.89 for EqNew to 4.86% for EqNA. Lastly, corrected milk yield based on EqNew was 12.8 (±3.17), being 1.1 to 1.6 kg less than estimated with the selected Eqn. Overall, the differences were small between Eqn, where EqS, EqTR, and EqNA resulted in a slight overestimation of milk GE (by 3.2% to 4.9%), and estimates from EqNew were slightly more accurate but underestimated (by 2.9%). Further analysis will investigate the contribution of individual milk FA and casein and whey fractions to milk GE.

Key Words: milk energy prediction, milk component, dairy cow

1617 Milk infrared predictions of blood biomarkers for metabolic stress response: Assessing sources of variation in different dairy systems and breeds.

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This study aimed to explore the associations between milk infrared predictions of blood biomarkers for metabolic stress (hepatic enzymes, ceruloplasmin [CP], total reactive oxygen metabolites [ROMt], and paraoxonase [PON]) and different dairy systems within Parmigiano Reggiano Consortium. All farms were surveyed and data on structures, management and feeding were collected. Blood metabolites were predicted (p) using Fourier-transform infrared spectroscopy (FTIR) applied to milk with equations developed previously ($0.48 \leq R^2 \leq 0.87$) on an independent calibration data set (1,351 cows, 5 herds). Calibration equations were then applied to a population database of 1,355,730 test-day FTIR milk spectra pertaining to 176,174 cows reared in 980 multibreed farms, collected by the Breeders Association of Emilia Romagna (Reggio Emilia, Italy) and made available by the Italian Breeders Association (Roma, Italy). Four farming systems were identified using a nonhierarchical clustering approach: traditional plain, traditional mountain, modern with TMR, and modern without TMR. All FTIR predictions of blood metabolites were analyzed using a linear mixed model that included the systematic effects of parity, DIM, year, month, farming system, breed and the random effects of herd (farming system) and cow (breed). DIM and parity were relevant sources of variation for the traits of concern ($P < 0.01$), with pPON showing opposite direction over lactation compared with pCP and pROMt, thus confirming their contrasting effects on inflammation/oxidation status. Concerning DIM and breed interaction, Brown Swiss cows displayed reduced adaptability to hepatic overload throughout lactation compared with all other breeds. Traditional mountain farms exhibited higher levels of pPON compared with modern farms ($P < 0.01$). About breed effect, Holstein cows displayed the lowest levels of pPON (<96 U/mL) and the highest of pCP (>2.29 μmol/L) and pROMt (>14.6 mgH^{2}O^2/100 mL) across all farming systems ($P < 0.01$). Our findings highlight the potential of using these predictions for monitoring purposes within milk recording schemes.

Key Words: blood metabolites, milk Fourier-transform infrared spectroscopy (FTIR), machine learning

Reproduction Symposium: Improving Pregnancy Survival in Dairy Cows—Challenges and Opportunities

1619 The dichotomy between pregnancy survival and loss: Challenging "post"-conceived notions. M. C. Wiltbank*[1], J. P. Andrade[1], V. Gomez-Leon[2], A. Garcia-Guerra[3], P. L. J. Monteiro[1,4], R. R. Domingues[3], P. M. Fricke[1], and R. Sartori[5], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Kansas State University, Manhattan, KS,* [3]*Ohio State University, Columbus, OH,* [4]*University of Florida, Gainesville, FL,* [5]*University of São Paulo, Piracicaba, SP, Brazil.*

Pregnancy loss is a prevalent and expensive problem in lactating dairy cows and in recipients of in vitro produced (IVF) embryos. Unfortunately, the causative etiology, outside of health and environmental challenges, and consistent methods to predict and prevent pregnancy loss still mostly elude reproductive physiologists. Previous research reported ~50% of embryos were degenerate by d 7 after AI. In contrast, 2 recent studies that used the DoubleOvsynch protocol reported >80% high-quality embryos on d 7 (85.1%) suggesting early embryonic problems can be reduced by preovulatory environment. This talk will focus on recent results using early pregnancy diagnosis by measurement of interferon-stimulated genes (ISGs) in cervical epithelial cells and circulating pregnancy-associated glycoproteins (PAGs). Based on ISGs on d 17 after AI, P/AI in lactating dairy cows is over 80% (81.4%) with only 12.8% loss during the interferon-tau (IFNT) period (d 17–22). Nonetheless, soon after embryonic attachment (initial PAG increase) is the greatest period of pregnancy loss (d 22–32) in both lactating cows (24.6%) and heifer recipients of IVF embryos (25.0%). From d 32 to d 62, there is another 9–12% pregnancy loss. In one study the pregnancy loss was reduced in lactating cows by producing a new accessory corpus luteum (CL) by GnRH treatment on d 5 but only if the new accessory CL was ipsilateral to the pregnancy. In heifers, treatment with intravaginal P4 has been successful in decreasing pregnancy loss in some, but not all, experiments apparently by improving P/ET in embryos with suboptimal placentation, based on PAGs. Recent experiments indicate that about half of pregnancy losses are initiated by CL regression in the presence of a functional embryo. Our initial results indicate that these potential pregnancy losses can be avoided by providing elevated P4 from an intravaginal implant and inducing a new accessory CL to maintain the pregnancy. Thus, these results offer optimism that an understanding of the underlying causes of pregnancy loss can allow development of physiologically-rational methods for overcoming this reproductive problem.

Key Words: pregnancy, progesterone, interferon-tau

1620 Developing and testing a novel model for conceptus attachment to gain a greater understanding of pregnancy loss in lactating dairy cows. J. R. Pursley*, A. Santos, and T. Minela, *Michigan State University, East Lansing, MI.*

Reducing pregnancy losses is critical for ensuring longevity of dairy cows. This presentation will outline the creation of an innovative approach to assess the timing of conceptus attachment and discuss recent breakthroughs in identifying factors influencing this crucial stage of pregnancy. Serum samples collected daily from d 17 to 28 post-AI were used to detect the onset of pregnancy-specific protein B. Initial studies indicated that nulliparous heifers had earlier conceptus attachment compared with lactating cows and primiparous cows had earlier conceptus attachment compared with multiparous cows. Most cows have conceptus attachment on d 20 or 21 post-AI. Thus far, all studies agree that cows with conceptus attachment >21 d post-AI have greater chances for pregnancy loss compared with cows that have earlier conceptus attachment. Treating multiparous cows post-AI with human chorionic gonadotropin to increase progesterone during elongation reduced the percentage of cows with conceptus attachment and prolonged time to attachment. Serum concentrations of PSPB were consistently reduced following conceptus attachment in cows with pregnancy loss compared with cows that maintained pregnancy. This model has enhanced our understanding of the fertility potential of AI following estrus when compared with Double Ovsynch as the gold standard for dairy cow fertility. Cows that received AI, and ovulated, following estrus had decreased chances for conceptus attachment compared with Double Ovsynch. Timing of AI related to the onset of estrus determined with automated activity monitoring did not affect outcomes. Cows with a previous estrus had greater chances for conceptus attachment compared with cows without a previous estrus. Cows treated with Double Ovsynch benefitted from at least 2 previously detected estruses with an increase of ≥30% in percent of cows with conceptus attachment. This paper will discuss how gaining a greater understanding of conceptus attachment may lead to new manipulative approaches to increase fertility and longevity of lactating dairy cows.

Key Words: conceptus attachment, dairy cow, fertility

1621 Pregnancy loss in embryo transfer recipients: Risk factors and mitigation strategies. P. Baruselli*[1], B. Catussi[1], E. Gricio[1], M. Viziack[1], L. Factor[1], and C. Rodrigues[2], [1]*Department of Animal Reproduction, University of São Paulo, São Paulo, SP, Brazil,* [2]*Clinica Veterinária SAMVET, São Carlos, SP, Brazil.*

Early embryo development, implantation and pregnancy involve a complex dialog between the embryo and mother. Our research group studied the risk factors that affect pregnancy loss (PL) in Holstein cows that received embryos. During heat stress (HS), the overall conception rate (CR) decreased for Holstein cows submitted to either AI or ET (season effect). However, CR was always higher following ET compared with AI (breeding technique effect), especially during HS (interaction season × breeding technique). Moreover, PL was higher during HS than in the cooler season and for ET than for AI, with no interaction season × breeding technique. Despite greater PL, cows receiving ET had higher pregnancy rates (PR) after 60 d than AI. The embryo grade quality was linearly associated with PR. As the quality of the grade decreased, the PR per ET also decreased. However, the PL only tended ($P = 0.07$) to be influenced by embryo quality with the smallest loss for recipients receiving an embryo graded as 1 compared with grades 2 to 3. Lactating Holstein cows that received AI, in vivo ET and in vitro ET were studied to analyze gestational outcomes: gestation loss, abortion, and birth rates. ET showed higher PR, with similar PL when compared with AI. Additionally, in vivo ET showed similar PR and PL than in vitro ET. Comparing abortion rates (60 d to calving), pregnancies from in vivo ET had a higher rate (26.8%) than AI (23.5%), whereas in vitro ET (26.4%) had no difference. Holstein cows pregnant with in vivo ET had a lower birth rate of 66.1% compared with AI at 70.8%. However, in vitro ET (67.5%) did not differ significantly from in vivo ET or AI. We conducted a study to analyze the reproductive performance of lactating Holstein cows (milk yield 33.0 L) and crossbred beef cows (cross Bos indicus) after they received a Holstein in vitro ET. The birth rate was

higher for beef recipients (83.1%) than for lactating milk recipients (67.5%). To summarize, the incidence of PL is higher in ET than in AI. Various factors such as HS, embryonic quality, embryo production (in vivo or in vitro) and the recipient can impact PL.

Key Words: embryo transfer, reproductive efficiency, biotechnology

Ruminant Nutrition 7: General

1622 The impact of genomic database choice on microbiome analysis: Why should the dairy community care about it? H. F. Monteiro*, C. S. Schlesener, R. Profeta, M. Van Heule, B. C. Weimer, P. Dini, C. T. Brown, and F. S. Lima, *Department of Population Health and Reproduction, University of California, Davis, Davis, CA.*

Most of the current technologies for microbiome evaluation rely on databases for identifying microbes. Ideally, everything that is present in a sample should be in a database as well. However, the advent of shotgun metagenomics has uncovered a myriad of organisms yet to be cataloged in existing databases, presenting a significant challenge for comprehensive microbiome analysis. This study underscores the importance of database choice in rumen microbiome research, comparing the widely utilized RefSeq against the more extensive GenBank database. It was hypothesized that database comprehensiveness can affect study outcomes, especially from the rumen microbiome that is largely uncharacterized. A total of 24 rumen samples from dairy cows were sequenced using ultra-deep shotgun metatranscriptomics. Quality-controlled sequences were used in *kraken2* and *Bracken* for RefSeq with software default settings. GenBank was used in s*ourmash* to handle the comprehensiveness of the database. The total species identified across samples within the method was similar (Chao1 index $r = 0.98$, $P < 0.001$). However, there were differences in identifying microbes depending on the method (10,987 species in RefSeq vs. 17,137 in GenBank, $P < 0.001$). The shifted microbial composition affected their abundance distribution, as seen by the lower similarity in the Inverse Simpson ($r = 0.51$, $P = 0.01$) and Shannon ($r = 0.81$, $P < 0.001$) indexes. This discrepancy highlights GenBank's superior ability to better represent the broader array of organisms found in the rumen, often overlooked by less comprehensive databases. For instance, while RefSeq predominantly identified *Cryptosporidium parvum* (49.3% relative abundance), GenBank revealed *Entodinium caudatum* (29.4% relative abundance) as the most abundant protozoa. Concerning, those reads of *E. caudatum* and other protozoa were potentially shifted to their least common ancestor *C. parvum,* ahighly pathogenic protozoa. In summary, despite RefSeq being widely used in microbiome analysis, it was mostly curated for medical studies, and care should be given when attempting to use it in ruminant studies.

Key Words: nutrition, protozoa, metatranscriptomics

1623 Predicting the dry matter intake of dairy cow pens with machine learning. P. M. Lucey* and H. A. Rossow, *Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California Davis, Tulare, CA.*

The quantity of feed required by a pen of dairy cows is the sum of the DMI of each member, and its distribution is influenced by the pen demographics. This is not directly measured and DMI requires variables such as BCS and milk energy for an individual's prediction. Precision feeding is not achieved if an "average" cow's DMI is extrapolated to the pen. Instead, the distribution shape of DMI could be predicted from the pen demographics to improve diet formulation inputs. Our objective was to train a machine learning model that classifies the distribution shape of DMI, then predicts a DMI value for a pen. A set of 5,203,685 pens ranging from 25 to 250 cows in size were sorted together from 9,029 weekly observations of 609 cows. Milk yield, milk fat, milk protein, parity, week in milk, body weight, and BCS were described by pen in percentiles, mean, median, skew and kurtosis. The DMI was fit to either the Beta or Generalized Normal distribution by the Kolmogorov-Smirnov goodness of fit test. These data were split 75:25 for training and testing, then trained to 3 machine learning algorithms: Decision Tree, Random Forest, Extreme Gradient Boosting (XGB). The XGB model was chosen by 5-fold cross-validation of accuracy, precision, recall and F1-score. All descriptive statistics were initially included in model training; only milk yield, parity and week in milk were retained in the final model. Hyperparameters were tuned by Randomized Grid Search. Two models were constructed: a classification model to predict the distribution shape (Beta or Generalized Normal), a regression model to predict the parameter values of this shape. Both models were assessed with the testing data. The classification had an accuracy of 71%, and the regression model had a RMSE of 11.31 for Beta and 0.55 for Generalized Normal. These models were used to calculate a DMI for each pen with its predicted distribution. The R^2 of DMI prediction for all pens in the testing data was 0.99. The percentage error of Beta and Generalized Normal pens was 2.45% and 0.99% respectively. This model results in an accurate prediction of DMI using only milk yield, parity and week in milk of the pen.

Key Words: machine learning, precision feeding, dry matter intake

1624 The metabolic fate of deuterium-labeled choline in gestating and lactating Holstein dairy cows. T. France*, A. Ortega, A. Richards, M. Farricker, and J. McFadden, *Cornell University, Ithaca, NY.*

Our objective was to evaluate changes in choline metabolism including bacterial degradation to trimethylamine (TMA), methyl group transfer, and bioavailability in pregnant and lactating cows. Six multiparous, rumen-cannulated Holstein cows were enrolled in a study design where they were abomasally infused 18 g/d choline chloride (CC; dissolved in water; 170 mL/h) for a 5-d experimental period at −3 wk from expected due date and again at 2 wk postpartum. The stable-isotope trimethyl-deuterium (d9)-CC replaced 20% of daily unlabeled CC to study methyl group transfer. Cows were fed a Met deficient diet. Plasma, milk, liver, urine, and fecal samples collected on d 4 and 5 of each period (gestation and lactation) were analyzed for choline and choline metabolite concentrations and isotope enrichment using mass spectrometry. Data were analyzed using a general linear model including the fixed effect of period. Plasma betaine ($P < 0.01$), liver choline and betaine concentrations were lower during lactation ($P < 0.01$). Lactating cows had greater circulating enrichment of d3-choline, d3-betaine, d9-betaine, and d9-phosphatidylcholine (PC; $P \leq 0.05$), and greater liver enrichment of d3-glycerophosphorylcholine and d9-sphingomyelin ($P \leq 0.02$). Liver d3-PC was detected but not modified by period. Greater urinary and fecal losses of d3- and d9-TMA, and d3- and d9-TMA *N*-oxide (TMAO) were observed during pregnancy ($P \leq 0.05$). Total choline metabolites detected in urine from infused CC were greater during pregnancy, relative to lactation (17 vs. 9%; $P < 0.01$). Total fecal choline metabolites from infused CC were greater during lactation, relative to pregnancy (5 vs. 1%; $P = 0.03$). We estimate that 25% of infused CC was used for milk choline metabolite enrichment. We conclude that choline is a methyl donor in the cow (e.g., detected d3-choline), and choline is utilized by the cytidine diphosphate-choline and phosphatidylethanolamine *N*-methyl transferase pathways. We also provide evidence for endogenous recycling of absorbed choline via bile (i.e., presence of d3-TMA).

Degradation of choline to TMA and TMAO must be considered when evaluating choline bioavailability.

Key Words: choline, methyl donor, transition period

1625 Effects of live yeast *Saccharomyces cerevisiae boulardii* CNCM I-1079 supplementation during the late dry and early postpartum periods on feed intake and production responses.

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The study aimed to evaluate the effects of *Saccharomyces cerevisiae boulardii* CNCM I-1079 supplementation (SCB; 1.0×10^{10} cfu/cow per d) from d −28 to 70 postpartum on dry matter intake (DMI) and production responses of Holstein cows. Eighty-three multiparous (n = 52; MP) and primiparous (n = 31; PP) cows were blocked by previous 305-d milk yield, parity, body condition score (BCS), and body weight (BW), and randomly assigned to either prepartum and postpartum control (CON; n = 43) or SCB (n = 40) dietary treatments. The BCS and BW prepartum were measured weekly. Individual DMI using automatic feeders, milk yield, and postpartum BW were recorded daily. Milk samples were collected weekly and analyzed for fat, protein, and lactose. Data were analyzed using the Mixed procedure of SAS (v.9.4). Prepartum and postpartum DMI, BW, and milk yield were similar ($P > 0.17$) among dietary treatments. However, 3.5% fat-corrected milk (FCM; 43.3 vs. 40.7 ± 0.97 kg/d) and energy-corrected milk (ECM; 42.8 *vs*. 40.2 ± 0.95 kg/d) were greater ($P = 0.05$) in SCB than CON cows. The MP cows had greater ($P < 0.01$) milk yield (41.8 vs. 33.5 ± 1.12 kg/d), FCM (49.4 vs. 34.6 ± 1.07 kg/d), and ECM (48.5 vs. 34.5 ± 1.05 kg/d) than PP. Milk fat content was greater in SCB than CON (4.44 *vs*. 4.24 ± 0.07%; $P = 0.05$) and in MP than PP cows (4.39 vs. 4.29 ± 0.08%; $P < 0.01$). Similarly, milk fat yield was greater in SCB than CON (1.65 vs. 1.53 ± 0.04 kg/d; $P = 0.05$) and in MP than PP cows (1.90 vs. 1.29 ± 0.05 kg/d; $P < 0.01$). Content and yield of milk protein and lactose were similar ($P > 0.45$) among dietary treatments. The MP cows had greater yields of milk protein (1.35 vs. 0.98 ± 0.03 kg/d; $P < 0.01$) and lactose (2.01 *vs*. 1.45 ± 0.05 kg/d; $P < 0.01$) than PP. Prepartum BCS was greater in CON than SCB cows (3.39 vs. 3.32 ± 0.02; $P = 0.01$), but no difference was observed postpartum ($P = 0.11$). Overall, supplementation of SCB during the dry period and early lactation in dairy cows increased FCM, ECM, and milk fat content and yield and did not impact DMI.

Key Words: probiotic, live yeast, transition cow

1626 A comprehensive analysis of organic trace minerals effects on the rumen microbiome of dairy cows from transition period to peak of lactation.

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Our objective was to evaluate the effects of complete replacement of supplementary inorganic salts of trace minerals (STM; Co, Cu, Mn, and Zn sulfates and Na selenite) by organic trace minerals (OTM; Co, Cu, Mn, and Zn proteinates and selenized yeast) on the rumen microbiome. Heifers and cows (n = 72) were enrolled at 45 ± 3 d before expected calving, blocked by parity and BCS, and allocated randomly to STM or OTM supplementation of a basal diet. Treatments continued until 156 DIM; diets were formulated to meet 100% of trace minerals recommended levels. Individual automatic feeding gates were used to assign treatments. Rumen contents were collected on −21, 23, and 65 ± 3 DIM using an oro-ruminal sampling device and immediately frozen in liquid N_2. Rumen samples DNA were sequenced using deep shotgun metagenomics. Host reads were removed, and microbes classified using *sourmash* software against the GenBank database, controlling for false discovery rate (FDR). Microbial normalized abundances (n = 3,616 species) were analyzed in a mixed model that included the fixed effects of sampling day, parity, treatment, and their interactions using the *ASReml* software. The random effect of sire was included to correct for a potential host effect. Post-hoc FDR adjustment was performed. The largest effects were observed in a 3-way interaction ($P < 0.05$) between treatment, parity, and day for 365 microbes, mostly due to a decreased abundance of microbes under the OTM treatment in primiparous cows on 23 and 65 DIM. The OTM reduced several *Bifidobacterium* and *Prevotellaceae sp.* that thrives in starch and high concentrate diets and produce acetic acid, while increased some fibrolytic *Fibrobacter, Ruminococcus,* and *Prevotella sp.* Other 88 species were affected by treatment and parity, while 84 by treatment and day, with OTM mostly negatively affecting them in primiparous cows and 23 DIM. Lastly, 145 microbes were affected by treatment regardless of day and parity. In summary, complete replacement of supplementary STM by OTM largely affected the rumen microbiome, especially in primiparous cows early postpartum.

Key Words: transition, primiparous, fermentation

1651 Effect of palmitic acid and a blend of palmitic and stearic acid on milk production of Holstein cows when fed in a low-fat diet.

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Fatty acid supplements are fed to dairy cows to increase dietary energy intake and milk and milk fat yield, but differential responses have been observed between supplements that contain mostly palmitic acid (PA) and those that are blends of PA and stearic acid (SA). The effect of supplement FA profile on yield of milk and milk components and milk FA profile was investigated in a replicated 3 × 3 Latin square design with 21-d periods using 12 post-peak multiparous Holstein cows (DIM = 186 ± 57, MY = 42.1 ± 4.41 kg/d; mean ± SD). Cows were fed a basal diet that was low in total FA (~2.5%) balanced to ~32% NDF for a 14-d pretrial period. Treatments were the low fat basal diet (CON) and the basal diet supplemented with 2% of a fat supplement high in PA (~85% 16:0) or a blend of PA and SA (BLEND). Oleic acid was ∼10% FA in both supplements. Data were analyzed with JMP Pro 17, and the model included random effects of cow, period, and the fixed effects treatment and the pretrial observation as a covariate. Supplementing PA and BLEND increased DMI by 1.5 and 1.4 kg/d compared with CON, respectively ($P < 0.01$). Milk yield was not modified by treatment (35.7, 37.3 and 36.7 kg for CON, BLEND, and PA, respectively; $P = 0.26$). Milk fat concentration was increased 0.43 percentage units by PA compared with CON ($P = 0.02$), and milk fat yield was increased 130 g/d in BLEND and 180 g/d in PA compared with CON ($P = 0.01$) but did not differ between PA and BLEND ($P = 0.29$). The concentration of de novo FA in milk fat (<16C) was reduced by both FA supplements ($P = 0.04$), although yield of de novo FA was not changed. The concentration of 16C FA was increased 4.6 percentage units and 110 g/d by PA ($P < 0.01$), but BLEND was not different from CON. The BLEND did increase the concentration and yield of preformed FA (3.2 percentage units and 81 g/d, $P = 0.01$). Milk protein concentration and yield were not changed by treatment. Overall, the PA supplement increased milk fat content and yield largely through increased synthesis of 16C FA, whereas the blend of PA and SA increased milk fat yield by increasing preformed FA in milk fat.

Key Words: milk fat, fat supplement, dairy nutrition

1628 Withdrawn.

1629 Effect of administering a *Saccharomyces cerevisiae* fermentation product from dry-off through 90 days of lactation on the metabolic adaptations of dairy cows with previous lactation low or high somatic cell counts.

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A group of 60 Holstein cows was enrolled at −20 d from dry-off (DFD) and classified based on the daily SCC during the week before from an automated milking system. The separation threshold for low (L, n = 46) and high (H, n = 14) classifications were 100 K/mL for primiparous and 200 K/mL for multiparous cows. Cows were then assigned to 2 homogeneous groups and received diets supplemented with 19 g/d of a *Saccharomyces cerevisiae* fermentation product (SCFP; NutriTek, Diamond V) or without supplementation (CTR) until 90 d from calving (DFC). Disease incidence, milk yield and composition, plasma metabolic profile and whole blood count were monitored. Data were analyzed utilizing ANOVA and mixed models for repeated measures. During dry period, SCFP cows had greater plasma thiol and albumin compared with CTR ($P = 0.04$ and 0.05). SCFP-L cows had greater plasma protein and globulin than CTR-L ($P = 0.05$). SCFP-H cows had heightened hematocrit, reduced plasma globulin and haptoglobin, and higher albumin, albumin-to-globulin ratio (AGR) and thiol than CTR-H ($P = 0.05$). SCFP-H cows had greater concentration of leukocytes and lymphocytes and lower plasma protein and ceruloplasmin at 2 DFD, lower reactive oxygen species to ferric ion reducing antioxidant power ratio at 12 DFD, and greater concentration of lymphocytes and plasma GGT at −7 DFC than CTR-H ($P = 0.05$). After calving, SCFP cows had a lower incidence of mastitis and higher butterfat ($P < 0.01$ and 0.03), greater plasma haptoglobin and glutamate oxaloacetate transaminase (GOT) and reduced Mg compared with CTR ($P < 0.01$). SCFP cows had lower SCC between 1 and 7 DFC and a greater ECM between 41 and 60 DFC compared with CTR ($P = 0.05$). SCFP-H cows had lower SCC between 1 and 7 DFC, greater hemoglobin and plasma GOT than CTR-H ($P < 0.05$). Ameliorated immune system functions due to SCFP lowered the chronic SCC increase in SCFP-H cows and prevented the onset of new intramammary infections across both L and H SCC groups, improving the cow's productive performances.

Key Words: inflammation, postbiotic, mammary gland

1630 Comparative availability of five magnesium sources in nonpregnant nonlactating Holstein cows.

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This study assessed the relative availability of 4 commonly used inorganic Mg sources and a novel proprietary mineral blend (PMB; Multesium). The study was a duplicated 6 × 6 Latin Square, with 12 nonlactating, non-pregnant Holstein dairy cows assigned to square based on BW and parity. Cows were fed 90% of their voluntary DMI (0.21% Mg). Each experimental period included 7 d. On d 2, urinary catheters were fitted for total urine collection at 12 h intervals for 48 h, starting one day before treatment administration (d 3). On d 4, 30 g of Mg were administered as boluses with gelatin capsules: negative control (one empty capsule), PMB [a blend of $Mg(CO_3)_2$, $Mg(OH)_4$, and MgO], magnesium oxide (MgO), magnesium sulfate ($MgSO_4$), calcium magnesium hydroxide [$CaMg(OH)_4$], calcium magnesium carbonate [$CaMg(CO_3)_2$]. Blood samples were collected at 0, 1, 2, 3, 12, and 24 h after treatment administration on d 4. Urine and blood samples were analyzed for Mg and Ca concentration. Statistical analyses were conducted with PROC GLIMMIX including treatment, time, period, square, treatment × time, treatment × period, and time × period as fixed effects, and cow nested within square as a random effect in the model. A similar increase in urinary Mg excretion was observed for 4 of the Mg sources studied relative to control [PMB, MgO, $CaMg(OH)_4$, and $MgSO_4$] representing an increase of at least 40.8%. However, $CaMg(CO_3)_2$ supplementation caused a non-significant increase above control of 8.1%. There were no significant changes in blood Mg concentration in supplemented cows. However, a significant treatment × time effect was observed. Calcium rich sources [PBM, $CaMg(OH)_4$, $CaMg(CO_3)_2$] had lower blood Mg concentrations at 12 or 24 h after treatment. Based on urinary Mg excretion 24 h after treatment, PMB had a similar relative availability to 3 commonly used inorganic Mg sources, and it was superior to the $CaMg(CO_3)_2$ commercial source included in our study. However, considering the possible variation in availability within inorganic sources of the same type, further studies are advised to design future nutritional guidelines.

Key Words: magnesium availability, dairy cow, Multesium

1631 Does isoacid supplementation at varying dietary forage levels affect nutrient digestibility and milk fatty acid profile in dairy cows?

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Our objective was to examine the effects of isoacid (ISO) supplementation on the performance, nutrient digestibility, and milk fatty acid (FA) profile of lactating cows under 2 forage NDF levels (FL). In a randomized complete block design trial (10 wk), 64 Holsteins (662 ± 71 kg BW, 119 ± 51 DIM) were blocked by parity, DIM, and prior milk yield (MY) or genetic merit; and randomly assigned to 1 of 4 diets (n = 16). Diets were arranged as 2 × 2 factorial with 2 FL comprising 18 (LF) and 23% forage NDF (HF), without or with ISO supplementation (40 g/d per cow). All diets had 16.5% CP, 28% NDF, and 1.72 Mcal/kg DM of NEL. Feed intake and MY were recorded daily. Nutrient digestibility for each cow was calculated using indigestible NDF as a marker with 8 time points fecal grab samples (4-h interval between samples). Individual cow milk samples composited over a 10-wk were analyzed using gas chromatography for FA profile. The statistical model included FL, ISO, and FL × ISO as fixed effects and block as a random effect (lme4 in R). The ISO did not affect DM intake ($P = 0.13$), whereas LF had greater DMI than HF diets (27.8 vs 26.0 kg/d; SEM = 0.81; $P < 0.01$).

However, ISO increased MY by 7% in HF (34.7 vs 37.2 kg/d; SEM = 1.68; $P < 0.01$) but not in the LF diet, suggesting FL × ISO interaction ($P = 0.04$). As expected, LF diets had greater DM, OM, and NDF digestibility than HF diets ($P < 0.01$), while CP digestibility was similar ($P = 0.73$) between the FL. In contrast, ISO increased DM (74.8 vs. 67.7%, SEM = 0.92, $P < 0.01$) and OM digestibility (75.7% vs. 69.1%, SEM = 0.87, $P < 0.01$) and tended to increase NDF ($P = 0.07$) and CP ($P = 0.06$) digestibility only in HF diets (i.e., FL × ISO interaction). Compared with LF, HF diets had greater C18:1 n-9 ($P = 0.03$) but lower C20:1 n-11 milk FA concentration. Besides, ISO increased *trans*-10 C18:1 FA ($P = 0.04$), odd-chain FA C15:0 ($P = 0.03$), and C17:0 ($P = 0.05$), irrespective of FL. Overall, ISO improved MY and nutrient digestibility in HF diet, while also increased certain *trans* and odd-chain FA (C15:0 and C17:0) in both FL.

Key Words: odd-chain fatty acid, milk production, isoacid

1632 Assessing serum fatty acids associations with genomic prediction for feed saved in dairy cows. W. M. Coelho Jr.*[1], H. F. Monteiro[1], R. S. Bisinotto[2], J. E. P. Santos[3], F. Schenkel[4], E. S. Ribeiro[4], F. Penagaricano[5], B. C. Weimer[1], P. Vahmani[6], and F. S. Lima[1], *[1]Department of Population Health and Reproduction, University of California, Davis, Davis, CA, [2]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, [3]Department of Animal Sciences, University of Florida, Gainesville, FL, [4]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [5]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, [6]Department of Animal Sciences, University of California, Davis, Davis, CA.*

Improving the sustainability of dairy farms continues to pose a significant challenge, with the optimization of feed and milk production efficiency in dairy cows at its forefront. Prior research conducted by our team suggests that serum fatty acids (FA) hold promise in enhancing the accuracy and predictability of residual feed intake (RFI). This discovery prompted our investigation into the association between feed saved (FS) and serum fatty acids. We hypothesized that genomic prediction for FS correlates with serum fatty acids. Our objective was to investigate if the genomic prediction of FS correlates with serum FA. A total of 495 lactating dairy cows from the United States and Canada had DMI measured daily between 50 ± 13 to 99 ± 13 d in milk. During this period, production parameters were measured. A total of 24 cows from the extremes of RFI (12 high vs. 12 low) were selected to assess serum fatty acids using a 2-step assay. The fatty acid methyl ester was done using solid phase extraction and quantified using chromatographic peak area and internal standard-based calculations. Cows were categorized as positive (pFS, n = 10) and negative (nFS, n = 14) for genomic prediction of feed saved to compare the group's FA profiles further. Statistical analyses were performed using the MIXED procedure of SAS (SAS/STAT version 9.4; SAS Institute Inc., Cary, NC), and Spearman rank correlation coefficients (r_s) were generated. From 32 different categories of FA, 8 FA correlated with the genomic prediction of FS: C14:0 ($P < 0.001$, $r_s = 0.69$), C15:0 ($P = 0.05$, $r_s = -0.41$), C17:0 anteiso+t13–16:1 ($P = 0.02$, $r_s = -0.48$), C17:0+C13–16:1 ($P = 0.03$, $r_s = -0.45$), C18:0 ($P = 0.02$, $r_s = -0.49$), C18:3n-3 ($P = 0.02$, $r_s = -0.47$), C20:5n-3 ($P = 0.03$, $r_s = -0.45$) and n3-PUFA ($P = 0.04$, $r_s = -0.42$). Cows in pFS had higher concentrations of C18:2n-6 and n6-PUFA than cows in nFS. Cows in nFS had higher concentrations of C15:0, cis9–16:1, C17:0+c13–16:1, and C18:0 than cows in the pFS group. These differences and correlations between FA concentration and genomic prediction of FS suggest that serum FA needs to be further investigated as a biomarker for the FS trait and feed efficiency in dairy.

Key Words: feed efficiency, ruminant nutrition, fatty acids

Ruminant Nutrition Symposium: Advances in Analytical Procedures Affecting Dairy Nutritionists

1634 Forage and feed laboratory focus to support industry advancement. R. Ward*, *Cumberland Valley Analytical Services, Waynesboro, PA.*

Forage and feed characterization underlies the ability to monitor nutrient variation at the farm, provide inputs for ration modeling, understand nutritional contribution to cow response, and execute animal and agronomic research. While different applications of data have different underlying requirements, data users all benefit from standardization and definition of methods by which data are produced and reported. Forage laboratories in the US over the last 40 years have provided huge competitive advantage to the feed industry through fast execution of analysis at very affordable prices. Quality of execution has improved significantly over this time. To support the needs of technology development and AI applications, defined method protocols, sampling variation, lab variation, and sample relationship to population data become more important considerations. This underlying information supporting the generated lab data should be readily available to the user of the data. Systems required to improve lab performance and support provision of a significant amount of additional information require investment by labs that the industry must be willing to support. In this talk we will consider (1) current status of defining lab methods, (2) defining primary method lab methods for applicability, precision and accuracy, (3) a brief discussion of sampling theory and sample presentation for given assay needs, (4) consideration of the sensitivity of the assay, (5) qualification of NIR equation models and predictions, (6) reporting and data access considerations, (7) cooperative relationships with private and academic institutions to develop new methodologies, and (8) current investigations of technologies to improve feedstuff characterization.

Key Words: forage analysis feed analysis, NIR, laboratory methods

1635 Reliability of near-infrared reflectance spectroscopy (NIRS) to accurately predict nutrient composition of forage and feed ingredients. D. W. McIntosh*[1], B. J. Husmoen[2], E. A. Eckelkamp[1], P. Berzaghi[3], and G. E. Bates[1], [1]*The University of Tennessee, Knoxville, TN,* [2]*NIRS Consortium, Berea, KY,* [3]*University of Padova, Padova, Veneto, Italy.*

Forage and feed ingredients contain valuable nutritive content information that often-expensive wet chemistry testing is not conducted, or the distrust in near infrared spectroscopy (NIRS) predictions hinder the researcher or producer submitting samples to laboratories for analysis. Overcoming this hurdle is a must to understand the accuracy and reliability of NIRS. With an overwhelming reminder that calibrations have improved immensely while being developed with accuracy and the validation necessary to change this. Identification of best practices from sampling, identification, particle size, and dry matter at scanning all affect the final analyte data in both the primary (wet chemistry) and secondary method NIRS. Additionally, reporting these data correctly and validating for scientific journal publication is becoming increasingly important. To enhance trust with researchers, nutritionists, and producers, laboratories are encouraged to communicate more fully and openly about the procedures used to generate accurate NIRS predictions. This discussion will provide an in-depth identification of an ideal dry matter (DM) range when scanning samples with essential techniques implemented across laboratories. Techniques for presentation of samples to instrumentation should be appropriate to achieve the best possible prediction, whether a one-time sample or an entire project. The impact of this is significant along with the other factors affecting NIRS predictions in bench-top or portable instrumentation. Improving results for research and provided to laboratory clientele will be discussed with specific examples for across laboratory comparison, calculations using analyte predictions, and proper validation techniques.

Key Words: forage, feed, NIRS

1636 Impact of alternative methods on measurement of NDF compared to the official method. M. B. Hall*, *U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Neutral detergent fiber (NDF) is an important metric of fiber in dairy cattle nutrition that is used in diet formulation and feed valuation. An empirical method, NDF is defined by the procedure used to measure it. AOAC Official Method 2002.04 (AOAC) is the current definitive method for NDF treated with amylase (aNDF). It is performed on dried samples ground through the 1-mm screen of a cutting mill with refluxing and then filtration through coarse porosity Gooch crucibles without or with a filtration aid. Method variants with modifications such as grinding materials through the 1-mm screen of an abrasion mill or using different filtration or extraction systems must give analytical values equivalent to AOAC or they do not measure aNDF. Comparisons must rigorously adhere to recommended procedures. Extraction of samples and recovery of the residue on filters may be affected by method variants. Grinding though a 1-mm screen with an abrasion mill versus a cutting mill gives a finer particle size, possibly allowing greater extraction or escape. Extracted particles may pass through filters when liquid carrying the particles flows through; this is affected by a filter's particle retention value (PR). Performance of filters and filtration methods must be assessed within the procedure used. Simultaneous extraction and filtration used in filter bag systems gives particles the time of extraction and rinsing to interact with the filter, whereas decanting, rinsing, and soaks give less time in other filtration systems. In a limited study, filter bags gave values closer to AOAC when bags with finer PR were used. Crucibles that are plugged or degraded can also deviate from AOAC. In a limited study, best agreement between AOAC and aNDF method variants was achieved with the 1-mm screen cutting mill grind. The 1-mm abrasion mill grind produced more aNDF% results that were lower than AOAC but with fewer differences when filter PR was smaller. The use of filters that retain finer particles could be explored to improve comparability of NDF method variants and grinds with AOAC. Further evaluation with an expanded set of materials is needed.

Key Words: fiber, feed analysis, methods

1637 Impact of laboratory feed digestibility methods on nutrition models. M. Van Amburgh* and A. LaPierre, *Cornell University, Ithaca, NY.*

Accurate predictions of nutrient flows in formulation models rely on a comprehensive description of all ingredients within a diet. To best describe this behavior requires a series of chemical and enzymatic assays to estimate characteristics, including solubility and insolubility, ruminal digestibility, rates of degradation, and intestinal digestibility. When characterizing carbohydrate digestibility, the detergent chemistry methods of Van Soest and Mertens serve as the foundation for most models, describing fiber and cell soluble fractions for most cellulosic-based feeds. These fractions behave uniformly when analyzed chemically; however, several methods exist to assess fiber degradability, each leading to different interpretations of an ingredient's digestibility. A time series analysis to measure fiber disappearance is equally imperative, as pools of potentially degradable fiber can be fractionated into different pools, each with its rates of degradability. The use of these fractions has improved predictions in energy supply, microbial yield, DMI, and the role of particle size in rumen fill. Laboratory analyses for sugar, starch, and soluble fiber also exist and are routinely performed, yet the interpretability and use of these data can be biased based on the allocation of results and the user-preferred modeling approach. The correct appropriation of nutrients into digestible fractions becomes important because although the predicted total-tract digestibility for the sum of sugars and soluble fiber are different, the location of degradation, either ruminally or postruminally, will alter the predicted microbial supply and alter rumen outflow of protein. The measurement of the digestibility of sugar and soluble fiber are not routinely conducted due to lower uniformity of recovery. Finally, methods for intestinal protein digestibility are important and independent of the estimation of RUP. Acid detergent insoluble protein has been a relevant measure of undigestible protein in forages and requires no estimation of RUP. Similar approaches are needed for non-cellulosic, proteinaceous feeds for ruminants and should be independent of any estimation of RUP.

Key Words: digestibility, modeling, feeds

ADSA-GSD Competition: Dairy Foods Poster Semifinalists

2000 Shelf-stability of microwave-vacuum dried cream powder. B. Gong*[1], J. Dumpler[2], A. Kalenak[1], G. Sacks[1], and C. I. Moraru[1], *[1]Cornell University, Ithaca, NY, [2]ETH Zürich, Zürich, Switzerland.*

Microwave vacuum drying (MVD) can be used to dehydrate cream and preserve its functionality. The objective of this study was to assess the shelf-stability of MVD cream powder, achieved by characterizing the quality and functionality of MVD cream powder over a 7-mo storage period at ambient and elevated temperatures. Pasteurized, homogenized heavy cream was dehydrated by MVD at 2.5 ± 0.3 kPa and 1.3 W/g for 20 min, 1.0 W/g for 10 min, and 0.67 W/g for 30 min using an Enwave MVD unit. The MVD cream powder was packaged into metalized barrier pouches in 60-g portions and flushed with nitrogen gas. Samples were stored at 23°C and 40°C for 7 mo. A positive control sample was packaged in a transparent plastic pouch, without a nitrogen gas flush, and stored under natural light. Water activity, color, headspace volatiles, reconstituted cream particle size, and whipped cream firmness were evaluated monthly, and peroxide value was measured initially and at the end of the storage period. Analyses were performed in triplicate and data were analyzed statistically for differences between means. Water activity remained below 0.6 for all samples, which prevented microbial growth. Powder samples stored at 40°C became significantly more yellow (b*) after 6 mo ($P < 0.01$), indicating Maillard browning, but no significant changes in color were observed after reconstitution. N-alkanals increased significantly ($P < 0.01$) after 4 mo in samples stored at 40°C, indicating lipid oxidation. Particle size and whipped cream firmness remained stable throughout the storage period at both storage temperatures, and the peroxide value remained below the detection limit. The positive control sample had a peroxide value 27.0 mEq/kg fat and was much whiter than the other samples, due to light oxidation, and 30 to 100 times higher concentration of n-alkanals than the other samples, indicating high rancidity; after reconstitution, the positive control completely lost its whippability. This study shows that the quality of MVD cream powder is stable for at least 7 mo if stored in the absence of light and oxygen, which recommends MVD as a unique method of preserving cream solids without refrigeration.

Key Words: microwave vacuum drying (MVD), cream, shelf-stability

2001 Impact of proteolytic and crosslinking enzymes on the functional properties of micellar casein concentrate. K. Dubey*, M. Akter, and P. Salunke, *South Dakota State University, Brookings, SD.*

Micellar casein concentrate (MCC) is derived from the microfiltration of skim milk. Due to its diverse functional properties, it plays an important role in dairy and food products. This study aims to further modify these functional properties by hydrolyzing and then crosslinking enzymes. Pasteurized skim milk was subjected to microfiltration to produce MCC retentate. The MCC retentate was standardized to 12% protein concentration. The MCC retentate was heated to 55°C, hydrolyzed by adding alcalase enzyme for 15 min to form MCC hydrolysate (MCC-H), and the enzyme was deactivated at 90°C for 10 min. This hydrolysate was crosslinked using transglutaminase enzyme with an activity of 110 U/g used at the rate of 2U/g protein at 55°C for 15 min to produce a crosslinked MCC hydrolysate (MCC-HC) sample. The enzyme was deactivated by heating the sample to 72°C for 10 min. The control sample (MCC-C) was subjected to all heat treatments without any enzyme. Care was taken not to coagulate the retentate. The control and treatment samples were subjected to analysis for particle size, foaming ability, emulsifying ability, heat stability, and rheology. The experiment was replicated thrice, and data were analyzed using Costat software. The heat stability varied significantly ($P < 0.05$) among the samples, with the MCC-C sample at 878.1 s showing the highest heat stability and the MCC-HC sample showing the lowest heat stability at 307.7 s. In rheological tests, sample MCC-HC showed lower viscosity than the other 2 treatments which were close. The samples' G′ and G″ values increased as the shear rate increased; however, MCC-HC showed more variation at higher shear rates. The foam overrun of MCC-H was the highest, followed by MCC-HC, and the control sample had the lowest foam overrun; however, the foam stability was highest in the control sample but was not significantly different from other treatment samples. In other tests, the differences between the samples were too small to be significant. In conclusion, the combination of proteolytic and crosslinking enzymes can tailor certain functional properties.

Key Words: micellar casein concentrate (MCC), enzymes, functional properties

2002 The role of animal welfare perception and consumer trust in the dairy industry. M. E. Watson*[1], D. Rovai[1], D. M. Barbano[2], and M. A. Drake[1], *[1]North Carolina State University, Raleigh NC, [2]Cornell University, Ithaca, NY.*

Consumer definitions of sustainable, organic, local and healthy all include an aspect of animal welfare. The underlying emotional connotations of animal welfare can inform food-choice decisions. This study identified consumer implicit and explicit attitudes toward dairy cattle animal welfare practices and trust in the dairy industry. In-depth interviews (n = 50) were conducted to map consumer knowledge, concerns, and emotions as they related to dairy cattle animal welfare. Subsequently, dairy and non-dairy consumers completed 2 online surveys. Survey 1 (n = 300) used MaxDiff and Projective Mapping exercises to quantify ethical opinions and knowledge of dairy husbandry practices, whereas Survey 2 (n = 325) focused on attitudes, trust, and implicit bias through implicit association tests (IAT), check-all-that-apply (CATA), and attitudinal Likert questions. Credibility of dairy farming statements counter to consumer assumptions and the social narrative were also assessed. Qualitative beliefs and attitudes from interviews were leveraged to construct consumer personas. Univariate and multivariate statistics were used to evaluate survey data. Consumer knowledge of dairy husbandry practices was limited, and unfamiliarity drove negative assumptions. A small percentage (15%) of consumers reported trust in the dairy industry. Lack of knowledge, skepticism and mistrust as they pertained to animal welfare were reasons cited. Top consumer physical welfare concerns centered around confinement, pasture access, grass vs grain feed, and antibiotic/hormone use ($P < 0.05$). Additionally, the anthropomorphism of dairy cattle elicited strong negative emotions toward cow/calf separation. Consumers intrinsically stereotyped dairy farms as either corporate/large or local/small, and local/small farms were assumed to adhere to a higher level of animal welfare practices ($P < 0.05$). Targeted consumer education to address animal welfare in the dairy industry can provide a more accurate map of societal expectations for the dairy industry to redirect negative assumptions toward more positive associations and increased trust.

Key Words: animal welfare, consumers, consumer trust

2003 Impact of homogenization on microfiltration of whole milk.

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Microfiltration (MF) of whole milk (WM) can improve the quality of dairy products such as cheese and yogurt by separation of milk components ranging from pathogens to casein micelles. However, the fat in the milk can clog the membrane pores and cause early fouling which leads to processing inefficiency. The homogenization can reduce the size of the fat uniformly. Hence the study was designed to assess the differences in processing characteristics between homogenized and unhomogenized whole milk using MF under identical conditions. The trials were conducted using two 0.1-μm flat sheet polyvinylidene difluoride (PVDF) MF membranes at 19°C using pasteurized homogenized WM (WM-H) and unhomogenized WM (WM-UH). The flux rate was calculated to measure the process efficiency throughout the process. The initial, 2×, 3×, final, retentate, and permeate samples were analyzed for proximate composition, particle size, and minerals. The trials were replicated 3 times and data were analyzed using Costat. The flux rate of MF in WM-UH rapidly decreased throughout the trials due to clogging of the MF membrane surfaces. The WM-UH milk could be run optimally up to 2× only even though WM-H was run till 3× concentration. Hence all the comparisons were made at 2× concentration. The fat loss in WM-UH permeate was 0.70% ± 0.53% which was almost double that of the WM-H sample. At 2× concentration, the WM-H retentate samples had higher values for proximate and mineral composition and lower particle size than in WM-UH retentate however, the differences were non-significant ($P < 0.05$). In WM-H milk could be concentrated up to 3× without encountering fouling. This study concludes that WM-UH can be concentrated up to 2× using MF, while WM-H can be concentrated up to 3× and retentate can be used in yogurt, cheese, or other dairy products.

Key Words: whole milk, homogenized, microfiltration

2004 Whey protein combinations largely shift individual whey proteins' melting temperatures.

C. C. Jennings*, J. C. Devenport, and J. D. Kenealey, *Brigham Young University, Provo, UT.*

Whey proteins are increasingly used for their unique health properties in supplements and foods. This study identifies how whey proteins (β-lactoglobulin [β-LG], α-lactalbumin [α-LA], immunoglobulin-G [IgG], serum albumin [SA], lactoferrin [LF] and lactoperoxidase [LP]) thermally stabilize and destabilize each other around physiological pH (≈7). This will help supplement and food manufacturers understand how they can stabilize the unique protein they are interested in when preparing products. Further, thermal stabilization of a protein may indicate that the protein is more bioactive as it may last longer in the human body and exert more of its activities. Differential scanning calorimetry was used to measure changes in the denaturation temperature of the whey proteins when in combination with each other and data were analyzed using paired *t*-tests. We found at pH 7 that a combination of β-LG and LF significantly shifted both melting temperatures (Tm) greater than 3°C ($P \leq 0.003$). Further, apo-LF Tm significantly increased in combination with IgG and LP ($P = 0.001$, Tm increased more than 5°C), while holo-LF significantly decreased in combination with SA and LP ($P \leq 0.03$; Tm decreased more than 6°C). LP's Tm significantly shifted in combination with every protein but only shifted more than 1.5°C in combination with IgG ($P = 0.001$, Tm decreased more than 5°C). We conclude that apo-LF was generally stabilized, while holo-LF was destabilized in combination with other whey proteins; this warrants further research to see if LF supplements should include other whey proteins to enhance LF's antimicrobial functions which are largely due to its apo-form. Lastly, LP Tm significantly decreased in combination with IgG which may indicate that LP's enzyme activity is diminished in the body where IgG is present and thus LP's antimicrobial activities may be dampened. These findings show that whey proteins do affect each other thermally and encourage further research to fully understand how thermal shifts influence whey proteins' biological functions.

Key Words: melting temperature, whey proteins, calorimetry

2005 The impact of storage time and temperature on the protein fraction of UHT shelf-stable milk.

J. Pranata*[1], D. C. Cadwallader[2], D. M. Barbano[1], and M. A. Drake[2], [1]*Cornell University, Ithaca, NY,* [2]*North Carolina State University, Raleigh, NC.*

Ultrahigh temperature (UHT) pasteurization combined with aseptic filling produces a shelf stable milk. With storage, there is gradual sedimentation of protein, and after 12 mo of storage, the beverage may form a weak gel. Our objective was to determine the effect of storage temperature (4°C vs. 21°C) and storage time (12 mo) on the Kjeldahl nitrogen content and SDS-PAGE protein composition of UHT milk. Grade A milk with 1% fat was heat treated (142°C for 3 s) on 2 different dates at a commercial facility and aseptically filled into 946-mL cardboard containers, stored quiescent, and sampled each month. The milks were analyzed by Kjeldahl to determine total nitrogen (TN), noncasein nitrogen (NCN), and nonprotein nitrogen (NPN) content, and analyzed by SDS-PAGE to determine the protein degradation. Univariate statistical analyses were applied to evaluate the data. TN of the UHT milk decreased ($P < 0.05$) with time of storage at both 4°C and 21°C, whereas NCN and NPN increased ($P < 0.05$) with time of storage, especially at 21°C. Casein as a percentage of true protein and casein content of the UHT milk decreased ($P < 0.05$) with time of storage, and the concentration of casein proteolysis product remained constant ($P > 0.05$) at about 0.2%. The decrease in TN and casein content was due to aggregation and sedimentation of proteins that stuck to the bottom of the container over storage time resulting in a decrease in protein concentration of product poured from the container. The SDS-PAGE results clearly showed that protein sediment and formation of gel layer on the bottom of the container were not driven by proteolysis of caseins but were due to nonenzymatic changes in the milk. We hypothesize that there was an interaction of Maillard browning products from lactose that polymerized with milk proteins and these glycosylated proteins have more solubility at pH 4.6 (NCN) and in 12% TCA (NPN), similar to proteolysis products of casein. Therefore, controlling peak temperature and not having excessively long hold times will result in less Maillard browning products and produce shelf stable milk that may be less prone to age gelation during storage.

Key Words: UHT milk, protein, sedimentation

2006 Stabilizing high-protein dairy dispersions with CO_2 nanobubbles: Technological and rheological implications.

S. Irfan* and M. Mohan, *South Dakota State University, Brookings, SD.*

High-protein milk products are increasingly in demand for their health and wellness relevance. High-protein products require additives to prevent technological issues such as sedimentation and graininess. The effect of CO_2 nanobubbles (CO_2-NB) as a method for improving the technological properties of high-protein dairy dispersions, without resorting to the use of additives, was studied. Milk protein concentrate powders (MPC; 80% protein) were hydrated in ultrapure water (18 h at 18 ± 5°C) to prepare 5%, 7.5%, and 10% (wt/vol) protein dispersions, which were treated with CO_2-NB (NB size 148–448 nm, 40 psi and 0.75 SCHF gas flow, 10 min at 20 ± 5°C). These were compared with control dispersions (without CO_2-NB treatment) for pH, ionic calcium (calcium ion-selective electrode), colloidal stability (centrifugation method), particle size (dynamic light scattering), zeta potential (electrophoretic light scattering), and rheological properties. Data were analyzed as a 2 × 3 factorial design using ANOVA for the treatment, protein concentration, and interaction effects, with 3 treatment replicates. The CO_2-NB treatment led to a reduction in pH (6.72 ± 0.02 to 6.15 ± 0.32), which resulted in a significant increase in ionic calcium (112.5 ± 15.0 to 165.2 ± 7.9 ppm), partially destabilizing casein micelles, at all protein concentrations ($P \leq 0.05$). Compared with the control dispersions, the CO_2-NB treatment also reduced the protein aggregate sizes and viscosity, reflecting the increase of absolute zeta potential at 7.5 and 10% protein concentrations. The CO_2-NB treated dispersions also had higher colloidal stability, particularly for the 10% dispersion (92.3 ± 0.7%), compared with its control (77.1 ± 0.7%) dispersion ($P \leq 0.05$). The loss moduli viscoelastic behavior (at angular frequency 1 and 100 rad/s) showed lower values for 10% CO_2-NB treated dispersion as compared with control dispersions ($P \leq 0.05$). Overall, CO_2 nanobubbles significantly improved the technological and rheological properties of high-protein dairy dispersions, suggesting their potential for producing stable dairy beverages and with potential for tailoring for diverse applications.

Key Words: nanobubbles, dispersions, milk protein concentrate (MPC)

2007 Comparison of liquid and rehydrated milk protein concentrates for protein beverage manufacture. Y. Liu*[1], K. Ow-Wing[1], D. M. Barbano[2], and M. A. Drake[1], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Consumers want great tasting protein beverages with simple ingredient lists. The objective of this study was to determine the impact of utilizing milk protein concentrate (MPC) in liquid versus rehydrated powder form on the physical, chemical and sensory properties of 7.5% milk protein beverages. Duplicate lots of liquid or spray dried milk protein concentrate were used to manufacture 7.5% protein beverages. Dipotassium phosphate (DKP) (0.15% wt/wt) was added to increase heat stability. Protein beverages were heat processed by direct steam injection ultra high temperature (DSI-UHT: 141°C for 3 s) pasteurization or autoclave (121°C for 6 min). Subsequently, pH, color, viscosity, proximate analyses, furosine, trained panel sensory properties, and sulfur volatiles were measured. The entire experiment was replicated twice. Univariate and multivariate statistics were used to evaluate data. The DSI-UHT heat treatments (liquid or powder) with no added DKP were not heat stable and coagulated in the UHT system, while the DSI-UHT beverages with DKP were heat stable, as were both autoclave treatments with and without added DKP. Addition of DKP to the 7.5% protein beverages decreased whiteness (L-value: 81 vs. 75.1), increased the pH (6.42 vs. 6.62) and decreased sensory opacity (14.9 vs. 12.3) before heat treatment ($P < 0.05$), respectively. The impact of using fresh liquid MPC versus rehydrated MPC on the protein beverage L-values, pH (21C), and sensory opacity were distinct ($P < 0.05$) 81.0 versus 75.1, 6.50 versus 6.62, and 13.5 versus 14.1, respectively. Beverages made with liquid MPC had lower cardboard flavor than those made from rehydrated MPC (1.5 vs. 2.0; $P < 0.05$). Beverages made from liquid MPC had higher sulfur/eggy flavor and were also higher in H_2S and dimethyldisulfide ($P < 0.05$). Beverages made from rehydrated MPC had more caramelized flavor intensity and contained more carbon disulfide ($P < 0.05$). Furosine was not different among treatments ($P > 0.05$). This study provides an understanding of how ingredient format effects physical and sensory properties of milk protein beverages. These results are crucial for product development and process scale-up.

Key Words: beverage, protein, flavor

2008 Predictive modeling of rehydration behavior of high protein powder using machine learning approach. S. Roy*, J. Amamcharla, and N. Subhash, *University of Minnesota, Saint Paul, MN.*

Milk protein concentrates (MPC) are high protein dairy ingredients produced from membrane filtration of milk followed by spray drying. Rehydration of MPC involves simultaneous processes starting with penetration of water through particles followed by the transfer of water into pores, swelling of particles, and dispersion of solids. Solubility is affected by factors as protein content, processing conditions, storage time and temperature, rehydration temperature, ionic strength, etc. Predicting rehydration properties of high-protein powders is important for different product applications. Solubility index and rapid dissolution index (RDI) by focus beam reflectance measurement are predicted using dynamic surface adsorption of water. For modeling 2 batches of MPC 80, 85, and 90 are procured from a commercial manufacturer and stored at 25°C and 50°C for 2 mo. A water activity meter (HC2-AW-USB) and measurement chamber are used to accommodate water and MPC separated by a water vapor permeable mesh. The powder-to-water ratio and data acquisition settings for continuous water activity measurement are optimized. Representative sample of powder and water is placed in measurement chamber and water activity is continuously recorded for 30 min at 25°C. To assess predictive performance 2 metrics are used: Residual Prediction Deviation (RPD) and R^2. A data set comprising 212 samples is partitioned into calibration set (70%) and validation set (30%) using Caret package in R. Feature scaling and dimensionality reduction are done before modeling. Performance is assessed using multiple models, such as Gradient Boosting Regressor (GBR), Support Vector Regression, Multilayer Perceptron (MP), and Random Forest Regression. For Solubility Index, the best performance is observed using a GBR with Standard Scaling and FastICA. An R^2 value of 0.83 and RPD value of 2.00 is observed. For RDI, best performance is observed using an MP with Standard Scaling and PCA. An R^2 value of 0.87 and RPD value of 2.36 is observed. Therefore, predictive modeling using machine learning can be used for predicting rehydration behavior.

Key Words: rehydration, machine learning, predictive modeling

2009 Characterization of milk fermentation using lactic acid-producing yeasts. P. M. Gamboa-Moreno*, T. A. DeMarsh, and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Cultured dairy products represent a major segment of the dairy market; although they utilize a wide variety of microorganisms, the application of yeasts has received little attention. This work aims to characterize milk fermentation using one bioengineered commercial strain of *Saccharomyces cerevisiae* and 4 *Lachancea thermotolerans* strains, to lay the groundwork for the development of novel cultured dairy products that use lactic acid-producing yeasts instead of lactic acid bacteria, which are susceptible to bacteriophage. For each yeast strain, 3 pairs of bottles of 200 mL UHT milk received lactase (120 ALU/g lactose) and 10^8 cfu/mL of that strain; one of each pair also received 50 g/L of glucose. Each pair was then incubated for 24 h at one of 3 temperatures: 25°C, 30°C, and 35°C. Spread plate counts on potato dextrose agar with chloramphenicol (25 mg/L) executed at 0 and 24 h revealed a consistent cell population. After measuring pH every 15 min, 3-parameter exponential decay modeling and analysis of means ($\alpha = 0.05$) were used to compare the acidification rates and the pH levels at equilibrium. For most strains, the acidification rate was faster at 35°C, but pH at equilibrium was lower at 30°C. At 30°C and 35°C, glucose addition decreased acidification rates, but it led to a lower pH at equilibrium. Concentrations of lactose, glucose, galactose, ethanol, and lactic acid were determined via HPLC. At 30°C, without glucose addition, the *S. cerevisiae* strain produced the lowest ethanol concentration (0.72%), while still providing the fastest acidification rate (−0.25 pH units/h) and lowest final pH (3.31) among all strains. Additional *S. cerevisiae* fermentations with these conditions were sampled at 0.5, 1, 1.5, 2, 3, 5, and 10 h. Results indicated complete lactose hydrolysis and significant lactic acid production, with glucose but not galactose utilization. Finally, the same model predicted that the milk's concentration of ethanol would only exceed 0.5% after 12 h. Understanding these yeasts' effects on milk's physicochemical characteristics provides a solid foundation for exploring the development of novel cultured dairy products.

Key Words: milk, fermentation, yeast

2010 Characterization of a model milk protein concentrate containing pre-aggregated whey proteins.

A. Schnurr* and J. Amamcharla, *Midwest Dairy Foods Research Center, Food Science and Nutrition, University of Minnesota, St. Paul, MN.*

Milk protein concentrate (MPC) can be customized based on the end-use application. Denaturation of whey protein in MPC results in casein-whey protein interactions. Whey proteins can be selectively modified through heat induced pre-aggregation to reduce their interaction with caseins. This work aims to characterize model MPC developed using pre-aggregated whey proteins and micellar casein concentrate (MCC). Two lots of 3 treatments of MPC were produced to investigate functionality of model MPC with pre-aggregated whey proteins. mWPI was hydrated to 8% (wt/wt) protein and divided into 3 equal portions. The first batch was used as control and had a particle size of 160.9 ± 2.6 nm. The pH of the second and third portions was adjusted to 3 and 7, respectively and heated at 85°C for 10 min under continuous shear and cooled back to room temperature. Subsequently, the pH of the samples was adjusted to 6.8. Mean particle size of samples heated at pH 3 and pH 7 were significantly ($P < 0.05$) different at 1580.34 ± 25.3 nm and 99.4 ± 9.9 nm, respectively. Reconstituted MCC was combined with each batch to make MPC with a ratio of 80:20 casein to whey. MPC containing mWPI heated at pH 7 (MPC7) had an emulsion capacity of 4.5 ± 0.3 g oil per mg protein, significantly ($P < 0.05$) higher than both control MPC (3.8 ± 0.1) and MPC containing mWPI heated at pH 3 (MPC3) (3.9 ± 0.1). MPC7 had a solubility significantly ($P < 0.05$) lower than both other treatments. Apparent viscosity of MPC7 was significantly ($P < 0.05$) higher than that of control MPC. There was no significant ($P < 0.05$) difference in foaming capacity or foaming stability between treatments. To determine particle size of whey proteins after spray drying, samples were hydrated and casein was acid precipitated. Filtered WPI (FWPI) heated at pH 3 had a significantly ($P < 0.05$) larger particle size (436.6 ± 13.2 nm) than FWPI heated at pH 7 (203.8 ± 27.0 nm). Neither were significantly ($P < 0.05$) different from control FWPI (249.5 ± 57.1 nm). Based on these findings, future studies should investigate modifying the proportion of aggregated whey to tailor model MPC to different applications.

Key Words: functionality, functional modification, aggregation

2011 Heat stability of rehydrated milk protein concentrates at 6.8% protein.

J. Hargrove*[1], M. A. Drake[1], and D. M. Barbano[2], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

Shelf-stable milk protein beverages have increased in popularity but heat induced protein aggregation remains a challenge. This study compared the heat stability of reconstituted milk protein concentrate (MPC) powders using a new oil bath heat treatment method followed by a centrifugal sedimentation test. Eight commercial MPC powders (duplicate lots) from 3 manufacturers were tested. The MPC powders were rehydrated (6.8% protein wt/wt) overnight at 4°C, heated to 50°C, smoothed with a 2-stage homogenizer (pressure: 19,500 mPa; 2,830 psi) to reduce particle size and then cooled to 20°C for pH measurement. The oil bath heat treatments were 145, 150 or 155°C for 30 s rapidly cooled to < 4°C. The 8 unsmoothed and smoothed liquid MPC were heat processed and tested for sedimentable protein and particle size. Heat-induced protein aggregation was measured as the amount of sedimentable protein and reduction in the percentage of casein micelle (CM) size particles after heating. The experiment was replicated twice. Univariate statistical analyses were applied to evaluate the data. Smoothing of rehydrated MPC reduced particle size of protein aggregates in the unheated products. The percentage of particles in the size range of casein micelles (0.1 to 0.26 microns) increased ($P < 0.05$) from 45.2 to 88.6% of the particle volume due to smoothing before heating. The range of pH (20°C) of rehydrated powders before heating was 6.58 to 6.91. Heat stability of unsmoothed rehydrated MPC was lower ($P < 0.05$) than smoothed liquid MPC. Among the 8 smoothed liquid MPC, the weight of sedimentable protein varied from 3.4 to 7.2% of the sample weight and the proportion of particles in the size of CM range varied from 63 to 84%. After heat treatment, sedimentable protein increased ($P < 0.05$) and proportion of particles in the CM size range decreased ($P < 0.05$) with lower starting pH of the rehydrated MPC powder. The effect of low starting pH on heat stability of the unsmoothed liquid MPC was larger ($P < 0.05$) than for smoothed, with sedimentable protein values as high as 14% for unsmoothed liquids. The MPC manufacture factors that influence pH of rehydrated MPC need further study.

Key Words: MPC, heat stability, oil bath

2012 Preliminary studies on impact of low and high heat processing of nonfat dry milk on powder rheology.

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Nonfat dry milk (NDM) is a versatile dairy ingredient extensively used in the food industry. Skim milk undergoes different heat treatments to produce high heat (HH) and low heat (LH), each customized for specific purposes. These treatments significantly affect NDM properties, ultimately determining its suitability for various applications. This study investigates the influence of HH and LH treatments on powder rheology of (NDM). Two lots of HH and LH NDM were procured from a US commercial manufacturer and characterized in terms of specific energy, basic flow energy, moisture content, bulk and tapped densities, Carr's compressibility index, Hauser ratio, particle size distribution parameters, wall friction angle, and powder shear and compression tests. No significant differences were observed in wall friction angle, A_w and moisture content between HH and LH NDM ($P > 0.05$), however HH NDM showed a significantly lower specific energy (0.78 ± 0.0 mJ) and basic flow energy (239.75 ± 3.53 mJ) compared with LH NDM (0.95 ± 0.04 mJ and 307.03 ± 21.10 mJ, respectively; $P < 0.05$), indicating a better flowability of HH NDM. Additionally, HH NDM exhibited a lower Carr's compressibility index (19.14%) compared with LH NDM (21.32%), suggesting reduced cohesion. It was observed that

HH have low than LH particle size distribution analysis reveals finer particle size distribution in HH-treated NDM, with smaller D50, values (74.63 ± 2.81 μm) compared with LH NDM (82.395 ± 0.29 μm). The powders underwent 3-point shear failure testing using a powder shear cell attached to an MCR302e rheometer and flow function coefficients (ffc) were derived through Mohr circle analysis at both pre-shear and shear-to-failure stages. However, due to their spherical shape and larger particle dimensions, both powders exhibited a stick-slip behavior. This study offers valuable insights into how heat treatment affects powder rheology, emphasizing the significance of heat treatment in shaping flow behavior for optimizing processing parameters.

Key Words: nonfat dry milk (NDM), thermal processing, rheological characterization

2013 Flavor chemistry of browned butter. A. R. Best*[1], Y. Liu[1], D. M. Barbano[2], and M. A. Drake[1], *[1]North Carolina State University, Raleigh, NC, [2]Cornell University, Ithaca, NY.*

Browned butter has gained popularity as an ingredient. The flavor chemistry of this product has not been characterized. The objective of this study was to characterize the flavor chemistry of browned butter. Duplicate lots of salted fresh butter (<3 weeks) were obtained from a manufacturer. Butter was browned by heating in a saucepan with stirring to a temperature of 138° for 3 min. The solids were removed by filtration and the browned butter (BB) was then cooled in an ice bath. Unbrowned (control) butters were included in the experiment. Instrumental color L, a, and b* values were used to document consistent browning. Descriptive sensory analysis (DA) was conducted on all butters. Volatiles were evaluated by headspace (SPME) and solvent extraction followed by gas chromatography mass spectrometry (GCMS). Aroma-active volatiles from headspace and solvent extractions were identified by gas chromatography olfactometry (GCO). High intensity compounds by GCO were quantified using external standard curves. Univariate analyses were applied to evaluate data. BB color was consistent across all browned samples ($P > 0.05$). BB had characteristic caramel and toasted flavors while control butters were characterized by cooked and milkfat flavors ($P < 0.05$). BB had higher concentrations and prevalence of sulfur volatiles produced from Maillard reactions (dimethyl disulfide, methional, and dimethyl trisulfide; $P < 0.05$), whereas control unheated butters had more hydrogen sulfide and dimethyl sulfide ($P < 0.05$), which were associated with the cooked flavor of fresh butter. A combination of aroma-active compounds was responsible for the caramel and toasted flavors of BB. They included compounds with fruity (octanal, 2-pentanone), green (2-heptanone), nutty (furfural, 2-pentylfuran, 1-(2-furanyl)-ethanone), caramel (maltol, 2-furanmethanol) and sharp (acetic acid, hexanoic acid) aromas. In contrast, the unheated butters had more aroma-active compounds with milkfat and sweet aromas, such as waxy/coconut (γ-decalactone), creamy (δ-dodecalactone), sweet (acetophenone), and fatty (nonanal). An understanding of the flavor chemistry of fresh and browned butters can pave the way for product innovation.

Key Words: browned butter, flavor, flavor chemistry

2014 Flavor chemistry of aseptic milk. D. C. Cadwallader*[1], Y. Liu[1], D. M. Barbano[2], and M. A. Drake[1], *[1]North Carolina State University, Raleigh, NC, [2]Cornell University, Ithaca, NY.*

Previous research has demonstrated that ultrapasteurization (UP) of fluid milk creates distinct sensory and volatile compound profiles than high-temperature, short-time (HTST) pasteurization. Aseptic milk has a sensory profile that is distinct from UP or HTST milk with a characteristic caramelized flavor. Our objective was to identify and quantify the aroma-active compounds that contribute to the typical flavor of aseptic milk. Aseptic fluid 1% fat milk (946 mL containers, duplicate lots) were collected from the process line of a commercial facility and cooled to 21°C. Neutral (SPME GC/MS) and sulfur volatiles (SPME GCMS/MS) and descriptive sensory analysis were conducted monthly for 12 mo. Solvent assisted flavor evaporation (SAFE-GC/MS) with aroma extract dilution analysis (AEDA-GC/FID) and gas chromatography olfactometry (GC-O sniffing) were conducted at 0, 6, and 12 mo. Aroma active compounds were identified with authentic standards and selected compounds were quantified using standard curves. Univariate statistical analyses were applied to evaluate the data. Thirty-two aroma-active compounds were identified, and 12 high aroma impact volatiles were quantified in aseptic milks. The major aroma-active compounds in aseptic milks initially were hydrogen sulfide, carbon disulfide, dimethyl disulfide, dimethyl trisulfide, and methional, consistent with high intensities of sulfur/eggy and cooked/milky flavors by descriptive analysis. Both sulfur volatile concentrations and sensory intensities of sulfur/eggy flavor decreased with storage ($P < 0.05$). The characteristic aroma-active compounds of aseptic milk by 3 mo at 21°C were 2-heptanone, hexanal, 3-methyl butanal, benzaldehyde, maltol, furaneol, and (z)-6-dodecen-4-olide. The characteristic caramelized flavor of aseptic milk was due to increased concentrations ($P < 0.05$) of Maillard reaction volatiles maltol and furaneol. An understanding of the inherent flavor chemistry of aseptic milk provides the necessary knowledge platform for flavor improvement.

Key Words: aseptic milk, flavor, flavor chemistry

2015 Impact of low moisture part skim (LMPS) mozzarella shred geometry on the functional properties. Z. Modi* and P. Salunke, *Dairy Science Department, South Dakota State University, Brookings, SD.*

Low-moisture part-skim (LMPS) mozzarella cheese is widely used on pizza as a topping because of its texture, melting, stretch quality, longer shelf-life, and functionality. The LMPS is generally shredded before use. Companies sell shredded LMPS for convenience to customers. Many types of shred quality (thick, fine, medium, coarse, "V," and flat-V shreds) are available in the market and vary in functionality and pizza baking performance. The study was designed to analyze the different shreds available in the market and their functional properties. Two commercial samples, each of finely shredded (FS), medium shredded (MS), and thickly shredded (TS) LMPS, were collected. The experiment was replicated thrice with different lots of cheese. All the samples were analyzed for proximate composition, shred size, density, Schriber melt test, pizza bake test, stretchability, and image analysis (IR thermal image camera) before and after the pizza bake to check heat distribution and melting behavior. The data collected were statistically analyzed using Costat. The image analysis was carried out using the Fluke Connect software. The proximate composition of shred samples differed significantly ($P < 0.05$). The moisture of TS samples was significantly ($P < 0.05$) higher (45.39%) and lower in protein content (18.06%), whereas FS samples had lower moisture (42.28%) and significantly ($P < 0.05$) higher protein content (24.04%). All the samples had different lengths and thickness. The loose density values differed according to the cuts' size, mass, and volume. The TS samples showed the lowest loose and tapped (100×) density. The meltability and stretchability (33 cm) were significantly lower ($P < 0.05$), in MS samples, whereas the TS samples showed a significantly ($P < 0.05$) higher value of stretchability (83 cm). In conclusion, geometric cuts significantly affect the functionality of the finished product. The product manufacturers should select proper cutting and processing equipment to extract the full functional benefits.

Key Words: Low moisture part skim, shred geometry, functional properties

2016 Impact of different types of cellulose anticaking agents on the functionality of shredded low-moisture part-skim Mozzarella cheese during different storage conditions. P. M. Eberly*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], R. A. Ibáñez[2], M. E. Johnson[2], and J. A. Lucey[1,2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

Anticaking agents (ACA), such as cellulose, are commonly mixed with shredded cheese to prevent clumping during storage. However, there is a lack of information on how various types of cellulose affect the functionality of low-moisture part-skim (LMPS) Mozzarella under different storage conditions. LMPS Mozzarella from industry was stored for 2 wk at 4°C, then shredded. Five ACA treatments were applied at 2% wt/wt: control (no ACA), powdered cellulose (CEL), carboxymethylcellulose (CMC), hydroxypropyl methylcellulose (HPMC) and methylcellulose (MC; n = 3). Within each treatment, shreds were split into different storage temperatures (ST): refrigerated (4°C), superchilled (−2°C) and frozen (−20°C). Composition, work to extend (using a texture analyzer with an extensibility rig), sensory properties of un- and melted shreds on pizza (quantitative descriptive analysis; 10 trained panelists) were tested during storage (0, 1, and 3 mo). Samples stored at −2 and −20°C were tempered for 1 wk at 4°C before testing. Split-split plot design ($\alpha = 0.05$) was used for statistical analyses. Composition of shreds was similar during storage. Work to extend was similar during storage at −20°C but decreased at −2 and 4°C ($P < 0.05$). ACA affected the pizza and shred attributes of the cheese. Pizzas baked with LMPS Mozzarella cheese containing ACA showed increased blister quantity (BQ) and blister color (BC) and decreased shred melt due to ACA imbibing water that should be released during baking ($P < 0.01$). ST affected the BQ and BC; these attributes increased moderately at 4°C and slightly at −2°C, but not at −20°C. Compared with the control, CEL and CMC had reduced matting of shreds and adhesiveness scores, whereas HPMC and MC increased them ($P < 0.0001$). Although modified celluloses enhance water binding, they were water soluble; HPMC and MC gelled in the presence of surface moisture on the shreds, suggesting that insoluble ACA were more effective on shredded cheese. Additionally, freezing with a 1-wk thaw time retained the functionality of the cheese during storage.

Key Words: shredded cheese, anticaking agent, functionality

ADSA-GSD Competition: Production (MS) Poster Semifinalists

2017 Use of triticale silage as an alternative to corn silage in dairy cow diets. V. A. Achziger*[1], A. M. Cezar[1,3], I. R. R. Castro[1,2], L. N. C. Silva[1,3], G. B. C. Leite[1], A. Zaragoza[1], and M. I. Marcondes[1], *[1]Department of Animal Sciences, Washington State University, Pullman, WA, [2]Universidade Federal de Viçosa, Viçosa, MG, Brazil, [3]Escola Superior de Agricultura Luiz de Queiroz,Universidade de sao paulo, Piracicaba, São Paulo State, Brazil, [4]Universidade Federal de Norte se Tocantins, Tocantins, Brazil.*

Corn silage (CS) requires ample irrigation and high-quality soil, current limitations in water availability and quality land pose challenges for CS production. The economic and environmental feasibility of utilizing CS in dairy operations is constrained. Triticale, a hybrid of wheat and rye, has an enhanced stress tolerance, making it an effective crop for preventing soil erosion and thriving in regions with limited irrigation. Thus, we aimed to evaluate triticale silage (TS) as a replacement for CS in lactating cow diets of an artificial rumination system. We hypothesized that TS could completely replace CS in diets for high-producing cows while keeping the same dietary energy (NE_L = 1.52 Mcal/kg) and starch (27%). We used 16 fermenters with 4 fermenters per treatment. Four diets were formulated with adjusted levels of TS as a replacement for CS (0%, 33%, 66%, and 100%). Rumen fluid used was collected from harvested cows. The apparatus was consistently supplied with artificial saliva (dilution rate of 2.9%/h), periodically agitated, fed daily, and kept O_2-free to mimic rumination. Liquid effluent produced by the fermenters was recorded daily. The experimental period lasted 10 d, allowing fermenters 7 d to stabalize. The sampling period went from d 8 to 10. We evaluated the system's fermentation parameters pH, volatile fatty acids, dry matter disappearance, digestibility, gas production, and methane synthesis. Data were run as a completely randomized design. Treatments showed no significant impact on pH ($P > 0.101$), methane ($P > 0.150$), digestibility of dry matter ($P > 0.316$), protein ($P > 0.351$), and starch ($P = 0.067$). No effect was found in either VFA proportions or production (acetate, propionate, and butyrate; all $P > 0.050$). However, we observed a significant effect on NDF digestibility ($P < 0.044$), where a positive linear trend was present as the TS increased. This trend provides additional evidence endorsing TS as increased NDF digestibility encourages a greater intake, consequently promoting a higher performance level. We accepted our hypothesis that TS could replace CS in lactating diets while maintaining dietary energy and starch.

Key Words: triticale, digestibility, Rusitec

2018 Evaluating strategies for dairy farm sustainability using a virtual farm model. J. C. Becker*, V. A. Ishler, T. J. Beck, and L. A. Holden, *The Pennsylvania State University, State College, PA.*

Feed cost is the largest dairy expense and long-term viability is dependent on managing the feeding program within the whole farm system. The objective of this project was to evaluate cropping and feeding strategies to enhance dairy farm sustainability. To evaluate the whole farm's efficiency and profitability, a virtual farm model was used with 2 diverse cropping rotations (injected manure with reduced herbicide [IMRH] or broadcast manure with standard herbicide [BMSH]) and producing most of the forages and feed for a simulated 65-milking cow herd on 240 acres. The 6-yr, no-till crop rotations were managed and harvested in research plots and the crop and feed quality data were used in the virtual farm model for lactating and dry cows and youngstock with extensive financial data for the whole farm system. Results indicated that the biggest financial impact was savings in feed cost when growing all forages and corn grain versus buying forages and corn grain. The average cost of production/cwt for the BMSH rotation was \$16.26 + 1.850, while the cost for the IMRH rotation was \$17.80 + 1.663. The total feed cost per cow was \$1,779 + 191.228 for BMSH and \$1,908+286.270 for IMRH, which was competitive for Pennsylvania dairy farms. The main reason for the financial advantage of the BMSH versus the IMRH was the increased revenue from crop sales of excess feeds. The average for BMSH had \$65,614 in sales compared with IMRH with a \$51,657 average over the 6 yr. A *t*-test was performed on the crop sales data for BMSH and IMRH. The t-statistic [LH1] was 1.2279 and the *P*-value was 0.2469 indicating no significant difference in the means of BMSH and IMRH for crop sales. Another *t*-test was performed on the Cost of Production data for BMSH and IMRH. The t-statistic was −0.42224, and the *P*-value was 0.6803 indicating no significant difference between treatments for cost of production. This study demonstrates that small farms can achieve profitability if milk production and feed cost are carefully monitored and there is a match of crop acreage to cow numbers that allows the farm to grow both corn grain and forages.

Key Words: dairy sustainability, feed costs, double cropping

2019 Effects of maternal supplementation of *Saccharomyces cerevisiae* var. *boulardii* CNMCM I-1079 during late gestation on neonatal Holstein calf immune status. M. Boerefyn*[1], S. Cartwright[1], S. Jantzi[1], K. Dekraker[1], A. Pineda[1], S. K. Kvidera[2], R. Sargent[3], L. R. Cangiano[4], C. Villot[5], and M. A. Steele[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Elanco Animal Health, Granger, IA, [3]Saskatoon Colostrum Company Ltd., Saskatoon, SK, Canada, [4]University of Wisconsin–Madison, Madison, WI, [5]Lallemand SAS, Blagnac, France.*

The objective of this study was to evaluate the effect of *Saccharomyces cerevisiae* var. *bouldarii* CNCM I-1079 (SCB) maternal supplementation during late gestation on offspring's immune function. Eighty Holstein cows were blocked by previous 305-d milk yield, parity, body condition score, and body weight, and were randomly assigned to dietary SCB supplementation (SCB; n = 40) or a control (CON; n = 40). After birth, calves were fed colostrum replacer at a liquid volume of 15% of birth weight in 2 feedings with a target IgG of 300 g. There were 40 bulls (SCB; n = 19; CON; n = 24) and 40 heifer calves (SCB; n = 22; CON; n = 21) enrolled. Blood samples were obtained at 0 h, 12 h, 24 h, d 1, d 4, and d 7 of life, and analyzed for IgG concentrations. Polymorphonuclear neutrophils were isolated from blood on d 1 and 7 and were analyzed by flow cytometry to determine the oxidative burst and phagocytic capacity of circulating neutrophils in each treatment group. Peripheral blood mononuclear cells were isolated on d 1 and 7 and were stained with fluorescent antibodies CD21, CD 32, WC 1.1, and WC 1.2 and analyzed through flow cytometry to determine B and T cell function. Data was analyzed using the GLIMMIX procedure of SAS (v9.4). No differences ($P > 0.05$) in IgG concentration were observed between treatment groups. Oxidative burst capacity was similar ($P > 0.05$) between treatment groups. Phagocytic capacity did not differ ($P > 0.05$) between treatments. The percentage of WC 1.1 (34.5% vs. 23.1%) and WC 1.2 (36.3% vs. 21.4%) in T cells was higher ($P < 0.05$) in CON calves than SCB calves. Female calves had a higher ($P < 0.05$) percentage of CD21 (7.0% vs. 4.3%) and CD32 (8.14% vs. 5.1%) than male calves. No treatment effects ($P > 0.05$) were found in B cell functions. This data suggests that SCB supplementation during late gestation of dairy cows does not affect offspring phagocytic or oxidative burst capacity, but may reduce T cell populations.

Key Words: Probiotic supplementation, calf immunity, flow cytometry

2020 Group housed dairy calves have greater body weight gain during preweaning than individually housed calves: A meta-analysis. J. P. Donadio*[1,2], K. T. De-Sousa[1], R. N. S. Torres[1], T. C. Alves[3], M. J. Hotzel[4], and M. Deniz[1], [1]*Grupo de Estudos em Bovinos Leiteiros, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil,* [2]*Programa de Pós-Graduação em Zootecnia, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil,* [3]*Embrapa Pecuária Sudeste, São Carlos, São Paulo, Brazil,* [4]*Laboratório de Etologia Aplicada e Bem-Estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.*

Social isolation during preweaning has negatively affected dairy calf welfare, but its impact on their performance remains uncertain. In this study, using a meta-analytic approach, we assess the effect of the housing system (group or individual) on dairy calf feed intake and performance during preweaning. The analyzed database consisted of 27 peer-reviewed studies written in English, published from 1979 to 2023, and recovered from Web of Science, PubMed, and CabDirect. Only studies providing means and mean errors of at least one feed intake or performance variable were included. The weighted mean differences between group and individual housing systems were analyzed for each variable using the Der-Simonian and Laird method. Heterogeneity between calf housing systems was evaluated by the chi-squared test and I^2 statistics. A meta-regression analysis was conducted to identify categorical covariate effects for variables with high heterogeneity. The most frequently studied variables regarding calf feed intake and performance were daily concentrate intake (kg/d; n = 10), average daily gain (kg/d; n = 27), and weaning weight (kg; n = 19). Group housed calves (n = 225/764/374) exhibited higher concentrate intake (P = 0.021; 0.037 kg/d; confidence interval (CI) = 0.01–0.07), average daily gain (P = 0.001; 0.056 kg/d; CI = 0.03–0.08) and weaning weight (P = 0.037; 1.436 kg; CI = 0.09–2.78) compared with individually housed calves (n = 190/529/266). Concentrate intake and average daily gain had high (>50%) and significant ($P < 0.05$) heterogeneity among studies. Categorical covariates such as age at weaning, sex class and pen area per calf affected ($P < 0.05$) the average daily gain; in contrast, no covariates affected ($P > 0.05$) the concentrate intake. These covariates should be considered in future subgroup analyses to verify the effect of each rearing system on the average daily gain of calves. In conclusion, housing dairy calves in groups positively impacts their performance, resulting in higher weaning weights; this is due to increased concentrate intake and average daily gain.

Key Words: dairy cattle, growth, positive experience

2021 Comparative analysis of milk protein prediction in automatic milking systems. K. Frady*[1], L. M. Campos[2], S. Wimer[1], S. Campbell[1], X. Sun[1], M. D. Hanigan[2], and A. G. Ríus[1], [1]*The University of Tennessee, Knoxville, TN,* [2]*Virginia Polytechnic Institution and State University, Blacksburg, VA.*

Automatic milking systems (AMS) on a dairy farm use sensors, algorithms, and interfaces to gather and process biological data, including measurements of milk components to enhance decision-making. This work aimed to study the milk protein content analyzed by AMS (AMS-MPrt) compared with milk protein analysis conducted by a commercial laboratory (LAB-MPrt). We hypothesized that the AMS could be a good indicator of milk protein (MPrt). Fifty-five Holstein cows (44.7 ± 12.7 kg of MY/d, 101 ± 62 DIM, 3.2 ± 1.3 parity) were randomly assigned in a replicated 5 × 5 Latin square design balanced for carry over effects with 10-d experimental periods. In 5 periods, cows were submitted to 1 of 5 dietary CP levels (14%, 14.75%, 15.5%, 16.25%, 17%). Treatments were developed using NASEM (2021) and the diet was optimized to maximize profit. Cows were fed an 80:20 feed ratio (%DM) in the partial mixed ration (PMR) and AMS, respectively. The PMR contained corn silage, straw, and a grain mix and the AMS pellets contained corn, wheat middlings, and various levels of soybean meal to achieve treatments. Milk production and components were recorded every milking via AMS. Milk samples were collected 2× daily on d 9 to 10 of each treatment period and sent to a commercial laboratory. A linear mixed model was developed in RStudio to determine the relation between AMS-MPrt with LAB-MPrt measurements, with significance declared at $P < 0.05$. Fixed effects included AMS-MPrt, CP treatment, and AMS-MPrt × CP treatment interaction. Random effects included animal and period. Analysis showed that the AMS-MPrt × CP treatment interaction was nonsignificant ($P > 0.05$), indicating that dietary CP level had no effect on the regulation of LAB-MPrt by AMS-MPrt. There was a significant linear relationship between AMS-MPrt and LAB-MPrt ($P < 0.001$), leading to the conclusion that AMS-MPrt predictions are a good indicator of MPrt.

Key Words: precision, milk protein, automatic milking system

2022 Characterizing the temporal changes in mammary tissue microstructure from the nonlactating to lactating period in multiparous Holstein dairy cattle. J. R. Petrou*[1], A. J. Fischer-Tlustos[1], K. Klein[1], W. Silva[1], J. P. Cant[1], J. Mergh Leao[2], M. C. Guenther[3], J. Laporta[3], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Saskatoon Colostrum Co. Ltd., Saskatoon, SK, Canada,* [3]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The aim of this study was to characterize changes in mammary tissue microstructure during the transition from the nonlactating to lactating period in multiparous dairy cattle. Starting from d −58 ± 2.9 before calving, parity 2 (n = 6) and 3 (n = 4) Holstein cows were dried off and fed a controlled energy dry diet (NE_L = 1.48 Mcal/kg DM) until calving, after which cows were switched to a standard lactating diet (NE_L = 1.67 Mcal/kg DM). Mammary gland biopsies were performed at wk −6, −4, −2, and −1 before expected calving date, d +1 (within 10 min of colostrum collection), and d +7 postcalving. Mammary tissue was fixed in formalin for 24 h, dehydrated, embedded in paraffin, sectioned (5 µm), and stained with hematoxylin and eosin to visualize tissue microstructure. Three images/cow/time point at 40× magnification chosen randomly were used to quantify the number of alveoli/image, whole alveoli and inner lumenal areas, and the number of mammary epithelial cells (MEC)/alveolus using ImageJ software. Data were analyzed using PROC GLIMMIX considering the fixed effect of parity, time, and their interaction, and the random effect of cow. At wk −1, d +1 and d +7, greater ($P < 0.04$) inner lumenal and whole alveoli area was observed than at wk-6, and a 222% increase in inner lumenal area from wk −6 to d +7 was observed. At wk −6, −4, and −2 the number of alveoli per image was greater ($P < 0.02$) than on d +1 and +7, and there was a negative correlation ($r = -0.64$, $P < 0.0001$) between alveoli number and whole alveoli area. Total number of MEC was unaffected (P = 0.11) by time; yet, the number of MEC/alveolus increased (P = 0.03) by 35% from wk −6 to d +7. Week −6 had a greater ratio of inner

Table 1 (Abstr. 2023). In vitro ruminal fermentation parameters at 48 h

	Treatment								
Item	CON	B15	B30	T15	T30	W15	W30	SEM	*P*-value
pH	6.32^{b}	6.35^{ab}	6.33^{ab}	6.35^{ab}	6.36^{a}	6.34^{ab}	6.34^{ab}	0.04	0.02
DMD, %	62.6	62.4	64.2	63.2	62.4	63.0	64.6	3.02	0.20
Gas, mL	431^{a}	410^{ab}	408^{ab}	369^{bc}	353^{c}	381^{bc}	439^{a}	26.4	< 0.01
CH_4, mg	26.8^{bc}	30.8^{abc}	32.1^{ab}	21.4^{c}	26.1^{bc}	31.4^{ab}	37.3^{a}	6.89	< 0.01
CH_4, mg/g DMD	0.013^{ab}	0.011^{b}	0.011^{ab}	0.015^{a}	0.013^{ab}	0.025^{ab}	0.010^{b}	0.003	< 0.01

$^{a–c}$Within a row, means with different superscripts differ ($P \leq 0.05$).

lumenal area:MEC than wk −1, d +1 ($P < 0.09$), and d +7 ($P = 0.01$). The number of MEC/alveolus was positively correlated with the inner lumenal area and whole alveoli area ($r = 0.56$ and 0.59, respectively; $P < 0.0003$). These results highlight the dynamic temporal alterations that occur in mammary tissue microstructure during the dry period in preparation for the onset of lactation in Holstein cattle.

Key Words: mammary, microstructure, transition period

2023 Hydroponic sprouts inclusion in lactating dairy cow diets: Effects on methane production, rumen fermentation, and nutrient degradability in vitro. G. K. Salas-Solis*[1], J. A. Arce-Cordero[2], R. R. Lobo[1], M. U. Siregar[1], M. L. Johnson[1], K. S. Alves[1], A. C. S. Vicente[1], T. Bilchik[1], and A. P. P. Faciola[1], *[1]University of Florida, Gainesville, FL, [2]Universidad de Costa Rica, Montes de Oca, San Jose, Costa Rica.*

This study evaluated the effects of 3 hydroponic sprouts at 2 inclusion levels on CH_4 production and rumen fermentation in lactating dairy diets in vitro. A batch culture experiment was conducted as a randomized complete block design with 3 fermentation runs (blocks). Treatments were a control diet (CON), and the replacement of dietary ingredients with sprouts: barley at 15 (B15) and 30% (B30), wheat at 15 (W15) and 30% (W30), and triticale at 15 (T15) and 30% (T30); DM basis. Experimental diets have 16.0% CP, 32.2% NDF, 24.1% starch, and 10.1% water-soluble carbohydrates; DM basis. Rumen content was collected from 2 rumen-cannulated lactating Holstein cows, filtered, and mixed in a 1:2 ratio with Van Soest medium. Three replicates per treatment, per incubation setup (24 and 48 h) were carried out in serum bottles with 52 mL of solution and 0.5 g DM of the experimental diets (within Ankom bags) at 39°C. Headspace pressure gas was measured at 0, 2, 4, 8, 16, 24, 36, and 48 h to obtain a pooled sample at 24 and 48 h for CH_4 and total gas. Dry matter degradability (DMD), pH, and CH_4 intensity (mg/g DMD) were determined at 24 and 48 h. Data were analyzed using the mixed procedure of SAS, with hydroponic source as fixed effect, and run and bottle as random effects. Treatment differences were determined using Tukey-Kramer multiple comparisons. At 24 h, we found that pH, CH_4, and CH_4 intensity were similar between CON and all hydroponic sprout treatments. At 48 h, Table 1 shows treatment effects on in vitro fermentation. Total CH_4 was the lowest in T15; however, T15, and T30 had the greatest CH_4 intensity. Further effects on VFA will improve our understanding of hydroponic fodder fermentation characteristics.

Key Words: hydroponics, methane, fermentation

ADSA-GSD Competition: Production (PhD) Poster Semifinalists

2024 Lipogenic effects of palmitic and oleic acids in subcutaneous and visceral adipocytes. U. Abou-Rjeileh*, M. Grusczynski, and G. A. Contreras, *Michigan State University, East Lansing, MI.*

Excessive lipid mobilization due to negative energy balance (NEB) in periparturient dairy cows increases their risk for diseases and decreases their productivity. Improving energy intake during the immediate postpartum limits NEB. One way to enhance energy density of the diet is through fatty acid (FA) supplementation. However, the extent to which distinct adipose tissue (AT) depots store energy in response to differences in diets is poorly understood. Our objective was to assess lipid accumulation in subcutaneous (SC) and omental (OM) derived bovine adipocytes following FA supplementation. Pre-adipocytes were isolated from SC and OM AT (n = 4, nonlactating, nongestating Holstein dairy cows) and induced to differentiate. Mature adipocytes were cultured with standard differentiation media (CON) supplemented with mixture of palmitic (PA) and oleic (OA) acid (60% PA–40% OA; 60–40) at 300 µ*M*. FA were solubilized in albumin (10% BSA). Adipogenesis was evaluated using a neutral lipid stain, Bodipy 493/503. Triglyceride levels were quantified using Triglyceride-Glo Assay. Expression of lipogenic gene networks was evaluated using RT-qPCR. The statistical model included the random effect of cow and fixed effect of treatment, AT depot, and their interaction. OM adipocytes had higher adipogenesis efficiency compared with SC adipocytes ($P < 0.01$). FA supplementation did not affect adipogenesis ($P = 0.10$). However, FA supplementation increased triglyceride accumulation ($P < 0.01$) compared with CON. This increase tended to be higher in SC versus OM adipocytes ($P = 0.06$). Compared with SC, 60–40 tended to increase *FABP4* expression in OM ($P = 0.08$). No difference was observed between depots or treatments on *PPARA*, *AGPAT2*, *PLIN3*, and *ACSL1* gene expression ($P \geq 0.12$). Our results show that AT depots differ in their response to FA supplementation. Additional studies are needed to further characterize lipid metabolism in different depots in dairy cows.

Key Words: lipid metabolism, adipose tissue depots, fatty acid supplementation

2025 Feeding whole milk enriched with transition milk improves the performance and health of young calves. I. F. Carrari*[1], M. H. Safaee[2], M. Kanani[2], L. V. Kladt[1], S. Kargar[2], and M. I. Marcondes[1], [1]*Washington State University, Pullman, WA,* [2]*Shiraz University, Shiraz, Fars, Iran.*

We investigated the effects of feeding whole milk (WM) enriched with transition milk (milk from the first 3 d in milk; TM) for different periods on the performance and health of calves. We used 64 female Holstein calves (3 d of age; 37.6 ± 1.7 kg BW), randomly assigned to one of the following 4 treatments: T0 (6 L of WM daily); T1 (4 L of WM + 2 L of TM daily during one week); T2 (4 L of WM + 2 L of TM daily during 2 weeks); T3 (4 L of WM + 2 L of TM daily during 3 wk). After ending the TM period, calves in all treatments were fed 6 L of WM daily until d 57. The animals were weaned in a step-down system decreasing 1 L per day until d 64, fed starter feed, and kept until d 91. Calves' growth indices were measured on the first day of the trial, weekly until d 21, and every 2 wk after the d 21. Calf health and fecal scores were also monitored daily. Blood samples were collected to assess health indices. Data were evaluated as a completely randomized design in a repeated measures scheme. Period was significant for all variables ($P < 0.01$). Calves on T2 and T3 had increased DMI, MEI, and ADG while receiving TM and on d 91. IgG was higher for T3 and T2 until d 63 but was higher for T3 at d 91. T3 increased withers height, body length, βHBA, and total protein ($P < 0.01$). BUN was higher during the time calves were receiving TM but did not differ afterward. T0 had higher likelihood of fever and diarrhea than T2 and T3 ($P < 0.01$). Overall, our results indicate that feeding WM enriched with TM for 3 wk improves the performance and health of young calves (Table 1).

Key Words: diarrhea, growth, intake

2026 Liver transcriptomic profile of lactating dairy cows fed spent hemp biomass. A. Irawan* and C. T. Estill, *Oregon State University, Corvallis, OR.*

Including spent hemp biomass (SHB), an extracted byproduct from industrial cannabidiol (CBD) production, in the diets of ruminants has provided evidence of possible long-term beneficial effects. Our prior studies suggested improved production performance especially after the withdrawal of SHB from the diet, that could be partly associated

Table 1 (Abstr. 2025). Performance and health parameters as influenced by feeding whole milk enriched with transition milk (2:1 ratio) to Holstein calves

	Treatment					*P*-value	
Item	T0	T1	T2	T3	SEM	Treatment	T × P
DMI, g/d	1,400.3	1,411.9	1,479.3	1,501.7	45.009	0.29	0.01
MEI, Mcal/d	5.3	5.4	5.7	5.8	0.146	0.18	0.01
ADG, g/d	758.1	807.4	858.9	877.3	192.4	0.01	0.01
Body weight, kg	67.2	71.2	71.9	69.6	0.762	0.01	0.01
Withers height, cm	88.1^{c}	88.7^{bc}	89.4^{ab}	89.5^{a}	0.258	0.01	0.16
Body length, cm	88.1^{c}	88.7^{bc}	89.4^{ab}	89.6^{a}	0.259	0.01	0.12
IgG, g/L	15.4	21.0	25.3	26.4	0.349	0.01	0.01
BUN, mg/dL	27.0	30.0	32.8	34.6	1.047	0.01	0.01
βBHA, mmol/L	0.17^{c}	0.18^{bc}	0.21^{ab}	0.23^{a}	0.008	0.01	0.69
Total protein, g/dL	5.9^{c}	6.1^{bc}	6.3^{ab}	6.4^{a}	0.085	0.01	0.15
Fever, d	2.4^{a}	1.3^{b}	1.1^{b}	0.6^{b}	0.234	0.01	—
Diarrhea, d	3.7^{a}	2.3^{b}	1.4^{bc}	1.2^{c}	0.182	0.01	—

$^{a–c}$Superscript letters indicate different means between treatments.

with cannabinoids contained in the SHB. However, it remains largely unknown how cannabinoids play a role in affecting gene regulation in the liver, a central hub of metabolic processes. This study aims to study the transcriptional profile of liver of dairy cows fed SHB. We conducted a liver biopsy in 18 lactating Jersey cows (2.0 ± 1.1 in parity and 245 ± 37 DIM) at the end of the 4-wk of feeding up to 13% SHB (TRT) or 13% alfalfa meal (CON) (intervention period; IP) and 4-wk after the SHB was withdrawn from the diet (withdrawal periods; WP). RNA was extracted from the liver and sequenced using Illumina NextSeq 2000. After quality control, trimming low-quality reads, and mapping to *Bos Taurus* reference genome, transcripts were normalized, and the ones with <4 raw counts for at least 50% biological samples within the treatment group were removed. The final data set was analyzed using the DESEq2 pipeline. Bioinformatics analyses were conducted, including over-representation analysis using gene ontology (GO) enrichment analysis using DAVID, GO Gene Set Enrichment Analysis using (GSEA; ClusterProfiler), and the dynamic impact approach (DIA) to identify affected pathways. We identified only 20 differentially expressed genes (DEG; FDR ≤ 0.05) during the IP and 3 DEG during the WP between TRT and CON. None of the GO biological processes and cellular components were significantly enriched. Through GSEA, cholesterol binding was inhibited. DIA analysis revealed that SHB activated terpenoid backbone biosynthetic pathway and inhibited lipid metabolism especially related to primary bile acid biosynthesis subcategory and, although with less impact, suppressed immune-related pathways. Our data reveal a minor effect of SHB feeding on liver transcriptome with some effects on genes related to lipid metabolism and the immune system.

Key Words: cannabinoids, transcriptomic, ruminant

2027 Rumen microbiome signature-based machine learning model for heat stress prediction. H. Joshi*, L. Reon, M. Caprio, and P. Fan, *Mississippi State University, Starkville, MS.*

Heat stress has a huge negative impact on the global dairy industry, leading to a reduction in milk production and reproduction, as well as alteration of rumen microbiota which can affect rumen fermentation functions. However, the reported heat-stress-associated rumen microbes were not consistent across studies, partially due to the varied microbiota analysis pipelines. To evaluate whether heat stress could be detected from specific rumen microbiota features and identify potential microbial markers, we collected publicly available 16S sequencing data of 55 rumen samples of dairy cows under heat stress or thermoneutral conditions from 3 studies and built machine learning models with the rumen microbiota profile to predict the heat stress. All the raw sequencing data were analyzed with QIIME2 software and the Silva 138.1 database. The sequencing depth and matrix of absence/presence of the 183 bacterial genera served as independent variables to predict heat stress as the response. The important independent variables that contribute to the model were first selected using Boruta with 5-fold cross-validation. The feature-selected dataset containing 10 important bacterial signatures was further trained using several binary algorithms, including Random Forest, Support Vector Machines (SVMs), and Logistic Regression. The trained models predicted heat stress with an area under the receiver operating characteristic curve (AUROC) of 0.86 to 0.93 (interquartile range, IQR, 0.80–0.94). SVMs outperformed other algorithms with a mean accuracy of 92.8%, and a mean AUROC of 0.86–0.93. Moreover, it has been found that certain selected important features, such as *Bacteroidales* BS11 and *Bacillus*, have also been reported to be enriched in heat-stressed cattle in multiple studies, suggesting their potential as bacterial markers for heat stress. In conclusion, the high accuracy of the built machine learning model indicates a unique rumen microbiota feature in heat-stressed cows, which may be specifically targeted to mitigate the heat stress responses in dairy cows.

Key Words: heat stress, rumen microbiota, machine learning

2028 Histidine, lysine, and methionine effects on milk components production and nitrogen efficiency. M. Killerby*[1], K. Ruh[1], L. A. C. Ribeiro[1], V. Pszczolkowski[1], A. Larsen[1], E. Cohan[1], A. Benn[1], D. Sherlock[2], and S. I. Arriola Apelo[1], *[1]Animal and Dairy Science, University of Wisconsin–Madison, Madison, WI, [2]Adisseo USA Inc., Alpharetta, GA.*

This study aimed to determine the effect of balancing lactation diets for His, in addition to Met and Lys, on milk production and N efficiency. Thirty-two lactating Holstein cows (77 ± 17 DIM) were enrolled by calving date in a replicated 4×4 Latin square study with 28-d periods. The 4 dietary treatments were arranged in a 2×2 factorial with low (LH; $\overline{x} = 56$ g/d) or high (HH; $\overline{x} = 79$ g/d) metabolizable His (H), and low (LMK; $\overline{x} = 61 + \overline{x} = 197$ g/d) or high (HMK; $\overline{x} = 77 + \overline{x} = 243$ g/d) metabolizable Met+Lys (MK), respectively, by substituting corn gluten meal with blood meal or soyhulls or supplementing it with rumen protected Met+Lys (Smartamine M and ML). The 4 diets contained 30.7% corn silage, 27.7% alfalfa silage, 9.2% cottonseed, and 32.3% concentrate (% DM). Cows were milked and fed twice daily. Milk yield and DMI were recorded daily, body weight (BW) was recorded weekly, milk samples (×10) and urine samples (×8) were collected on the last 5 d of each period. Results were averaged by cow-period and analyzed with a mixed model containing fixed effects of H, MK, HxMK interaction, period, and square, and the random effect cow within square. Factors did not affect BW change ($P > 0.10$). Both factors increased DMI to a similar extent (+1 kg/d) compared with LH-LMK, but had no further effect when combined, indicating non-additive effects (H×MK; $P = 0.02$). Unlike DMI, energy-corrected milk (ECM) was additively increased by H (+1.68 kg/d, $P < 0.001$) and MK (+0.85 kg/d, $P = 0.008$). The increases on ECM were driven by the additive effects of H and MK on milk protein yield (H: +74 g/d and MK: +45 g/d, $P < 0.001$), and effects of H on milk fat (+45 g/d; $P = 0.008$) and lactose (+67 g/d, $P < 0.001$). MK tended to increase milk fat yield (+31 g/d; $P = 0.072$) but had no effect on lactose yield ($P = 0.5$). Both factors additively increased MUN (H: +0.56 mg/dL and MK: +1.62 mg/dL, $P < 0.001$), but only MK increased total and urea N excretion in urine (+16 and +28 g/d, respectively; $P < 0.008$), and decreased N use efficiency (NUE; −0.7%, $P < 0.001$). Overall, balancing lactation diets for His, in addition to MK, increases milk components yield without negatively affecting NUE.

Key Words: histidine, amino acids, N efficiency

2029 Continuous abomasal infusion of ^{15}N-Lys to determine metabolizable Lys supply and Lys utilization for milk protein synthesis in dairy cows. J. Kim*[1,2], H. Hu[1], K. Park[1,2], and C. Lee[1], *[1]Department of Animal Sciences, The Ohio State University, Wooster, OH, [2]Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH.*

Amino acids (AA) undergo complex metabolism for protein synthesis or excretion after oxidation. We investigated the fate of metabolizable Lys via continuous abomasal infusion of ^{15}N-Lys. Four rumen-cannulated Holstein cows (147.0 ± 63.2 d in milk, 49.4 ± 9.5 kg milk yield) received 0.6 g/d of ^{15}N-Lys into the abomasum for 5 d. Cows were offered a common diet (16.6% crude protein; 89% of metabolizable Lys requirement) once daily for an ad libitum intake. Total feces, urine, and milk were collected 1 d before the infusion and for 5 d during the infusion. All samples collected were analyzed for total N and ^{15}N (atom percent excess) for the mass balance of ^{15}N. Milk samples were determined for ^{15}N enrichment of milk protein AA. Total Lys absorbed was estimated by NASEM (2021) and calculated using ^{15}N enrichment of milk Lys and ^{15}N absorbed (infused – total ^{15}N excreted in feces) assuming that net utilization of absorbed Lys by peripheral tissues other than mammary tissues is zero. The statistical analysis was conducted using the Mixed procedure of SAS with a random effect of cow. On d 5, ^{15}N excretion in feces was 19.8% of ^{15}N infused, and ^{15}N secretion in milk and excretion in urine were 33.2 and 13.5% of ^{15}N absorbed, respectively, indicating that 53.3% of absorbed ^{15}N was retained. Total Lys absorption calculated using ^{15}N of milk Lys was not different (200 vs. 183 g/d, SEM = 17.7, $P = 0.41$) from that estimated by NASEM (2021). ^{15}N enrichment was observed in various milk protein AA except Ile, Thr, Pro, Phe, and Tyr+His. Milk Leu and Met tended to be enriched with ^{15}N ($P < 0.10$). The distribution of ^{15}N among milk AA was determined. Milk AA with the largest ^{15}N enrichment was Lys (76.3 ± 3.2% of total ^{15}N in milk AA) followed by Glx (10.8 ± 1.4%), Asx (4.3 ± 0.5%), Ser (2.2 ± 0.3%), Ala (1.9 ± 0.4%), Val (1.8 ± 0.5%), Leu (1.5 ± 0.3%), Gly (0.8 ± 0.2%), and Met (0.4 ± 0.1%). The results showed potential use of ^{15}N for AA metabolism and confirmed the flexibility of mammary tissues in producing other AA from Lys to synthesize milk protein.

Key Words: milk amino acids, stable isotope, 15N enrichment

2030 Prepartum serum immunoglobulin G dynamics and the potential use of mammary arteriovenous difference technique to estimate mammary immunoglobulin G uptake. K. Klein*[1], A. J. Fischer-Tlustos[1], J. R. Petrou[1], C. McQuaig[1], J. P. Cant[1], R. Sargent[2], and M. A. Steele[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Saskatoon Colostrum Co. Ltd., Saskatoon, SK, Canada.*

At present, there is a lack of understanding regarding the process of mammary IgG accumulation before calving. The objective of this study was to investigate arteriovenous difference as a measure for mammary IgG uptake throughout the dry period. From d −58 ± 2.9 before calving, parity 2 (n = 10) and 3 (n = 5) Holstein cows were fed controlled energy pre- and postpartum rations. Serum samples were collected from the tail vein (A) and milk vein (V) on d −49, −35, −21, −7 relative to expected calving, every 4 d until actual calving, and in the first milking interval after calving. Total milk vein blood flow was measured by Doppler ultrasound. Colostrum was collected within 1.4 ± 0.76 h of calving and cows were milked again at 12.1 ± 0.61 h. Colostrum and serum were analyzed for IgG via radial immunodiffusion. Mammary uptake of IgG was estimated as arteriovenous concentration difference (AVD) × serum flow rate. Data were evaluated using PROC GLIMMIX of SAS considering repeated effects of time and the random effect of cow. Tail vein IgG concentrations increased 19.1% ($P = 0.024$) from wks −9 to −3 then decreased 42.3% ($P < 0.01$) 1 d before calving. AVD of IgG at wk −8 (−1.34 ± 0.74) and −1 (−1.47 ± 0.68) were lower than 0 ($P < 0.07$), indicating net IgG output from mammary glands to blood at these time points. At all other time points, mean AVD of IgG were negative but not different from 0 ($P > 0.44$). Based on colostrum and milking-2 yields, mammary IgG accumulation was a total of 769 ± 199 g. Assuming mammary IgG accumulation is active 7 d before calving, the expected daily mammary IgG accumulation was approximately 100 g/d, on average. The SE of observed IgG uptake fluctuated between 3,616 g/d at wk −5 and 1886 g/d at milking 1 which makes detection of uptakes near 100 g/d difficult. However, the significantly negative AVD suggest that colostral IgG have an intramammary origin and are not taken from serum.

Key Words: mammary arteriovenous difference, immunoglobulin G, colostrum

2031 Dietary crude protein is independent of rumen microbial community ecology in lactating dairy cows. M. Liao*[1], A. Ramirez[2], J. Duncan[1], K. Alward[1], C. Owens[1], L. Campos[1], M. Hanigan[1], and R. Cockrum[1], *[1]Virginia Tech, Blacksburg, VA, [2]Universidad Austral de Chile, Valdivia, Chile.*

High-protein diets maximize dairy cow production, but reduced crude protein (CP) diets are also effective, improving economics, nitrogen efficiency, and environmental impact. This study aimed to examine the effect of dietary CP on rumen microbial dynamics in lactating dairy cows using shotgun metatranscriptome analysis. We hypothesized that decreased dietary CP would have minimal effect on rumen composition and function. Fistulated lactating Holstein cows (n = 4,806 ± 38 kg of body weight, 260 ± 62 d in milk, and 26.5 ± 12.0 kg milk/d) were randomly distributed to 2 dietary treatments differing in dietary CP concentrations: 16.6% CP (Control) versus 13.2% (lowCP; Table 1). lowCP was formulated to fulfill all nutrient needs and maximize nitrogen efficiency through tailored post-ruminal amino acids supply according to the National Academies of Sciences, Engineering, and Medicine (NASEM, 2021) model. The main amino acids content in the diets was sourced from ingredients such as soybean meal, canola meal, distillers grains, and blood meal. A crossover design with 2 periods (18 d each) was implemented with a 2-wk washout between periods. A permutational multivariate ANOVA (PERMANOVA) test was used to evaluate differences in the microbial community and function in RStudio. The results showed a similarity in the rumen microbial community ($P = 0.24$) and function ($P = 0.13$). This suggests that decreased dietary CP does not alter the balance of rumen microorganisms in the rumen. This study will contribute significantly to the understanding of rumen microbial ecology and its implications for dairy cow feed management.

Key Words: crude protein, rumen microbiome, shotgun metatranscriptomic

2032 Impact of breed and colostrum intake on IgG kinetics in Holstein and Holstein-Angus crossbred bulls. H. McCarthy*[1], T. Chapelain[1], M. Kovacs[1], D. L. Renaud[1], J. M. Leão[2], and M. A. Steele[1], *[1]University of Guelph, Guelph, ON, Canada, [2]Saskatoon Colostrum Company Ltd., Saskatoon, SK, Canada.*

Beef calves report higher serum immunoglobulin G (IgG) compared with dairy calves; however, little is known with regard to crossbred dairy calves. The objective of this study was to explore the effect of colostrum intake and breed on IgG kinetics in Holstein and Holstein-Angus crossbred bulls. Holstein (n = 12/TRT) and crossbred (n = 12/TRT) bulls

Table 1 (Abstr. 2031). Phenotypic performance in different diet groups

Item[1]	Low CP (Mean ± SE)	Control (Mean ± SE)
DMI	20.31 ± 3.42	22.01 ± 3.44
MY	21.21 ± 5.53	25.79 ± 5.51
RFI	−0.20 ± 2.43	0.14 ± 2.44
REI	5.61 ± 4.71	5.21 ± 4.75

[1]DMI = dry matter intake; MY = milk yield; RFI = residual feed intake; REI = residual efficiency index; REI = net energy intake − (milk energy + maintenance energy + body tissue energy + pregnancy energy).

were assigned to 1 of 2 diets for the 0 h and 12 h meals: low colostrum (LOW; 2.5 g of IgG/kg birth weight; BW) or high colostrum (HIGH; 5 g of IgG/kg BW). Calves received milk replacer fed at 6% BW at 24 h, 36 h, and 48 h. Calves were catheterized at birth and blood samples were taken hourly from 0 h to 6 h, every 2 h from 6 h to 12 h, and at 15 h, 18 h, 21 h, 24 h, 36 h, and 48 h post-colostrum feeding to assess serum IgG concentrations and average efficiency of absorption (AEA). At 24 h, calves were dosed with chromium and blood sampled every 2 h from 0 h to 12 h postdosing to estimate gut permeability (GP). All data were analyzed using the GLIMMIX procedure in SAS 9.4. Serum IgG concentrations were increased ($P < 0.01$) in the HIGH calves and there was no impact ($P = 0.17$) of breed, yet there was an interaction of diet and breed, where crossbred LOW calves had increased ($P = 0.04$) serum IgG concentrations when compared with Holstein LOW calves. LOW calves had increased ($P = 0.03$) AEA; however, AEA was not impacted ($P = 0.28$) by breed. Crossbred LOW calves tended to have increased ($P = 0.08$) AEA compared with Holstein LOW calves. HIGH calves and crossbred calves had reduced ($P = 0.02$ and 0.04, respectively) GP. These results suggest that increased colostrum allowance leads to increased serum IgG concentrations; however, breed does not appear to impact serum IgG concentrations. The breed by diet interactions in IgG concentrations and AEA suggest that crossbred calves may have a physiological mechanism that allows them to better absorb IgG when fed low levels of colostrum; however, more research must be done to further understand this difference.

Key Words: dairy beef, colostrum, IgG absorption

2033 Effects of supplementing a feed additive containing bioactive peptides on performance in Holstein cows. M. Nehme Marinho*, T. H. R. Souza, J. Rasia, T. M. Adeoti, Z. Sarwar, M. Perdomo, and J. E. P. Santos, *University of Florida, Gainesville, FL.*

Objectives were to assess the effects of supplementing a feed additive containing bioactive peptides on production performance in dairy cows. Eighteen multiparous cows at 65 ± 14 d postpartum were enrolled in a sextuplicate 3 × 3 Latin square design with treatment sequence balanced for carry-over. Cows were ranked by energy-corrected milk (ECM) yield and assigned to one of the 6 squares. Cows within square were assigned to a treatment sequence with treatments top-dressed onto the diet once daily at 0 (T0), 1 (T1), or 2 (T2) g/kg of diet DM. The feed additive contained 50% of 16 different bioactive peptides extracted from a blend of 8 cereals. Each experimental period lasted 28 d with data collection from d 22 to 28. Cows were milked thrice daily and dry matter intake (DMI), body weight (BW), and milk yield and composition were measured daily (Table 1), whereas body condition was scored (BCS) in the last 3 d of each period. Data were analyzed fitting mixed-effects models and orthogonal polynomial contrasts evaluated the linear (T0 vs. T2) and quadratic (T1 vs. [1/2 T0 + 1/2 T2]) effects of the amount of feed additive supplemented. Supplementing bioactive peptides linearly increased the content and yield of milk fat which resulted in increases in all 3 groups of milk fatty acids. True protein yield tended to increase quadratically with treatment; however, treatment did not affect DMI, BW (729 vs. 726 vs. 727 ± 13 kg), BCS (3.15 vs. 3.08 vs. 3.12 ± 0.06), true protein content, and urea N in milk (9.9 vs. 10.2 vs. 9.9 ± 0.4 mg/dL). Supplementing bioactive peptides improved milk fat yield that resulted in a 4.4% increase in yield of ECM.

Key Words: dairy cow, bioactive peptides, production

Table 1 (Abstr. 2033). Effect of supplemental bioactive peptides on performance of dairy cows

	Treatment				*P*-value	
Item	T0	T1	T2	SEM	Linear	Quadratic
DMI, kg/d	26.8	26.7	26.8	0.6	0.96	0.85
Milk, kg/d	52.3	52.8	52.5	0.9	0.70	0.21
ECM, kg/d	47.6	49.7	49.6	1.0	0.02	0.09
ECM/DMI, kg/kg	1.80	1.89	1.86	0.06	0.32	0.25
Fat, %	2.91	3.07	3.13	0.14	0.01	0.41
Fat, kg/d	1.50	1.63	1.63	0.06	0.01	0.12
Fatty acids, kg/d						
<16 C	0.38	0.41	0.41	0.02	0.01	0.10
16 C	0.51	0.56	0.56	0.02	0.02	0.09
>16 C	0.46	0.50	0.51	0.02	0.004	0.19
Protein, %	2.77	2.80	2.80	0.04	0.20	0.37
Protein, kg/d	1.45	1.48	1.47	0.04	0.14	0.08

2034 Impact of protein source and provision day of the different milk replacer on growth, health, and feeding behavior of Holstein CAlves. N. Noroozi*[1], G. Berzoini Costa Leite[1], A. Hooshyar[2], S. Kargar[2], M. Kanani[2], and M. Inácio Marcondes[1], *[1]Department of Animal Sciences, Washington State University, Pullman, WA, [2]Department of Animal Science, School of Agriculture, Shiraz University, Shiraz, Fars, Iran.*

Whether derived from animal protein (AP) or plant protein (PP), the choice of milk replacer impacts growth, performance, and health of calves. This study investigated the effect of milk replacer protein sources and the provision day (PD), starting at d 4 or d 24 of life, on the above-mentioned metrics. A total of 58 female Holstein calves housed in individual pens were assigned to 4 treatment groups: calves receiving AP milk replacer from d 4 (#1), and from d 24 (#2); calves receiving PP milk replacer from d 4 (#3), and from d 24 (#4). Calves starting with milk replacer at d 24 were fed raw milk from d 4 to 24. Regardless of treatment, all the calves received liquid feed from d 1 to 55, were weaned gradually from d 55 to d 61, and were evaluated until d 91. Nutrient intake, body weight (BW), and diarrhea incidence were measured. Results were analyzed by a randomized block design in a 2 × 2 factorial scheme with repeated measures. Significant interactions were observed between PD and time in BW ($P = 0.001$) and between protein source and time in BW ($P = 0.004$). Regardless of the PD, calves fed with AP exhibited higher body measurements than those fed with PP. We observed similar average daily gain (ADG) for all animals until d 24; however, when animals started milk replacer intake, regardless of the protein source, a reduction in ADG was observed. By d 90, all calves exhibited comparable body measurements, regardless of the PD. Significant interactions were observed in starter dry matter intake between PD and time ($P = 0.048$), and between protein source and time ($P = 0.010$). Significant interactions were noted in ADG between PD and time ($P = 0.013$) and between protein source and time ($P = 0.003$). Our results revealed an enhancement in feed efficiency, and average daily gain in calves fed with AP. We did not see any difference between the treatments on the incidence of diarrhea; however, calves fed with PP had a longer duration of diarrhea. We concluded that providing calves with milk replacers derived from AP improves growth, health, and performance.

Key Words: dairy management, nutritional planes, preweaning period

2035 The impact of maternal nutrition on calf performance during the weaning phase. K. R. Oliveira*, A. P. Oliveira Neto, A. L. Silva, S. E. F. Guimarães, P. T. R. Salgado, L. E. G. Souza, and P. P. Rotta, *Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*

A calf born to a malnourished heifer may encounter challenges, impeding the realization of its maximum genetic potential and resulting in financial losses. Our objectives are evaluating the effects of maternal nutrition on calf performance. We hypothesized that calves from dams with high average daily gain (ADG) during gestation would demonstrate enhanced performance during the weaning phase. Sixteen 3/4 Holstein × Gyr calves were included in this study, with their dams randomly assigned to 2 distinct treatments: (1) moderate ADG during pregnancy (0.37 kg; MOD) or (2) high ADG during pregnancy (0.72 kg; HIG). Colostrum, constituting 15% of their body weight (BW) with a Brix of 25%, was administered within the first 2 h of life. From birth to weaning, the calves received 6 L of transition milk for the initial 2 d, followed by an equivalent amount of raw milk. Commencing on d 3, the calves were granted ad libitum access to starter, and hay was introduced at d 40. Blood samples and BW measurements were collected on d 1, 31, 61, and 91 to evaluate animal performance, IGF-1, and glucose concentrations. Data were subjected to analysis using the MIXED procedure of SAS (SAS Institute Inc., 2008), there were submitted to Tukey test with a significance level of 0.05 and the trend was declared at $0.05 < P \leq 0.10$. No interaction ($P > 0.10$) was observed between varying performance of dams and the days of life of calves concerning ADG. Similarly, no interaction ($P > 0.10$) was found between dam ADG during gestation and intake of starter or hay, or the dry matter intake of their calves. IGF-1 exhibited a day effect ($P < 0.01$), with concentrations increasing from birth until weaning, while glucose concentration decreased ($P < 0.01$). No differences ($P > 0.10$) were noted in albumin, globulin, total protein, and urea. The 3/4 Holstein × Gyr calves from dams with high or moderate ADG during gestation did not demonstrate differences in development during the weaning phase. These calves exhibited similar intake, ADG, and metabolites levels, suggesting that they were capable of achieving comparable performance at the time of weaning.

Key Words: fetal programming, calves, performance

2036 Whole cottonseed and fatty acid supplementation impact milk fatty acid yields and plasma metabolites and hormones during the immediate postpartum in dairy cows. J. E. Parales-Giron*, J. M. dos Santos Neto, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of whole cottonseed (WCS) and fatty acid (FA) supplementation on milk FA yields and plasma metabolites and hormones of early-lactation cows. Fifty-two multiparous cows were used in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Dietary treatment were fed from 1 to 24 DIM and contained 0% or 10% DM WCS (NC and CS) and 0% or 1.5% DM supplemental FA (LF and HF). The FA supplement was a Ca-salt containing 60% palmitic and 30% oleic acid. Treatment diets were formulated to contain 18% CP, 21% forage NDF, and 24% starch. The statistical model included the random effect of block, cow within block and treatment, Julian date, and the fixed effects of WCS, FA supplementation, time, and their interactions. Results are presented in the following order: NCLF, CSLF, NCHF and CSHF. WCS increased the yield (g/d) of preformed FA (799, 962, 920, 988; $P < 0.01$), C18:0 (201, 287, 228, 288; $P < 0.01$), and *t*11 C18:1 (14.1, 21.6, 15.7, 20.8; $P < 0.01$), tended to increase the yield of *c*9, *c*12 C18:2 (42.6, 48.5, 45.3, 48.1; $P = 0.07$), but did not affect de novo or mixed FA ($P \geq 0.36$). FA supplementation increased the yields of mixed FA (556, 603, 664, 656; $P = 0.09$) and C16:0 (515, 563, 615, 617; $P < 0.01$), decreased de novo FA (406, 422, 365, 349; $P < 0.01$), but had no effect on preformed FA ($P = 0.11$). The only interaction we observed between WCS and FA supplementation was for the yield of C4:0, with FA only increasing it in a diet without WCS (60.2, 71.9, 71.4, 72.2; $P = 0.09$). WCS increased the concentration of plasma BHB (11.3, 12.8, 9.17, 11.5, mg/dL; $P = 0.04$) but did not affect insulin or NEFA ($P \geq 0.58$). FA supplementation did not affect plasma insulin, NEFA, or BHB ($P > 0.12$). WCS and FA interacted to modify the yield of C4:0. WCS increased the yield of preformed FA, C18:0, and t11 C18:1. FA supplementation increased the yields of C16:0 and mixed FA and decreased de novo FA. Finally, WCS increased plasma BHB, but FA supplementation did not affect plasma metabolites and hormones.

Key Words: whole cottonseed, early-lactation, fatty acid

2037 Effects of rumen-protected His at different levels of metabolizable protein under adequate Met and Lys supply on production and blood metabolites in lactating cows. K. Park*[1], J. Kim[1], H. Hu[2], M. Kambara[3], K. Nakagawa[3], and C. Lee[2], [1]*Interdisciplinary Nutrition Program, Department of Animal Sciences, The Ohio State University, Wooster, OH,* [2]*Department of Animal Sciences, The Ohio State University, Wooster, OH,* [3]*Ajinomoto Co., Inc, 1-1 Suzuki-Cho, Kawasaki-Ku, Kawasaki-Shi, Kanagawa, Japan.*

Balancing for limiting amino acids may prevent reduced production when deficient metabolizable protein (MP) is provided. The objective of the study was to determine the effects of rumen-protected (RP) His supplementation at different levels of MP on production when Lys and Met supplies meet the requirements. Sixty lactating cows (days in milk, 112 ± 27; milk yield, 50 ± 8 kg/d) were blocked by parity and days in milk, and randomly allocated to one of the following diets: a MP-adequate diet that meets the MP, Met, Lys, and His requirements (HCP; CP, 17.1%); a MP-moderately deficient diet supplemented with RP-Met and RP-Lys that meets 90% of MP, 105% of Met and Lys, and 90% of His required (LCP+LM; CP, 15.6%); LCP+LM diet supplemented with RP-His to meet the His requirement (LCP+LMH; CP, 15.6%); a MP-deficient diet supplemented with RP-Met and Lys that meets 80% of MP, 105% of Met and Lys, and 81% of His required (LLCP+LM; CP, 14.5%); LLCP+LM diet supplemented with RP-His to meet the requirement of His (LLCP+LMH; CP, 14.5%) according to NASEM (2021). All cows were fed HCP for 2 weeks for covariate followed by 6 weeks of the experimental period. Blood samples were collected in the last week of the experiment and analyzed for blood metabolites. Data were analyzed using PROC MIXED of SAS. Milk yield (45 ± 0.6 kg/d), DMI (27 ± 0.4 kg/d), and body weight (676 ± 18 kg) were not affected by treatments. Milk fat and protein concentrations and yields did not differ among treatments. Blood urea-N decreased

($P < 0.01$) as MP supply decreased but was not affected by RP-His. Blood hemoglobin was lower for LLCP+LM ($P < 0.05$) compared with HCP and LCP+LM, but the decrease was eliminated with RP-His (i.e., LLCP+LMH vs. HCP and LCP+LM). In conclusion, deficient MP supplies did not decrease milk and component yields when Met and Lys supply met the requirements. Although production was not affected, RP-His supplementation increased erythrocyte parameters in blood, suggesting that additional His supply supported body His reserves when MP supply was deficient.

Key Words: metabolizable protein, histidine, methionine

2038 Effect of antral age of the ovulatory follicle on fertility of lactating dairy cows. A. Santos*, T. Minela, L. F. Danrat, and J. R. Pursley, *Michigan State University, East Lansing, MI.*

Reproductive management strategies must maximize P/AI at first service and minimize pregnancy losses to increase the longevity of lactating dairy cows (Middleton et al., 2019). This study was designed to assess the effect of different antral ages of the ovulatory follicle (OF) at first AI on ovarian dynamics and fertility of lactating Holstein cows. We hypothesized that differences in antral age of the OF before timed-AI may lead to differences in fertility of lactating dairy cows. Cows were randomly assigned to 3 different period lengths of pre-ovulatory follicle development. Lactating cows were synchronized to d 6 of the estrous cycle using Ovsynch. On d 6 following the 2nd GnRH of Ovsynch, synchronized cows received a third treatment of 100 µg of GnRH to initiate a new follicular wave and 1 mg of cloprostenol sodium to regress corpora lutea 5 (D5), 7 (Control), or 9 (D9) d later. Cows then received a final GnRH 56 h after final cloprostenol and received AI 16 h later. Ovarian dynamics were evaluated with transrectal ultrasonography. Only cows that were synchronized to Ovsynch and had ovulation to both the 3rd and final GnRH were used in this study ($n = 321$). Pregnancy was diagnosed on d 34 post-AI. Data were analyzed using the MIXED (continuous) and GLIMMIX (binomial) procedures in SAS® 9.4. D9 reduced ovulation rates to the final GnRH (82%) compared with Controls (96%) and D5 (100%). Five cows in D9 had spontaneous ovulation before the final GnRH compared with 0% in both D5 and Control. D5 had a greater occurrence of multiple ovulations (20%) than Control and D9 (9 and 7%). D5 reduced the diameter of the OF in cows with single ovulation (13.60 ± 0.2 mm) compared with Control (15.63 ± 0.2) and D9 (15.98 ± 0.2). D9 (43%) and D5 (45%) reduced P/AI on d 34 compared with Control (59%). These results revealed notable differences in ovulation rates, number of ovulation, and diameter of OF when modulating the antral age of the OF before timed-AI. It was clear that antral age of the ovulatory follicle is a limiting factor in fertility of lactating dairy cows.

Key Words: ovulatory follicle, dairy cow, fertility

2039 Effects of partially replacing soybean meal with sprouted peas or sprouted soybean on ruminal fermentation in vitro. A. C. S. Vicente*[1], G. K. Salas-Solis[1], M. U. Siregar[1], M. L. Johnson[1], J. E. P. Santos[1], J. Rohrer[2], and A. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*RP Nutrients, Inc., East Troy, WI.*

The objective of this study was to evaluate the effects of partially replacing soybean meal (SBM) with sprouted peas or sprouted soybean on ruminal fermentation in a batch culture experiment. A complete randomized block design was used, with 3 runs (blocks) and 3 treatments. The treatments tested were a control diet (CON) with SBM at 20.8% of the diet; and 23% replacement with sprouted peas (SP) or 30% replacement with sprouted soybean (SS). The basal diet was formulated to meet the requirements of a 700 kg Holstein dairy cow producing 43 kg/d of milk with 3.8% fat and 3.3% protein. All diets had a similar nutritional composition (17% CP; 31% NDF; 42% NFC-DM basis). Diets were weighed (0.5 g of DM) into Ankom bags, that were placed in glass serum bottles and incubated in buffered ruminal fluid for 24 and 48 h. Gas pressure was measured at 0, 3, 6, 9, 12, 24, 36, and 48 h to estimate kinetics of gas and methane production. At 24 and 48 h the bottles were opened to measure pH, DM, OM, and NDF degradability; NH_3-N concentration; and total gas and total methane production. Statistical analysis was performed with the mixed procedure of SAS, data using time points were analyzed using repeated measures. The treatment effect was depicted using multiple comparisons, the P-values were adjusted using Tukey correction, and significance was declared when $P \leq 0.05$. At 24 h, there was an effect of treatment on DM degradability and total gas, in which CON had the lowest degradability (57%) and gas production (318.9 mL), with no effects on pH. At 48 h, there was an effect of treatment on pH and total gas, in which CON had the lowest pH (6.44) and total gas production (454.3 mL), with no effects on DM degradability. Preliminary data indicates that sprouted ingredients appear to have the potential to partially replace SBM, improving DM degradability and pH, but increasing total gas production. Further analyses are needed to assess the effects of these ingredients on ruminal fermentation, nutrient utilization, and ruminal microbial protein growth.

Key Words: gas production, methane, ruminal pH

ADSA-USD Competition: Original Research Poster Presentations

2040 Lactose-free ice cream: Evaluation of the enzyme kinetics and physiochemical properties. N. S. Bolinger*[1], D. R. Stroinski[1], A. Lokken[1], A. Gruman[1], G. E. Lewis[1], S. Rankin[2], and C. Nicholson[2], [1]*University of Wisconsin–River Falls, River Falls, WI,* [2]*University of Wisconsin–Madison, Madison, WI.*

To properly digest lactose, β-galactosidase (or lactase), an enzyme secreted by the epithelial cells in the small intestine, must hydrolyze lactose into its component sugars: β-D-glucose and β-D-galactose. Without this enzyme, lactose cannot be broken into its component sugars in the small intestine, allowing lactose to resist digestion until the large intestine and causing a variety of uncomfortable gastrointestinal symptoms. This research aims to analyze the kinetics of β-galactosidase in ice cream mix at varying temperatures (5°C, 15°C, 25°C, 35°C, 45°C, 55°C) and sucrose concentrations (0%, 6%, 12%) as a means of establishing economically suitable conditions for lactose hydrolysis. Ice cream mixes (13% fat, 10% milk-solids-not-fat) of differing sucrose concentrations (0, 6, or 12%) were prepared and incubated at varying temperatures (5–55°C) with β-galactosidase (0.01% vol/wt). To evaluate the hydrolysis of lactose into glucose, ice cream mixes were tested every 30 min with a ReliOn Premier Blood Glucose Monitor over a 4-h time span. The rate of lactose hydrolysis varied with both temperature and sucrose concentration. For temperature variations, lactose hydrolysis was found to occur fastest at 45°C, resulting in 443.7 ± 32.5 mg/dL glucose after 4 h for the mix with 0% sucrose. Comparatively, at 5°C and 55°C for the same mix, this hydrolysis was slower with 156.3 ± 36.1 mg/dL and 27.7 ± 3.1 mg/dL glucose after 4 h, respectively. With no sucrose, lactose hydrolysis occurred at a relatively fast rate compared with the sucrose-containing samples. For example, at 45°C the glucose concentrations of the 0% and 12% sucrose samples were 443.7 ± 32.5 mg/dL and 174.7 ± 20.2 mg/dL after 4 h, respectively. These results reflect the impact temperature and sucrose concentration have on lactose hydrolysis, indicating the possibility of altering lactose hydrolysis processing parameters to optimize the efficiency of ice cream production. The next steps in this research involve the evaluation of lactose-free ice cream produced with maximized β-galactosidase efficiency.

Key Words: lactose-free ice cream, lactase, sucrose

2041 Response of mid-lactation Holsteins cows to the supplementation of rumen-protected methionine during the summer. V. A. de Oliveira*[1], C. R. Monteiro[1], L. H. Silva[1], G. M. de Souza[1], T. L. Resende[2], J. Petrini[3], and M. A. C. Danes[1], [1]*Department of Animal Science, Federal University of Lavras, Lavras, MG, Brazil,* [2]*Evonik Industries, Sao Paulo, SP, Brazil,* [3]*Instituto Clínica do Leite, Piracicaba, SP, Brazil.*

Heat stress has a negative impact on dairy cow performance and health status. Methionine (Met) is an essential amino acid involved in protein synthesis and antioxidant function that can help alleviate the negative effects of heat stress. Our objective was to evaluate the effect of rumen-protected Met (RPM) supplementation on milk production and composition in lactating dairy cows during the summer. The experiment was carried out in randomized blocks for 9 weeks in Brazil. Eighty primiparous Holstein cows (43.7 ± 4.0 kg/d of milk and 182 ± 63 DIM) were paired by milk yield and DIM and distributed into a diet either without or with 0.75 g/kg of RPM (Mepron®, Evonik Industries) in the TMR. Cows were housed in a compost barn and were divided into 2 pens (one pen per treatment). Milk yield and composition were measured in wk 3, 6, and 9. The model included treatment, week and week × treatment interaction as fixed effects, in addition to block and cow as random effect. Least squares means were compared using Tukey's test. Significance was declared when $P \leq 0.05$ and tendency with $0.05 < P \leq 0.10$. During 68.4% of the time, the temperature-humidity index was greater than 68. Supplementation with RPM increased production of milk (44.9 vs 42.9 kg/d, $P < 0.01$), protein (1,464 vs. 1,414 g/d, $P = 0.03$), lactose (2,109 vs. 2,001 g/d, $P = 0.03$) and total solids yields (5,331 vs. 5,155 g/d, $P = 0.03$). Energy-corrected milk (42.2 vs. 40.7 kg/d, $P = 0.06$) and casein yield (1,156 vs. 1,121 g/d, $P = 0.06$) tended to increase for the RPM treatment. Fat yield was not affected by RPM supplementation (1,316 vs. 1,269 g/d, $P = 0.36$), but there was an interaction ($P = 0.02$) between treatment and week for fat content. For the RPM treatment, fat content was greater in wk 9 compared with wk 3, while for the control treatment it remained constant. This was an interesting finding, since the heat increased throughout the experiment (THI average of 69, 72, and 76 for wk 3, 6, and 9, respectively). The inclusion of RPM in the diet resulted in greater milk and milk protein production, indicating greater productive resilience of cows during the summer.

Key Words: heat stress, amino acid, primiparous

2042 Development of whey protein-lignin based film materials for dairy food packaging applications. Y. Deng*, S. Kolodjski, G. Lewis, and Y. Kim, *University of Wisconsin–River Falls, River Falls, WI.*

Whey protein (WP) and lignin are by-products of cheese production and the pulp and paper industry, respectively. This project aims to combine WP, glycerol, and lignin to develop biodegradable films for food packaging applications. WP solutions underwent denaturation at temperatures between 60°C and 90°C with glycerol and lignin. SDS-PAGE showed that the film-forming solution prepared at 90°C resulted in 88.7% WP denaturation, 51.1% for WP made by mixing 20°C and 90°C in a 1:1 ratio, and 12.3% for WP prepared at 60°C. Results revealed that WP denaturation increased available thiol groups, which were likely responsible for forming the polymeric structure of WP films. With altered polymeric structures, different levels of WP denaturation altered film properties such as water vapor permeability, water solubility, glass transition temperature, and mechanical strength. Water solubility, glass transition temperature, puncture force, and tensile strength were inversely correlated with the percent denaturation of WP. The 90°C WP film had the highest tensile strength ($5.77 \pm 0.\ 31\ \text{N.mm}^{-2}$), and puncture force ($67.57 \pm 6.88\ \text{N.mm}^{-1}$). The addition of lignosulfonate and alkaline lignin (at 5% wt/wt of WP) provided antioxidant functionality equivalent to 6.25 ± 1.00 mg ascorbic acid/g film and 6.71 ± 1.12 mg ascorbic acid/g film, respectively. The thermal stability of WP films was improved with the addition of lignin as indicated by the increased glass transition temperature (T_g with alkaline lignin = 77°C, T_g with lignosulfonate = 80°C vs. T_g of 90°C WP film = 62°C) Adding lignosulfonate improved water barrier properties by 10%. WP films with alkaline lignin demonstrated improved light barrier properties than those with WP only. Overall, the results highlight the importance of WP denaturation in film properties and the potential benefits of different types of lignin for improving film properties.

Key Words: whey protein, lignin, food packaging

2043 Wildfire-PM2.5 triggers immune responses in dairy calves.

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Wildfire smoke is a courier for respiratory pollutants such as particulate matter (PM$_{2.5}$). Wildfires are common in the western United States, where many dairy calves are raised. PM$_{2.5}$ in wildfire smoke may be hazardous to dairy calf health, as they are born immuno-naive. Previously, our group has observed lung consolidation, systemic inflammation, and metabolic responses triggered by PM$_{2.5}$ inhalation in calves. With a focus on gene expression alterations in peripheral blood mononuclear cells (PBMC), this study aims to elucidate the underlying mechanisms of inflammation triggered by wildfire-PM$_{2.5}$. PM$_{2.5}$ and meteorological data were acquired from a local monitoring station to calculate temperature-humidity index (THI). Daily PM$_{2.5}$ concentrations were traced to active wildfires using HYSPLIT modeling. Holstein heifers (n = 17) were sampled from birth to 90-d. Rectal temperature (RT), heart rate (HR), respiration rate (RR), and health scores were taken 3× weekly. On a subset of calves (n = 13), blood was taken before and during smoke exposure. Immune cell populations and serum albumin were analyzed, and PBMC were isolated to assess the expression of *IFN-γ* and *IL1-β* using RT-PCR. Data were analyzed in SAS. RT-PCR data were analyzed using *t*-tests to assess relative changes in expression before and during smoke exposure. All other variables were analyzed using mixed models with calf as a random effect, and lags of up to 7 d with PM$_{2.5}$, THI, and interactions as fixed effects. PM$_{2.5}$ reached 113.5 μg/m^3. RT and HR were greater but RR was reduced, with concomitant elevated PM$_{2.5}$ and THI ($P \leq 0.01$). PM$_{2.5}$ and THI interactions increased eye scores, but reduced cough scores ($P \leq 0.02$). PM$_{2.5}$ and THI interactions increased eosinophil and basophil counts ($P \leq 0.04$). Serum albumin, neutrophil, monocyte, and lymphocyte concentrations were lower until lag d-3 but then increased until lag d-6 ($P \leq 0.04$). No difference was detected in PBMC expression of either *IFN-γ* or *IL1-β* relative to smoke exposure ($P = 0.26$ and $P = 0.15$, respectively). Although a systemic inflammatory response in calves was observed after exposure to PM$_{2.5}$, these changes do not appear to be mediated by *IFN-γ* or *IL1-β* gene expression in PBMCs.

Key Words: smoke, Holstein, inflammation

2044 Processing-induced caffeine and taurine encapsulation for energy drink and nutraceutical applications.

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Taurine is a sulfur-containing β-amino acid abundant in the milk of mammals, and caffeine is a relatively hydrophobic and naturally occurring stimulant of the central nervous system. Encapsulation of these compounds with milk proteins has the potential to slow their release and absorption, as well as potentially increase their bioavailability. The objectives of the present work were (1) to develop a standardized procedure for determining taurine and caffeine concentration via fluorescence spectroscopy and (2) to apply high-pressure homogenization (HPH) and emulsifying salts (ES) to skim milk to alter protein structures and enhance interactions between milk proteins and target compounds (i.e., taurine and caffeine). Using fluorescence methodologies, successful standard curves were developed for the concentrations of 0.12 to 25.17 μ*M* taurine and 0 to 100 μ*M* caffeine ($R^2 = 0.9984$ and $R^2 = 0.9837$, respectively). For encapsulation potential, the intrinsic fluorescence associated with hydrophobic amino acids ($\lambda_{ex} = 275$ nm, $\lambda_{em} = 300$–500 nm) was monitored before and after taurine/caffeine addition. Specifically, a decrease in fluorescence was associated with target compounds (i.e., caffeine, taurine) interacting with the fluorescent amino acid residues, reflecting an enhanced encapsulation potential. A 20% skim milk "control" sample was prepared and compared with a "processed" sample containing 20% skim milk and 100 m*M* ES and processed using HPH at 300 MPa. All samples were prepared in triplicate. After evaluating these samples, "processed samples" were shown to have more amino acids exposed for interaction with caffeine based on the difference in intrinsic fluorescence peak height before and after caffeine addition (difference = 459.79 ± 26.70) compared with the control (difference = 205.83 ± 19.56). This interaction between milk proteins and caffeine provides insight into the feasibility of milk protein-based caffeine encapsulation, with the potential to use this process for novel, value-added food ingredients with continued research.

Key Words: casein, encapsulation, high-pressure homogenization

2045 The salty chemistry on anhydrous milk fat and corn oil.

T. Katz*, S. Ginsburg, and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

Fats and oils play a crucial role in flavor and function. Fatty acids are an important dietary component providing both energy and vitamins. However, the type of fat used can have a drastic impact on human health. In recent years, consumption of polyunsaturated fatty acids (PUFA), which can come from plant oils, has increased due to their health benefits. Due to these health benefits, many plant oils have seen incorporation into butter blends, resulting in a more spreadable butter. Combining plant-based oils, and salts with anhydrous milk fat (AMF) alters the structure of the butter fat, allowing for unique chemical characteristics, ultimately producing a more desired product for the consumer. While oils such as canola and olive have been studied, the ability to use corn oil to create a blend is still to be discovered. Corn is a major crop and product within the United States, however, ironically, since it is so widely used it is also a major waste product. To minimize waste and use waste product of the corn industry, the oil derived could be used in the dairy industry. In the presence of corn oil, additional nucleation of the AMF occurs creating larger and more defined butter crystals, while also decreasing the amount of energy required for melting and crystallization ($P < 0.05$). Additionally, the presence of corn oil reduces β crystal formation while retaining β prime and α crystals. However, the addition and presence of salts in AMF promotes β crystal formations, sometimes rendering the butter harder. The presence of salts in butter oil and butter-corn oil blends increased the size and number of prominent crystals. This study uses analytical techniques to gain a greater understanding of the chemistry-based changes that occur when calcium chloride, sodium chloride, and corn oil are added to butter. This work is imperative to understand how these additives affect AMF for future consumer applications.

Key Words: butter, oil, salt

2046 Effects of weekly access to a playpen on growth, intake, and standing behavior of individually housed preweaning dairy calves.

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In the US dairy industry, dairy calves are commonly raised individually before weaning. Literature supports the idea of group housing; however, alternatives should be explored in the chance that social housing cannot be achieved on dairy farms. The objective of this study was to measure the effect of weekly access to a playpen on the growth, intake, and standing behavior of calves before and during weaning. Calves were assigned to either a Solo (S; n = 17) or Play Group (PG; n = 17, 3–4 calves/play group) treatment beginning at 3 weeks of age. The playpen was 24 × 12 ft and contained a plastic chain, ball, and hay as enrichment items. Calves in the PG treatment were moved to the playpen for 3 to 4, 90-min sessions per week. Grain and milk intake were measured daily, and body weights were measured weekly from wk 2 to 10 of age. Data loggers were used to measure standing behavior, throughout the observation period. Data were analyzed in SAS using a mixed model. Body weight was similar between S and PG calves before weaning, but during weaning (wk 8 to 10) PG calves weighed more (73.4 ± 1.4 vs. 68.1 ± 1.4 kg; $P = 0.01$). Grain intake did not differ between treatment groups except during wk 10, when S calves were observed to consume about 300 g/d more grain than PG calves ($P = 0.02$). Among PG calves, as age increased, time spent consuming hay in the playpen increased ($P < 0.001$), which may help to explain their higher body weights at weaning compared with the S calves. There were no differences in the standing behavior of S and PG calves throughout the observation period ($P \leq 0.64$); across the 10-week observation period, calves spent on average 396 ± 10 min/d standing and had 18 ± 0.4 standing bouts/d. Additional research is necessary to determine if providing opportunities for socialization via a playpen can serve as an effective alternative to group housing calves. Future investigation on this subject will aim to explore how calves in each treatment transition to group living after weaning.

Key Words: behavior, playgroups, dairy calves

2047 A burning issue: Effects of in utero wildfire-PM$_{2.5}$ exposure on dairy calf growth and metabolism.

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Wildfires produce smoke, which contains pollutants such as particulate matter (PM$_{2.5}$). Wildfire-PM$_{2.5}$ affects lactating dairy cows and neonatal calf metabolism. Wildfire-PM$_{2.5}$ has also been linked to lower birth weights and greater preterm births in humans. However, the effects of intrauterine wildfire-PM$_{2.5}$ exposure on calves are unknown. To investigate this, calves exposed to wildfire-PM$_{2.5}$ in utero (WFS, n = 17) were monitored from birth to 7 mo and compared with calves not exposed to wildfire-PM$_{2.5}$ *in-utero* (CON, n = 26). Every 2 wks, calf growth and metabolic parameters were analyzed. Diet and management was the same between groups. Plasma concentrations of B-hydroxybutyrate (BHB), glucose, nonesterified fatty acids (NEFA), and cortisol were measured. Statistical analysis was performed in SAS. Cortisol concentrations at birth were compared between groups using *t*-tests. For all other variables, mixed models were used with time, treatment, and their interactions as fixed effects, and calf within time as a repeated effect. WFS dams were exposed to smoke for 2 wks during mid-gestation. WFS calves had lower wither height at wk 6, 24, and 26 ($P \leq 0.02$) compared with CON. Hip height was higher at birth for WFS (5.07 ± 0.15cm vs. 4.42 ± 0.11, $P = 0.0004$) relative to CON, but lower at wk 14 (4.64 ± 0.13cm vs. 5.40 ± 0.13cm, $P < 0.0001$). Cortisol tended to be lower in WFS than CON at birth ($P = 0.1$). Glucose was greater for WFS from 4 wk to 12 wk ($P \leq 0.005$), and lower at 16 wk (90.04 ± 5.11 mg/d vs. 103.4 ± 4.21mg/d, $P = 0.04$) compared with CON. BHB was lower in WFS calves for wks 14, 18, 20, and 22 ($P \leq 0.006$), but greater at 26 wk ($P < 0.0001$) relative to CON. No difference was detected in NEFA concentrations or average daily gain between groups. These results suggest that in utero wildfire-PM$_{2.5}$ exposure affects dairy calf weight and metabolism postnatally. Future work should investigate if the performance of prenatally exposed calves is impaired later in life.

Key Words: air quality, blood metabolites, livestock

2048 Optimization of casein micelle nanoparticle formation using high-pressure homogenization and emulsifying salts.

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Casein proteins, accounting for 80% of milk protein, are known to form a loosely bound aggregation called a micelle. The casein micelle is highly stable against typical food processing procedures (e.g., pasteurization, homogenization). This stability along with the propensity to orient itself with a hydrophobic core makes casein ideal for many applications, but this functionality can be further enhanced with the dissociation of the micelle structure and exposure of this hydrophobic core. This research aims to evaluate the dissociation of casein micelles following emulsifying salt-based dissociation coupled with high pressure homogenization (HPH). Two emulsifying salts, sodium hexametaphosphate (SHMP) and sodium citrate (SC), were evaluated at varying concentrations (0–100 m*M*) in skim milk solutions (20% wt/wt) without HPH and at varying HPH treatments (100–300 MPa). The absorbance (at 400 nm) and intrinsic fluorescence (λ_{ex} = 285 nm, λ_{em} = 300–400 nm) of the samples were assessed using a spectrophometer and spectrofluorometer, respectively. Using these methods, a lower absorbance was indicative of casein micelle dissociation, whereas a higher intrinsic fluorescence value suggested the exposure of more hydrophobic amino acid residues. The control sample, containing just skim milk, had an initial absorbance of 0.850 ± 0.018 and fluorescence of 432.96 ± 10.6. Without HPH, samples with 100 m*M* SHMP exhibited an absorbance of 0.111 ± 0.010 and a fluorescence of 770.71 ± 42.2. The application of HPH in addition to SHMP furthered this casein micelle dissociation ($P = 0.008$). Specifically, at the highest level of HPH (300 MPa), samples with 100 m*M* SHMP had absorbance values of 0.069 ± 0.006. With this extensive dissociation, these samples were found to be highly functional in foaming applications, with ca. 2-fold more foam expansion than the control. These results indicate that, while higher concentrations of emulsifying salts are efficient at dissociating the casein micelle, dissociation can be further optimized using HPH, and this dissociation has many functional applications.

Key Words: high-pressure homogenization, casein, encapsulation

Animal Behavior and Well-Being 1

2049 Familiarity influences social proximity for some groups of dairy calves. K. C. Burke*, K. N. Gingerich, and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Social relationships play an important role in cattle, however research is lacking on the social development of group-housed calves. The aim of this study was to assess the influence of prior familiarity on social interactions of dairy calves in group pens. Within the first 48 h of birth, Holstein calves were housed in pairs of consecutively born calves. At 15.7 ± 2.2 (mean ± SD) days of age, consecutively enrolled pens of pair-housed calves were moved to group pens (10 calves/pen including 5 former pairs; 5.9 m^2/calf). Using continuous positional data obtained from a real time location tracking system, non-directed weighted matrices representing the social networks of 9 groups of calves (55 heifers and 32 bulls) were constructed using frequency of pair-wise interactions (within 1 m) over consecutive 4-d periods per week, aggregated over 6 weeks. To determine if calves' association was related to prior familiarity, we labeled dyads of calves "1" if they were previous pair housed penmates and "0" if they were not, and used MRQAP-DSP tests (which perform regressions with matrices) to test whether the assigned dyad-level explanatory variable explained the structure of the dependent matrix. Separate tests were run for each group with 10,000 permutations. The results indicated that prior pair assignment was a significant predictor of the proximity networks in 3 of the 9 groups (group 5: $R^2 = 0.19$, $P = 0.002$; group 7: $R^2 = 0.12$, $P = 0.01$; group 8: $R^2 = 0.42$, $P < 0.001$), indicating that dyads of calves previously housed in the same pair pen before grouping had more associations. We did not find any significant relationships between prior pair assignment and the proximity networks in 6 of the 9 groups (group 1: $R^2 = 0.06$, $P = 0.10$; group 2: $R^2 = 0.02$, $P = 0.33$; group 3: $R^2 = 0.04$, $P = 0.15$; group 4: $R^2 = 0.03$, $P = 0.25$; group 6: $R^2 = 0.01$, $P = 0.37$; group 9: $R^2 = 0.01$, $P = 0.39$). Overall, these results suggest that prior familiarity may partially explain some proximity interactions, but that social network structure of group-housed dairy calves varies considerably between pens.

Key Words: social networks, group housing, social behavior

2050 Associations between health status and social network centrality in group-housed dairy calves. K. N. Gingerich*, K. C. Burke, F. P. Maunsell, and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Evidence across species suggests that the expression of social behavior may relate to health status. The objective of this study was to assess how social network centrality in group-housed dairy calves is associated with clinical and sub-clinical bovine respiratory disease (BRD). At 2 weeks of age, calves (n = 90; 58 heifers and 32 bulls) were group housed (9 groups; 10 calves/group) and received 8 L/d milk via an automated feeder. Calves were weaned over 10 d beginning at 47 ± 2.5 d of age. Using continuous positional data obtained from a real time location tracking system, non-directed weighted matrices representing the social networks of each group were constructed using the frequency of pair-wise interactions (within 1 m) over consecutive 4-d periods per week. Twice weekly, we recorded health scores (Wisconsin calf health scoring chart) to characterize clinical BRD. Thoracic ultrasounds were performed weekly to detect sub-clinical cases of BRD (lung lesion >1 cm). We tested effects of BRD status (clinical, sub-clinical, none) on social network centrality measures (strength, sum of an individual's connections, and eigenvector centrality, sum of the centralities of an individual's direct and indirect connections) during preweaning (wk 4–6 of age) and weaning (wk 7–8 of age), in general linear mixed models including week of age, sex, body weight upon grouping, and calf and group as random effects. Preweaning BRD status (mean weekly prevalence: 34% clinical, 11% sub-clinical) was unrelated to social network centrality ($P > 0.2$). During weaning, when BRD prevalence was numerically greater (44% clinical, 20% sub-clinical), eigenvector centrality was negatively related to severity of disease (0.91 vs. 0.90 vs. 0.86; none vs. sub-clinical vs. clinical; SE = 0.01; $P < 0.01$ for clinical vs. none; $P = 0.03$ for clinical vs. sub-clinical; $P = 0.1$ for sub-clinical vs. none). Strength centrality did not differ by BRD status. These results suggest a relationship between social behavior and BRD status in group-housed calves which depended on stage of development.

Key Words: bovine respiratory disease, social behavior, health

2051 The impact of a maternal bovine appeasing substance on productivity and treatment frequency in Holstein dairy calves after caustic paste disbudding. A. Hajny*[1], J. A. Spencer[1,2], D. Duhatschek[1], J. Piñeiro[1,3], C. Daigle[1], T. Hairgrove[1], and J. Cleere[1], [1]*Texas A&M University, College Station, TX,* [2]*Texas A&M AgriLife, Stephenville, TX,* [3]*Texas A&M AgriLife, Amarillo, TX.*

To investigate the impact of a pheromone (MBAS; maternal bovine appeasing substance) and pain mitigation on productivity (ADG) and disease prevalence, dairy calves from 3 dairies were weighed upon arrived at a heifer rearing ranch (d 0) and were disbudded according to one of 4 randomly assigned disbudding protocols: (1) CON (n = 30) disbudding using caustic paste only; (2) MBAS (n = 27) disbudding 24 h after topically administering 5 mL of MBAS (FerAppease®) above muzzle and 5 mL behind poll; (3) AA-CON (n = 25): disbudding using caustic paste 10 min after administering pain mitigation (AA: analgesic (3.3 mg/kg BW transdermal flunixin meglumine) and anesthetic (2 mL per horn bud of 2% Lidocaine); (4) AA-MBAS (n = 28) administering MBAS and AA at respective time points relative to disbudding. BW was measured on d 0, 14, and 28 and ADG calculated. Frequency of treatments for health concerns (e.g., pneumonia, diarrhea, bloat, and naval infection) from d 0 to 14 relative to disbudding were recorded. The impact of pain mitigation (AA), pheromone (MBAS) and their interaction were analyzed using a Generalized Linear Mixed Model (PROC GLIMMIX) that included farm and arrival BW as covariates and a random effect of calf within treatment. Least squared means corrected with Bonferroni's method was used to identify pairwise differences ($P < 0.05$) and tendencies ($P < 0.10$). The d 0–14 ADG (lb) was greater ($P = 0.03$) for MBAS (1.14 ± 0.08) calves compared with CON (0.9 ± 0.08), AA-CON (0.9 ± 0.09), and AA-MBAS (1.0 ± 0.08) calves. Calves receiving MBAS treatment tended ($P = 0.09$) to have the greatest ADG throughout the study. The percentage of calves that received treatment during d 0 to 14 did not differ among treatments (CON 60%; MBAS 56%; AA-CON 56%; AA-MBAS 61%). In this study, MBAS had a positive effect on ADG potentially due to MBAS threat-reducing properties, regardless of pain mitigation, during the first 14 d after disbudding. These results suggest that pheromones have the potential to improve calf welfare by minimizing the negative effects of disbudding. Funded by FERA Diagnostics & Biologicals.

Key Words: maternal bovine appeasing substance, disbudding, calf

2052 Mitigating castration pain in cattle: A systematic review and meta-analysis. E. Nogues*, J. Stojkov, B. Jonoska Stojkova, M. A. G. von Keyserlingk, and D. M. Weary, *The University of British Columbia, Vancouver, BC, Canada.*

Castrating cattle is a common painful procedure, but there is a lack of consensus on which methods of pain control are most effective. We conducted a systematic review and meta-analysis of studies using 3 common methods for castrating cattle: surgical, elastration, crushing. Studies were eligible for inclusion if they included a control group (no pain mitigation) and at least one treatment group (local anesthetic alone, or in combination with a non-steroidal anti-inflammatory drug), and authors reported at least one of these outcomes: cortisol, body weight gain, foot stomping, wound licking, a subjective assessment of pain (visual analog scale), or stride length. Completed in March 2023, our search identified 383 publications. Two reviewers independently screened all publications, identifying 17 as eligible. Surgical castration techniques were the most frequently researched (14 publications), and blood cortisol was the most frequently reported outcome (15 publications). We conducted a risk of bias assessment on each outcome for all eligible studies; none were assessed as low risk. Only one study stated that observers were blind to the analgesia treatment. Only one study reported blinding for outcome assessors. Meta-analysis could only be performed on blood cortisol after surgical castration, as too few data were available for other outcomes and methods. We used a 3-level random effect model fitted at 1, 3, 4, 6, 12, and 24 h after castration, with cortisol concentration as response variable and treatment as fixed effect. Multimodal analgesia reduced blood cortisol concentrations in the first hour following surgical castration in comparison to the control. This effect diminished at 3 and 4 h after castration, and was no longer evident beyond this time. Overall, studies varied greatly in their choice of castration method, analgesia, outcomes, and time points, hindering the comparison of results. Replication studies consolidating current trends are needed to provide evidence-based recommendations for effective pain control, but we encourage considering negative pain controls using multimodal analgesia following best practices for other procedures.

Key Words: castration, pain mitigation, cortisol

2053 Investigating dairy calf behavior after being fed electrolytes. M. Longer*[1], G. Figueroa[2], M. Woodrum Setser[2], D. L. Renaud[1], J. H. C. Costa[3], and K. C. Creutzinger[2], *[1]University of Guelph, [2]University of Wisconsin-River Falls, [3]University of Vermont.*

Electrolytes are often used as a source of hydration instead of milk for preweaning calves; however, the impact of a single electrolyte meal in place of milk on the calf's subsequent behavior is unclear. Therefore, the objective of this study was to investigate the effect of feeding preweaning dairy calves electrolytes or milk on their lying behavior and activity. Healthy preweaning dairy calves (n = 69) were enrolled at 23 ± 1 d of age and outfitted with an automated accelerometer. At 0500 h, calves were fed 2.7 L of either a novel electrolyte solution (252 mOsm/L) or milk at 39.5°C via bucket. Calves were excluded from analysis if they did not eat at least 20% of their morning meal. Testing occurred twice per week over a 2-week period with each calf receiving 2 milk feedings and 2 electrolyte feedings in random order as their morning meal. Calves were individually housed in hutches and had ad libitum access to water and starter. The accelerometer data, including motion index, steps (no.), lying time (m), and lying bouts (no.) during the 8 h period between feedings (0600 h to 1800 h), were summarized on each test day. One-way ANOVAs were used to determine if locomotor activity was different between treatments. Compared with calves that were fed milk, calves that received electrolytes took fewer steps (89.2 ± 3.0 vs. 65.7 ± 3.6 steps; $P < 0.01$), had a lower motion index (568.8 ± 15.7 vs. 452.2 ± 19.2; $P < 0.01$), and spent more time lying (390.2 ± 3.5 vs. 369.7 ± 5.0; min $P < 0.01$). Interestingly, there was no difference in the number of lying bouts between the 2 groups ($P = 0.11$). The results of this study suggest that providing electrolytes in replacement of a milk meal even one time may cause calves to be more fatigued as they were less active between feedings compared with calves that received milk.

Key Words: electrolyte, dairy calf, hunger

2054 The effect of meloxicam administration on the behavior of transported surplus dairy calves. M. Longer*[1], C. K. Creutzinger[2], H. Goetz[1], and D. L. Renaud[1], *[1]University of Guelph, Guelph, Canada, [2]University of Wisconsin–River Falls, River Falls, WI.*

Transportation introduces surplus calves to novel stressors that can affect them both physiologically and behaviorally. While there is evidence that behavioral differences exist due to transport, there has been little work on ways to mitigate those differences. Therefore, the objective of this study was to investigate the effects of meloxicam, a non-steroidal anti-inflammatory drug, on the movement and lying behavior of surplus calves following transportation. Surplus dairy calves (n = 160) were enrolled upon arrival to a single calf raising facility in Ontario where they were outfitted with an automated accelerometer and randomly assigned to receive an injection of either meloxicam (0.5 mg/kg body weight SQ) or saline with treatments balanced for sex. Daily lying time (h/d), transition bouts (no./d), steps (no./d), and motion index were continuously recorded starting 1 d after transport and ending 12 d following transport. The data were summarized into 24 h time periods by day and changes in the behaviors over time were evaluated using a mixed model with repeated measures. Calves that received meloxicam spent less time lying down than control calves (−0.34 h, $P = 0.01$, 95% CI −0.61 to −0.08). However, there was no difference in the total number of transition bouts, steps, or total motion index between treatment groups. Interestingly, male calves performed fewer transitions than female calves (−4.79 bouts, $P < 0.01$, 95% CI −8.08 to −1.50). The results of this study suggest that providing meloxicam minimizes fatigue as the calves spent more time standing after transport potentially indicating that they were able to recover quicker from the transport stressors however, the mechanism requires further exploration.

Key Words: veal calf, fatigue, transport

2055 Effects of pair-housing and season on dairy calf health and growth. J. Bonney-King*[1], E. E. Lindner[1], C. M. M. Bittar[2], and E. K. Miller-Cushon[1], *[1]University of Florida, Gainesville, FL, [2]University of São Paulo, Piracicaba, SP, Brazil.*

Given variable previous findings regarding effects of dairy calf social housing on health, the objective of this study was to examine interactive effects of social housing and season on calf performance. Within 24 h of birth, Holstein heifer calves were randomly assigned to either individual (IH, n = 50 calves) or pair-housing (PH, n = 50 pairs). The enrollment of calves occurred during warm (May–October) and cool seasons (November–April) in the subtropical climate in north-central Florida. Calves were provided 8 L/d milk replacer (2×/d feeding) and were weaned gradually over 10 d beginning at 42.6 ± 2.1 d of age (mean ± SD). Onset and duration of scouring was determined by daily visual inspection using the Wisconsin Calf Health Scoring Guide (score 2 or 3 indicating presence of scours). Calves were weighed at birth and at the beginning and end of weaning. Data were analyzed at the pen level (data averaged across pair-housed calves) in general linear models with fixed effects of housing, season, and their interaction. The duration of scouring (100% prevalence) did not differ between treatments ($P = 0.7$) but was greater during the warmer season (9.2 vs. 7.2 d, warm vs. cool; SE = 0.6; $P = 0.01$). The age at first scouring event was subject to an interaction between housing treatment and season (PH = 6.6 [warm] and 9.4 [cool], IH = 6.5 [warm] and 6.9 [cool] days of age; SE = 0.5; $P < 0.01$), where PH calves began scouring later during the cooler season, as well as overall effects of housing ($P = 0.02$) and season (scouring began later in cool season; $P = 0.04$). The difference in the onset of scours between pair-housed calves within the same pen was 1.5 ± 0.2 d (mean ± SD). Housing treatment did not affect weight gain before weaning ($P = 0.8$) but PH calves tended to have greater weight gain during weaning (0.6 vs. 0.5 kg/d; PH vs. IH; SE = 0.04; $P = 0.08$). These findings contribute to evidence suggesting that early-life social housing does not appear to impair, and may enhance, calf health and performance, and provide novel evidence to support possible interactive effects between season and social housing on calf health which warrant further exploration.

Key Words: social housing, performance, welfare

2057 Effects of preweaning social housing on behavior of pregnant dairy heifers experiencing a housing change. D. A. Clein, E. E. Lindner, J. Bonney-King, and E. K. Miller-Cushon*, *University of Florida, Gainesville, FL.*

In this study, we characterized effects of preweaning social housing on behavior of pregnant dairy heifers upon initial introduction to a freestall barn. Heifers raised during the preweaning period in individual (IH; n = 20) or paired pens (PH; n = 20 heifers; 1 focal animal/pair) were mingled between housing treatments following weaning and subsequently raised and managed according to standard farm procedures. At 4 weeks before expected calving, heifers were introduced to a freestall barn and behavior was recorded continuously for 24 h beginning at 0000 h on the day after introduction, to characterize feeding time, stall usage, and locomotor activity. Observed heifers were balanced across seasons (cool [November to April] or warm [May to October]) and varying stocking densities (low [≤75%], medium [>75%, <100%], or high [≥100%]). Data were analyzed in general linear models with fixed effects of treatment, stocking density, season, and 2-way interactions. Heifer age and body weight were considered for model inclusion with the more significant, if either, retained; age did not differ (669 d of age; SE = 10.9; $P = 0.64$), and BW was slightly greater for PH heifers (670.9 vs. 632.8 kg; PH vs. IH; SE = 15.3; $P = 0.078$). Stall use and feeding behavior were subject to interactions between previous housing and stocking density. At high stocking density, IH heifers spent more time in the stall (48.7 vs. 30.8 min/h; IH vs. PH; SE = 3.6; $P = 0.015$) due to longer average stall visits ($P = 0.0078$) and spent less time feeding (3.8 vs. 8.9 min/h; IH vs. PH; SE = 0.9; $P = 0.014$). Previously IH heifers were more likely to be displaced from a stall (7.4 vs. 3.0% of visits were displaced; IH vs. PH; SE = 1.4; $P = 0.044$). Locomotor activity was subject to interactions between treatment and season; in the cool season, IH heifers spent less time walking (2.3 vs. 1.0 min/h; IH vs. PH; SE = 0.3; $P = 0.02$) whereas activity did not differ in the warm season ($P > 0.7$). These results suggest that preweaning social housing had long-term effects on behavior and ability to adapt to a novel environment which became most apparent under heightened competitive pressure.

Key Words: social behavior, pair housing, long-term welfare

2058 Association of temperament with retrospective growth performance in Nili Ravi buffalo heifers. S. K. Gorsi and M. Q. Shahid*, *Department of Livestock Management, University of Veterinary and Animal Sciences, Lahore, Pakistan.*

The present study aimed to investigate the association between the temperament of dairy buffalo heifers and retrospective growth rates. The study enrolled 84 Nili Ravi buffalo heifers aged 18 to 24 mo and assessed chute score and exit velocity to categorize their temperament. Scoring was conducted twice with a 7-d interval. Retrospective data on average daily weight gain were retrieved from farm records, which were recorded every month after birth. After the second scoring, blood samples were collected to measure cortisol levels. Temperament was classified as calm (= 3) or nervous (>3) based on the chute score and exit velocity. The chute score ranged from 1 to 5, with 1 indicating standing very calm and 5 indicating frightened jumping in the chute. Exit velocity was measured in meters per second. The average of these 2 measures was used to determine the temperament of the heifers. To assess the association between temperament and retrospective growth performance, the collected data were subjected to ANOVA using the MIXED procedure of SAS. The temperament trait, age group of heifers, season of birth, and year of birth were considered as fixed effects, with birth weight as a covariate. The results indicated that 48 heifers were classified as calm, while 36 were classified as nervous. Calm heifers had an average daily gain 240 g/day higher than nervous heifers during the post-weaning period (4–6 mo of age; $P < 0.001$). Similarly, from 6 to 12 mo of age, calm heifers exhibited a higher average daily gain (420 g/d) compared with nervous heifers (230 g/d; $P < 0.001$). However, this difference disappeared at the age of 18–24 mo ($P = 0.144$). The cortisol level of calm heifers tended to be lower (0.96 μg/dL) than the nervous heifers (1.27 μg/dL; $P = 0.11$). Calm dairy heifers demonstrated superior performance during the post-weaning period. This highlights the potential significance of temperament in breeding programs aimed at enhancing growth rates and welfare.

Key Words: temperament, buffalo heifers, average daily gain

2767 Hiding behavior of dairy calves kept with their dams for the first week of life. A. Roussac*[1], H. B. Spitzer[1], R. K. Meagher[2], and K. L. Proudfoot[1], [1]*University of Prince Edward Island, Charlottetown, PE, Canada,* [2]*Dalhousie University, Truro, NS, Canada.*

In a natural environment, newborn calves are often hidden in tall grass or tree cover during the first few days of life. Indoor-housed dairy calves do not face the same natural pressures as those outdoors, so their hiding tendencies are unknown. The objective was to describe the use of a hiding space provided to indoor-housed dairy calves kept with their dams during the first week of life. A total of 15 calves (9 female and 6 male) were given a U-shaped hiding area, created using 3 plastic panels (107 × 76 cm each). To date, data from 6 calves (5 female, 1 male) have been analyzed; the remaining calves will be analyzed before data are presented. Behavioral data were collected continuously from video during d 1 to 7 of life, including bouts and duration of time that calves spent with any part of their bodies in the hide (“inside hide”) and time spent within one calf-length of the hide (“near hide”). Duration of time spent lying in these locations was recorded. Descriptive statistics were calculated for hide use and lying time across the week. To describe individual variability in hide use, an area under the curve (AUC) was calculated for the duration of time each calf spent inside the hide across the week. Overall, calves were inside the hide for 22% of their day (mean = 319 min/d; range = 50 to 711; SD = 249) across 11 bouts/d (range = 6 to 19; SD = 6). They spent an additional 17% of their day near the hide (240 min/d; range = 52 to 474; SD = 177) across 28 bouts (range = 18 to 34; SD = 7). Hide use over the week was highly variable between calves (inside the hide AUC = 1,980; range = 210 to

4,530; SD = 1,641), as some calves used the hide often during the first few days and reduced hide use over time, whereas others increased their hide use over the week. Calves spent 27% of their daily lying time inside the hide (range = 3 to 63%; SD = 22%) and 17% of their lying time near the hide (range = 0 to 35%; SD = 15%), suggesting that some calves used the hide for resting. These preliminary data provide initial evidence that some dairy calves valued the presence of a hide during the first week of life, although the use of the hiding space was inconsistent between and within calves.

Key Words: hide, isolation, seclusion

Animal Health 1

2059 Effect of postbiotic *Saccharomyces cerevisiae* fermentation product on experimentally induced digital dermatitis infections in Holstein Friesian steers. D. Döpfer[1], S. Ordaz Puga[2], M. Aviles[1], S. Henschel[1], J. Buettner[1], M. Henige[1], I. Yoon[3], G. Dawson[3], J. Wheeler[3], and K. Anklam*[1], [1]*School of Veterinary Medicine, University of Wisconsin, Madison, WI,* [2]*College of Veterinary Medicine, University of Minnesota, St. Paul, MN,* [3]*Diamond V, Cedar Rapids, IA.*

Bovine digital dermatitis (DD) is a disease encountered worldwide causing severe lameness in cattle. This debilitating disease is characterized by circumscribed ulcero-proliferative lesions typically located on the plantar aspect of the hoof. The consequences of DD are decreased animal welfare and economic loss due to reduced milk production, decreased reproductive performance and premature culling. The objective was to evaluate the efficacy of a postbiotic *Saccharomyces cerevisiae* fermentation product (SCFP, NutriTek®, Diamond V, Cedar Rapids, IA) supplemented in the diet in reducing the number of experimentally induced M2 (ulcerated/active) DD lesions in Holstein Friesian (HF) steers. A blind complete randomized design study was conducted using a DD infection model with 49 HF steers (24 Control and 25 SCFP). Pellets containing no supplement (Control) or 12 g/d NutriTek (SCFP) were fed in the steers' respective total mixed ration for 49 d before induction of DD. After 7 d of hydropic skin maceration under exclusion of oxygen with a wrap, the hind feet of steers were inoculated with homogenate of bovine DD lesions. Hooves remained wrapped until clinical signs of DD were observed at pre-determined time points until 4 weeks post challenge. Data analysis was performed using R and the relative risks along with their 95% confidence intervals were calculated. All 49 steers developed M1 (early focal bacterial keratolysis of the epidermis) or M2 lesions on at least one hind foot. Results indicated that the relative risk for developing experimentally induced M2 lesions in the Control group was 1.53 (1.09–2.15, 95% CI) times greater ($P < 0.05$) compared with the SCFP group. *Treponeme* spp. were detected by polymerase chain reaction and invasive spirochetes were observed by histology in skin biopsies of the experimentally induced DD lesions. These results suggest that postbiotic SCFP supplementation provides a protective effect on the development of M2 lesions in dairy steers. Enhanced protection from DD is advantageous to the longevity, performance, and well-being of cattle.

Key Words: digital dermatitis, postbiotic, M2 lesions

2060 Evaluation of an automated lameness detection system's accuracy in identifying cows with foot lesions. N. Siachos*, A. Anagnostopoulos, B. E. Griffiths, J. M. Neary, R. F. Smith, and G. Oikonomou, *Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, United Kingdom.*

Our aim was to evaluate the performance of an automated lameness detection system in identifying cows bearing foot lesions (CattleEye Ltd., Belfast, UK). A veterinarian (VET) was present during 46 trimming sessions in 5 dairy farms (milking herd size range: 1,000–2,800). The VET recorded the presence and graded the severity of any sole hemorrhage (SH), sole ulcer (SU), white line (WL), toe ulcer (TU), digital dermatitis (DD), and interdigital phlegmon (IP) cases. Cows were binary classified, with those bearing at least one case of severe SU, severe WL, any TU, DD stage M2 and severe IP, being considered as having potentially painful lesions. From an initial data set of 2,212 cows with lesions records, 2,008 cows were matched with the weekly average scores produced by the system (CE). The VET also performed several mobility scoring sessions using a 0-to-3, 4-grade system (scores 2 and 3 were considered as lame). From the lesions records dataset, 654 cows were also assigned mobility score by the VET (MAN) 1 to 3 d prior trimming. Lameness prevalence in the cows with a CE and a MAN was 29.4% and 27.2%, respectively. Confusion matrices were created to calculate accuracy (ACC), sensitivity (SE) and specificity (SP) for both CE and MAN in predicting the presence of severe cases, using VET records as the gold standard. SE and SP were also calculated for the presence of severe SH, any SU, severe WL, and for DD stage M2, separately; only cases without severe coexisting lesions were considered as negative controls. Overall, ACC, SE and SP of CE and MAN in predicting the presence of severe cases were 0.72, 0.53 and 0.75, and 0.77, 0.60 and 0.80, respectively. Both CE and MAN obtained lower SE in primiparous cows (0.23 and 0.33, respectively). The CE and MAN could predict the presence of severe SH, any SU, severe WL, and DD stage M2, with SE/SP combinations of 0.41/0.76 and 0.50/0.83, 0.51/0.76 and 0.62/0.83, 0.57/0.76 and 0.67/0.83, and 0.45/0.76 and 0.37/0.83, respectively. Our results suggest that CE can detect the presence of foot lesions with an adequate accuracy, comparable to that of an experienced human scorer.

Key Words: artificial intelligence, hoof lesions, mobility

2061 Use of machine learning techniques to detect subclinical mastitis using temperature and milk variables. B. Shrestha*, R. Neupane, and S. Paudyal, *Department of Animal Science, Texas A&M University, College Station, TX.*

The objective was to evaluate the potential for the use of a thermal imaging system for identification of subclinical mastitis in dairy cows. Multiparous Holstein dairy cows within 150 d in milk were enrolled in the study at a commercial dairy farm in central Texas. Milk samples were collected from each quarter separately and evaluated for somatic cell counts (SCC; n × 1,000), fat (%), protein (%), lactose (%), solid not fat (SNF %), Beta-hydroxybutyrate (BHBA, m*M*/L), acetone (m*M*/L), and milk urea nitrogen (MUN; mg/dL). A minimum of 4 thermal images per cow representing all 4 quarters were captured using a handheld infrared camera (Fluke TiS 75+®) from 1-m distance from the cow at the milking parlor and after the morning milking during February 2023 to minimize the effect of daily ambient temperatures. The surface and skin temperatures from the udder (maximum [Max], minimum [Min], average [Avg] were extracted using Fluke Connect® software). A total of 776 quarter-level milk samples and corresponding udder images were obtained, and 10 images were discarded from analyses due to lack of appropriate resolution and focus. The data were loaded into the Padas Dataframe in Python Programming Framework and subjected to Random Forest Regression (RFR) and Classification (RFC) using Sklearn library. The RFR models had r^2 of 0.43, 0.27, 0.43, for prediction of LogSCC using milk and temperature extracted variables for subclinical mastitis-infected quarters ($SCC > 250,000$; $n = 399$), healthy quarters ($n = 377$), and all quarters, respectively. Subsequently, for the prediction of subclinical mastitis utilizing both temperature and milk variables, RFC models had accuracy and f1 scores of 0.76 and 0.74, with high feature importance to Lactose% and SNF%. However, when the model was tested with only temperature features, it had accuracy and f1 of 0.50 and 0.53, respectively, with high model importance to average and minimum temperature extraction variables. These findings conclude that thermal imaging is a promising tool for subclinical mastitis detection when used with other milk variables and relevant machine-learning techniques.

Key Words: mastitis, thermal-imaging, machine-learning

2062 *Streptococcus uberis*: Subtyping and analysis of genotypic and phenotypic antimicrobial resistance.

A. Romanò*, L. Vazquez Rojo, L. Egger, and H. U. Graber, *Agroscope, Bern, Switzerland.*

Streptococcus uberis is a common environmental pathogen causing clinical and subclinical mastitis in cattle. Although clinically very relevant, *S. uberis* is still poorly characterized in terms of subtypes and its genotypic and phenotypic antimicrobial resistance (AMR) profiles. To identify the genotypes and address the AMR issue, 109 strains isolated from clinical bovine mastitis from 6 different Swiss districts were analyzed. All strains were subjected to whole genome sequencing (Illumina) and tested for phenotypic AMR using the minimum inhibitory concentration (MIC) assay following the Comitè de l'Antibiogramme de la Sociètè Française and EUCAST breakpoints. The WGS data were used for subtyping the strains by MLST (PubMLST), for evaluating their genotypic AMR profile (Resfinder, CARD, AMRFinder Tool and Resistance Gene Database) and their mobile genetic elements (open-source bioinformatics tools). With a total of 80 different sequence types (ST), the number of subtypes was high. Thirty-five STs were present in the PubMLST database, 45 were new. The strains were attributed to 3 different ST complex (ST5 complex 32%, ST86 complex 4%, ST143 complex 2%) but for the majority of the strains, no ST complex was defined. Seventy strains (64%) were susceptible to all the 23 antibiotics tested. Thirty-nine strains were resistant to cephalosporins (2%), lincosamides (12%), macrolides (12%), and tetracycline (33%). Eleven strains (10%) were resistant to 3 different classes of antibiotics (multi-drug resistant strains). All strains, however, were sensitive to penicillin. Regarding genotypic AMR analysis, 15 AMR genes (ARG) were detected with most ARG related to tetracycline resistance (*tetL*, *tetM*, and *tetO*). In 16 strains, the presence of tetracycline ARG was associated with the occurrence of the *repUS43* plasmid integrated on a transposon indicating a possible transmission among *S. uberis*. The present study nicely explains the observed phenotypic tetracycline resistance of *S. uberis* at the genomic level and demonstrates a non-understood phenomenon of a high ST diversity.

Key Words: Streptococcus uberis mastitis, antimicrobial resistance (AMR), WGS

2063 Effects of a transdermal nonsteroidal anti-inflammatory drug on productive and inflammatory responses of Holstein cows challenged intramammarily with *Escherichia coli*.

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The objectives were to determine the effects of a non-steroidal anti-inflammatory drug (i.e., flunixin transdermal) on local and systemic markers of inflammation and productive responses of Holstein cows challenged intramammarily with *Escherichia coli*. Eighty-eight cows (272 ± 89.2 DIM), free of clinical mastitis, and negative for gram-negative bacterial growth in milk samples from individual quarters were enrolled in the experiment. At enrollment (d −11; d 0 = day of challenge), cows were blocked by parity. Within block, cows were assigned to one of 3 treatments: PCON – one quarter infused with sterile PBS (n = 30), NCON – one quarter challenged with *E. coli* + one quarter infused with sterile PBS (n = 29), and TFM – one quarter challenged with *E. coli* + one quarter infused with sterile PBS + flunixin transdermal 12 h after the challenge (n = 29). The bacterial strain used (*E. coli* P4, USDA) resulted in moderate/severe cases of clinical mastitis. Milk samples were obtained before each milking and blood samples and rectal temperature (RT) were collected after each milking from 0 to 168 h relative to the challenge. Data were analyzed by ANOVA. Average milk yield in the 7 d preceding challenge were NCON = 33.6 ± 0.6, PCON = 33.2 ± 0.6, TFM = 33.7 ± 0.6 kg/d. Treatment tended ($P = 0.10$) to affect *E. coli* counts in challenged quarters (NCON = 5.1 ± 0.5, TFM = 4.0 ± 0.5 logN cfu/mL). Treatment had ($P = 0.03$) an effect on somatic cell score of challenged quarters (NCON = 7.7 ± 0.2; TFM = 7.2 ± 0.2). Treatment did not ($P = 0.46$) affect bovine serum albumin concentration in the challenged quarter. Treatment with transdermal NSAID affected ($P < 0.01$) RT, milk yield, and haptoglobin concentration but these effects were dependent on time relative to challenge. Treatment with flunixin transdermal reduced markers of inflammation among cows intramammarily challenged with *E. coli*.

Key Words: mastitis, milk production, nonsteroidal anti-inflammatory drug (NSAID)

2064 Effect of chitosan on viability of bovine immune and mammary epithelial cells and neutrophil chemotaxis.

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Infusion of chitosan into mammary gland after dry-off was reported to induce immune cell infiltration. But, the mechanism remains unclear. We aimed to evaluate the effect of chitosan on viability of bovine immune and mammary epithelial cells and neutrophils chemotaxis in vitro. Blood was collected from 6 heathy mid-lactation Holstein dairy cows to isolate peripheral blood mononuclear cells (PBMC) and neutrophils. Monocytes were isolated from PBMC using plastic adhesion. Non-adherent cells were centrifuged to obtain lymphocytes. MAC-T, an established clonal cell line produced from primary bovine mammary alveolar cells, was used in this study. Monocytes, lymphocytes and MAC-T were cultured with different concentrations of chitosan (340, 34, 3.4, 0.34, 0.034, 0 μg/mL) and LPS (100, 10, 1 μg/mL). Cells were harvested at 1, 3, 6, 12 h to assess viability via ATP assay, and supernatant collected for cytokine analysis. Neutrophil chemotaxis in response to chitosan (340, 34, 3.4, 0.34, 0.034, 0 μg/mL) was determined via a Transwell chamber using 100 ng/mL bovine interleukin 8 as positive control. Viability assays were repeated 3 times for each cell type and neutrophil chemotaxis assays were performed on 6 cows. Data were analyzed using mixed procedure of SAS. Regardless of time, lymphocytes stimulated with 340 μg/mL chitosan had greater ($P = 0.01$) viability than other concentrations. In contrast, chitosan has no effects on viability of monocytes ($P = 0.57$). The viability of MAC-T stimulated with 340 μg/mL chitosan decreased ($P \leq 0.01$) as the culture time increased. Similar time effects were not observed for other chitosan concentrations. Addition of LPS did not affect the viability of lymphocytes, monocytes, or MAC-T ($P \geq 0.07$). The migration rate of neutrophils was similarly enhanced by both interleukin-8 and 340 μg/mL chitosan (61% vs. 48%, $P \leq 0.01$), but not by chitosan of other concentrations ($P \geq 0.36$). In conclusion, chitosan with high concentration improves bovine lymphocyte viability and induces neutrophils chemotaxis but inhibits MAC-T activity in vitro.

Key Words: chitosan, immune cell, chemotaxis

2065 Proteomics analysis reveals changes in plasma protein abundance of dairy cows from pre- to postpartum.

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The study aimed to assess the changes in plasma proteomic profiles, identifying biological signatures associated with the transition from prepartum to postpartum in dairy cows. Six multiparous Holstein dairy cows were enrolled in the study, and all cows received the same close-up diet from −21 DIM until calving (1.59 Mcal/kg of DM and 14.6% CP) and the same lactation diet from calving until 30 DIM (1.82 Mcal/kg of DM and 18.4% CP). Cows were housed in a compost-bedded pack barn before calving, then moved to a freestall barn after calving. Cows were fed once a day and blood samples were collected from the coccygeal vein in the morning before the feeding at −10 (PRE) and 7 (POST) days relative to parturition. After blood collection, the lithium heparin tubes were placed on ice, and the plasma was obtained by centrifugation at 1,300 × *g* for 15 min at 4°C. Three hundred micrograms of protein from each sample was precipitated and duplicate injections of 5 µg of the resulting peptide mixture were analyzed utilizing an LC-MS/MS DDA method on an Orbitrap Fusion Lumos Tribid mass spectrometer. Data were analyzed using a generalized mixed model followed by a comparison of adjusted means and an estimation of fold change (FC) using a *t*-test. Significant differences were declared when FDR ≤ 0.05 and absolute Log2(FC) ≥ 1. In total, 346 proteins were quantified, and out of those, 39 were differentially abundant in the comparison of POST/PRE. Twenty-five proteins were downregulated in POST, and 14 were upregulated in PRE. The 3 proteins most upregulated in POST were Cilia And Flagella Associated Protein 53 (Log2[FC] = 6.18), haptoglobin (Log2[FC] = 3.89), and inter-α globulin inhibitor H4 (Log2[FC] = 2.59). The 3 proteins most downregulated in POST were keratin type I cytoskeletal 16 (Log2[FC] = −5.02), keratin type I cytoskeletal 14 (Log2[FC] = −4.17), and myosin regulatory light polypeptide 9 (Log2[FC] = −3.55). Overall, our study confirms well-known postpartal inflammatory conditions in dairy cows characterized by increased haptoglobin. However, less is known about keratin proteins as potential blood biomarkers of peripartal adaptations in dairy cows.

Key Words: proteomics, keratin, plasma

2066 Effect of supplementation of *Saccharomyces cerevisiae boulardii* CNCM I-1079 on cell-mediated immunity during early lactation period in Holstein dairy cows.

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The objective of this study was to evaluate the effect of supplementation of *Saccharomyces cerevisiae boulardii* CNCM I-1079 (SCB) on systemic cell-mediated immunity and to characterize its changes during the postpartum period of dairy cows. Holstein cows (n = 83) were blocked by parity and, within block, randomly assigned to one of the 2 treatments, 100 g of cornmeal (CON, n = 43) or 87.5 g of cornmeal with 12.5 g of SCB (n = 40) to deliver 1×10^{10} cfu/head/d. Treatments were fed from d −28 prepartum to d 70 postpartum. Blood samples were collected on d 7, 21, and 70. Lymphocytes were isolated and stained with fluorescent antibodies against CD3, CD4, CD28, and CD8 to determine proportions and receptor expression of T cell subsets using flow cytometry. Data were analyzed using a linear mixed effects model with repeated measures in SAS. The statistical model included the fixed effects of treatment, time, parity, and all possible interactions and the random effect of block. An overall time effect was observed in the CD4 receptor expression with a decrease from d 7 to d 21 and recovery from d 21 to d 70 ($P < 0.01$). Additionally, the percentage of $CD4^+$ T cells increased from d 21 to d 70 ($P < 0.01$). The expression of CD28, a costimulatory molecule that aids in T cell activation, decreased in $CD4^+$ T from d 7 to d 21 ($P < 0.01$). Supplementation of SCB resulted in a reduction in receptor expression of CD4, and the percentage of $CD28^+$ in $CD4^+$ T cells ($P < 0.03$). The percentage of $CD8^+$ T cells in SCB increased from d 7 to d 21 and remained elevated until d 70 ($P < 0.01$). However, the expression of the CD8 receptor cells decreased from d 7 to d 21 ($P < 0.01$), whereas no change was observed in the expression of CD28 in $CD8^+$ ($P > 0.80$), nor an effect of treatment was observed. These findings suggest that markers associated with activation of cell-mediated immunity experience a downregulation in the early postpartum period, with subsequent recovery in the late postpartum phase. Supplementation with SCB leads to a further reduction in CD4 T cell activation during the early transition period.

Key Words: Transition cow, probiotic, health

2068 Colostrum supplementation as a therapy for neonatal calf diarrhea when administered at the time of a diarrhea alert generated by an automated milk feeder.

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This randomized clinical trial aimed to determine if intervening with colostrum at the time of a diarrhea alert could reduce diarrhea duration and improve feed intake and performance in Holstein dairy calves. Fourteen calves were offered 15 L/d of milk replacer by an automated milk feeder from 3 to 28 d of age. An alert validated to identify calves at risk for diarrhea was programmed using milk feeder data (≤60% rolling dividends in milk intake and/or drinking speed over 2 d). At the first alert, calves were randomly allocated to receive either 1 L of colostrum replacer mixed at 130 g/L (CR) or 1 L of milk replacer mixed at 150 g/L (MR) by bottle for 4 consecutive d. Treatments were balanced for metabolizable energy. Fecal consistency was scored daily, and diarrhea was diagnosed when calves had loose feces for ≥2 d or watery feces for ≥1 d. Body weight was collected at birth, on alert day, and 2× weekly thereafter. Mixed linear models evaluated the association of treatment with duration of diarrhea after the alert, milk intake, drinking speed, and ADG for 7 d after the alert, and final body weight at 7 and 14 d after the alert. Alert weight and alert age were included as covariates. Calves triggered the alert at (mean ± SD) 10 ± 3 d of age and were diagnosed with diarrhea at 11 ± 3 d of age. Duration of diarrhea after the alert was similar between treatments (CR: 3.8 ± 0.87 vs MR: 5.2 ± 0.87 d, $P = 0.27$). Similarly, there was no association of treatment with milk intake (CR: 8.3 ± 0.74 vs MR: 7.2 ± 0.74 L/d, $P = 0.29$) or drinking speed (CR: 0.38 ± 0.04 vs MR: 0.33 ± 0.04 L/min, $P = 0.36$). Calves provided CR tended to have greater ADG (CR: 1.03 ± 0.17 vs MR: 0.59 ± 0.19 kg/d, $P = 0.06$) and weighed 4 kg more at 7 d after alert (CR: 55.2 ± 1.94 vs MR: 51.3 ± 2.10 kg, $P = 0.05$). Weights were similar between treatments 14 d after alert (CR: 64.0 ± 1.62 vs MR: 61.1 ± 1.62 kg, $P = 2.66$). Preliminary results from this study suggest that providing colostrum at the time of a diarrhea alert could improve weight gain over the 7 d after the alert.

Key Words: precision livestock farming, sickness behavior, nutraceutical intervention

2069 The influence of diarrhea on growth, intake, and metabolic profiles in neonatal dairy calves. A. F. Souza Lima*[1], G. Goncalves Begalli[1], M. H. Oliveira[1], R. Chaves Barcellos Grazziotin[1], J. Halfen[1], E. Trevisi[2], and J. S. Osorio[1], [1]*School of Animal Science, Dairy Science, Virginia Tech, Blacksburg, VA,* [2]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Sciences, Universita Cattolica del Sacro Cuore, Piacenza, Italy.*

Our objective was to investigate how diarrhea influences the growth, intake, and metabolic profiles of neonatal dairy calves. Twenty-seven Holstein dairy calves were retrospectively classified into healthy (HC; n = 13) or diarrhea (DC; n = 14) groups. From 7 and 14d of age, calves with 2 consecutive days with fecal (FS) ≥ 3 and rectal temperature (RT) ≥ 39.5°C were classified as DC (at 11 ± 0.21 d of age), and calves below these thresholds were HC calves. All calves were housed in individual hutches and received 3.8 L of colostrum (Brix ≥21%) within 4 h after birth, followed by a second feeding of 2 L of colostrum. Calves received 2 L of milk replacer (MR, 24% CP, 20% fat, 15%DM) 2×/d for the first 2 d and 3 L of the same MR 2×/d until 21 d. The calves had ab libitum access to starter grain (21% CP) from d 3 and had free access to water. Daily records were kept for MR and starter intakes, respiratory scores, and RT. The BW was recorded at birth and then weekly. Blood samples were collected at 0 (before colostrum), 2, 7, 14, and 21d to evaluate metabolism biomarkers. Statistical analysis was performed using the MIXED procedure of SAS. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. A group × time (G × T) interaction ($P = 0.02$) on starter intake was due to greater ($P < 0.01$) starter intake in HC calves compared with DC at 20 and 21d. This was reflected in greater ($P = 0.02$) overall starter intake in HC calves compared with DC. Greater birth BW ($P = 0.02$) was observed in HC calves compared with DC (47.3 vs. 44.0 kg). A G × T interaction ($P = 0.10$) in glucose was explained by greater ($P \leq 0.03$) glucose in HC calves compared with DC at 2 and 7d. The latter was reflected in greater blood glucose ($P = 0.04$) in HC calves compared with DC. The G × T effect ($P \leq 0.01$) in glucose/insulin ratio was explained by a greater ($P = 0.04$) ratio in HC calves compared with DC at 14 d, followed by a lower ($P = 0.01$) ratio in HC calves compared with DC at 21 d. Overall, our study suggests that greater stress in DC calves led to lower starter intake and consequently impaired nutrient absorption, such as carbohydrates from the starter grain.

Key Words: diarrhea, fecal score, dairy calves

2070 Evaluating case definitions of respiratory disease in calves: A scoping review. K. Spohn*, C. Winder, H. Goetz, D. Wilson, and D. Renaud, *University of Guelph, Guelph, ON, Canada.*

The objective of this scoping review was to describe case definitions and diagnostic methods used to diagnose bovine respiratory disease (BRD) in preweaning dairy calves. This scoping review is the first step in developing a core outcome set (COS). Seven electronic databases were searched for relevant published and gray literature. This search returned 14,047 unique articles, which after screening against pre-determined eligibility criteria, resulted in 278 publications providing 352 case definitions. Most studies were observational (57%) or controlled trials (29%), with some challenge (9%) and diagnostic accuracy (5%) studies. Most studies used dairy-specific breeds (80%), with the rest using cross-bred or a mix of dairy and beef calves (18%), or did not report breed (2%). Diagnostic methods were varied and included clinical scoring system (CSS) alone (40%), physical exam parameters alone (26%), thoracic ultrasound (TUS) (8%) alone, CSS with additional methods (11%), farm treatment record (2%), lung imaging other than TUS (1%), diagnostic method used not apparent (1%), and other methods (11%). Case definitions between and within diagnostic methods were diverse in the level of detail provided and criteria used to define a BRD case; frequently this information was not cited (37%). Further, validity and accuracy of the diagnostic method used were only reported in 1% and 28% of studies, respectively. The Wisconsin Respiratory Scoring Systems were most cited, with a case definition of "a calf was considered to have respiratory disease when they had a total score of >4" (McGuirk, 2008) (18%) or ≥5′ (McGuirk and Peek, 2014) (10%). Examples of other definitions include "showed depression, anorexia, dyspnea, cough, nasal discharge and temp > 39.5°C," "severely increased respiratory sounds at lung auscultation," and "TUS examination with ≥1, ≥2 or ≥3 cm lung consolidation present." This review identified numerous BRD case definitions across various study designs, populations, and diagnostic methods, suggesting a need for standardization through a COS.

Key Words: bovine respiratory disease, case definition, synthesis

2071 Farm-specific machine learning models for early detection of respiratory disease in Holstein dairy calves using automatic milk feeder data. A. Bousselmi[1,2], E. Poulin[1,2], É. Charbonneau[1], A. Bélanger-Roy[1], D. E. Santschi[3], S. Buczinski[4], and É. R. Paquet*[1,2], [1]*Département des sciences animales, Université Laval, Québec, QC, Canada,* [2]*Institut intelligence et données (IID), Québec, QC, Canada,* [3]*Lactanet, Centre d'expertise en production laitière, Ste-Anne-de-Bellevue, QC, Canada,* [4]*Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, Canada.*

Respiratory diseases (RD) pose a significant threat to the health of Holstein dairy calves. Early detection and management strategies are paramount in mitigating the impact of such diseases. In this study, we investigate the use of machine learning to enhance RD management on dairy farms. We selected 5 commercial farms covering a broad range of calf-related management strategies in Quebec, Canada. For one year, we recorded consumption, drinking speed, and number of rewarded/unrewarded visits for all individual female calves using automatic milk feeder (AMF). In parallel, treatments associated with RD were monitored to define the moment when RD was detected by producers. In total we obtained 105,759 AMF data points on 2,358 unique calves of which 1,710 (73%) were healthy (i.e., received no treatment) and 648 (27%) had at least one episode of a RD. To detect early signs of RD from the AMF data, farm-specific random forest models were built to predict if a calf has RD using AMF data from −5 d to 0 d before the treatment by the producer. We also build one model using all the data from all the farms. Accuracy of the different models were estimated using 10-fold cross-validation. Performance of the different models varied greatly from one farm to another. Best performances were obtained at 0 d (60% accuracy), −1 d (65% accuracy), −2 d (72% accuracy), −1 d (63% accuracy) and −2 d (77% accuracy) on Farm 1 to 5, respectively. Except for Farm 1, farm-specific models outperformed the models built using all the farms where the best accuracy is at 62%. The results suggest that higher performance is obtained on farms with more homogeneous RD detection either because only one employee is dedicated to calf management (Farm 3) or because a well-defined chart of RD detection exists on that farm (Farm 5). This study represents an important step toward a proactive and data-driven approach to RD management on commercial farms using machine learning and highlight the importance of developing models that are farm-specific targeting farms that have already in place a well-define protocol for RD detection.

Key Words: calf health, disease, machine learning

2072 Impacts of bovine respiratory disease and lung consolidation at weaning on growth performance in beef × dairy calves. I. L. B. Fernandes*[1], A. Welk[2], B. L. Basiel[1], D. Sockett[3], D. L. Renaud[2], T. L. Felix[1], and M. C. Cantor[1], *[1]Penn State University, State College, PA, [2]University of Guelph, Guelph, Canada, [3]University of Wisconsin–Madison, Madison, WI.*

The objective of this study was to evaluate the impact of lung consolidation at weaning on the ADG of 145 beef × Holstein calves. Calves were sourced from 2 dairies and raised in 3 cohorts. Calf health (diarrhea, bovine respiratory disease) was assessed after arrival and postweaning at 57 d, 83 d (short-term), and 238 d (long-term). Thoracic ultrasonography (TUS) was performed to assess the lung consolidation score (LCS; none, 1 to 2 cm^2, or 3 cm^2) and TUS+ ($\geq$1 cm^2 in one lobe) status at 57 d (4 d) postweaning and the short-term check. Calves were fed milk replacer (0.84 kg/d), had ad libitum access to starter and water in buckets, and were weaned at 57 ± 14 d. At 4 ± 2 d postweaning, calves were moved to a separate rearing facility. Calf ADG was calculated into periods: preweaning ([arrival wt − weaning wt]/d), short-term ([weaning wt – 83 d wt]/d), and long-term ([83 d wt − 238 d wt]/d). Mixed linear models were used to assess the association of TUS+ status and LCS with ADG, and the ADG × period interaction. Period and sire breed were fixed effects, serum total protein and birthweight were covariates, and calf nested within the cohort was a random effect. There were 36 calves with LCS+ at weaning (109 healthy, 10 = 1 to 2 cm^2, 26 = 3 cm^2), and 11 LCS+ at the short-term check (132 healthy, 4 = 1 to 2 cm^2, 7 = 3 cm^2). Period interacted with TUS+ status and LCS ($P \leq 0.01$). Preweaning ADG was similar between healthy and TUS+ calves (0.56 ± 0.02 and 0.56 ± 0.03 kg/d, $P = 0.99$). Over the short-term, healthy calves grew 0.12 (±0.03) kg/d more than calves with TUS+ ($P = 0.01$) and had greater ADG (1.18 ± 0.02 kg/d) than calves with 3 cm^2 LCS (1.05 ± 0.04 kg/d $P = 0.02$). However, calves with 1 cm^2 LCS had similar ADG to healthy calves over the short-term ($P = 0.62$). Long-term ADG was similar between healthy and calves with TUS+ (1.20 ± 0.02 and 1.28 ± 0.03 kg/d, $P = 0.19$). The final weight for the cattle was 323.51 ± 36.63 kg (mean ± SD). We suggest that lung consolidation at weaning has short-term effects on beef × dairy calf growth, but compensatory growth nullifies the impact within 238 d post-weaning.

Key Words: health, pneumonia, growth

2073 Factors associated with reduced growth of Angus x Holstein dairy calves. E.F. Ferdman*[1], M. A. Steele[2], J. H. C. Costa[3], and D. L. Renaud[1], *[1]Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada, [2]Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph, Guelph, ON, Canada, [3]Department of Animal and Veterinary Science, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT.*

The objective of this prospective cohort study was to identify risk factors associated with average daily gain (ADG) of cross-bred dairy calves. Angus × Holstein dairy calves (n = 1,124) that arrived at a commercial calf-raising facility in Ontario, Canada, were enrolled in the study from May to November 2023. Calves were weighed upon arrival and 7 weeks later using a digital body weight. Information regarding calf sex, number of treatments administered, season at enrollment, the calf transporter and the calves' farm of origin, were extracted from farm records. A mixed linear regression model with a random effect for calf lot was utilized to determine associations between ADG during the 7-week period and number of treatments administered, calf sex, season, initial arrival weight and calf transporter. The mean body weight upon arrival and at 7-wk 50.83 ± 0.18 kg and 75.64 ± 0.25 kg, respectively, with an ADG of 0.61 ± 0.17 kg/d over this period. With regard to factors impacting ADG, calves that received 2 (−0.06 kg/d, $P < 0.001$, 95% CI: −0.86 to −0.27), 3 (−0.11 kg/d, $P < 0.001$, −0.15 to −0.07), and 4 or more (−0.18 kg/d, $P < 0.001$, −0.26 to −0.14) treatments for disease were associated with lower ADG compared with those that received no treatments. Male calves tended to have higher ADG compared with females (0.02 kg/d, $P = 0.07$, 0.01 to 0.04) and calves that arrived in the summer had lower ADG (−0.05 kg/d, $P < 0.05$, −0.08 to −0.01) than those arriving in the fall. The arrival weights were divided into quartiles, with calves arriving with a body weight greater than 60.14 kg tended to exhibit a lower ADG compared with calves weighing less then 43.01 kg (−0.02 kg/d, $P = 0.08$, −0.05 to 0.003) at arrival. Calf transporter was also associated with ADG with different transporters having varying impacts on ADG ($P < 0.001$), indicating potential differences in management practices. The results from this study suggests that disease may have the largest impact on ADG in crossbred dairy calves. Disease mitigation strategies should be further studied in crossbred dairy calves to reduce the need for multiple treatments.

Key Words: surplus calf, pneumonia, crossbreeding

2074 Factors associated with transfer of passive immunity in dairy calves. K. Y. Edwards*[1], J. H. C. Costa[2], S. J. LeBlanc[1], A. K. McNichol[3], T. J. DeVries[4], M. A. Steele[4], and D. L. Renaud[1], *[1]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, Vermont, [3]Tavistock Veterinary Services, Tavistock, ON, Canada, [4]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this retrospective cohort study was to explore factors associated with transfer of passive immunity (TPI) in dairy calves fed maternal colostrum. Complete farm-recorded calf health records were available from 2,022 female Holstein calves from 11 dairy farms in Ontario, Canada. Whole blood was collected from calves at 1–7 d old for serum total protein (STP) analysis using an optical refractometer. The mean (±SD) STP of all samples was 6.03 ± 0.82 g/dL. Variables screened in univariable analysis included season of birth, bodyweight at birth, calving ease, first colostrum feeding volume, colostral Brix, colostrum feeding method, number of colostrum feedings, and time of birth. Season of birth was categorized as winter (Dec-Feb), spring (Mar-May), summer (Jun-Aug), and fall (Sept-Nov). Due to lack of linearity, Brix was categorized into quantiles. Variables with $P < 0.20$ in the univariable analysis were included in a multivariable mixed-effects linear regression model of STP, with a random effect for farm, and manual backward stepwise elimination was used until all variables had $P \leq 0.05$ or were identified as confounders. Calves born in the summer (+0.16 g/dL; 95% CI: 0.09 to 0.22; $P < 0.001$) or fall (+0.23 g/dL; 95% CI: 0.13 to 0.32; $P < 0.001$) had greater STP compared with calves born in winter (5.79 ± 0.83 g/dL, mean ± SD). Calves fed colostrum with 24–25% Brix (+0.22 g/dL; 95% CI: 0.19 to 0.25; $P < 0.001$), 26% Brix (+0.28 g/dL; 95% CI: 0.24 to 0.32; $P < 0.001$), or $\geq$27% Brix (+0.28 g/dL; 95% CI: 0.13 to 0.43; $P < 0.001$) had greater STP than calves fed colostrum with $\leq$23% Brix (5.87 ± 0.81 g/dL, mean ± SD). Calves that were recorded as suckling colostrum from the dam had lower STP (−0.43 g/dL; 95% CI: −0.80 to −0.07; $P = 0.02$) than calves recorded as being bottle fed (5.89 ± 0.69 g/dL, mean ± SD). Calves born in the afternoon had lower STP compared with calves born in the morning (−0.15 g/dL; 95% CI: −0.21 to −0.09; $P < 0.001$). Calves born in the morning had a mean STP (±SD) of 6.09 ± 0.74 g/dL. These results illustrate manageable variables that are associated with STP and, thus, TPI in dairy calves.

Key Words: colostrum, calf health, serum total protein

2075 Colostrum quality and yield on Dutch dairy farms. J. B. Veneman*[1], N. Kleinveld[2], and J. O. Goelema[1], *[1]De Heus Animal Nutrition, Ede, the Netherlands, [2]Animal Nutrition Group, Wageningen University, Wageningen, the Netherlands.*

The objective of this study was to investigate associations between maternal dry period nutrition and transition period health with colostrum quality and yield on Dutch dairy farms. In total, 37 dairy farms were selected based on the ability to determine intake and composition of the dry cow diet fed. Ten to 15 dry cows per farm, entering lactation ≥2 and calving within a 2-mo period were enrolled. Per cow, a prepartum (−15 to −1 d) and postpartum (pp; 24 to 72 h) blood sample was taken from the coccygeal vessels for serum analysis of β-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA) and urea. Clinical disorders (retained placenta, metritis, ketosis, and mastitis) up to 21 d pp were recorded. In total, 401 colostrum yield samples from all farms and 254 Brix readings from 22 farms were collected. Pen-level intake and composition of the far-off (FO) and close-up diet (CU) was determined on 3 to 4 consecutive days during the 2 mo period. Colostrum yield was square root transformed before statistical analysis. Multivariable regression models were built to identify factors associated with colostrum yield and Brix. Mean ± SD of colostrum yield and Brix were 6.2 ± 0.6 L and 22.9 ± 4.2%, respectively. In the final model for colostrum yield, cows with a clinical disorder had higher colostrum yields compared with cows without (6.9 and 6.0 L, SE 0.6, $P = 0.10$), and increased pp NEFA concentrations increased colostrum yield ($P < 0.01$). In addition, cows that received the CU >14 d had higher colostrum yields than cows receiving the CU for 14 d, with <14 d being intermediate (8.0, 5.4, and 6.2 L, SE 0.8, $P < 0.01$). In the final model for colostrum Brix, pp serum urea concentration ($P = 0.06$), the difference in DCAD between the CU and FO ($P = 0.04$), FO crude fat level ($P < 0.01$) and diet proportion of concentrates + minerals ($P = 0.07$) were positively associated with Brix, whereas colostrum yield ($P = 0.01$) and pp serum BHBA concentration (0.06) were negatively associated with Brix. In addition, second parity cows had lower colostrum Brix values than parity ≥5 (21.8 and 24.1%, SE 0.7, $P = 0.01$). In conclusion, colostrum quality and yield are associated with nutritional and health factors.

Key Words: colostrum, Brix%, dry cow nutrition

2076 Factors associated with morbidity and mortality in preweaned dairy calves. K. Y. Edwards*[1], J. H. C. Costa[2], S. J. LeBlanc[1], A. K. McNichol[3], T. J. DeVries[4], M. A. Steele[4], and D. L. Renaud[1], *[1]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT, [3]Tavistock Veterinary Services, Tavistock, ON, Canada, [4]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this retrospective cohort study was to explore factors associated with diarrhea, respiratory disease (BRD), and mortality in preweaning dairy calves. Complete calf health records from birth to 56 d of age were available from 2,361 female Holstein calves from 11 dairy farms in Ontario, Canada. Variables screened in univariable analyses for each morbidity model included season of birth, birth bodyweight, calving ease, first colostrum feeding volume, colostral Brix, number of colostrum feedings, time of birth, and transfer of passive immunity (TPI) among total protein categories of poor (PR; < 5.1 g/dL), fair (FR; 5.1–5.7 g/dL), good (GD; 5.8–6.1 g/dL), and excellent (EX; ≥ 6.2 g/dL). Variables screened in the mortality model included the aforementioned variables as well as 2 additional potential predictors: any health event and presence of lung consolidation (≥1 cm^2). Multivariable mixed-effects logistic regression models with a random effect for farm were built using a manual backward stepwise elimination process until all variables had a $P \leq 0.05$ or were identified as confounders. Calves born in the summer had greater odds of diarrhea (odds ratio [OR] = 1.54; 95% CI: 1.08–2.20; $P = 0.02$) compared with winter. Calves had lower odds of BRD if born in summer (OR = 0.67; 95% CI: 0.51–0.88; $P = 0.005$) or fall (OR = 1.51; 95% CI: 1.12–2.04; $P = 0.007$) compared with winter. Furthermore, calves with EX TPI had lower odds for BRD (OR = 0.71; 95% CI: 0.52–0.98; $P = 0.04$) compared with PR TPI. For mortality, the odds were lower in calves born in fall (OR = 0.23; 95% CI: 0.05–0.97; $P = 0.05$) compared with winter and if TPI was GD (OR = 0.13; 95% CI: 0.07–0.78; $P = 0.042$) or EX (OR = 0.34; 95% CI: 0.12–1.00; $P = 0.05$) compared with PR. Moreover, the odds of mortality were higher for calves with a health event compared with those with no health events (OR = 20.18; 95% CI: 4.24–96.13; $P < 0.001$) and if calves had lung consolidation (OR = 2.65; 95% CI: 1.02–6.88; $P = 0.05$) compared with no lung consolidation. These results highlight the importance of improving TPI to reduce preweaning morbidity and mortality, and the effect of season on the incidence of morbidity.

Key Words: calf health, colostrum, illness

2077 Factors associated with lung consolidation in preweaned dairy calves. K. Y. Edwards*[1], J. H. C. Costa[2], S. J. LeBlanc[1], A. K. McNichol[3], T. J. DeVries[4], M. A. Steele[4], and D. L. Renaud[1], *[1]Department of Population Medicine, University of Guelph, Guelph, ON, Canada, [2]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT, [3]Tavistock Veterinary Services, Tavistock, ON, Canada, [4]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this retrospective cohort study was to explore factors associated with lung consolidation (LC) in preweaned dairy calves. Complete calf health records from birth to 56 d of age were available from 2,276 female Holstein calves from 11 dairy farms in Ontario, Canada. Thoracic ultrasound was performed at 30 d (±7 d) using a linear ultrasound probe set at a depth of 8 cm and 8.5 mHz. Lung tissue was considered consolidated if hypoechoic areas were present eliminating the bright white band at the pleural interface and reverberation aspect found in normal lung tissue. The extent of lung consolidation was determined with the 1-cm gridlines of the ultrasound, summing the affected areas of all lung lobes. Calves were considered LC positive if they had = 1 cm^2 of consolidated lung. The overall prevalence of LC was 50.8% and ranged from 0 to 81.8% by farm. The mean (±SD) birth weight of all calves was 42.3 (±4.8 kg). Variables screened in univariable analysis included season of birth, birth weight, calving ease, first colostrum feeding volume, colostral Brix, number of colostrum feedings, time of birth, incidence of diarrhea, and serum total protein categories of poor (PR; < 5.1 g/dL), fair (FR; 5.1–5.7 g/dL), good (GD; 5.8–6.1 g/dL), and excellent (EX; = 6.2 g/dL). Variables with $P < 0.20$ in the univariable analysis were included in a multivariable mixed-effects linear regression model of LC, with a random effect for farm, and manual backward stepwise elimination was used until all variables had $P = 0.05$ or were identified as confounders. The odds of LC were lower if TPI was GD (OR = 0.56; 95% CI: 0.38–0.82; $P = 0.003$) or EX (OR = 0.41; 95% CI: 0.28–0.61; $P < 0.001$) compared with PR. Calves born in the summer had lower odds of LC than calves born in the winter (OR = 0.72; 95% CI: 0.51–1.00; $P = 0.05$). Birth weight was associated with LC, where every 1 kg increase reduced the odds for LC (OR = 0.96; 95% CI: 0.94–0.98; $P < 0.001$). These results emphasize the importance of achieving good TPI to reduce respiratory damage. The seasonal effects are likely associated with temperature, ventilation, and air quality oscillation.

Key Words: calf health, colostrum, respiratory

2078 Farm-level risk factors for digital dermatitis in dairy cows in mountainous regions.

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Reduction of risk factors for bovine digital dermatitis (BDD) is crucial in current disease control. However, risk factors that might arise especially in mountainous regions are unknown until now, and an adapted BDD control program is consequently missing. The objective of this observational case-control study was to identify farm-level risk factors for BDD in dairy herds in mountainous regions. To investigate predictors for the occurrence of BDD, one hundred farms located in mountainous districts of Switzerland were visited and information about herd characteristics and management practices, potentially relevant explanatory variables for either external or internal biosecurity, were gathered by completing a questionnaire with the farmer or herd manager. Cases and controls were defined using within-herd prevalences of BDD assessed during the previous 3 routine claw trimmings taking place with an interval of 6 mo. Two seperate models were built using stepwise backward elimination according to either internal or external biosecurity. Results of model 1 (i.e., aspects related to BDD introduction) revealed that access to mountain pastures during the summer season (odds ratio, 95% confidence interval: 0.12, 0.04–0.35), participation in dairy shows (0.32, 0.11–0.94), and the number of new animals introduced into the farm during the last 2 yr (1.28, 1.12–1.52) were significantly associated with the occurrence of BDD. Model 2 (i.e., aspects related BDD establishment) showed that cows kept in freestalls were at higher risk for BDD compared with those kept in tiestalls (20.65, 1.59–649.37). Furthermore, number of days between diagnosis and treatment of a BDD lesion (10.31, 3.55–81.21) and the amount of concentrate feeding (median 5 kg) per cow and day (7.72, 2.46–6.47) were positively associated with BDD occurrence. In conclusion, the findings of this study provide a set of risk factors that are associated with BDD status within herds in mountainous regions. These results may help in development of adapted control programs for BDD in dairy cows.

Key Words: biosecurity, cattle, lameness

2079 *Staphylococcus aureus* modulins in clinical and subclinical mastitis.

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Staphylococcus aureus is one of the main pathogens associated with mastitis cases, which is an inflammation of the mammary gland and can manifest in clinical or subclinical form. In both forms, there are many virulence factors involved. Phenol-Soluble-Modulins (PSMs) play various roles in the pathogenesis of *S. aureus*, such as modulating host immune responses by promoting inflammation and inhibiting phagocytosis, cell lysis, and biofilm formation. Delta (d)-Hemolysin (*hld* gene) in one of the PSM and it forms pores in cell membranes, leading to cell lysis and tissue damage. It also modulates host immune responses by activating pro-inflammatory pathways. The aim of this study was to evaluate whether the presence of *S. aureus* modulins genes mastitis-causing are associated with the development and severity of mastitis in dairy cows. Therefore, we investigated the genetic variations of *S. aureus*, the prevalence of those genes, and their potential impact on the severity of mastitis. A total of 103 isolates from cows with subclinical mastitis and 103 clinical isolates (72 mild, 28 moderate, and 2 severe cases) were used. *S. aureus* were seeded on sheep blood agar (5%) to DNA extraction (MiniSpin kit) for PCR tests. Taq Green Master Mix was used for nucleic acid amplification. Positive control (*psmA, psmB*, and *hld: S. aureus* USA 300) and negative control (*E. coli* ATCC 25.9222) were included in all reactions. In cases of clinical mastitis, the *psmA*, *psmB*, and *hld* genes were present in 93 (90.3%), 84 (81.6%), and 81 (78.6%) isolates, respectively. All differences were statistically significant in the Chi-squared test ($P > 0.0001$) when comparing the presence of these genes among subclinical isolates, which occurred at frequencies of 70 (68%), 47 (45.6%), and 52 (50.5%), respectively. It was a surprising fact considering that most modulins genes are core genome-encoded. Understanding the role of modulins in *S. aureus* pathogenesis is essential for developing new strategies to combat *S. aureus* infections, including the development of vaccines and novel therapeutics targeting these virulence factors.

Key Words: virulence, severity, PCR

Breeding and Genetics 1

2080 Approximated reliabilities of breeding values in single-step GBLUP for Holstein dairy cattle. G. Vargas*[1], N. Vukasinovic[1], C. A. Przybyla[1], M. Bermann[2], S. N. S. Sierra[2], D. Lourenco[2], and D. Gonzalez-Peña[1], [1]*Zoetis Genetics, Kalamazoo, MI,* [2]*University of Georgia, Athens, GA.*

The program ACCF90GS2 included in the BLUPF90 software suit allows to approximate reliabilities of estimated breeding values for large-scale genomic data in single-step GBLUP (ssGBLUP) with the algorithm for proven and young (APY). Most recently, the program has included a method for approximating reliabilities of indirect predictions and random regression models. Two data sets were used to obtain accuracy estimates and to test the software performance for a Holstein dairy population. Traits studied included 8 cow wellness traits: mastitis (MAST), lameness (LAME), metritis (METR), retained placenta (RETP), displaced abomasum (DA), ketosis (KETO), milk fever (MFEV), respiratory disease (RESP); 3 fertility traits: cystic ovary (CYST), abortion (ABRT), and twinning (TWIN); and 3 calf wellness traits: calf livability (CLIV), respiratory disease (CRESP), and scours (SCOUR). The phenotype and pedigree records ranged from 952K for CYST to 5.8M for RETP and from 4.5M for CYST to 6.5M for CRESP, respectively. A single-trait model was fitted for each trait using the APY with 25,000 core animals randomly selected to obtain genomic breeding values (GEBV) for the full data set consisting of 2.3M animals genotyped for 45K SNP. Indirect predictions were obtained for the subset of data consisting of 430K genotyped animals and adjusted to the same scale of GEBV enabling comparison with animals from regular ssGBLUP. Correlations between approximated reliabilities obtained from both data sets were over 0.98 for all analyzed traits. The computing requirements of ACCF90GS2 increase with the number of genotyped animals considered for creating the APYGi matrix, but an ongoing project aims to reduce time and memory usage. The reliabilities obtained based on indirect predictions were very similar to those based on ssGBLUP using a large number of genotyped animals. Therefore, the approximation of reliabilities for large-scale genomic data in ssGBLUP and indirect predictions is possible.

Key Words: Holstein, reliabilities, ssGBLUP

2081 Pattern of linkage disequilibrium in Nili Ravi buffalo. H. Mustafa*[1], I. Anwar[2], M. Akhter[2], G. Bilal[3], and K. Farooq[4], [1]*University of Veterinary and Animal Sciences, Lahore, Lahore, Pakistan,* [2]*Livestock Production Research Institute Bahadurnagar, Okara, Okara, Pakistan,* [3]*PMAS-Arid Agriculture University, Murree Road, Shamsabad, Rawalpindi, Murree Road, Shamsabad, Rawalpindi, Pakistan,* [4]*Buffalo Research Institute, Bhunekey Tehsil Pattoki District Kasur, Pattoki, District Kasur Pakistan.*

Buffalo serves as the predominant dairy animal in Pakistan, contributing 65% to the total milk production share and securing the country's position as the 3rd largest milk-producing nation. Among the 5 buffalo breeds, Nili Ravi stands out as the most prominent globally. However, challenges such as low production rates and a protracted generation interval impede its potential as a competitive dairy animal, impacting its market competitiveness. Genomic selection emerges as a valuable tool for accelerating breed improvement. In this study, we investigate the pattern of linkage disequilibrium (LD) in Nili Ravi buffalo, employing 96 samples from diverse herds and analyzing them with the 90k Axiom chip. The r^2 and D methods are utilized to measure LD. A significant observation is the decreasing trend in average r^2 values as the genomic distance between single nucleotide polymorphism (SNP) pairs increases. Specifically, the highest r^2 values are found in the 0–25 Kb interval, gradually decreasing in subsequent intervals (25–50 Kb, 50–100 Kb, and 100–500 Kb). This pattern suggests that SNPs in close proximity on the chromosome exhibit stronger linkage, while those farther apart demonstrate weaker correlations, aligning with genetic recombination principles. Notably, this study represents the first application of the SNP chip to evaluate the informativeness of Nili Ravi buffalo in Pakistan. There is a critical need to establish a reference population for Nili Ravi buffalo, which is currently absent from the country's buffalo improvement program. This research provides valuable insights into the genomic landscape of Nili Ravi buffalo, laying the groundwork for improved breeding strategies and the overall enhancement of the buffalo population in Pakistan.

Key Words: buffalo, Nili Ravi, linkage disequilibrium

2082 Performance comparison of Holstein cows with different breeding values for heat tolerance. M. A. Sánchez-Castro*[1], N. Vukasinovic[1], F. Di Croce[1], S. Dikmen[2,3], and P. J. Hansen[2], [1]*VMRD Genetics, Zoetis Inc., Kalamazoo, MI,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*Department of Animal Science, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, Turkey.*

Thermotolerance can be estimated as the slope of a reaction norm model because it represents the performance change of cows in response to increments in the temperature-humidity index. Such a trait is heritable and can be used to generate breeding values for heat tolerance (HT). Our objective was to compare the performance of Holstein cows with different breeding values for HT. Genomic predicted transmitting abilities (gPTA) for 2,616 cows located in hot climates of Florida, California, and Turkey were calculated based only on their genomic information using the single-step GBLUP method. A total of 168,439 test-day records of these cows were available for analyses. The Wood's model was used to fit a lactation curve per each cow-lactation combination (4,466 observations) and calculate the milk yield adjusted to 305-d of lactation (MY305, kg). Two general linear models (M1 and M2) that included the effects of farm-year (25 levels), lactation number (1, 2, ≥ 3), genetic group (T1 = top third, T2 = middle third, T3 = bottom third, based on the gPTA for HT), calving season (winter, spring, summer, fall), and the interaction between genetic group and calving season were tested. The difference between models was that in M1 the intercept of the reaction norm was included as linear covariate while in M2 it was not. Significant differences ($P < 0.05$) between genetic groups were found for all calving seasons except for spring in M1. Least squares means ± SE for winter-calved cows were 12,105.5 ± 126.2, 11,995.4 ± 124.9, and 11,692.9 ± 125.9 for T1, T2, and T3, respectively. For summer-calved cows results for T1, T2, and T3 were 11,788.9 ± 141.7, 11,860.8 ± 140.9, and 11,583.4 ± 144.5, respectively. For fall-calved cows, results were 11,819.0 ± 128.9, 11,821.3 ± 126.8, and 11,515.4 ± 123.6 for T1, T2, and T3, respectively. For M2, no differences between the mean performance of T1 and T3 for any season were identified ($P > 0.05$). These results suggest that the inclusion of the intercept as adjustment for the base genetic merit for milk production is important when evaluating the utility of gPTA generated using the slope of reaction norm model for HT in Holstein cattle.

Key Words: thermotolerance, heat stress, reaction norm

2083 Discovery of the SLICK2 variant in West African taurine and Zebu crossbred cattle. S. Gebeyehu*[1], E. S. Kim[2], T. S. Sonstegard[2], and B. J. Heins[1], [1]*University of Minnesota, St. Paul, MN,* [2]*Acceligen, Eagan, MN.*

In many regions worldwide, cattle are vital for agricultural production and provide essential resources such as milk, meat, and draft power. African cattle breeds face challenges related to environmental stressors, such as heat tolerance and disease resistance, which can affect their productivity and overall health. The SLICK coat allele in cattle is a naturally occurring mutation associated with the prolactin receptor (PRLR) gene associated with heat resistance of the animals. The study investigated the prevalence and distribution of the SLICK variants in West African taurine, and Zebu crossbred compared with Criollo cattle breeds. Four breeds of Criollo cattle, (Caracu [n = 29], Colombian Blanco Orejinegro [n = 40], Hartón del Valle [n = 71], and Mexican Criollo Lechero tropical [n = 20]), 4 Zebu breeds (Brahman [n = 5], Bunaji [n = 30], Gir [n = 245]), and a Zebu crossbred with West African taurine breeds (n = 50), and 2 West African taurine breeds (Muturu [n = 30] and Baoulé [96]), and the Sanga breed Mashona (n = 37) were compared for allelic frequency of SLICK variants. The frequency of the dominant allele (A), represented as p, and the frequency of the recessive allele (a), represented as q, was calculated from the following formulas: $P = [2 \times f(AA) + f(Aa)] / (2 \times N)$ and $q = [2 \times f(aa) + f(Aa)]/(2 \times N)$, where N denotes the sample size. The Criollo breeds had high allelic frequencies for multiple SLICK variants. Genotypic analysis revealed the presence of SLICK2 variants in both West African taurine and Zebu crossbred cattle. Specifically, among West African taurine breeds, Baoulé cattle had an allelic frequency of 0.01% for the SLICK2 variant. The West African Zebu crossbreds had an allelic frequency of 0.02%. Despite the low frequency, the discovery of the SLICK2 variant in these populations is unprecedented in the literature, highlighting a novel finding in the genetic diversity assessment of Baoulé and Zebu crossbred cattle. This discovery contributes to a deeper understanding of West African cattle genetic composition and potential adaptive traits.

Key Words: Baoulé, SLICK2, Zebu, crossbred

2084 Assessing genetic variants as indicators of susceptibility to digital dermatitis infection in lactating Holstein cattle. Z. Macon*[1], B. Jones[2], and J. Waddell[1], [1]*Tarleton State University, Stephenville, TX,* [2]*Eastern Kentucky University, Richmond, KY.*

Digital dermatitis infection in dairy cattle is a major economic drain on producers and causes morbidity deficits in production animals. Infections are difficult to control, and active lesions often return after initial onset. The shortcomings of prevention and treatment methods offer an opportunity to look to genetic predisposition in affected and healthy animals. This study aimed to design and test qPCR assays to target regions with previously identified single nucleotide polymorphisms (SNP) that affect susceptibility or resistance to digital dermatitis, to then evaluate effectiveness of assays to predict susceptible or resistant animals. A sample (n = 45) of lactating Holstein cows, 35 infected and 10 noninfected, were selected for DNA collection and were assigned foot scores. Results of chi-squared analysis indicate that designed assays for TLR4 ($P = 0.042$), IL8 ($P = 0.0003$), and CMPK2-associated ($P = 0.021$) SNPs exhibit non-random relationships between amplicon melting temperature and foot score of individuals. Therefore, there is evidence to support the idea of a qPCR-based assay to potentially incorporate into producers' selection criteria for production herds to combat and prevent digital dermatitis infection.

Key Words: digital dermatitis, genetic susceptibility, PCR assay

2085 Investigating the genetic architecture of dairy calf disease traits and their relationships with economically important traits. C. Lynch*[1], B. O. Makanjuola[1], F. S. Schenkel[1], F. Miglior[1,2], D. Kelton[3], and C. F. Baes[1,4], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Lactanet Canada, Guelph, ON, Canada,* [3]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [4]*Institute of Genetics, Department of Clinical Research and Veterinary Public Health, University of Bern, Bern, Switzerland.*

The dairy industry faces the challenge of improving calf health as it works to maintain and enhance animal health standards. The 2 most prevalent calf diseases on Canadian farms are respiratory problems (RESP) and diarrhea (DIAR), and genetic selection is one tool that can help reduce their incidences. However, there has been limited investigation into the genetic architecture of dairy calf disease traits and how selection on these traits may impact genetic evaluation for other traits. To address this gap, we estimated genetic correlations among calf disease traits and other economically important traits, including production, health, and fertility, and conducted a genome-wide association study (GWAS) for the calf disease traits. Producer-recorded calf disease data were provided by Lactanet Canada and included 69,695 Holstein calf disease records for RESP and DIAR from 62,361 calves in 1,617 Canadian dairy herds from 2006 to 2021. Additionally, we explored 2 scenarios based on minimum herd-year disease incidence thresholds (1% and 5%) to highlight the influence of data filtering criteria on selection potential for RESP and DIAR. Phenotypes related to production, fertility and health were also provided by Lactanet Canada. Genetic correlations from multivariate analyses between calf disease traits and production traits were low (0.03 to 0.08) but were stronger with both fertility and health traits (−0.66 to 0.35). For the GWAS, 71,428 genotyped animals with a medium-density panel (50K) were available across the relevant pedigree, and SNP effects were estimated via single-step genomic BLUP. Preliminary results demonstrate 4 SNPs and 2 genes (CAPN5 and ACER3) associated with DIAR, while 21 SNPs and one gene (SIRPA) were found to be associated with RESP after a chromosome-wise false discovery rate correction. Our results provide novel information regarding the genetic architecture of calf disease traits and how inclusion of these traits in national evaluations may impact other economically important traits.

Key Words: calf health, genetic correlations, GWAS

2086 A transgenerational study to detect the effect of calving season of ancestors on Mediterranean dairy buffalo performances. N. P. P. Macciotta*[1], A. Cesarani[1], R. Cimmino[2], M. Gomez Carpio[2], R. Negrini[3], and M. Fioretti[3], [1]*Dipartimento di Agraria, University of Sassari, Italy,* [2]*Associazione Nazionale Allevatori Specie Bufalina ANASB, Napoli, Italy,* [3]*Associazione Italiana Allevatori AIA, Roma, Italy.*

Transgenerational studies on dairy cattle have reported an effect of the calving month of ancestors on cow dairy performances. These results have been explained by suggesting a transgenerational inheritance of epigenetic markers caused by the heat stress during great grand dam pregnancy. In this work, the effect of calving season of dam (F_2), grand dam (F_1), and great grand dam (F_0) on EBV for dairy production traits of their great granddaughters (F_3) was investigated in the Italian Mediterranean River Buffalo. Data were EBV for milk, fat and protein yields, of 112,625 buffalo cows born in the period 1985–2022, provided by the Italian Buffalo Association (ANASB). Data were analyzed with a linear model that included the fixed effects of F_0, F_1, and F_2 calving seasons, their 3-way interaction, and the fixed covariables of F_1 birth date and F_0 EBV for the considered trait. The calving season of the 3 generations of ancestors affected all the considered traits. Calving seasons that had the largest positive effects on F_3 EBV were spring for F_0, winter for F_1, and fall for F_2, respectively. Lowest LS means of EBV were obtained for F_3 cows whose ancestors calved in summer (F_1 and F_2) and fall (F_0), respectively. These results confirm previous report in cattle on the effect of the period of pregnancy on performances of their offspring. In particular, a positive effect of late pregnancies carried out in winter-early spring and a negative effect of those carried out in summer-fall has been highlighted. These figures suggest that also in buffalo could be hypothesized a transgenerational epigenetic inheritance due to heat stress experienced by ancestors during pregnancy.

Key Words: buffalo, transgenerational, epigenetic inheritance

2087 Genomic predictions for feed efficiency traits in Holstein cows. G. Oliveira Jr., M. Borchers, K. Borchers, D. Liang, M. Li, A. Kulkarni, G. Vargas, N. Vukasinovic, and M. A. Sánchez-Castro*, *Zoetis Inc., Parsippany-Troy Hills, NJ.*

Feed is a major component of variable costs associated with dairy systems and is, therefore, an important consideration for breeding objectives. The objective of this project was to estimate genetic parameters for dry matter intake (DMI; kg/day) and residual feed intake (RFI; kg/day) using data from commercial Holstein herds. The data were collected in 4 commercial herds across the US using the *SmartFeed* equipment. A total of 32,824 weekly averages of daily DMI measured on 3,742 Holstein cows from June 2022 to November 2023 were available for this study. RFI was defined as the residual of a linear regression of DMI on energy-corrected milk, metabolic body weight, and other environmental effects. Genetic parameters were estimated using a univariate animal model with repeated records using the AIREMLF90 software. This statistical model included year-season at calving, week of lactation, lactation number, and herd-year at calving as fixed effects. The predicted variance components were then used to estimate breeding values (EBV) including genomic data of over 2 million animals using single-step genomic BLUP methodology. Reliabilities of EBV were estimated using accf90GS v2.54 program. The phenotypic mean (SD) of DMI and RFI was 21.0 (3.87) and 0.0 (2.46) kg/day, respectively. Heritability estimates (SD) for DMI and RFI were 0.27 (0.04) and 0.14 (0.04) respectively. EBV of phenotyped animals had mean (SD) of 0.20 (0.97) and 0.29 (0.52) kg/day for DMI and RFI, respectively. The reliability of EBV for DMI had mean (SD) of 0.26 (0.19) when accounting for all animals, 0.49 (0.05) for phenotyped animals, and 0.40 (0.05) when considering only animals with genotype information. Likewise, the reliability of EBV for RFI had mean (SD) of 0.18 (0.14) across all animals, 0.35 (0.05) for phenotyped animals, and 0.29 (0.05) when including only animals with genotype data. The results of this study contribute to a better understanding of the genetics of feed efficiency related-traits, providing insight into potential selection strategies to include feed efficiency in breeding programs.

Key Words: feed intake, heritability, SmartFeed

Dairy Foods 1: Chemistry

2088 A functionality study of whey protein aggregates derived from fibril/ribbon-shaped assemblies induced by antisolvent precipitation. H. Shi*[1], O. Velev[1], and H. Zheng[1], [1]*Southeast Dairy Foods Research Center, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC,* [2]*Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC.*

The current research aims to 1) characterize mesoscopic sized whey protein particles (WPPs) formed via antisolvent induced aggregation followed by heat treatment and microfluidization; 2) investigate WPP functionality with regards to food applications such as interfacial behavior, foaming properties and heat stability. Two types of whey protein particles (WPP1 and WPP2) were manufactured in laboratory scale through modulation of the injection rate of whey protein isolate (WPI) solution to the antisolvent. The resultant WPPs were characterized for sulfhydryl (SH) contents, protein composition and interaction using gel electrophoresis, internal structural feature using small angle X-ray scattering (SAXS), air-water interfacial behavior using Drop Shape Analyzer (DSA), and foaming properties using Dynamic Foam Analyzer (DFA). Heat stability was indicated by the changes in optical density (OD at 500 nm) and particles size distribution before and after heat treatment (95°C/5 min). The results showed that the total and exposed SH group contents in WPP1 and WPP2 were lower than those in WPI ($P < 0.05$). Native-PAGE and SDS-PAGE results indicated that disulfide bonding is the major intermolecular force mediating the formation of WPP particles. Moreover, a multilevel unified fit was used to interpret the structural information using SAXS scattering data of WPPs. Three levels of internal structure were revealed for WPP particles based on the computed power law exponent (P) and radius of gyration (R_g): a smooth surface with a spherical shape (P1~4.00, R_{g1}~21.82Å), a mass fractal (P2~2.62, R_{g2}~74.13Å), and a surface fractal (P3~3.29). Additionally, WPPs also demonstrated a significant improvement in foam stability and heat stability compared with WPI ($P < 0.05$). Therefore, the WPP particles investigated in the current work have a great potential to be used as a functional ingredient for food applications requiring heat stability and foam stability.

Key Words: whey protein isolate, small angle X-ray scattering, foam

2089 Effect of genetic polymorphism on the physicochemical and coagulation properties of bovine milk. Z. Zhang*[1], F. J. Muñoz[1], H. Huang[1], M. Faust[2], S. Hulgan[1], N. Park[1], A. Hassan[1], and B. Nelson[1], [1]*Daisy Brand, Garland, TX,* [2]*Agri Innova LLC, Waunakee, WI.*

The impacts of genetic polymorphisms in milk proteins on physicochemical and coagulation properties were investigated. Milk samples from 10 individual cows differing genetically were selected, and their milk protein polymorphisms were confirmed by HPLC-MS. Milk composition (lactose, protein, fat, and total solids) was determined by FTIR. To avoid the potential confounding effects of multiple factors, comparisons were made using only milk samples from cows exhibiting differences for a single milk protein. All milk samples were defatted and standardized to the same protein level (2.8%) by the addition of milk ultrafiltration permeate. Physicochemical properties including pH, particle size, surface charge, and buffering capacity were assessed. The κ-casein AA genotype milk samples exhibited larger average casein micelle size (>200 nm) than milk with other κ-casein genotypes ($P < 0.05$). Milk with the β-casein A2A2 genotype had a lower buffering capacity and total and colloidal calcium than milk with other β-casein genotypes. Rennet and acid gels from milk samples differing only in one protein variant were characterized by measuring the coagulum firming rate, firmness, yield stress, and water holding capacity. The acidification profile was also monitored using a CINAC® system. A strong correlation was found between size of the casein micelle and coagulum firming rate and firmness. The correlations between milk pH and particle size or gelation properties were not statistically significant ($P > 0.05$). Among all tested milk samples, 2 rennet non-coagulating milks were identified. These 2 samples had the lowest buffering capacity and calcium content among all samples tested. Because one of the 2 non-coagulating milk samples was A2A2, future study will test more samples of this milk type to determine whether the noncoagulation property is associated with the protein variant or calcium deficiency. The study suggests that differences in the coagulation properties among samples from cows producing different protein variants may be related to variations in casein micelle particle size and calcium content.

Key Words: genetic polymorphism, coagulation, physicochemical properties

2090 Enzymatic synthesis of calcium lactobionate from cheese whey permeate as a potential food ingredient. U. Amin*[1], C. K. Yeung[2], and H. Zheng[1], [1]*Southeast Dairy Foods Research Center, Department of Food, Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC,* [2]*Animal Science Department, California Polytechnic State University, San Luis Obispo, CA.*

Lactobionic acid (LBA) is of great interest in food and pharmaceutical applications as a functional ingredient e.g., acidulant, antioxidant, metal chelator etc. and a carrier in drug delivery systems. LBA and its salt-form (lactobionate, LBN) can be produced by the enzymatic oxidation of lactose through ping-pong reaction mechanism. Oxidation reduces the pH of permeate solution during LBA/LBN production and needs to be maintained by an alkaline solution for an efficient enzyme activity. Although the production process of LBNs using different alkaline solutions has been studied, operational parameters for the scalable biosynthesis of calcium lactobionate (Ca-LBN) using $Ca(OH)_2$ dispersion as base-titrant are scarce in literature. In current study, oxidase (400 U/kg, LactoYield, Chr. Hansen) was used to produce Ca-LBN using reconstituted cheese whey permeate solution (300 g/L) as substrate and $Ca(OH)_2$ dispersion as titrant. The production process was conducted using a laboratory bioreactor coupled with human-machine-interface analogous to industrial control panel. Two-independent replicated batches were processed and target critical control parameters (CCPs) were adjusted as: pH 6.40; O_2 level: 44%,; temperature: 38°C. The amount of $Ca(OH)_2$ consumed was used to calculate real-time molar conversion rate (MCR) and accumulative molar conversion yield ($MCY_{LBN/lactose}$) according to pH-stat method. Initially, MCRwas rapidly increased from 0 to 472.0 ± 37.6 m*M*/h within 20 min of experiment. MCR declined continuously throughout the oxidation process to ~150 m*M*/h after 6.5 h and to ~0 m*M*/h after 7 h. Enzymatic oxidation reaction continued for 7 h and $MCY_{LBN/lactose}$ was observed 49.85 ± 5.04% (equivalent to ~99.7% lactose molar conversion). In-process CCPs e.g., pH remained 6.40 ± 0.04, while O_2 level, initially dropped to ~15% after the addition of enzyme, remained stable at 44 ± 5% during the experiment. These obtained results provide the ingredient manufacturers with practical knowledge on the processing parameters for scalable production of

Ca-LBN, which may be utilized as functional ingredient in diverse food and pharmaceutical formulations.

Key Words: Ca-lactobionate, oxidation, cheese whey permeate

2091 Preliminary studies on developing low-acid high protein beverages with pre-aggregated whey proteins.

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Consumer focus on health and wellness products is the driving force behind high protein dairy-based beverage growth. They can be used as meal replacements, to increase overall protein intake, as a part of a sports nutrition diet, or to improve satiety. Casein-based ingredients such as micellar casein concentrate (MCC), caseinates, and milk protein concentrates are used in shelf-stable low-acid (pH ~6.8) beverages. Whey proteins are not typically used in the formulation due to their poor heat stability. The work aims to understand the use of whey protein aggregates in high protein systems. Two lots of milk whey protein isolate (mWPI) were procured from a commercial manufacturer. The mWPI was reconstituted to 8% protein (wt/wt) and heated at 80°C for 10 min at pH 3. The particle size of mWPI decreased significantly ($P < 0.05$) to 116 ± 10.67 nm from 160 ± 3.82 nm. The pH was adjusted back to 6.8 after cooling. The particle size increased to 1,426 ± 179.80 nm at pH 6.8 with a high polydispersity index of 0.89 ± 0.11 due to the formation of aggregates. This pre-aggregated WPI was used as an ingredient in a high protein model beverage. MCC was reconstituted to 14% protein solution and mixed with treated WPI to create 8%, 9%, and 10% total protein solutions resulting in 80:20, 60:40, and 50:50 casein to whey protein ratio, respectively. The particle size of the samples with increasing protein content did not change significantly ($P > 0.05$). The viscosity increased significantly ($P < 0.05$) when protein content was increased to 9% and 10% due to the larger aggregates. The presence of pre-aggregated WPI did not further improve the heat stability. Sodium hexametaphosphate was added at 0.1% wt to all the samples. The viscosity and particle size did not change significantly ($P < 0.05$) after adding salt. The 8% protein sample with salt showed a higher HCT of 30.6 ± 1.25 min but HCT for 9% and 10% did not improve. Therefore, controlling the size of the whey protein aggregates is critical for improving the stability of the samples.

Key Words: whey protein aggregates, sodium hexametaphosphate, heat stability

2092 Dissociating casein micelles by ethanol and temperature to bind triglycerides.

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Casein micelles (CM) are known as natively disordered proteins that can self-assemble and are stable against temperature, pressure, and changes in the environment. This allows CM to encapsulate small hydrophobic molecules. The objective of this study was to form a CM-triglyceride complex by dissociating CM using ethanol and temperature to expose the hydrophobic regions for triglycerides to bind with. To form the complexes, 1 g of micellar casein was mixed with 9 mL of ethanol (0–60%) and held at 60°C for 30 min. Hydrogenated palm kernel stearin (HPKS) was added at 0.1 mL. The dispersions were mixed for 15 h at 60°C. Ethanol and water were removed by (1) centrifugal vacuum concentrator (CVC) at 60°C and freeze-dried at −105°C, or (2) only freeze-dried. The dried samples were ground into a fine powder using a mortar and pestle. All treatments were evaluated by differential scanning calorimetry. The samples (~5 mg) were weighed into aluminum pans and subjected to the following: starting at 20°C, the samples were heated at 20°C/min to 70°C and held for 3 min. Next, they were cooled at 20°C/min to 0°C and held for 3 min. Finally, they were heated at 10°C/min to 60°C. The melting curves from the third cycle were integrated from 21.5 to 40°C to determine the enthalpy (J/g). When the CM-HPKS was made with 10% ethanol and processed using the CVC, it resulted in a significantly less ($P < 0.05$) enthalpy (1.52 J/g) compared with the control with 0% ethanol (3.02 J/g). Likewise, when the CM-HPKS was made with 60% ethanol and processed using only the freeze dryer, it resulted in a significantly less ($P < 0.05$) enthalpy (1.19 J/g) compared with the control (3.47 J/g). Despite the fact that the *same* level of HPKS was added to all treatments, there is evidence that certain ethanol levels and processing methods promote more binding between HPKS and CM. This is shown by the lower enthalpy values; hence a smaller melting peak implies that HPKS is bound and unable to melt. We believe that this complexation behavior may enhance casein-based food ingredients (e.g., emulsifiers) or act as a way to replace unwanted fats with healthier options.

Key Words: casein micelles, dissociation, differential scanning calorimetry

2093 Characterization of the seasonal variability of minor compounds in cow milk.

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Variability remains a major problem in the cheese industry, despite the standardization of milk protein and fat on an industrial scale. This variability could be due to variations in the minor constituents of cow milk, which could affect the acidification rate of the lactic bacteria and the curd permeability during the cheese-making process. Therefore, this research project aims at determining if there is a relationship between the variability of minor constituents in cow milk and the growth of lactic acid bacteria. Milk samples were collected every 2 weeks for one year at the Centre de recherche en sciences animales de Deschambault (CRSAD, QC, Canada). The first objective was to characterize milk composition throughout the year. Minor compounds targeted in this study are amino acids and B vitamins. They were both analyzed using UPLC-MS/MS, using different separation conditions. Fourier-transform infrared spectroscopy (FTIR) characterized the contents of major constituents (protein, fat, lactose). Multivariate analysis was used to establish the link between the milk composition and the environmental data (e.g., sampling date, weather, health status). The principal components analysis (PCA) on correlation revealed 4 principal components (CP) with explained variances percentages of 35.65%, 26.36%, 14.72%, and 10.13% respectively for a total variance in the data of 86.86%. The CP 1 is mostly definite by fat and solids in the milk with a negative correlation with temperature. The CP 2 is defined by the correlation between true protein and casein contents. The CP3 revealed a negative correlation of vitamins B2 and B3 with non-protein nitrogen/calculated urea (NPN/CU.) This work will improve our understanding of factors contributing to cheese variability and pave the way for new milk standardization strategies considering the nutritional requirements of the lactic bacteria used in cheese production.

Key Words: milk characterization, minor compounds, vitamin B

Dairy Foods 1: Dairy Products

2094 Investigating potential application of rare sugars obtained from whey permeate in ice cream production. L. Peck*[1], P. Sharma[2], J. Waite-Cusic[1], H. D. Goff[3], and Z. Atamer[1], *[1]Oregon State University, Corvallis, OR, [2]Utah State University, Logan, UT, [3]University of Guelph, Guelph, ON, Canada.*

Underutilization of whey permeate has been a major issue within the dairy industry. Efforts are being made to add value to it. One of the potential uses of this low value stream is to produce a non-nutritive sweetener, such as tagatose. Tagatose is enzymatically isomerized from galactose, a breakdown of lactose. Tagatose has many benefits, including prebiotic traits, sweetening, and a low glycemic index, making it a great sucrose substitute. Tagatose is on its way to becoming more commercialized. For a suitable application and usage level, it is important to understand its thermal properties associated with crystal formation and melting behavior so it can commercially be added to frozen desserts such as ice cream. The objective of this study was to determine a difference between the melting characteristics of tagatose and sucrose if any and then to evaluate the performance of tagatose when used in ice cream. To achieve this a differential scanning calorimetry (DSC) was performed with the protocol being: start at 4°C ramp 5°C min^{-1} to −50°C and hold at −50°C for 10 min, then ramp 2°C min^{-1} to +20°C. The tagatose powder was prepared at 10%, 20% and 40% in solutions of water. The following parameters such as the onset temperatures (T0), peak temperatures (Tp), melting points (Tm) and enthalpies (ΔHf) were determined for each solution. For a 10% tagatose solution in water, T0 was −3.62°C, Tp was −0.29°C, and ΔHf was 221.8 J g^{-1}. At a higher concentration of 20% tagatose, T0 was −5.97°C, Tp was −1.65°C, and ΔHf was 191.1 J g^{-1}. An increase in tagatose levels to 40% resulted in following values: T0 was −15.58°C, Tp was −8.14°C, and ΔHf was 96.07 J g^{-1}. Comparing these with 40% sucrose led increased onset (−9.66), peak temperature (−4.24) and ΔHf 124.80 J g^{-1}. The dose dependency of the enthalpy of melting was significant (P-value = 0.0055), with sucrose being significantly higher than tagatose at 40%. These results suggest that increasing concentration of tagatose led to the melting of ice crystals in comparison with sucrose solution. These findings are important in relation to avoiding grittiness defect in ice cream.

Key Words: tagatose, freezing, alternative sweeteners

2095 Impact of proteolytic and crosslinking enzymes on the functional properties of milk protein concentrate. M. Akter*, K. Dubey, and P. Salunke, *Department of Dairy & Food Science, South Dakota State University, Brookings, SD.*

Milk protein concentrate (MPC) has become popular for increasing protein content and improving functionalities in dairy and food products. However, MPC functionality still needs to be fully expressed in certain products. Hence, enzymatic treatment has been used to alter the functionality. The study was planned to determine the effect of proteolytic hydrolysisand then crosslinking treatment to produce novel ingredients and study functionality. MPC retentate was produced from pasteurized skim milk via ultrafiltration. MPC retentate was divided into 3 parts: no treatment (control), alcalase hydrolysis of protein followed by deactivation of the enzyme (90°C/90 min) to manufacture milk protein hydrolysate (MPH), and then crosslinking hydrolyzed proteins using transglutaminase (TGase) at a concentration 2 U/g protein, followed by deactivation of the enzyme (72°C/10 min) to manufacture cross-linked milk protein hydrolysate (CMPH). The functional properties studied at 12% protein solution included particle size, emulsion properties, heat stability, viscosity, and foaming properties. Statistical analysis was performed using Costat software. The experiment was replicated twice. No significant ($P < 0.05$) was observed in the particle size of the treatments. The heat stability of the MPH at 537.25 s and CMPH at 188 s was significantly ($P < 0.05$) lower than the control at 895 s. MPH 61.25% and CMPH 67% emulsion properties were significantly ($P < 0.05$) lower than the control 70.75%. The foam overrun of MPH (41.25%) was significantly ($P < 0.05$) higher, and CMPH (26.25%) was significantly ($P < 0.05$) lower than the control sample (31.25%). However, the foaming stability of the control sample was significantly ($P < 0.05$) higher than the MPH and CMPH samples. MPH emulsion activity and stability were significantly lower than control and SMPH samples. In the frequency sweep test, the viscosity of MPH and CMPH samples was significantly ($P < 0.05$) lower than the control samples. In conclusion, the functionality of the MPC can be tailored using the combination of proteolytic and crosslinking enzymes.

Key Words: milk protein concentrate, enzyme, functionality

2096 Kinetics of digestion on selected whey-based milk fat globule membrane ingredients in a model infant formula. L. Wilmot*[1,2], A. Kelly[1], J. A. O'Mahony[1], J. O'Regan[1,3], D. Goulding[3], and R. Jimenez-Flores[2], *[1]University College Cork, Cork, Ireland, [2]The Ohio State University, Columbus, OH, [3]Nestlé Development Centre Nutrition, Limerick, Ireland.*

In recent years, there has been a growing interest in the use of milk fat globule membrane (MFGM) ingredients in infant nutrition. Many clinical studies have demonstrated the physiological benefits of adding such ingredients to infant formulae. However, there are considerable compositional differences between commercially produced MFGM ingredients and there is lack of knowledge on the impact this has during infant digestion, with previous studies showing that the interfacial composition and fat globule size controls the rate of lipolysis. The kinetics of digestion differs in breast-fed infants compared with formula-fed infants, which is mostly attributed by the size of the milk fat globule and presence of MFGM. By understanding the kinetics of digestion, the release, bioaccessibility and bioactivity of some bioactive compounds may be controlled to some extent via alteration of the interfacial composition with MFGM which is rich in both phospholipids and proteins. The objective of this study was to assess whether the protein profile of selected whey-based MFGM ingredients influences gastroduodenal digestion. First stage model infant formulas were supplemented with commercially available MFGM ingredients and subjected to a static in vitro infant digestion model, and the rates of proteolysis and lipolysis were studied. Microscopy was used to examine the protein structures and fat globules in the digesta. SDS-PAGE also showed differences in resistance and susceptibility of whey and MFGM proteins to digestion. Lipolysis was measured by free fatty acid release and lipolysis degree. The results of this study may allow infant formula manufacturers to formulate products to achieve more desirable rates of proteolysis and lipolysis based on selection of MFGM ingredient.

Key Words: digestion, milk fat globule membrane, infant nutrition

2097 Temperature variability throughout e-commerce distribution pathways and its impact on dairy product quality. Z. D. Wasserlauf*[1], A. Trmcic[1], J. Su[2], M. Wiedmann[2], and N. H. Martin[1], *[1]Milk*

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The rise of e-commerce distribution has grown drastically in recent years, a trend that was accelerated by the COVID pandemic. The types of perishable products being purchased as well as the distance over which these products will travel to consumers has also increased considerably. Perishable goods are commonly available through various e-commerce channels (e.g., third-party grocery shopping and delivery, direct processor to consumer delivery, and overnight shipment via centralized distribution centers). New or expanded distribution methods have surfaced to accommodate the increasing demand of grocery delivery, which can introduce the potential for temperature abuse of these perishable goods, ultimately leading to premature spoilage. To determine the impact of e-commerce distribution on the microbial quality of dairy products, we assessed the variability of simulated and real e-commerce time-temperature profiles of fluid milk and Greek yogurt ($n = 54$, $n = 51$, respectively). We assessed 3 unique transportation channels associated with e-commerce distribution, including; (1) processor direct to consumer, (2) distributor to consumer, and (3) third-party shopper and delivery. Our results showed that product temperature at the time of delivery ranged from 0.2 to 10.1°C for processor direct to consumer pathway, −0.9 to 19.2°C for distributor to consumer pathways, and 3.1 to 18.3°C for third-party shopper and delivery. The temperature profiles measured during 1.89 L milk transportation were incorporated into a previously developed computation model that predicts milk spoilage based on major groups of bacterial contaminants. These models can be used by the dairy industry to predict the quality and shelf-life of dairy products after moving through the e-commerce distribution chain and to quickly assess intervention strategies to reduce temperature abuse during e-commerce distribution.

Key Words: e-commerce, temperature abuse

2098 Microbial and sensory assessment of novel probiotic dairy products.

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This study aims to explore the potential of incorporating *Lacticaseibacillus rhamnosus* GR-1 (*L. rhamnosus* GR-1) in 5 dairy samples with or without quinoa, chickpea, soybean, and rice flour over various fermentation periods and refrigerated storage durations. These flours contain resistant starches known for their prebiotic properties and potential stabilizing effects in dairy products. Additionally, the study aims to evaluate consumer preferences using a hedonic scale. The samples were inoculated with *L. rhamnosus* GR-1 and were fermented for 0, 2, 4, and 6 h, followed by refrigerated storage for 1, 15, and 30 d. Microbial enumeration was performed throughout fermentation and storage to assess the viability of *L. rhamnosus* GR-1. A sensory evaluation involving 86 participants was conducted to assess consumer acceptability. Notably, *L. rhamnosus* GR-1 achieved viable counts of 10^8 cfu/mL in all treatments. The change in microbial counts over the duration of fermentation was significant for control sample ($P < 0.029$) and yogurt with soy flour ($P < 0.007$) as they had higher counts compared with other treatments. Throughout the 30-d storage period, there were no significant differences ($P > 0.05$) in the average microbial counts or pH values among the 5 different treatments. Treatment 4 which included rice flour, received significantly higher hedonic scores ($P < 0.05$) from sensory panelists in terms of appearance, flavor, texture, and overall acceptability. These findings indicate promising potential for successfully incorporating various functional flours into dairy mixtures with *L. rhamnosus* GR-1.

Key Words: probiotic, sensory, novel

2099 Innovative utilization of orange peel powder in *burfi* preparation: Circular economy strategies.

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This research investigates the potential enhancement of the nutritional quality and functional characteristics of *burfi* (a traditional Indian sweet) through the incorporation of orange peel powder, a byproduct from juice companies known for its rich phenolic, antioxidant, flavonoid, fiber, and ascorbic acid content. The orange peel powder was derived from pre-blanched (steam) peels, followed by drying using a convective tray drier at 90°C for 5 h. It imparts a distinctive citrus flavor and antioxidant profile for potential health benefits. The *burfi* formulations were prepared by varying the proportions of orange peel powder at 5%, 10%, and 15% in sweetened khoa which was further compared with the control (without any addition). It is then subjected to a comprehensive analysis of key parameters for moisture, total solids, fat, protein, acidity, crude fiber, antioxidant activity, ascorbic acid, and total phenolic content. The data were statistically analyzed by one-way ANOVA using SPSS 28.0 software and revealed significantly higher physico-chemical properties and phytochemical content ($P \leq 0.05$) with orange peel powder inclusion in burfi compared with the control. Total solids increased with powder levels, and functional constituents such as fiber, total phenols, ascorbic acid, and antioxidant activity also rose with higher inclusion. The sample contained 15% orange peel powder has 9.26% moisture, 2.8% fat, 8.16% protein, 0.69% LA acidity, 1.85% crude fiber, and 17.07 mg GAE/100g. Fifteen semi-trained panelists evaluated various formulations of *burfi* on the basis of sensory evaluation, which was conducted at regular analysis of 3-d intervals for 10 d. The mean sensory score of color and appearance, texture, flavor, and overall acceptability was 7.93, 8.21, 8.3, and 8.15 on the 9-point hedonic scale for 15% contained orange peel powder *burfi*. The inclusion of orange peel powder not only enhanced the functional properties and nutritional value of *burfi* but also promotes sustainability by reducing food waste and enhancing flavor diversity.

Key Words: orange peel powder, burfi, waste utilization

2101 Stability of various sugars in simple and complex yogurts.

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Innovation in whey upcycling has significant potential in the food industry. Multi-step enzyme treatment of whey lactose leads to the production of mixed sugar-syrups with increased sweetness yet decreased calories. These syrups could be reapplied in dairy foods, such as yogurt or ice cream, as a sucrose replacement. Yogurt contains live cultures, and consequently their enzymes, including those capable of sugar hydrolysis or isomerization, suggesting that sugar profiles might not be stable throughout product shelf life. Our objective was to evaluate the stability of tagatose, fructose, lactose, galactose, glucose, and sucrose in yogurt under typical storage conditions (4°C, 6 weeks). Two commercial nonfat Greek yogurt products with different bacterial profiles (simple: *Streptococcus thermophilus* and *Lactobacillus delbreukii* ssp. *bulgaricus*; complex: *S. thermophilus*, *Lb. bulgaricus*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Lactoccocus lactis*) were supplemented with each of the sugars at 10% (wt/wt) in duplicate and analyzed every 2 weeks. Individual sugar concentrations and lactic acid were measured using a combination of chromogenic enzyme kits (Megazyme) and high-performance liquid chromatography. The pH of all yogurts slightly decreased initially but remained constant throughout remaining storage, and there was no significant difference in pH between yogurts with different added sugars ($P > 0.05$). Lactic acid content was at 0.8% (simple) and 1.3% (complex) and no changes were observed. For simple type yogurt, measured fructose, lactose, galactose, glucose, and sucrose contents after 6 weeks was 11.3%, 9.9%, 11.9%, 11.2%, and 9.8% respectively, noting that the control yogurts contained galactose and lactose. For complex type yogurt, measured fructose, lactose, galactose, glucose, and sucrose contents after 6 weeks was 10.7%, 13.8%, 11.4%, 9.9%, and 10.5% respectively, noting that the control yogurts contained glucose, galactose, and lactose. The results showed no significant change in sugar contents or lactic acid over the course of 6 weeks ($P > 0.05$). This study supports that sugars remain stable throughout yogurt storage.

Key Words: yogurt, sugar, stability

Dairy Foods 1: Microbiology

2102 Transgalactosylation activity of *Lactobacillus bulgaricus* YB1 in sweetened nonfat milk. G. K. P. Guron*, A. T. Hotchkiss, and J. A. Renye, *USDA, ARS, Eastern Regional Research Center, Wyndmoor, PA.*

β-galactosidase is a vital enzyme used in the food industry to reduce lactose from dairy ingredients through hydrolysis and to synthesize galacto-oligosaccharides (GOS), a known bifidogenic prebiotic, from galactose moieties through transgalactosylation. To favor transgalactosylation, high carbohydrate concentrations must be available to accept the galactose moiety. High lactose concentrations are often used for transgalactosylation reactions, but because many dairy products rely on other sweeteners, this study examines the use of a GRAS status organism, *Lactobacillus bulgaricus* YB1, that produces β-galactosidase to determine if this reaction can still be driven in high concentrations of other sweeteners such as granulated sugar or corn syrup dissolved in milk. Nonfat dried milk (10%, wt/wt) was dissolved in water along with 16% lactose, 16% granulated sugar, 16% corn syrup, or 11% granulated sugar, and 5% corn syrup, then heated with stirring for 30 min at 70°C for batch pasteurization. YB1 whole cells were inoculated at 6.8 log cfu/mL, or the same quantity of cells were lysed before adding to the milk and carbohydrate mixtures. All samples were incubated for 18 h at 50°C (n = 3). The final glucose, galactose, and lactose concentrations were measured to calculate % transgalactosylation (%TG) from the proportion of galactose that was hydrolyzed from lactose or polymerized with another carbohydrate. There were no differences in %TG (mean ± SD = 25.1 ± 9.9%, $P > 0.05$). All treatments had lower free galactose compared with the lysate with 16% lactose (93.7 ± 15.0 m*M*, $P < 0.001$) by over 31 m*M*. Free glucose was the lowest in 16% lactose (56.4 ± 2.4 m*M*, $P < 0.022$) and 16% granulated sugar (45.8 ± 3.6 m*M*, $P < 0.003$) with live whole cells. High-performance anion-exchange chromatography with pulsed amperometric detection was used to visualize the presence of oligosaccharides with degrees of polymerization of 3 or above. Live YB1 whole cells can potentially produce GOS or other unidentified oligosaccharides as a starter culture for sweetened fermented dairy products.

Key Words: Lactobacillus bulgaricus, transgalactosylation, galacto-oligosaccharides

2103 Ethanol production by yeast using ice cream by-product as the fermentation substrate. A. L. Miller*[1], J. A. Renye Jr.[1], J. Thomas[2], D. Johnston[2], R. Garcia[1], and P. Tomasula[1], *[1]Dairy and Functional Foods Research Unit, Agricultural Research Service, USDA, Wyndmoor, PA, [2]Sustainable Biofuels and Co-products Research Unit, USDA, Wyndmoor, PA.*

Ice cream is a popular frozen dairy dessert consumed worldwide, with the US producing 1.38 billion gallons of ice cream in 2022; however, an estimated 10 to 20M pounds of waste is generated annually. The economic burden associated with ice cream waste results from disposal fees and the loss of valuable ingredients that could be transformed into novel products. Fermentation has been shown to successfully convert dairy food waste into novel consumable products such as alcohol. This study aimed to investigate the potential for using an aqueous by-product recovered during fat extraction from vanilla ice cream as a fermentation substrate for production of ethanol by 2 strains of *Saccharomyces cerevisiae*: a brewer's yeast, Lalvin EC-1118 (LEC), and an industrial yeast, Ethanol Red (ETR). Fat extraction was performed using a modified butter churning procedure, with the aqueous by-product collected after butter grains were strained though cheesecloth. The aqueous by-product was aliquoted into 100 mL samples and inoculated with yeast (1×10^7 cfu/mL). Fermentation was carried out in a shaking incubator at 30°C, with a sterile syringe needle inserted through the rubber stopper to allow for CO_2 release. Flasks were monitored intermittently for weight loss which correlates with ethanol production. Fermentations were stopped when weight loss plateaued at 72 h. Cell free supernatants were collected following centrifugation and filtered for analysis by high performance liquid chromatography (n = 3). The churned by-product contained approximately 18.05 ± 0.05% fermentable sugars. Fermentation with LEC resulted in the production of 6.88 ± 0.49% ethanol, while the use of ETR yielded 7.54 ± 1.37% ethanol. Ethanol production was further tested after enzymatic treatment with lactase (75 µL/100 mL) and/or glucoamylase (50 µL/100 mL). The highest ethanol yield was observed when enzymes were used simultaneously, resulting in 9.18 ± 1.10% and 8.95 ± 1.08% ethanol for LEC and ETR, respectively ($P < 0.0001$). Results from this study suggest that this aqueous by-product from wasted ice cream can be used as a fermentation substrate for future studies to optimize ethanol production.

Key Words: yeast, ice cream, ethanol

2104 Developing a sweet potato medium for the cultivation of *Lactobacillus* sp. A. S. Eddin*, R. Tahergorabi, and S. Ibrahim, *North Carolina A&T State University, Greensboro, NC.*

Purpose: High cell mass production of lactic acid bacteria (LAB) has become increasingly important in the food industry. The supplementation of culture media with various nutrients is crucial for the optimal growth of LAB strains. Nitrogen, for example, is an essential component of growth culture media. However, traditional nitrogen sources used in LAB media can be expensive and contribute significantly to the overall cost of the media. To address the challenges, researchers have started exploring alternatives such as the potential of lower-cost food items and surplus agricultural products such as sweet potatoes as substitutes for expensive nitrogen sources. Sweet potato is a root tuber that is rich in carbohydrates, minerals, and vitamins. The objective of this study was thus to investigate the use of sweet potato as a primary component in developing a medium for the cultivation of *Lactobacillus* sp. Methodology: Total of 7 strains (3 strains of *Lactobacillus delbrueckii* ssp. *bulgaricus*, 3 strains of *Limosilactobacillus reuteri*, and 1 strain of *Lactobacillus rhamnosus* GG) were used in this study. Three batches of sterilized sweet potato juice were mixed separately with a group of nitrogen sources (X-Seed Nucleo Max, X-Seed KAT, and NuCel 780 MG) to form SPM1, SPM2, and SPM3. MRS medium broth was used as a control. The samples of media were inoculated and incubated for 12 h at 37°C. The bacterial populations were determined at 12 h of incubation by plating appropriate dilution, and the turbidity (OD_{610} nm) was measured at (0, 6, 9, and 12 h) of incubation. Findings: The results for the bacterial populations accorded with log cfu/mL and OD_{610} indicated that the growth of the tested *Lactobacillus* sp. strains in SPM3 was higher than in SPM1, SPM2, or MRS. Consequently, SPM3 is suitable for the growth of the tested *Lactobacillus* sp. strains. Conclusion: Our findings demonstrated that SPM3 could be a suitable medium for the growth of *Lactobacillus* sp. and could thus be used as low-cost an alternative medium of standard MRS medium.

Key Words: lactic acid bacteria, growth medium, sweet potato

2105 Screening and selection of commercial human probiotic lactic acid bacteria strains for biotechnological milk applications.

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Probiotics can improve host health when ingested in adequate amounts. These microorganisms are highly employed in biotechnology processes, such as fermentation, food technology, and detoxification. In this context, this study aimed to investigate and characterize the potential of commercial human probiotic strains for biotechnological milk applications. Twenty lactic acid bacteria were investigated concerning kinetic growth curves, gastrointestinal assays, lactose consumption, antimicrobial resistance, and bioremoval, as well as aflatoxin M1 (AFM1). Following an initial screening, 5 *Lactobacillus* spp. strains were selected as potential biotechnological agents (Table 1). All achieved the desired concentrations within 24 h and presented antimicrobial resistance, as well as good intestinal adhesion, consisting of viable probiotics with high lactose consumption, thus enabling their application in food technology and organic acid production. The observed lactose consumption rates indicate the possibility of obtaining low lactose content products while setting a precedent for studies focusing on the production of secondary metabolites of industrial interest, such as lactic acid. Although no AFM1 and antimicrobial biometabolization was observed, the strains presented AFM1 adsorption rates of up to 65.9%, reducing AFM1 bioavailability in food matrices. The selected strains therefore display the necessary properties for biotechnological milk applications and potential for decontamination, allowing for LAB application in bioremoval technologies, especially in the fermentation processes of low lactose content products.

Table 1 (Abstr. 2105). Kinetic parameters and correlation coefficient of selected lactic acid bacteria[1]

Lactobacillus strain	μ max (h^{-1})	gt (h)	r^2	*P*-value
L. acidophilus ACID GB®	0.0206	33.65	0.93	<0.05
L. bulgaricus LB-G40®	0.0405	17.11	0.99	<0.05
L. casei NTU 101	0.0137	50.59	0.97	<0.05
L. gasseri LG08®	0.0423	16.39	0.90	<0.05
L. rhamnosus CRL 1505®	0.0291	23.82	0.92	<0.05

[1] μ max = maximum growth speed; gt = generation time; r^2 = Spearman's correlation coefficient.

Key Words: contaminant bioremoval, AFM1, lactose

Forages and Pastures 1

2106 Effect of urea addition on the microbiology and quality of corn stover (*Zea mays*) silage. A. Sanchez Laiño*[1], E. Torres Navarrete[1], J. Sanchez Torres[1], I. Espinoza Guerra[1], and D. Verdecia Acosta[2], *[1]Universidad Técnica Estatal de Quevedo, Quevedo, Los Ríos, Ecuador, [2]Universidad de Granma, Bayamo, Granma, Cuba.*

The knowledge about the influence of urea on the conservation and nutritional quality of fibrous feed silages is crucial. This study evaluated the impact of urea addition on the microbiology and quality of corn stover (*Zea mays*) silage. A factorial arrangement of 4 (urea levels: 0%, 1%, 2%, and 3%) × 8 (silage opening days: 7, 14, 21, 28, 35, 42, 49, and 56 d) and 4 replications per treatment and mean differences were analyzed using the Tukey test ($P \leq 0.05$). Measurements of total bacteria, lactobacilli, molds (cfu g^{-1}), pH, and temperature (°C) were conducted. Additionally, chemical composition, including dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (HEM), was evaluated. Significant interactions were observed between urea levels and silage days ($P < 0.05$). Notably, treatment T3 (2% urea) showed higher values for total bacteria and lactobacilli (22.21 and 15.60 cfu g^{-1}, respectively). For molds, treatment T4 (3% urea) exhibited a less defined pattern with constant variations, though all treatments showed a tendency to increase over time. Treatment T1 (control, 0% urea) demonstrated a decrease in molds count from 7 to 35 d, followed by an increase up to 56 d (8.53 cfu g^{-1}). Similarly, pH and temperature showed significant interactions between urea levels and silage days ($P < 0.05$). A pH of 5.48 and a temperature of 23.75°C were observed with 1% urea from d 35 of fermentation. The interaction between urea addition and ensiling days promoted the increase of total bacteria, lactobacilli, DM (72.72%), and CP (9.25%), as well as the decrease of molds and cell wall components (NDF: 63.94%, ADF: 29.81%, and HEM: 35.46%) up to 35 d. In conclusion, treatment T3 containing 2% urea added to corn aggregate silage improves microbiological (total bacteria, lactobacilli) and nutritional quality (DM, CP) up to 35 d of fermentation. It reduces the amount of molds and cell wall components (NDF, ADF, HEM).

Key Words: bacteria, fungi, lactobacilli

2107 Effect of zero-grazing herbage on ruminal fermentation and methane yield in dairy cows. N. P. Hansen, M. R. Weisbjerg, P. Lund, and M. Larsen*, *Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark.*

The effect of zero-grazing grass-clover or grass on ruminal fermentation and methane yield as compared with grass-clover silage was investigated. Six rumen-cannulated Holstein cows were used in a replicated 3 × 3 Latin square design with 14-d periods. Short periods were chosen to reduce the variation in fresh herbage among periods. Treatments were (1) first-cut ryegrass-white clover silage (SiGC), or 3-wk regrowth of (2) fresh ryegrass-white clover (FrGC), or (3) fresh ryegrass (FrG). Fresh herbages were harvested every morning with a mover-wagon giving minimal physical herbage handling. Forages were allocated ad libitum, and 4.5 kg DM/d of pelletized concentrate was offered separately. After 10 d adaptation, 6 ruminal ventral samples were taken over 48 h with 8 or 12 h intervals. Hereafter, one square of cows was placed in respiration chambers for 48 h and 3 rumen evacuations were performed over 48 h with cows in the other square. The statistical model contained the fixed effects of Period, Treatment, and when relevant, Time and Treatment × Time (Time within Cow × Period was considered as repeated measure). Cow was considered a random factor. Forage DMI was lower for FrG as compared with FrGC and SiGC (13.6 vs. 15.1 kg/d, SEM = 0.71, $P = 0.02$). Concentrate DMI did not differ among forages (4.3 ± 0.12 kg/d, $P = 0.51$). Average and minimum ruminal pH did not differ among forages (6.2 ± 0.06, $P = 0.99$, and 5.8 ± 0.08, $P = 0.62$, respectively). The ruminal VFA proportion of acetate did not differ among forages (0.60 ± 0.005, $P = 0.25$), whereas the proportions of propionate and butyrate were greater and lower, respectively, for FrGC and FrG as compared with SiGC (0.23 vs. 0.20, SEM = 0.011, $P = 0.02$, and 0.13 vs. 0.15, SEM = 0.009, $P = 0.04$, respectively). The methane yield was lower for FrGC and FrG as compared with SiGC (19.4 vs. 22.3 g/kg DMI, SEM = 0.59, $P = 0.01$). Ruminal pool sizes of water and DM did not differ among forages (65 ± 1.9 kg, $P = 0.98$, and 8.1 ± 0.75 kg, $P = 0.95$, respectively). In conclusion, zero-grazing did not affect ruminal pH, but the fermentation pattern shifted from butyrate to propionate, likely explaining the lower methane yield.

Key Words: fresh grass, VFA, methane

2108 Effect of oat variety and oat type (feed-type vs. milling-type) on intestinal digestion of dry matter, protein, starch, and fiber in dairy cows. G. Deng*, M. E. Rodriguez Espinosa, and P. Yu, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.*

The objectives of this study were to investigate the effects of new oat variety and oat type (feed-type vs. milling type) on intestinal digestion of protein and starch in dairy cows. The oat varieties were provided by Crop Development Center, including Arborg (Milling-type), Haymaker (Feed- and Forage-type), Nasser (Feed-type), and Summit (Milling-type), which were grown and harvested at the university crop research fields for 3 consecutive years. The intestinal digestion of protein and starch was determined using 3-step in vitro technique with fistulated lactating dairy cows. The experiment design was a RCBD in which the data were analyzed using mixed-model procedure of SAS with the oat varieties as a fixed effect and the years as a random block effect. Treatment means were compared using Tukey methods. The comparison between milling-type oat and feed-type oat was used orthogonal contrasts of SAS. The results showed that the oat variety had significant effect ($P < 0.05$) on intestinal digestibility of rumen undegraded dry matter (%dBDM, ranged from 17.5% to 32.2%) and crude protein (%dBCP, ranged from 65.6% to 85.3%) but did not affect starch (%dBST) and NDF (%dBNDF). For intestinal digestion (ID) of rumen undegradable nutrients, the oat variety significantly affected ($P < 0.05$) IDBDM (ranged from 161 to 186 g/kg DM), IDBCP (ranged from 46 to 67 g/kg DM), IDBST (ranged from 55 to 92 g/kg DM), and IDBNDF (ranged from 4 to 22 g/kg DM). For total-tract digestion (TD), the oat variety significantly affected TDDM (ranged from 401 to 625 g/kg DM), TDCP (ranged from 129 to 158 g/kg DM), TDST (ranged from 233 to 381 g/kg DM), TDNDF (ranged from 37 to 75 g/kg DM). In conclusion, oat variety significantly impacted intestinal digestion of nutrients in dairy cows.

Key Words: oat variety, feed-type and milling-type, intestinal digestion of nutrients

2109 Characterization of ryegrass silage (*Lolium perenne*) from Southern Chile ensiled in piles or bales, and nutritional value estimation by the Cornell Net Carbohydrate and Protein System model. P. Melendez[1], I. Castro[2], A. Adesogan[3], P. Pinedo*[4], and O.

Nekouei[1], [1]*City University of Hong Kong, Kowloon, Hong Kong SAR, China,* [2]*Cooprinsem, Osorno, Chile,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [4]*Department of Animal Sciences, Colorado State University, Fort Collins, CO.*

The objectives were to evaluate the nutritional characteristics of *Lolium perenne* silage from Southern Chile using the CNCPS model and to compare these characteristics between pile and bale ensiling methods. Between 2019 and 2021, 880 perennial ryegrass silage samples (445 from piles and 435 from bales) submitted as part of the routine nutritional management of dairy farms were studied from 158 dairies in Southern Chile. Samples were analyzed by NIRS and dairy cow performance was assessed using the CNCPS model. Average of all samples within each quintile of DM distribution within conservation method (5 values per cluster) were calculated for each nutritional fraction and entered into the CNCPS model. The NIRS and CNCPS outcomes were compared between pile and bale silos using a GLM ANOVA. Least squares means were adjusted for year and farm. Significance was considered at $P \leq 0.05$. DM and crude protein were significantly lower in pile than in bale silages; 35.2% versus 42.2%, and 13.3% versus 13.9%, respectively. aNDFom and lignin were significantly higher in pile than in bale silages; 51.43% versus 49.1%, and 4.52% versus 3.85%, respectively. uNDF residue in the rumen at 240 h postfermentation was higher in pile (14.80%) than in bale silages (12.45%). NDF digestibility at 30, 120, and 240 h was not statistically different between the 2 methods. Lactic acid was lower in bale (4.53%) than in pile silages (6.27%), resulting in a higher pH for bale silages (4.69) than pile silages (4.37). Assuming a cow consuming 25 kg of DM of silage conserved as bale versus piles, the metabolizable energy (ME; Mcal/day) and protein (MP) (g/day) to produce milk were higher for bale silages (41.8 Mcal and 462.3 g) than pile silages (40.3 Mcal and 296.6 g), respectively. Fecal N, P, and K (g/day), and CH_4 yield (L/day) in the rumen were higher in bale silages than in pile silages. In conclusion, perennial ryegrass silage conserved as pile had better nutrition composition than silage conserved as bale in the south of Chile.

Key Words: ryegrass, bale silage, pile silage

2110 Measurement of the productive potential of *Megathyrsus maximus* grass under tropical conditions by using TaurusWebs®2023 software.

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The objective of this study was validating TaurusWebs®2023 software as a management tool in the productive system of a tropical dairy cattle unit through integrated analysis of biomass production and nutritional quality of the pastures with respect to production potential. A total area of 8.44 ha was used, divided into 19 paddocks of ~0.44 ha each, under an intensive rotational grazing system. Cows grazed at each paddock for one day, followed by an 18-d resting period, for a total of 19 d per cycle. Seventy-four Holstein and Brown Swiss cows were used (second or third lactation cows), with an average milk yield of 18 to 24 kg/d. Variables evaluated were biomass production, fresh matter intake (FMI), milk production (kg) and bromatological analysis (CP%, NEL [Mcal], ADF%, NDF%). A completely randomized design (CRD) was used with 2 treatments: conventional system (Estimated carrying capacity and NIRS analyses) and TaurusWebs®2023 software. Nineteen experimental units per treatment, each experimental unit corresponding to each paddock used in the experiment. All paddocks were subjected to both methods. Eight grass samples were collected from the center of each paddock via the quadrat method to avoid a border effect. The grass was cut at a height of 20 cm. Drone images were taken from the same quadrants to compare both methods. An ANOVA with repeated measures over time, and a SNK test for minimum differences were performed by using the Statistical Analysis System SAS® version 9.4 ($P \leq 0.05$). Differences were found in the average grass biomass offered (0.15 and 1.55 kg/m^2). Likewise, for the bromatological analysis, significant differences were found in CP (9.83% and 17.06%), NEL (0.77 and 1.23 Mcal), NDF (69.77% and 54.10%) and ADF (48.99% and 26.27%) for conventional method and TaurusWebs®2023, respectively. There was no difference found between FMI and milk production ($P \geq 0.05$). Differences between the TaurusWebs®2023 software and the conventional method differed in a minimal range, because the diets were adjusted according to the cows' requirements.

Key Words: biomass production, Livestock 4.0, pasture quality

2111 Effect of supplementation on production performance of grazing dairy cows: A systematic review and meta-analysis.

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Supplementation of grazing dairy cows is key to enhancing productivity in pasture-based systems. We aimed to assess the effect of different supplementation strategies on DMI, milk yield (MY), and concentrations of milk fat and protein in grazing dairy cows through a systematic review and a meta-analysis (R software). The selection criteria included population (grazing dairy cows), intervention (supplementation strategy), and individual DMI. Out of 925 articles screened from PubMed, ScienceDirect, and CABI Direct, 14 were selected. Supplementation types were energy-based (e.g., oil, corn) and energy-protein blend (e.g., soybean meal-corn). Covariates were subjected to meta-regression and, when significant, subgroup analyses were performed. Total DMI and MY averaged 16.7 kg/d (range from 11.0 to 24.9 kg/d) and 23.9 kg/d (range from 14.0 to 31.6 kg/d), respectively. Data are presented in Table 1. Compared with pasture as the sole diet, total DMI (+2.26 kg/d) and MY (+3.58 kg/d) increased with supplementation at expense of pasture DMI (−1.60 kg/d) due to the substitution rate effect. Milk fat concentra-

Table 1 (Abstr. 2111). Effect of pasture supplementation on production performance

		RMD[1] (95% CI)		Heterogeneity (I^2)	
Item	n[2]	Random effect	*P*	*P*	I^2 (%)
Total DMI, kg/d	32	2.26 (1.47; 3.05)	<0.001	<0.001	86.8
Pasture DMI, kg/d	30	−1.60 (−2.21; −1.00)	<0.001	<0.001	74.9
Milk yield, kg/d	29	3.58 (2.75; 4.41)	<0.001	<0.001	70.4
Milk fat, %	33	−0.41 (−0.60; −0.21)	<0.001	<0.001	89.0
Milk protein, %	31	0.04 (−0.02; 0.09)	0.21	<0.001	83.1

[1] RMD = randomized mean difference.

[2] n = number of comparisons.

tion decreased (−0.41 percentage unit) in response to supplementation, due to dilution effect caused by increased milk volume. Contrarily, milk protein concentration was not affected by supplementation. Based on the subgroup analysis, milk fat concentration decreased more pronouncedly in cows supplemented with energy-based sources (−0.66 percentage unit) than energy-protein blend (−0.06 percentage units) with no change in MY. In brief, supplementation increased total DMI and MY, and supplementation type modulated milk fat concentration.

Key Words: grazing, pasture, supplement

2112 Chemical composition of corn silage with varying levels of inclusion of cocoa maguey residues (*Theobroma cacao* L.). M. L. Medina*[1], F. A. Ortiz[2], I. F. Espinoza[1], A. E. Barrera[1], D. A. Romero[1], and G. R. Alvarez[1], *[1]Universidad Tecnica Estatal de Quevedo, Quevedo, Los Rios, Ecuador, [2]Escuela Superior Politecnica del Chimborazo, Riobamba, Chimborazo, Ecuador.*

The maguey is the placenta where the cocoa beans are attached and this research aimed to determine the nutritional value of corn silage with different levels of inclusion of dehydrated cocoa maguey residues (*Theobroma cacao* L.). For the chemical analysis evaluated, Corn forage harvested at 90 d and cocoa maguey residue, cut into 1 cm pieces, were used, and stored together in 40 kg capacity plastic bags. A completely randomized experimental design was employed with 5 treatments and 5 replications: T1 (100% corn forage silage), T2 (90% corn forage silage + 10% cocoa maguey), T3 (80% corn forage silage + 20% cocoa maguey), T4 (70% corn silage + 30% cocoa maguey), and T5 (60% corn silage + 40% cocoa maguey). The significance of the collected data was evaluated through ANOVA and Tukey's multiple range test ($P < 0.05$). The contents of dry matter (DM), organic matter (OM), inorganic matter (IM), crude fiber (CF), neutral detergent fiber (NDF) and acid detergent fiber (ADF) did not significantly affect ($P > 0.05$) the inclusion of cocoa maguey in forage corn silage. However, crude protein (CP) in T5 (10.87%) differed significantly ($P < 0.05$) when compared with T1, T2, and T3 (9.45%, 9.47%, and 9.57%, respectively). Additionally, fat (EE) in T5 (3.43%) exceeded T1, T2, and T3 (2.49%, 2.08%, and 2.26%, respectively). Levels of cocoa maguey residue inclusion corn forage silage did not have a significant impact on the contents of DM, OM, IM, CF, NDF, and ADF of corn silage with varying levels of inclusion of dehydrated cocoa maguey residues. However, the silage of forage corn and the inclusion of cocoa maguey contributed to higher CP and EE content.

Key Words: agricultural residues, chemical composition, nutritional value

2113 The effects of a new grain processing technology on forage sorghum processing. D. Duhatschek*[1], A. Grando Pilati[1], L. Luckasson[2], J. Goeser[3,4], E. Coons[3], L. F. Ferraretto[4], and J. M. Piñeiro[1], *[1]Department of Animal Science, Texas A&M University, College Station, TX, [2]Scherer Inc., Sioux Falls, SD, [3]Rock River Laboratory, Inc., Watertown, WI, [4]Department of Animal & Dairy Sciences, University of Wisconsin, Madison, WI.*

The processing of forage sorghum and in situ starch digestibility with a novel grain processing technology was evaluated on a commercial dairy farm in New Mexico under center pivot irrigation. The objectives were to assess the effect of a novel kernel processing (KP) technology and ensiling time (ET) on whole plant sorghum silage processing and rumen in situ starch digestibility 7 h (isSD7). A forage sorghum hybrid was harvested at the hard dough stage with 3 different harvesting settings (HS): (1) Low noKP: cut height of 20 cm without KP; (2) Low+KP: cut height of 20 cm with KP; or (3) High+KP: cut height of 122 cm with KP. Processed samples were obtained from 3 replicates (plots) randomly selected and screened to determine particle size distribution using the Penn State Particle Separator (PSPS). Duplicate samples of the harvested material were kept frozen or ensiled in vacuum sealer plastic bags for 90 d and sent to a commercial laboratory for analysis. Data were analyzed using mixed linear regression models with HS, ET and HS × ET as predictor variables and plot as the random effect. High+KP had the highest amount of material passing through all PSPS sieves followed Low+KP and Low no KP (43.7%, 20.7% ,and 6.8%, SEM 2.1%, $P < 0.0001$). The isSD7 increased after ensiling both cut heights when using KP for 90 d but not when KP was not applied (Table 1). Our results suggest that this new processing technology combined with a 90 d ensiling time increases sorghum processing and isSD7.

Key Words: forage sorghum, grain processing, cut height

2114 Chemical composition of forage corn silage with cocoa mucilage residue in different proportions. I. Espinoza*, A. Barrera, A. Sanchez, M. Medina, E. Torres, and R. Bastidas, *Universidad Tecnica Estatal de Quevedo, Quevedo, Los Rios, Ecuador.*

Cocoa mucilage, a mucilaginous substance that coats cocoa seeds, is traditionally used as a substrate in the cocoa bean fermentation process and plays a crucial role in the formation of flavor and aroma precursor substances. The objective was to determine the chemical composition of forage corn silage with the addition of different proportions of cocoa mucilage. A completely randomized experimental design was employed with 5 treatments and 5 replications: T1 (100% Forage Corn), T2 (90% Forage Corn + 10% Cocoa Mucilage), T3 (80% Forage Corn + 20% Cocoa Mucilage), T4 (70% Forage Corn + 30% Cocoa Mucilage), and T5 (60% Forage Corn + 40% Cocoa Mucilage). The evaluated variables were subjected to ANOVA and the Tukey test at a 5% probability level. The silage was subjected to a fermentation time of 30 d. The significance of the collected data was evaluated through ANOVA and Tukey's multiple range test ($P < 0.05$). The results revealed significant differences between treatments; dry matter (DM) content increased ($P <$

Table 1 (Abstr. 2113). LSM particle size distribution of forage sorghum harvested with a novel grain processing technology assessed with the Penn State Particle Separator

	Low noKP		Low+KP		High+KP			*P*-value		
Item	0 d	90 d	0 d	90 d	0 d	90 d	SEM	HS	ET	HS × ET
ADF, % DM	24.3	23.9	22.1	22.0	15.9	16.8	1.13	<0.01	0.91	0.85
aNDFom, % DM	34.2	33.3	30.4	27.5	22.2	22.4	1.05	<0.01	0.14	0.25
Starch, % DM	33.1	30.6	37.2	36.7	52.1	51.2	1.67	<0.01	0.29	0.76
BPS,[1] %	7.7	8.6	55.8	56.7	70.1	71.6	1.28	<0.01	0.22	0.93
isSD7, % starch	29.0	25.8	39.9	65.9	36.2	66.7	4.62	<0.01	<0.01	<0.01

[1] BPS was defined as the % of starch passing a 1.7-mm sieve.

0.05) in T5 (25.76%) compared with T1, T2, and T3 (15.73%, 16.85%, and 14.56%, respectively), indicating that the inclusion of cocoa mucilage was associated with higher DM content. T5 (10.02%) showed a significant ($P < 0.05$) increase in crude protein (CP) compared with T1 (8.90%), while T2, T3, and T4 presented similar values. Regarding neutral detergent fiber (NDF), T1 (68.96%) exceeded ($P < 0.05$) T3, T4, and T5 (53.54%, 56.09%, and 54.25%, respectively). Other components such as organic matter (OM), inorganic matter (IM), crude fiber (CF), acid detergent fiber (ADF), ether extract (EE), and energy did not show significant differences between treatments ($P > 0.05$). The inclusion of cocoa mucilage in forage corn silage positively influenced the content of DM, CP, and NDF. However, there were no significant variations in components such as CF, ADF, EE, and energy among the evaluated treatments. These findings provide valuable information for improving silage formulation, highlighting the importance of cocoa mucilage in the nutritional quality of forage.

Key Words: agricultural residues, silage, mucilage

2115 Effect of hybrid type and region on the neutral detergent fiber digestibility of whole-plant corn forage.

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The objective of this retrospective study was to evaluate the effect of corn hybrid type and region on NDF digestibility (NDFD) of whole-plant corn forage. Corn forage samples (n = 6,323) from different corn hybrids grown multiple years (2017 to 2021) were analyzed for in vitro NDFD at 12, 24, 30, and 48 h. For each time point, the rate of digestion (kd) for NDF was calculated by the linear slope over time of the natural logarithm of the appropriate NDFD as a percentage of the total NDF concentration, assuming 3 h of lag, and using the potentially digestible pool of NDF (corrected for 240 h uNDF concentration). Estimated ruminal NDFD (rNDFD) was calculated with first order kinetics and estimated NDF kd along with NDF kp calculated from literature. Corn forage samples differed in hybrid type, including conventional or brown midrib (BMR), and region, including the Upper Midwest (UM; Wisconsin and Minnesota), Northeast (NE; New York, Pennsylvania, and Vermont), Mideast (ME; Michigan and Ohio), and California (CA). Data were analyzed as a completely randomized design with fixed effects of hybrid type and region, while hybrid (year) was the random effect. Interactions between hybrid type and region were observed ($P = 0.001$) for all NDFD time points. Regardless of time point, BMR had greater NDFD than conventional hybrids. Additionally, ME had greater NDFD than other regions within hybrid type. Similarly, interactions between hybrid type and region were observed for 24, 30, and 48 h NDF kd ($P = 0.01$, $P = 0.001$, and $P = 0.01$, respectively). Likewise, BMR hybrids had greater kd than conventional hybrids, except when BMR hybrids were grown in CA. The 12 h NDF kd was not affected by hybrid type ($P = 0.48$), but ME was greater ($P = 0.001$) than NE or UM. There was an interaction between hybrid type and region observed for 12, 24, 30, and 48 h rNDFD ($P = 0.001$), which was greater for BMR than conventional hybrids, though there were region differences within hybrid type. Overall, BMR had greater NDFD and estimated digestibility than conventional hybrids. Additionally, most time points had similar patterns for NDFD and estimated digestibility.

Key Words: NDF digestibility (NDFD), brown midrib (BMR), digestibility

Physiology and Endocrinology 1

2116 Abomasal infusion of docosahexaenoic acid alters plasma oxylipin profiles in lactating dairy cows. G. A. Contreras*[1], M. Chirivi[1], U. Abou-Rjeileh[1], H. Reisinger[2], M. Miller[2], A. L. Lock[2], and B. Bradford[2], [1]*Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI,* [2]*Department of Animal Science, Michigan State University, East Lansing, MI.*

During periods of negative energy balance, such as the periparturient period of dairy cows, metabolic stress driven by excessive lipolysis and production of reactive oxygen species induces inflammatory responses that predispose cows to inflammatory and metabolic diseases. Docosahexaenoic acid (DHA; C22:6 n-3) supplementation to cattle may dampen inflammatory responses because its oxylipin products have pro-resolving and anti-inflammatory properties. However, it is currently unknown if supplementing n-3 fatty acids effectively increases plasma oxylipins. We evaluated the effects of DHA supplementation on plasma oxylipin profiles in lactating dairy cows. DHA (0, 2, 4, or 6 g/d) was abomasally infused to ruminally cannulated multiparous Holstein cows (n = 3; 97 ± 37 DIM, 49 ± 3 kg/d milk) for 11 d using an incomplete 4 × 4 Latin square design with a 10-d washout period between infusion periods. DHA was from an enriched algal oil (64.5% DHA) suspended in ethanol (~200 g/d). Plasma was collected on d 11 of each period for targeted lipidomic analysis using HPLC–MS/MS. Data for 77 n-3- and n-6-derived oxylipins were analyzed using mixed models in JMP Pro using cow as the random effect. DHA did not alter the concentration of linoleic acid (n-6) derived 9-, 13-HODE ($P > 0.2$). Similarly, DHA did not affect plasma content of arachidonic acid (n-6) products 5-, 8-, 11-, 12-. 15-, and 20-HETE, prostaglandins A_2, E_1, E_2, E_3, J_2, F1α, and F3α ($P > 0.2$). However, DHA at 6 g/d increased PGF2α concentration compared with the other treatments ($P = 0.001$). DHA at all doses increased DHA-derived oxylipins 14-HDoHE, 16-HDoHE, 20-HDoHE, 19,20-DiHDoPE, and the DHA resolvins, oxo-Resolvin D1, and 4–17-DiHDoHE (all $P < 0.01$). 19,20 DiHDoPE concentration was higher ($P < 0.004$) for 6 g/d DHA (0.26 ± 0.03 ng/mL plasma) than DHA at 4 and 2 g/d (0.16 and 0.15 ± 0.03 ng/mL plasma, respectively). These data demonstrate that delivering at least 2 g/d of DHA postruminally effectively increases the concentrations of DHA-derived anti-inflammatory and pro-resolving oxylipins. DHA supplementation has the potential to limit inflammatory responses driven by metabolic challenges.

Key Words: omega-3 fatty acids, docosahexaenoic acid, oxylipins

2117 Effect of controlled ionized calcium status during the first 24 hours postpartum on neutrophil function of periparturient multiparous Holstein cows. T. O. Cunha*[1], W. S. Frizzarini[1], A. L. Vang[1], S. Mann[2], and J. A. A. McArt[2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Cornell University, Ithaca, NY.*

Delineation of physiological mechanisms underlying possible interactions between postpartum hypocalcemia and immune function is critical to improvement of cow health. Our objective was to evaluate the effect of ionized calcium (iCa) concentrations during the first 24 h postpartum on neutrophil phagocytic capacity (PC) and oxidative burst (OB) of multiparous Holstein cows. At calving, cows were randomly assigned to receive one of 3 treatments (tx) administered via infusion pump over the first 24 h postpartum (1) saline (CON; n = 11), (2) 5% calcium gluconate to achieve normocalcemia (iCa 1.0–1.1 mmol/L, CALC; n = 10), or (3) 5% egtazic acid to induce subclinical hypocalcemia (iCa 0.8–0.9 mmol/L, EGTA; n = 10). Phagocytic capacity was measured by engulfment of fluorescent beads, and OB was quantified by median fluorescence intensity after exposure to a combination of phorbol myristate acetate and the reactive oxygen species sensitive dye 2’,7’-dichlorodihydrofluorescein diacetate. Assays were performed on blood samples collected immediately after calving and at the end of the 24-h infusion period. Data were analyzed using PROC MIXED in SAS with the fixed effects of tx, the repeated effect of time, and tx × time. Concentrations of iCa (1.12 mmol/L) at calving did not differ among groups ($P = 0.73$). At the end of infusion period, CALC, EGTA, and CON groups averaged 1.18 ± 0.02, 0.86 ± 0.03, and 1.06 ± 0.05 mmol/L iCa, respectively ($P < 0.01$). There was no interaction between circulating neutrophil count and time among groups ($P = 0.59$); however, there was an effect of time ($P < 0.01$), with neutrophil counts decreasing at 24 h postpartum. Phagocytic capacity did not differ among groups ($P = 0.15$), and no tx × time interaction was detected ($P = 0.92$). Oxidative burst capacity was elevated in CALC cows at calving and at the end of infusion ($P < 0.01$), but no differences were found between CON and EGTA groups ($P = 0.90$) and no tx × time interaction was observed ($P = 0.51$). In summary, iCa the first 24 h postpartum did not appear to influence PC or OB of neutrophils from postpartum cows.

Key Words: hypocalcemia, peripartal period, neutrophil function

2118 Transcriptomic analysis reveals the important role of aromatic amino acids in primary bovine hepatocytes. J. R. Daddam*, C. Collings, M. Sura, and Z. Zhou, *Animal Sciences, Michigan State University, East Lansing, MI.*

Around parturition, circulating amino acid (AA) concentrations reach their lowest levels in a lactation cycle. As integral components for protein synthesis, aromatic amino acids (AAA, phenylalanine, tyrosine, and tryptophan) supplementation may alleviate the negative impact of AA deficiency, thereby contribute to a healthy and productive lactation cycle. The objective of this study was to determine the transcriptomic changes in response to AAA supplementation in primary bovine hepatocytes (PLEH). These PLEH were cultured in customized medium mimicking cow plasma AA and FA profile on d 4 postpartum with AAA (150% of physiological circulating AAA concentration) or without AAA (CON). After 72 h, PLEH were harvested for single-end RNA sequencing. Raw reads were trimmed (Trimmometry), aligned to the bovine reference genome (STAR), assembled and quantified (Salmon). Differential gene expression (FDR <0.1) analysis was performed with DESeq2. Compared with CON, a total of 385 genes were differentially expressed in PLEH receiving AAA supplementation. Differentially expressed genes were used to identify functional enriched pathways using String 12.0. Pathway analysis revealed that 14 pathways were enriched (FDR <0.10) in AAA supplemented PLEH compared with CON, including oxidative phosphorylation, apoptosis, endocytosis, ubiquitin-mediated proteolysis, and ribosome biogenesis, which might positively impact hepatocyte function and metabolism. Overall, these results suggest that various aspects of PLEH transcriptome were altered by AAA supplementations. Future work will be required to determine the functional implications for these changes in vivo.

Key Words: aromatic amino acids, primary bovine hepatocytes, transcriptomics

2119 Daily rhythms of glucose tolerance following an abomasal bolus infusion of glucose.

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Glucose (GLU) is a metabolic fuel that is required for milk lactose and fat synthesis. Circadian rhythms (CR), repeating cycles of ~24 h govern aspects of physiology, including GLU metabolism. Although it has been established that blood GLU concentration (CONC) follows a CR, and previous research shows that GLU tolerance (GT) follows a CR when administered with an intravenous GT test, it is unknown if a CR persists when GLU is administered via the gastrointestinal tract (GIT). Successful administration of GLU in the GIT of cows must be done postruminally to avoid metabolism of GLU by rumen microbes. Thus, the objective of this experiment was to compare GT in cows that were abomasally infused with a 50% wt/vol D-glucose solution bolus at 4 time points representing 24 h across the day (0300, 0900, 1500, and 2100 h). Eight ruminally cannulated cows were divided into 2 blocks based on days in milk (107 ± 18 DIM; Block 1 n = 5; Block 2 n = 3). Each block consisted of 2 GT tests conducted 36 h apart (300 and 1500 h) with an 11-d washout, followed by the final 2 GT test (2100 and 900 h). Jugular catheters were placed to allow for frequent blood collection. Beginning 12 h before the first GTT, cows were fed every 2 h to eliminate the effect of the daily pattern of feed intake. Blood was collected into NaF and K-EDTA tubes before infusion and at 5, 10, 15, 20, 30, 45, 60, 90, 120, and 240 min after infusion. Cosinor rhythmometry was used to determine the fit, amplitude and acrophase of a 24-h rhythm. For outcomes of clearance rate, area under the curve (AUC), average (AVG), basal (BL), and peak (PK) CONC a mixed linear model including effects for sine, cosine and the random effect of cow within block was used to obtain CR residuals. A reduced model which included the fixed effect of TIME was used as a comparison. Based on a zero-amplitude test, AVG ($P = 0.18$), BL (P = NA), and PK ($P = 0.55$) GLU CONC did not follow CR. The clearance rate and AUC for GLU did not follow a CR (P = NA; $P = 0.77$). NEFA AVG (P = NA) and BL CONC ($P = 0.33$) and AUC ($P = 0.67$) did not follow CR. Overall, no effects of CR were observed for GT across different time points in the day.

Key Words: NEFA, clearance rate, daily cycles

2120 Feeding spent hemp biomass does not affect rumen fermentation and inflammatory cytokines in lactating dairy cows.

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The extracted byproduct (spent hemp biomass; SHB) from cannabidiol oil (CBD) of industrial hemp possesses excellent nutritional value for ruminants. In monogastric animals, CBD has anti-inflammatory properties. In our recent study, we observed a possible sub-clinical inflammatory situation in dairy cows fed SHB, as indicated by a higher level of ceruloplasmin in cows fed SHB. Furthermore, the withdrawal of SHB from the diet revealed an effect on blood BHBA indicating a role of SHB on the rumen fermentation. Therefore, this aimed to investigate the effects of dietary SHB supplementation on rumen fermentation and inflammatory responses of dairy cows. Eighteen late-lactating multiparous Jersey cows were randomly enrolled to receive isonitrogenous and isoenergetic TMR diets containing either 13% alfalfa pellet (CTR) or 13% pelleted SHB (TRT) for 4 weeks of intervention period (IP) followed by 4 weeks of SHB withdrawal (WP). Rumen content was collected via rumenocentesis during the IP only. Volatile fatty acids (VFA) of the rumen fluid were analyzed using gas chromatography and molar quantity of individual VFA was calculated using a standard mixture. Blood collected at the end of the IP and WP was treated with lipopolysaccharide for 3h before isolation of the plasma. The latter was used to measure 10 inflammatory cytokines using G-Series Bovine Cytokine Array (RayBiotech). Data were analyzed using GLIMMIX model in SAS following a 2 × 2 factorial arrangement (group × period interaction). The proportion of VFA was not statistically different ($P > 0.05$) between CTR and TRT, except a tendency ($P < 0.10$) of higher proportion of isovalerate and acetate-to-propionate ratio in TRT versus CTR. No cytokines were affected by feeding SHB during the IP. During WP, higher concentration ($P < 0.01$) of IFN-α, IFN-γ, IL-1 F5, IP-10, MIG, and MIP-1β were found in TRT versus CTR. Overall, our results indicate that feeding SHB does not affect rumen fermentation and has a minor effect on inflammation during the IP; however, some effect on pro-inflammatory cytokines are evident during the withdrawal of SHB from the diet.

Key Words: cannabinoids, inflammation, rumen fermentation

2121 *Saccharomyces cerevisiae boulardii* CNCM I-1079 effects on inflammatory response following systemic lipopolysaccharide challenge in dairy cows.

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The aim of this study was to investigate effects of *Saccharomyces cerevisiae boulardii* (SCB) supplementation on inflammatory response after an acute endotoxin challenge. Cows were enrolled and blocked by parity (30 primiparous [PP] and 45 multiparous [MP]), previous 305-d milk yield, BW, BCS, and randomly assigned to 1 of 2 dietary treatments from −28 to 77 d, relative to calving: (1) control (CON; n = 38) or (2) dietary SCB supplementation (1.0×10^{10} cfu/d; n = 37). On d 72, cows underwent a jugular intravenous infusion of lipopolysaccharide (LPS; 0.1 mg/kg of BW over 1 h). Plasma cytokine concentrations were analyzed at 0, 1, 2, 3, 4, 6, 12, and 24 h, and complete blood cell counts at 0, 3, 6, 12, 24, 48, and 72 h, relative to LPS infusion. Clinical signs were monitored for 7 h and vaginal temperature (VT) was recorded every 5 min for 12 h using a VT logger. Data were analyzed using mixed linear regression models accounting for repeated measures using SAS (v.9.4). Respiratory rate was lower in SCB than CON cows (44.7 vs. 48.0 bpm; $P = 0.01$). Heart rate was higher in PP than MP cows (89.7 vs. 87.0 bpm; $P = 0.02$) but was not affected by treatment. There was a treatment × parity interaction where temperature was higher for PP SCB cows compared with PP CON from 290 to 680 min ($P < 0.01$). All cytokines (TNF-a, IL-6, IFN-y, IL-10) were increased following the challenge ($P < 0.01$) but were not affected by treatment. Whole blood cell count was lower in SCB cows than CON (3.6 vs. 3.9×10^9 g/L; $P = 0.03$), and greater in PP than MP (4 vs. 3.5×10^9 g/L; $P < 0.01$). Neutrophil count was lower in SCB than CON cows (2.1 vs. 3.0×10^9 g/L; $P < 0.01$). Lymphocyte count was lower in SCB than CON cows (2.1 vs. 2.3×10^9 g/L; $P < 0.01$) and greater in PP than MP (2.5 vs. 1.9×10^9 g/L; $P < 0.01$). Monocyte count was lower in SCB than CON cows (0.10 vs. 0.12×10^9 g/L; $P = 0.05$) and greater in PP than MP (0.12 vs. 0.10×10^9 g/L; $P < 0.01$). Overall, supplementation of SCB may reduce the concentration of immune cells in blood and reduce respiratory rate following an LPS challenge.

Key Words: immunity, probiotic, endotoxin

2122 Changes in concentrations of serum calcium and inflammatory markers in Holstein and Jersey cows in the periparturient period.

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There is currently great interest in the relationship between immune activation and inflammation with subclinical hypocalcemia in early postpartum cows. Therefore, blood serum samples from 47 cows (19 Holsteins, 28 Jerseys) of second (22) or third (25) parity obtained from 5 d prepartum to 5 d postpartum were analyzed for the concentrations of Ca and the inflammatory markers tumor necrosis factor-α (TNFα), haptoglobin (Hap), and intestinal fatty acid binding protein (IFABP). An average of 4 blood samples per cow were obtained and metabolite concentrations were analyzed as a mixed linear model including the effects of breed, lactation, day, and their interactions, with day as a repeated factor. All inflammatory markers were log-transformed before statistical analyses to stabilize the variances. Serum Ca concentrations tended to be lower with increasing parity for Jerseys, but not for Holsteins (interaction of lactation with breed, $P = 0.10$). Serum Ca concentrations decreased to a nadir at d 1 postpartum, the decrease being greater for Jersey than Holstein cows (interaction of breed with day, $P < 0.001$). Serum TNFα concentrations tended to increase with increasing parity for Jersey cows but not for Holsteins (interaction of breed with lactation, $P = 0.066$). Serum haptoglobin concentrations increased sharply from the day of parturition until d 2 to 3 postpartum and remained above prepartum levels (main effect of day, $P < 0.001$). This increase from the prepartum to postpartum period was greater for second parity versus third parity animals (interaction of day with parity, $P = 0.016$). Serum IFABP concentrations were unaffected ($P > 0.10$) by the main effects or any interactions involving breed or parity but tended to be affected ($P = 0.07$) by the main effect of day, decreasing throughout the prepartum period but remaining unchanged throughout postpartum sampling days. Among the inflammatory markers tested, TNFα appeared to be most reflective of Ca status.

Key Words: dairy cows, hypocalcemia, inflammation

2123 Mitochondrial function of dairy calf lymphocytes from birth to maturity.

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Dairy calves have the necessary immune cell machinery to mount an effective immune response. However, their lymphocytes often fail to fully respond to parenteral vaccination until 6 mo of age, limiting vaccine effectiveness during the first months of life. Lymphocytes' mitochondrial functionality drives effector functions critical for vaccine responsiveness. However, the extent to which lymphocyte mitochondrial function changes with age and could explain this poor vaccination response remains unknown. Thus, we aimed to determine how the mitochondrial function of dairy calf lymphocytes changes with age from birth to immunologic maturity. Given lymphocyte dependency on mitochondrial efficiency, we hypothesized that the mitochondrial efficiency of dairy calf lymphocytes increases with age. In this cross-sectional study, groups of 4 Holstein calves were sampled at birth before colostrum consumption (0), 1, 2, 3, 4, 6, 8, 16, and 24 wk of age. Mid-lactation cows (n = 4) were sampled as controls. Whole blood was collected, and B, CD4, CD8, and γδ T lymphocytes were isolated using immunoprecipitation. Mitochondrial function was assessed with extracellular flux analysis, reporting non-mitochondrial oxygen consumption, basal respiration, maximal respiration, spare respiratory capacity, proton leak, and the oxygen consumption rate (OCR) to extracellular acidification rate (ECAR) ratio. Significant differences ($P < 0.05$) were determined using Kruskal-Wallis tests and the Dunn method for all pairwise comparisons. CD4+ cells had higher non-mitochondrial oxygen consumption at 3 wk relative to 0 and 16 wk. Also, the OCR:ECAR for 0 wk was lower than in adults. CD8+ cells had higher OCR:ECAR at 3wk than at 2 wk. γδ T-cells had higher basal respiration at 2 wk compared with 24 wk and maximal respiration of 3 wk was higher than 16 wk. B-cells had higher maximal respiration at 1 wk than 16 wk, and the proton leak of 1 wk was higher than 0 wk. Overall, there was no pattern of changes in any of the studied mitochondrial outcomes across age groups. In conclusion, our results do not support the existence of differences in mitochondrial function of dairy calf lymphocytes associated with age.

Key Words: T-cell, B-cell, immunity

2124 Characterization of an in vitro model to study CD4$^+$ T cell metabolism in dairy cows.

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Dairy cows are susceptible to several health disorders throughout their lactation and methods to understand immune cell metabolism are needed. The objective was to characterize an in vitro model to study bioenergetic measures in CD4$^+$ T cells. Twenty-four healthy multiparous Holstein dairy cows were enrolled at a mean ± standard deviation (SD) of 234 ± 22 d in milk (DIM). Cows were blocked by DIM and blood was collected to isolate peripheral blood mononuclear cells followed by magnetic separation of CD4$^+$ T cells using bovine specific monoclonal antibodies. The isolated cells from each cow were split into 2 tubes and randomly assigned to incubate in an assay medium as control (resting state; CON) or with a combination of phorbol myristate acetate and ionomycin (activated state; ACT) to evaluate metabolic function. Mitochondrial and glycolytic functional kinetics were recorded in real-time measuring oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Data were analyzed by mixed-effects models (PROC MIXED, SAS). The statistical models included the fixed effects of treatment, time, and interaction between treatment and time, and the random effects of block and tube nested within treatment. The mean ± SD viability and purity of CD4$^+$ T cells was 92.5 ± 2.9% and 95.2 ± 2.9%, respectively. The basal OCR in ACT CD4$^+$ T cells was greater than CON (CON = 16.0 vs. ACT = 18.7 ± 1.8 pmol/min; $P = 0.05$); nevertheless, the sparing respiratory capacity rate was decreased in ACT CD4$^+$ T cells (CON = 42.0 vs. ACT = 28.7 ± 4.2 pmol/min; $P = 0.001$). The ECAR in CD4$^+$ T cells increased ($P < 0.0001$) progressively over time in ACT compared with CON, which indicated an increase in aerobic glycolysis in ACT compared with CON (CON = 46.9 vs. ACT = 86.4 ± 7.0 pmol/min). Activated CD4$^+$ T lymphocytes exhibited a metabolic switch from oxidative phosphorylation to aerobic glycolysis, which may support rapid cell proliferation. The results observed in this experiment demonstrate the sensitivity of the technique to detect changes in metabolic function under different cellular conditions, providing a robust framework to study immuno-metabolism in ruminants.

Key Words: metabolism, immune system, seahorse

Production, Management, and the Environment 1

2125 Evidence for varying degrees of asymmetry in circannual rhythms of milk fat in the United States. A. E. Pape* and H. M. Dann, *William H. Miner Agricultural Research Institute, Chazy, NY.*

Models of the circannual rhythms of milk fat (and other components) have conventionally assumed that no asymmetry is present (i.e., assumed that fat percentage is increasing for exactly 6 mo and decreasing for exactly 6 mo). Our objective was to test this assumption using fat percentage data from the Federal Milk Marketing Order (FMMO) program between 2000 and 2023 sourced through the USDA Agricultural Marketing Service. Considering each of the 10 orders separately, we measured asymmetry by applying periodic regression in R v. 4.2.2 and comparing the relative amplitude and phase shift of the annual and semiannual components (i.e., first and second harmonics). The assumption of no asymmetry was violated for 8 out of 10 orders. For these orders, there tended to be a relatively short rapid increase in fat percentage in the autumn months and a relatively long slow decrease in the spring months. Most orders showed little or no weakening or strengthening of asymmetry from year to year, which is noteworthy given the contemporaneous increase in overall fat percentage (Table 1). For order 131 (Arizona), the 6-mo component of the periodic regression was significant ($P < 0.001$) but there was no evidence of asymmetry, indicating that it deviated from a sinusoidal pattern in other ways. An understanding of the causal mechanisms underlying circannual rhythms of milk fat and other milk components would be aided by considering their asymmetry and other forms of non-sinusoidal structure.

Key Words: fat, circannual rhythms, seasonality

2126 Predicting plasma nonesterified fatty acid concentrations in early-lactation Holsteins using milk FTIR-estimated fatty acids, spectra, and machine learning. F. Huot*[1,3], D. Warner[4], D. E. Santschi[4], E. R. Paquet[1,3], and R. Gervais[1,2], *[1]Département des sciences animales, Université Laval, Québec, QC, Canada, [2]Dairy Science and Technology Research Centre (STELA), Québec, QC, Canada, [3]Institute Intelligence and Data (IID), Québec, QC, Canada, [4]Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

Elevated plasma concentrations of nonesterified fatty acid (NEFA), indicative of energy deficit in postpartum dairy cows, are associated with impaired health and production. To develop predictive models of plasma NEFA concentrations based on milk Fourier-transform infrared spectroscopic (FTIR) analysis, 297 early-lactation Holsteins ($4 < \text{DIM} <65$) were recruited from 24 Canadian dairy farms. Between April and August 2022, each farm was visited once ($n = 20$) or twice ($n = 4$) during routine morning milk recording. In addition to each official milk sample, blood was withdrawn at 0 (PRE) and 4 h post-prandial (POST) from the coccygeal vessel. Plasma NEFA concentrations (m*M*) were determined by enzymatic method. The mean (MEAN) and the difference (DIFF) between PRE and POST were subsequently calculated. Different partial least squares (PLS) models were developed to predict each plasma NEFA metric (PRE, POST, MEAN and DIFF) by using either milk FTIR estimated fatty acids (FA) or spectra as predictors. A leave-one-herd-out cross-validation (CV) was used for training PLS models and prediction accuracy was evaluated by computing the coefficient of determination (R^2) achieved with each PLS model on each herd during CV. Obtained R^2 were then treated as dependent variable and were analyzed by a mixed model including predictors (milk FA or spectra) and plasma NEFA metrics (PRE, POST, MEAN, and DIFF) as fixed effects, and herd as a random effect. There was no predictor × NEFA metric interaction. The model R^2 were similar regardless of the predictor used (0.49 ± 0.040, LSM ± SEM; $P = 0.68$). However, achieved R^2 was higher ($P < 0.01$) when predicting MEAN ($0.65^a \pm 0.042$), lower when predicting DIFF (0.22^c), and intermediate when predicting PRE (0.55^b) or POST (0.55^b). Based on these findings, prediction performance is better when predicting the average of pre- and post-prandial plasma NEFA concentrations in early-lactation cows ($R^2 = 0.65$), but this prediction can be established as efficiently based on either milk FTIR estimated FA or spectra.

Key Words: nonesterified fatty acid, machine learning, Fourier-transform infrared spectroscopy

2127 Predicting feed intake for dairy cows using an RGB image-based approach. M. Wang*, S. Li, B. Zandona, S. E. Räisänen, A. M. Serviento, and M. Niu, *Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zurich, Switzerland.*

The prediction of individual cow feed intake (FI) is crucial for optimizing production efficiency. There is an increasing interest in applying computer vision techniques to measure feed intake, but most developed models are based on 3D images, which are not practical in farm conditions. Therefore, this study aimed to predict FI using RGB images by

Table 1 (Abstr. 2125). Estimated asymmetry in circannual rhythms of milk fat and change in asymmetry over time for Federal Milk Market Orders from 2000 to 2023

		Asymmetry		Change in asymmetry per year	
Order name	Order number	Estimate	*P*-value	Estimate	*P*-value
Northeast	1	0.14	<0.001	−0.0029	0.38
Appalachian	5	0.11	<0.001	0.0038	0.20
Florida	6	−0.02	0.71	−0.0054	0.34
Southeast	7	0.08	<0.001	0.0071	0.005
Upper Midwest	30	0.21	<0.001	−0.0071	0.023
Central	32	0.18	<0.001	−0.0014	0.55
Mideast	33	0.13	<0.001	−0.0026	0.38
Pacific Northwest	124	0.17	<0.001	−0.0019	0.69
Southwest	126	0.13	<0.001	0.0056	0.11
Arizona	131	−0.02	0.61	0.0045	0.39

implementing a convolutional neural network (CNN) for spatial feature extraction and a long short-term memory module to capture the eating patterns of individual cows. RGB images were collected from 2 cows housed in a tie-stall barn, with an RGB camera (DAHUA, model: DH-SD1A404XB-GNR, 2.8–12 mm lens) positioned above the feeding plate for each cow. The camera, capturing the feed pile on the plate, recorded images at a resolution of 2,560 × 1,440 pixels. The plate was equipped with a weighing scale to provide the ground truth for FI. Cows were fed 2×/d at 0800 and 1730 h respectively, creating 2 feeding sessions per day. Data from 5 feeding sessions were collected, and for each session images without cow's head were selected at 10-min intervals to establish the time series data set for training and evaluation of the model. In total, 144 images were collected throughout the day, with 56 images generated from the a.m. feeding session and 78 images from the p.m. feeding session. The baseline CNN model used for this study was EfficientNet-B0. For the purpose of adapting the model to our dataset, we first trained and evaluated on data from 3 feeding sessions of 1 cow with a time step of 30 min (3 images), resulting in a mean absolute error (MAE) of 0.44 kg and a root mean square prediction error (RMSPE) of 7.99% on the test set. Model parameters were then retained and fine-tuned using data from another cow, which included 2 feeding sessions. This resulted in an MAE of 1.89 kg and an RMSPE of 32.4%. The suboptimal performance of the second cow's data might be due to insufficient data, particularly the lack of a complete full day feeding period in the training set. More data are being collected to enhance the model's robustness and performance.

Key Words: dairy cows, feed intake, computer vision

2128 Heat stress negatively influences milk yield, somatic cell count, and body surface temperature of Girolando cows?

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The aim was to evaluate the respiration rate (RR), vaginal temperature (VT), eye and mammary surface temperature, milk yield (MY), and somatic cell count (SCC) of lactating Girolando cows under heat stress (HS). Twenty-four Girolando cows (3/4 Holstein × 1/4 Gir), averaging 561 ± 74 kg of body weight, 111 ± 54 d in milk, 19.4 ± 4.5 kg/d of MY, and 183,000 ± 138,000 cells/mL of SCC. Twelve cows were subjected to HS for 8 h/d in a climate chamber regulated to maintain a temperature-humidity index (THI) of 84 ± 0.94, while the other 12 (CTRL) were housed in a freestall barn with a cooling system during 8 d, with an average THI of 71 ± 3.92. VT was measured using an intravaginal device with data loggers (iButton®, Whitewater); RR was measured by counting the number of movements on the flank, twice a day (9:00 a.m. and 2:00 p.m.). The surface eye and mammary gland temperature were measured using an infrared thermography camera (FLIR T420; FLIR Systems Inc., Wilsonville, OR). Data were analyzed using a completely randomized design (SAS Institute Inc.) with repeated on time measures (1, 2, 4, 6, and 8 d) with significance of $P \leq 0.05$ and marginal significance $P > 0.05$ $P \leq 0.10$. Heat stress did not influence eye ($P = 0.98$) and vaginal temperature ($P = 0.54$). However, cows under heat stress tended to have greater temperature in the left quarter mammary ($P = 0.07$) at d 4 and 6 of evaluation, and on right quarter mammary there was a trend ($P = 0.09$) only at d 6. The respiration rate was not influenced by treatments. The HS cows had lower MY (17.3 ± 3.36 vs.18.1 ± 3.97 kg/d; $P < 0.09$) during the experimental period. However, there was no significant difference in milk SCC ($P = 0.20$). Heat stress negatively influences milk yield in Girolando cows, but does not change eye and vaginal temperature, respiratory rate and somatic cell count. Nevertheless, the mammary gland temperature tended to be greater from d 4.

Key Words: infrared thermography, milk somatic cell, milk yield

2129 Protein intake from corn silage and alfalfa hay and their influence on milk urea nitrogen and nitrogen excretions in dairy cows.

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Usually, alfalfa, as hay or haylage (AH), and corn silage (CS) are the main forage sources for lactating cows in Idaho. A shift in the inclusion of these forages in diets has occurred in the last decades though. AH inclusion in lactating cow diets has consistently decreased and CS has increased. Nitrogen (N) efficiency of feeding CS to cows compared with AH is not well known. Our study aimed to evaluate the relationships between protein intake from CS and AH with milk urea N (MUN) and N excretions, using a meta-analytical approach. A database with 450 treatment means from 124 studies published in peer-reviewed journals from 2018 to 2022 was used. Eligible studies included lactating Holstein dairy cows fed diets with CS, AH, or both as the primary forage sources, and available information on MUN, milk production, and dry matter intake (DMI). Meta-regression was used to identify the relationships between protein intake (from CS and AH) and MUN and N excretions, considering the random effect of study. Backward stepwise was used to remove nonsignificant factors until all remaining factors in the final model had a $P < 0.05$. All analyses were conducted using R studio. We also considered lowest AICc and independent variables that had variance inflation factor <10. Including milk production in addition to CP intake from CS did not improve MUN prediction models (9.59 vs. 9.66 RMSE, % mean). An increase in CP intake from CS resulted in lower MUN (slope −0.0181 mg/dL per 1 g increase in CP intake) and higher urinary N excretion (slope 0.3565 g per 1 g increase in CP). Similar patterns were found in MUN when RDP and RUP intakes from CS were considered in the models (slope −0.02698 mg/dL per 1 g increase in RDP intake and −0.05473 mg/dL per 1 g increase in RUP intake). The CP intake from AH with or without CP intake from CS was not a good predictor of MUN or N excretions. Substituting AH for CS in diets affected MUN, NUE, and N excretions. Further studies will be conducted to better understand the effects of different alfalfa maturities on protein intake and their influence on MUN, N efficiency, and N excretions in dairy cows.

Key Words: alfalfa, corn silage, milk urea nitrogen (MUN)

2130 Relationship between behaviors, vaginal temperature, and environment of lactating dairy cows in freestall barns.

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Understanding the environmental impacts on behavior is critical to enhance welfare and performance. Our aim was to identify associations between meteorological variables (MV), vaginal temperature (VT) and behaviors of Holstein dairy cows during summer. Mid- to late lactating cows in freestall system from 2 farms were randomly enrolled: farm A (n = 26; 130 milking cows; milked 2×/d, fans and misters for cooling) and farm B (n = 57; 950 milking cows; milked 3×/d; fans and sprinklers for cooling). The VT was recorded every 5 min and behaviors were recorded every 15 min using wearable sensors (Nedap Smarttag Neck and Leg sensors) for 6 consecutive days. Air temperature (AT) and relative humidity (RH) were measured every 15 min and temperature-humidity index (THI) calculated. Data from each farm were averaged or summed every 24 h and analyzed separately for simple linear regression using PROC GLM of SAS. Dependent variables included behavioral measures and MV and VT considered as independent variables. In farm A, lying time was correlated with daily average AT (= 1706 – 39AT, $r^2 = 0.11$, $P < 0.01$) and daily average VT (= 2325 – 40 VT, $r^2 = 0.04$, $P = 0.01$). Standing bout (= −56 + 1.7 VT, $r^2 = 0.11$, $P < 0.01$) and ruminating time (= −994 + 38 VT, $r^2 = 0.04$, $P = 0.02$) were correlated with daily average VT, but not with any MV. Eating time was correlated with max THI of a day (= 860 – 8.5 THI, $r^2 = 0.04$, $P = 0.02$) and was not correlated with VT. In farm B, lying time was correlated with max THI of a day (= 2184 −18 THI, $r^2 = 0.11$, $P < 0.01$) but was not correlated with VT. Standing bout was not correlated with any MV or VT. Rumination time was only correlated with max RH of a day (= −839 + 14 RH, $r^2 = 0.026$, $P = 0.01$). Eating time was only correlated with max RH of a day (= −945 + 12 RH, $r^2 = 0.3$, $P = 0.01$) and VT (= −531 + 19 VT, $r^2 = 0.03$, $P = 0.01$). In conclusion, changes in environment explain more of the variation of lying behavior; however, the correlations between behaviors and MV or VT are farm specific and may be influenced by factors such as intensity of cooling and housing.

Key Words: vaginal temperature, behavior, environment

2131 Difference of animal traits between nitrogen users in grazing dairy cows. E. Tavernier*[1,2], L. Delaby[3], I. C. Gormley[2], M. O'Donovan[1], and D. Berry[1], *[1]Department of Animal Bioscience, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland, [2]School of Mathematics and Statistics, University College Dublin, Dublin, Ireland, [3]INRAE, Institut Agro, UMR Physiologie, Environnement et Génétique pour l'Animal et les Systèmes d'Elevage, Saint-Gilles, France.*

Excess nitrogen contributes to environmental issues with ramifications for human health. Grazing dairy cows are among the contributors to potential nitrogen pollution. Differences among dairy cows in how much nitrogen they excrete, as well as the efficiency by which they use nitrogen, are known to exist. Characterizing such cows is of interest. Two types of nitrogen utilization metrics were explored in the present study: nitrogen use efficiency (i.e., nitrogen used by the cows for milk, growth, and pregnancy divided by the nitrogen available from that ingested and mobilized from body tissues) and nitrogen balance (i.e., the nitrogen available less the nitrogen used). Nitrogen utilization metrics were computed for 491 lactations from 184 grazing Holstein-Friesian dairy cows, resulting in 1,249 weekly estimates for each nitrogen metric. Linear mixed models with cow as a random effect were used to compute the nitrogen use efficiency and nitrogen balance for each cow; values were then used to classify cows as sustainable if their nitrogen use efficiency was above the average nitrogen use efficiency while their nitrogen excretion was below the average nitrogen excretion, or inadequate if their nitrogen use efficiency was below the average nitrogen use efficiency while their nitrogen excretion was above the average nitrogen excretion. Mean differences in performance between the sustainable and the inadequate strata were computed using a linear mixed model. The sustainable dairy cows, on average, were 20 kg lighter (SE = 5) and ate 53 g of nitrogen per day less per day (SE = 12) than the inadequate dairy cows. Milk yield, protein content, and milk urea nitrogen did not differ between the 2 groups of cows. Thus, sustainable dairy cows were interesting for a more sustainable milk production as they ate less and without a consequence for milk production. However, the higher nitrogen use efficiency could be explained by the higher use of the cow reserves, which are important for dairy cow health or reproduction capabilities.

Key Words: nitrogen use efficiency, nitrogen balance, milk urea nitrogen

2132 Bromide, iodine, bromoform, and fatty acids of milk from certified-organic dairy cows supplemented with *Asparagopsis taxiformis.* B. J. Heins[1,2], K. T. Sharpe[2], J. Felton*[1], I. J. Salfer[1], E. Bell[3], and M. C. Honan[4], *[1]University of Minnesota, Saint Paul, MN, [2]West Central Research and Outreach Center, Morris, MN, [3]CROPP Cooperative, LaFarge, WI, [4]Symbrosia Inc., Kailua-Kona, HI.*

The objective of the study was to evaluate bromide, iodine, bromoform, and fatty acids of milk from certified-organic Holstein and crossbred cows that were supplemented with *Asparagopsis taxiformis*. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, Minnesota, organic dairy. Forty Holstein and crossbred certified organic cows were supplemented with *A. taxiformis* (SeaGraze, Symbrosia, Inc., Kailua-Kona, HI) from July 2023 to January 2024 in a randomized complete block design with 2 treatment levels (Mean DIM = 122 d). Cows were paired by parity, breed, and milk production and randomly assigned to either the control (CON; 0% SeaGraze) or treatment group (SGR; 0.1% SeaGraze per cow/d on a DM basis). Cows were supplemented with 3.6 kg/head/day of corn grain and a mineral premix. SeaGraze was mixed with the corn grain for the SGR group. Milk was sampled monthly during the morning milking. Bromide and iodine concentrations were analyzed at Michigan State Vet Diagnostics Laboratory (Lansing, MI). Bromoform was analyzed at Bigelow Laboratory for Ocean Sciences (East Boothbay, ME) with gas chromatography mass spectrometry. Milk fatty acid concentrations were provided via fourier-transform infrared spectroscopy (Minnesota DHIA, Buffalo, MN) from test days of July 2023 to January 2024. Independent variables for statistical analysis were the fixed effects of breed group, parity, and treatment group. The SGR cows had higher ($P < 0.05$) milk iodine compared with CON (293.86 µgL vs. 111.43 µgL). Furthermore, the SGR had higher ($P < 0.05$) bromide (18.3 µgL vs. 1.55 µgL) in milk compared with the CON cows, respectively. Bromoform ($\mu g\ L^{-1}$) levels were all lower than the limit of detection except for 1 sample (6.6 $\mu g\ L^{-1}$). Total fatty acid, denovo fatty acid, mixed fatty acid, and preformed fatty acids were not different ($P > 0.10$) for SGR (3.6, 0.95, 1.32, 135 g/100 g) and CON cows (3.62, 0.92, 1.33, 1.4 g/100 g), respectively. Feeding *Asparagopsis taxiformis* at 0.03 kg/cow/day to certified organic dairy cattle resulted in higher iodine and bromide levels in milk.

Key Words: organic, methane, asparagopsis

2133 Safety and pathogen suppression by *Bacillus velezensis* LL11 in compost-bedded pack material. A. Tarrah*, V. Owosangba, and G. LaPointe, *University of Guelph, Guelph, ON, Canada.*

Mastitis-related microorganisms, including *Staphylococcus*, have recently been found in compost-bedded packs (CBP). *Bacillus velezensis* strains have been recognized for their use as biocontrol agents, showcasing antimicrobial activity through the synthesis of diverse compounds such as bacteriocins, non-ribosomal polypeptides, and surfactants. In this study, the safety of *B. velezensis* LL11, with antimicrobial activity against *Staphylococcus aureus*, was assessed through genomic and phenotypic analyses. The findings indicate the absence of safety concerns related to blood hemolytic activity and no acquisition of antimicrobial resistance, as determined through both in vitro and *in silico* analyses. Plasmids and transposons were absent in the genome. Through cell culture testing, strain LL11 did not show any cytotoxicity against Vero cells. Autoclaved CBP material (0.5 g per Falcon tube) was co-inoculated with vegetative cells of *B. velezensis* LL11 at 5 log cfu/g and *S. aureus* ATCC 29213 at a concentration of 4 log cfu/g, then incubated for 6 mo at 2 temperatures of 20°C and 37°C, representing the average CBP temperature at the surface and depth (15 cm), respectively. Sampling was carried out in triplicate at 9 time points. The microbiota was eliminated through autoclaving to determine the specific influence of the biocontrol strain without interference from the CBP microbiota. At 37°C, *S. aureus* ATCC 29213 dropped below the detection limit within 1 week in the presence of strain LL11, remaining absent until 6 mo. In contrast, *S. aureus* survived for up to 3 mo in the control CBP material with the absence of strain LL11, reaching a maximum level of 8 log cfu/g at one week. Conversely, at 20°C, there was no significant reduction in *S. aureus* when the biocontrol strain LL11 was inoculated. With the practice of daily tilling of CBP, the surface bacteria including pathogens, will be transferred to the deeper layers where biocontrol agents may actively reduce their numbers. This process could contribute to the efficacy of the biocontrol strain to enhance the overall safety of CBP.

Key Words: compost-bedded pack, biocontrol, Staphylococcus aureus

2134 Comparison of bulk tank milk free fatty acid concentration among farm types and seasons.

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Triacylglycerol (TAG) hydrolysis yields free fatty acids (FFA) and > 1.2 mmol FFA/100 g of milk fat in bulk tank milk is associated with off-flavor, rancidity, reduced foam stability, and inhibited cheese coagulation. The objective of this study was to compare milk FFA concentrations among conventional (CON), organic (ORG), and certified grass-fed (CGF) dairy farm types in Ontario (ON), Canada and describe monthly and yearly FFA patterns. Bulk tank FFA levels at every ON farm milk pick-up from August 2018 until December 2022 were measured using infrared spectroscopy in units of mmol/ 100 g of fat and averaged by month. A mixed linear regression model of monthly average FFA with herd as a random effect was used to investigate associations with month, year, and farm type. There were 171,843 observations from 3,771 farms over 53 mo. Ninety-seven percent (n = 166,355) of observations were from CON farms (n = 3,659), while the other 3% (n = 5,488) were from ORG (n = 72) and CGF (n = 40) herds. Conventional farms had the lowest overall average FFA (0.83 mmol/100 g of fat) with 7% (n = 11,645) of elevated (1.2 mmol/100 g of milk fat) monthly averages, while CGF herds had the highest overall average FFA (1.10 mmol/100 g of fat) with 23% (n = 842) of elevated FFA months. Seventy-five percent (n = 30) of CGF farms had at least one elevated monthly average FFA over the 53 mo. In the mixed linear regression model, monthly average FFA levels in May were lower than in other months ($\beta = -0.02$ to -0.21, $P < 0.001$) and monthly average FFA levels in July were higher than in other months ($\beta = 0.01$ to 0.12, $P < 0.001$). Conventional herds were associated with lower monthly average FFA levels than CGF herds ($\beta = -0.27$, 95% CI [−0.18, −0.35], $P < 0.001$) and ORG herds ($\beta = -0.08$, 95% CI [−0.01, −0.14], $P = 0.016$). These results suggest that the ON dairy industry can expect higher FFA levels in non-CON herds and in the late summer and fall months. The year should also be considered in future FFA studies.

Key Words: milk fat, lipolysis, milk quality

2135 Assessing dairy manure on the emitting potential of N_2O and NH_3 in an in vitro manure incubation system.

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This study aimed to refine the in vitro manure incubation conditions for assessing the potential of dairy manure to emit N_2O and NH_3. Fresh manure rarely emits N_2O due to a lack of nitrification and denitrification microbes in feces while NH_3 emission from fresh manure is considerable. To evaluate dairy manure on the emitting potential of N_2O in addition to NH_3, we investigated manure mixed with varying amounts of soil. Feces and urine were collected from 2 lactating cows and composited to have 1 fecal sample and 1 urine sample. Soil samples were collected from a local corn field (10 cm below the surface). The following manure treatments were prepared for a 14-d in vitro incubation: blank (empty; n = 1), manure only (M: 160 g feces, 80 mL urine; n = 3), mixture of manure and 1× soil (MS: 160 g feces, 80 mL urine, 960 g soil; n = 3), mixture of manure and 2× soil (MS2: 160 g feces, 80 mL urine, 1,920 g soil; n = 3), 1× soil only (S: 960 g soil, 80 mL water; n = 3), and 2× soil only (S2: 1,920 g soil, 80 mL water). A continuous air flux manure incubation with 16 manure chambers was used. Air samples exiting each chamber were sampled for 30 s every 30 min. The samples were collected in a sample bag over 24 h and the daily samples were analyzed for N_2O, NH_3, and CH_4. Data of gas emission rates and cumulative emissions were analyzed using PROC MIXED of SAS. The N_2O emissions were markedly elevated for MS2 from d 5 (4.4 mg/d and 95.9 mg/14 d, $P < 0.01$) while no N_2O emission was observed for other treatments. As expected, decreases in NH_3 emission when manure was mixed with soils, were observed ($P < 0.01$) where NH_3 emissions were lowest for MS2 (58 mg/d and 0.6 g/14 d) followed by MS (128 mg/d and 1.3 mg/14 d) and M (283 mg/d and 2.3 mg/14 d). However, CH4 emissions were not affected (41 mg/d and 0.59 g/14 d). In conclusion, the presence of soil and the amount of soil was critical to stimulate N_2O emission from during the in vitro manure incubation. However, the presence of soil did not affect CH_4 emission in the short-term incubation of fresh manure.

Key Words: dairy manure, in vitro manure incubation, nitrous oxide (N_2O) emissions

2136 Milk production, composition, and energy content during lactation in Angus Brangus, Braford, and Hereford cows under grazing conditions.

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Knowledge of lactation curves and milk composition in beef cows makes it possible to know the energy expenditure destined to this activity, as well as to foresee management during lactation (early weaning, creep feeding, etc.). The objective of this work was to analyze the production curves, composition and energy content (NE) of milk produced by Hereford-H, Angus-A, Braford-Bf, and Brangus-Ba cows grazing in native pastures throughout lactation period. Monthly from calving to weaning of calves (5 mo), milk production of 24 first-calf cows (2 years old) was determined by daily milking with a portable milking machine, and individual samples were taken to analyze their composition (fat, protein and lactose). Milk energy was estimated using the NRC formula (2001). Analysis used repeated-measures time series and a mixed model with the fixed effects of calving date (covariate), calf sex, month of determination, breed and the interaction breed × month, and the cow(breed) as random. Adjusted means of daily milk production at each month were modeled with linear and quadratic regressions using the r^2 as fitting coefficient. The effects of month, breed and their interaction were significant for milk production and composition. Lactation curves were fitted to quadratic models for A, Bf and H with r^2 ranging from 0.84 to 0.92 while Ba had a lower fit (0.36). At the beginning of lactation, Bf and H breeds had the highest daily milk yields (10 kg/d), and Ba and A lower yields (7 kg/d). Throughout lactation (fourth month), the trends reversed, with the highest production in Ba and A (9 kg/d) and the lowest in H and Bf (5 kg/d). A similar trend was observed for milk energy, with 6 Mcal NE/d in Bf and 5 Mcal NE/d in A, Ba and H breeds. At the end of lactation A and Br produced more NE (5.5 MCal/d), while Bf and H averaged 3.5 Mcal/d. In conclusion, breeds differed in their milk production and composition throughout lactation, with similar trends observed in A and Br and in H and Bf.

Key Words: milk energy, lactation curves, beef breeds

2137 Pasture-raising heifers reduced fat deposition compared with confinement heifers at similar body weights. C. H. P. Camisa Nova*[1], D. Jaramillo[2], L. de Oliveira Lima[3], M. Akins[2], and K. Kalscheur[4], [1]*University of Wisconsin, Madison, WI,* [2]*US Dairy Forage Research Center, Marshfield, WI,* [3]*University of Florida, Gainesville, FL,* [4]*US Dairy Forage Research Center, Madison, WI.*

Body composition of prepubertal dairy heifers can impact lactation performance and health. Information regarding body composition of heifers raised under different conditions is limited. Thus, this study evaluated the effects of rearing prepubertal dairy heifers on pasture or in confinement on body composition and growth. Holstein heifers (n = 63; 5.2 ± 0.45 mo) were raised under 2 systems: 32 heifers (4 groups; 8 animals/pasture) raised on pasture (PAST), and 31 heifers (3 pens of 8 and 1 pen of 7) raised in a bedded-pack barn (CONF) for 5 mo. CONF was fed a high-forage diet with corn and grass silage, while PAST was supplemented with a ground corn and corn gluten mix (0.5% BW/d). Diets were targeted for an average daily gain (ADG) of 0.91 kg/d. Body weight (BW), condition score (BCS), hip height (HH), wither height (WH), hip width (HW), body length (BL), and heart girth (HG) were taken at the beginning and end of the grazing season. Backfat and rump fat ultrasounds were done to assess subcutaneous fat depth at the end of the season. Statistical analysis was performed based on a completely randomized design using PROC MIXED in SAS. BW, ADG, and body measures were similar between the treatments ($P \geq 0.22$; Table 1). However, backfat was greater in CONF versus PAST (7.83 vs. 6.97 mm, $P = 0.025$, respectively), indicating different types of gain. Rump fat was similar in CONF versus PAST (10.7 vs. 9.8 mm, $P = 0.23$, respectively). Initial BCS diverged between CONF and PAST (3.21 vs. 3.31, $P = 0.035$), however final BCS was similar (3.43, $P = 0.90$). Overall, PAST heifers performed similar to CONF heifers, but with less backfat accumulation. This may reduce the risk of metabolic disorders associated with high body fat content when animals begin lactation.

Key Words: dairy heifer, growth, performance

Table 1 (Abstr. 2137). Initial (i) and final (f) weights (kg or kg/d) and measures (cm) of heifers for each treatment

Item	Pasture	Confinement	SE	*P*-value
iBW	179.3	180.3	4.48	0.84
fBW	338.5	335.6	7.65	0.71
ADG	0.94	0.92	0.03	0.53
iHH	118.6	118.8	0.90	0.83
fHH	130.2	129.5	0.74	0.37
iWH	112.6	113.1	0.83	0.61
fWH	123.7	124.3	0.68	0.46
iBL	116.4	115.7	1.04	0.57
fBL	135.5	135.5	1.09	0.98
iHW	30.2	30.2	0.42	0.94
fHW	40.2	40.3	0.47	0.78
iHG	126.4	126.5	1.18	0.90
fHG	158.6	156.8	1.32	0.22

2138 Blood glucose concentrations of unfed newborn dairy calves exposed to different periods of intrauterine heat stress. A. D. Del Río-Avilés*[1], D. C. Valles-Ibarra[1], M. G. González-Valdéz[1], M. I. Morales-Pablos[1], J. C. Leyva-Corona[1], and M. A. Sánchez-Castro[2], [1]*Instituto Tecnológico de Sonora, Ciudad Obregón, Sonora, Mexico,* [2]*Zoetis, Veterinary Medicine Research and Development, Animal Genetics, Kalamazoo, MI.*

Intrauterine heat stress (IUHS) has been reported to alter neonatal dairy calves' metabolism. Although calves born to heat-stressed cows have shown an improved glucose uptake after weaning, the effects of IUHS on the blood glucose levels of unfed newborn dairy calves remains unclear. Additionally, according to the conception date of their dams, dairy calves might experience different periods of IUHS during the last 60 d of pregnancy. The objective of this study was to determine the effects of different periods of IUHS on the blood glucose levels of unfed newborn Holstein calves. To assess the influence of the number of days of heat stress during late gestation (e.g., days with an average THI >72 units), 3 IUHS groups of calves were formed: G1 = calves born to dams exposed to HS less than 20 d within the last 60 d of gestation (n = 30), G2 = calves born to dams exposed to HS between 21 and 40 d within the last 60 d of gestation (n = 20), and G3 = calves born to dams exposed to HS for more than 40 d within the last 60 d of gestation (n = 16). Upon calving and before the calves were fed, the glucose levels of each newborn were determined using a hand-held glucometer. A general linear model including the effects of sex of the calf and intrauterine HS calves' group was implemented to analyze the blood glucose levels. Harmonic means were compared via the Tukey-Kramer test. Calves in G1 exhibited the lowest blood glucose concentrations (99.37 ± 5.26 mmol/dL) in comparison to G2 (164.50 ± 14.75 mmol/dL) and G3 (140.31 ± 15.47 mmol/dL) calves ($P < 0.01$). No differences were observed between G2 and G3 calves ($P = 0.25$). Results of this study suggested that IUHS exposures of more than 21 d during the last 60 d of gestation alter the metabolism of newborn dairy calves generating an increment of their blood glucose levels at birth.

Key Words: maternal heat stress, glucose, newborn

2139 Association between milk yield and traits measured via automatic milking systems and rumination collars across lactation stages. J. M. Hooker* and A. A. C. Alves, *University of Georgia, Athens, GA.*

Dairy cows exhibit different nutritional requirements and physiological phases across lactation, potentially affecting the relationship between milk yield (MY) and other economically important traits. This study aimed to analyze the associations between average MY (AMY) and traits measured via automatic milking systems and wearable sensors across lactation stages. A total of 160,963 daily information for MY, conductivity (Cond), milk flow (MF), rumination time (RT), and activity time (AT) from 801 lactating Holstein cows in different lactation periods were collected from July 2023 to January 2024. This information was summarized per individual in weekly intervals and grouped by days in milk (DIM) in early (4–31 DIM), peak (32–60 DIM), and late (277–305 DIM) lactations. RT consistently showed a positive correlation with AMY across all stages, with the strongest correlation in the early stage (0.42), followed by peak (0.26) and late stages (0.22). Conductivity (Cond) and milk flow (MF) correlated positively with AMY in the early (0.22 and 0.25, respectively) and peak (0.47 and 0.27, respectively) stages. AT was negatively associated with AMY in peak lactation (−0.28), while negligible associations were found in the other periods. A *t*-test suggested significant differences in RT and AMY correlation estimates between early and late periods ($P = 0.0224$). Not enough evidence was found to support a change in associations between RT and AMY from early to peak periods ($P = 0.0647$). Additionally, differences in correlation coefficients between peak and late periods for AT, Flow, and Cond with AMY were significant ($P < 0.05$). After correcting for the systematic effects of age, lactation order, reproduction status, week, and the random animal effect, a positive effect of RT on AMY was found for the early (+0.01838 ± 0.002 kg/min of RT), peak (+0.01326 ± 0.001 kg/min of RT) and late (+0.01071 ± 0.002 kg/min of RT) periods. These findings underscore the importance of considering the lactation stage in dairy cow management based on AMS and collar sensor data.

Key Words: early lactation, milk conductivity, rumination time

2140 Cost of production of dairy heifers in Brazil. L. H. R. Silva[1], G. S. Laud[1], E. P. Lima Neto[2], M. I. Marcondes[3], A. L. Silva[1], and P. P. Rotta*[1], [1]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil,* [2]*SEBRAE, Belo Horizonte, Minas Gerais, Brazil,* [3]*Washington State University, Pullman, WA.*

Assessing the accurate cost of producing dairy heifers, along with benchmarking, holds paramount significance for the success and efficiency of this agricultural activity. This study aimed to estimate the production costs of dairy heifers in the state of Minas Gerais, Brazil. Minas Gerais stands as the largest milk-producing state in Brazil. The dataset utilized herein encompasses financial and zootechnical information from 311 farms, graciously provided by the EDUCAMPO/SEBRAE-MG group. The farms were categorized into 3 groups based on the average milk production (AMP) per cow per day. The lowest one-third AMP (12.8 L/cow/d) fell under the low technological level (LTL), the highest one-third AMP (25.3 L/cow/d) under the high technological level (HTL), and the middle range AMP (17.7 L/cow/d) as the medium technological level (MTL). These groups comprised 103, 103, and 105 farms, and 4331, 6456, and 8168 animals, respectively. Statistical analyses, conducted through the GLIMMIX procedure in SAS Studio 2024, indicated no significant differences in the final cost from birth to first calving across AMP, amounting to US$1,955.97, $1,874.74, and $1,805.29 for LTL, HTL, and MTL, respectively (*P*-values: HTL × MTL = 0.36; HTL × LTL = 0.29; MTL × LTL = 0.06). However, the age at first calving (AFB) in months varied significantly ($P < 0.01$), standing at 34.9, 29.9, and 26.4 for LTL, MTL, and HTL, respectively. Thus, farms in the LTL group demonstrated inefficiency in raising dairy females, attributed to their herds having lower AMP and an elevated AFB. An alternative approach in such scenarios could involve selling calves shortly after birth and buying genetically superior heifers from other herds when they are closer to calving.

Key Words: calves, cost of production, heifers

Reproduction 1

2141 Characterizing a 5-day CIDR synch protocol without GnRH at the end in Holstein heifer recipients. V. E. Gomez-Leon*[1], S. A. Paez Hurtado[1], J. V. Chaves Silva[1,2], J. F. Marquez Alvarez[1], A. A. Perez Carrillo[1], P. Medellin[3], B. Medellin[3], J. D. Guimarães[2], J. F. Moreno[4], M. Fosado[4], M. E. Hockett[4], and P. J. Ross[4], *[1]Kansas State University, Manhattan, KS, [2]Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, [3]APM Dairy Service LLC, Syracuse, KS, [4]STgenetics, Navasota, TX.*

The current field-observational study aimed to characterize reproductive parameters of a 5-d CIDR synch protocol that favors expression of estrus by diminishing the use of GnRH to induce ovulation at the end of the protocol: d –8, GnRH$^+$CIDR insert; d –3, PGF+CIDR withdrawal; d –2, PGF; d –1, 0, and 1 daily ultrasound to detect ovulation, and estrus expression evaluation using tail chalk. Briefly, 33.1% and 44.6% of heifers were determined to have spontaneous ovulation on d 0 and 1, respectively. Additionally, 18.5% received GnRH due to lack of ovulation on d 1 regardless of estrus expression. Ovulation in this group was only determined on d 6 when all recipients were evaluated for corpus luteum presence. Finally, 3.8% of heifers were removed due to a lack of ovulation and absence of a follicle >9 mm. On d 7, IVP embryo transfer (ET) was performed randomly without any blocking factor. Embryo characteristics were not recorded. Pregnancy (P) was determined by ultrasound on d 36 and d 85. Data from 683 recipients was contrasted considering ovulation type (spontaneous Spont and GnRH) and expression of estrus (E and NoE) using Fisher's exact test (Table 1). The interaction of E × OV was associated with utilization rate. P/ET D36 was not associated with ovulation type, estrus, or their interaction. However, a tendency was detected for Spont (33.1%) to be associated with fewer pregnancy losses (PLOSS) when compared with GnRH-induced ovulations (42.8%). Finally, greater P/treated recipients was associated with estrus expression (28.4% vs. 11.8%). In summary, ~80% of heifers ovulated spontaneously on d 0 and d 1, and estrus expression was ~95%. Moreover, evidence indicates an association between ovulation type and estrus with efficiency of IVP embryo recipients.

Key Words: estrus, embryo transfer, pregnancy per embryo transfer (P/ET)

2142 Effects of gonadorelin hydrochloride dose on luteinizing hormone release in Holstein lactating dairy cows starting the breeding Ovsynch of a Double-Ovsynch. D. B. Melo*[1], R. G. S. Bruno[2], R. M. Cleale[2], P. L. J. Monteiro[3], M. C. Wiltbank[3], M. C. G. Menezes[1], W. M. Coelho Jr.[1], T. C. Marques[1], H. F. Monteiro[1], T. Castro[1], E. B. de Oliveira[2], and F. S. Lima[1], *[1]Department of Population Health and Reproduction, University of California, Davis, Davis, CA, [2]Zoetis Inc., Parsippany, NJ, [3]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Recent studies have shown that increasing the dose of gonadorelin hydrochloride from 100 μg to 200 μg at 1st GnRH of the Breeding-OvSynch of a Double-OvSynch (DO) increases ovulation risk and pregnancy per AI (P/AI) in Holstein dairy cows. The improvement in ovulatory response and P/AI has been attributed, at least in part, to a potential increase in luteinizing hormone (LH) release. However, the LH response in cows treated with different doses of gonadorelin hydrochloride at 1st GnRH of the Breeding-OvSynch of a DO has not yet been assessed. Our hypothesis was that increasing the dose of gonadorelin hydrochloride from 100 μg to 200 μg would increase LH release in Holstein cows at the first GnRH of the Breeding-OvSynch of a DO. A total of 17 Holstein cows (multiparous = 5; primiparous = 12) were blocked by parity and randomly allocated to receive either 100 or 200 μg of gonadorelin hydrochloride (Factrel, Zoetis Inc., 50 μg of gonadorelin hydrochloride per mL). Blood samples were collected at 0 (before treatment) 30, 60, 90, 120, 150, 180, 210, 240, 300, and 360 min after treatment. Statistical analysis was performed using ANOVA with repeated measures with models including treatment effects, time, parity, and their interactions with cow nested within treatment as the random term for the test of effects of treatment. The mean LH concentration over time was greater ($P < 0.01$) for cows receiving the higher dose (100 μg = 2.2 ± 0.2 ng/mL vs. 200 μg = 3.0 ± 0.2 ng/mL). Similarly, the area under the curve was also greater ($P < 0.01$) for cows treated with the higher dose (100 μg = 740.6 units vs. 200 μg = 1040 units). There were no effects ($P > 0.05$) of parity for LH responses. There were no statistical differences in the timing of LH peak (100 μg = 73.3 min vs. 200 μg = 75.0 min, $P = 0.92$) and peak concentration (100 μg = 4.9 ± 0.9 ng/mL vs. 200 μg = 6.3 ± 0.9 ng/mL, $P = 0.28$). Increasing the dose of gonadorelin hydrochloride led to increased release of LH over time, but peak time and peak concentration did not differ.

Key Words: luteinizing hormone (LH) surge, ovulatory response, estrous synchronization

2143 Effects of adding meloxicam to the treatment of metritis on subsequent performance. G. C. S. Pontes[1], G. T. P. Ferreira[1], and E. S. Ribeiro*[2], *[1]Rehagro, Belo Horizonte, MG, Brazil, [2]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Our objectives were to evaluate if the inclusion of meloxicam to the treatment of metritis improves clinical cure and subsequent lactation and reproductive performance. Holstein and Gyr-Holstein crossbred cows (n = 2,771) from 2 herds in southeastern Brazil (8,000 kg of milk/cow/year) were examined for diagnosis of metritis at 4, 6, 8, 10, 12, and 14 DIM.

Table 1 (Abstr. 2141). Reproductive parameters

Item	Incidence, n (%)	Utilization rate, n (%)	P/ET D36, n (%)	PLOSS, n (%)	P/recipients, n (%)
Spont+E	528 (77.3)	477 (90.3)^{a}	157 (32.9)	52 (33.1)	157 (29.7)
GnRH+E	121 (17.7)	87 (71.9)^{b}	27 (31.0)	12 (44.4)	27 (22.3)
Spont+NoE	24 (3.5)	19 (79.2)^{ab}	3 (15.8)	1 (33.3)	3 (12.5)
GnRH+NoE	10 (1.5)	7 (70)^{ab}	1 (14.3)	0 (0)	1 (10)
Estrus (E)	—	0.12	0.08	0.60	0.046
Ovulation (OV)	—	<0.01	0.72	0.06	0.08
E × OV	—	<0.01	0.32	0.11	0.06

^{a,b}Superscript letters indicate differences within a column when $P < 0.05$ for the E × OV interaction.

Cows with fetid vaginal discharge (n = 501) were considered metritic and randomly assigned to 1 of 2 treatments: a) 2.2 mg of Ceftiofur/kg of BW once daily for 3 d (CEF, n = 251); or b) 0.5 mg of meloxicam/kg BW once daily for 3 d in addition to the CEF protocol (CEFMel, n = 250). Cows without metritis served as non-metritic control (NOMET, n = 2,270). Cure was evaluated 3 d after onset of treatment, and those not cured from both groups received another 2 d of treatment with antimicrobial only. Cows were followed until 120 DIM for evaluation of lactation and reproductive performance. Milk yield was tested once a month. The reproductive program included timed AI for all cows at 53 ± 3 DIM, and a combination of estrous detection and timed AI for subsequent breeding. Statistical models considered the effects of experimental group, parity, breed, time (milk yield only), their interactions, season and farm. Orthogonal contrasts assessed the effect of metritis (NOMET vs. CEF+CEFMel) and treatment protocol (CEF vs. CEFMel). No difference in cure were observed (CEF = 52.5 ± 3.7% vs. CEFMel = 59.8 ± 3.6%; $P = 0.13$). Milk production by 120 DIM was reduced ($P < 0.01$) by metritis, and improved ($P = 0.07$) by CEFMel in metritic cows (NOMET = 24.8 ± 0.2 vs. CEF = 22.9 ± 0.5 vs. CEFMel = 24.1 ± 0.5 kg/d). Rate of pregnancy (adjusted hazard ratio [95% CI]: NOMET = 1.64 [1.35–1.99] vs. CEF = 1.0 [ref] vs. CEFMel = 1.35 [1.00–1.67]) was affected by metritis ($P < 0.01$) and treatment protocol ($P = 0.05$), resulting in different proportions of cows pregnant by 120 DIM among all groups (NOMET = 72.7 ± 1.5 vs. CEF = 53.2 ± 3.7 vs. CEFMel = 64.4 ± 3.6%). In conclusion, metritis impaired subsequent lactation and reproductive performance but its consequences were lessen by the incorporation of meloxicam into the treatment protocol.

Key Words: transition, health, cow

2144 Effect of source of trace minerals on conceptus development and reproduction in dairy cows. Z. Sarwar*[1], T. Adeoti[1], M. N. Marinho[1], J. Rasia[1], L. R. S. Oliveira[1], B. S. Simões[1], M. C. Perdomo[1], K. Griswold[2], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*Selko USA, Indianapolis, IN.*

Objectives were to evaluate the effects of replacing sulfate (STM) with hydroxychloride (HTM) sources of Zn, Cu, and Mn on reproduction in dairy cows. One hundred forty-one Holstein cows were blocked by parity group (lactation 0 vs. lactation >0) and randomly assigned to STM (30 nulliparous and 40 parous cows) or HTM (31 nulliparous and 40 parous cows). Supplemental Zn, Cu, and Mn were incorporated at, respectively, 30, 7, and 22 mg/kg of diet DM prepartum, and at 44, 8, and 36 mg/kg diet DM postpartum. The pre and postpartum diets contained, respectively, 64 and 77 mg/kg of Zn, 15 and 19 mg of Cu, and 58 and 65 mg of Mn. Treatments started at 245 d of gestation and stopped at 105 d postpartum. The estrous cycle was synchronized, and the uteri flushed on d 16 after AI. Reproduction was evaluated for the first 305 d postpartum. Data were analyzed by mixed-effects models fitting either normal or binary distribution with the GLIMMIX procedure of SAS. Days open were analyzed by the Cox's regression in SAS. Results are presented in Table 1. Replacing STM with HTM did not affect resumption of estrous cyclicity or ovulatory responses during the synchronization protocol. Cows fed HTM tended to have larger luteal area on d 7 of the cycle, but treatment did not affect pregnancy on d 16, length of the conceptus and the concentration of interferon-tau (IFNt) in the uterine flush. The rate of pregnancy did not differ between treatments (adjusted hazard ratio = 1.18; 95% CI = 0.79–1.76), but feeding HTM tended to increase the proportion of pregnant cows by 305 d postpartum. Feeding HTM might benefit reproduction in dairy cows.

Key Words: hydroxychloride, reproduction, trace minerals

Table 1 (Abstr. 2144). Effect of source of trace minerals on reproduction in dairy cows

Item	STM (n=70)	HTM[1] (n=71)	SEM	*P*-value
Cyclic by 38 d postpartum, %	62.2	59.3	0.08	0.73
Synchronized ovulation, %	82.7	93.0	4.2	0.12
Ovulatory follicle, mm	12.7	13.4	4.7	0.18
Luteal area d 7, mm^2	344	386	20	0.08
Pregnant d 16, %				
All cows	56.2	67.7	7.7	0.29
Synchronized cows	63.6	76.6	7.6	0.23
Conceptus length, cm	8.17	7.60	1.14	0.70
Flush IFNt, ng/mL	11.6	17.6	6.3	0.47
Pregnant by 305 d, %	70.1	82.2	5.1	0.08

[1]IntelliBond® Z, C, M; Selko USA.

2145 Effect of different luteolytic strategies at the end of the Breeding-Ovsynch on luteal regression and endometrial diameter of lactating dairy cows submitted to first and subsequent artificial insemination. I. M. R. Leão*[1], D. Ponce-Aguilar[1,2], F. P. J. da Silva Jr.[1], L. G. Wichman[1], L. J. Montiel-Olguin[3], and J. P. N. Martins[1], [1]*School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [2]*Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Juriquilla, Querétaro, Mexico,* [3]*CENID Fisiología y Mejoramiento Animal-INIFAP, Colón, Querétaro, Mexico.*

Our aim was to determine the effect of doubling the cloprostenol (PGF) dose administered once or delaying the 2nd PGF treatment at the end of Ovsynch on luteal regression and endometrial diameter between PGF treatment and TAI. Lactating Holstein cows (n = 1,223) submitted to a Breeding-Ovsynch [GnRH(G1) –7d– PGF(PGF1) –56h– GnRH(G2) –16h– AI] for 1st or = 2nd AI were randomly assigned to receive either CON, DPG, or PG56. Cows enrolled in CON received 2 PGF treatments (0.5 mg) 1 d apart (PGF1 –1d– PGF2), while cows in PG56 received a 2nd PGF at the same time as G2 (PGF1 –56h– PGF2+G2). Cows in DPG were treated with a double dose of PGF (1 mg) only at PGF1. In a random sub-sample of 413 cows, we recorded ultrasonographic examination videos of ovaries and uteri at PGF1, G2, and TAI. In this preliminary data, we measured total luteal area (TLA) and endometrial diameter of 107 cows. Endometrial diameter was measured from both uterine horns 2–3 cm from the uterine body and averaged. If fluid was present in the horn, the lumen diameter was subtracted from the endometrial diameter of the horn. Response variables were analyzed by ANOVA using the MIXED procedure of SAS. The proportional reduction in TLA between PGF1 and TAI did not differ among treatments (*P*

> 0.20) and the overall average was 73%. Endometrial diameter at the time of PGF1 (18.4 ± 0.2 mm), GnRH (21.7 ± 0.2 mm), and TAI (23.0 ± 0.2 mm) was similar among treatments. Yet, primiparous cows had a smaller endometrial diameter than multiparous cows at PGF1 (17.9 ± 0.3 vs. 18.7 ± 0.2 mm, $P = 0.01$), GnRH (21.2 ± 0.3 vs. 22.0 ± 0.2 mm, $P = 0.02$), and TAI (22.4 ± 0.3 vs. 23.3 ± 0.2 mm, $P = 0.02$). Also, the proportional increase in endometrial diameter between PGF1 and TAI was not affected by treatment and parity (overall 26.3%). In summary, based on our preliminary data, extending the interval between PGF treatments from 48 to 56 h or using a single double PGF dose at the end of the Breeding-Ovsynch did not affect structural luteal regression and endometrial diameter changes before ovulation.

Key Words: luteolysis, prostaglandin $F_{2\alpha}$, endometrium

2146 Effect of large follicle (= 10 mm) number at prostaglandin $F_{2\alpha}$ administration on the reproductive performance of lactating dairy cows. R. Miura*, K. Bandai, S. Watanabe, T. Tajima, and T. Ajito, *Faculty of Veterinary Science, School of Veterinary Medicine, Nippon Veterinary and Life Science University, Musashino, Tokyo, Japan.*

Prostaglandin $F_{2\alpha}$ (PGF) administration is a useful treatment for inducing estrus. However, the efficiency of PGF treatment is limited because of variability in the stage of follicle development among cows at PGF treatment. From our previous study, more than 75% cows during the early diestrous had only one large follicle (LF, = 10 mm) (1F), and more than 75% during late diestrous had 2 or more LFs (2F). Therefore, it was speculated that the first-wave dominant follicle (DF) is developing in cows with 1F, and second or larger-wave DF is developing in cows with 2F. Also, it is reported that the conception rate (CR) in artificial insemination (AI) of the first-wave DF is lower than second-wave DF. Therefore, we hypothesized that reproductive performance after PGF administration will differ between cows with 1F or 2F. The objective was to evaluate the effect of LF number at PGF administration on the reproductive performance of lactating dairy cows. Holstein cows (n = 277, DIM = 117.3 ± 61.4, Parity = 3.0 ± 0.3, Milk yield = 33.2 ± 8.7 kg/d; Mean ± SD) were examined using ultrasonography and the LF number (1F: n = 170, 2F: n = 107) was determined in those with a functional corpus luteum (= 20 mm). Cows included in the 2F had more than one LF. PGF was administered intramuscularly and estrus detection performed from d 1 to 6 after PGF treatment. Cows identified in estrus with visual observation were artificially inseminated and pregnancy diagnosis was conducted at 60 d after AI. Service rate, CR, and pregnancy rate (PR) were evaluated using logistic regression, while the interval between PGF treatment and estrus was evaluated using Student's *t*-test. Service rate was lower ($P < 0.01$) in 1F (78.8%) than 2F (94.4%) cows, CR tended to be lower ($P = 0.05$) in 1F (26.9%) than 2F (39.0%) cows, and PR was lower ($P < 0.01$) in 1F (21.2%) than 2F (36.4%) cows. Interval between PGF administration and estrus was not different between 1F (3.1 ± 0.9 d) and 2F (3.3 ± 0.9 d) cows. These results indicate that cows with 2 or more LFs at the time of PGF treatment have improved reproductive performance.

Key Words: large follicle number, prostaglandin $F_{2\alpha}$, reproductive performance

2147 A novel method to synchronize luteolysis in lactating nonpregnant dairy cows 20 days after induced ovulation. L. M. Duarte*, J. P. N. Andrade, A. Beard, P. J. L. Monteiro, R. R. Domingues, E. M. Cabrera, M. R. Pupo, C. H. P. C. Nova, and M. C. Wiltbank, *University of Wisconsin–Madison, Madison, WI.*

The goal was to synchronize luteolysis (P4 < 0.1 ng/mL) by d 20 of the estrous cycle with a long-term goal of developing a program to rebreed dairy cows every 21 d (ReBreed21-dairy). Holstein cows (n = 83) were induced to ovulate with Double-OvSynch (last GnRH = d0) but were not bred to ensure nonpregnant synchronized cows. Ovulated cows (n = 76) were blocked by parity and randomized into 1 of 2 treatments: oxytocin (Oxt-50 IU IM) and saline (Sal-2.5 mL i.m.). On d 17, 18, and 19 cows received either Oxt (n = 39) or Sal (n = 37), and blood samples were collected daily from d 14 to 30. On d 17 to 19, a blood sample was collected before treatment, and a second sample was taken 2h later. Prostaglandin 2-α metabolite (PGFM) was analyzed by ELISA assay. A significant increase in circulating PGFM was judged using a threshold of 2 standard deviations from the first sample on d 17. Statistical analysis was conducted using linear regression and generalized linear models with treatment and parity as fixed effects. Circulating PGFM in pg/mL (±SEM) for Oxt vs. Sal was not different on any day before treatment: d 17 = 36.6 vs. 31.3, $P = 0.86$; d 18 = 36.7 vs. 34.4 $P = 0.64$; and d 19 = 41.7 vs. 32 $P = 0.22$. In contrast, PGFM was greater for Oxt than Sal in the samples 2 h after treatment: d 17 = 136 vs. 25.6, $P < 0.01$; d 18 = 121.1 vs. 24 $P < 0.01$; and d 19 = 112.2 vs. 29.2 $P < 0.01$. The percentage of cows that had an increase in PGFM at 2 h after treatment was 66.6% (26/39) vs. 2.7% (1/37) $P < 0.01$;51.28% vs. 0% (0) $P < 0.01$; and 56.41% (22/39) vs. 8.1% (3/37) $P < 0.01$, respectively for Oxt vs. Sal on d 17, 18, and 19. In the Oxt group, 69.2% (27/39) of cows underwent luteolysis between d 18 and 20, while 37.8% (14/37) in Sal group. Average day to luteolysis was advanced for Oxt compared with Sal (19.6 d ± 0.3 vs. 21.3 ± 0.5; $P < 0.01$), with 76.9% (30/39) of Oxt-treated cows having undergone luteolysis by d 20, as opposed to 43.2% (16/37) in the Sal group. Thus, Oxt treatment synchronized luteolysis in ~80% of non-bred lactating dairy cows by d 20 after induced ovulation, making this a potential method to explore in the development of early rebreeding programs.

Key Words: luteolysis, synchronization, oxytocin

2148 Proteomic profiling reveals potential mechanisms regulating prostaglandin $F_{2\alpha}$ responsiveness in bovine corpora lutea. J. M. Hughes* and C. H. K. Hughes, *Department of Animal Science, Pennsylvania State University, University Park, PA.*

A mature corpus luteum (CL), crucial for pregnancy maintenance through progesterone production, undergoes luteolysis in response to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), while a developing CL is resistant to $PGF_{2\alpha}$. This transition, termed acquisition of luteolytic capacity (ALC), occurs between d 4 and 6 in the cow. The goal of this study was to develop an improved understanding of the physiological changes in the CL during ALC. Estrous cycles of 8 Holstein cows were synchronized, with ovulation induction via GnRH (and confirmed via ultrasound) designated d 0. On d 4 or 6, CL were collected and frozen (n = 4/group). Plasma progesterone was assessed to confirm functionality of CL. Total luteal proteins were extracted, quantified, and tested for differential abundance using proteomics (Q-Exactive HF mass spectrometry; Data Independent Acquisition mode). Among the total 5,179 proteins identified, 63 proteins were more abundant on d 4 and 394 were more abundant on d 6 (Padj <0.10). The prostaglandin F receptor (PTGFR) was not detected, but previous reports indicate that in bovine CL PTGFR expression does not change during ALC. Gene ontology and pathway analysis were used to identify key cellular mechanisms that may regulate luteal PGF2α responsiveness. Several members of the apoptosis-regulating BCL-2 gene family, including BASP1 (log2FC = 1.806), BAK1 (log2FC = 1.220) and BCL2L13 (log2FC = 1.297) were upregulated in d 6 CL. Additionally, proteins forming the nuclear pore complex (NPC) were upregulated on d 6. NPCs are channels that allow for transport into and out of cell nuclei, including transport of apoptotic proteins to induce cell death. Changed NPC proteins included NUP37 (log2FC = 1.26), NUP43 (log2FC = 0.825), and NUP93 (log2FC = 1.582). Upregulation of NPCs and BCL-2 family proteins may indicate increased potential for uptake of apoptotic signaling in the nucleus, which is essential for cellular death by apoptosis. Overall, this study contributes to our knowledge of $PGF_{2\alpha}$ responsiveness and could be used to develop technology to address pregnancy loss caused by luteal regression in dairy cattle.

Key Words: corpus luteum, luteolysis, prostaglandin $F_{2\alpha}$

2149 Embryo quality and yield improved in nulliparous Holstein heifers supplemented with OmniGen® AF.

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The aim of this study was to evaluate the effects of feeding OmniGen® AF (OG) nutritional specialty product, a combination of all-natural silicates, yeast components, and B vitamins, on embryo quality and yield in nulliparous Holsteins in a retrospective observational study, at a dairy undergoing an FSH-$PGF_{2\alpha}$ superovulation protocol with embryos harvested 7 d post insemination with either sexed or unsexed semen. Embryos were collected from 68 control (CT, no OG) heifers from Jan 1–Aug 1, 2022, and 79 OG fed (8.8 g/100 kg BW added to the TMR for > 30 d before superovulation) heifers from Jan 1–Aug 1, 2023. Heifers were fed a corn silage, small grain silage based TMR with a protein-mineral supplement. Heifers (363 kg BW) were 11 ± 4 mo of age at first flushing and were flushed between 1 to 5 times. Embryos were graded by morphological quality, those graded 1, 2, and 3 were considered viable and UFO and DEG were considered non-viable (unfertilized or degraded). Data were analyzed using GLM procedure of SAS v 9.4 (Cary, NC) comparing the treatment effects sorted by semen type on embryo viability. Significance was declared at $P = 0.05$. Heifers fed OG produced more viable embryos (7.8 and 5.9; OG and CT, respectively, $P < 0.01$) and fewer non-viable embryos (3.1 and 5.1, OG and CT, respectively, $P < 0.05$), resulting in greater viable embryo (%) per flush (73 and 57; OG and CT, respectively, $P < 0.01$). For unsexed semen, viable embryos (%) per flush were not different between OG and CT but heifers fed OG produced more viable embryos per flush (9.2 and 4.9; OG and CT, respectively, $P < 0.01$). The number of non-viable embryos from unsexed semen did not differ. For sexed semen, OG fed heifers produced more viable embryos (7.6 and 5.9; OG and CT, respectively, $P < 0.01$) resulting in an increase in viable embryo (%) per flush for OG (69.6 and 56.2; OG and CT, respectively, $P < 0.01$). These data indicate feeding OG before initiating a superovulation protocol may help improve the quality and number of viable embryos.

Key Words: embryo quality, embryo yield, OmniGen AF

2150 Clinical cure failure of metritis is not associated with differences in the uterine metabolome of dairy cows.

F. N. S. Pereira*[1], D. Z. Bisinotto[2], K. N. Galvão[2], R. S. Bisinotto[2], and C. C. Figueiredo[1], [1]*Washington State University, Pullman, WA,* [2]*University of Florida, Gainesville, FL.*

The objective was to characterize differences in uterine metabolome associated with clinical cure failure of metritis in lactating Holstein cows. This prospective cohort study was conducted from February to November 2018 in 2 dairy herds in Florida. Vaginal discharge was evaluated within 15 DIM using the Metricheck device and metritis was characterized by the presence of fetid, watery, reddish-brownish vaginal discharge (study d 0). Cows with metritis (n = 24) were paired with cows without metritis of similar DIM and parity (NoMET; n = 24). On d 0, the uterine lumen of all cows was flushed with 15 mL of saline solution using a plastic infusion pipette connected to a syringe and cows with metritis were treated with ceftiofur. On d 5, clinical cure failure of metritis, characterized by the persistence of fetid, watery, reddish-brownish vaginal discharge (Cure; n = 8 and NoCure; n = 16), was assessed before the performance of a second uterine flush in all cows. The uterine metabolome was evaluated using untargeted gas chromatography time-of-flight mass spectrometry. Autoscaled and normalized data were analyzed in Metaboanalyst using ANOVA and principal component analysis. Enriched pathway analysis was performed whenever differences in uterine metabolome composition between Cure and NoCure cows was observed. False discovery rate (FDR) was used to adjust P-values for multiple comparisons. A total of 188 annotated primary metabolites, characterized by different peak intensities and charge, composed the data set in the study. On d 0, the abundance of 107 metabolites differed (FDR ≤0.05) between the uterus of cows with and without metritis. Most compounds were associated with the metabolism of amino acids, carbohydrates, and fatty acids. No differences in the uterine metabolome were observed between Cure and NoCure cows on d 0. No differences in the uterine metabolome were observed among any of the groups on d 5. This demonstrates that although metritis is associated with differences in the uterine metabolome on the day of diagnosis, clinical cure failure of metritis is not associated with differences in the uterine metabolome.

Key Words: uterine health, disease, cure

2151 Analysis of commercial *Acacia mearnsii* extract to investigate the potential use of flavonoid constituent as an antioxidant in semen.

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This study aimed to identify a potential compound in the commercial extract of *Acacia mearnsii* bark (ATE) that could be used as an antioxidant to protect semen during preservation. Flavonoid compounds are tentatively identified with ultra-performance liquid chromatography coupled with high-resolution mass spectrometry (UPLC-HRMS). ATE was extracted with ethanol, filtered, and dried to yield a filtrate powder (MTE_0). A portion of MTE_0 (1.4 g) was solubilised in methanol and fractionated using column chromatography with Amberlite XAD-16 resin. Four fractions (MTE_1, MTE_2, MTE_3, and MTE_4) were collected and stored at –20°C. Extracts were subjected to UPLC-HRMS identifying sucrose and 7 flavonoid compounds among which was gallocatechin. Antioxidant activity was determined using 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays. The ABTS, DPPH of extract MTE_0 (0.00 ± 0.00; 0.02 ± 0.00) and fraction MTE_3 (0.00 ± 0.00; 0.02 ± 0.00) had antioxidant activity comparable to that of ascorbic acid (0.00 ± 0.0.00; 0.01 ± 0.00). Effects of MTE_0 and gallocatechin compound on chilled semen were determined. Six ejaculates were obtained from a mature merino ram, allocated to 10 groups (2 × control, MTE_0, and gallocatechin assigned into different aliquots at concentrations of 12.5, 25, 50, and 100 µ*M*), chilled, and maintained at a temperature of 4 to 10°C for 10 d. The motility of the semen was analyzed using CASA, viability, acrosomal integrity (lectin PNA), and capacitation by flow cytometry. Data were analyzed by one-way ANOVA procedure of IBM SPSS 21.0. Gallocatechin at 25 µ*M* significantly ($P = 0.001$) improved proportions of live spermatozoa (32.38 ± 0.55%; 19.54 ± 0.26%), reduced ROS (35.38 ± 0.55%; 25.60 ± 0.55%), and % capacitation status (31.48 ± 0.81%; 20.46 ± 0.81%) compared with control. Gallocatechin addition to semen recorded a significant ($P = 0.001$) effect compared with MTE_0 extract. It is concluded that gallocatechin is a promising candidate antioxidant for use in semen during preservation.

Key Words: *Acacia mearnsii*, antioxidant, semen

2152 Comparisons of the uterine metagenome on the day of calving in dairy cows with or without metritis. A. D. Ravelo*, P. Ferm, N. R. Noyes, and L. S. Caixeta, *Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN.*

Metritis is a dairy cow postpartum disease associated with decreased milk production and fertility. Our primary objective was to compare the uterine metagenome at calving in cows that were later diagnosed with or without metritis, and secondarily, compare the uterine metagenomes based on the onset of diagnosis. Uterine microbiome samples were collected in the first 24 h after parturition, using double-guarded swabs. Upon the diagnosis of metritis (defined as red-brownish vaginal discharge, fever, and signs of systemic illness before 21 DIM), samples from 11 cows with metritis (MET) were matched to 2 samples from cows without metritis (CON; n = 22). Cows were matched by parity, calving difficulty, calving date, antimicrobial treatment, and occurrence of other disease. Within MET, 6 cows had an early (1–3 DIM) and 5 a late (4–10 DIM) diagnosis. DNA was extracted from all samples and subjected to shotgun metagenomics. Microbial species were identified using Kraken2 and their potential functions using Humann3.8. There was no difference in α diversity for bacterial species ($P > 0.32$). Beta diversity of bacterial species was not significantly different ($P = 0.10$) when comparing CON and MET; however, there were 63 differentially abundant bacterial species ($P < 0.05$). The β diversity of potential functions was different between the 2 groups ($P = 0.01$). Wilcoxon rank sum test was used to compare CON and MET pathway abundance and no functions were different ($P > 0.05$). When comparing cows based on onset of metritis diagnosis (early, late, control) the β diversity was significantly different ($P = 0.01$), and *Clostridium perfringens* ($P = 0.03$) and *Peptoniphilus* sp. *ING2.D1G* ($P < 0.01$) were differentially abundant present with greater abundance in MET early. Beta diversity comparing pathway abundance for onset was different ($P < 0.01$) and when Kruskal-Wallis test was used to compare the pathway abundance 22 functions were different among the 3 groups ($P < 0.05$). The microbiome of the uterus at calving was different between cows that were diagnosed as MET or CON, especially when considering the onset of diagnosis.

Key Words: bacteria, postpartum disease, bacteria functions

Ruminant Nutrition 1: Calves and Heifers

2153 Phytogenic antioxidant supplementation improved efficiency, growth, and health outcomes in dairy calves under 8 weeks of age regardless of vitamin E intake. T. S. Dennis*, K. E. Cowles, M. A. Messman, A. N. Hafla, and A. H. Souza, *Cargill Animal Nutrition, Lewisburg, OH.*

The objectives of this study were to determine if a phytogenic antioxidant (AOX) could partially replace synthetic vitamin E and if pre-weaning calf growth and health improve with additional AOX intake. Holstein heifer calves (n = 50; 42 ± 4.3 kg BW) were randomly assigned to 1 of 4 treatments and blocked by initial BW, serum total protein, and pen location. Treatments were positive control (PC; 3.8 IU/kg BW vitamin E intake), negative control (NC; 2.0 IU/kg BW vitamin E intake), PC+AOX (additional 1.8 IU/kg BW vitamin E equivalent from AOX), and NC+AOX. Calves were individually housed for 56 d in a barn with natural ventilation and straw bedding. A common milk replacer (MR; 24% CP, 17% fat fed up to 910 g/d AF) was used and calves were weaned on d 49. Treatments were added to MR using supplements (20 g/d) and formulated into pelleted starters. Starter intake was measured daily, BW and BCS weekly, and hip height (HH) and hip width (HW) at d 0 and 56. Antioxidant status was assessed via serum malondialdehyde (MDA) and vitamin E at d 28 and 56, and metabolic profile via serum BUN, BHB, and NEFA at d 42, 50, and 56. Data were analyzed as a GLMM with a randomized incomplete block design and 2 × 2 factorial treatment arrangement, repeated measures, block as a random effect, and calf as experimental unit. Vitamin E intakes averaged 383 and 234 IU/d for PC and NC targeted vitamin E intakes, respectively. Total DM intake and ADG were similar among treatments, but gain:feed tended to improve for NC+AOX (0.479) vs. PC (0.449; $P = 0.07$). AOX tended to increase HH ($P = 0.08$) but not HW change ($P = 0.48$). Total medical days were reduced 67% when calves were fed AOX regardless of vitamin E level (1.5 vs. 3.8 d; $P = 0.05$). Serum vitamin E was similar among treatments, but MDA was greater at d 28 for NC+AOX vs. PC ($P = 0.03$). Serum BUN ($P = 0.07$) and BHB ($P = 0.03$) were elevated on d 50 for calves fed AOX. Under study conditions, phytogenic AOX can safely replace synthetic vitamin E in pre-weaning calf feeds and may improve health outcomes by reducing the need for medical intervention.

Key Words: calf growth, oxidative stress, antioxidant

2154 The effects of transition milk replacers with different IgG levels on growth performance of neonatal dairy calves. K. Shimada*[1,2], R. Harada[2], H. Sato[3], R. Fukumori[3], M. A. Steele[1], and K. Izumi[3], [1]*University of Guelph, Guelph, ON, Canada,* [2]*The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Tokyo, Japan,* [3]*Rakuno Gakuen University, Ebetsu, Hokkaido, Japan.*

The effects of transition milk replacer (TR) with 3 levels of IgG contents on growth performance of neonatal calves was investigated. Forty-eight Holstein-Friesian neonatal calves (male = 19, female = 29, 42.64 ± 0.88 kg) were randomly assigned to 3 groups; Control (TR with no IgG), Mid (12 g IgG/L of TR), and High (24 g IgG/L of TR). All calves were fed the first colostrum replacer within the first 2 h of their lives and then offered a second meal 8 h later. The colostrum replacer was given via esophageal catheter and all calves consumed 240g IgG total. Following the colostrum feedings, d 1 to d 5, calves were fed their respective transition milk replacers, which were isoenergetically formulated, twice a day (4.9 L/d, 0.816 kg/d on powder basis), then, from d 6, all calves were fed the same calf milk replacer until 56 d of age. Calf starter, hay, and water were offered from d 1. DMI and fecal scores were measured on a daily basis, and body weight and size were measured in 5–14 d intervals. All data were presented as the least squares means ± SE. Data were analyzed using the Fit model procedure of JMP. The model included treatment as fixed effects and calves as random effects. For all statistical estimates, age, treatment, and age × treatment interaction, $P < 0.05$ indicates a significant difference. There was no difference in DMI among the groups throughout the trial period. The ADG among the groups were similar, except between d 56 to 65, during which the High group was higher than the Con and Mid groups (1.07 ± 0.08 kg/d, 1.08 ± 0.08 kg/d, and 1.33 ± 0.08 kg/d for Con, Mid, and High, respectively). There was no difference in the health score among the groups. There was no difference in the feed efficiency among the groups. These results suggest that the transition milk replacer containing 24 g IgG/L positively affects growth of calves after weaning. However, further investigation is needed to find the effects of the formulation on enhancing their growth.

Key Words: neonatal calves, transition milk, growth performance

2155 Impact of casein addition in colostrum replacer on neonatal calf serum immunoglobulin G concentration and dynamics. J. M. V. Pereira[1], G. Mazon[1], J. V. R. Lovatti[1], E. V. Lopez-Bondarchuk[1], A. J. Geiger[2], and J. H. C. Costa*[1], [1]*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT,* [2]*Zinpro Corporation, Eden Prairie, MN.*

The impact that casein has on colostrum immunoglobulin G (IgG) absorption and serum IgG concentration in neonatal calves remains unclear. Thus, this study aimed to assess the impact of casein presence in colostrum replacer on serum IgG concentration dynamics in neonatal dairy-beef calves. Holstein-Angus calves (n = 72 [24/treatment]; 45.3 ± 5.9 kg) were randomly assigned to one of 3 colostrum treatments at birth: Colostrum Replacer: CR-C (85 g/L of IgG; Premolac Plus, Zinpro), Colostrum Replacer with casein reconstituted: CR+C (85 g/L of IgG; Premolac Plus, Zinpro + 95 g/L of casein), and maternal colostrum: MC (85 g/L of IgG). Calves received 3.7 L of solution via tube feeding at 3.1 ± 0.2 h postbirth, solution had 300 g total of IgG, and casein addition was calculated to match the MC treatment. Blood samples were collected at colostrum feeding and at 6, 12, 18, 24, 36, 48, and 72 h post feeding. Serum was analyzed for IgG concentration (g/L) and maximum IgG concentration (g/L; Cmax), time to maximum IgG concentration (h; Tmax), IgG area under the curve (AUC), and the apparent IgG persistency (%; AIP) were calculated. The IgG AUC was calculated using the trapezoidal rule, and the AIP was obtained by the difference of the Cmax and the serum IgG concentration at 72 h, expressed proportionally to the Cmax. Mixed linear models were used to evaluate the effect of colostrum treatments on Cmax, Tmax, AUC, and AIP. Treatment affected the Cmax, with CR+C treated calves exhibiting reduced Cmax compared with CR-C, and MC calves (CR-C = 29.5 ± 1.3a, CR+C = 21.1 ± 1.3b, MC = 26.2 ± 1.4a g/L; $P < 0.01$). For Tmax no differences were observed between treatments (CR-C = 23.7 ± 1.1, CR+C = 29.1 ± 1.1, MC = 24.9 ± 1.1 h; $P = 0.44$). The AUC was lower for CR+C compared with CR-C and MC treated calves (CR-C = 2,641.2 ± 117.5a, CR+C = 1,719.3 ± 120.6b, MC = 2,197.5 ± 124.4b g/Lxh; $P < 0.01$). Different letters (a,b) indicate significant differences between treatments. Treatment did not affect AIP (CR-C = 60.3 ± 6.1, CR+C = 48.02 ± 6.3, MC = 59.8 ± 6.5%; $P = 0.21$). These findings suggest that casein presence in CR affected maximum IgG concentration and total AUC in neonatal calves; casein plays a role in IgG dynamics in neonate calves.

Key Words: passive transfer, area under the curve (AUC) of IgG, casein

2156 Effects of fat content of high-protein milk replacer on insulin sensitivity in dairy calves.

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The objective of this study was to evaluate the effects of fat content of high-protein milk replacer (MR) on insulin sensitivity of Holstein calves. Forty-two Holstein heifer calves (born between October 31 and December 22, 2022) were fed one of the 3 MR (96% dry matter and 29% crude protein): (1) 20.4% fat, 41% lactose, and 4.7 Mcal/kg of metabolizable energy (ME) (LF; n = 14), (2) 26.2% fat, 36% lactose, and 5.0 Mcal/kg of ME (MF; n = 14), (3) 31.8% fat, 31% lactose, and 5.3 Mcal/kg (HF; n = 14), on a DM basis. The treatment MR were offered at 600 g/d (powder basis) from 7 to 13 d, 800 g/d from 14 to 20 d, 1,200 g/d from 21 to 41 d, 800 g/d from 42 to 48 d, and 600 g/d from 49 to 55 d, and then calves were weaned at 56 d of age. All the calves were fed calf starter and chopped hay ad libitum from 7 d of age. Insulin sensitivity was calculated according to the following equation: homeostatic model assessment for insulin resistance (HR) = plasma insulin (μIU/mL) × plasma glucose (mmol/L) / 22.5, and revised quantitative insulin sensitivity index (RQ) = 1/[log plasma glucose (mg/dl) + log plasma insulin (μIU/mL) + log plasma fatty acids (mmol/L)]. A high HR or low RQ value indicate decreased insulin sensitivity. Data were analyzed by Linear Contrast using Fit Model procedure of JMP® 17 pro (SAS Institute Inc., Cary, NC). Plasma total cholesterol, fatty acids and triglyceride concentrations of preweaning calves increased linearly ($P < 0.05$) as the fat content of MR increased. Plasma urea nitrogen concentration decreased linearly ($P < 0.05$) as the fat content of MR increased, and this reduction continued even after weaning ($P < 0.05$). As the fat content of MR increased, HR increased linearly ($P < 0.05$) in 22 to 56 d of age, and RQ decreased linearly ($P < 0.05$) in 43 to 56 d of age. These results suggested that higher fat contents of high-protein milk replacer increase nitrogen efficiency in dairy calves, but decrease insulin sensitivity in preweaning dairy calves.

Key Words: fat, insulin sensitivity, nitrogen efficiency

2157 Colostrum from cows with high or low somatic cell count at dry-off: Effects on the development of dairy calves during the neonatal period.

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This study aimed to evaluate the transfer of passive immunity (TPI), performance, metabolism, and organs weight of calves receiving colostrum from cows with high (≥300,000 cfu/mL) or low (<200,000 cfu/mL) SCC at dry-off. Ten Holstein calves were distributed in randomized blocks according to weight and date of birth, and received high-quality maternal colostrum (>22% Brix) in a volume corresponding to 10%–12% of live weight in the first 2 h of life. The colostrum used was obtained from 2 groups of cows: High SCC: ≥ 300,000 cfu/mL at dry-off; Low SCC: < 200,000 cfu/mL at dry-off. After the first colostrum meal, the calves received 6 L/d of milk replacer in 2 meals and had access to water and concentrate ad libitum from the first day. Blood collections were carried out at 24, 48, 72 h of life, and at 7 d to determine selected metabolites (glucose, protein, and lactate). TPI was also evaluated in the collections carried out in the first 72 h. On the seventh day, the calves were weighed to monitor weight gain, and approximately 11 h after the last feeding, the calves were slaughtered for digestive tract and internal organs evaluation. Both treatments reached excellent TPI in the evaluation with a protein refractometer (High: 7.3 mg/dL; Low: 6.9 mg/dL, SEM: 0.28) and Brix (High: 10.8%; Low: 10.2%, SEM: 0.37) without differing from each other ($P > 0.05$). Liquid diet intake, slaughter weight, fecal score, metabolic profile, and weight of all organs were also not influenced by treatments ($P > 0.05$). However, calves fed colostrum from the low SCC group present greater intestinal weight as a percentage of birth weight (7.18 *vs.* 6.12, SEM: 0.29; $P = 0.03$) and a tendency toward greater weight gain (414.3 *vs.* 177.1 g/d, SEM: 78.35; $P = 0.06$). The supply of maternal colostrum from cows with low SCC at dry-off increased the intestinal weight of calves and short-term weight gain, suggesting an improved nutrient absorptive capacity and, potentially, their long-term performance.

Key Words: metabolism, organ weight, transfer of passive immunity (TPI)

2158 Effect of water quality and milk replacer composition on performance and health of dairy calves.

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This study aimed to evaluate the performance and health of calves fed 2 milk replacers (MR) with different composition and offered water with different treatments (tap water and treated purified water). Forty-five colostrum-fed Holstein calves were used in a randomized block design, considering sex, birth body weight, and date as blocking criteria. The calves were individually housed with free access to water and a commercial pelleted starter. Calves were assigned to 3 treatments: (1) VP: MR mostly with vegetable protein (20% CP;17% Fat) diluted in tap water; (2) MT: MR mostly with milk protein (22% CP; 18% Fat) diluted in tap water; and (3) MP: MR mostly with milk protein (22% CP; 18% Fat) diluted in purified water. All MR were diluted to 14% solids. Calves were fed with 6 L/d of the respective MR, until they were gradually weaned from 49 to 53 d of age. Chopped hay was offered ad libitum from 49 until 70 d of age, when evaluation ended. Dry matter intake (DMI) and water intake (WI) were monitored daily, while average daily gain (ADG) was evaluated weekly. Data were analyzed as repeated measures comparing treatments and testing contrasts for MR and water quality. During the experimental period, there were no differences on DMI and WI among treatments ($P > 0.05$). The purified water (MP) tended to increase preweaning ADG (0.38 vs. 0.32 ± 0.03 kg/d, $P = 0.07$) and feed efficiency (0.39 vs. 0.31 ± 0.035, $P = 0.08$) and decrease fecal scores (1.44 vs. 1.57 ± 0.058; $P = 0.07$). However, final weight did not differ among treatments. Postweaning, MP resulted in higher ($P < 0.014$) hay consumption (135.9 g/d) than MT (100.0 g/d) and VT (103.2 g/d). The purified water also increased hay consumption ($P < 0.004$). The potential benefits of providing different milk replacers compositions were not observed in this short-term study, probably due to the similar nutrient levels. Offering purified water tended to improve performance and fecal score preweaning, and increased hay consumption

postweaning. However, no interaction between MR composition and water quality was observed, suggesting that water quality is important regardless of the MR composition.

Key Words: liquid diet, preweaning, postweaning

2159 Performance and health of calves fed corn and pellet starters with the inclusion of oats or a fiber pellet.

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Due to inconsistent ingredient availability and volatile commodity pricing, many calf raisers have transitioned to more economical calf starters (CS) in the form of corn and pellet (CP) mixes, ideally with the inclusion of oats, where available and cost-competitive, or utilizing a more cost-effective fiber pellet (FP). With limited research available on CP CS with the inclusion of FP, the objective of this study was to evaluate calf performance and health of these diets compared with a more conventional CP CS formulated with oats, and with or without additional yeast-based gut health technology (GH). Holstein heifer calves (n = 105) were randomly assigned to 1 of 4 treatments in a randomized complete block design from birth to d 56 of age. Treatments included CP CS with 1) FP and GH (FWG); 2) oats and GH (OWG); 3) FP without GH (FNG); and 4) oats without GH (ONG). All CS was fed ad libitum throughout the study. Crude protein and NDF of all CS were as follows, respectively (% DM): (1) 21.7%, 16.0%; (2) 23.4%, 11.7%; (3) 22.1%, 14.2%; and (4) 23.2%, 11.0%. Calves received 283.5 g of 20% protein, 20% fat milk replacer (MR) 2×/d from d 1–35, and 1×/d from d 36–42. Body weight (BW) was measured at d 0, 14, 28, 42, 49, 56, and 84. Fecal scores were measured weekly. Feed intake was measured daily and calculated bi-weekly. Initial BW was used as a covariate for BW, gain and feed intake. Data were analyzed using the PROC MIXED procedure of SAS with fixed effects of fiber source and GH technology and random effects of source herd and barn. No effects of treatment on MR intake, CS intake, or scouring frequency were observed throughout the entirety of the study. During the post-weaning period (d 43 to 49), there was an interaction of fiber source and GH on feed efficiency ($P = 0.02$), with FWG reporting 20% improved G:F over FNG. This coincided with a tendency for an interaction of fiber source and GH on BW gain in the same period ($P = 0.09$). Under conditions of this study, replacing oats with a FP in a CP-type CS may be a viable and economical option for calf raisers, with improved performance and efficiencies seen post-weaning when implementing GH.

Key Words: calf starter, fiber, gut health

2160 Health and growth of weaned Holstein and crossbred calves fed bovine plasma proteins in grower grain.

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Plasma protein has been shown to have health benefits for preweaning calves. We hypothesized that plasma proteins would improve health and growth of calves during post-weaning commingling. The objectives of this study were to evaluate health and growth response of newly weaned calves under stress fed bovine plasma proteins in grower feed. During experiment 1, 21 male Holstein calves were housed outdoors in groups of 3 from 10 to 14 wk of age. Experiment 2 included 40 male and female Holstein-Angus cross calves, housed outdoors in groups of 6–7 from 9 to 13 wk of age. Calves were weaned on d −1 for both experiments. Calves were weaned over 1 wk (Exp. 1) or 2 wk (Exp. 2). Both experiments were randomized complete block designs. Data from experiments 1 and 2 were pooled. Treatments were a control grower diet with no bovine plasma protein (CON; 20% CP, 2.4% fat, 32% starch) and a grower diet including plasma protein (PP; 20% CP, 2.9% fat, 32% starch). Experiment 1 had 3 CON pens and 4 PP pens. Experiment 2 had 3 CON pens and 3 PP pens. Pen was the experimental unit. Both experiments provided ad libitum water and grower grain with a 2.5% inclusion of chopped wheat straw. Data collection was the same during both experiments. Feed intake and health scores, including assessment of fecal, ocular, nasal, ear, and respiratory, were recorded daily. Body weight (BW) was recorded weekly, and skeletal growth measurements were recorded on d 0 (Exp. 1) or d −1 (Exp. 2), and d 28. Average daily gain and feed intake data were analyzed using the MIXED and UNIVARIATE procedures, while health data were analyzed using the FREQ and GLIMMIX procedures in SAS. Calves fed PP tended to have greater final hip height ($P = 0.07$) and greater hip height ADG ($P = 0.09$), but no other differences were reported for frame growth, BW, or ADG. No differences were observed between treatments for health scores. Grower intake was not different between treatments. Overall, there is potential for bovine plasma proteins fed to weaned calves in grower to improve skeletal growth, but they did not benefit health or feed intake.

Key Words: plasma proteins, growth, health

2161 Effect of extended colostrum feeding on the performance of Jersey heifers.

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Research into extended colostrum feeding in Holstein calves has pointed toward beneficial effects in growth, and feed efficiency. However, there has been no research into its effect of feeding extended colostrum on Jersey calves. This experiment is being conducted to determine the short- and long-term effects of an extended colostrum feeding on Jersey calves and heifer performance. The data are being gathered on 1 commercial Jersey Farm in Maryland. The calves are separated into a control and treatment group with 60 calves per treatment (n = 120). The control group is fed 2 feedings of colostrum while the treatment group is given 6 feedings of colostrum. Both groups receive 2 feedings of colostrum at 0–4 h and 12 h after birth. Afterward, the control group is fed milk replacer powder at 12-h intervals, while the treatment group receives 4 more feedings of colostrum at 12-h intervals before being switched to milk replacer. After 7 d all calves are transferred to a group pen with a free choice automatic milk feeder where they remain until weaning (77 d). Calves are measured on body weight, heart girth, hip height, rectal temperature, and fecal scores. These measurements are recorded at birth and then every other week until weaning. Our preliminary results were determined with a smaller sample size (control n = 34 and treatment n = 31). Data were analyzed using mixed model analysis, with statistical significance set at $P \leq 0.05$. Calves in the treatment group increased ($P = 0.04$) ADG compared with control (0.75 and 0.66 kg, respectively). Weaning weight increased ($P = 0.03$) for the treatment group compared with control (81 and 74 kg, respectively). Weaning heart girth, and height did not differ among treatments. While the results are not statistically significant, they do indicate a trend toward increased growth and feed efficiency for the treatment group. These findings can provide a baseline understanding for future research.

Key Words: ruminant nutrition, calves, growth and development

2162 Effect of probiotic fed in milk and starter feed to preweaning Holstein Angus calves on health.

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Feeding probiotics to preweaning calves can reduce gastrointestinal related health challenges. The objective of this study was to determine the effects of feeding a *Lactobacillus* probiotic supplement (PS; 1 × 10^9 cfu) in milk and starter grain on Holstein × Angus cross calves health and growth from 24 to 48 h to weaning at 60 d of age. Calves at a California commercial calf ranch were randomly enrolled into 4 treatment groups: 1. Control with serum total proteins (TP) < 5.2 g/dL (n = 56), 2. Control with serum TP ≥5.2 g/dL (n = 114), 3. Treated with 50 mg/calf/d PS added to milk, 50 mg/calf/d added to grain and TP <5.2 g/dL (n = 56) and 4. Treated with 50 mg/calf/d PS added to milk, 50 mg/calf/d added to grain and TP ≥5.2 g/dL (n = 110). Calves were weighed upon entry into the hutch and at weaning to determine total gain. The hutches were wooden with slatted floors, and each housed 3 divided calves. Starter grain was fed beginning at 14 d of age. Calves were scored daily with fecal scoring on a scale from 1 to 3 (FS) with 1 being normal and 3 being scours, respiratory score (RS) and general appearance scores (GA) were recorded on a scale of 1 to 5 with 1 being normal. Data were analyzed using general linear models (SAS v. 9.4, 2024). Difference was determined by $P < 0.05$. There were no differences among treatments for total gain, ADG, cause of death as determined by necropsy, days of FS = 3, days of RS ≥4 and days of GA ≥4. Total gain was affected positively by initial body weight, TP and negatively by GA. Average daily gain was affected positively by TP, initial bodyweight and negatively by FS and GA. Of the total number of calves necropsied (n = 8), cause of death was predominantly pneumonia (n = 7) and was affected positively by RS and GA. Treatment did not affect calf health or death, but TP positively affected overall gain and ADG for control calves indicating that the probiotic may have helped low TP calves maintain growth.

Key Words: fecal score, health score, *Lactobacillus*

2163 Temporal differentiation of enterotypes of young ruminants during early life shapes the characteristic growth phenotype.

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Colonization and evolution of the gut microbiota in young animals can lead to lifelong consequences and are closely associated with host health. The relationship between the hindgut microbiota and growth of young ruminants has been explored, but representative characterization of the microbiota in a large cohort is lacking. The internal mechanism of calf growth driven by enterotypes of beneficial core bacteria is also unclear. In this study, we sequenced 1,045 fecal samples from 408 newborn calves to characterize the invasion and dynamic changes of core taxa. Multi-omics analyses and fecal microbiota transplantation (FMT) in mice were used to reveal and verify the mechanism by which the enterotype shapes the calf growth phenotype. Four calf enterotypes were identified, 2 of which (dominated by *Bifidobacterium* and dominated by *UCG-005*, respectively) were representative. The frequency of conversion between enterotypes shifted from variable to stable, and was mainly influenced by age and diet. The functional characteristics of the different enterotypes were distinct. The close relationship observed between phenotype and enterotype, revealing a potential pro-growth effect of *Bifidobacterium*, may be implemented by promoting the use of carbohydrate, activating the synthesis of volatile fatty acids, amino acids and vitamin B6, and inhibiting methane production in the gut. The FMT confirmed the beneficial effect of a *Bifidobacterium*-dominated microbiota on animal growth and gut development. Collectively, our characterization of the gut microbiota enterotypes in calves highlights the potential beneficial effect of *Bifidobacterium* on calf growth, and will contribute to screening and manipulation of other beneficial gut colonizers to improve growth performance and productivity of young ruminants.

Key Words: fecal microbiota, calf, growth performance

Ruminant Nutrition 1: Carbohydrates and Lipids

2164 Effects of sodium acetate and high oleic acid soybeans on milk production of high-producing dairy cows. A. M. Bales*, V. Ribeiro Pinheiro Coelho, and A. L. Lock, *Michigan State University, East Lansing, MI.*

Milk fat is comprised of fatty acids (FA) originating from de novo and preformed sources. Our objective was to determine how the supply of sodium acetate (NaAC) and high oleic acid soybeans (HOSB) altered the yields of milk fat and milk FA sources of high-producing dairy cows. Twenty multiparous Holstein cows (52.3 ± 5.5 kg/d of milk; 123 ± 42 DIM) were randomly assigned to treatment sequences in a replicated 4×4 Latin square design with 14-d periods. Treatments were (1) control (Trt1), (2) 3% DM NaAC (Trt2), (3) 16% DM HOSB (Trt3), and (4) 3% DM NaAC + 16% DM HOSB (Trt4). NaAC replaced soybean hulls and HOSB replaced conventional soybean meal and hulls to maintain diet nutrient composition (% DM) of 21% forage NDF, 27% starch, and 17% CP. Total FA content of each treatment was 3.45, 3.42, 5.62, and 5.58% DM, respectively. The statistical model included the random effect of cow within square, the fixed effects of period, square, NaAC, and HOSB, and the interaction of NaAC and HOSB. Results are presented in the following order: Trt1, Trt2, Trt3, and Trt4. There was an interaction for DMI as Trt2 increased DMI compared with the other treatments (33.3, 35.3, 33.2, 33.4 kg/d; $P < 0.01$). There were no interactions between NaAC and HOSB for milk production responses. However, NaAC increased the yields (kg/d; all $P < 0.001$) of 3.5% FCM, ECM, and milk fat, but did not affect yields (kg/d) of milk ($P = 0.51$) or milk protein ($P = 0.48$). HOSB increased yields (kg/d; all $P < 0.001$) of milk (47.4, 48.1, 50.0, 50.0), 3.5% FCM (49.8, 52.3, 54.1, 56.2), ECM (50.2, 52.0, 53.9, 56.2), and milk fat (1.81, 1.95, 2.00, 2.16) and tended to increase milk protein (1.55, 1.55, 1.59, 1.57 kg/d; $P = 0.06$). In summary, milk production responses were increased due to NaAC and HOSB, with an additive effect when fed together (Trt4). These additive effects are shown in milk fat yield, as compared with Trt1, there was an increase of 140, 190, and 350 g/d of milk fat for Trt2, Trt3, and Trt4, respectively. This highlights the benefit of increasing both de novo and preformed FA supply to the mammary gland. In conclusion, responses to HOSB are improved when more acetate is available.

Key Words: oleic acid, acetate, milk fat

2167 A meta-analysis examining the effect of supplemental palmitic acid during the immediate postpartum on production responses of multiparous dairy cows. J. M. dos Santos Neto* and A. L. Lock, *Michigan State University, East Lansing, MI.*

Our objective was to perform a meta-analysis to evaluate the effects of supplementing palmitic acid (C16:0) during the immediate postpartum period on multiparous dairy cows. Our data set included 4 studies from MSU with 2 dietary treatments: (1) control diet without fatty acid (FA) supplementation (CON, n = 312) and (2) treatment diet containing a C16:0 supplement (≥70% C16:0, n = 396) with an average inclusion level of 1.41 ± 0.38% (mean ± SD), ranging from 0.87 to 2.00% diet DM. CON treatments contained (mean ± SD) 31.2 ± 2.01 NDF, 17.2 ± 0.53 CP, and 2.44 ± 0.34 FA %DM, and C16:0 treatments contained 29.9 ± 1.66 NDF, 17.0 ± 0.51 CP, and 4.02 ± 0.32 FA %DM. Treatments were fed for the first 3 wk of lactation in a complete block design. Our statistical model included the fixed effects of treatment, wk, and their interactions, and the random effects of study, block within study, and cow within study. We used repeated measures for production variables but estimated body weight (BW) loss using linear regression. We observed an interaction between treatment and wk for DMI ($P = 0.01$); however, there were no differences between treatments within weeks ($P \geq 0.17$). Compared with CON, C16:0 had no effect on DMI ($P = 0.85$), yields of milk ($P = 0.41$) and milk protein ($P = 0.36$), milk protein content ($P = 0.92$), BW ($P = 0.65$), body condition score (BCS; $P = 0.82$), or energy output to maintenance ($P = 0.64$). Compared with CON, C16:0 increased the yields of milk fat (0.17 g/d, $P < 0.01$) and energy-corrected milk (ECM, 2.81 g/d, $P < 0.01$), milk fat content (0.32 percentage-units, $P < 0.01$), feed efficiency (ECM/DMI, 0.11 kg/kg, $P = 0.03$), energy output to milk (1.82 Mcal/d, $P < 0.01$), and tended to increase BW loss (0.41 kg/d) and body energy mobilization (2.3 Mcal/d, $P = 0.07$). In conclusion, our meta-analysis demonstrates that feeding a C16:0 supplement to multiparous dairy cows for the first 3 wks of lactation increases the yields of milk fat and ECM without changing DMI. Although C16:0 tended to increase daily BW loss, it was not enough to affect overall BW or BCS across the first 3 wk of lactation.

Key Words: early lactation, fatty acid, transition period

2168 Meta-analysis examining the effect of dietary inclusion of oils and oilseeds on methane production of lactating dairy cows. M. A. Karpyn Esqueda, J. M. dos Santos Neto*, and A. L. Lock, *Michigan State University, East Lansing, MI.*

Our objective was to determine the impact of dietary inclusion of oils and oilseeds on methane (CH_4) production of lactating dairy cows. The database used 23 peer-reviewed publications containing at least one diet that included oils or oilseeds (OS, n = 50) and a control diet (CON, n = 32). The OS treatments comprised whole cottonseed, rapeseed, linseed, sunflower, or oils from oilseeds. CON treatments contained (% DM; mean ± SD) 17.7 ± 7.40 starch, 36.0 ± 5.14 NDF, 16.7 ± 1.94 CP, and 2.89 ± 0.81 fat, and OS treatments contained 15.8 ± 7.24 starch, 36.1 ± 5.28 NDF, 16.2 ± 1.80 CP, and 5.81 ± 1.47 fat. Within each study CH_4 was determined using the same method; across studies respiratory chambers, tracers, and GreenFeed methods were used. Our statistical analyses used PROC MIXED of SAS with the fixed effect of treatment (CON vs. OS) and the random effect of study. We used SEM as a weighting factor. Compared with CON, OS tended to decrease DMI (0.55 kg/d, $P = 0.10$), increased the yields of milk (2.22 kg/d), milk protein (0.05 kg/d), and milk lactose (0.16 kg/d), had no effect on milk fat yield ($P = 0.17$), and tended to increase energy-corrected milk yield (ECM, $P = 0.06$). Compared with CON, OS increased milk lactose content (0.05 percentage-units, all $P < 0.01$), and decreased milk fat content (0.21 percentage-units) and milk protein content (0.09 percentage-units, both $P < 0.01$). Compared with CON, OS decreased total daily enteric CH_4 emissions (55 g/d) and CH_4 emissions per kg/DMI (1.77 g/kg, both $P < 0.01$), per kg/milk (1.78 g/kg, $P = 0.01$), per kg/ECM (1.19 g/kg), and per Mcal of energy output to milk (2 g/Mcal, both $P < 0.01$). In conclusion, including oils and oilseeds in the diet of dairy cows tended to decrease DMI and increased the yields of milk and milk protein while reducing total daily enteric CH_4 emissions and CH_4 emissions per kilogram of DMI and ECM. The available data are too limited for a comprehensive meta-analysis of the literature comparing different types of oils and oilseeds. Further studies are needed to evaluate the effects of different fatty acid supplements and individual fatty acids on CH_4 emissions.

Key Words: greenhouse gases, environment, fatty acid

2169 Effects of dietary fatty acids concentrations on milk fatty acids yield by dairy cows—A meta-analysis. C. Heinzen Jr.*, J. R. Dorea, and L. F. Ferraretto, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The objectives of this study were to investigate the association of dietary fatty acids concentration (C16:0, C18:0, C18:1, C18:2, C18:3) in corn silage-based diets on milk fatty acids yields. A meta-regression was conducted to model the effects of dietary fatty acids concentrations (% of DM) on milk fatty acids yield (g/d) or dietary fatty acids, forage, and starch concentrations (% of DM) on milk fatty acids yield (g/d). Only studies with cows fed corn silage in the diet, and treatments containing fatty acids supplements were included. Twenty-three studies were part of the final data set (76 treatment means and 1,662 dairy cows). Based on CCC and RMSE evaluation, predictions of milk fatty acids yields were reliable. Dietary concentrations C16:0 had a positive effect on mixed ($P < 0.001$), preformed ($P = 0.04$), and total ($P < 0.001$) milk fatty acids yield. Dietary concentrations of C18:0 had a positive effect on preformed fatty acids yield ($P = 0.03$) and tended to positively affect de novo ($P = 0.07$) and total ($P = 0.06$) milk fatty acids yields. Dietary concentrations of C18:1, C18:2, and C18:3 had negative effects on yield of de novo ($P < 0.01$), mixed ($P < 0.001$), and total ($P < 0.03$) milk fatty acids. When starch and forage concentrations were included in the model, saturated fatty acids did not influence or tended to influence de novo milk fatty acids yield (C16:0, $P = 0.18$; C18:0, $P = 0.06$), but had a positive effect on preformed (C16:0, $P = 0.02$; C18:0, $P = 0.02$) and total (C16:0, $P < 0.001$; C18:0, $P = 0.02$) milk fatty acids yield. Unsaturated fatty acids had a negative effect or tended to have a negative effect on de novo (C18:1, $P = 0.06$; C18:2, $P < 0.001$; C18:3, $P < 0.001$) and mixed (C18:1, $P = 0.03$; C18:2 $P < 0.001$; C18:3, $P < 0.001$) and total (C18:2, $P < 0.001$; C18:3, $P < 0.001$) milk fatty acids yield. Dietary starch had a positive effect on de novo ($P = 0.02$), mixed ($P < 0.001$) and total milk fatty acids yield ($P < 0.001$). These results suggest that energy contributions from saturated fatty acids are crucial on milk fat predictions, while supporting unsaturated fatty acids role on milk fat depression.

Key Words: fatty acids, starch, milk fat depression

2170 Impact of elevated dried distillers grains levels in conjunction with low and high forage fiber contents on performance of dairy cows. V. S. S. Viana[1], J. M. V. Pereira[2], M. I. Marcondes[3], S. C. Valadares Filho[1], E. Detmann[1], E. A. C. Lopes[2], and P. P. Rotta*[1], *[1]Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, [2]University of Vemont, Burlington, VT, [3]Washington State University, Pullman, WA.*

The heightened incorporation of byproducts from the ethanol industry raises a notable concern regarding its potential effects on animal performance, especially when utilizing increased levels of forage fiber in the diet. The primary aim of this study was to evaluate the effects of escalating levels of dried distillers grains (DDG) in diets with varying forage neutral detergent fiber (fNDF) content on performance of dairy cows. Twelve first-lactation Holstein cows (DIM 99 ± 24.5, BW 502 ± 27.9 kg, and MY 30.5 ± 1.36 kg/d) were assigned to replicated 6 × 6 Latin squares using a 3 × 2 factorial arrangement of treatments. Diets were composed of 0, 20, and 40% DDG on a DM basis, combined with either low forage (21% fNDF) or high forage (31% fNDF). Feed intake, MY, and milk composition were assessed from d 15 to d 18 of each period. For all analyses, multiple comparisons between treatments were performed using the *t*-test, and differences were declared when $P < 0.05$. A significant interaction was noted between DDG and fNDF levels concerning fat-corrected milk (FCM). When cows were provided with low fNDF diets, higher FCM values were observed for 0 and 20% DDG inclusions. Conversely, in diets with high fNDF levels, the inclusion of DDG resulted in decreased FCM. Additionally, a notable difference emerged in FCM/DMI efficiency. The highest value was observed in high fNDF diets without DDG, followed by low fNDF diets with 20% DDG. Another crucial interaction was observed for milk protein percentage, with the highest value observed in low fNDF diets without DDG inclusion, and the lowest value in low fNDF diets with 40% DDG inclusion. It is essential to highlight that milk protein levels in this study were consistently low across all diets, hovering around 2.9%. The performance of dairy cows fed with DDG exhibited variations based on the fNDF levels in the diet, underscoring the significance of this interaction in diet formulation.

Key Words: byproducts, fat milk, protein milk

2171 Increasing indigestible neutral detergent fiber dietary levels on ruminal fermentation and productive performance in dairy cows. N. T. Scognamiglio Grigoletto[1], D. J. Cavalli Vieira[1], N. Pereira Martins[1], R. G. Chesini[1], G. Poletti[1], B. de Magalhães Ceron[1], O. P. Sbaralho[1], M. S. R. Serrasqueiro[1], N. Terassi da Silva[1], C. S. Takiya[2,1], and F. P. Rennó*[1], *[1]University of São Paulo, Pirassununga, SP, Brazil, [2]Federal University of Technology–Paraná, Pato Branco, PR, Brazil.*

This study aimed to evaluate the impacts of increasing dietary levels of indigestible NDF (iNDF) on performance, apparent digestibility, and ruminal fermentation in cows. Eight rumen-cannulated Holstein cows (33.7 ± 3.0 kg/d milk yield, 151 ± 61 DIM, and 4 primiparous and 4 multiparous) were blocked by parity, and enrolled in a 4 × 4 Latin Square (LS) design experiment, consisting of 21-d periods with sampling conducted during the last 7 d. Cows within blocks were allocated to treatment sequences with increasing levels of iNDF (9.0, 9.7, 10.4, and 11.1% diet DM). Dietary iNDF was manipulated using corn silage (CS) made from plants harvested at 2 heights from the ground level (40 cm [CS40] and 65 cm [CS65]). Diets were fed as TMR with 48:52 forage-to-concentrate ratio. CS was the sole forage source. The forage proportion of diets with 9.0, 9.7, 10.4, and 11.1% iNDF comprised 100, 66.6, 33.3, and 0% of CS65, respectively, while the remainder consisted of CS40. Milk samples were collected for 3 d during the sampling periods and analyzed for fat, protein, and lactose. Total fecal collection was performed during 3 consecutive d of each period. Ruminal digesta samples were collected on the first day of each sampling period (before feeding and every 2 h until 16 h) and were analyzed for pH and VFA concentrations. Data were analyzed by Mixed procedure of SAS modeling the fixed effects of treatment, square, and their interaction. Cow within square was considered as a random effect. Treatment differences were analyzed by linear and quadratic contrasts. DMI and NDF digestibility were not affected. Intake of CP and OM exhibited a positive quadratic response to increasing levels of iNDF. Intake of NDF linearly increased with greater iNDF concentrations. DM digestibility quadratically decreased with increasing iNDF (67.9, 65.9, 65.7, and 65.8%). ECM yield linearly decreased with increasing iNDF (36.6, 36.9, 36.5, and 34.9 kg/d). Milk composition and feed efficiency were similar among treatments. Ruminal pH and acetate-to-propionate ratio linearly increased with greater concentrations iNDF. Increasing dietary iNDF content above 9% may reduce ECM yield.

Key Words: carbohydrate, fiber, indigestibility

2172 Changes in the fatty acids of abomasal digesta of sheep supplemented with a blend of babassu oil and sunflower oil. M. O.

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Diet composition is the major modulator of the rumen biohydrogenation (RBH) pathways, which determines the fatty acid (FA) profile in ruminants' product. Babassu oil (BAO) is rich in lauric acid which induces stress on rumen bacteria and favors the *trans* FA accumulation of 18:1 *t*10 instead 18:1 *t*11. The use sunflower oil (SO) as precursor of FA for RBH could be used to minimize this stress. Thirty-five sheep (BW 28 ± 3.9 kg) were used in a complete randomized block design to determine the effects of BAO and SO blends on FA profile of abomasal content and RBH estimate. Control diet (CON) consisted of 70% concentrate and 30% Tifton hay. In the remaining 4 oil supplemented (4.5%, DM basis) diets, the SO replaced BAO in 0% (0SO), 33% (33SO), 50% (50SO), and 66% (66SO). After 50 d in feedlot, sheep were slaughtered and a representative sample of abomasal content was collected, for FA determination. The RBH estimates for 18:1 *c*9, 18:2 *n*-6 and 18:3 *n*-3 were obtained using the diminishing abundance of these FA, proportional to the sum of C18-carbon FA, between diet and abomasal digesta. The contrasts (CON vs. 0SO and CON vs. diets containing SO blend) were performed. The effects of SO blend content added in the diets as the replacement for BAO were evaluated using linear and quadratic effects ($P < 0.05$). The experimental diets did not change the sum of saturated (SFA), polyunsaturated (PUFA) and *trans*-monounsaturated (MUFA) in abomasal content, but 0SO reduced ($P < 0.001$) the sum of branched-chain FA (BCFA) compared with CON and increased the sum of *cis*-MUFA ($P = 0.03$) and the proportion of 18:1 *t*10 and 18:1 *t*12. Feeding SO blend exert a quadratic effect ($P = 0.02$) on 18:0 proportion (final product of RBH) and CLA 18:2 *c*9*t*11, with a maximum point observed for 50SO. The sum of *cis*-MUFA had a quadratic effect ($P = 0.03$) with SO blend, but no changes ($P > 0.05$) were found for the sum of BCFA and RBH of 18:1 c9, 18:2 n-6 and 18:3 n-3. The partial replacement of BAO with SO blend was shown to be an efficient strategy for increasing desirable FA in abomasal content, but it did not change the RBH.

Key Words: t10 shifted, rumen biohydrogenation, *Attalea speciosa*

Ruminant Nutrition 1: General

2173 Comparison of zinc sources on increased tissue zinc enrichment using stable isotopes. H. Tucker*, C. Foran, T. Rode, and D. Hancock, *Novus International, Chesterfield, MO.*

With limited knowledge of Zinc (Zn) nutrition in dairy, use of stable isotopes to compare tissue enrichment across various molecular structures is warranted. The objective of this trial was to determine the effect different molecular structures have on absorption and subsequent tissue enrichment of Zn when comparing Zn-nonspecific amino acid complex (Zn-AAC) and Zn-methionine hydroxy analog chelate (Zn-MHAC; Novus International, Inc.). Twenty weaned male Holstein calves (BW = 65.9 ± 1.3 kg) were fed a starter diet formulated to meet nutrient requirements for 32 d. On d 30, calves were orally administered 8 mg of Zn from each of 2 sources: 67Zn-AAC and 70Zn-MHAC at 0 h. Before isotope administration and over a 48-h period following, blood (16 time points), feces, and urine were collected. At 48 h, tissue samples were collected. All samples were assayed for Zn enrichment via ICP-MS. Data were analyzed with MIXED procedure of SAS 9.4, and significance declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$. Although not significant ($P = 0.12$), plasma Zn area under the curve (AUC) was numerically increased (10%) with Zn-MHAC (0.61 ± 0.031 ppm·h) relative to Zn-AAC (0.54 ± 0.031 ppm·h). Zn-MHAC resulted in greater Zn enrichment for rumen (0.75 vs. 0.52 ± 0.07%; $P = 0.03$), abomasum (0.65 vs. 0.44 ± 0.04%; $P < 0.01$), ileum (0.79 vs. 0.56 ± 0.04%; $P < 0.01$), jejunum (0.94 vs. 0.67 ± 0.05%; $P < 0.01$), kidney (2.19 vs. 1.61 ± 0.19%; $P = 0.04$), heart (0.63 vs. 0.50 ± 0.04%; $P = 0.04$), and bone (0.12 vs. −0.36 ± 0.18%; $P = 0.06$). Counter to this, Zn-AAC resulted in greater Zn enrichment for muscle (0.10 vs. 0.05 ± 0.01%; $P < 0.01$) and thymus (0.37 vs. 0.26 ± 0.02% $P < 0.01$). For all other tissues, enrichment did not differ ($0.10 < P < 0.94$) between Zn sources. Fecal Zn AUC was greater ($P = 0.01$) with Zn-MHAC (2.90 ± 0.16 ppm·h) compared with Zn-AAC (2.31 ± 0.16 ppm·h). Urinary Zn AUC did not differ ($P = 0.83$) with Zn source (−0.04 vs. 0.01 ± 0.14 ppm·h, Zn-MHAC vs. Zn-AAC). In conclusion, providing a Zn source with a bis-chelate structure results in greater Zn enrichment of tissues primarily associated with nutrient digestion and absorption.

Key Words: absorption, stable isotope, zinc

2174 Effects of source of trace minerals on production performance in dairy cows. T. Adeoti*[1], Z. Sarwar[1], M. N. Marinho[1], J. Rasia[1], L. R. S. Oliveira[1], B. S. Simões[1], M. C. Perdomo[1], K. Griswold[2], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*Selko USA, Indianapolis, IN.*

Objectives were to determine the effects of replacing sulfate sources of Zn, Cu, and Mn (STM) with hydroxychloride sources (HTM). One hundred forty-one Holstein cows were assigned to a randomized complete block design. Cows were stratified by parity group as nulliparous (lactation 0) or parous cows (lactation > 0) and, within parity, cows were blocked by genomic breeding value for energy-corrected milk (ECM) yield (nulliparous) or recently completed lactation 305-d ECM (parous) and then randomly assigned to STM (30 nulliparous and 40 parous cows) or HTM (31 nulliparous and 40 parous cows). Supplemental Zn, Cu, and Mn were incorporated at, respectively, 30, 7, and 22 mg/kg of diet DM prepartum, and at 44, 8, and 36 mg/kg diet DM postpartum. The pre and postpartum diets contained, respectively, 64 and 77 mg/kg of Zn, 15 and 19 mg of Cu, and 58 and 65 mg of Mn. Treatments started at 245 d of gestation and stopped at 105 d postpartum. Milk yield, body weight (BW), and dry matter intake (DMI) were measured daily, whereas body condition (BCS) and milk samples (AM and PM) were evaluated twice weekly. Data were analyzed with mixed-effects models with repeated measurements within experimental units. Results are depicted in Table 1. Replacing sulfate sources of supplemental Zn, Cu, and Mn with hydroxychloride sources did not affect DMI pre- or postpartum, but tended to increase yields of colostrum, milk, and milk fat, and increased yields of ECM and true protein by 3.3 and 4.3%, respectively, in the first 15 wk of lactation, which resulted in a 3.6% increase in the estimated diet net energy for lactation content.

Key Words: dairy cow, hydroxychloride, trace minerals

Table 1 (Abstr. 2174). Effect of source of trace minerals on performance of dairy cows

Item	STM	HTM[1]	SEM	*P*-value
Prepartum DMI, kg/d	11.7	11.9	0.3	0.38
Postpartum DMI, kg/d	21.9	22.4	0.3	0.21
Colostrum, kg	5.2	6.2	0.6	0.08
Colostrum IgG, g/kg	114	113	6	0.93
Milk, kg/d	41.7	43.0	0.5	0.07
ECM, kg/d	41.8	43.2	0.5	0.04
Fat, kg/d	1.53	1.58	0.03	0.08
Protein, kg/d	1.15	1.20	0.02	0.01
Somatic cell score	2.48	2.56	0.25	0.76
BW change, kg/d	−0.67	−0.65	0.06	0.73
BCS, 1 to 5	3.14	3.16	0.06	0.62
ECM/DMI, kg/kg	1.95	1.99	0.02	0.20
Estimated diet NE_L, Mcal/kg	1.65	1.71	0.02	0.02

[1]IntelliBond® Z, C, M; Selko USA, Indianapolis, Indiana.

2175 Effect of supplying a portion of trace mineral amino acid complexes on serum folate concentrations from dry period to early lactation. M. Duplessis*[1], A. L. Kerwin[2], D. H. Kleinschmit[3], M. T. Socha[3], and T. R. Overton[2], [1]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [2]*Cornell University, Ithaca, NY,* [3]*Zinpro Corporation, Eden Prairie, MN.*

Folates (B9) are required in single-carbon metabolism and DNA methylation. In early lactation, B9 supplementation has been found to increase milk yield, suggesting that B9 requirements increase during this period. However, it is unclear whether additives which increase milk yield, such as trace mineral amino acid complexes (AATM), may further increase B9 demands. Thus, study objectives were to evaluate serum B9 concentrations of cows receiving a portion of AATM. With dietary trace minerals (TM) being the same between treatments, a total of 36 Holstein cows were assigned to either: (1) an inorganic TM blend consisting of Zn (75 mg/kg of DM), Mn (65 mg/kg), and Cu (10 mg/kg) as hydroxichlorides and Co (1 mg/kg) as carbonate (ITM) or (2) partial replacement of inorganic TM with AATM of Zn (40 mg/kg), Mn (20 mg/kg), Cu (3.5 mg/kg), and Co glucoheptonate (1 mg/kg; AATM, Availa-Dairy, Zinpro Corp.) from 1 wk after dry off through 22 wk of lactation. Blood samples were taken at wk −8 (before treatment administration), −1, 1, 2, and 8 relative to calving for B9 analysis. Statistical analyses were performed using Proc MIXED of SAS and repeated measures for serum samples. Folates of the far-off diets without treatment averaged

0.32 mg/kg of DM. There were no differences in dietary B9 between treatments, and averaged 0.28 and 0.35 mg/kg of DM for close-up and lactation diets, respectively ($P > 0.12$). Milk yield increased for AATM compared with ITM within the first 8 wk of lactation (46.8 vs. 44.9 (SE: 0.7) kg/d; $P = 0.05$). Before treatment administration, serum B9 averaged 10.3 (SE:0.8) ng/mL ($P = 0.86$). Serum B9 were not different between treatments throughout the sampling period ($P = 0.42$) but vary according to the lactation stage ($P < 0.01$). It reached a nadir from wk −1 to 2 relative to calving (7.3 [SE: 0.5] ng/mL) and the highest concentration was obtained at wk 8 of lactation (10.7 [SE: 0.8] ng/mL). Results suggested that, even with the milk yield increase, B9 metabolism was not affected when a portion of inorganic TM supplement was replaced by AATM.

Key Words: vitamin B9, cattle, trace element

2176 Preference for and acceptability of a novel, fat-microencapsulated anionic salt by dry dairy cows.

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The objective of this study was to determine if dry dairy cows prefer or not to consume a dry cow TMR that contains a novel, fat-microencapsulated anionic salt and their level of acceptability of a diet with that product. Twenty-four multiparous, pregnant, Holstein dairy cows were enrolled at dry-off (56 d to calving), in cohorts of 6, and housed in a tie-stall facility. After adapting to a base dry cow diet (35% corn silage, 24% straw, 17% haylage, 24% dry cow supplement, DM basis) for 14 d, cows were exposed to each of 2 treatment diets in a crossover design (with 7-d periods): (1) base dry cow diet with added fat-microencapsulated anionic salt (ANIC; Jefo, St. Hyacinthe, Quebec) at 0.30 kg/d DM (formulated dietary DCAD = −120 mEq/kg DM), or (2) base dry cow diet with fat placebo (PLCB) at 0.15 kg/d DM (formulated dietary DCAD = +45 mEq/kg DM). Following the crossover design, cows were offered both diets simultaneously in a 3-d preference test; feed was offered in a split bunk, in large enough quantities to ensure ad libitum daily access to both diets. The side of the bunk in which each diet was provided was constant within cows, and randomized across cows. DMI was calculated for each day, and urine pH was measured 2×/wk for every cow throughout the study. Data (DMI from the crossover study and preference ratio from the preference test) were analyzed using mixed-effect, repeated measures linear regression models. During the baseline period cows consumed 19.9 ± 0.59 kg/d DM and had a urine pH of 8.9 ± 0.04. When cows consumed only the ANIC diet, they had lower urine pH values than when on the PLCB diet (7.6 vs. 8.2; SE = 0.10; $P < 0.01$). Accounting for baseline DMI ($P < 0.001$), no difference in DMI was detected between the ANIC and PLCB diets (19.3 vs. 19.5 kg/d DM; SE = 0.33; $P = 0.6$). During the preference phase, cows chose to eat a similar proportion of their total daily DMI for the ANIC and PLCB diets (48.6 vs. 51.4% of total DMI; SE = 1.73; $P = 0.26$). The results demonstrate that dry cows showed no preference between a TMR containing a fat-microencapsulated anionic salt or a fat placebo, and demonstrated similar acceptability of both diets.

Key Words: preference test, anionic salt, behavior

2177 Effect of feeding zeolite versus a negative DCAD diet during the close-up period to multiparous Jersey cows on blood minerals and colostrum.

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The objective was to evaluate the effect of feeding zeolite (ZLT) vs. negative DCAD diet during the close-up period on blood mineral levels and colostrum production. Parous Jersey cows (n = 1,506) were randomized to one of 2 close-up pens (CUP) fed the respective diet (ZLT or DCAD). Dietary DCAD was 31.6 and −10.1 for XZT and DCAD. Colostrum was collected within 24 h of calving (ZLT n = 475, DCAD n = 461 cows). The diet offered to each CUP switched every 4 wk (6 periods). In a subset sample, blood was collected at 0, 24, and 96 h after calving (ZLT n = 63, DCAD n = 70). Outcomes of interest were blood Ca, P, Mg, Cl, and TCO$_2$, and colostrum weight (CW) and quality (CQ). Mixed linear models included the fixed effects of treatment, days in close-up (DCUP), their interaction, and parity (2 vs. 3+). Repeated measures were considered for the blood minerals. A random effect of diet within period accounted for the pen effect (degrees of freedom < 12). Blood Cl and TCO$_2$ were different ($P < 0.01$) between groups at 0 h (Cl: ZLT 100.9 [95% CI: 99.5–102.0]; DCAD 103.2 [101.8–105] mmol/L; TCO$_2$: ZLT 25.6 [25.0–26.3]; DCAD 23.2 [22.6–23.8] mmol/L). Blood P and Mg were reduced ($P < 0.01$) at 0 h (−0.72 and −0.18 mmol/L, respectively) and 24 h (−0.46 and −0.16 mmol/L, respectively) in the ZLT compared with the DCAD group. Blood Ca was greater ($P < 0.01$) in the ZLT group than in the DCAD at 0 h (ZLT: 2.05 [2.00–2.11]; DCAD: 1.84 [1.78–1.89]) and 24 h (ZLT: 1.96 [1.90–2.02]; DCAD: 1.78 [1.73–1.84] mmol/L); no differences observed at 96 h. An interaction of treatment by DCUP was detected for CW and CQ. In the ZLT group, cows with DCUP >28 d had greater CW than cows with ≤28 d (4.39 [3.70, 5.21] vs. 3.35 [2.91–3.90] kg; $P < 0.01$). Overall, CW in the ZLT group was lower than the DCAD (3.74 [3.22–4.30]; 4.30 [3.74–4.95] kg; $P < 0.01$). In cows with ≤28 DCUP, CQ was greater in the ZLT (23.6 [22.3–24.9]) than in the DCAD (22.3 [21.0–23.6] Brix; $P = 0.05$). Overall, ZLT increased blood Ca levels immediately postpartum compared with DCAD but decreased levels of P and Mg. Compared with DCAD, ZLT yielded colostrum in lower quantity with greater quality.

Key Words: mineral binder, acidogenic diets, hypocalcemia

2178 Classifying Holstein dairy cows by body condition score and its associations with milk production, dry matter intake, and inflammation during the transition period.

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Monitoring body condition score (BCS) to classify cows into high (H), intermediate (I), or low (L) over the course of lactation may be used to predict decreases in dry matter intake (DMI) and milk yield (MY) in early postpartum. The objective of this study was to determine if there is an association of BCS with DMI, MY, milk solids and circulating inflammatory and energy metabolism markers. Fifty-two multiparous and 31 primiparous Holsteins were BCS (1 to 5 scale) weekly by 3 individuals from −4 to +10 wk relative to calving and classified as H (>3.5), I (2.75 to 3.25), and L (<2.75). Classification at −4 to +1 wk (H = 54.8, I = 45.1%), +1 to +10 wk (H = 2.6, I = 85.4, and L = 11.9%). Both DMI and MY were recorded daily. Milk samples were collected weekly from +1 to +10 wk relative to calving. Blood samples were taken from −4 to +10 wk relative to calving for the assessment of biomarkers of energy metabolism and inflammation. Data were analyzed using proc MIXED procedure of SAS (v.9.4). Prepartum DMI did not differ between H, I, and L. Postpartum DMI was lower in H, intermediate in I and highest in L (15.2, 19.9, and 21.3 kg/d; $P < 0.01$). Milk yield was significantly lower in H, intermediate in I, and highest in L from +1 to +10 wk of lactation (31.1, 36.7, and 39.9 kg/d; $P < 0.01$), but fat-corrected milk (FCM) did not differ between categories. Milk fat content was greater in H compared with L (4.73 vs. 4.11%; $P = 0.05$). Protein content was greater in H, and I compared with L (3.41, and 3.19, vs. 2.98%; $P = 0.04$). Yields of milk fat and protein were similar among BCS categories. Concentrations of NEFA was lowest in L compared with I and H groups (0.28, 0.33, and 0.35 mmol/L; $P < 0.01$). Haptoglobin concentration was lowest in I compared with H and L (0.29, 0.46, 0.41 mmol/L; $P < 0.01$). Similar BHBA concentration was lowest in I compared with H and L (526.6, 571.8, and 579.3 mmol/L; $P < 0.01$). Results from our study show that an association between BCS categories and DMI, milk production, milk solids, and biomarkers of energy metabolism and inflammation in Holstein cows.

Key Words: body condition, transition cows, production

2179 Effect of glycerol supplementation in early lactation on metabolic health, milking activity, and production of dairy cows in automated milking system herds. C. J. McWilliams[1], T. F. Duffield[2], M. N. Pereira[3], K. M. Woods[1], and T. J. DeVries*[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada, [2]Department of Population Medicine, University of Guelph, Guelph, Ontario, Canada, [3]Department of Animal Science, University of Lavras, Lavras, MG, Brazil.*

The objective of this study was to quantify the effects of supplementing early-lactation cows with a dry pure glycerol product, delivered through the automated milking system (AMS) concentrate, in the first 21 DIM on metabolic markers, milking behavior, and milk production. In 5 AMS dairy herds, 389 dairy cows were randomly assigned to 1 of 2 treatments, within farm: (1) control group (CON) receiving the standard AMS pellet (n = 213) from 1 to 150 DIM, or (2) glycerol group (GLY) receiving the treatment AMS pellet (n = 176) formulated to deliver 250 as fed g/d of glycerol product from 1 to 21 DIM (actual = 249 g/d DM) and then receiving the standard AMS pellet from 22 to 150 DIM. Blood samples were collected postpartum from each cow to determine BHB. Cow BCS was recorded every 21 d from −21 to 63 DIM. Data were analyzed for the treatment period (1 to 21 DIM) and a follow-up period (22 to 150 DIM), separately, using repeated measures, mixed-effect regression models. There was no treatment ($P = 0.8$) effect on serum NEFA concentrations in the first week of lactation. There was an interaction of BCS with treatment on the incidence of BHB ≥1.2 mmol/L, whereby overconditioned CON cows (BCS ≥3.5) were 3.5× more likely ($P = 0.002$) to have a high BHB test than CON cows with normal prepartum BCS. During the 21-d treatment period GLY cows had 0.1 ± 0.05 more successful milkings per day ($P = 0.03$), were delivered 0.27 ± 0.05 DM kg/d more AMS concentrate ($P < 0.01$), and tended to yield 0.8 ± 0.47 kg/d more milk ($P = 0.09$). During the follow-up period GLY cows had 0.1 ± 0.04 more successful milkings per day ($P = 0.047$), were delivered 0.18 ± 0.06 DM kg/d more AMS concentrate ($P = 0.004$), and yielded 1.5 ± 0.53 kg/d more milk than CON cows ($P < 0.01$). Glycerol supplementation allowed cows to maintain better BCS, as GLY cows had higher BCS at 63 DIM than CON cows (BCS = 2.9 vs. 2.8, SE = 0.04; $P = 0.05$). These results demonstrate that supplementing pure glycerol through the AMS concentrate for the first 21 DIM can improve BCS in early lactation, improve milking behavior, and increase milk yield to mid lactation.

Key Words: glycerol, transition cow, robotic milking

2180 The use of *Bacillus licheniformis* and *Bacillus subtilis* in the feeding of dairy cows during the peripartum period and their effects on dry matter intake and productive parameters. B. E. Velasquez[1], M. S. Nicola[1], U. S. Londero[1], B. I. Cappellozza[2], M. N. Correa[1], and O. Queiroz*[2], *[1]Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil, [2]Chr. Hansen A/S, Hoersholm, Hovedstaden, Denmark.*

The present study aimed to evaluate the supplementation of *Bacillus licheniformis* and *B. subtilis* (Bovacillus, Chr. Hansen, Denmark) on the feeding behavior and productive parameters of dairy cows during the peripartum period. Sixty multiparous Holstein dairy cows were divided into 2 groups: control group (CG, without additive, n = 30) and *Bacillus* group (BG, 3 g of Bovacillus/animal per day in TMR, equivalent to 9.6×10^9 cfu of *B. licheniformis* and *B. subtilis*, n = 30). Cows were supplemented and monitored from 30 d prepartum to 60 d postpartum, matched by previous lactation production, number of lactations, and initial weight. Postpartum feeding comprised 44% concentrate and 56% roughage, provided twice daily with free access to water. After calving, the animals were milked 3 times a day, and milk samples were collected weekly for the evaluation of fat and SCC. Milk production was recorded daily by the electronic milking system (Alpro, DeLaval, USA), corrected to 3.5% fat (FCM), and individual DMI assessed daily by smart feeders (Intergado, Brazil). Feed efficiency (FCM/DMI) was calculated. The results were analyzed using JMP Pro 14 software (SAS Institute Inc., 2018). The DMI, FCM, SCC (transformed to $\log_{10}$) and feed efficiency were treated as repeated measures using the PROC MIXED procedure. Statistical significance was declared at $P < 0.05$. Results showed higher FCM in BG (39.9 ± 0.3 kg/d) compared with CG (39.0 ± 0.3 kg/d), $P = 0.03$. The DMI was also higher in BG (20.6 ± 0.2 kg/d) compared with CG (20.1 ± 0.2 kg/d), $P = 0.02$, whereas feed efficiency did not differ between groups (BG = 1.95 ± 0.05; CG = 1.94 ± 0.06; $P = 0.90$). Regarding SCC, BG presented lower values compared with CG, $528.98 \times 10^3 \pm 87.9 \times 10^3$ cells/mL versus $819.1 \times 10^3 \pm 85.7 \times 10^3$ cells/mL, respectively, $P = 0.04$. In conclusion, supplementation with *B. licheniformis* and *B. subtilis* during the peripartum period in dairy cows reduced SCC, increased milk production and DMI, and did not affect postpartum feed efficiency.

Key Words: milk quality, probiotic, production

2181 Urinary volume of nonlactating cows: Observations versus estimates. J. Bermeo*[1], N. Silva-del-Rio[1], and L. C. Solórzano[2], *[1]UC Davis Veterinary Medicine Teaching and Research Center, Tulare, CA, [2]GLC Minerals, Green Bay, WI.*

In lactating dairy cows, creatinine and BW have been used as predictors of urine volume based on the following equation: Urine volume = [29 × BW (kg)]/[urinary creatinine (mg/L)]. Our objective was to evaluate whether this equation could be reliably used to estimate urine volume output in nonpregnant, nonlactating Holstein cows, and to identify factors that could influence that association. Data from a prior study evaluating the relative availability of different Mg sources were used. Briefly, the study was a duplicated 6 × 6 Latin square with cows (n = 12) assigned to the square based on low (square 1) or high (square 2) BW with 6 periods. Cows were of second (n = 8) or ≥third (n = 4) parity, and BW ranged from 590 to 831 kg. Data of total urine collection and creatinine concentration from 24 h before treatment administration was used in this study. Two linear mixed-effects models were built to estimate slope bias (with estimated urinary volume as outcome) and mean bias (with difference between estimated and observed urine volume as outcomes). Both models included observed urinary volume, experimental period, square, and the interactions between volume and period and volume and square as fixed effects, with cow as random effect. There was evidence of an overall slope bias ($P < 0.001$) with an estimated slope of 0.86 ± 0.06 when comparing estimated and observed urinary volumes. Furthermore, bias was more pronounced in square 1 than in square 2 (slope 0.75 ± 0.11 vs. 1.03 ± 0.10). Period II showed a substantial bias (slope 0.45 ± 0.10) compared with the range of slopes observed in other periods (0.85 ± 11 to 1.14 ± 0.16). Overall, there was a significant mean bias ($P < 0.001$) with the estimated urinary volume 0.97 ± 0.26 kg greater than observed. This mean bias was particularly evident ($P < 0.05$) for square 1 (1.14 ± 0.42 kg) compared with square 2 (0.81 ± 0.41 kg), and during periods II and VI (1.84 ± 0.53 and 1.62 ± 0.50 kg, respectively) compared with all other periods (−0.60 ± 0.50 to 1.25 ± 0.52 kg). Our results suggest further research is needed to refine the predictive equation using creatinine and BW for nonpregnant, nonlactating Holstein dairy cows.

Key Words: urinary volume, creatinine, body weight

2182 Relationship between iodine intake and milk iodine concentration and yield in dairy cows. M. M. H. Khandakar, E. A. Cruz, and A. F. Brito*, *Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH.*

Milk is one major source of iodine (I) for humans, and the concentration of I in the diet is known to affect the I levels in milk. We aimed to investigate the relationships between I intake and milk I concentration (MIC) or milk I yield (MIY) by summarizing data from the literature using a systematic review approach. Data were sourced from Web of Science, PubMed, and CAB Direct, with selection of peer-reviewed articles based on the report of the following information: individual DMI, dietary I concentration and intake, MIC, and MIY. A data set using 13 out of 944 studies published between 1981 and 2023 was developed (n = 49 treatment observations). Pearson correlation and regression analyses were conducted in R to test the relationships between dietary I concentration or dietary I intake and MIC, MIY, or I recovery rate in milk (MIY/I intake × 100). Reported MIC ranged from 92.8 to 2,471 µg/L (mean ± SD = 536.3 ± 489 µg/L) and calculated I recovery rate from 6.03 to 92.8 (mean ± SD = 30.7 ± 23.7). Positive correlations ($P < 0.001$) were observed between dietary I concentration and MIC (r = 0.77), and between dietary I intake and MIC (r = 0.75) or MIY (r = 0.66). Furthermore, dietary I concentration and dietary I intake were negatively correlated (r = −0.33; $P < 0.05$) with the I recovery rate in milk. Regression analyses showed that MIC and MIY increased linearly ($P < 0.001$) in response to increases in dietary I concentration (r^2 = 0.59 and 0.42, respectively) and dietary I intake (r^2 = 0.56 and 0.44, respectively). The I recovery rate in milk decreased linearly ($P < 0.05$) with increasing dietary I intake, albeit with lower explanatory power (r^2 = 0.11). In brief, our results showed positive relationships between dietary I concentration and I intake on MIC or MIY. However, dietary factors such as the presence of goitrogenic compounds in certain feeds possibly explain the poor relationship between I intake and its transfer to milk, indicating that goitrogens intake should be accounted for when developing tools for predicting MIC and MIY.

Key Words: dairy cow, iodine intake, milk iodine

2183 The effects of feeding a cinnamaldehyde and garlic oil blend with or without monensin on feed efficiency of lactating dairy cows. S. L. Rigert*[1], P. Hartoonian[1], H. A. Tucker[2], and J. A. D. R. N. Appuhamy[1], *[1]Department of Animal Science, Iowa State University, Ames, IA, [2]Novus International Inc., Chesterfield, MO.*

Cinnamaldehyde and garlic oil modified the rumen function in vitro similar to the modifications associated with monensin-induced feed efficiency improvements. The study objective was to investigate the effects of a novel cinnamaldehyde and garlic oil blend (NECGO; Novus International Inc.) fed alone or with monensin (Rumensin; Elanco Animal Health) on milk production and feed efficiency of dairy cows. Sixty Holstein cows (40 multiparous + 20 primiparous) at 81 ± 29 DIM were balanced for parity and DIM and assigned to treatments in a 2 × 2 factorial arrangement (NECGO: 0 and 710 mg/cow per day and Rumensin: 0 and 406 mg/cow per day). After a 10-d baseline measurement period, cows were allowed 21 d to adapt to their respective dietary treatments, which were top-dressed daily. The DMI, milk yield, milk composition, BW, and BCS were collected over the next 49 d during which cows continued to receive their respective treatments. Treatment effects were analyzed by using the MIXED procedure of SAS with the REPEATED option. When fed alone, NECGO tended to decrease DMI by 1.7 kg/d ($P = 0.06$) without affecting milk yield and improved gross feed efficiency (milk yield:DMI) by 8% ($P < 0.01$). NECGO increased milk fat concentration (4.7% to 5.0%, $P = 0.02$) regardless of Rumensin in the diet. Rumensin increased milk yield (1.3 kg/d, $P < 0.01$), milk fat yield (0.1 kg/d, $P = 0.03$), energy-corrected (ECM; 2.4 kg/d, $P = 0.03$) and fat-corrected milk yields (FCM; 2.7 kg/d, $P = 0.02$), independent of NECGO, which did not affect those variables ($P > 0.30$). When fed alone, NECGO increased milk protein efficiency (milk protein yield:CP intake, $P = 0.02$) and tended to increase the feed efficiency related to ECM (ECM:DMI, $P = 0.06$) and FCM (FCM:DMI, $P = 0.09$) by 10% to 11%. Those improvements disappeared when NECGO was fed with Rumensin. NECGO did not affect BW, daily gain, or BCS individually ($P > 0.50$) or in interactions with Rumensin ($P > 0.25$). NECGO has the potential to increase the feed efficiency of cows when fed in a diet devoid of monensin.

Key Words: cinnamaldehyde, feed efficiency, monensin

Ruminant Nutrition 1: Protein and Amino Acids

2184 Effects of a plant extract-based rumen modifier and rumen-degraded protein levels on production performance and nutrient digestibility in lactating dairy cows. K. V. Almeida*[1], D. C. Reyes[1], A. L. Konopka[1], M. H. Khandakar[1], R. A. Menezes[1], M. A. Rahman[1], P. Piantoni[2], G. F. Schroeder[2], K. Cowles[2], and A. F. Brito[1], *[1]Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH, [2]Cargill Animal Nutrition and Health, Innovation Campus, Elk River, MN.*

The objective of this study was to investigate the effect of adding a rumen modifier (RM) containing condensed tannins and spice extracts in diets with 2 levels of RDP on production performance and apparent total-tract digestibility of nutrients in dairy cows. Sixteen multiparous Holstein cows (81 ± 33 DIM and 750 ± 79 kg of BW) were used in a replicated 4 × 4 Latin square design experiment with a 2 × 2 factorial arrangement of treatments and 28-d periods. Data and sample collection were done during the last 7 d of each period. Treatments were 2 levels of RDP (9.0% or 10.6% DM basis) and 2 levels of RM (0 or 15 g/d; Valido PRO®, Cargill Inc.): (1) low RDP without RM (LRDP), (2) LRDP plus 15 g of RM top-dressed, (3) high RDP without RM (HRDP), and (4) HRDP plus 15 g of RM top-dressed. All diets contained (DM basis) 46.6% of corn silage, 11.4% of alfalfa-grass hay, and 42% of a concentrate pellet and were formulated to provide the targeted levels of RDP and similar levels of metabolizable AA. The main effects of dietary RDP level and RM, and their interactions, were tested using ANOVA with the MIXED procedure of SAS. Feeding RM increased ($P \leq 0.03$) DMI (28.9 vs. 28.1 ± 0.79 kg/d) and yields of milk fat (1.57 vs. 1.52 ± 0.06 kg/d) and ECM (46.4 vs. 45.2 ± 1.28 kg/d) but did not affect milk yield, milk protein yield, MUN, or feed efficiency. Decreasing dietary RDP did not affect DMI or yields of ECM, milk fat, and protein but decreased ($P < 0.01$) MUN (10.9 vs. 14.7 ± 0.61 mg/dL). In addition, LRDP diets increased ($P < 0.01$) milk N efficiency (30.2 vs. 27.9 ± 0.94%) compared with HRDP diets, regardless of RM supplementation. Diets did not affect DM, OM, and aNDFom digestibility, but HRDP increased ($P < 0.01$) CP digestibility (72.5 vs. 70.5 ± 0.66%) versus LRDP, and feeding RM tended ($P = 0.08$) to decrease it (70.9 vs. 72.0 ± 0.66%). Overall, feeding a plant-extract-based RM increased DMI and ECM yield but did not affect OM or aNDFom digestibility in dairy cows. Feeding lower RDP diets while maintaining AA supply did not negatively affect production and increased N efficiency.

Key Words: rumen degradable protein, rumen modifier, milk performance

2185 Effects of prepartum supplementation of rumen-protected methionine on productive performance in transition dairy cows. N. P. G. Viana[1], L. C. Obialeski[1], M. Bugoni[1], C. G. Cordeiro[2], T. L. Resende[3], and R. Almeida*[1], *[1]Universidade Federal do Paraná, Curitiba, PR, Brazil, [2]Agropecuária Regia, Palmeira, PR, Brazil, [3]Evonik Brasil, São Paulo, SP, Brazil.*

The aim of this study was to evaluate the effects of prepartum supplementation of rumen protected methionine (RPM) on milk yield (MY) and composition, BW and BCS, and blood BHB and glucose concentrations in early-lactation dairy cows. A total of 214 prepartum Holsteins from a commercial herd were blocked based on parity (84 heifers, 76 primiparous and 54 multiparous), MY in the previous lactation (for parous only), and expected calving date. In a randomized block design, the treatment consisted of daily supplementation of 15 g of RPM (Mepron®, Evonik Industries, Germany) mixed with 85 g of corn meal provided top-dressed from 26 ± 4 d before calving, whereas control (CT) animals received 100 g of corn meal. Both groups received 15 g of RPM postpartum until 19 ± 3 DIM. Data were analyzed through MIXED procedure of SAS containing the fixed effects of treatment, time, parity, and their interactions, and the random effects of block and cow within treatment. BW and BCS were not affected ($P > 0.05$) by prepartum RPM supplementation. Likewise, prepartum RPM did not affect ($P > 0.05$) calf birth weight, colostrum IgG concentration, and calf serum Brix. RPM-treated and CT cows also showed no differences ($P > 0.05$) for BHB at d 3, 7, and 14, and glucose at d 7. A difference for MY was found from 1 to 30 DIM, with an interaction ($P < 0.01$) between treatment and parity; no differences were detected for heifers (31.18 vs. 33.15 ± 0.56 kg/d; $P = 0.13$) or multiparous cows (45.67 vs. 43.58 ± 1.04 kg/d; $P = 0.48$), but a difference was found for primiparous cows (39.56 vs. 46.34 ± 0.54 kg/d; $P < 0.01$), respectively, for RPM and CT. Concerning milk composition, prepartum RPM animals showed greater protein content (3.84 vs. 3.71 ± 0.02%; $P < 0.01$), greater casein content (3.07 vs. 2.96 ± 0.02%; $P < 0.01$), and lower milk urea nitrogen (18.29 vs. 19.06 ± 0.23 mg/dL; $P = 0.02$). No differences ($P > 0.05$) were detected for the remaining milk components. It was concluded that RPM supplementation during the prepartum period increased milk protein and casein contents but, unexpectedly, decreased milk yield for the younger animals.

Key Words: amino acid, milk protein, transition period

2186 Milk fat synthesis from dairy cows receiving a jugular infusion of isoleucine or leucine. T. Fernandes*[1], Y. C. T. Taguti[1,2], A. Hruby-Weston[1], D. Luchini[3], M. D. Hanigan[1], and I. A. M. A. Teixeira[2,4], *[1]School of Animal Sciences, Virginia Tech, Blacksburg, VA, [2]Departament of Animal Science, UNESP, Jaboticabal, São Paulo, Brazil, [3]Adisseo North America, Naperville, IL, [4]Department of Animal, Veterinary, and Food Sciences, University of Idaho, Twin Falls, ID.*

Previous studies identified Leu and Ile as positive drivers of milk protein production through mTOR stimulation, and some AA that stimulate mTOR may also affect milk fat synthesis. Therefore, we aimed to assess milk fat synthesis responses in cows supplemented with isoleucine or leucine. Six Holstein cows (117 ± 29 DIM and 41 ± 5.3 kg/d of milk) were randomly assigned to treatment sequences within orthogonally replicated 4 × 3 Youden squares (6 repetitions per treatment). Treatments were jugular infused: CON = 2.5 L/d 0.9% saline; Ile = 28.6 g of Ile/d; Leu = 41.0 g of Leu/d; and IL = 28.6 g of Ile/d and 41.0 g of Leu/d. Cows were fed a base diet (1.77 Mcal of NE_L/kg and 9.55% of MP). On 10 d of each period, 2-$[^{13}C]$-acetate was infused for 24 h as a tracer for milk fat synthesis. Plasma samples were collected every 3 h, and milk samples were collected every 12 h during the acetate infusion. Data were analyzed using R software, with AA, hour, and their interaction as fixed effects and period, and cow nested in square as random effects. No changes were observed for DMI (18.3 kg/d) or milk production (34.6 kg/d). Plasma Ile increased ($P < 0.01$) by 1.8 μM per g/d of Ile infused per day. Plasma Leu ($P = 0.04$) and Asp ($P = 0.03$) increased by 1.1 and 0.1 μM per g/d of Leu infused per day. Plasma Ile ($P = 0.01$), Lys ($P = 0.04$), and Met ($P = 0.04$) declined by 0.9, 0.3, and 0.1 μM per g/d of Leu infused per day, respectively. Leu also decreased Gly (1.0 μM, $P = 0.03$) and tended to decrease plasma NEAA (1.6 μM, $P = 0.06$). No interaction between Ile and Leu was observed for plasma AA. To assess milk fat synthesis, we evaluated the percentage of $[^{13}C]$ infused that was recovered in milk fatty acids. We observed a trend ($P = 0.09$) for increased [^{13}C] recovery in de novo fatty acids of milk. The range was 10.8% for CON to 23.9% for Ile and 22.3% for Leu, and there was no difference for IL (12.5%) at 24 h of infusion. Also, [^{13}C] recovery in mixed fatty acids (C16) in milk at 12 h increased ($P = 0.04$) from 10.9% to 13.7% for the Ile treatment. This indicated that increased blood Ile or Leu concentrations individually stimulated milk fat synthesis, but there was no combined effect of Ile and Leu on milk fat synthesis.

Key Words: milk fat, EAA, de novo FA

2187 Evaluation of rumen undegradable protein and intestinal digestibility on a new commercial rumen-protected methionine product. O. R. Drehmel*, A. E. Hirsh, B. N. Rowbotham, J. J. Albrecht, and M. F. Scott, *Milk Specialties Global, Eden Prairie, MN.*

Methionine is one of the essential limiting AA in cattle, and rumen protection and availability for absorption in the small intestine are critical for the cow. There are many different rumen-protected methionine products in the market, including a newly released product called RPMet (Milk Specialties Global, Eden Prairie, MN). Our objective was to evaluate RPMet for rumen undegradable protein (RUP) using the in situ rumen bypass method, and intestinal digestibility using the Ross Assay. Over a 10-mo period, March to December 2023, 12 different manufactured lots of RPMet (RPM) were compared with an uncoated methionine pellet (CON) and Smartamine® M (Adisseo Inc., France) (SMM). The minimum methionine contents for CON, RPM, and SMM were 87%, 80%, and 75%, respectively. The in situ rumen bypass method was conducted using 2 post-peak multiparous Holstein cows fitted with rumen cannulas. Duplicate treatment samples were placed in Dacron bags in each cow for 16 h, removed, and prepped for further analysis. Rumen fluid was used from the same 2 cannulated cows for the Ross assay as well. Samples were tested in triplicate using the complete Ross assay that includes rumen fermentation and intestinal digestion steps. Samples were analyzed for nitrogen content using a Kjeltec 8420. Statistical analysis was completed in R, and significance was declared at $P < 0.05$. Compared with the CON (0.4 ± 0.59% RUP), RPM significantly increased ($P < 0.01$) RUP (93.6 ± 0.35%) and was similar ($P = 0.34$) to SMM (94.5 ± 0.59%). Intestinal digestibilities were similar ($P = 0.86$) between RPM and SMM, whereas CON was higher (94.7 ± 0.29%, 94.4 ± 0.51%, and 98.4 ± 0.51%, respectively). The calculated metabolizable methionine (RUP % × intestinal digestibility % × methionine level %) for RPM was 70.6% and 66.9% for SMM. From these data, it can be concluded that RPMet provided at least 90% protection from rumen degradation and at least 90% readily available methionine for absorption in the small intestine.

Key Words: methionine, rumen undegradable protein, intestinal digestibility

2188 A meta-analysis to identify factors impacting milk component responses to metabolizable methionine fortification with Smartamine M or MetaSmart evaluated based on CNCPS. L. Bahloul[2] and N. L. Whitehouse*[1], [1]*University of New Hampshire, Durham, NH,* [2]*Adisseo, Commentry, France.*

The objective was to establish response equations on milk protein and fat contents and yields (MPC, MFC, MPY, and MFY) to rumen protected methionine (RPM) supplementation and to determine factors affecting the responses. Meta-analysis was applied on peer-reviewed data (82 treatments, 26 publications). Lactating dairy cows consumed 22.5 ± 3.99 kg of DM, 161.5 ± 18.6 g of CP/kg of DM, 2.59 ± 0.09 Mcal of ME/kg of DM, and 1.04 ± 0.17 g of mMet/Mcal of ME. Robust within-study models were obtained to predict MPC ($R^2_{adj} = 0.89$, root mean squared error [RMSE] = 0.05%), MPY ($R^2_{adj} = 0.94$, RMSE = 45.0 g/d), MFC ($R^2_{adj} = 0.88$, RMSE = 0.15%), and MFY ($R^2_{adj} = 0.97$, RMSE = 58.0 g/d) to RPM based on metabolizable methionine as g/Mcal of metabolizable energy (mMet, g/Mcal of ME). The MPC and MPY models suggested an increase of 0.29 g/Mcal of ME mMet increased 0.14% and 51 g/d, respectively. Within-study slopes were positively affected by ether extract, mHis (%MP), and mLys/mMet ratio ($P < 0.05$) for both MPC and MPY and by ME, mLys (%MP), and mVal (%MP) for MPY ($P < 0.05$). For a same g/Mcal of ME mMet content, MPC decreased with DMI, starch, ME, and mLeu (g/Mcal of ME) and increased ($P < 0.05$) with MP, RUP, mArg, mLys, mIleu, and mThr (as %MP), whereas MFY increased in early lactation, DMI, ME, MP, ether extract ($P < 0.05$), mHis (g/Mcal of ME), mLys/mMet ratio ($P < 0.1$) and decreased with mIle (g/Mcal of ME, $P < 0.1$). MFC and MFY indicated an increase of 0.16% and 66 g/d, respectively, by increasing 0.29 g/Mcal of ME mMet. Within-study slopes were positively affected by ME for MFC ($P < 0.1$) and by lactation stage and mLys/mMet ratio for MFY ($P < 0.01$). For a same g/Mcal of ME mMet content, MFC increased in early lactation ($P < 0.05$) and decreased by ME, RUP, mLy (%MP), mHis, mIle, mLeu, mPhe, mVal (as g/Mcal of ME, $P < 0.05$), whereas MFY increased in early lactation and by ME ($P < 0.05$) and decreased by mIle (g/Mcal of ME, $P < 0.01$). In summary, it is possible to predict the responses of lactating dairy cows as a function of RPM and determine factors that could affect these responses. These results provide deeper insight into biological processes and may help improve dietary formulation.

Key Words: meta-analysis, methionine, amino acids

2189 Rumen-protected amino acids for fresh cows. M. M. Efil*[1], F. C. Cardoso[1], L. Bahloul[2], and J. K. Drackley[1], [1]*University of Illinois Urbana-Champaign, Urbana, IL,* [2]*Adisseo France S.A.S., Malicorne, France.*

The goal of this experiment was to establish the effect of supplementation of the rumen protected AA (RPAA) Met and Lys (Adisseo Inc., France) to the diet of fresh cows on lactation performance. Primiparous (n = 40) and multiparous (n = 48) Holstein cows were blocked by calving date, lactation number, previous lactation data (multiparous cows), and BW (primiparous cows) and within blocks were assigned randomly to 1 of 4 treatments beginning at calving. During the far-off and close-up dry periods, all cows received the same diets. Treatments were as follows: 1) control (CON), basal diet AA supply without RPAA, 45 g/d of Met and 137 g/d of Lys (3.06 Lys:Met), 2) MET, supplemental Met, 53 g/d Met and 137 g/d Lys (2.61 Lys:Met), 3) ML, supplemental Met and Lys, 53 g/d Met and 162 g/d Lys (3.04 Lys:Met), and 4) HML, high supplemental Met plus Lys, 62 g/d Met and 162 g/d Lys (2.61 Lys:Met). Cows were on experiment from 24 d before expected calving, and treatments continued from calving until 63 d post-calving. Daily DMI, milk yield, energy-corrected milk yield (ECM), fat-corrected milk yield (FCM), weekly BW, BCS, and milk composition (protein, fat, MUN, SCC) were measured. Data were analyzed using a randomized block design with the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model contained the fixed effects of treatment, parity, week or day, and all interactions, with the random effect of block. No statistical difference was detected in weekly BW, BCS, SCC, or MUN($P > 0.10$). Cows fed ML tended to have greater DMI and greater milk production ($P = 0.08$ and $P = 0.06$, respectively) than CON (19.68 kg/d vs. 17.35 kg/d and 40.4 kg/d vs. 36.6 kg/d, respectively). Both FCM and ECM were greater for cows fed ML than CON (40.9 kg/d vs. 36.0 kg/d, $P = 0.02$; and 39.6 kg/d vs. 34.9 kg/d, $P = 0.03$, respectively). In addition, protein and fat yields were higher for cows in ML than CON (1.22 kg/d vs. 1.07 kg/d, $P = 0.04$; and 1.41 kg/d vs. 1.63 kg/d, $P = 0.03$, respectively). Protein and fat percentages were not different among treatments. Addition of RPMet and RPLys to the fresh cow diet had a positive effect on milk production.

Key Words: methionine, lysine, transition cow

Animal Behavior and Well-Being 2

2190 Temperature-humidity index, behavior and respiratory rate in high yielding dairy cows. G. M. Martinez[1], G. Frossasco[2,3], P. Viretto[2], P. Turiello*[4,5], and V. H. Suarez[1], *[1]Instituto Nacional de Tecnologia Agropecuaria, Salta, Salta, Argentina, [2]Instituto Nacional de Tecnologia Agropecuaria, Rafaela, Santa Fe, Argentina, [3]Universidad Nacional de Villa Maria, Villa Maria, Cordoba, Argentina, [4]Universidad Nacional de Rio Cuarto, Rio Cuarto, Cordoba, Argentina, [5]Instituto de Formacion e Investigacion en Nutricion Animal—IFINA, Rio Cuarto, Cordoba, Argentina.*

Heat stress is associated with temperature-humidity index (THI) values greater than 68 in lactating cows, although recent studies suggested a limit of 60 for high yielding dairy cows (>35 kg/d). The aim of this study was to evaluate the association between THI, respiratory rate (RR), and behavior of lactating cows housed in a compost barn during summer time. Forty high yielding Holstein cows (34.4 ± 8.75 kg/d; 183.6 ± 96.80 DIM) from a commercial dairy farm in Santa Fe province in Argentina were randomly enrolled in the study to be monitored during 3 consecutive days each week in February 2023. We calculated THI according to Thom (1958), based on data from a meteorological station located near the farm, and we categorized it into 2 levels: low (= 68) and high (>68). We evaluated behavior at 11 a.m. and 2 p.m. (n = 918) by direct observation and using a partial ethogram recording whether the cow was lying down, standing, or other (eating, interacting with other cows or the environment, and so on). We assessed RR (abdominal muscle movements per minute; AMM) and classified it into normal (RR = 50 AMM) or accelerated (RR >50 AMM). We analyzed the results using a contingency table and compared them using a Pearson's chi-squared test ($P = 0.05$). In low (n = 240), 38.3% and 16.3% of cows preferred to be lying down or standing, respectively, whereas in high (n = 678), 27.7% and 35.5% of the cows were lying down and standing, respectively ($P < 0.0001$). In high, 406 cows with accelerated and 272 cows with normal RR were identified, whereas in low this ratio was 83 and 157, respectively ($P < 0.0001$). No association between behavior and RR was found ($P = 0.37$). These results show lactating cows generally express their discomfort through changes in behavior and RR under high THI conditions. In our study the lowest THI was 60. Some cows could have been affected under low, and this could explain some accelerated cows found in low. We conclude that there is an association among THI, behavior, and RR, although all the 3 variables should be considered to identify animals under heat stress.

Key Words: welfare, milk, heat stress

2191 Dairy cow behavior related to temperature-humidity index and temperature inside compost barn. P. Viretto[1], M. Martinez[2], G. Frossasco[1,3], P. Turiello*[4,5], and V. H. Suarez[2], *[1]Instituto Nacional de Tecnologia Agropecuaria, Rafaela, Santa Fe, Argentina, [2]Instituto Nacional de Tecnologia Agropecuaria, Salta, Salta, Argentina, [3]Universidad Nacional de Villa Maria, Villa Maria, Cordoba, Argentina, [4]Universidad Nacional de Rio Cuarto, Rio Cuarto, Cordoba, Argentina, [5]Instituto de Formacion e Investigacion en Nutricion Animal—IFINA, Rio Cuarto, Cordoba, Argentina.*

The temperature-humidity index (THI) is a useful tool to detect risk of heat stress in dairy cows. A THI above 68 means the cow is under heat stress, no heat is dissipated and cows tend to spend less time lying down. The aims of this study were 1) to evaluate behavior of high-producing cows (>35 kg/d) according to THI and compost barn inside temperature (IT) and 2) to explore the relationship between THI and IT. Twice a week during February and March 2023, we focally observed 29 cows from a commercial farm in Santa Fe, Argentina. Measurements were taken at 11, 12, 13, and 14 h (n = 1,840). We calculated THI according to Thom (1958), based on data from a meteorological station located on the farm. We registered barn IT using a datalogger located inside a black globe in the middle of the barn, 2 m above the floor. We evaluated behavior by direct observation using a partial ethogram recording whether the cow was lying down (L), standing (S), or other (eating, interacting with other cows or the environment, and so on). We classified THI into 2 levels: no stress (NS; THI = 68) and with stress (WS, THI >68). For IT, we considered it as adequate (A; IT = 21°C) or not adequate (NA; IT >21°C). Results were analyzed using a contingency table and compared using a Pearson's chi-squared test ($P = 0.05$). Spearman coefficient ($P = 0.05$) was used to evaluate the correlation between THI categories and IT. Results show behavior changes according to the considered variables ($P < 0.001$). When cow comfort was affected by THI, the proportion of L and S cows was 31.7% and 32.6%. Similar results (31.1% and 31.5% for L and S cows, respectively) were obtained under NA IT. However, under NS conditions or A IT, the proportion of cows L were higher (42.9% and 47.1%) than those S (16.1% and 18.8%). We also found a high (r = 0.79) and significant ($P < 0.0001$) correlation between THI and IT. According to these results we conclude that cows changed their behavior, increasing the proportion of animals S and diminishing those L when THI and IT increased. Measurement of IT could be useful to monitor dairy cows' comfort when no meteorological data are available.

Key Words: heat stress, comfort, thermoneutral zone

2192 Effect of shade availability on the physiological responses, water intake, and feeding behavior of grazing cows with an increasing heat load. A. Pontiggia[1,2], L. Eggerschwiler[1], A.-M. Reiche[1], N. M. Keil[2], and F. Dohme-Meier*[1], *[1]Ruminant Nutrition and Emissions, Agroscope, Posieux, FR, Switzerland, [2]Centre for Proper Housing of Ruminants and Pigs, Swiss Federal Food Safety and Veterinary Office, Ettenhausen, TG, Switzerland.*

This study investigated the effect of shade availability on pastures in a temperate zone on the physiological traits, water intake, and feeding behavior of grazing dairy cows in relation to increasing heat load. Twenty-four lactating Holstein cows (108 ± 32 DIM, 2.8 ± 1.7 lactation) in 6 subgroups of 4 cows grazed all day on 6 paddocks equipped either with or without a shade sail. Data were collected across 24 d in 4 crossovers (with/without shade). Each crossover lasted 3 d twice. Heat load was expressed as a comprehensive climate index (CCI in °C). Each subgroup had a water station available. Cows' water intake, reticular temperature, heart rate, and feeding behavior were continuously recorded for each cow, and respiration rate and use of shade were recorded by visual observation between 1300 and 1500 h. The data were analyzed using generalized linear mixed models (fixed effects: shade provision, CCI, interaction; random effects: crossover, subgroup, cow; experimental unit: cow). The CCI varied from 16.7 to 28.2°C and the temperature-humidity index from 56.2 to 71.1. Over 24 h, water intake and reticular temperature increased, and time spent ruminating decreased with increasing CCI (all $P < 0.01$). However, no treatment × CCI interactions (all $P > 0.05$) were detected for these traits. From 1300 to 1500 h, cows used the available shade less (10%–25% of the time), but the use increased with increasing CCI as did reticular temperature, heart rate, and respiration rate (all $P < 0.01$). No treatment and treatment × CCI interactions (all $P > 0.05$) were observed for heart rate (80.1 ± 8.9 beats/min), water intake (9.4 ± 4.6 L), and time spent eating (81.3 ± 16.7 min) and ruminating (15.7 ± 9.4 min) between 1300 and 1500 h. However, cows offered shade had a lower reticular temperature (38.9 ± 0.2 vs. 39.1 ± 0.3°C, $P < 0.05$) and a lower respiration rate (50.5 ± 7.3 vs. 56.3 ± 10.4 breaths/min, $P < 0.01$) than cows without shade. In conclusion, offering shade seemed to lower the body temperature and respiration rate of grazing dairy cows, although the cows did not use it much in the afternoon.

Key Words: cow, pasture, heat stress

2193 Behavioral and physiological differences of dry dairy cattle under heat stress based on median core body temperature.

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Heat stress results in significant economic losses to the dairy industry. Here, we investigated behavioral and physiological differences of dairy cows with above or below median core body temperature (CBT) assessed for 1 wk during late gestation. Temperature data were collected every 5 min for 7 d using a temperature logger attached to an intravaginal insert between d 225 and 239 of gestation. Cows having above-median CBT were classified as high temperature (HT) and those below median CBT were classified as low temperature (LT). Behavioral data from 50 cows (10 cows per replicate; primiparous [n = 19] and multiparous [n = 31] Holstein cows) were collected using automated activity monitors equipped with accelerometers in addition to visual observations. Accelerometer data were evaluated from d −21 to 21 relative to calving. Cows were visually observed for 8 h in the far-off pen and 8 h in the close-up pen. Repeated measures ANOVA was employed to analyze the accelerometer data. For each visual observation, the percentage of time allocated to each behavior was analyzed by fitting generalized linear mixed models. Morning and afternoon behavioral observations (lying, standing, eating, drinking, and perching [only recorded in the far-off pen]) of both far-off and close-up pens showed no detectable differences ($P \geq 0.12$). An interaction between temperature and parity ($P = 0.02$) showed that HT primiparous cows spent the most time eating in the afternoon visual observations (30.9%), followed by LT multiparous cows (25.4%) and LT primiparous cows (22.5%), whereas HT multiparous cows spent the least time eating (19%). Accelerometer data indicated that HT cows were more active ($P < 0.01$) than LT cows. Multiparous cows were more active than primiparous cows during both prepartum ($P = 0.02$) and postpartum ($P < 0.01$) periods. Results from the present study indicate distinct behavior patterns between HT and LT cows; however, further research is required to determine precise thresholds for classifying cows as HT and LT, as well as to assess behavior indicators that could predict the likelihood of postpartum health issues.

Key Words: thermal stress, activity monitors, transition period

Animal Health 2

2067 Survival of a four-strain probiotic for dairy calves in whole milk prepared at different temperatures. G. Copani*[1], N. Milora[1], R. Gresse[1], K. Morrill[2], O. C. M. Queiroz[1], and B. I. Cappellozza[1], [1]*Novonesis, Hørsholm, Denmark,* [2]*Novonesis, Milwaukee, WI.*

Probiotics, by definition, are live microorganisms that confer a health benefit to the host when consumed in adequate amounts. Probiotics are administered to livestock animals in different ways, being mixed directly into the final diet, incorporated in the whole milk/milk replacer, or included in premixes. During milk feeding, the probiotic bacteria could be exposed to a wide range of temperatures used for dissolving the powder. In this study, we investigated the survivability of a bacteria-based probiotic containing *Bacillus licheniformis* 809, *B. subtilis* 810, *Ligilactobacillus animalis* 506, and *Propionibacterium freudenreichii* 507 (BOVAMINE® Dairy Plus; Novonesis, Denmark) when added to whole milk at different temperatures, mimicking the preparation of milk replacer at: Exp. 1 - 45°C, Exp. 2 - 55°C, Exp. 3 - 65°C, and Exp. 4 - 75°C for the duration of the entire experiment (90 min post-probiotic addition). The probiotic was included (T0) at 1.0×10^6 cfu/mL of whole milk. Samples were stored at the respective temperatures and analyzed for total cfu counts at 0, 30, 60, and 90 min. In all conditions, at the different time points, samples were enumerated using MRS (37°C, anaerobic, 3 d) and TSA plates (37°C for 24 h). All data were analyzed using SAS (version 9.4; SAS Inst., Cary, NC, USA) and comparisons were performed vs. time 0 (T0). The total cfu recovered after 90 min did not differ from T0 in any of the experimental conditions in Exp. 1 ($P = 0.38$) and Exp. 2 ($P = 0.69$). However, an increase of 0.5% in the cfu was observed in Exp. 3 ($P = 0.03$) at 90 min and a trend was reported in Exp. 4 ($P = 0.07$), as cfu counts at 30 and 90 min increased by 1% ($P = 0.03$ vs. T0). In summary, these experiments demonstrate that BOVAMINE® Dairy Plus remain stable in the whole milk for up to 90 min post-mixing in temperatures that ranges from 45 to 75°C.

Key Words: milk replacer, calf, probiotics

2194 Descriptive characteristics of suckle physiology, milk intake, and health in neonatal dairy calves. C. R. Seely[1], K. R. K. Gottwald*[1], T. Bhattacharjee[2], and T. E. von Konigslow[1], [1]*Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine, Ithaca, NY,* [2]*Department of Computer Science, Cornell University, Ithaca, NY.*

Despite technological advancements in precision animal health monitoring, morbidity and mortality in neonatal dairy calves remains pervasive. In calves experiencing diarrhea, suckle reflex can be measured to estimate disease severity by encouraging the calf to suckle on a finger. This diagnostic method is highly subjective. To date, suckle physiology is not well described, and sensors to measure the behavior are not available. Our overarching goal was to inform novel sensor design. Our objective in this observational cohort study was to describe patterns in suckle pressure in neonatal dairy heifer calves. Female Holstein calves (n = 50) from a single New York dairy were enrolled and followed from 1 to 21 d of life. Calves were raised in pens of 5 from 1 to 5 d and then moved to pens of 20 fed by automated milk feeder (11 L/d whole milk allowance). Suckle pressure was measured at 1, 3, 5, 7, 10, 14, and 21 d using impression film wrapped around a nipple that calves suckled for 15 s. Impression image density (scale: 0 to 1), saturation (scale: 0% to 100%), and entropy (scale: infinite) were measured using Python. Milk intakes were recorded daily from 5 d and health scores from 1 d. Statistical analysis were computed in R using *summary* and *lme* for fixed effect model to measure least squares means by age. Means with standard deviation across sampling days for density, saturation, and entropy were 0.4 ± 0.1, 65.4 ± 27.8%, and 346 ± 124 bits, respectively. All suckle pressure metrics peaked at 3 d and declined to 21 d. Density, saturation, and entropy means between d 3 and 21 were 0.5 and 0.4 ($P < 0.01$), 1.8% and 1.7% ($P < 0.01$), and 372 bits and 275 bits ($P < 0.01$), respectively. Milk intake increased from 5 to 21 d (3.1 ± 0.6 kg/d vs. 7.2 ± 0.3 kg/d, respectively; $P < 0.01$). Diarrhea and respiratory disease incidence were highest between 9 and 13 d, and 10 and 21 d, respectively. Consistent patterns in suckle pressure and intake were observed in relation to health outcomes. These findings suggest that further exploration of suckle physiology might be useful for early disease detection in neonatal calves.

Key Words: suckle physiology, automated health monitoring systems, calf health

2195 Detection of neonatal calf diarrhea using suckle pressure. B. Xu*[1], C. Seely[2,1], K. Gottwald[1], T. Bhattacharjee[3], and T. von Konigslow[1], [1]*Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY,* [2]*Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH,* [3]*Department of Computer Science, Cornell University, Ithaca, NY.*

Neonatal calf diarrhea (NCD) poses a significant challenge to calf health, welfare, and production economics. Suckle strength has been used as a measure of NCD severity and to guide NCD treatment. Conventional suckle strength measurement involves a subjective assessment of the calf's response to finger placement in its mouth. Sensors designed to measure suckle strength are not currently available. Our objective in this observational cohort study was to evaluate suckle pressure impression images for NCD classification. Group housed female Holstein calves (n = 50) from a single dairy in New York were enrolled and followed from 1 to 21 d. Calves were fed by bottle until 5 d then moved to automated milk feeders. Daily health scores were performed, and NCD was defined as watery feces that can sift through bedding. Suckle pressures were measured using pressure scale sensor film wrapped nipples at 1, 3, 5, 7, 10, 14, and 21 d and daily following NCD diagnosis until fecal consistency was no longer watery. A total of 349 images were gathered with 54 from calves with NCD. Python was used for data analysis. Image features were extracted (pixel density, color saturation, grayscale difference-based features, and Histogram of Oriented Gradients). Data were normalized into high-dimensional vectors and applied to classifiers for NCD detection. Testing and training data sets (2:8) were generated through random partitioning. Multiple machine learning (ML) classifiers were explored including Support Vector Machine, K-Nearest Neighbors, Random Forest, Gradient Boosting, and the Easy Ensemble (EE). Performance was assessed using accuracy, precision, and recall based on a confusion matrix. The EE classifier demonstrated the best performance (average accuracy = 87%, average precision = 90%, average recall = 87%). Accuracy was calculated as the ratio of correctly predicted images to the total number of predictions. Notably, even in the presence of imbalanced data, the EE classifier achieved accuracies of 82% for NCD and 88% for non-NCD. These findings indicate that suckle pressure and ML techniques might provide a novel approach for NCD detection in preweaned dairy calves.

Key Words: NCD, suckle pressure, machine learning

2196 Prepartum acetylsalicylic acid effects on colostrum and passive immune transfer in neonatal Holstein calves.

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The objective of this study was to assess the effects of dam's acetylsalicylic acid administration 14 d before the expected calving date on colostrum quantity and quality and newborn immune passive transfer. Prepartum cows, blocked by BCS and parity, were randomly allocated to 1 of 2 groups: 1) ASA (n = 102): cows were treated with 4 acetylsalicylic acid boluses (480 g/bolus; equivalent to 125 g/d) or 2) PLC (n = 103): cows were treated with 4 gelatin capsules filled with water. Treatments were administered 14 d before expected calving date. Colostrum samples (random subgroup of cows; ASA n = 51; PLC n = 54) were collected during the first milking (~0–8 h after calving) for assessment of colostrum quantity and colostrum quality (i.e., Brix refractometer percentage; immunoglobin G [IgG]). Singleton alive female calves (ASA-CLF = 47; PLC-CLF = 46) born from this cohort of cows were enrolled in the study. A blood sample was collected within 3 d after birth for assessment of IgG concentration. The data were analyzed using the MIXED procedure of SAS as a randomized complete block design. There was no difference in colostrum quantity between treatment groups (ASA = 13.89 ± 1.37 kg; PLC = 13.52 ± 1.32 kg; $P = 0.84$). Similarly, the colostrum quality, using the Brix refractometer (ASA = 26.14 ± 0.49%; PLC = 26.37 ± 0.47%; $P = 0.73$) or IgG concentration (ASA = 130.27 ± 6.09 g/L; PLC = 133.22 ± 5.79 g/L; $P = 0.72$) did not differ between treatment groups. Nevertheless, calves born from cows treated with ASA tended ($P = 0.08$) to have a higher concentration of IgG compared with calves born from cows treated with PLC (ASA-CLF = 37.04 ± 2.04 g/L; PLC-CLF = 31.79 ± 2.25 g/L). Similarly, when assessed with the Brix refractometer, calves born from ASA cows had higher immune passive transfer compared with calves born from PLC cows (ASA-CLF = 6.67 ± 0.09 g/dL; PLC-CLF = 6.24 ± 0.10 g/dL; $P = 0.002$). These results suggest that although prepartum acetylsalicylic acid administration did not affect colostrum quantity or quality, it may positively influence immune passive transfer in neonatal calves through in-uterus mechanisms.

Key Words: prepartum acetylsalicylic acid, colostrum quality and quantity, immune passive transfer

2197 Prepartum acetylsalicylic acid effects on metabolic status and systemic inflammation in preweaned Holstein calves.

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The objective of this study was to assess the effects of dam's acetylsalicylic acid administration 14 d before the expected calving date on metabolic status and systemic inflammation in Holstein calves during the first 4 wk of life. Prepartum cows, blocked by BCS and parity, were randomly allocated to 1 of 2 groups: 1) ASA (n = 102) = cows were treated with 4 acetylsalicylic acid boluses (480 g/bolus; equivalent to 125 g/d); or 2) PLC (n = 103) = cows were treated with 4 gelatin capsules filled with water. Treatments were administered 14 d before the expected calving date. Singleton alive female calves (ASA-CLF = 47; PLC-CLF = 46) born from this cohort of cows were enrolled in the study. Blood samples were collected within 3 d of birth and weekly for the first 4 wk of life to assess levels of nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHB), haptoglobin (HP), and serum total protein (TP). The data were analyzed using linear regression models with the MIXED procedure of SAS as a randomized complete block design. Calves born from cows treated with ASA had higher NEFA concentrations within 3 d after birth compared with calves born from cows treated with a placebo (ASA-CLF = 384.68 ± 16.35 mEq/L; PLC-CLF = 306.63 ± 17.59 mEq/L; $P = 0.02$). There was no difference in BHB concentrations between ASA-CLF and PLC-CLF. Furthermore, ASA-CLF showed higher TP levels during the first 4 wk of life compared with PLC-CLF (ASA-CLF = 6.07 ± 0.07 g/dL; PLC-CLF = 5.81 ± 0.08 g/dL; $P = 0.01$). In accord, there was a statistical tendency ($P = 0.09$), where ASA-CLF had lower HP concentrations compared with PLC-CLF (ASA-CLF = 42.11 ± 12.43 μg/mL; PLC-CLF = 72.21 ± 13.09 μg/mL). Although ASA-treated calves may experience higher metabolic stress in the first week after birth, this process may be associated with an improved passive immunity early in life.

Key Words: prepartum acetylsalicylic acid, preweaned calves, metabolic status and systemic inflammation

2198 Prepartum acetylsalicylic acid effects on growth and health during the preweaning period in Holstein calves.

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This study aimed to assess the effects of acetylsalicylic acid administration 14 d before the expected calving date on calf growth and health in the first 8 wk of life. Prepartum cows, blocked by BCS and parity, were randomly allocated to 1 of 2 groups: 1) ASA (n = 102) = cows were treated with one administration of 4 acetylsalicylic acid boluses (480 g/bolus; equivalent to 125 g/d) or 2) PLC (n = 103) = cows were treated with one administration of 4 gelatin capsules filled with water. Treatments were administered 14 d before the expected calving date. Singleton alive female calves (ASA-CLF = 47; PLC-CLF = 46) born from this cohort of cows were enrolled in the study. An initial calf weight was measured within the first 3 d after birth. Subsequently, calves were weighed weekly for 8 wk. Disease incidences were documented using on-farm computer records and weekly observations by the research team. The data were analyzed using linear (MIXED procedure) and logistic (GLIMMIX procedure) regression models with SAS as a randomized complete block design. With regard to calf weight at birth, there was an interaction between treatment and parity ($P = 0.03$), where calves born from parous (PAR) cows treated with ASA had a higher weight compared with calves born from PLC PAR cows. Similarly, PAR ASA-CLF tended to be heavier during the first 8 wk of life compared with

PAR PLC-CLF ($P = 0.05$; PAR ASA-CLF = 56.35 ± 0.85 kg; PAR PLC-CLF = 53.98 ± 0.87 kg). Interestingly, a higher percentage of ASA-CLF became sick compared with PLC-CLF ($P = 0.04$; ASA-CLF = 63.36 ± 7.26%; PLC-CLF = 41.81 ± 7.57%). However, when analyzing diseases separately (i.e., pneumonia, lameness, scours), ASA-CLF had a higher incidence only for scours ($P = 0.04$; ASA-CLF = 46.81 ± 7.37%; PLC-CLF = 26.08 ± 6.54%). These findings suggest that this ASA treatment approach may affect late fetal development in multiparous cows and calf intestinal health in the first weeks of life.

Key Words: prepartum acetylsalicylic acid, preweaning period, calf growth and health

2199 Measuring immunoglobulin G and immunoglobulin G specificity in milk replacers.

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Milk replacers (MR) are formulated to provide nutrition to calves. Ingredients like dried whey and other milk components contain IgG antibodies that can provide an immune boost to calves by binding pathogens for removal from the GI tract. The objective of this study was to determine the amount of IgG present in 5 commercial MR as well as the specificity of the IgG to common calf enteric pathogens. Five commercial MR were randomized and reconstituted following manufacturer's instructions. The total IgG titers were measured using a bovine IgG ELISA Kit (ICL, Inc.). The IgG was purified from each MR by (1) acidifying the sample to remove casein, (2) precipitating the IgG with ammonium sulfate, (3) purification through a protein A column, and (4) dialysis against PBS. The purified IgG was then conjugated to a horseradish peroxidase (HRP) and used in direct ELISA to measure antigen specificity against the following pathogens: bovine rota- and coronaviruses, *Cryptosporidium parvum*, *E. coli* K99, and *Clostridium perfringens* Types C and D. The amount of IgG tested for each MR was equivalent to a 10-oz. dose of MR. Specificity results were determined using A450 values (absorbance at 450 nm). This provided a measure of the amount of antibody bound to the pathogen. Analysis of variance comparison of least significant difference was performed at a significance level of 0.05. Statistical groups are indicated by different superscripts (Table 1). The 5 MR were different in terms of total IgG and specificity of the IgG. Importantly, the total amount of IgG did not always correspond to levels of specific pathogen IgG; for example, MR A and MR E had comparable total IgG but had significant specificity differences.

Key Words: calves, IgG, pathogen

2200 Perceived producer barriers to the implementation of best management practices for the control of *Salmonella* Dublin.

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Salmonella Dublin is an emerging pathogen on Canadian dairy farms making biosecurity practices crucial. However, the barriers to implementing best management practices (BMP) to control *S.* Dublin are unknown. The objectives of this study were to understand Ontario dairy farmers' awareness, perceptions, and barriers to implementation of established BMP for control of *S.* Dublin. In total, 28 dairy farmers participated in 5 focus groups. Four themes were developed from the data: 1) informational preparedness, 2) structural factors that influence disease mitigation, 3) motivational drivers of disease mitigation, and 4) shifts in biosecurity engagement. There was variation in participants' perceived awareness and preparedness for an outbreak of *S.* Dublin. Most participants were aware of the pathogen, not concerned about their farm becoming infected, and stated that their farm was not prepared to handle an outbreak. All focus groups discussed how regulatory organizations (e.g., Dairy Farmers of Ontario) and governments could improve disease surveillance and academia could create new on-farm best practices to mitigate *S.* Dublin transmission. All focus groups also discussed intrinsic motivational barriers (e.g., biosecurity does not register as a daily priority) and the belief that the risk of *S.* Dublin infecting animals on their farms is low. Participants also discussed extrinsic barriers (e.g., money, labor, practicality) as challenges to change on-farm biosecurity practices. Finally, participants who have experienced a *S.* Dublin outbreak expressed a shift in their perspective toward biosecurity (e.g., proactive rather than complacent) and risk management (e.g., wanting to limit spread to other farms). Our results suggest that participants viewed the responsibility to mitigate the spread of *S.* Dublin to be with regulators, government, and academia rather than themselves. Participants also did not view *S.* Dublin as a substantial risk to their farm. Therefore, until their perceived risk increases, the motivation to overcome extrinsic and intrinsic barriers to adopt best management practices will likely remain low.

Key Words: dairy cattle, focus groups, qualitative

2201 Comparison of health and performance of Holstein and Holstein-Angus crossbred calves reared under the same conditions.

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Over the past several years, there has been a notable increase in the number of beef-on-dairy calves reared for veal and beef production. The objective of this cohort study was to compare health and growth performance of Holstein and Holstein × Angus calves reared under the same conditions from arrival until wk 12 at a commercial calf-rearing facility. The records from 384 calves (344 Holstein and 40 crossbred)

Table 1 (Abstr. 2199).

Sample	Total IgG (mg/g MR)	IgG specificity (A450 value)				
		Rotavirus	Coronavirus	*Crypto. parvum*	*E. coli* K99	*C. perfringens* Types C/D
MR A	11.09^{d}	0.136^{c}	0.075^{c}	0.084^{c}	0.131^{c}	0.345^{c}
MR B	2.01^{a}	0.009^{a}	0.004^{a}	0.006^{a}	0.012^{a}	0.026^{a}
MR C	3.76^{b}	0.031^{b}	0.024^{b}	0.022^{b}	0.044^{b}	0.054^{b}
MR D	5.65^{c}	0.128^{c}	0.117^{d}	0.116^{d}	0.203^{d}	0.355^{c}
MR E	12.16^{d}	0.301^{d}	0.277^{e}	0.305^{e}	0.494^{e}	0.703^{d}

were analyzed. Calves arrived at the facility between 3 and 10 d of age and were housed individually. Up to 8 L of milk replacer (130 g/L) was fed per day, divided in 2 meals. Water and grain were provided ad libitum, and consumption was recorded weekly. Serum total protein (STP) and BW were assessed upon arrival, and BW was recorded weekly thereafter for 12 wk. Fecal consistency was evaluated twice a day for the first 15 d, and a respiratory score was determined twice daily for the entire study to identify the presence of respiratory disease. A mixed linear regression model was used in Stata to evaluate the effect of breed on ADG, whereas a mixed Poisson model was used to evaluate the effect of breed on health. Initial BW was assigned as a fixed effect, and group (based on arrival date) as a random effect. Body weight upon arrival did not differ (47.5 kg) between Holstein and crossbred calves. However, STP tended to be higher ($P = 0.10$) for Holsteins (5.44 g/dL) than crossbreds (5.27 g/dL). No differences were detected for ADG between Holsteins (1.07 kg/d) or crossbreds (1.09 kg/d). Holsteins had a higher number of days with a respiratory score of >5 (incidence rate ratio: 2.44; 95% CI: 1.73 to 3.42; $P < 0.01$), which may be indicative of respiratory disease. Specifically, Holsteins exhibited a high respiratory score (≥5) for an average of 2.36 d (SD = 7.39), whereas crossbreds had an average of 0.98 d (SD = 2.41). Breed, however, had no effect on the number of days with diarrhea. The results of this study suggest that crossbred calves may experience a lower level of respiratory disease. Future studies should investigate the reasons for this finding.

Key Words: health, performance, calves

2202 Predictors of bovine respiratory disease and its effect on growth and hazard of mortality in dairy calves.

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The objective of this cohort study was to investigate predictors of bovine respiratory disease (BRD) and how BRD affects growth and mortality in calves. Beginning at arrival to a commercial calf-raising facility in southern Ontario, Canada, Holstein calves estimated to be 3 to 10 d old were examined twice daily for signs of BRD using the UC Davis Respiratory Scoring chart and weighed weekly for 77 d until leaving the facility (n = 1,100). Serum total protein (STP) was also determined using a digital refractometer upon arrival. A mixed logistic regression model was used to identify predictors associated with occurrence of BRD, a mixed linear regression model was used to identify variables associated with ADG, and a Cox proportional hazards model was built to assess BRD association with hazard of mortality throughout the 77 d at this facility. A total of 100 calves died (9.1%), and 485 calves (44.1%) were diagnosed at least once with BRD (score >5). Compared with calves sourced directly from dairy farms, calves from auction (OR = 1.49, $P < 0.01$, 95% CI = 1.11 to 2.00) and drovers (1.51, $P < 0.001$, 95% CI = 1.00 to 2.29) had higher odds of BRD. Additionally, having diarrhea resulted in higher odds of BRD compared with calves that did not have diarrhea (1.88, $P < 0.001$, 95% CI = 1.42 to 2.49). Calves that arrived with STP between 5.1 and 5.7 g/dL (0.67, $P = 0.03$, 95% CI = 0.47 to 0.95), 5.8 and 6.1 g/dL (0.58, $P = 0.005$, 95% CI = 0.39 to 0.85), or = 6.2 g/dL (0.52, $P = 0.001$, 95% CI = 0.34 to 0.78) had reduced odds of BRD compared with calves with STP <5.1 g/dL. Calves with BRD had reduced ADG in the 77 d at the facility compared with calves without BRD (−0.13 kg/d, $P < 0.001$, 95% CI = −0.16 to −0.10). Furthermore, calves with BRD had a greater hazard of mortality throughout the 77 d compared with calves that did not (hazard ratio = 10.30, $P < 0.001$, 95% CI = 5.32 to 19.92). These findings demonstrate that predictors of BRD can be identified upon arrival to a calf-raising facility and that BRD negatively affects calf success.

Key Words: surplus calf, morbidity, dairy beef

2203 Evaluation of automatic milk feeders for early detection of respiratory disease in dairy calves on 5 commercial farms.

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Respiratory diseases (RD) in calves pose a significant health challenge, with potential increase in morbidity and mortality rates for the dairy industry. Early detection of RD could mitigate its effect, and our study aimed to evaluate the use of an automatic milk feeder (AMF) for early detection of RD on commercial farms. Five dairy commercial farms located in Quebec, Canada, were selected for this study to cover a broad range of calf management practices. The AMF data, comprising daily calf consumption (mL/d), consumption speed (mL/min), and number of rewarded or unrewarded visits, were collected on all farms for a year, totaling 105,759 data points on 2,358 unique female Holstein heifers. Calves had access to the AMF between 7 and 14 d of age, depending on the farm. The RD treatment data were gathered, and calves were considered sick when they first received RD-associated treatment from the producer. The number of calves with RD varied from 27 (22%) on farm 2 to 225 (25%) on farm 4. Healthy and RD-affected calves were matched by season and days at the AMF (±2 d). One linear mixed model with the health status of calves, days relative to treatment, season, and interactions between day and health status used as fixed effects, as well as the calf as a random effect, was built per farm. Heterogeneity between farms was evident in the results, and at least one farm had a significant difference between healthy and RD calves' days before treatment by the producer for each of the studied parameters. The earliest significant differences between healthy and RD calves were −1 d for the number of rewarded visits ($P < 0.001$), −2 d for unrewarded visits ($P = 0.048$), −5 d for the drinking speed ($P < 0.001$), −5 d for consumption ($P = 0.003$), and −4 d for unrewarded visits ($P = 0.033$) on farms 1 to 5, respectively. The AMF showed promise for early RD detection under commercial conditions. However, the number of days before detection and usefulness of individual AMF metrics vary between farms, emphasizing the importance of developing future early RD detection approaches that are specifically tailored to individual farms.

Key Words: dairy calf, respiratory disease, automatic milk feeder

2204 Comparative analysis of gut microbiome diversity in Holstein cattle: Focus on age-related variation.

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The gut microbiome plays pivotal roles in the health and performance of livestock; however, limited information is available on the gut microbiome of Holstein cattle. Therefore, this study aimed to examine the gut microbiota of Holstein cattle in Korea, with a specific focus on age-related variations. Fecal samples were collected longitudinally twice from the same Holstein females (n = 4) at the ages of 5 and 12 mo. The first sampling was conducted in January and the second in August of 2022. We analyzed a total of 8 fecal samples with 4 samples from each age group. The microbial profiles were examined through 16S rRNA gene sequencing targeting the V5-V6 regions. The generated sequencing data underwent processing and analysis using the QIIME2 (Quantitative Insights Into Microbial Ecology) software, with statistical analyses performed using STAMP (Statistical Analysis of Metagenomic Profiles) and GraphPad PRISM. The average α diversity of the samples was higher at 12 and 5 mo. Beta diversity analysis revealed distinct clustering patterns between the 2 groups, suggesting significant differences in microbial composition between 5 and 12 mo. The LEfSe (Linear discriminant analysis Effect Size) analysis revealed significant differences primarily at the phylum level in microbial abundance. Notably, *Bacteroidetes* and *Campylobacterota* phyla were more abundant at 5 mo, whereas *Actinobacteria*, *Verrucomicrobiota*, *Euryarchaeota*, and *Firmicutes* were more abundant at 12 mo of age. *Actinobacteria*, known for their involvement in acetate synthesis, are typically found in the rumens of cows that produce high levels of SFA. The taxonomic classification of sequences at the genus level unveiled the 3 most abundant genera at 5 mo of age as *Treponema*, *Alistipes*, and *Succinivibrio*, whereas at 12 mo of age, *Alistipes*, *Dysosmobacter*, and *Clostridium IV* dominated. This study contributes to our understanding of the gut microbiota in Holstein cattle and highlights the age-related variations in microbial communities.

Key Words: Holstein cattle, microbiome, metagenomics

2205 Liver microbiome community in Holstein dairy cows from pre- to postpartum period.

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Our objective was to investigate the presence of a microbiome community in the liver of dairy cows during the periparturient period. Eight multiparous Holstein dairy cows were monitored from −21 to 30 DIM. All cows received the same close-up diet from −21 DIM to calving (1.59 Mcal/DM and 14.63% CP) and lactation diet (1.82 Mcal/DM and 18.39% CP) from calving until 30 DIM. Liver biopsies were performed at −10 and 7 d relative to calving. Bacterial DNA was isolated from 20 mg of liver tissue using the QIAamp Fast DNA Stool mini kit (Qiagen). The DNA quantity and purity were measured using a Nanodrop spectrophotometer. MiSeq Micro V2 300-Cycle SR Cluster Gen and Seq Per Lane of the bacterial 16S rRNA gene were generated by the Virginia Tech Genomics Center. Alpha diversity indices (Shannon and Simpson) were determined using the "diversity" command from "vegan" on the data set for each sample. Comparisons of abundance for phylum, class, and order among −10 and 7 d relative to calving were performed by a log-linear regression framework-based package, ANCOM-BC, and an ANOVA-like, Aldex2, followed by the Benjamini–Hochberg *P*-value correction for multiple comparisons. Although there were no overall differences ($P > 0.05$) between microbial abundance at −10 and 7 d relative to parturition for phylum, class, and order, a high abundance of *Proteobacteria* phylum was observed among both periods, followed by *Firmicutes* and *Bacteroidetes*. The most abundant class at −10 and 7 d relative to parturition was *Gammaproteobacteria*. The second most abundant class at −10 d was *Alphaproteobacteria*, and *Clostridia* was the second most abundant at 7 d. *Bacilli* was the third most abundant class at 7 d. The most abundant order at −10 d was *Pseudomonadales* followed by *Clostridiales* and *Bacteroidales*. On the other hand, the most abundant order at 7 d was *Clostridiales*, followed by *Pseudomonadales* and *Bacteroidales*. Although no difference was observed between pre- and postpartum, the results indicates the presence of a microbiome community within the liver of peripartal dairy cows, which could potentially affect metabolic and immunological responses.

Key Words: microbiome, liver, dairy cow

2207 Milk component ratios and their associations with energy balance indicators and serum calcium concentration in early-lactation spring-calving grazing dairy cows.

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The objectives of this study were to 1) describe milk component ratios (MCR; fat-to-protein [FPR], fat-to-lactose [FLR], protein-to-lactose [PLR]) at 0 to 45 d postpartum, 2) evaluate the association between MCR and indicators of energy balance (EB; serum BHB concentration at 5–45 d postpartum and BCS change), and 3) evaluate the association between MCR and serum Ca concentration at 0 to 4 d postpartum in spring-calving cows from 27 pasture-based dairy farms. This observational study was conducted using information attained 1 or 2 times from 548 cows (970 MCR, 851 BCS change observations, and 918 and 50 serum BHB and total Ca wet chemistry determinations) analyzed by multiple linear regression with farm as a random effect. Overall, milk FPR, FLR, and PLR were 0.70 ± 0.02, 0.53 ± 0.02, and 0.72 ± 0.01 during the study period (±standard error). Milk FPR linearly increased while FLR decreased at a rate of 0.003 and 0.004 units/d, respectively; milk PLR decreased 0.02 units/d for the first 30 d postpartum and moderately increased afterward. Milk FPR and FLR were 0.69 and 0.52 units lower by a.m. than p.m. milking; milk PLR was similar before a.m. and p.m. milking. Milk PLR was 0.02 higher for ≥fourth-parity cows compared with second- to third-parity and first-parity cows, whereas FPR and FLR were not affected by parity. Furthermore, crossbred cows had 0.07, 0.08, and 0.03 higher milk FPR, FLR, and PLR than Holstein-Friesian cows. No associations were observed between MCR and indicators of EB. A positive linear association was observed between milk FPR and serum Ca concentration within 4 d postpartum; milk FPR increased 0.31 units per each mmol/L increase in serum Ca concentration. Cows with low serum Ca concentration (<2.12 or < 2.00 mmol/L) within 4 d postpartum had 0.27 units and tended to have 0.15 units lower milk FPR compared with cows above the threshold. In conclusion, MCR determined before milking vary with milking time and may not be associated with EB indicators in early-lactation grazing dairy cows. The potential of milk FPR for monitoring blood Ca status warrants further investigation.

Key Words: calcium, hypocalcemia, hyperketonemia

Forages and Pastures 2

2208 Ethanol and organic acid content in sugarcane silage with anti-yeast antibodies. G. B. Neto*[1], A. W. de Paula Freitas[1], R. B. Botelho[1], C. A. Rosa[2], and J. P. Sampaio[3], *[1]Animal Science Institute of Department of Agriculture and Food Supply, Ribeirão Preto, São Paulo, Brazil, [2]Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [3]Universidade Nova de Lisboa, Lisboa, Portugal.*

Although sugarcane produces a large amount of energy, the sugarcane silages are characterized by extensive ethanol production. This study was conducted to evaluate an additive containing anti-yeast antibodies (requested patent number: BR 10 2022 017453 9, FAPESP 2017/01646-2) on ethanol and organic acids in sugarcane silage. The treatments were sugarcane silage without additive, sprayed with the inoculant *Lactobacillus buchneri* (2.5×10^{-10} cfu/g) or homogenized with 26 kg/t of additive containing anti-yeast in a completely randomized design with 4 replicates. After 114 d of ensiling, the silage juice was extracted with a hydraulic press (2 kgf/cm^3). Gas chromatography was used to determine the content of organic acids and ethanol, and lactic acid content was measured with HPLC. The 3M Petrifilm yeast count plates were used to determine yeast concentrations. The data were analyzed using one-way analysis, and means were compared using the Tukey test ($P < 0.05$). Compared with the control, the addition of *L. buchneri* increased acetic and lactic acids levels, whereas ethanol production was decreased (Table 1). The additive containing anti-yeast increased the acetic, propionic, and lactic acid levels, whereas the level of ethanol production decreased. The higher levels of acetic acid reduced yeast counts and decreased ethanol production resulting in a decline of DM losses. The additive containing anti-yeast can inhibit yeast multiplication, prevent spoilage, and allow for more efficient production of sugarcane silage by farmers.

Key Words: dry matter losses, yeast, ethanol

2209 Fiber degradability in whole-plant corn and corn stover as affected by maturity. H. Scardini Jr., M. Cardoso, E. Medeiros, A. Matias, and T. Bernardes*, *University of Lavras, Brazil, Lavras, MG, Brazil.*

The hypothesis of this study is that starch in kernels hides the negative effects of maturity on NDF degradability of whole-plant corn (WP). Thus, our objective was to assess the effects of hybrid, maturity, and kernels remotion on NDF degradability of fresh corn plants. Over 2 crop years, 2 hybrids (H1 and H2) were grown at 70,000 plants/ha on plots (6 × 5 m) in 3 blocks. Hybrids were harvested at the target maturity levels of 30% of DM (M1), 35% of DM (M2), and 40% of DM (M3). Plants were harvested manually and bundled. Then, they were split into 2 subsamples (WP and plants without kernels [stover]). For the last, kernels were removed manually from the ears. Then, cob and husks were joined to the stem and leaves to be processed. Measurements included DM, NDF concentration, and in situ NDF degradability at 24 h (isNDFD_{24h}). The experimental design was randomized complete blocks using a mixed repeated measures model. Hybrids, maturities, and kernels remotion were considered as fixed effects, and blocks and years were considered as random effects. Data were analyzed using the MIXED procedure of SAS, followed by Student's *t*-test at $P \leq 0.05$. At M1, M2, and M3 the DM concentrations were 32.2 and 33.8, 37.2 and 38.5, and 44.6 and 44.3 for H1 and H2, respectively. Regarding the NDF concentrations, there was an interaction between hybrid and maturity, and effects of hybrid and kernels remotion. Fiber concentrations were greater in stover than WP. Fiber concentrations increased in stover and decreased in WP from M1 to M3. For isNDFD_{24h}, there were interactions of hybrid, maturity, and kernels remotion, and maturity and kernels remotion. Hybrid, maturity, and kernels remotion also affected the isNDFD_{24h}. The isNDFD_{24h} in WP decreased from M1 to M2 for both hybrids (no differences between M2 and M3). Conversely, in stover, the isNDFD_{24h} decreased from M1 to M3 for both hybrids. In WP, at M3, isNDFD_{24h} was greater than in stover. Overall, maturity at harvest impairs NDF degradability in H1 and H2. Starch in kernels at delayed maturity, may have led previous studies to conclude that there is no effect of maturity on NDF degradability.

Key Words: corn silage, maturity, NDF digestibility

2210 The effects of triticale silage maturity and dietary forage on nutrient digestibility and methane emissions. H. Galyon*[1], L. Robinson[1], K. Payne[2], W. Thomason[1], S. Stewart[1], and G. Ferreira[1], *[1]Virginia Tech, Blacksburg, VA, [2]Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA.*

The study analyzed triticale silage maturity and dietary forage effects on nutrient digestibility and methane emissions. Holstein cows (8 primiparous, 16 multiparous) were assigned to 1 of 4 diets in a replicated 4 × 4 Latin square design over 21-d periods. The diets contained triticale silages harvested at either boot stage (BS) or soft-dough stage (SDS) and either high- (HF) or low-forage (LF) inclusions (50% and 38% dietary forage, respectively). Digestibilities of DM (DMD), CP (CPD), NDF (NDFD), and starch (StchD) were determined using an external marker. Methane production (CH_4P), yield (CH_4Y), and intensity (CH_4I) were calculated via GreenFeed system. Using PROC MIXED of SAS, the model included the fixed effects of square, silage maturity, dietary forage, and their interaction and the random effects of period and cow

Table 1 (Abstr. 2208). Ethanol, organic acids, yeast, and DM losses (DML) of sugarcane silage with *Lactobacillus buchneri* or additive containing anti-yeast antibodies

Item	Control	*L. buchneri*	Anti-yeast	SEM	*P*-value
Acetic acid (m*M*)	58.65^{c}	152.44^{b}	212.74^{a}	8.720	<0.0001
Propionic acid (m*M*)	0.00^{b}	1.34^{a}	1.39^{a}	0.474	0.012
Latic acid (% DM)	1.60^{b}	2.13^{b}	6.10^{a}	0.314	<0.0001
Ethanol (% DM)	8.27^{a}	3.89^{b}	2.51^{b}	0.663	<0.0001
DML (% DM)	32.26^{a}	19.68^{b}	11.47^{b}	4.758	0.005
Yeast (0 h), log cfu/g	4.54^{a}	1.65^{b}	0.00^{c}	1.627	0.093
Yeast (24 h), log cfu/g	4.87^{a}	4.04^{a}	0.00^{b}	1.477	0.528

$^{a–c}$Means with different superscripts are significantly different.

within square. Significance was declared at $P < 0.05$. The BS resulted in greater DMI than SDS when feeding HF (25.3 vs. 22.4 kg/d; $P < 0.01$), but no difference was seen when feeding LF (27.7 kg/d). Feeding LF had greater ECM yield than HF (49.1 vs. 45.6 kg/d; $P < 0.01$), but maturity did not affect ECM yield (47.3 kg/d). The BS had greater DMD than SDS (68.2% vs. 66.0%; $P = 0.02$) and HF had greater DMD than LF (68.8% vs. 65.4%; $P < 0.01$). The BS had greater CPD than SDS when feeding LF diets (60.0% vs. 48.3%; $P < 0.01$) and tended to be greater than SDS when feeding HF (58.3% vs. 52.8%; $P = 0.06$). The BS had greater NDFD than SDS (58.2% vs. 53.0%; $P < 0.01$) and HF had greater NDFD than LF (58.8% vs. 52.4%; $P < 0.01$). While a difference between BS and SDS was not seen (95.0%), HF had greater StchD than LF (95.2% vs. 94.7%; $P = 0.02$). The SDS had greater CH_4P than BS (391 vs. 368 g/d; $P < 0.01$), but no difference was seen between HF and LF (380 g/d). The SDS resulted in greater CH_4Y than BS (17.5 vs. 15.2 g/kg DM) when feeding HF, but no difference was seen when feeding LF (13.6 g/kg DM). The SDS had greater CH_4I than BS (8.4 vs. 7.7 g/kg ECM, $P < 0.01$), and HF had greater CH_4I than LF (8.5 vs. 7.7 g/kg ECM; $P < 0.01$). In conclusion, the maturity of triticale at harvest for silage affects methane emissions and nutrient utilization.

Key Words: triticale, forage maturity, methane

2211 Amino acid composition in forages.

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A data set containing NIR nutrient analysis and AA composition (MET, LYS, HIS, and ILE) was extracted from Dairyland results. Samples included alfalfa hay (5,976 samples), alfalfa silage (9,266), corn silage (32,595), grass hay (3,597) and grass silage (5,037). Outliers were excluded using K nearest neighbor methodology with K equal to 8. All statistics were done with JMP ver. 17.2.0. Pairwise correlations were done for MET, LYS, ILE, HIS, CP, true protein (CP – ammonia), ADICP, uNDFom240, aNDFom, ash, lactic acid, and acetic acid. Dry hays generally had different correlations then silages. In alfalfa and grass hay, AA were highly correlated with CP (r ranging from 0.80 to 0.93). In hay silages, correlations were more variable and significantly lower ranging from 0.37 (grass silage LYS and CP) to 0.81 (alfalfa silage, ILE and CP). The aNDFom and uNDFom240 correlations with AA in alfalfa hay ranged from −0.63 (uNDFom240 and HIS) to −0.84 (aNDFom and MET). These correlations decreased in alfalfa silage (−0.40 to −0.61). In grasses, MET and ILE correlation were similar either as hay or silage (−0.70 to −0.78). LYS was greatly impacted by preservation method with r decreasing from −0.78 to −0.30 with uNDFom240. Corn silage LYS and lactic acid resulted in bimodal distributions. Lactic acid also was moderately to highly correlated with LYS and HIS (−0.66 and −0.64, respectively). Generally, the ensiling process greatly alters the AA contents and increases variability in silages. The potential economic impact was calculated using an example diet in AMTS.Cattle.Professional ver. 4.18.6. Commercial bypass LYS and MET using current NY market prices to represent iso-MET and iso-LYS MP supply. The results suggest LYS and MET variability could represent 20 to 30 cents per cow shifts in diet cost. Forage AA content was found to be variable with potential for large economic and efficiency impacts during formulation. Adopting routine AA analysis, including forages, is warranted. Additional feeds and statistics will be presented.

Key Words: forages, amino acids, NIR

2212 In situ ruminal degradability of a biodegradable plastic bale wrap.

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Involuntary plastic consumption can lead to digestive problems in ruminants. Utilization of biodegradable plastic bale wrap (BioPBW), developed in the Department of Agricultural and Biological Engineering at Penn State, may alleviate chances for accumulation of foreign material within the rumen and related animal health concerns. We hypothesized that BioPBW, both with and without polyvinyl alcohol (PVA), would be highly degradable in the rumen. The objective of this study was to determine the in situ (IS) degradability of 2 BioPBW, with (BioPBW+PVA) and without PVA (BioPBW-PVA). The IS incubation was performed with a ruminally cannulated lactating Holstein cow. The cow was 137 DIM, producing 54 kg/d milk, and consuming 26 kg/d feed DM of a corn silage-based lactation diet consisting of 60% forage and 40% concentrate feeds. The BioPBW materials were cut into pieces of approximately 8 × 9 mm, and 5.5 g were weighed into 10 × 20 cm nylon bags with 50 ± 10 µm porosity (R1020, ANKOM Technology Corp.) in triplicates. Bags were sealed using zip ties, placed into a mesh bag (polyester, 40 × 20 cm), and incubated in the rumen for 6, 12, and 36 h. Samples were removed from the rumen at the end of each time point, rinsed with cold water, dried for 24 h and the dry weight was recorded using an analytical scale. Degradation curves were fitted to a 3-parameter exponential growth to a maximum model ($R^2 = 0.99$; SigmaPlot 13.0; Systat Software). Ruminal effective degradability (ED, % of DM) of the BioPBW products was determined as follows: $ED = a + b \times [c/(c + k)]$, where a (%) is the washout or rapidly degraded fraction; b (%) is potentially degradable fraction; c (%/h) is the rate of degradation of fraction b; and k is passage rate (assumed to be 3%/h). Fraction b was 92.5% for BioPBW+PVA and 98.4% for BioPBW-PVA; rate of degradation (c) was 26 and 19%/h, and ED was 83 and 85%, respectively. Results show that both BioPBW materials had high ruminal degradability with 95% to 100% of the material being degraded or lost through the bag pores in 36 h rumen incubation.

Key Words: biodegradable bale wrap, in situ, ruminal fermentation

2213 Performance of dairy heifers on contrasting meadow fescue grazing systems.

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This study explored the potential of clover as a nitrogen (N) fertilizer alternative in meadow fescue pastures, assessing key pasture and animal productive parameters. The experiment was conducted at the University of Wisconsin Marshfield Agricultural Research Station, in Stratford, Wisconsin, from May to October 2023. The study will be replicated in 2024. The experiment used a randomized complete block design with 3 treatments and 3 replications, yielding 9 pastures (experimental units). The treatments comprised meadow fescue without N fertilizer (MF), meadow fescue + N fertilizer (MF+N; 157 kg N/ha per year and meadow fescue + clover (MF+C). Eighteen "tester" heifers, 2 per pasture, were allocated to each pasture and remained in treatment throughout the experimental period. Stocking rates (SR) were adjusted bi-weekly using put-and-take animals, to obtain a target herbage allowance of 2.2 kg DM/kg BW. Average daily gain assessments occurred every 28 d, with fasting BW obtained after a minimum 12-h feed and water withdrawal. All pastures were rotationally stocked, following a 27-d rest period per paddock, allocating animals to new paddocks every 3 to 4 d across 10 paddocks per pasture. Data were analyzed using PROC GLIMMIX of SAS. Pregraze herbage mass was greater for MF+N ($P = 0.02$), across most weeks. While ADG and gain per area showed no significant differences among treatments (0.63 kg/d, 218 kg/ha, respectively; $P > 0.05$), SR was greater for MF+N and MF+C than MF (4.1, 4.0, 3.1 animal units/ha [1 animal unit = 300 kg]; $P = 0.038$). There was also a treatment by time of evaluation interaction for in vitro digestible OM concentrations ($P = 0.01$), where these decreased as the season progressed, ranging from 79% to 65% from May to September, respectively. However, IVDOM concentrations decreased at a faster rate for MF, relative to other treatments. The MF+N treatment displayed no substantial divergence compared with other treatments, suggesting that strategic grazing management and legume integration offer viable alternatives for sustaining grazing systems without compromising productivity.

Key Words: grazing, integration, ruminant

2214 Nutrient utilization by lactating Holsteins and Jerseys with different forage sources. V. L. Pszczolkowski*[1], K. F. Kalscheur[1], H. C. Wilson[1], and A. J. Bowers[2], [1]*US Dairy Forage Research Center, Madison, WI,* [2]*University of Wisconsin–Madison, Madison, WI.*

Alfalfa and corn are contrasting forage sources, at both ecological and nutritional levels. Holsteins and Jerseys also offer distinct production qualities, ergo we hypothesized they may differ in ability to utilize forages for milk synthesis. Our objective was to determine if responses to these forages differ between breeds, particularly in nutrient utilization. Holstein (n = 30, 146 ± 29 DIM) and Jersey (n = 30, 136 ± 45 DIM) cows were used in a crossover design with two 6-wk periods. Cows were fed diets formulated as either high corn silage (CS), containing 47.2% CS and 11.8% alfalfa silage (AS), or as high AS, with the opposite ratio of CS and AS. While diets were formulated to be equivalent for MP supply, MP for the CS diet averaged 2.39 kg/d while the AS diet averaged 2.65 kg/d. Data were analyzed using multiway ANOVA, with week as the repeated measure. Intake and production responses were normalized to metabolic BW (MBW). Compared with Holsteins, Jerseys' intake changed with diet, with lower DMI/MBW when fed high AS (194 g/kg) versus CS (211 g/kg) in wk 2 to 4 ($P \leq 0.02$), whereas Holsteins did not differ between diets (202 g/kg). Diet had no effect on milk yield/MBW, but Holsteins (320 g/kg) had consistently greater milk yield/MBW compared with Jerseys (281 g/kg; $P < 0.01$). Protein yield/MBW was unaffected by diet or breed, but milk protein content was greater for high CS (3.38%) versus AS (3.26%) during wk 5 to 6 ($P \leq 0.04$) and overall for Jerseys (3.55%) versus Holsteins (3.01%; $P < 0.01$). The MUN was greater in wk 1 and 4 for Holsteins (18.2 mg/dL) compared with Jerseys (17.2 mg/dL; $P < 0.01$) but not different between diets. Urinary allantoin was not different between forage or breed, but uric acid was greater for Holsteins (5.8 mM) versus Jerseys (4.2 mM; $P \leq 0.02$). Efficiency as ECM/DMI was greater for AS (1.67 kg/kg) versus CS (1.59 kg/kg; $P = 0.01$), but MP gross efficiency (MP supply/protein yield) was greater for CS (0.51 kg/kg) versus AS (0.44 kg/kg; $P < 0.01$). Overall, while breeds had distinct DMI/MBW responses to forages, there was no evidence of other diet-breed interactions. Cows fed high CS were more efficient from a N perspective but less efficient by ECM.

Key Words: efficiency, forage, breed

2215 Effect of defoliation frequency on the nutritive value of the regrowth of tall fescue. F. B. Scheeren[1], M. Fernández-García[2], A. C. Sabbatella[1], L. Lasa[1], F. Berti[1], F. A. Lattanzi[1], M. Sánchez*[3], and J. M. Arroyo[1], [1]*Instituto de Producción Animal de Veterinaria, Facultad de Veterinaria, Universidad de la Republica, Libertad, San Jose, Uruguay,* [2]*Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay,* [3]*Dellait Research Center, Brookings, SD.*

Knowledge of the factors that influence the composition of grass is essential for its proper management in pasture-based dairy systems, thus increasing its productivity. An experiment was carried out to study the evolution of chemical composition and nutritional value of a regrowth of tall fescue as affected by the defoliation regimen around flowering time. The experiment was conducted on a 2-yr-old tall fescue (*Festuca arundinacea* 'INIA Fortuna') pasture, at the experimental station "La Estanzuela" (INIA, Uruguay). Two defoliation regimens—cut at 1.5 leaf-stage before flowering and 2.5 leaf-stage (T1) afterward, and 2.5 leaf-stage (T2)—were established in one paddock. Defoliation treatments were arranged in 6 × 2 m plots in a completely randomized block design with 4 repetitions (blocks). After two 2-harvest events (1.5 leaf stage) at T1 and one (2.5 leaf stage) at T2, in the following regrowth, samples of the pasture were taken every 0.5 leaves until 2.5 leaf-stage for both treatments. For each sample, CP, NDF, and in vitro digestible NDF (NDFD; Ankom Technology Corp., Macedon, NY) were analyzed. The evolution during the regrowth of each variable was studied through linear regression including the block as a random effect, the treatment as an independent categorical variable, and the leaf-stage as a continuous independent variable. The slopes of the regressions were compared using the "ESTIMATE" statement of the MIXED procedure. The CP content of the regrowth decreased (−2.50 percentage units/leaf on average of both treatments; $P < 0.05$), whereas for NDF content linear regression was not significant ($P > 0.01$) without differences between treatments for both fractions. The DNDF decreased in all treatments (−4.85 percentage units/leaf, average of both treatments; $P < 0.01$). The average content of CP, NDF, and DNDF was 24.1%, 51.2%, and 66.5%, average for both treatments, respectively. Defoliation frequency of a tall fescue pasture in spring did not modify the nutritional value during the regrowth. The variations observed were exclusively due to the accumulation of leaves in the plants.

Key Words: tall fescue, defoliation regime, nutritive value

2216 Assessing the effects of preservative type, dose, and storage phase on nutrient losses, heating kinetics, and microbial populations of high-moisture alfalfa hay. J. Poblete*[1], B. Escudero-Alejos[1], M. Chusho-Guevara[1], D. Zamudio-Ayala[1], A. P. Jimenez[1], K. Nishimwe[1], A. S. Mindiola[1], M. V. Cardoso[1], C. Knight[2], S. Annis[3], J. Garzon[2], and J. J. Romero[1], [1]*Animal and Veterinary Science, University of Maine, Orono, ME,* [2]*University of Maine Cooperative Extension, Orono, ME,* [3]*School of Biology and Ecology, University of Maine, Orono, ME.*

Our objective was to determine the effects of preservative type (TY), dose (DO), and storage phase (SP) on preserving alfalfa hay baled at high moisture (29.18%). The TY were 1) propionic acid (61% vol/vol; PRP); 2) PRP buffered with 5% vol/vol NH_3OH (AMP); and 3) Fresh Cut® Plus, a commercial mixture of organic acids (including propionic and acetic acids) buffered with NH_3OH (FC). These were applied at 0%, 0.25%, and 0.5% (fresh basis) on a volatile organic acid equivalent basis (i.e., propionic plus acetic acids) at 2 SP (d 0 and 78). Data were analyzed as a randomized complete block design (5 blocks) with a 3 TY × 3 DO × 2 SP factorial arrangement using the PROC GLIMMIX of SAS v.9.4. Blocks were 5 areas within the field. Differences were declared at $P < 0.05$. After 78-d storage, no differences in DM losses were observed. The DM increased from d 0 to 78 (70.82 to 85.92 ± 0.37%; SP). For all TY at d 0, as the DO increased (0% to 0.5%), pH decreased linearly (6.32 to 5.72 ± 0.05; SP × DO), but it increased quadratically at d 78 (7.25 to 7.36 ± 0.15; SP × DO). After 78-d storage, for all TY, the visual moldiness score (0–10 ranking) linearly increased from 0% to 0.5% DO (5.1 to 7.8 ± 0.44; DO). For all TY and SP, 0.5% DO had

lower mold counts than 0.25% and 0% (5.96 vs. 6.27 and 6.26 ± 0.13 log cfu/g fresh, respectively) in malt extract agar (DO). For all TY and SP, the 0.5% and 0.25% DO had lower yeast count than 0% DO (6.25 and 6.15 vs. 6.52 ± 0.13 log cfu/g fresh, respectively) in malt extract agar. During the 78 d of storage, as DO increased (0% to 0.5%), the maximum temperature in FC decreased linearly (32.68 to 29.10 ± 0.87°C, respectively; TY × DO) and it decreased quadratically in PRP and AMP (32.68 to 27.54 ± 0.37°C and 32.67 to 27.52 ± 0.42°C, respectively; TY × DO). For all TY, the 0.5% DO had higher aerobic stability than 0.25% and 0% (41.67 vs. 3.30 and 1.50 ± 13.82 h, respectively; DO) during storage. In conclusion, applying buffered TY (AMP and FC) at 0.5% DO reduced heating and microbial counts in high-moisture alfalfa hay without affecting DM losses.

Key Words: propionic acid, ammonium hydroxide, spoilage

Physiology and Endocrinology 2

2217 Oxidized linoleic acid metabolites modulate proliferation, adipogenesis, and lipogenesis in bovine adipocytes. G. A. Contreras*, M. Chirivi, and U. Abou-Rjeileh, *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Oxidized linoleic acid metabolites (OXLAM) are products of lipolysis in adipocytes. Among OXLAM, 9-, 12-, and 13-hydroxy-octadecadienoic acids (HODE) are more abundant, especially during the periparturient period when their biosynthesis peaks in adipose tissue (AT) due to intense lipolysis. In monogastrics, 9- and 13-HODE can promote the formation of new adipocytes and increase lipogenesis. However, the effects of 12-HODE are unknown. Our goal was to evaluate the adipogenic and lipogenic potential of 9-, 12-, and 13-HODE in primary bovine adipocytes. Adipocyte progenitors (AP) were isolated from subcutaneous AT ($n = 6$, nonlactating nongestating multiparous Holstein dairy cows) by nonenzymatic isolation. AP were expanded and induced to differentiate into adipocytes using standard adipogenic media. During differentiation, AP were exposed (4 d) to 9-, 12-, or 13-HODE at low (L9 = 5, L12 = 0.1, L13 = 5 n*M*) and high (H9 = 100 n*M*, H12 = 10 n*M*, H13 = 100 n*M*) concentrations, or adipogenic media only (control). Adipogenesis was evaluated by using the live cell imaging IncuCyte S3 system using a neutral lipid stain (Bodipy 493/503) and a nuclear stain (NucSpot Live). Adipogenesis efficiency was calculated as the percentage of differentiated adipocytes relative to the total cell count, using ImageJ software. Triglyceride accumulation was evaluated with Triglyceride-Glo assay (Promega). Compared with control, H9 tended to increase adipogenesis efficiency (49.44 vs. 59.21 ± 3.21%; $P = 0.06$). Compared with control, L9, H9, and L12 increased triglyceride accumulation by 32.6%, 42.9%, and 41.8%, respectively ($P < 0.05$). No differences were observed between control and H12, L13, and H13 ($P > 0.20$). These results suggest that the biosynthesis of 9- and 12-HODE during lipolysis in AT may function as a negative feedback loop, enhancing the re-esterification of free fatty acids released by lipases. The specific role of 13-HODE during lipolysis has yet to be determined.

Key Words: adipogenesis, adipocytes, linoleic acid

2218 Branched-chain amino acids and branched-chain keto acid supplementation altered abundance of proteins in various metabolic pathways in primary bovine hepatocytes. J. R. Daddam*, C. Collings, M. Sura, and Z. Zhou, *Animal Sciences, Michigan State University, East Lansing, MI.*

During early lactation, accumulation of triglycerides (TG) in hepatocytes render dairy cows at high risk of fatty liver disease and consequent compromised lactation performance. Previous results from our group demonstrated that branched-chain AA (BCAA) supplementation improved lactation performance whereas branched-chain keto acid (BCKA) supplementation decreased liver TG in fresh cows. The objective of this study was to determine the proteomic changes in response to BCAA and BCKA supplementation in primary bovine hepatocytes. Primary bovine hepatocytes (PLEH) were cultured in customized media mimicking cow plasma AA and FA profile on d 4 postpartum with BCAA (133% of physiological circulating BCAA concentration), BCKA (equal molar amount as BCAA), or CON (100% of physiological BCAA). After 72 h, PLEH were harvested for iTRAQ-based proteomics with multi-dimensional LC-MS. A total of 963 proteins were identified and quantified. Compared with CON, BCAA and BCKA treatments result in 204 and 130 differentially abundant proteins ($P \leq 0.05$ and fold change >±1.5). Pathway analysis revealed that BCAA supplementation leads to >50 enriched pathways (FDR <0.05), including various pathways regulating AA metabolism (biosynthesis of AA; arginine and proline metabolism; and alanine, aspartate, and glutamate metabolism), Pentose phosphate pathway, oxidative phosphorylation, and Apoptosis. In contrast, only TCA cycle, ribosome, and lysosome pathways were enriched in response to BCKA supplementation. Overall, these results suggest that BCAA and BCKA supplementation altered various aspects of hepatic AA, fatty acid, and carbohydrate metabolism, which likely contribute to the favorable changes in lactation performance and liver TG concentrations observed previously. Future work should determine the specific changes in key genes and proteins in these pathways.

Key Words: branched chain amino acids, branched chain keto acids, primary bovine hepatocytes

2219 Mammary blood flow and lactational response of the udder to air inflation. E. M. Shangraw* and T. B. McFadden, *University of Missouri, Columbia, MO.*

Prolonged intervals between milkings reduce milk production. As the milking interval increases, accumulation of milk causes intramammary pressure (IMP) to exceed 40 mmHg. We tested the hypothesis that elevated IMP would reduce milk yield and mammary blood flow. For milk yield responses, udder halves of 4 cows were inflated with air via teat cannula to low (20–30 mmHg) or high (40 mmHg) IMP in both ipsilateral glands immediately after morning milking; contralateral glands were cannulated but not inflated. After treatment, the glands remained inflated for 12 h and then cows were remilked. All quarters were milked every 12 h. To monitor quarter SCC, we collected milk samples from bulk milk and strip milk samples 6 h after inflation. For mammary blood flow responses, all 4 glands of 4 cows were inflated to 60 mmHg shortly after milking. Mammary blood flow through both pudendoepigastric trunks was measured by transrectal color Doppler ultrasound immediately after milking but before inflation, 10 min after inflation, and 2 h after inflation. In milk yield studies, all quarters remained culture negative 24 h after inflation and no clinical mastitis was observed. There was no difference ($P > 0.2$) in milk yield between control and low IMP treatment. After 12 h, high IMP treatment caused a 23% reduction in milk yield relative to control quarters ($P = 0.06$), and milk yields remained lower ($P < 0.05$) compared with preinflation until 48 h after inflation. After both low and high IMP treatment, SCC increased ($P < 0.001$) by 6 h after inflation compared with control glands. The SCC recovered to <250,000 cells/mL in 3 or 5 d for low and high treatment, respectively. In mammary blood flow studies, IMP declined over 2 h but udders remained distended and palpably hard. There was no difference in blood flow between left- and right-sides. Relative to preinflation, blood flow tended to be higher after 10 min ($P = 0.06$) and had increased by 50% after 2 h ($P < 0.001$). Heart rate did not change over time. Results suggest that negative effects of elevated IMP on milk yield are not mediated by reduced blood flow to the udder.

Key Words: milk stasis, insufflation, Doppler sonography

2220 Physiological and productive responses to a natural short-term heat wave in Holstein Friesian and Brown Swiss lactating cows. P. De Palo*[1], E. Trevisi[2], A. Aloia[1], U. Bernabucci[3], C. Lecchi[4], V. Landi[1], and A. Maggiolino[1], [1]*Department of Veterinary Medicine,*

University of Bari, Bari, Italy, [2]Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, Piacenza, Italy, [3]Department of Agriculture and Forests Sciences, University of Tuscia, Viterbo, Italy, [4]Department of Veterinary Medicine and Animal Science, Università degli Studi di Milano, Lodi, Italy.

The aim of the study was to assess the different adaptative physiological and productive responses of Brown Swiss (BS) and Holstein Friesian (HF) dairy cows to a 4-d heat wave. Forty multiparous BS (n = 20) and HF (n = 20) dairy cows were involved, balanced for parity, DIM, ECM yield and BCS. All the animals were kept in the same barn and fed with the same diet. The barn was provided with sprinklers and fans that were switched off at the start of the trial (and at the beginning of the first day of the heat wave) and remained switched off for 4 consecutive days. Barn temperature-humidity index (THI) was monitored every 5 min with 4 data loggers. Before the switching off, THI was always under 68. Respiration rate (RR), rectal (RT), skin, eye, muzzle, and vaginal temperatures were collected at 0400, 1500, and 2000 h of each trial day. Blood samples were collected the moment before turning off the cooling systems, before the heat wave, at 0400 h (the thermal comfort condition [TC]; THI = 63.4). Blood samples were also collected at the end of the heat wave, fourth day, at 1500 h (the heat stress condition [HS]; THI = 81.3). Serum biochemical profile, plasma oxidative profile and antioxidant capacity and some inflammatory patterns were detected. Milk yield (MY) and some milk qualitative patterns were recorded at each milking session (0500 and 1700 h). A GL Model was applied, considering as fixed effects the thermal-hygrometric environmental condition and the breed. The RT increased each day from 0400 to 1500 h in both breeds, and HF showed greater values ($P < 0.01$) during the whole trial. While these changes were more marked in HF, it is noteworthy that they are capable of more effective recovery and return to physiological levels during nighttime hours. No differences between breeds were observed for RR ($P > 0.05$). The BS did not change MY, whereas in HF cows, MY decreased ($P < 0.05$) day by day. However, metabolic and inflammatory patterns did not show differences among the breeds. These results emphasize the necessity for additional research to comprehensively grasp the underlying differential mechanisms between the breeds.

Key Words: heat stress, Brown Swiss, Holstein Friesian

2221 Genomic predicting transmitting ability for cow fertility is associated with resumption of cyclicity and early pregnancy in Holstein cows.

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Our objectives were to evaluate the associations between genomic predicting transmitting ability (GPTA) for cow fertility with ovarian responses and conceptus development in Holstein cows. A total of 304 cows were genotyped with a HD50K SNP chip. The GPTA for cow conception rate (GCCR) and daughter pregnancy rate (GDPR) were assessed before 12 mo of age. The GCCR and GDPR were highly correlated (r = 0.90; $P < 0.001$); thus, GCCR was used. The GCCR was centered around the mean, and cows were categorized as low (GCCR ≤0; L-GCCR; n = 143) or high (GCCR >0; H-GCCR; n = 161) GCCR. Ovulation was synchronized, cows were inseminated, and uterine flush and conceptus were collected on d 16 after first AI. Cows were resynchronized and pregnancy evaluated 75 d after AI for 305 d postpartum. Data were analyzed by mixed-effects models fitting normal or binary distribution in SAS. Days open were analyzed by the Cox's regression in SAS. Genomic CCR was fitted either as a continuous (GCCR) or as a categorical (L-GCCR vs. H-GCCR) variable. Cows in H-GCCR had increased rate of resumption of postpartum cyclicity (AHR = 1.63; 95% CI = 1.15–2.31) that resulted in a greater proportion of cyclic cows by 40 d postpartum (Table 1). Synchronization of ovulation and diameter of the ovulatory follicle did not differ between GCCR category. Cows in H-GCCR had greater pregnancy on d 16 after first AI. For each 1 unit of centered GCCR increase, the interferon-tau (IFNT) in the uterine flush increased 1.34 ng/mL (SE = 0.71). The rate of pregnancy increased ($P = 0.02$) 13% with each 1 unit of GCCR (AHR = 1.13; 95% CI = 1.02–1.25), although the proportion of pregnant cows by 305 d postpartum did not differ between L-GCCR and H-GCCR. Genomic CCR is associated with resumption of cyclicity and early pregnancy in dairy cows.

Table 1 (Abstr. 2221). Genomic cow conception rate (GCCR) and reproduction in dairy cows

Item	GCCR ≤0 (n = 143)	GCCR >0 (n = 161)	SEM	*P*-value
Cyclic by 40 d, %	64.1	82.3	4.9	<0.01
Synchronized ovulation, %	88.7	88.4	2.9	0.92
Ovulatory follicle, mm	14.7	14.6	2.6	0.85
Pregnant d 16, % all cows	50.4	64.3	5.6	0.06
Pregnant d 16, % synchronized cows	55.1	72.0	4.7	0.02
Conceptus, cm	10.6	9.6	0.7	0.33
Flush interferon-tau, ng/mL	7.23	8.20	3.40	0.80
Pregnant by 305 d, %	72.3	79.6	3.5	0.15

Key Words: embryo, genomics, reproduction

2222 Effects of intermittent lipopolysaccharide administration and nutrient restriction on the hepatic proteome of lactating dairy cows.

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Our objectives were to evaluate the effects of chronic inflammation and subsequent nutrient restriction on the hepatic proteomic profile of lactating dairy cows. Cows (631 ± 16 kg BW; 124 ± 15 DIM) were enrolled in a study with 3 experimental periods (P). During P1 (5 d), cows were fed ad libitum, and baseline data were obtained. During P2 (7 d), cows were assigned to 1 of 2 treatments: 1) saline-infused and pair-fed (PF; 5 mL i.v. saline on d 1, 3, and 5; n = 6) or 2) LPS-infused and ad libitum-fed (LPS; i.v. *Escherichia coli* O55:B5 at 0.2, 0.8, and 1.6 µg/kg of BW on d 1, 3, and 5, respectively; n = 6). In P3 (7 d), all cows were feed restricted to 50% of P1 feed intake. On d 3 of P3, LPS cows received an LPS bolus (0.8 µg of LPS/kg of BW), and PF cows received saline. Liver biopsies were obtained on d 1 of P1 and on d 7 of P2 and P3. Hepatic protein was isolated for LC-MS/MS analysis, and Uniprot identifiers were used to obtain biological and molecular information. Relative to PF, LPS administration during P2 affected hepatic proteins with molecular and biological functions involved in immune activation, fatty acid and AA metabolism, and calcium binding ($P < 0.05$). During P2, the hepatic abundance of 26 and 33 proteins in LPS and PF cows, respectively, were differentially altered compared with P1 ($P \leq 0.05$). In P2, liver abundance of 8 proteins involved in immune activation processes (MAPK, neutrophil chemotaxis, apoptosis signaling) increased in both LPS and PF, relative to P1 ($P \leq 0.05$). During P3, LPS altered the hepatic abundance of 16 proteins relative to PF cows ($P \leq 0.05$), of which 10 decreased (involved in cell energetics, gluconeogenesis regulation, lipid transport, and oxidation) and 6 increased (involved in ATP synthesis, glycolysis). In P3, the hepatic abundance of 52 and 49 proteins in LPS and PF cows, respectively, were differentially altered compared with P1 ($P \leq 0.05$). Additionally, 27 liver proteins were mutually altered between LPS and PF cows during P3, relative to P1 ($P \leq 0.05$). In summary, LPS followed by nutrient restriction altered the abundance of hepatic proteins involved in cellular energetics, metabolism, and inflammation.

Key Words: inflammation, hypophagia, proteomics

2223 In vitro carbon flux and proteome analysis reveals α-linolenic acid ameliorates palmitic acid lipotoxicity while promoting tricarboxylic acid carbon flux.

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High circulating nonesterified fatty acids (NEFA) increase risk for fatty liver disease in peripartum cows. Palmitic acid (C16:0) is one of the most abundant NEFA, and in vitro treatment of Madin Darby Bovine Kidney (MDBK) cells with C16:0 decreased expression of genes that regulate carbon flow through the tricarboxylic acid (TCA) cycle, whereas α-linolenic acid (C18:3n-3 *cis*) prevented this effect. Therefore, we hypothesized that C16:0 modifies abundance of TCA cycle enzymes and decreases flux of carbons. The objective was to measure the flux of carbon substrates and proteome of MDBK cells incubated with different ratios of C16:0 (P) to C18:3n-3 *cis* (L): 1:0 m*M* (1P:0L), 0.75:0.25 m*M* (0.75P:0.25L), 0.50:0.50 m*M* (0.5P:0.5L), 0.25:0.75 m*M* (0.25P:0.75L), 0:1 m*M* (0P:1L), or vehicle control (BSA) for 21 h. Cells were incubated with either [U-^{13}C] pyruvate, [1-^{14}C] palmitic acid, or [U-^{14}C] lactate for 2 h. Incorporation of ^{13}C into TCA cycle intermediates and ^{14}C into CO_2 was quantified. Protein was collected from separate cultures and proteome was measured. Compared with BSA, more carbon from ^{13}C pyruvate enriched citrate M+5 in 0.5P:0.5L, and 0P:1L decreased enrichment of citrate M+5 and increased succinate M+2. 1P:0L had the lowest oxidation of [U-^{14}C] lactate to CO_2. Relative to BSA, 1P:0L, 0.5P:0.5L, 0P:1L increased abundance of 175, 110, and 153 proteins, respectively, and decreased 229, 177, and 213 ($P < 0.05$). Functional annotation analysis of proteins increased by 1P:0L indicated enrichment of TCA cycle, fatty acid degradation, and cellular stress. Proteins increased by 0.5P:0.5L enriched protein folding and decreased pyruvate metabolism. 0P:1L showed increased enrichment of DNA replication and decreased enrichment of lipid metabolism. Flux measurements indicate more efficient carbon flux in C18:3n-3 *cis*, but proteome analysis indicates C16:0 increased abundance of TCA cycle and fat metabolism enzymes, whereas C18:3n-3 *cis* increased proteins that abate cellular stress. Findings support in vivo studies to optimize circulating ratio of C16:0 to C18:3n-3 *cis* to promote carbon flux to reduce fatty liver risk.

Key Words: palmitic acid, flux, proteome

2536 Tumor necrosis factor alpha (TNFα) as a marker for intestinal permeability in dairy cattle.

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Stress can negatively affect gastrointestinal tract (GIT) function (e.g., cause microbial dysbiosis, increase gut permeability), resulting in an inflammatory response, compromised animal health, and partitioning of nutrients away from productive functions. We evaluated the effects of long-term (21 d) of oral aspirin administration (200 mg/kg of BW per day) on the intestinal permeability of Holstein and Jersey heifers using the urinary excretion of cobalt from the nonabsorbable marker, Co-EDTA and serum analysis of markers for inflammation. Six Jersey (10.4 ± 0.3 mo of age, 269 ± 21 kg BW) and 6 Holstein (10.5 ± 0.9, 362 ± 25 kg BW) heifers were each randomly allotted to receive either 0 or 200 mg aspirin/kg of BW per day for 21 d. Blood samples were collected on d 21 and heifers then were dosed orally with gelatin capsules containing 40 g of Co-EDTA using a balling gun. Urine was sampled at 0, 1, 3, 6, 8, 12, 18, 24, 30, and 36 h postdosing, frozen, and subsequently analyzed for cobalt and creatinine. Serum was evaluated by ELISA for markers of inflammation and intestinal permeability; TNFα, lipopolysaccharide binding protein (LBP), and intestinal fatty acid binding protein (IFABP), respectively. Serum concentrations of markers were analyzed by ANOVA to determine the effects of aspirin, breed, and the interaction of aspirin with breed. Aspirin treatment resulted in an increase in TNFα ($P = 0.04$) and IFABP ($P = 0.02$), but not LBP ($P = 0.06$). Analysis of covariance indicated that serum TNFα ($P = 0.009$) but not LBP ($P = 0.6$) or IFABP ($P = 0.9$) increased linearly with increasing urinary Co excretion. These data indicate that TNFα plays an important role in intestinal permeability in dairy cattle and is an effective marker for gut inflammation.

Key Words: aspirin, tumor necrosis factor-α, leaky gut

Production, Management, and the Environment 2

2224 Dynamic pricing based on shelf-life can reduce fluid milk waste: Evidence from laboratory and grocery store experiments. A. Trmcic*[1], S. Desai[1], L. Kemmerling[1], N. Martin[1], A. Adalja[2], and M. Wiedmann[1], *[1]Department of Food Science, Cornell University, Ithaca, NY, [2]SC Johnson College of Business, Cornell University, Ithaca, NY.*

Food waste has increasingly become an issue both globally and nationally, and fluid milk represents a sizeable part of this issue. Past studies have identified consumers' willingness to pay (WTP) for additional days of milk shelf-life, presenting an opportunity to use shelf-life-based dynamic pricing to improve inventory management and reduce food waste at the retail level. A series of incentivized laboratory-based auction experiments were performed to quantify consumers' WTP for additional shelf-life of milk. A total of 152 participants were included in 9 in-person experiments in which participants were presented with several different purchase options related to price and shelf-life and asked to engage in bidding auction using the Becker–DeGroot–Marschak auction method. Based on the findings from the laboratory experiments, a 2-wk field experiment was performed at a local supermarket. In wk 1, half-gallon milk was arranged on the shelves based on 3 fat levels and 3 categories of remaining shelf-life, but no price changes took place. In wk 2, the milk remained organized as in wk 1, but dynamic (shelf-life-based) pricing was implemented. The number of half-gallon milk units sold each day was recorded for each product category. Laboratory-based experiments determined consumers are willing to pay a price premium for each additional day of shelf-life; however, the amount they are willing to pay as premium diminishes with each additional day of shelf-life. Censored regression models based on the experimental data indicated an average marginal WTP of $0.37 for one additional day of half-gallon milk shelf-life. The field experiment confirmed that consumers' WTP for longer shelf-life can compensate for price reduction of product with shorter shelf-life, resulting in more product with reduced shelf-life being sold while maintaining a revenue-neutral or even improving outcome for the retailer. The work provides a foundation for introducing new dairy retail pricing models that will reduce food waste, promote development of new digital solutions, and revolutionize how the dairy industry operates.

Key Words: food waste, fluid milk, price

2225 Withdrawn.

2226 Withdrawn.

2227 Assessment of heat stress response of dairy cows previously stress phenotyped using bacterial lipopolysaccharide endotoxin. N. R. Komadan*[1], U. K. Shandilya[1], Á. Cánovas[1], B. A. Mallard[2], and N. A. Karrow[1], *[1]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

Heat stress can affect gut barrier integrity, leading to leaky gut and elevated enteric bacterial LPS endotoxin levels in the circulation. This LPS can induce systemic inflammation manifesting as the acute-phase response, which includes a febrile response that may compromise heat dissipation from the body during heat stress. We hypothesize that animals resilient to LPS-induced inflammatory stress will be more resilient to heat stress, and that LPS contributes to heat stress pathology. To test this hypothesis, peripheral blood mononuclear cells (PBMC) were isolated from dairy cows previously stress phenotyped based on their 4-h serum cortisol response to *Escherichia coli* LPS challenge (400 µg i.m). After stress phenotyping a population of dairy calves (n = 140), 10 high (HSR; +1 SD from the mean, >956.0 nmol/mL), 10 middle (MSR; ±SD around the mean, 573.4 nmol/mL), and 10 low (LSR; −1 SD from the mean, <190.8 nmol/mL) stress responding animals were selected for the study. The PBMC were later isolated and subjected to heat shock (HS) ex vivo at 42°C for 4 h and then returned to 37°C where the control plates remained. Cell viability and proliferation were assessed, and total RNA was extracted and culture supernatant collected to assess candidate gene expression and cytokine secretion, respectively. There was a significant ($P \leq 0.05$) increase in apoptosis of PBMC in the HSR group as compared with the LSR and MSR groups. However, there was a significant decrease ($P \leq 0.01$) in necrosis of the MSR and HSR groups. The proliferation of PBMC from HSR cows was attenuated compared with MSR and LSR cows. The LSR cows displayed a cytokine profile significantly ($P \leq 0.01$) favoring a T-helper (Th)-1 immune response when compared with MSR and HSR cows. Last, there was a significant increase ($P \leq 0.001$) in gene expression of *HSP90* and *HSP70* in the MSR group compared with the HSR and LSR groups. Overall, these results suggest that variable stress responding cow PBMC respond differently to HS, and future research is warranted to investigate if this phenotype can be used to improve HS resilience of dairy cattle through selective breeding.

Key Words: heat stress, cortisol, LPS

2228 Late-gestation heat stress alters placental vasculature in dairy cows. L. T. Casarotto*[1], H. N. Jones[2], P. Chavatte-Palmer[3], and G. E. Dahl[1], *[1]University of Florida, Gainesville, FL, [2]University of Florida College of Medicine, Gainesville, FL, [3]Université Paris-Saclay, Jouy-en-Josas, France.*

Global climate change is significantly impacting productivity of the dairy sector. Previous research indicates that heat stress during the last 6 to 8 wk of gestation reduces the number of placental cotyledons but increases their size, along with a decrease in calf weight at birth consistent with placental dysfunction. This study aimed to understand the mechanisms involved in vascularization by quantifying placental cotyledonary structures through histology analysis. Pregnant Holstein cows were randomly assigned to either cool (CL) or heat stress (HT) treatment during their dry period of 54 ± 5 d. The CL treatment group had access to the shade of the barn, forced ventilation via fans, and water soakers, whereas the HT group had access to the shade of the barn only. Whole placentas were collected within 4 ± 1.5 h after birth (n = 10/treatment). The cotyledonary tissues were washed in sterile saline, trimmed, and dissected; fixed in 4% PFA + 2.5% PVP-40 for 48 h; washed for 48 h in PBS; and stored in 70% ethanol until processing at 4°C. The tissues were paraffin embedded, sectioned at 5 µm, and then stained with H&E and Picro Sirius Red before imaging. Three random fields of view across 1 slide per stain from each cow:calf placental unit were analyzed with a threshold on ImageJ software for each cotyledon sample. The data were analyzed using the GLIMMIX procedure in SAS, with the ID, treatment, and parity as fixed effects. Cotyledons exposed to HT during late gestation had a higher vascular area compared with the CL group with H&E staining (43.1% vs. 31.8 ± 10.4% of total area; $P = 0.04$). However, no significant differences were found between the groups in the percentage of extra-cellular-matrix area with Picro Sirius Red staining (47.9% vs. 46.9 ± 9.6%; $P = 0.87$), indicating that structural components were unaffected by heat stress. In conclusion, our findings suggest that heat stress induces changes in vasculature, likely associated with thermoregulation. Further investigation focusing on the interhemal barrier and fetal-placental vasculature is warranted to elucidate the effects of these modifications on placental transfer and fetal development

Key Words: hyperthermia, placenta, fetal development

2229 Effects of thermal range on animal physiology, intake, nutrient digestibility, and performance of dairy calves.

M. B. Gomes[1], S. G. Coelho[1], L. F. M. Neves[1], J. P. Campolina[1], and M. M. Campos*[2], *[1]Departamento de Zootecnia, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, [2]Empresa Brasileira de Pesquisa Agropecuária—Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil.*

Farms located in continental areas are more susceptible to temperature fluctuations, making the rearing phase of dairy calves more challenging due to the detrimental effects of daily temperature variations. Thus, the aim of this study was to evaluate animal physiology, intake, performance, and digestibility in dairy calves subjected to thermal amplitude in a climate chamber with controlled temperature and humidity. Thirty-four Holstein calves were divided into 2 treatments starting at d 1 of life: control (CON, temperature-humidity index [THI] of 66 during 24 h, n = 17) and thermal range (TR, THI of 84 for 9 h + THI of 66 for 6 h + THI of 54 for 9 h, n = 17). Animals were exposed to treatments 24 h from 1 to 28 d of age. Respiratory rate (RR), heart rate (HR), rectal temperature (RT), intake, and health were evaluated daily. Performance was assessed weekly and rumen parameters biweekly. Digestibility trials were conducted at 9 to 12 and 23 to 26 d of age. Data were analyzed using R software with ANOVA and 95% confidence in a completely randomized design, where treatment was the main plot, week was the subplot, and hour was the sub-subplot. The TR group increased the RR by 82% ($P < 0.05$) during THI = 84 exposure. The HR was similar between treatment groups but was affected by week and THI exposure time ($P < 0.05$). The RT was higher for the TR group, averaging 0,49°C at THI exposure 84 ($P < 0.05$). There were no treatment effects on feed and nutrient intake ($P > 0.05$). However, water intake was 32% higher in the TR group ($P < 0.01$), with more pronounced differences on wk 3 and 4. Apart from a higher C2:C3 proportion for the TR group ($P = 0.01$), ruminal parameters were similar. The TA group had lower ether extract digestibility ($P = 0.01$) in the first digestibility trial and higher fecal nitrogen on the second digestibility trial ($P = 0.03$). No differences were observed for final weights ($P = 0.96$), ADG ($P = 0.10$), withers height ($P = 0.96$), chest circumference ($P = 0.99$), and width ($P = 0.47$). Thus, calves subjected to thermal range show an increased RT and RR but no differences in intake or performance.

Key Words: preweaning, temperature, thermal variation

2230 Evaluating effects of heat stress on the efficacy of robotic milking systems.

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The objective of this study was to examine the associations between the efficiency metrics of robotic milking systems, milk yield within the robots, and temperature-humidity index (THI) in robotic milking operations. This retrospective study was conducted using data collected from March to October 2023, at a commercial dairy farm in Dublin, Texas, USA, milking 1,500 cows with 22 robots in a batch milking system. Temperature and humidity data were collected from the nearest weather station. Daily idle time per robot was compared with different levels of daily THI (low <72 THI, moderate 72 to 79 THI, and high >79 THI). Statistical analysis was conducted using ANOVA and associated post hoc tests in SAS 9.4. The average robot idle time during the study period was 220 ± 1.27 min per day per robot. Robot idle time (minutes; mean ± SE) was higher in the high THI days compared with low THI days ($P < 0.05$; 192 ± 1.88, 231 ± 1.74, 280 ± 2.52 for low, medium, and high groups, respectively). The idle time per robot was lower in morning milking compared with evening milking ($P < 0.05$; 115 ± 0.76, 106 ± 0.75 min/12 h for morning and evening milkings, respectively). Robot idle time was not associated with robot direction or robot location (front, mid, and back) in the barn. Average milk yield per cow per milking was also reduced with increase in THI ($P < 0.05$; 7.4 ± 0.01, 7.8 ± 0.01, 9.09 ± 0.01 kg, for high, moderate, low THI levels, respectively). The average milk flow rate (kg/min) was also reduced during heat stress ($P < 0.05$; 0.89 ± 0.01, 0.93 ± 0.01, and 0.99 ± 0.01 for high, moderate, and low THI groups, respectively). Furthermore, the average duration of milking (seconds) was also reduced in heat-stressed cows ($P < 0.05$; 315.1 ± 0.25, 323.0 ± 0.17, and 334.4 ± 0.14 for high, moderate, and low levels of THI, respectively). The data from this preliminary exploratory study demonstrate a relationship between elevated THI values and decreased milking efficiency metrics in robotic milking systems, suggesting that heat stress conditions have a detrimental effect on robot efficiency due to the effect on cow behavior.

Key Words: heat stress, robotic milking, efficiency

2231 Evaluation of voluntary milking system metrics in a small North Carolina Jersey herd.

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The adoption of the voluntary milking systems (VMS) in the Southeast United States is increasing as new generations come onto the farm and labor availability lessens. A VMS was recently installed at North Carolina Agricultural & Technical State University, the only operating dairy farm at an 1890s land-grant institution. The objective of this study was to evaluate the data gained from the VMS including milk conductivity, number of visits, concentrate consumed, and milking time on a teat, cow, and herd basis across seasons for a small Jersey herd with access to pasture. Primiparous and multiparous Jersey cows (n = 30) were equipped with an radio-frequency ID, electronic ear tag, to gain access to the VMS and connect data gained. Cows were fetched twice a day at 0600 and 1400 h to ensure all cows received at least 2 milkings. The VMS was left open in between these fetch times to allow free access for the cows while in the free-stall barn for 12 h or pasture for 12 h. Data obtained from the VMS in daily offloads included milk conductivity, number of visits, concentrate consumed, and milking time on a teat, cow, and herd basis with time stamps and days in milk for all milkings and major events such as breeding and mastitis treatments. The number of visits to the VMS increased 3 mo after startup to an average of 2.3 ± 0.6 (mean ± SD) visits per day. Concentrate consumed varied by cow and stage of lactation with an average of 1.84 ± 0.98 (Mean ± SD) per visit to the VMS. Further evaluation of milk conductivity and VMS visits could result in increased alerts for attention cows.

Key Words: automated milking system, voluntary milking, pasture

2232 Seasonal effect on growth of dairy calves in the southeastern United States. A. M. Roper*[1], R. M. Orellana Rivas[1], T. N. Marins[1], J. Gao[1], C. G. Savegnago[1], P. Melendez[2,4], J. K. Bernard[3], and S. Tao[1], *[1]Department of Animal and Dairy Science, University of Georgia, Athens, GA, [2]Department of Population Health, College of Veterinary Medicine, University of Georgia, Tifton, GA, [3]Department of Animal and Dairy Science, University of Georgia, Tifton, GA, [4]Department of Veterinary Clinical Services, City University of Hong Kong, Hong Kong SAR.*

Our aim was to evaluate seasonal effects on growth of dairy calves in Georgia. Holstein calves (n = 48/season) from UGA research dairy located in Tifton, Georgia, were enrolled at birth during summer (June to August, BW = 40 ± 4 kg) and winter (November to January, BW = 42 ± 4 kg). Similar general management and feeding practices were applied to calves in both seasons. Calves were housed in individual polyethylene hutches with sand as bedding material. Starting at 2 d of age (DOA), calves were fed a 26:17 milk replacer (MR) and calf starter (CS) was offered ad libitum. Weaning started at 43 DOA by reducing MR by 50% until 49 DOA when MR feeding ceased. Calves remained enrolled until 63 DOA. Calf starter intake was recorded daily. Ambient temperature (AT) and relative humidity (RH) were recorded hourly. Rectal temperature and respiration rate were assessed 3× per week, and BW was measured weekly. The mixed procedure of SAS was used to analyze repeated measures data. Season, time, and their interactions were included as the fixed effect. The coefficient of variation (CV) of hourly AT within a day was calculated as a proxy of within-day AT variation. The CV of daily average AT within week was used as the proxy of day-to-day AT variation. The correlation analysis between ADG and environmental parameters was performed using GLM procedure. Summer months had greater AT (27 vs. 13°C) but similar RH (83% vs. 81%) compared with winter, respectively. Calves in summer had greater ($P < 0.01$) rectal temperature and respiration rate than winter calves. Seasons had no effect on CS intake before weaning, but summer calves had greater CS intake at 9 wk of age than winter calves (season × time: $P < 0.01$). No seasonal effect was observed for BW, but summer calves had greater ADG at wk 3, 5, and 9 of age (season × time: $P < 0.01$). Among environmental parameters, preweaning ADG is negatively correlated with within day AT variation during winter ($P < 0.01$, $r^2 = 0.11$) and negatively correlated with day-to-day AT variation during summer ($P < 0.01$, $r^2 = 0.06$). In conclusion, in Georgia, the within-day and day-to-day AT variations significantly affect preweaning calf growth.

Key Words: season, temperature variation, calf

2233 The effects of lunar phases on fertility in dairy cattle. M. A. Schatte*, T. Grandin, P. Pinedo, and K. Keller, *Colorado State University, Fort Collins, CO.*

Myths and old farming legends have circulated the belief that the full moon affects livestock reproduction. To assess this effect in dairy cattle, we looked at 72,353 records from 2019 to 2021 at an organic dairy farm in Colorado. These records included lactation number, AI date, and pregnancy result. The AI date was categorized into season and lunar phases. Lunar phases were separated into 4 equal categorizations: new moon, first quarter, full moon, and third quarter. The objective of this study was to identify what phase during the lunar cycle was the most probable for pregnancy to occur. R studio was used to complete this objective by comparing the pregnancy result of lunar phases through logistic regression. The week of the new moon was found to have the least probable chance for pregnancy to occur ($P < 0.0001$). The week of the full moon was found to have the highest probability of pregnancy at 43.3% more likely than the week of the new moon ($P < 0.0001$). The week of the first quarter closely followed this result at 39.0% more likely compared with the week of the new moon ($P < 0.0001$). Using an analysis of deviance, the breeding season was found to have a statistically significant effect on pregnancy ($P < 0.0001$). Estimated marginal means were found and showed that winter had the highest probability of pregnancy at 52.0% and summer had the lowest probability of pregnancy at 39.9%. This analysis of records suggests an optimal time to breed toward the light half of the month (first quarter to full moon to third quarter) and to avoid breeding the week of the new moon if possible.

Key Words: lunar cycle, dairy cows, conception rate

Reproduction 2

2234 ISGylation of proteins in the ovine corpus luteum may function in stabilizing steroidogenesis for continued production of progesterone during pregnancy. A. Guzeloglu*[1], J. V. Bishop[1], H. Van Campen[1], J. S. Davis[2], and T. R. Hansen[1], *[1]Animal Reproduction and Biotechnology Laboratory, Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, [2]VA Medical Center and Department of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, NE.*

Interferon stimulated gene 15 (ISG15) is a ubiquitin paralog that is induced by pregnancy and conceptus-derived interferon-tau (IFNT) and becomes covalently conjugated (ISGylated) to targeted intracellular proteins in the endometrium and corpus luteum (CL). It was hypothesized that determination of the identity of ISGylated proteins would lead to inference on function in the CL. The CL was collected from nonpregnant ewes (n = 4; not exposed to rams) on d 10 of the estrous cycle (EC) or on d 17 of pregnancy (n = 4; presence of conceptus) to ensure that both groups had high circulating progesterone concentrations. Protein extracts from CL were immunoprecipitated by using rabbit anti-bovine ISG15 polyclonal antibody. Immunoprecipitated proteins were loaded on SDS-PAGE and gel fragments containing proteins above 50 kDa were subjected to in-gel trypsin digestion and LC-MS/MS. Proteins (n = 372) with affinity to the anti-ISG15 antibody (ISGylated conjugates) were identified based on the following criteria: 1) adjusted P-value = 0.05 at 2-fold change or greater in pregnant d 17 compared with d 10 EC CL (288 proteins) and 2) proteins that were present only in the CL from pregnant (63 proteins) or EC (21 proteins) ewes. Hierarchical and principal component analysis revealed that ISGylated proteins were clustered into distinctive populations in pregnant compared with EC CL. By using ingenuity pathway analysis, 274 proteins were assigned into specific pathways. The top 4 ranked canonical pathways were ISG15 antiviral mechanism, cholesterol biosynthesis, activation of gene expression by sterol regulatory element binding transcription factor, and interferon α/β signaling. It is concluded that pregnancy activated type I IFN pathways and ISGylation in the CL. ISGylation appears to be associated with steroidogenesis that may contribute to sustained production of progesterone during pregnancy.

Key Words: corpus lutem, pregnancy, interferon stimulated gene 15

2235 Unveiling putative causal variants for stillbirth in Holstein dairy cattle. P. A. S. Fonseca*[1,2], G. A. de Oliveira Jr.[2], C. F. Baes[2], F. Schramm Schenkel[2], and A. Cánovas[2], *[1]Departamento de Producción Animal, Facultad de Veterinaria, Universidad de León, León, Spain, [2]Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, Canada.*

Reduced reproductive performance is a primary cause of economic losses and indicates animal welfare concerns in dairy farming, with stillbirths significantly contributing to these issues. Identifying causal variants for stillbirths offers the potential for enhancing management and breeding strategies, thereby mitigating this adverse gestation outcome. Here, we imputed genomic coordinates of 41 haplotypes linked to stillbirth in Holstein cattle, which were previously identified by our research group, to sequence level. The identified variants were fit to a repeated-measures linear model to evaluate their association with 50,541 birth records (frequency of 11.47% stillbirth cases) from 10,570 cows (only cows with 3 or more birth records), revealing 1,744 significantly associated single nucleotide polymorphisms after a 5% false-discovery rate adjustment. To focus on novel causal variants, intergenic and intronic variants and those with higher homozygosity frequency for the alternative allele were filtered out, resulting in 100 variants across 25 genes on BTA 1, 14, 17, and 25. The identified putative causal variants located in functional candidate genes, including *FAM110B*, *PCMTD1*, *SDCBP*, *PXN*, *TBL3*, *ZNF598*, *NOXO1*, and *TSC2* genes, crucially regulate biological processes related to embryonic development and perinatal lethality. Additionally, previous associations of these genomic regions and fertility-related traits were identified by annotating QTL records available in the Cattle QTLdb. These findings highlight potential causal variants for stillbirth in Canadian Holstein cattle, offering avenues for functional investigations to deepen our understanding and develop strategies for reducing stillbirth frequency in this population.

Key Words: causal, variants, stillbirth

2236 Reproductive performance and herd removal of lactating dairy cows with anatomical defects affecting the cranial vagina and cervical ostium. M. B. Ugarte Marin*[1], M. L. Newman[1], M. E. Hernandez[1], T. D. Gonzalez[1], C. Rouillon[2], E. Schmitt[2], T. Allard[3], N. Bliznyuk[4], K. N. Galvão[1], and R. S. Bisinotto[1], *[1]Department of Large Animal Clinical Sciences, D. H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL, [2]IMV Technologies, L Aigle, France, [3]IMV Imaging, Angoulême, France, [4]Department of Agricultural and Biological Engineering, Biostatistics and Statistics, University of Florida, Gainesville, FL.*

Our objectives were to evaluate the prevalence of cervical and vaginal conditions in dairy cows using a digital vaginoscope and its association with the risk of receiving AI, pregnancy outcomes, reproductive ineligibility, and risk of leaving the herd. Lactating Holstein cows (n = 3,618) were evaluated from 0 to 7, 8 to 21, 22 to 35, 36 to 50, and 51 to 70 DIM in a cross-sectional study. Anatomical anomalies (i.e., adhesions, double cervical ostium, masses, scar tissue, urovagina and vaginal septum), cervical fold definition, bruising, and cervical prolapse were evaluated. Vaginal discharge (VD) was scored (0: no discharge; 1: clear mucus; 2: flecks of pus; 3: mucopurulent with <50% pus; 4: mucopurulent with = 50% pus; 5: watery brownish/reddish). Metritis was defined as VD 5 within 21 DIM and purulent vaginal discharge (PVD) as VD = 3 after 21 DIM. Independently of DIM, 5.6% of cows presented anatomical anomalies. Binary outcomes were analyzed using Fisher's exact test and logistic regression. Metritis prevalence was 5.1% (0–7 DIM) and 7.4% (8–21 DIM). Prevalence of PVD was 11.9% (22–35 DIM), 8.5% (36–50 DIM), and 6.3% (51–70 DIM). Prevalence of cervical prolapse ranged from 52.3% to 67.7% among DIM categories. Proportion of cows with well-defined cervical folds increased ($P < 0.01$) and bruising decreased ($P < 0.01$) as lactation progressed. Cows with urovagina tended to ($P = 0.07$) have smaller odds of becoming pregnant by 305 DIM (61.5% vs. 78.3%; AOR = 0.47, 95% CI = 0.21–1.06) and had greater odds ($P = 0.02$) of becoming reproductive ineligible (34.6% vs. 14.5%; AOR = 2.76, 95% CI = 1.19–6.38). Cows with PVD had reduced risk of receiving AI (51–70 DIM: 91.1% vs. 98.2%; $P = 0.01$), fewer pregnancies per AI at first AI (36–50 DIM: 28.6% vs. 46.7%; $P = 0.07$), smaller proportion of cows pregnant by 305 DIM (36–50 DIM: 62.5% vs. 82.1%; 51–70 DIM: 59.0% vs. 80.9%; $P = 0.03$), and increased odds of reproductive culling (36–50 DIM: 33.3% vs. 11.5%; 51–70 DIM: 33.9% vs. 11.9%; $P < 0.01$). To conclude, a consistent pattern of association between visual characteristics and outcomes of interest was not observed.

Key Words: reproduction, cervix, vaginoscopy

2237 Pregnancy associations using skin thermal patterns in dairy cows.

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Infrared thermography can visualize and measure temperature (°C) changes attributed to different physiological stages. The objective was to evaluate associations between reproductive states (cyclic–pregnancy), skin thermal pattern sizes (Patterns), and skin maximum temperature (Tmax). This study followed a complete block design to record thermal images from 40 lactating cows (n = 40) at different reproductive states (Cyclic = [nonpregnant n = 10] and pregnant [Early, 60 to 90 d in gestation, n = 10; Mid, 90 to 180 d in gestation, n = 10; and Late, >180 d in gestation, n = 10]) represented in 3 ambient settings data collection dates (June [Amb1], July [Amb2], and August [Amb3]). Images were recorded using a Flir E40 hand-held camera facing the lateral right side of the abdomen at 1 m in a perpendicular angle. In addition, temperature (Temp) and relative humidity (RH) inside the lactation barn were recorded. Patterns (#Pixels) and Tmax (°C) were processed using Flir ReaserchIR for each image and analyzed using Proc Glimmix and Proc Corr in SAS 9.4. Reproductive state, cow location in the barn (barn area [A, B, C, and D]), and ambient settings were considered independent variables. Reproductive stage had a significant effect on Pattern size (Cyclic: 12.7 ± 2.14 Pixels; Early: 15.29 ± 1.96 Pixels; Mid: 16.52 ± 2.00 Pixels, and Late: 25.25 ± 1.96 Pixels). However, the reproductive state did not affect Tmax significantly (Cyclic: 35.25 ± 0.25°C; Early: 34.71 ± 0.25°C; Mid: 34.86 ± 0.25°C, and Late: 34.44 ± 0.25°C). Ambient temperature (Temp) and relative humidity (RH) fluctuated significantly for the 3 ambient settings, but no strong correlations were found in Patterns (Temp [$P = 0.01$]: r = 0.39; RH [$P = 0.07$]: r = −0.18) and Tmax (Temp [$P = 0.01$]: r = 0.43 and RH [$P = 0.01$]: r = −0.32). Furthermore, no significant effects were found in ambient settings for Patterns; however, the ambient settings and reproductive state interaction were significant only in Tmax. In addition, there was no correlation between Patterns and Tmax (r = −0.12; $P = 0.24$). The size of skin thermal patterns can be indicative of reproductive states in lactating dairy cows.

Key Words: infrared, gestation, contactless

2238 Effects of prepartum acetylsalicylic acid administration on local reproductive inflammation in postpartum Holstein dairy cattle.

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The objective of the study was to assess the effects of prepartum acetylsalicylic acid on cytokine concentrations in vaginal discharge of non-metritic (non-MET) and metritic (MET) cows. At −21 ± 3 d relative to calving (d.r.c.), cows were randomly allocated to 1 of 2 treatment groups for administration on expected −14 ± 3 d.r.c. (−10 ± 5d before the true calving date): 1) ASA (n = 31): cows received one oral administration with 4 boluses of acetylsalicylic acid (125 g/dose); or 2) PLC (n = 29): cows received one oral treatment with 4 gelatin capsules filled with water. Vaginal discharge was sampled from each cow at 7 ± 3 d and 14 ± 3 d after calving, using a Metricheck device fitted with a sterile cup, to allow for diagnosis of MET or non-MET. Cows were categorized as having MET if the discharge was reddish-brown and watery. Discharge was diluted with phosphate-buffered saline and homogenized with a pestle. Cytokine analysis was performed at Eve Technologies Corp. using Luminex xMAP technology for multiplexed quantification. Differences in cytokine concentrations for each cow's sample were analyzed in R using a Mann-Whitney test and Bonferroni correction with outliers over 3 standard deviations removed only from that cytokine. MET incidence for the study was ASA = 20.84 ± 4.35%; PLC = 31.02 ± 5.49% ($P = 0.07$). On d 7, there were 25 MET cows, and on d 14, there were 18 MET cows. There were no significant differences between treatments based on day sampled and metritis status. Overall concentration averages (in pg/mL) by treatment were IL-1β (ASA: 1,754 vs. PLC: 2,471), IL-6 (ASA: 420 vs. PLC: 414), and TNFa (ASA: 234 vs. PLC: 221) (adjusted $P > 0.05$, values reflect diluted samples). Regardless of treatment and day, MET cows had higher concentrations of IL-1β (MET: 3,378 vs. non-MET 1,360 pg/mL, adjusted $P < 0.0001$, values reflect diluted samples). There were no differences in TNFa or IL-6 concentrations between non-MET and MET cows. Overall, cows with MET had increased vaginal discharge inflammatory biomarkers. However, ASA treatment did not affect local inflammation in MET cows.

Key Words: acetylsalicylic acid, metritis, reproductive inflammation

Ruminant Nutrition 1: Gut Physiology, Fermentation, and Digestion

2239 In search of a better way: Exploring the effects of enrichment and DNA extraction on long-read sequencing of rumen protozoa. J. McClure*[1], K. Panke-Buisse[1], E. Lai[1,3], R. A. de Hegedus[1], G. Zanton[1], E. French[1], T. Smith[2], G. Suen[4], and D. Bickhart[1,5], *[1]USDA-ARS DFRC, Madison, WI, [2]USDA-ARS-MARC, Clay City, NE, [3]ABS Global, DeForest, WI, [4]University of Wisconsin, Madison, WI, [5]Hendrix Genomics, CK Boxmeer, the Netherlands.*

Protozoa constitute up to 50% of the living biomass in the rumen, though the roles they play are largely unknown. While advances in long-read sequencing and metagenomics have provided resources for rumen bacteria, fungi and archaea, protozoa continue to present unique challenges. Their genomes' high AT-richness and repeat regions result in biases during DNA extraction, sequencing and downstream analysis. Enrichment of protozoa from rumen liquid (RL) can mitigate biases from extraction and sequencing by increasing the proportion and quality of extracted DNA. A gravity-based separation and low-speed spin method optimized for downstream long-read sequencing was developed to enrich protozoa in RL samples. DNA from enriched and unenriched samples from 2 multiparous Holstein cows were extracted via 7 distinct protocols (kits from Invitrogen [INV], Millipore [MIL], Promega [PRO], Qiagen [QIA], Roche [ROC], ZYMO [ZYM], and a modified SDS lysis [YM]) to identify suitability for downstream Oxford Nanopore Technologies (ONT) sequencing and potential biases. Extracted DNA samples were sequenced on ONT R9.4.1 flow cells, reads were classified and annotated with Kaiju, and results were visualized in Krona. Fragment length distribution of extracted DNA was sufficient for downstream ONT sequencing for 6 extraction protocols. For the 6 protocols, enrichment resulted in an average increase in AT-richness of 13.8 ± 0.17%. The proportion of classified Eukaryotic reads in enriched samples were 5-fold greater than unenriched samples (~32% and ~6%, respectively). There were statistically significant differences between sequenced fragment length for all kits ($P < 0.05$). YM and ROC produced the longest reads on average, 4,889 bp and 4,275 bp, respectively, though ROC was more consistent between animals. The proportions of sequenced reads that could be classified were relatively small and highlight the need for additional information on rumen protozoa to improve classification databases. However, these results indicate enrichment and ONT long-read sequencing are viable methods for obtaining high-quality protozoa metagenomic data from RL.

Key Words: rumen, protozoa, DNA

2240 The effects of multiple freeze-thaw cycles on ruminal microbial populations. A. C. B. Johnson*[1], M. Mojica[2], N. Indugu[1], and D. Pitta[1], *[1]University of Pennsylvania, Kennett Square, PA, [2]Westtown School, West Chester, PA.*

Preservation methods to maintain populations for reuse are sought after. However, it is unclear what possible biases arise from preservation time and whether multiple freeze-thaw cycles (FTC) may affect the microbial populations. This study investigated the effects of multiple FTC on the metabolically active (cDNA) and total bacterial rumen solid bacterial populations (DNA). Rumen contents from lactating dairy cows (n = 8) were sampled and aliquoted for cDNA and DNA amplification in 6 batches. Batch 1 was processed immediately after collection, which represented 0-FTC (fresh), and the remaining tubes were stored at −80°C. For 1-FTC, batches 2, 3, and 4 were thawed once and processed immediately on d 30, 60, and 90, respectively. Batch 5 was thawed for the first time on d 30 and returned to −80°C storage before it was rethawed and processed on d 60 to represent 2-FTC. Last, batch 6 was thawed twice on d 30 and 60, stored at −80°C between thaws, and finally rethawed and processed on d 90 to represent 3-FTC. Results showed that DNA- and cDNA-based communities differed between the fresh and frozen samples. Within DNA, microbial richness (Observed/Shannon indices) were reduced ($P < 0.05$) in 60 d of 1-FTC and 2-FTC compared with 0-FTC. Similar patterns were also observed for cDNA communities for weighted Unifrac (adonis, $P > 0.05$). At the phyla level *Fibrobacteres* DNA was decreased at 60 d of 1-FTC onward ($P < 0.001$), but cDNA was not affected. *Firmicutes* DNA and cDNA were 1.3-fold increased ($P < 0.001$) in 3-FTC compared with 0-FTC. Meanwhile, *Bacteroidetes* DNA was 1.2-fold decreased but cDNA was 1.2-fold increased in 3-FTC compared with 0-FTC ($P < 0.05$). At the genus level, *Succinivibrionaceae* cDNA was 9-fold increased at 60 d of 1-FTC and 2-FTC, and the DNA content was only increased at 2-FTC compared with 0-FTC ($P < 0.001$). Other genera also showed unique responses to FTC, suggesting certain bacteria may gain the competitive opportunity to utilize substrates when others are hindered during sample handling, but their functional contributions to the rumen microbiome require further investigation.

Key Words: freeze-thaw, cDNA, DNA

2241 Seasonal and feeding effects on microbial populations and volatile fatty acids in lactating dairy cattle. A. C. B. Johnson*, K. Narayan, A. Castaneda, T. Webb, J. Bender, L. Baker, S. Rassler, A. Post, R. Kashyap, N. Indugu, and D. Pitta, *University of Pennsylvania, Kennett Square, PA.*

Seasonal and feeding time effects on the rumen microbiota of lactating dairy cows are poorly understood. We aim to determine how season and sampling time postfeeding (2 vs. 8 h) and their interaction will affect the microbial population and VFA profiles in lactating dairy cattle. Over the course of 1 yr, a TMR diet was fixed and fed to 159 lactating dairy cattle that were enrolled to the study. After a 4-wk adaptation period, rumen contents were collected for 16S populations and VFA. A total of 101 animals (average DIM = 103) were retained to the current analysis spanning fall (FA, n = 41), spring (SP, n = 23), summer (SU, n = 29), and winter (WI, n = 8). Season affected microbial richness and diversity ($P < 0.001$) with FA being the least rich (Observed index) but the most variable (indicated by weighted Unifrac distances). Seasonal effects were also seen in the VFA profile with the FA having the highest total VFA concentrations followed by SP, SU, and WI ($P < 0.05$), indicating that bacterial activities are slowed during the more extreme seasons. Sampling time (2 vs. 8 h) also affected microbial richness and diversity metrics ($P < 0.001$) but had no effect on VFA except for butyrate within FA (11.5% vs. 13.5%, $P = 0.03$). Time point and seasonal interactions ($P < 0.05$) were noted in some key bacteria including *Fibrobacter*, *Coprococcus* (Co), *Mogibacteriaceae* (Mo), *Ruminococcaceae* (Ru), and *Lachnospiraceae* (La). Notably, Co, Mo, Ru, and La abundances were positively correlated in SU, FA, and WI; however, in SP, but the correlation was absent in SP. All 4 bacteria are associated with feed efficiency and may indicate a unique metabolic trade-off between them that specifically occurs in SP. Fibrobacter abundance increased from 2 to 8 h in the FA and SP but decreased over time in SU and WI, suggesting that this bacterium is affected by environmental changes. In summary, rumen microbial populations and activities are significantly affected by season, but sampling time after feeding is most affected in FA and SP when the microbiome is more variable, likely due to increased animal activities during the milder temperatures.

Key Words: season, sampling, VFA

2242 Effect of inoculum processing technique on in vitro digestibility and kinetics.

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Blending ruminal solids to prepare inoculum for in vitro (IV) digestibility is presumed to increase the concentration of particulate-associated microbes in inocula but adds complexity during preparation. The objective was to evaluate the effect of inoculum preparation methods (Tx) on IV undigested ash-free aNDF residue (uNDFom) and digestion kinetics. Ten feeds were selected to provide a range of maturity and types, including ryegrass, mature grass, corn silage and stover, alfalfa, clover, distillers, canola meal, beet pulp, and soy hulls, and assigned to 1 of 2 blocks consisting of 5 feeds per block. Ruminal contents were collected from 3 donor animals fed a diet consisting of 30% NDF as DM to prepare inoculum using 4 Tx: strained ruminal liquid (S), strained liquid from ruminal solids blended with an equivalent volume of reduced media (B), composite of S and B (C), and strained liquid from ruminal contents blended in ruminal liquid (GVS). Each block was evaluated over 2 IV runs, for a total of 4 runs. All Tx were evaluated in each run. Incubation times were 0, 3, 6, 12, 24, 48, and 96 h. Data were fitted to an exponential decay model with Microsoft Excel Solver (Microsoft Corp., Redmond, WA) to determine discrete lag, indigestible NDF (iNDF), and fractional digestion rate (kd). The average uNDFom at 0 h for each feed type was used for the 0 h value, and starting values for kinetic parameters were determined by first fitting median data across Tx for each feed to the model. Data were analyzed using the "lm" function in R version 4.2.2 according to a model that included the main effect of Tx, feed × Tx, and feed-within-run. Although the effects of Tx were small, Tx C consistently obtained lowest uNDFom residues for all time points. The feed × Tx interaction was significant for all uNDFom time points and kinetic parameters ($P < 0.001$). Discrete lag for Tx C was generally shortest across feeds, except for beet pulp and soybean hulls, which were lowest for Tx S, and ryegrass, which was lowest for Tx GVS. Inoculum preparation method is an important consideration for in vitro systems used to evaluate diverse feed types.

Key Words: in vitro NDFD, fermentation, digestibility

2243 Validating rumen evacuation and sampling techniques for passage rate studies.

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The objectives of this study were to determine if ruminal pool size varies over time relative to feeding and if rumen grab samples represent the entire rumen. Six rumen-cannulated cows were randomly subjected to rumen evacuation 2 h before feeding (PR) and 2 h after feeding (PO) 7 d apart in a crossover design with 2 periods of 28 d. Ruminal pool sizes of DM, NDF, and undegraded NDF (uNDF) were estimated from rumen content weight and chemical analysis of pressed rumen solids. Rumen grab (RG) samples were taken from inside the rumen before evacuation and rumen contents (RC) samples were taken from evacuated and mixed rumen contents within a barrel to be analyzed for NDF and uNDF. Using PROC MIXED of SAS, the statistical model for pool size included the fixed effect of evacuation time and the random effect of cow within period. The statistical model for the sample type included the fixed effects of the technique, the evacuation time, and their interaction, and the random effect of cow within period. Total rumen contents averaged 70.7 ± 3.4 kg between evacuation times ($P = 0.68$). The PO samples tended to contain more DM compared with PR (13.9 vs. 12.7 ± 0.4%; $P = 0.08$) and increased DM pool size (9.9 vs. 8.9 ± 0.5 kg; $P = 0.04$). Relative to PO, PR samples contained more NDF (66.3 vs. 62.7 ± 1.4%, $P = 0.02$) and uNDF (27.0 vs. 24.7 ± 0.8%; $P = 0.02$). The pool sizes of NDF (6.1 ± 0.3 kg, $P = 0.42$) and uNDF (2.4 ± 0.1 kg, $P = 0.74$) did not differ between evacuation times. An interaction did not exist between the sampling technique and the evacuation time. The concentration of NDF did not differ between RG and RC samples (67.5 ± 1.8%; $P = 0.19$) but PR samples contained more NDF than PO samples (71.1 vs. 64.0 ± 1.8%; $P < 0.01$). The RG samples tended to have more uNDF (%DM) than RC samples (29.0 vs. 27.7 ± 1.2%; $P = 0.05$). The PR samples had more uNDF than PO samples (29.9 vs. 26.9 ± 1.2%; $P < 0.01$). In conclusion, rumen grab samples represent rumen fiber concentration. When determining the rumen pool size, at least 2 rumen evacuations (relative to mealtime) must be conducted to account for differences in the NDF and uNDF concentrations of rumen contents.

Key Words: passage rate, rumen sampling, rumen pool size

2244 A comprehensive overview of the archaea activity in the rumen of dairy cows and their impact on feed efficiency.

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In the quest to enhance dairy production sustainability, understanding the activity of the archaea microbial community in the rumen is pivotal. Despite archaeon groups being known to produce methane, their functional interplay in the rumen community needs further exploration. Here, a comprehensive metatranscriptomics approach of the active archaea community of the rumen of dairy cows was performed to test their association with other rumen microbes and RNA viruses and their effect on feed efficiency. Given the nature of methanogens' mutualistic activity in the rumen, it was hypothesized archaea are tightly associated with other microbes, especially rumen protozoa. Twenty-four cows only diverging in residual feed intake (RFI) were matched for this study. All RNA was extracted from rumen contents and analyzed using an ultra-deep dual-RNA sequencing approach. After quality control, sequences were cleaned from bovine reads in *kraken2*. The microbiome composition was assessed using *Sourmash* against the GenBank database. Overall, 47,277 bacteria, 6,690 archaea, and 7,947 eukaryotes genomes, and 504 RNA viruses, were identified. Methanogens from archaea accounted for 20.3% of total organisms in this domain. Least efficient cows were not correlated with archaea abundance (r = 0.05), whereas most efficient cows had a moderate to high correlation with the RFI ($P < 0.001$, r = 0.73, $R^2 = 0.54$), suggesting the greater efficiency, the lower the dependency on archaea. Regarding the association with other domains, archaea abundance almost did not have any correlations, but in least efficient cows it tended to be affected by RNA viruses ($P = 0.08$, r = −0.53, $R^2 = 0.28$). The previously tested association of *Entodinium caudatum* protozoa with archaea abundance was not significant. The association between archaea abundance and

RNA viruses in less efficient cows, with some viruses showing over 80% correlation, warrants further investigation as it could reveal a novel approach toward improving feed efficiency.

Key Words: methanogens, RNA viruses, metatranscriptomics

2245 Ellipsoid volumes of rumen protozoa decreased by exposure to selected mycotoxins.

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Mycotoxins (MYCO) can instigate productivity issues on farms; however, there is a dearth of knowledge about the contribution of protozoa to ruminal MYCO degradation. This study aimed to assess the effects of MYCO on rumen protozoa in vitro. We hypothesized that rumen protozoa would decrease in volume under MYCO exposure. Rumen fluid was sampled in 2 replicates from lactating Jersey cows, strained through 4 layers of cheesecloth, and flocculated in 45:55 rumen fluid:simplex buffer. Flocculated rumen fluid was dosed into 30-mL culture tubes maintained at 39°C and fed 1 g ground diet (50:50 alfalfa:concentrate). Treatments were as follows: CON (10 μL ethanol carrier), AFL (carrier delivering 600 ppb aflatoxin final concentration), or ZEA (carrier delivering 1,000 ppb zearalenone final concentration). At 0, 3, and 6 h after inoculation, samples were video recorded to quantify percent live protozoa and used to estimate protozoal volume by several equations. Tubes were terminated and protozoa enumerated. Data analysis included fixed effect of treatment, random effects of tube, video, and replicate, and covariate of 0 h for cell volume. There was no effect ($P > 0.10$) of MYCO on proportion of live protozoa at 6 h after inoculation, but AFL and ZEA decreased volume of *Isotrichidae* ($P < 0.01$). Dividing ellipsoid volume by area, *Isotrichidae* were shrunken 14% by both AFL and ZEA ($P < 0.01$) with no effect on entodiniomorphids ($P > 0.10$). Comparison of volume estimation methods indicate mean bias ($P < 0.01$) with ellipsoid method increasing estimates for *Isotrichidae* by 8.7% and decreasing for entodiniomorphids by 48.9%. A slope effect was also observed for both types that increased ($P < 0.01$) with longer cells. Simple means (in $\mu m^3 \times 10^4$) by protozoal type indicate widespread variation, encouraging further research into influencing factors: dasytrichids (8.67 ± 2.60), isotrichids (80.5 ± 27.3), spineless entodiniomorphids (1.86 ± 1.12), spined entodiniomorphids (3.70 ± 1.39), and *Ophyroscolex* (73.3 ± 15.8). Although protozoa appear to be negatively affected by MYCO in this study, future work still needs to quantify the contribution of protozoa to MYCO degradation.

Key Words: aflatoxin, ellipsoid, cell volume

2246 Using ultraviolet-spectroscopy to quantify peptide concentration in dual-flow continuous culture samples.

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Peptides stimulate microbial growth; however, dual-flow continuous culture fermenters (DFCC) recently used to contrast doses of a unique peptide (PEP) source versus urea demonstrated few effects of PEP. Quantification of PEP by absorbance (e.g., 280 nm) could improve our understanding of PEP degradation, but application of UV to rumen-fluid-based samples has been overshadowed by colorimetric assays. The objective of this study was to investigate UV for potential to quantify PEP concentration in DFCC effluent. Pilot screening indicated 280 nm as the most consistent wavelength and failure of a) soy-based standards due to poor filtration and b) tryptone standards due to 20-fold exaggerated PEP concentrations in recovery analysis. Thus, our hypothesis was that UV using a PEP standard would accurately quantify PEP concentration in DFCC samples. Treatments previously imposed to DFCC included the following: negative control (CON, 94 g of DM/d, 50:50 orchardgrass:concentrate) CON with urea (UL, UM, and UH at +1.5, 3.0, and 4.5% CP), CON with PEP (PL, PM, and PH at +1.5, 3.1, and 4.6% CP), and positive control (SOY, +8.4% CP from soy powder). Blank DFCC samples were spiked with PEP, filtered through a 0.45-μm filter, and serially diluted to evaluate a rumen fluid matrix effect. A 1:4 dilution removed inhibitory contaminants as judged by consistent curves across replicates and matching slopes to a water-based standard. The intercept for spiked blank dilutions was used to correct unknown samples. A mixed model included fixed effects of treatment and fermenter, random effects of period, and linear and quadratic contrasts for N sources. Adding PEP linearly increased ($P < 0.001$; SEM = 0.169) peptide concentration to 1.19, 1.27, 1.36, and 1.50 mg/mL for CON, PL, PM, and PH, respectively. Supplementary PEP also increased peptide concentration versus urea (1.38 vs. 1.18 mg/mL; $P < 0.001$; SEM = 0.169 vs. 0.170). These results demonstrate use of DFCC to estimate PEP degradability using a known standard of similar composition, with the current example approximately 25% undegraded. However, broader application of spectroscopy for PEP quantification is limited if there is no appropriate standard.

Key Words: peptides, protein degradation, spectroscopy

2247 Mixed rumen microbes grow more efficiently on cellulose versus glucose.

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Microbes growing in the rumen represent over half of the protein digested by ruminants. Despite the importance to the host, the efficiency of microbial growth can vary widely, and the reasons are not fully understood. Our objective was to measure the growth efficiency of rumen microbes using a single-flow continuous culture system fed with glucose or cellulose. We inoculated a system of 8 single-flow continuous culture fermenters with rumen fluid from 3 cows, fed them glucose or cellulose (46 mmol of hexose$\cdot d^{-1}$), and maintained them at a range of dilution rates (2% to 12%$\cdot h^{-1}$). We then measured the digestion of carbohydrates and the output of microbes and fermentation products. Our experiments followed a completely randomized design replicated over 3 periods. We found that microbes grew more efficiently (g cells$\cdot$[mol hexose digested]$^{-1}$) when fed cellulose versus glucose, especially at high dilution rates. We formally analyzed these differences in efficiency by fitting a statistical model to estimate maintenance requirements and maximum growth efficiency. In this model, inverse dilution rate and carbohydrate were fixed effects and trial and fermenter were random effects. All analyses were done with the nlme package and lme function within R. This model showed that maximum efficiency was higher ($P < 0.001$) when cells were grown on cellulose (132 [g cell]$\cdot$[mol hexose]$^{-1}$) versus glucose (70 [g cell]$\cdot$[mol hexose]$^{-1}$). Maintenance requirements did not differ ($P = 0.681$) by carbohydrate, and they averaged 399 (44 SEM) μmol of hexose$\cdot$(g cell)$^{-1}\cdot h^{-1}$. Similar relationships were observed when efficiency was expressed as g cell$\cdot$(mol of ATP)$^{-1}$. These results show that rumen microbes grow more efficiently on cellulose versus glucose, and this owes to higher maximum growth efficiency, not lower maintenance requirements. These results help explain why microbes grow with variable efficiency in the rumen and will help formulate diets to maximize this efficiency.

Key Words: rumen, microbes, growth efficiency

2248 Upcycling California agricultural byproducts as to reduce methane from ruminants.

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In the United States, California is the leading state in agriculture production in crops and livestock, both together accounting for 12.8% of total exports in 2021. The production of crops results in significant amounts of byproducts that cannot be sold to the consumer, and although they may still have the potential to be used as animal feed, they often end up at landfills, where they generate greenhouse gasses, such as carbon dioxide (CO_2) and (CH_4), that are released into the atmosphere. Previous work has shown that some commonly generated byproducts can contain phenolic compounds, tannins, saponins, or PUFA that can function as bioactive and inhibit the activity of methanogenic archaea, a group of microorganisms that are responsible for the production of methane in the rumen and other anaerobic environments. However, many of the previous results are inconclusive and often contradictory due to the lack of a consistent experimental procedure. In this project, we tested various agricultural byproducts such as grape pomace, agave, and onion waste. A standardized in vitro batch culture was incubated in serum bottles with 1 g of diet replaced with byproducts at different inclusion rates ranging from 0.5% to 20%, to evaluate their effects on fermentation and gas production over a 24-h period. Multiple inclusion rates were tested in the event that some byproducts may be toxic to animals at a higher dosage. The byproducts were tested alongside a negative control (no added byproducts) and a positive control (2% *Asparagopsis taxiformis*). Results were analyzed using ANOVA test to compare gas production parameters. None of the current tested byproducts showed significant reduction in methane production compared with the negative control. However, the results from this work is only the initial study and will provide the foundation to identify the biochemical key parameters of agricultural byproducts that drive the reduction of methane production in the rumen and to develop advanced feed additives to make the cattle industry more sustainable.

Key Words: methane, microbiology, fermentation

2249 The effects of low feed intake on regional permeability of the gastrointestinal tract, ruminal pH, and milk performance in lactating dairy cows.

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This study evaluated the effects of short-term feed restriction on regional permeability of the gastrointestinal tract (GIT), and the recovery for DMI, ruminal pH, and milk yield. Six lactating Holstein cows were blocked by parity (189 DIM ± 25.2) and enrolled in a crossover design. Experimental periods included 4 phases: 5-d baseline (BASE), 5-d challenge (CHAL), and 2 wk of recovery (REC1 and REC2). During the CHAL, cows received either ad libitum feed intake (AL) or 40% of their ad libitum feed intake (FR). On d 3 of CHAL, a ruminal dose of Cr-EDTA and an abomasal dose of Co-EDTA were infused (0.369 mmol/kg of BW). Plasma samples were collected and used to calculate the area under the curve (AUC) for Cr and Co over 48 h. Ruminal pH was measured using indwelling pH loggers. Data were analyzed with PROC GLIMMIX of SAS v9.4 considering treatment, phase, and their interaction as fixed effects and cow within period as a random effect. The BASE data were used as covariates. The DMI for FR was the least during CHAL and remained lower than AL during REC1 but was not different from AL in REC2 (interaction, $P < 0.01$). Mean ruminal pH for FR was greatest during CHAL and the least during REC1, with no differences detected between AL and FR in REC2 (interaction, $P < 0.01$). The duration that pH was <5.8 was the least for FR during CHAL (1.3 h/d) and greatest during REC1 (9.8 h/d), which were different from AL (5.1 h/d; interaction, $P < 0.01$). Milk, fat and protein yields were reduced by 29%, 29%, and 34%, respectively, with FR (treatment, $P < 0.01$). There was a tendency for milk, fat, and protein yields to be less for FR than AL during REC1 (interaction, $0.055 \leq P \leq 0.081$). Plasma Cr AUC was greater (34%; $P = 0.01$) and Co AUC tended to be greater (35%; $P = 0.06$) for FR than AL on d 3 of CHAL. Short-term FR in lactating dairy cows increases permeability of the total GIT and may increase permeability of the postruminal regions. In addition, upon return to voluntary feed intake, FR cows experienced ruminal acidosis and sustained reductions in milk and milk component yields.

Key Words: low feed intake, gut permeability, ruminal acidosis

2250 Effect of forage level and branched short-chain fatty supplementation on rumen bacteria in dairy cows.

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The objective of this work was to determine whether additional dietary forage in tandem with branched short-chain fatty acids (BSCFA) supplementation increases the abundance of rumen branched-chain fatty acid (BCFA)-producing bacteria. Lactating cannulated cows (n = 23; 169 ± 56 DIM) were randomly assigned to 1 of 4 diets for 9 wk in a 2 × 2 factorial arrangement within a randomized complete block design. Diets were composed of low forage (LF, 50% of the diet on a DM basis) or high forage (HF, 65% of the diet on a DM basis) supplemented with or without BSCFA (2.4 g/kg of DM of a BSCFA mix containing *iso*butyrate, 2-methylbutyrate, and *iso*valerate in a ratio of 2:1:1, respectively). Forage consisted of 70% corn silage and 30% alfalfa silage on a DM basis. Rumen samples were collected at the end of wk 8. The DNA was extracted from each rumen sample and analysis of 16S rRNA amplicons was performed using Nanopore sequencing technology. Analysis of variance was conducted for sample richness and α-diversity (Chao, Shannon, Simpson, and Observed OTUs). β-Diversity of sample groupings was measured by PERMANOVA with Bray-Curtis dissimilarity index in R using the adonis2 function in the "vegan" package. Individual taxonomic comparisons (relative abundance) were analyzed by 2-way ANOVA and Tukey's honestly significant difference using the fit model in JMP. Supplemental BSCFA increased Chao1 (Richness, $P = 0.002$), and HF decreased Shannon (Diversity, $P = 0.03$) and Simpson (Diversity, $P < 0.001$). There was an interaction effect on Simpson where LF and supplemental BSCFA were associated with a decrease in diversity, but HF and BSCFA were associated with an increase in diversity ($P = 0.02$). HF increased *Bacillus* (OTU: 1688, $P = 0.03$), whereas BSCFA decreased *Desulfovibrio* (OTU: 45006, $P = 0.02$). There was also an interaction effect for *Fibrobacter* (OTU: 45136, $P = 0.03$), which increased with BSCFA and LF but decreased with BSCFA and HF. In conclusion, HF increased the abundance of some BCFA-producing bacteria but decreased α-diversity, and BSCFA supplementation increased α-diversity but did not synergistically increase BCFA-producing bacteria with HF.

Key Words: 16S, α-diversity, ketoacids

2251 Effect of a blend of essential oils on ruminal fermentation and urinary excretion of nitrogenous metabolites in Jersey cows transitioning from confinement to grazing.

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We aimed to investigate the effect of supplementing a blend of essential oils (EO; Agolin® NATURU) on ruminal fermentation and urinary excretion of nitrogenous metabolites in dairy cows transitioning from confinement to grazing. Sixteen multiparous and 6 primiparous Jersey cows (83 ± 39 DIM and 468 ± 51 kg of BW) were assigned to 1 of 2 treatments in a randomized complete block design with repeated measures over time: 1) 0 g/d of EO (control = CON) or 2) 1 g/d of EO. The EO blend was mixed with 200 g of concentrate inside a rubber tub and offered immediately after the a.m. and p.m. feedings. The experiment lasted 12 wk, with 2 wk for covariate measurements and 10 wk for treatments evaluation (5 wk in confinement and grazing each). Data and samples collected during wk 5 (confinement) and wk 10 (grazing) were used in the statistical analyses in SAS, with block as a random effect in the model. Cows in confinement received (DM basis) a diet with 60% grass–legume baleage and 40% concentrate, and during grazing, 35% of baleage was replaced by grazed herbage in the diet. Ruminal fluid was collected once daily via esophageal tubing, and spot urine samples were collected twice daily (a.m. and p.m.) for 5 consecutive days. Treatment by week interactions were not significant. Diets did not affect the ruminal concentration of total VFA (mean = 76.9 m*M*) and the molar proportions of acetate (mean = 71.5%) and butyrate (mean = 11.3%). Contrarily, cows fed EO had greater ($P = 0.04$) ruminal propionate (14.4% vs. 13.9%) and lower ($P = 0.01$) NH_3-N concentration (4.13 vs. 4.87 m*M*) than CON. Feeding EO increased ($P = 0.02$) the urinary excretion of allantoin (489 vs. 443 mmol/d) and tended ($P = 0.06$) to increase that of total purine derivatives with no change in uric acid. Nitrogen intake (mean = 535 g/d) and the urinary excretion of total N (mean = 200 g/d) and urea N (mean = 122 g/d) were not changed by diets. In brief, EO supplementation increased the ruminal molar proportion of propionate and urinary concentration of allantoin and decreased the concentration of NH_3-N in the rumen.

Key Words: essential oils, ruminal fermentation, urinary N

2253 *Saccharomyces cerevisiae boulardii* CNCM I-1079 influence on gut permeability over the transition period in dairy cows.

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Inflammation in dairy cows, during the transition period, is thought to be related to increased gut permeability, and dietary microbial based technologies, such as the supplementation of *Saccharomyces cerevisiae boulardii* (SCB), may alleviate these challenges. The aim of the study was to investigate if SCB dietary supplementation during the transition period affects gut permeability in dairy cows. Eighty-three Holstein cows were enrolled; blocked by parity (31 primiparous [PP] and 52 multiparous [MP]), previous 305-d milk yield, BW, and BCS; and randomly assigned to 1 of 2 dietary treatments fed from 28 d before calving to 70 d after calving: 1) control diet (CON; n = 43) or 2) SCB supplemented diet (SCB; 1.0×10^{10} cfu/d; n = 40). Cows were dosed in their rumen with an indigestible marker Cr-EDTA (0.1g/kg of BW) on d −30, 7, and 70 relative to calving, and Cr serum concentration was evaluated at −1, 2, 4, 6, 8, 12, 18, 24, and 30 h relative to Cr-EDTA administration to evaluate marker kinetics. Data were analyzed using repeated measures in GLIMMIX procedure of SAS (v.9.4). The Cr area under the curve (AUC) was greater ($P < 0.01$) on d 7 than 70 (using d 30 as a covariate). The Cr delta AUC calculated for d 7 and 70 postpartum, relative to d 30 prepartum, tended to be higher ($P = 0.07$) in MP than PP cows and was higher ($P = 0.003$) on d 7 than 70. The delta Cr AUC tended to be lower ($P = 0.06$) in SCB compared with CON cows on d 7 and 70, suggesting SCB cows had decreased gut permeability. There was a treatment × parity interaction where MP SCB cows tended to have a lower maximum Cr concentration than MP CON ($P = 0.06$), but no differences were observed in PP cows. Overall, cows had an increase in gut permeability during their first week of lactation compared with 70 DIM, and our results suggest supplementation of SCB may reduce gut permeability in transition dairy cows.

Key Words: probiotic, leaky gut, transition cows

Ruminant Nutrition 2: Calves and Heifers

2254 Withdrawn.

2255 Effect of maternal or formulated transitional milk on gastrointestinal tract development and organ weight of neonatal dairy calves. I. C. R. Oliveira, N. I. Carvalho, A. F. Toledo, R. D. F. Barboza, J. G. Dantas, F. V. L. Barbosa, E. D. Marino, C. R. Tomaluski, and C. M. M. Bittar*, *Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil.*

The present study aimed to evaluate how the supply of maternal or formulated transition milk, using colostrum powder, affects the development of the gastrointestinal tract (GIT), ADG, and organ weight of neonatal dairy calves. Thirty Holstein male calves were colostrum fed 12% of the BW of a 23% to 25% Brix colostrum within 3 h after birth. Calves were then divided into randomized blocks, according to date of birth and birth BW, and distributed into treatments according to the liquid diet composition for the next 3 d: 1) whole milk (WM); 2) formulated transition milk (FTM): WM enriched with 70 g/L colostrum powder (resulting in 19.5% solids); 3) transition milk (TM): maternal transition milk with 16% Brix. Calves were fed 4 L/d of the respective diet, divided into 2 meals. The calves were individually housed with free access to water and a commercial pelleted starter. From the fourth day of age, calves were fed WM at 6 L/d. Five animals per treatment were slaughtered on d 21 for GIT evaluation, and data were analyzed using Proc Mixed of SAS 9.4. All treatments reached excellent levels of passive transfer measured at 48 h after birth (serum protein 11 ± 2.0% Brix). There were no differences in ADG from birth to 21 d (WM: 366.7 g/d; FTM: 292.3 g/d; TM: 370.5 g/d; SEM = 89.4; $P > 0.78$), BW at 21 d (WM: 48.1 kg; FTM: 46.7 kg; TM: 48.2 kg; SEM = 1.39; $P > 0.95$), as well as the weight of organs at d 21, such as the spleen (WM: 153 g; FTM: 154 g; TM: 174 g; SEM = 27.2; $P > 0.38$), liver (WM: 1,355 g; FTM: 1,337 g; TM: 1,220 g; SEM = 69.9; $P > 0.22$), and kidneys (WM: 281 g; FTM: 299 g; TM: 263 g; SEM = 24.0; $P > 0.42$). The total full GIT (WM: 6,292 g; FTM: 6,375 g; TM: 6,416 g; SEM = 450.2; $P > 0.93$), full intestines in (WM: 3,245 g; FTM: 3,710 g; TM: 3,195 g; SEM = 305.4; $P > 0.48$), and full forestomach weight (WM: 2,667 g; FTM: 2,690 g; TM: 2,872 g; SEM = 317.8; $P > 0.88$) were not affected by treatments. Potential benefits of providing TM or FTM were not observed in this short-term study, probably because of the excellent levels of passive transfer associated with the short period of TM diets supplied (3 d).

Key Words: colostrum, bioactive compounds, organ development

2256 Effects of vitamin B inclusion rates in milk replacer on health and growth of preweaned Holstein cross calves. K. Brost*[1], M. Pister[1], A. Saito[2], and J. Drackley[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL,* [2]*The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Tokyo, Japan.*

The objectives of this study were to determine the effects of different B vitamin inclusion rates on health and growth of preweaned calves through 9 wk of age. The experiment was a randomized complete block design with 42 male and female Holstein-Angus cross calves, purchased from a commercial dairy at ≤7 d old. Calves were housed in individual hutches bedded with straw. Milk replacer (MR; 28% CP, 15% fat) was fed in a step-up step-down protocol: 600 g/d for d 0 to 14 and d 57 to 63, 800 g/d for d 15 to 21 and d 50 to 56, and 1,125 g/d for d 22 to 49. Water was offered ad libitum from d 0, and starter was offered ad libitum from d 50. The B vitamins of focus for this study were B_1, B_3, B_6, and B_{12}. Treatments were basal MR with no added B vitamins (CON); MR with B vitamins above basal (BP; 1.9 mg/kg B_1, 1.68 mg/kg B_3, 0.32 mg/kg B_6, and 43 µm/kg B_{12} above basal); MR with B vitamins above the current NASEM (2021) recommendations (NP; 18.88 mg/kg B_1, 8.49 mg/kg B_3, 11.44 mg/kg B_6, and 292 µm/kg B_{12} above NASEM). Milk replacer intake and health scores were recorded twice daily. Starter intake, coprophagia, and fecal color scores were recorded daily. Skeletal measurements and BW were recorded weekly. Health data were analyzed using GLIMMIX procedures, and growth and intake data were analyzed using the MIXED procedure in SAS. For wk 1 to 9, days with ocular discharge were greater ($P = 0.02$) for CON compared with BP, and days with respiratory events tended to be greater ($P = 0.07$) for BP compared with CON. Starter intake for wk 8 to 9 was greater ($P < 0.0001$) for BP in comparison with CON. For wk 1 to 9, hip width tended to be greater ($P = 0.08$) for NP compared with CON. Fecal color scores noted a tendency ($P = 0.09$) for greater days with green feces for NP compared with CON. No other health scores, BW, growth measurements, or MR intake differed. In all, some health benefits were documented, but results were inconsistent among treatments. Increased starter intake for BP may suggest improved animal well-being but contributed minimally to ADG or growth performance. Further research with well-defined inclusion rates of B vitamins is needed.

Key Words: calves, B vitamins, performance

2257 Effects of fat content of high-protein milk replacer on dry matter intake and growth performance in dairy calves. K. Murayama[1], N. Kobayashi[1], R. Fukami[2], T. Sugino*[2], and M. Oba[3], [1]*Dairy Technology Research Institute, The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Nishi-shirakawa, Fukushima, Japan,* [2]*The Research Center for Animal Science, Graduate School of Integrated Science for Life, Hiroshima University, Higashi-Hiroshima, Japan,* [3]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.*

The aim of this study was to evaluate the effects of fat content of high-protein milk replacer (MR) on DMI and growth performance of Holstein calves. Forty-two heifer calves (born between October 31 and December 22, 2022) were fed one of the 3 MR (96% DM and 29% CP): (1) 20% crude fat (CF), 41% lactose, and 4.7 Mcal/kg ME (LF; n = 14); (2) 26% CF, 36% lactose, and 5.0 Mcal/kg ME (MF; n = 14); or (3) 32% CF, 31% lactose, and 5.3 Mcal/kg ME (HF; n = 14), on a DM basis. The MR were offered at 167 g/L, and osmolality was 488, 428, and 383 mOsm/kg (LF, MF, and HF, respectively). The MR were offered at 600 g/d (powder basis) from 7 to 13 d, 800 g/d from 14 to 20 d, 1,200 g/d from 21 to 41 d, 800 g/d from 42 to 48 d, and 600 g/d from 49 to 55 d, and then, calves were weaned at 56 d of age. All the calves were fed calf starter and chopped hay ad libitum from 7 d of age. Data were analyzed using the Fit Model procedure of JMP® 17 pro (SAS Institute Inc., Cary, NC). The average daily ambient temperature during experimental period was 5.1°C (ranging from −3.4°C to 14.4°C). The MR intake increased linearly ($P < 0.05$) as the fat content of MR increased in 21 to 41 d (1,135, 1,145, and 1,144 g/d for LF, MF, and HF, respectively) and 42 to 55 d of age (666, 669, and 671 g/d for LF, MF, and HF, respectively). For 7 to 55 d of age, calf starter intake was 250, 271, and 207 g/d, and hay intake was 54, 41, and 43 g/d, and ADG was 0.65, 0.68, and 0.66 kg/d, respectively, for LF, MF, and HF, and these response variables did not differ among treatments. However, withers height gain (0.11, 0.12, and 0.17 cm/d, respectively, for LF, MF, and HF) and hip width gain (0.01, 0.03, and 0.04 cm/d, respectively, for LF, MF, and HF) increased linearly ($P < 0.05$) in 7 to 20 d of age as the fat content of MR increased. These results suggested that the higher fat contents of high-protein MR may increase skeletal development of calves until 20 d of age under the cold stress.

Key Words: fat, lactose, skeletal development

2258 Influence of energy in late-lactation Holstein cow diets on female offspring growth efficiency preweaning and later prepubertal greenhouse gas emissions. Z. Wu*[1], T. O. Cunha[1], L. L. Hernandez[1], and E. A. French[2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Maternal nutrient restriction during late gestation has demonstrated lower BW gains, growth efficiency, and health of offspring. A study was conducted to evaluate if feeding late-lactation Holstein cows 2 different energy levels, control versus low (CTL vs. LO; 1.82 vs. 1.57 Mcal/kg of DM), affects female offspring BW, body measurements (MEAS), and prepubertal (PRP) greenhouse gas emissions (GHG). At 170 DIM, cows were blocked by calving date and BCS and randomly allocated to treatments to achieve a 4.0 or 2.75 BCS at the end of lactation (3.68 vs. 3.26, $P < 0.01$). Female calves were enrolled at birth, with 20 CTL and 18 LO. Preweaning BW, MEAS, and individual feed intakes (DMI) were collected weekly until 8 wk of age. At PRP, heifers (mean ± SD; 174 ± 6 d of age) were individually fed and observed for 17 d. Individual DMI, BW, MEAS, backfat depth via ultrasound, and daily enteric GHG by GreenFeed were collected. Data were analyzed by generalized linear mixed models (SAS 9.4) within preweaning and PRP phases separately. The fixed effects of maternal dietary energy level, age in weeks, and the interactions were used. Calf was a random effect, and a repeated measure was calf across age. In the PRP data, group was included as a random effect. Colostrum brix reading was similar ($P = 0.18$), and 24-h-old serum total proteins were higher in LO calves ($P = 0.02$). Calves were similar in birth (39 ± 2 kg), weaning (84 ± 1 kg), and 6-mo BW (191 ± 8 kg). Paunch and heart girth were greater for the CTL compared with LO animals in the preweaning phase (95 vs. 94 cm, $P < 0.07$; 94 vs. 93 cm, $P < 0.01$). Total milk (371.2 ± 15.0 L) and starter DMI (16.5 ± 1.9 kg) were similar at 8 wk of age, along with PRP DMI (5.6 ± 0.3 kg/d). Daily CH_4 and CO_2 PRP emissions (g/d) were similar (121 ± 5; 4,778 ± 163). The effect of improved serum total proteins at 24 h of age and influence to overcome prenatal nutrient restriction is unknown. Data suggest restricting maternal dietary energy affects preweaning growth and performance, but differences do not persist into the PRP stage.

Key Words: calf, prenatal nutrition, methane

2259 Preweaning transition milk supplementation does not improve productivity or calving intervals in first, second, or third lactation animals. P. O. McDonald*[1], B. Van Soest[2], M. S. Weber Nielsen[1], and B. J. Bradford[1], [1]*Michigan State University, East Lansing, MI,* [2]*University of Guelph, Guelph, ON, Canada.*

Calves are one of the most at-risk populations in dairy herds, with persistent morbidity and mortality rates over the past 2 decades. Transition milk (TM) may improve calf health and maturation, but no long-term studies have evaluated effects as heifers reach the productive herd. In this retrospective study, we investigated the role of calfhood TM supplementation on subsequent production, reproduction, and survival. Holstein heifers enrolled in the study by Van Soest et al. (2020) were randomly assigned to treatment (MR: milk replacer control; MR+CR: MR supplemented with colostrum replacer; TM) and fed their respective nutrition programs (3 feedings per day for 3 d) after colostrum feeding. The initial study revealed calves receiving MR+CR or TM did not have improved health scores compared with MR but had increased growth rates. Animal records were collected and used for analysis (31 MR, 30 MR+CR, 29 TM). Production data were analyzed with mixed models to account for the fixed effect of diet, random effect of block and covariates of PTA milk, fat, or protein, respective to the variable of interest. Time to first calving, subsequent time to pregnancy, and time to leave the herd were assessed using Cox proportional hazard analysis with herd exit censored at 64.5 mo of age. There were no differences among treatments for lactation 1 (n = 24–25) or 3 (n = 10–16) milk, fat, protein, or SCC (all $P > 0.2$). Lactation 2 (n = 16–18) had similar results, except TM calves tended to yield greater fat ($P = 0.09$). Treatment had no apparent effect on hazard of leaving the herd. The TM-supplemented calves tended to have a longer time to first calving than MR calves (686 vs. 669 ± 6.7 d, $P = 0.05$), with MR+CR calves intermediate (678 ± 6.4 d, $P = 0.29$ vs. MR). In lactation 1, there was no difference among treatments in time to pregnancy. This study was the first to investigate future production parameters following TM supplementation in the 3 d after colostrum feeding. Although few effects were observed, further research is necessary because this study was limited to one farm with decreasing sample size with increasing parity.

Key Words: transition milk, heifer, cow

2260 Incorporation of butyric and caproic acids in milk replacer improves gastrointestinal physiology and apparent fat digestibility in calves fed twice daily. J. N. Wilms*[1,2], J. Swaan[1], M. A. Steele[2], J. Martín-Tereso[1], I. van der Werff[1], and L. N. Leal[1], [1]*Trouw Nutrition R&D, Amersfoort, the Netherlands,* [2]*Department of Animal Bioscience, University of Guelph, Guelph, ON, Canada.*

The incorporation of alternative fat sources in milk replacers (MR) for calves leads to minimal levels of butyric (C4:0) and caproic acids (C6:0). This study evaluated the effects of incorporating tributyrin (TB) and tricaproin (TC) at the same levels as milk fat on gastrointestinal physiology and digestibility. Forty-five male Holstein calves (46.1 ± 4.6 kg of BW; 2.1 ± 0.63 d of age) were blocked by arrival day and age. Within each block of 3 calves, calves were randomly assigned to experimental treatments (15 calves each), including a MR with dairy cream (milk fat, MF), a MR with a blend of vegetable fats (CON), and a MR with the same fat blend as CON to which TB and TC were incorporated (TRI). All MR treatments were isoenergetic, with 36% lactose (%DM), 27% fat, and 24% protein. Calves were housed individually, and daily milk volumes were 6 L from d 1 to 5, 7 L from d 6 to 9, and 8 L from d 10 to 35, divided into 2 equal meals (13.5% solids). Starter feed was unavailable. Feces were scored daily for the first 21 d, and BW was recorded weekly. Feces collection took place on d 28 over 48 h for evaluation of apparent total-tract digestibility. Calves were euthanized on d 35 for histomorphometry measurements in the rumen (ventral sac) and ileum. Continuous variables were analyzed using a mixed-effects model in SAS, with the calf as the experimental unit. Treatment and time were treated as fixed effects, and the block was considered a random effect. Milk replacer intake and growth did not differ. Calves fed TRI had a lower percentage of days with abnormal fecal scores in wk 3 ($P = 0.07$). Apparent total-tract digestibility of crude fat ($P = 0.05$) and Ca ($P = 0.03$) were greater in calves fed MF than CON, whereas calves fed TRI had enhanced fat ($P = 0.10$) and Ca ($P = 0.07$) digestibility than CON. The width of polyps ($P = 0.06$) and the length of papillae ($P = 0.07$) in the rumen and villi in the ileum ($P < 0.01$) were greater in calves fed

TRI than other treatments. Incorporating TB and TC into MR enhanced gastrointestinal development and apparent fat digestibility.

Key Words: milk replacer, tributyrin, tricaproin

2261 Effects of different raw material additions on the protein intake and antioxidant capacity of Holstein heifers. R. Yao[1,2], T. Zhan[1], L. Ma[1], and D. Bu*[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science (IAS), Chinese Academy of Agricultural Sciences (CAAS), Beijing, China,* [2]*College of Animal Science and Technology, Beijing University of Agriculture, Beijing, China.*

The aim of this experiment was to investigate the effects of adjusting the dietary protein source by adding a common diet to dairy cows on protein intake and antioxidant status in Holstein heifers. Eight Holstein heifers (252.87 ± 5.28 kg) were randomly selected and assigned to 8 treatments in a 6 × 8 Youden square design with 6 periods of 13 d each. Treatment groups were the basal diet (BD; CP 17.55% DM, soybean meal 24.06%, corn silage 31.49%, and alfalfa hay 22.67%) and the diets supplemented with soybean meal (SM; CP 18.82%), cottonseed meal (CM; CP 18.57%), rapeseed meal (RM; CP 18.71%), expanded soybean (ES; CP 18.46%), alfalfa hay (AH; CP 16.98%), oat hay (OH; CP 16.52%), and corn silage (CS; CP 15.68%), which provided an additional CP 268 g/d, which was 12.1% of the total CP in the BD group. Daily feed intakes were recorded; TMR, residues, and feed materials were collected on d 11 to 13; and plasma samples were collected from the jugular vein on d 12 or 13 to determine conventional dietary components and plasma antioxidant indices. Data analysis was performed using the mixed model (treatment and period) of SAS. The results are as follows. 1) Compared with the BD group, the DMI of the AH group was significantly increased (8.46 ± 0.31 vs. 7.69 ± 0.41, $P < 0.05$), but the daily CP intake of all groups was significantly higher than that of the BD group ($P < 0.05$), except the CS group (1.44 ± 0.09 vs. 1.51 ± 0.10, $P < 0.01$). 2) Compared with the BD group, catalase activity of CM and superoxide dismutase activity of CS were significantly decreased (0.44 ± 0.25 vs. 1.09 ± 0.31, 16.75 ± 1.15 vs. 21.78 ± 1.52, $P < 0.01$), and malondialdehyde content of CS was significantly higher than that of the BD group (4.11 ± 0.83 vs. 2.4 ± 0.27, $P < 0.05$). In conclusion, the results of this study suggest that the increase of CS in the diet may affect the protein intake of heifers, and the addition of CM to 5.36% DM may have adverse effects on the antioxidant capacity of heifers.

Key Words: Holstein heifer, protein intake, antioxidant capacity

Ruminant Nutrition 2: General

2262 Defining a mixing quality marker in total mixed rations for dairy cows. E. F. Santos[2], E. Detmann[2], J. S. Pinheiro[2], P. S. D. Silva[2], M. M. Ferreira[3], J. M. R. Gesteira[2], and M. I. Marcondes*[1], *[1]Washington State University, Pullman, WA, [2]Federal University of Vicosa, Vicosa, MG, Brazil, [3]Cornell University, Ithaca, NY.*

When formulating diets for dairy cows, there are 3 main types: formulated, prepared, and provided diets. It's crucial for these diets to be homogeneous to ensure mixing quality (MQ). Poor mixing can lead to uneven nutrient distribution along the feedbunk, decreasing productivity. Although assessing the uniformity of each nutrient in TMR diets is impractical, using internal markers is common in the monogastric industry. However, there's a lack of data on markers for assessing mixing uniformity in TMR diets for dairy cattle. Our goal was to determine a methodology and marker to measure the MQ of TMR diets for dairy cows. We conducted a study on a commercial dairy farm, collecting TMR samples over 5 consecutive days. Three different diets were analyzed: fresh, high production, and low production, covering all 3 feeding times. At each feeding, we gathered 10 samples along the feed bunk. These samples underwent partial drying at 55°C for 72 h before being processed in a knife mill with a 1-mm sieve. Subsequently, we analyzed the samples for DM, CP, NDF, calcium (Ca), magnesium (Mg), zinc (Zn), iron (Fe), manganese (Mn), sodium (Na), potassium (K), and phosphorus (P) contents. We assessed the results using a model that treated diet as a fixed effect and considering day (diet) and feeding (day × diet) as random effects. This approach allowed us to anticipate that the residual variance would reflect the variation stemming from feed-bunk variability. Consequently, the nutrient exhibiting the highest proportion of variance in the residual variance could be identified as the most indicative marker for MQ. The proportions of variance in the residual variance were as follows: Zn (94.3%), Mn (81.9%), Fe (79.3%), Ca (77.9%), Mg (91.3%), P (93.0%), K (99.1%), and Na (77.4%). In conclusion, we successfully obtained a method to determine MQ in TMR for ruminants, and among the minerals analyzed, Zn (micromineral) and K (macromineral) stood out as best markers due to their substantial proportions of residual variance, indicating their heightened sensitivity to inadequate MQ.

Key Words: indicators, mixture quality, diets

2263 Evaluation of the effect of forage fragility on behavior, rumen environment, and digestibility in lactating Jersey cattle. K. Buse* and P. Kononoff, *University of Nebraska–Lincoln, Lincoln, NE.*

Forage fragility is the rate at which forage particles are broken down in size through chewing activity. The ratio of ADF to NDF is considered to be an indirect estimate of forage fragility. The objective of this study was to test the hypothesis that increasing forage fragility as measured by the ratio of ADF to NDF would reduce rumination activity, increase rumen rate of passage, and the combined effects would reduce rumen pH. Four multiparous, ruminally cannulated Jersey cows (88 ± 11 DIM) were used in a 4 × 4 Latin square with 4 periods of 28 d. Cows were randomly assigned to 1 of 4 treatments varying in inclusion of alfalfa hay (AH) and timothy grass hay (TH): 38.8% AH and 0% TH (ALF); 26.0% AH and 12.8% TH (HIALF); 12.8% AH and 26.0% TH (HITIM); and 0% AH and 38.8% TH (TIM). Data were analyzed with the GLIMMIX procedure of SAS (9.4) with treatment as the fixed effect and treatment and time for rumen pH repeated measure. Diets were similar in NDF content (34.3, 33.4, 32.4, and 31.5% for TIM, HITIM, HIALF, and ALF, respectively) but differed in ADF content (18.7, 19.3, 19.9, 20.5% for TIM, HITIM, HIALF, and ALF, respectively), resulting in differences in the ratio of ADF to NDF (0.55, 0.58, 0.61, and 0.65 for TIM, HITIM, HIALF, and ALF, respectively). No treatment difference ($P > 0.12$) was observed on total ruminating time (average 393 ± 36.2 min/d), but passage rate tended ($P = 0.08$) to increase linearly with increasing fragility (2.8, 2.81, 3.35, 3.22 ± 0.31%/h for TIM, HITIM, HIALF, and ALF, respectively). Contrary to our hypothesis, rumen pH linearly ($P = 0.01$) increased with increasing fragility (6.13, 6.20, 6.21, 6.30 ± 0.31 for TIM, HITIM, HIALF, and ALF, respectively). Digestibility of NDF linearly ($P = 0.01$) decreased with increased fragility (52.6, 51.4, 48.6, 44.4 ± 3.15 for TIM, HITIM, HIALF, and ALF, respectively), but ADF digestibility did not differ ($P > 0.36$; average 47.0 ± 3.49%). The results of our study indicate that rumen pH is not always inversely related to forage fragility, and we suggest pH may also be influenced by other factors such as buffering capacity of the forage itself.

Key Words: forage fragility, rumen pH, rumination

2264 Supplementing dairy cows' diets with algae-based feed additive reduces emissions, yield and intensity of enteric methane. C. Benchaar*[1] and F. Hassanat[2], *[1]Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, [2]Quebec Research and Development Centre, Agriculture and Agri-Food Canada, Quebec, QC, Canada.*

This study evaluated the effects of supplementing an algae-based feed additive on enteric CH_4 emissions and milk production of Holstein cows. Sixteen multiparous lactating cows (DIM = 162 ± 29.5; milk yield = 47.2 ± 4.09 kg) were used in a completely randomized design. The experimental period consisted of 5 wk preceded by 3 wk of covariate. Cows were fed (ad libitum) a TMR not supplemented (control), or supplemented (0.25% of DMI) with the algae additive (Synergraze Inc., Calgary, AB). The dried algae additive contained 4.65 ± 0.225 mg/g bromoform. The TMR consisted of 63% forages (corn silage, alfalfa silage, hay) and 37% concentrates, on a DM basis. Intake (DM), milk production, and enteric CH_4 production were determined over 4 consecutive days (respiration chambers) during the covariate period, at the beginning (wk 1), and after 35 d (wk 5) of feeding the algae. Data were analyzed (MIXED Procedure, SAS) using the repeated statement and adjusted for the covariate. Significant effect of algae supplementation or algae × sampling week interaction was declared at $P \leq 0.05$. There were no significant treatment by time interactions for CH_4 production, DMI, or milk production. Algae supplementation had no effect on DMI (25.8 kg DM/d, $P = 0.50$) or milk production (39.5 kg/d, $P = 0.76$). In contrast, compared with the control, feeding algae reduced ($P < 0.01$) enteric CH_4 emissions (g/d) by 47%. Consequently, methane yield (g CH_4/kg DMI) decreased from 19.2 g/kg to 10.4 g/kg, and CH_4 emission intensity (g CH_4 /kg milk) from 12.80 g/kg to 6.94 g/kg upon diet supplementation with algae. This study shows that including algae at 0.25% of DMI caused a marked reduction in daily CH_4 production without adversely affecting DMI or milk yield, thereby decreasing yield and emission intensity of CH_4.

Key Words: enteric methane, milk production, algae

2265 Evaluating acid detergent fiber insoluble ash as an intrinsic marker to estimate fecal outputs in lactating dairy cows. J.

Kim*, T. R. Grohnke, and C. Lee, *Department of Animal Sciences, The Ohio State University, Wooster, OH.*

Indigestible NDF (INDF) and acid-insoluble ash (AIA) as intrinsic markers have been widely used to estimate fecal outputs (FO) followed by total-tract DM digestibility (DMD). However, relatively large estimation errors have been reported for AIA. Although INDF has been considered a reliable marker, it requires cannulated cows and the procedure is relatively long. Recent studies have evaluated ADF insoluble ash (ADFIA) as an intrinsic marker. We evaluated ADFIA as an alternative marker to estimate FO and DMD in lactating cows. To validate ADFIA, samples were obtained from the previous 2 studies where total collection of feces was conducted: study 1 with 4 cows in a 4 × 4 Latin square design (n = 16; diet with 37.5% corn silage, 19.0% alfalfa silage, and 43.5% concentrate) and study 2 with 8 cows in a 4 × 4 Latin square design (n = 31, one cow was removed; diet with 49.0% corn silage, 6.3% alfalfa silage, and 44.7% concentrate). Feed ingredients and fecal samples were dried, ground, and composited by cow and period. All samples were assayed for ADF followed by ash (600°C for 8 h). Fecal output was calculated by DMI × dietary ADFIA concentration ÷ fecal ADFIA concentration. The statistical analysis was conducted using the Mixed procedure of SAS with random effects of study and cow (square and study). There was no difference between actual FO and estimated FO from ADFIA (8.31 vs. 8.00 kg/d, $P = 0.11$). However, the estimated DMD from ADFIA was lower (69.5% vs. 70.6%; $P = 0.04$) compared with the actual DMD. The linear relationship between actual and estimated FO was evaluated: Actual FO (kg/d) = 0.85 (SE = 0.077) × Estimated FO (kg/d) + 1.50 (SE = 0.631). The slope tended to differ from one ($P < 0.06$), and the intercept was not different from zero. The root mean squared prediction error was 11.55% of the mean, and the concordance correlation coefficient was 0.84. Results indicate that ADFIA has the potential for estimating FO and DMD in lactating cows, but the underestimation of DMD by 1.5% indicates that a correction factor might be necessary.

Key Words: digestibility, fecal output, intrinsic marker

2266 Evaluation of 48-hour ruminal dry matter digestibility of poly(butylene adipate-co-terephthalate) bioplastic bags. A. L. Carroll* and P. J. Kononoff, *University of Nebraska–Lincoln, Lincoln, NE.*

Compostable bags created from poly(butylene adipate-co-terephthalate) (PBAT) and corn starch are designed to be able to fully compost within 6 to 12 mo within the soil environment. Similar to soil, the rumen environment contains a host of different microorganisms and may provide a suitable environment to break down bioplastic materials. Our objective was to evaluate the 48-h rumen DM digestibility (DMD) of the bioplastic product. Two multiparous lactating Jersey cattle (140 ± 12.6 DIM) with a milk yield of 23.0 ± 9.07 kg/d fitted with flexible ruminal cannula were fed once daily at 0930 h and had an average intake of 20.2 ± 7.75 kg/d DMI. Samples of bioplastic and corn silage (8.4% CP, 32.5% aNDFom, 40.7% starch, and 2.39% TFA) were ground to 2 mm, and then, 1.5 g was weighed and placed in 5 and 3 R510 Ankom concentrate bags, for bioplastic and corn silage, respectively. Bags had a pore size of 50 µm and dimensions of 5 cm × 10 cm and were heat sealed. Dacron bags were placed in a mesh bag (8 Dacron bags per mesh bag) and then in a secondary bag that contained a 100-g weight and placed within the ventral sac of the rumen for a total incubation time of 48 h. Subsequent to this time, all bags were removed from the rumen and gently rinsed in a commercial washing machine using 5 cycles that were 1 min of agitation and 2 min of spin. After washing, each sample was dried in a 45°C oven for 48 h. After drying, bags were weighed to determine the weight of the residue. During the 48-h incubation, corn silage was also used as a standard and had a 48-h DMD of 70.6 ± 0.57%, which significantly differed ($P < 0.01$) from the bioplastic bag averaging 5.59 ± 0.441% with a range of 3.67% to 7.77% between the 2 animals, respectively. Although a 5.60% DMD may seem low, the average 48-h digestibility of this PBAT bioplastic during compositing ranges from 0.548% to 1.10% based on the 6- to 12-mo composability timeline. Thus, ruminal DMD was 5.09 to 10.2 times faster than composting. Therefore, the rumen microbiome may serve as a location to derive microorganisms for future bioplastic recycling strategies.

Key Words: bioplastic, rumen degradation, rumen microbiome

2267 Presence of multiple mycotoxins in the total mixed rations and subsequent milk of dairy cows in southeastern Brazil. A. M. Borowsky[1], R. D. Pires[1], R. C. Pires[2], R. E. Rosim[2], F. G. Tonin[2], and C. H. Corassin*[1,2], [1]*Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil,* [2]*School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil.*

Multiple mycotoxin contamination of animal feed is a problem that affects many sectors, from animal production to the food industry. Our aim was to investigate the presence of multiple mycotoxins in the TMR of dairy cows and in the milk produced by them, to identify and quantify the mycotoxins to assess the transfer from the cows to the milk. One sample of TMR and one sample of bulk tank milk were collected from 30 dairy farms in southeastern Brazil. An ultra-performance liquid chromatography system coupled to a mass spectrometer was used to determine the mycotoxins. Statistical significance was considered at $P = 0.05$. Mycotoxin analysis of TMR revealed fumonisins (FUM) in 96.7% of the samples, zearalenone (ZEN) in 93.3%, deoxynivalenol (DON) in 43.3%, and total aflatoxins (AF) in 26.6%. The mean concentration of positive TMR samples for FUM, ZEN, DON, and AF was 85.1 ± 60.7, 35.9 ± 24.7, 76.7 ± 56.6, and 5.2 ± 3.3 µg/kg, respectively. In the milk of cows fed with these TMR, α-zearalenol and β-zearalenol were detected in 56.7% of the samples, FUM in 53.3%, DON in 43.3%, and aflatoxin M_1 (AFM_1) in 13.4%. The average concentration of positive milk samples for α-zearalenol, β-zearalenol, FUM, DON, and AFM_1 was 1.958 ± 1.483, 0.218 ± 0.165, 1.353 ± 0.767, 0.294 ± 0.267, and 0.099 ± 0.081 ng/mL, respectively. Toxin T-2 and ochratoxins were not detected in TMR or milk. The results indicate that because multiple mycotoxins present in the diet were also detected in bulk tank milk samples, milk can be an interesting matrix for determining the contamination of dairy farms with multiple mycotoxins. However, further studies are necessary for the determination of risk levels for the presence of mycotoxins in milk.

Key Words: mycotoxin, contamination, milk

2268 Evaluating the total mixed ration stability of rumen-protected choline products. K. A. Estes[1,2], M. G. Zenobi*[1], C. A. Zimmerman[1], and M. D. Hanigan[2], [1]*Balchem Corporation, Montvale, NJ,* [2]*Virginia Tech, Blacksburg, VA.*

A technique has been established to determine the TMR stability of rumen-protected (RP)-lysine products, but the TMR stability of RP-choline (RPC) products has not yet been explored. The objective of this experiment was to evaluate the TMR stability of 5 RPC products using a modified technique. Triplicate Ziploc bags containing RPC (equivalent of 1 g of choline chloride [CC]), no RPC (negative control), or unprotected CC (positive control; 1 g of CC) mixed with 200 g of TMR (38% DM) were incubated at room temperature (22°C) and unsealed for 0, 6, 12, and 24 h. At the end of each time point, bag contents were transferred

Table 1 (Abstr. 2268). Rumen-protected choline (RPC) release (%) at different TMR exposure times

TMR exposure time (h)	RPC					
	A	B	C	D	E	SEM
0	14.5^{a}	10.9^{a}	2.9^{b}	0.8^{b}	1.2^{b}	0.9
6	70.1^{a}	74.8^{a}	10.7^{c}	1.7^{d}	24.0^{b}	1.6
12	81.7^{a}	88.1^{a}	16.0^{c}	2.5^{d}	43.8^{b}	1.7
24	98.8^{a}	103.8^{a}	25.5^{c}	4.9^{d}	62.5^{b}	1.4

$^{a–d}$Within a time point, values with different superscripts differ, $P < 0.05$.

to strainer bags and soaked in 1 L of distilled water for 1 min to solubilize any free CC released from the products. A sample of solution was filtered (0.45 µm) and stored at −20°C until analysis for CC content using a YSI biochemical analyzer. The CC content for samples containing RPC or unprotected CC were corrected for background CC measured in the negative control samples. Recovery of unprotected CC was influenced by TMR incubation time and was not complete (78%, 61%, 59%, and 51% at 0, 6, 12, and 24 h of TMR exposure, respectively). Thus, a correction factor was applied to samples containing RPC to account for unrecovered CC released from the products. Total CC release was calculated for RPC. Data were analyzed using the MIXED procedure of SAS. Means within an incubation time were separated using the Tukey adjustment. At time 0, products C, D, and E experienced minimal CC release (Table 1). Products A and B had a similar release pattern to one another across the 4 time points and were the 2 products with the highest CC release at all exposure times. Product D was the most stable from 6 to 24 h. The modified technique detected vast differences in TMR stability across RPC products over a 24-h period.

Key Words: rumen protected choline, TMR, choline

2269 Effect of dietary feed additives and organic trace minerals on nitrogen balance and blood metabolites of lactating cows.

G. Poletti[1], D. J. Cavalli Vieira[1], N. T. Scognamiglio Grigoletto[1], R. G. Chesini[1], N. Pereira Martins[1], B. de Magalhães Ceron[1], C. S. Takiya[1,2], C. S. Cortinhas[3], V. V. de Carvalho[3], and F. P. Rennó*[1], *[1]University of São Paulo, Pirassununga, SP, Brazil, [2]Federal University of Technology–Paraná, Pato Branco, PR, Brazil, [3]DSM-Firmenich, São Paulo, SP, Brazil.*

This study evaluated the effects of different feed additives on nitrogen balance and blood metabolites in dairy cows. Thirty-nine Holstein cows (201 ± 70 DIM; 651.6 ± 36.1 kg of BW and 33.4 ± 0.49 kg/d milk yield) were blocked (n = 13) according to parity, DIM, and milk yield and randomly assigned to one of the following treatments: 1) monensin (Rumensin 200, Elanco) at 15 mg/kg of DM (MON); 2) phytogenic feed additives (Digestarom Dairy, dsm-firmenich) at 2 g/cow per day (PFA); and 3) PFA + autolyzed yeast (AY) at 15 g/cow per day + organic trace minerals as carbo-aminofosfoquelates (OTM; dsm-firmenich) at 30 g/cow per day (PAC). Additives were provided mixed into the concentrate. Cows were allowed a 2-wk covariate period, and treatments were applied for the following 9 wk. Ingredients of the ration, milk, urine, and feces samples were sampled during the covariate period and at wk 3, 6, and 9 of the experiment for N analysis and determination of N balance. Blood samples were analyzed for glucose, urea, and hepatic enzymes using commercial kits. Data were analyzed using the MIXED procedure of SAS 9.4. Orthogonal contrasts evaluated treatment differences (MON vs. PFA + PAC; and PFA vs. PAC). Cows fed PFA and PAC had lower concentrations of BUN ($P = 0.002$) and glucose ($P = 0.040$) in the serum than cows fed MON. Additionally, cows of the PAC group had lower concentrations of BUN ($P = 0.003$) and tended to have lower concentrations of glucose ($P = 0.060$) than the cows fed PFA. No differences were detected for hepatic enzymes in the serum between the groups ($P = 0.05$). It was detected that cows fed MON tended to have greater intake of nitrogen ($P = 0.05$) and lower nitrogen excretion in milk ($P = 0.021$) than the groups supplemented with feed additives. In addition, cows fed PAC tended to have greater nitrogen excretion via urine ($P = 0.094$) and lower nitrogen excretion (MUN) in milk ($P = 0.080$) than the PFA cows. Feeding phytogenic feed additives, either alone or in combination with AY and OTM, resulted in decreased blood BUN and glucose levels, as well as improved nitrogen utilization compared with the MON treatment in dairy cows.

Key Words: blood urea nitrogen, glucose, nitrogen utilization

2765 Effect of an enzymatic xylanase complex in dairy cow diets on productive performance and dry matter intake.

A. Daudet[1], C. Rivoir*[1], V. Nenov[2], and P. Chilibroste[1], *[1]Universidad de la Republica, Facultad de Agronomía, Paysandú, Uruguay, [2]Huvepharma NV, Antwerp, Belgium.*

Feeding exogenous fibrolytic enzymes could be a nutritional strategy to increase fiber digestibility and maximize feed efficiency and sustain production performance. The objective of this study was to evaluate the effect of level of supplement enzymatic complex with main activity of xylanase (HX, Hostazym X, Huvepharma, Bulgary) on milk production (MP), dry matter intake (DMI) and energy efficiency (EF) of 16 Holstein multiparous dairy cows in mid lactation (165 d in milk). Four treatments (T) were compared according the dose of the enzymatic complex: 0 (T0), 5 (T5), 10 (T10) and 15 (T15) grams per day in an unbalanced 5 × 4 Latin square design with periods of 22 d (10 for adaptation and 12 for determinations). Cows were fed a total mixed ration (TMR) *ad libitum* (composed by sorghum silage, concentrate, moha hay and the xylanase carrier, DM bases: 46.5, 44.2, 4.7, and 4% inclusion, respectively) in a compost barn system. The enzymatic complex was supplied twice a day (a.m. and p.m.) with the carrier in a pellet form. Data were analyzed using the glimmix procedure of SAS, terms in model included square, period, cow(square) and T as fixed effects. Differences in MP were found among T (Table 1) with a

Table 1 (Abstr. 2765). MP, ECM, DMI, and feed efficiency with different doses of HX[1]

Variable	T0	T5	T10	T15	T	Lin	Qua
MP, kg/d	28.5 ± 0.3^{b}	28.8 ± 0.3^{ab}	29.8 ± 0.3^{a}	29.3 ± 0.3^{ab}	0.011	0.008	0.161
ECM, kg/d	29.5 ± 0.6	30.3 ± 0.6	31.3 ± 0.6	30.4 ± 0.6	0.223	0.182	0.15
DMI, kg DM/d	26.0 ± 0.6	25.5 ± 0.6	25.1 ± 0.6	25.8 ± 0.6	0.744	0.696	0.331
kg ECM/kg DMI	1.13 ± 0.08	1.17 ± 0.08	1.24 ± 0.08	1.15 ± 0.08	0.278	0.43	0.131
NE_L milk/NE_L intake	0.52 ± 0.02	0.54 ± 0.02	0.57 ± 0.02	0.53 ± 0.02	0.312	0.453	0.141

^{a,b}Different letters indicate significant differences between treatments.

[1] ECM = energy-corrected milk; NE_L = net energy of lactation; Lin = linear; Qua = quadratic response.

significant positive linear response to increasing HX dose. Knowing that the expression of the treatment effect may be affected by diverse factors in this study, it can be concluded a positive significant effect on HX use with a intermedium doses (T10).

Key Words: xylanase, doses responses, milk efficiency

Ruminant Nutrition 2: Gut Physiology, Fermentation, and Digestion

2270 Effect of red clover isoflavones on rumen microbial composition and fermentation in dairy cows. Y. Bu, J. Q. Wang, and S. G. Zhao*, *Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Red clover, enriched with phytoestrogens such as isoflavones, notably biochanin A and formononetin, has demonstrated potential in enhancing ruminant performance through improved feed efficiency and nitrogen utilization efficiency. Despite this, the precise effect of red clover isoflavones on rumen fermentation and bacteria diversity remained inadequately understood. Our study sought to comprehensively assess these effects in dairy cows using a combination of in vivo and in vitro experiments. In the in vivo experiments, employing a randomized complete block design, 40 Holstein dairy cows were divided into 4 groups, each fed red clover extracts at doses of 0, 2, 4, 8 g/kg containing a total of biochanin A and formononetin at 0, 0.46, 0.92, 1.83. Rumen fluid was collected for analysis of fermentation and microbial composition through shot-gun metagenomic sequencing. Concurrently, in an in vitro rumen fermentation trial, 4 treatments were designed to evaluate the effectiveness of biochanin A and formononetin. Rumen fermentation parameters data in experiments were investigated using MIXED model. Results from the in vivo experiments indicated that red clover isoflavones significantly decreased NH_3-N concentration and urease activity. These isoflavones also affected microbial composition by reducing the abundance of proteolytic bacterial species while enhancing cellulolytic species. The in vitro trial further demonstrated the ability of biochanin A and formononetin to decrease urea decomposition rates, with biochanin A exhibiting a stronger effect. This aligned with the reduction in ureolytic and proteolytic bacteria and an increase in cellulolytic bacteria observed in both in vivo and in vitro trials. In conclusion, biochanin A emerged as the predominant effective component of red clover isoflavones, influencing urea-N hydrolysis and rumen fermentation. This highlighted the potential of red clover isoflavones as urease inhibitors to enhance rumen fermentation, improve nitrogen utilization efficiency, and ultimately boost ruminant performance, suggesting a valuable tool for enhancing overall productivity in the future.

Key Words: red clover isoflavones, rumen microbiome, dairy cows

2271 Hydrolyzed yeast to influence rumen fermentation. H. Schulze*[1], T. Rinttilä[2], and J. Johnston[3], [1]*Livalta/AB Agri, Peterborough, UK,* [2]*Alimetrics Research Ltd., Espoo, Finland,* [3]*Fermentrics Technologies Inc., McNab-Braeside, ON, Canada.*

In vitro rumen fermentation studies have shown that supplementing diets with hydrolyzed yeast (HY; Livalta™Cell HY40, Peterborough, UK) can modify the rumen microbial activity (Schulze and Rinttilä, 2024), but missing is information on its prolonged effects on the rumen microbiota and metabolism. Therefore, this study aimed to investigate the effect of feeding HY (15 g/head per day) on ruminal microbial composition and activity. Two pairs of dry rumen-fistulated Holstein and Jersey cows were used in this study. Each pair was part of a 110-head milking group, jointly housed. Cows were fed a corn-silage-based TMR with or without HY supplementation in a 41-d switch-back design trial. At d 12, 21, and 41, duplicate samples of rumen fluid were collected at 0800 h from each cow into BioFreeze™ (Alimetrics Diagnostics Ltd., Espoo, Finland) sample preservation tubes for the analysis of short-chain fatty acids (SCFA) and quantification of microbial species, groups, or genera via real-time quantitative PCR according to Rinttilä et al. (2022). Data were analyzed using ANOVA on JMP 17.0 including fixed effects of diet, breed, and their interaction. Student's *t*-test was used to separate LSM. HY supplementation increased ($P < 0.05$) the total ruminal SCFA (176 to 206 mmol/kg), acetic, propionic, and valeric acid, and tended to increase ($P < 0.10$) butyric and branched-chain fatty acids but not relative proportions. Propionic and valeric acid concentrations for Holstein were higher ($P < 0.05$) than for Jersey. No significant diet × breed interaction was observed for total and individual SCFA. Ruminal eubacteria density tended ($P < 0.10$) to be increased with HY supplementation, and *Lachnospiraceae* and *Selenomonas ruminantium* density significantly increased. There were no breed effects on rumen eubacteria density; however, diet × breed interactions ($P < 0.05$) were observed for total eubacteria and protozoa counts. In addition, β-diversity analysis revealed differences in the analyzed rumen microbiota depending on diet and diet × breed interaction. It can be concluded that supplementing a TMR with HY can change the rumen microbiota density and composition leading to elevated SCFA concentrations.

Key Words: hydrolyzed yeast, rumen microbiome, rumen SCFA

2272 Effects of MicroLife® Rover direct-fed microbial on nutrient utilization in dairy heifers. A. O. Oyebade*, E. Hass, J. D. Chapman, B. D. Humphrey, and M. Garcia, *Phibro Animal Health Corporation, Teaneck, NJ.*

Bacillus-based direct-fed microbials (DFM) have demonstrated capability to enhance nutrient utilization in ruminants. This study evaluated the effects of MicroLife® Rover DFM (MR, Phibro Animal Health), a DFM containing *Bacillus subtilis* and *Bacillus licheniformis*, on in vivo nutrient digestibility and in vitro rumen fermentation (IVRF). Ruminally-cannulated Jersey heifers (n = 20) fed an early-lactation diet were blocked by pen and BW (433 ± 122 kg) and randomly assigned to treatments: control (CON; no DFM) or MR (fed at 1e10 cfu/d) for the 30-d study. Fecal grab samples were taken on d 28 and 29 for apparent nutrient digestibility and VFA estimations. The basal diet was ground (1-mm screen) and used for IVRF. Rumen fluid for IVRF was collected on d 28 and 30 of the study, corresponding to the first and second IVRF run, respectively. Rumen fluids were pooled by treatment separately for each day, buffered, and incubated for IVRF at 39°C for 24 h. Four IVRF treatments were tested in quadruplicates per run, using rumen fluids as is (CON, MR) and rumen fluids with 1e7 cfu of MR added (CON+, MR+). Data were analyzed by lme4 library of R v.4.0, with fixed effect of treatment. Block and run were considered random effects for in vivo and IVRF data, respectively. In vivo DMI (11.4 and 11.0 kg/d); ADG; and digestibility of DM, CP, starch, NDF, fat, and ash were not different ($P > 0.10$) between CON and MR. Fecal valerate (%) tended ($P = 0.07$) to be lower for MR, whereas other VFA and fecal NH_3-N (mg/dL) were not different. For the IVRF, MR and MR+ increased ($P \leq 0.05$) total VFA and increased ($P \leq 0.01$) acetate (m*M*), branched-chain VFA (isobutyrate and isovalerate), and NH_3-N (mg/dL) relative to CON. Treatment CON+ tended to decrease ($P = 0.08$) acetate (m*M*) relative to CON. No treatment differences ($P > 0.10$) were observed for ruminal VFA (%), except isovalerate and isobutyrate, which MR and MR+ increased ($P \leq 0.01$) or tended ($P = 0.10$ and 0.06) to increase, relative to CON, respectively. The results showed that MicroLife® Rover DFM increased concentrations of acetate, isobutyrate, and isovalerate in vitro, suggesting the potential to enhance rumen fermentation.

Key Words: *Bacillus*, DFM, rumen fermentation

2273 Preliminary study on some innovative rumen-bypass micro-encapsulation techniques for essential oils. A. Maggiolino*[1], G. Natrella[2], N. Denora[3], A. Lopedota[3], A. Di Grigoli[4], M. Ponte[4], M. G. Ciliberti[5], and R. Marino[5], *[1]Department of Veterinary Medicine, University of Bari, Bari, Italy, [2]Department of Soil, Plant and Food Sciences, University of Bari, Bari, Italy, [3]Department of Pharmacy, Pharmaceutical Sciences, University of Bar, Bari, Italy, [4]Dipartimento Scienze Agrarie Alimentari e Forestali, Università di Palermo, Palermo, Italy, [5]Department of Agriculture, Food, Natural Resources, and Engineering, University of Foggia, Foggia, Italy.*

The aim of this trial was to provide novel technologies for the production of rumen by-pass essential oils to be released in the abomasum. For this purpose, 99.98% of pure limonene (Lim) was microencapsulated. Optimal feed was determined by exploring 4 oil-in-water emulsions with Lim oil and alginate. The most stable emulsion was combined with Eudragit EPO 30% (wt/wt) or Edraguard® 30% (wt/wt) to produce 3 experimental formulations (F). Process parameters were set with a single (F1: EPO, 657 µL Lim/g) and concentric (F2: EPO, 558 µL Lim /g; F3: Edraguard®, 327 µL Lim/g) nozzles for microcapsules. The encapsulation yields ranged from 50% to 75% among the 3 different F. Rumen fluid was sampled at the slaughterhouse from animals from the same farm. In vitro digestion (IVD) trial for each F was performed into quadruplicate. In vitro digestion was conducted for 6, 12, 18, and 24 h using the Daisy II incubator system. A total of 14 bags were put in each jar, each bag containing 0.5 g of microcapsules. Bag content was weighed to calculate the disappearance (wt/wt), and rumen fluid was sampled for Lim quantification. Data were subjected to ANOVA analysis considering as fixed effects the experimental formulation and the digestion time. The concentration of Lim was carried out using SPME/GC-MS. The F1 and F2 had a disappearance of over 50% after 6 h ($P < 0.01$), increasing to more than 80% and 70%, respectively, after 24 h ($P < 0.01$), whereas in F3, no significant disappearance ($P > 0.05$) was observed, ranging between 0.5% (6 h) and 1.3% (24 h). Lim content ranged from 1,472 µg/L at 6 h to 1,959 µg/L at 24 h (64.5% vs. 86%) for F1, from 1,021 µg/L at 6 h to 1,488 µg/L at 24 h (52% vs. 77%) for F2, and from 2 µg/L at 6 h to 9 µg/L at 24 h (0.2% vs. 0.7%) for F3. Two of the 3 formulations (F1 and F2) in which EPO was used, both in matrix and core-shell, showed low resistance in the rumen environment. In contrast, the use of Endraguard® in core-shell exhibited a very high resistance up to 24 h, laying the groundwork for future research on the microencapsulation of substances characterized by rumen bypass.

Key Words: in vitro digestion, microincapsulation, rumen-bypass

2274 In vitro digestibility of a biofilm to be used in ruminant feed. D. Zhang[1], J. Russi*[2], A. Relling[1], and O. Campanella[1], *[1]The Ohio State University, Wooster, OH, [2]One Idea, Argentina.*

A biofilm derived from a blend of feather meal (FM), whey permeates (L), glycerol (G), and water (W), as outlined in PCT/US23/73843, was synthesized with varying amounts of raw materials. Treatments were 15 (150 g FM, 5 g L, 4.5 g G, and 45 g W), 17 (170 g FM, 3 g L, 5.1 g G, and 27 g W), 19 (190 g FM, 1 g L, 5.7 g G, and 9 g W), and 20 (200 g FM, 0 g f L, 6 g G, and 0 g W). Suspensions were heated (HT) 1 or 3 h at 90°C (to produce a Maillard reaction) and dried at 70°C for 5 h. The HT0 (control) had the same dispersions as other HT but was dried at 30°C for 48 h (to avoid a Maillard reaction). Films were prepared in triplicates (experimental units). Each triplicate was then subsampled in 4. From those 4 subsamples, 2 of each subsample were randomly added in a jar and incubated in a Daisy incubator for 12 or 24 h. Six jars were used with 24 samples per jar. For the incubation 1 g of film was placed in a filter bag (Ankom F57) and placed in jars containing 4:1 McDougall's buffer:rumen content solution. Samples were analyzed as a mixed model, considering the fixed effect of treatment, HT, digestibility time, and their interaction and the random effect of jar. Due to a triple interaction (treatment × HT × digestibility time, $P < 0.01$), we will present the data as the interaction of treatment × HT at different times of digestion (Table 1). The digestibility (expressed as %) of the biofilm at 12 h of digestibility was affected by the inclusion rate of the materials, decreasing the digestibility as the amount of FM increased. The HT affected digestibility at 12 h, with HT0 having the greatest digestibility. At 24 h the overall digestibility of the biofilm was greater than the 12 h. At 24 h HT did not affect the biofilm digestibility, but digestibility decreased as FM inclusion rate increased. The HT0 had greater digestibility. Treatment 15 had greater digestibility independent of the HT and at both 12 and 24 h.

Key Words: plastic, degradability, sustainability

Table 1 (Abstr. 2274). In vitro digestibility (%, 12 or 24 h) of biofilms with different concentrations of raw material and different processing times (HT)

		Treatment (Trt)					*P*-value	
Digestibility, %	HT	15	17	19	20	SEM	Trt	Trt × HT
12 h	0	47.7	46.0	37.1	35.6	0.78	<0.01	0.20
	1	42.8	36.7	31.1	32.7			
	3	44.2	39.8	35.5	35.3			
24 h	0	50.6	47.8	39.5	38.0	1.02	<0.01	<0.01
	1	45.4	41.9	36.7	36.7			
	3	47.7	40.9	39.1	39.3			

2275 Antimicrobial susceptibility of *Enterococcus* and *Bacillus* species isolated from probiotics. R. Branco-Lopes*[1], E. Okello[2], K. Tonooka[2], H. Okella[2], R. Pereira[2], and N. Silva-del-Río[2], *[1]University of California Agriculture and Natural Resources, Tulare, CA, [2]University of California, Davis, Tulare, CA.*

This study aimed to evaluate the phenotypic antimicrobial susceptibility of *Enterococcus* spp. and *Bacillus* spp. isolated from commercially available cattle probiotics. Thirty-five probiotics claiming to contain at least one type of *Enterococcus* spp. or *Bacillus* spp. were evaluated. Phenotypic antimicrobial susceptibility was evaluated by determining minimum inhibitory concentrations of 16 antimicrobials using broth microdilution (gram-positive panel plates—CMV3AGPF). The *Enterococcus* spp. (n = 16) were categorized as susceptible, intermediate, or resistant according to the breakpoints of the Clinical Laboratory and Standards Institute (2020) and the National Antimicrobial Resistance Monitoring System (2021). The *Bacillus* spp. (n = 23) were classified as susceptible or resistant using a cut-off from the European Food Safety Authority Panel on Additives and Products or Substances used in Animal Feed (2018). *Enterococcus*-based probiotics were identified as *Enterococcus faecium* (n = 15) and *Enterococcus hirae* (n = 1). *Bacillus*-based probiotics were identified as *Bacillus amyloliquefaciens* (n = 1), *Bacillus licheniformis* (n = 1), *Bacillus subtilis* (n = 7), and *Bacillus velezensis* (n = 14). All *Enterococcus* isolates exhibited susceptibility to chloramphenicol, streptomycin, tetracycline, tigecycline, and vancomycin. *Enterococcus* isolates were susceptible to all the antimicrobials tested (n = 4) or resistant to one antimicrobial (n = 9; ciprofloxacin, erythromycin, penicillin, and daptomycin) or 2 antimicrobials (n = 2; erythromycin and gentamicin and daptomycin and erythromycin) or were multidrug resistant (n = 1; ciprofloxacin, daptomycin, and quinupristin/dalfopristin). All *Bacillus* isolates showed susceptibility to both tetracycline and vancomycin, and 19 isolates were susceptible to all investigated antimicrobials with thresholds available. However, one *Bacillus* isolate displayed resistance to both chloramphenicol and erythromycin, and 3 *Bacillus* isolates were resistant to erythromycin. In conclusion, the antimicrobial susceptibility patterns among *Enterococcus* and *Bacillus* isolates highlight the need for safety assessments of commercially available probiotics.

Key Words: direct-fed microbials, antibiotics, resistance

2276 Fermentation and digestibility characteristics of *Ascophyllum nodosum*, *Alaria esculenta*, and an *Alaria esculenta* and *Palmaria palmata* blend in a continuous culture system.

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Bioactive compounds, such as polyphenols, differ among macroalgal species and have been shown to alter ruminal metabolism. Therefore, the fermentation profile and nutrient digestibility of diets supplemented with *Ascophyllum nodosum*, *Alaria esculenta*, or an *A. esculenta* and *Palmaria palmata* blend were assessed using a single-flow continuous culture system. Ruminal fluid was collected from 2 Holstein Friesian cows and inoculated in 4 prewarmed fermenter vessels. Treatments were randomly allocated in a 4 × 4 Latin square design and fed a corn-silage-based diet with a 60:40 forage:concentrate ratio. Each vessel received 1% (DM basis) of grass silage (control = CON), *A. nodosum*, *A. esculenta*, or a 50:50 blend of *A. esculenta* and *P. palmata* at the expense of corn silage. Periods lasted 10 d, with 7 d for diet adaptation and 3 d of sampling. Digestibility of DM, OM, aNDFom, and ADF and fermentation characteristics including pH and concentrations of VFA and NH_3-N were measured. Gaseous concentrations (CH_4 and CO_2) were measured daily 30 min before feeding, with a gas analyzer probe. Treatment and period were considered fixed effects and fermenter as the random effect, with the Tukey honestly significant difference test used to separate means in R. No difference ($P \geq 0.42$) was observed across treatments for nutrient digestibility. Furthermore, concentrations of NH_3-N and total VFA, and the molar proportion of acetate, propionate, and butyrate, were similar ($P \geq 0.18$). Daily pH was greatest ($P = 0.01$) in *A. nodosum* (5.48), lowest in CON (5.42), and intermediate in *A. esculenta* (5.44) and the seaweed blend(5.44). Fermenters supplemented with *A. nodosum* had a greater ($P = 0.04$) concentration of CO_2 (17.7 mg/dL) than CON (13.2 mg/dL) and increased ($P = 0.01$) concentration of CH_4 (0.13 mg/dL) compared with *A. esculenta* (0.05 mg/dL) or the seaweed blend (0.03 mg/dL). Our data showed similar fermentation profile (apart from pH) and digestibility characteristics across diets, and decreased methanogenesis with *A. esculenta* and the seaweed blend in comparison with *A. nodosum*.

Key Words: macroalgae, fermentation, methane

2277 The effects of dietary inclusion of LinPRO-R on ruminal fermentation and nutrient degradation in a dual-flow continuous culture system.

J. R. Vinyard[1], M. L. Johnson*[1], G. K. Salas Solis[1], A. C. Silva Vicente[1], M. U. Siregar[1], E. Sarmikasoglou[1], C. Hammond[1], S. W. Ma[1], R. R. Lobo[1], L. Girardin[2], R. Dreger[2], and A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*O&T Farms, Regina, SK, Canada.*

The inclusion of n-3 fatty acids (FA) in human diets has been demonstrated to be anticarcinogenic and prevent cardiovascular disease; therefore, there has been increasing interest in these FA in dairy products by including them in the diets of cows. Thus, the objective of this study was to determine the effects of feeding LinPRO-R, a rumen-protected matrix of fat and protein from flaxseed and peas (LIN; approximately 21% fat, 24% CP, and 5.4% n-3 FA; % of DM), using a dual-flow continuous culture system. Treatments were as follows: 0 (0P), 2.5 (2.5P), 5 (5P), and 7.5% of DM (7.5P) inclusion of LIN in a TMR with LinPRO-R replacing portions of dried distillers grains and Ca salts of long FA in the diet to maintain similar levels of CP and ether extract (EE). Twice daily, 53 g of DM of each TMR (16% CP, 33% NDF, 28% starch, 4.2% EE; as % of DM) was provided to each fermenter. The treatments were arranged in a replicated 4 × 4 Latin square using 8 dual-flow continuous culture fermenters. Four 10-d experimental periods (7 d adaptation and 3 d sample collection) were done. Samples were collected from fermenter effluents at 3, 6, 9, and 24 h after morning feeding and composited to determine lactate and VFA concentrations and nutrient degradation. Data were analyzed using the Glimmix procedure of SAS with square, fermenter within square, period, and the fermenter by treatment interaction being random effects. Butyrate (15.6%, 16.0%, 15.0%, and 13.2% of total VFA ± 1.45 for 0P, 2.5P, 5P, and 7.5P, respectively) and isobutyrate (0.74%, 0.69%, 0.70%, and 0.62% of total VFA ± 0.07) proportions decreased ($P \leq 0.04$), linearly, with increasing inclusion of LinPRO-R, potentially due to changing microbial communities within the fermenter due to the increasing concentration of PUFA. There were no differences ($P \geq 0.15$) for any other VFA proportions, total VFA or lactate concentrations, or the degradation of DM, OM, NDF, or CP. Thus, inclusion of LIN, with its higher concentration of n-3, can occur without impairing ruminal fermentation and nutrient degradation, and alters the fermentation profile to favor other VFA rather than butyrate or isobutyrate.

Key Words: flaxseed, fermentation, n-3

Ruminant Nutrition 2: Carbohydrates and Lipids

2278 The effect of prepartum dietary starch on colostrum fatty acid content in Holstein dairy cattle. L. E. Engelking*[1], A. J. Fischer-Tlustos[1], J. Yu[1], J. Haisan[2], W. Shi[3], M. Oba[2], and M. A. Steele[1], *[1]University of Guelph, Guelph, ON, Canada, [2]University of Alberta, Edmonton, AB, Canada, [3]Cargill Investments (China) Ltd., Shanghai, China.*

The fatty acid (FA) content of colostrum has been shown to influence neonatal calf health, growth, and immunity. However, little research has evaluated how prepartum dam nutrition influences colostrum FA composition; as such, the objective of this study was to investigate the effect of prepartum dietary starch content on colostrum FA profile. To assess this, 47 multiparous and 28 primiparous cows were fed 1 of 2 prepartum diets: 1) control (14.0% starch, 47.7% NDF; CON) or 2) high starch (26.1% starch, 37.8% NDF; HS). Diets were fed 28 ± 3 d before expected calving date until calving. First milking colostrum samples were collected and analyzed for FA content using gas chromatography. The proportion of individual FA was determined, relative to total FA; all data are expressed as FA percent (g/100 g). Data were analyzed using the GLIMMIX procedure of SAS and considered the fixed effects of prepartum diet, parity, and their interaction, and the random effect of cow. Cows fed HS, compared with CON, had higher total SFA (69.7% vs. 63.8%; $P < 0.0001$), including higher palmitic (C16:0; 42.7% vs. 39.3%; $P < 0.0001$) and myristic acid (C14:0; 13.6% vs. 10.8%; $P < 0.0001$); however, stearic acid was lower in HS (C18:0; 7.39% vs. 8.40%; $P = 0.01$). The proportion of total UFA was lower in HS, compared with CON (30.3% vs. 36.2%; $P < 0.0001$), including lower oleic (C18:1 *cis*-9; 17.5% vs. 23.6%; $P < 0.0001$) and arachidonic acid (C20:4n-6; 0.46% vs. 0.52%; $P = 0.03$); however, linoleic acid was higher in HS than CON (C18:2n-6; 2.62% vs. 2.38%; $P < 0.001$). The proportions of n-3 FA were reduced in cows fed HS, compared with CON, including EPA (C20:5n-3; 0.10% vs. 0.12%; $P < 0.01$), DPA (C22:5n-3; 0.27% vs. 0.31%; $P < 0.01$), and DHA (C22:6n-3; 0.05% vs. 0.06%; $P = 0.02$), although ALA (C18:3n-3) was unaffected by prepartum starch. Overall, these findings demonstrate that prepartum dietary starch contents greatly influence the FA profile of colostrum. Future research is required to determine if altered colostrum FA content, as a result of prepartum dietary starch, influences calf health and performance.

Key Words: colostrum, fatty acids, prepartum starch

2279 Impact of dietary essential fatty acid supply on the metabolism of polyunsaturated fatty acids as assessed by the study of milk fatty acids in mid-lactation dairy cows. P. Denis*, R. Gervais, and P. Y. Chouinard, *Université Laval, Québec, QC, Canada.*

We evaluated the effects of dietary essential fatty acid (EFA; 18:2n-6 and 18:3n-3) supply on milk PUFA profile and desaturase, elongase, and β-oxidation indexes using 10 rumen-cannulated Holstein cows (mean ± SD; 108 ± 30 DIM; 36 ± 5 kg of milk/d) in a 2 × 2 crossover design with 7-wk periods. Treatments consisted of a basal diet formulated to contain (DM basis) 0.42% of 18:2n-6, 0.18% of 18:3n-3, 35.5% of NDF, 15.1% of CP, 21.6% of starch, and 3.2% of total fatty acids, supplemented with (1) 100 g/d of free SFA (42% of 16:0 and 35% of 18:0) administered through the rumen cannula (LoEFA) or (2) 100 g/d of an equal mixture of soybean and linseed oil infused abomasally providing 34 g/d of 18:2n-6 and 33 g/d of 18:3n-3 (HiEFA). Milk fatty acids were analyzed from 3-d pools collected during the seventh week of each period. Indexes were calculated as product/(product + substrate). The model included the fixed effects of treatment (LoEFA vs. HiEFA), period (1 vs. 2), and sequence (LoEFA-HiEFA vs. HiEFA-LoEFA) and the random effect of cow. Milk concentrations (g/100 g of fatty acids) of both substrates 18:2n-6 and 18:3n-3 were higher for HiEFA than LoEFA (+84% for 18:2n-6 and +448% for 18:3n-3; $P < 0.01$). The concentrations of most 18:2n-6 derivatives were lower for HiEFA than LoEFA (20:3n-6, 24:5n-6; $P < 0.01$; 22:4n-6, 24:4n-6; $P < 0.10$). On the contrary, the concentrations of most 18:3n-3 derivatives were higher for HiEFA than LoEFA (20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3, $P < 0.05$; 18:4n-3, $P < 0.10$). Δ-6 Desaturase [18:3n-6/(18:3n-6 + 18:2n-6); 18:4n-3/(18:4n-3 + 18:3n-3)], elongase 2 [24:4n-6/(24:4n-6 + 22:4n-6); 24:5n-3/(24:5n-3 + 22:5n-3)], and elongase 2/5 [22:4n-6/(22:4n-6 + 20:4n-6); 22:5n-3/(22:5n-3 + 20:5n-3)] indexes were lower for HiEFA than LoEFA ($P < 0.05$), whereas the β-oxidation indexes [22:5n-6/(22:5n-6 + 24:5n-6); 22:6n-3/(22:6n-3 + 24:6n-3)] were higher for HiEFA than LoEFA ($P < 0.01$). This study shows that the dietary supply of EFA affects differently the milk concentrations of 18:2n-6 and 18:3n-3 derivatives.

Key Words: milk, PUFA, metabolism

2280 Influence of supplementation with medium-chain fatty acids on the modulation of gene expression in transition dairy cows. G. C. Aguiar[1], J. C. S. Lourenço[1], G. K. F. Merighe[2], E. W. Carneiro[3], D. E. Rico[4], J. A. Negrão[2], and R. Almeida*[1], *[1]Universidade Federal do Paraná, Curitiba, PR, Brazil, [2]Universidade de São Paulo, Pirassununga, SP, Brazil, [3]Royal Agrifirm Group, Curitiba, PR, Brazil, [4]CRSAD, Deschambault, QC, Canada.*

Medium-chain fatty acids (MCFA; 6:0, 8:0, 10:0, and 12:0) may offer health and metabolic benefits to dairy cows, but specific pathways leading to these effects remain unclear. The objective of this study was to investigate the effects of MCFA supplementation on different gene expression pathways in dairy cows in the transition period. A total of 168 Holstein cows (695 kg ± 4.09), 66 primiparous and 102 multiparous, were divided into 2 groups in a randomized complete block design for 35 d. Treatments administered from d 21 prepartum to d 14 postpartum were T1) control (without MCFA supplementation) and T2) 45 g of MCFA (0.25%–0.35% of dietary DM) based on coconut and palm oil (Aromabiotic Cattle®, Royal Agrifirm Group, NL), which was individually supplemented, top dressed, once a day, mixed with corn meal. Blood was collected from the coccygeal vessels from randomly chosen cows (n = 25 per treatment) on d 14 postpartum. Blood samples were centrifuged, and the white coat (i.e., leukocytes) was transferred for RNA isolation and subsequent qRT-PCR analysis to study oxidative stress, inflammation processes, glucose, and fat metabolism. Four control genes were used as endogenous reference genes: *GAPDH*, *YWHAZ*, *HPRT1*, and *UBC*. The mRNA relative fold-change of the target genes was performed based on the $2^{-\Delta\Delta CT}$ method. Data were analyzed using the MIXED procedure of SAS, with the model containing the fixed effects of treatment, parity, and their interaction, whereas block and animal within treatment were considered random effects. Differences ($P < 0.05$) were found for *PTX3*, *GPX1*, and *NF-kB1* genes. *PTX3* and *GPX1* were upregulated in MCFA-treated cows (1.0 vs. 1.58 and 1.0 vs. 3.2 mRNA $2^{-\Delta\Delta CT}$, respectively; $P < 0.05$). *GPX1* expression varied with parity, higher ($P < 0.05$) in multiparous cows. *NF-kB1* gene was downregulated in MCFA-treated cows (1.0 vs. 0.7 mRNA $2^{-\Delta\Delta CT}$; $P < 0.05$). In summary, gene expression results suggest that administration of a low dose of MCFA has the potential to attenuate oxidative stress and inflammation in cows during the transition period.

Key Words: inflammation, MCFA, oxidative stress

Animal Health 3

2206 Comparing oral versus intravenous calcium administration on production, metabolism, and inflammatory biomarkers during a lipopolysaccharide challenge in dairy cows. J. Opgenorth*[1], B. M. Goetz[1], S. Rodriguez Jimenez[1], A. D. Freestone[1], G. J. Combs[1], T. A. Flemming[1], J. L. McGill[1], P. J. Gorden[1], L. Tikofsky[2], and L. H. Baumgard[1], *[1]Iowa State University, Ames, IA, [2]Boehringer Ingelheim, Animal Health USA Inc., Duluth, GA.*

Route of Ca administration may influence recovery from hypocalcemia during disease. Our objectives were to evaluate the effects of oral relative to i.v. Ca administration on mineral and energetic metabolism and inflammatory parameters following an acute i.v. LPS challenge (0.375 µg of *Escherichia coli* O55:B5/kg of BW). Multiparous Holstein cows (124 ± 43 DIM) were assigned to 1 of 4 treatments: 1) saline control (CON; 4 mL saline; n = 4), 2) LPS control (CON-LPS; n = 6), 3) LPS with oral Ca (OCa-LPS; one 192 g bolus of Bovikalc [$CaCl_2$ and $CaSO_4$]; n = 8), and 4) LPS with i.v. Ca (IVCa-LPS; 500 mL Ca-gluconate, 23%; n = 8). Calcium treatments were administered twice (−0.5 and 6 h relative to LPS). During period (P) 1 (4 d), baseline data were obtained. At the initiation of P2, LPS was administered, and data were obtained the following 5 d. Data were analyzed with PROC MIXED in SAS. Hypocalcemia ensued in CON-LPS, but OCa-LPS and IVCa-LPS had increased ionized Ca compared with CON-LPS (16% and 34%, respectively; $P < 0.01$). Rectal temperature increased after LPS and was further elevated in OCa-LPS (0.4°C) and IVCa-LPS (0.8°C) from 3 to 5 h ($P < 0.01$). All LPS groups had decreased DMI and milk yield relative to CON (17%; $P < 0.01$). Glucose was decreased (21%; $P < 0.01$) in OCa-LPS compared with CON-LPS and IVCa-LPS during initial hyperglycemia at 1 h, but all LPS cows had similarly reduced glucose from 4 to 12 h (13% relative to baseline). Urea nitrogen increased after LPS but was attenuated in OCa-LPS compared with CON-LPS and IVCa-LPS (14%; $P < 0.01$). Glucagon increased in OCa-LPS and IVCa-LPS compared with CON-LPS (39%; $P < 0.01$) and insulin markedly increased over time regardless of LPS treatment. The LPS increased several acute phase proteins and cytokines ($P < 0.01$), but most temporal profiles did not differ by treatment. In summary, LPS activated the immune system, and both Ca delivery routes successfully ameliorated hypocalcemia. Oral and i.v. Ca treatments had differential effects on multiple metabolism variables and appeared to mildly influence production responses to LPS.

Key Words: mineral supplementation, delivery methods, immune activation

2300 Effects of early-lactation variables on first-lactation dairy cattle performance. M. L. Simonds*[1], I. G. M. A. Santos[1], L. F. Brito[1], K. Kalbaugh[2], J. S. Doucette[2], and J. P. Boerman[1], *[1]Department of Animal Sciences, Purdue University, West Lafayette, IN, [2]Agriculture Data Services, Purdue University, West Lafayette, IN.*

Our objective was to evaluate the effects of variables collected from genomic evaluations and during early lactation that influence first lactation performance. Data were collected from 3,093 first lactation Holstein cows that calved between January 2021 and December 2022 from one commercial dairy farm in Indiana. The variables analyzed included metabolic disease incidences, age at first calving (AFC), sex of the calf, BW at calving (BW_C), days until conception within first lactation, 305-d milk production (305M), and genomic predictions for milk production (GMilk) and body size (GBS). The average BW_C, 305M, and AFC for primiparous cows was 631.4 ± 57.4 kg (mean ± SD), 9,348.9 ± 1,293.4 kg, and 733.7 ± 51.6 d, respectively. Effects of BW_C, AFC, incidences of metabolic diseases, sex of the calf, days until conception, GMilk, and GBS on 305M were evaluated using a multiple regression model with stepwise selection criteria. The BW_C quadratically increased 305M in first lactation ($P < 0.0001$). GMilk had a linear effect on 305M in first lactation ($P < 0.0001$), with each additional kilogram of predicted milk resulting in 1.33 kg more 305M. The AFC quadratically increased 305M in first lactation ($P = 0.06$). Days until conception within first lactation increased 305M ($P < 0.0001$), each additional day until conception resulted in 3.8 kg more 305M. A metritis diagnosis tended to decrease 305M by 218 kg ($P = 0.07$) and a mastitis diagnosis reduced 305M by 441 kg ($P < 0.0001$). A ketosis diagnosis increased 305M by 181 kg ($P = 0.02$); however, a ketosis diagnosis also increased days until conception by 10 d ($P = 0.0001$). Interestingly, primiparous animals that produced bull calves yielded 78 kg more 305M than primiparous animals that produced heifer calves ($P = 0.06$). Metabolic incidences, BW_C, AFC, sex of the calf, GBS, and GMilk all affect total milk production during an animal's first lactation.

Key Words: primiparous, metabolic disease, body weight (BW)

2301 Analysis of calcium status of multiparous Holstein cows and its effect on milk production in pasture dairies. G. Graef*[1], A. M. Valencia[2], V. R. Cardona[3], A. C. Marquez[2], T. R. Overton[1], and F. L.Yepes[4], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Department of Agricultural Sciences, University of Caldas, Manizales, Caldas, Colombia, [3]School of Veterinary Medicine, University of Antioquia, Medellín, Antioquia, Colombia, [4]Population of Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY.*

Periparturient hypocalcemia in cows on pasture is understood to affect health and milk production significantly. The pasture-based dairy industry of Colombia is the fourth largest milk producer in Latin America, and rapidly growing. However, with renewed commitments to sustainable intensification, Colombia may be disproportionately affected by poor forage quality and periparturient diseases due to limited access to technology and infrastructure. The objective was to establish the prevalence of subclinical hypocalcemia (SCH) and its relationship with milk production outcomes in grazing dairy systems. Multiparous Holstein cows (n = 132) were enrolled in a cross-sectional study design on 3 farms in Antioquia, Colombia, from July to December 2023. Pre- and postpartum total Ca (tCa) and Mg (tMg) blood concentrations were analyzed at −7, 1, 2, 4, and 7 DIM. Cows were assigned to 1 of 4 SCH groups based on tCa at 1 and 4 DIM: normocalcemic (NC; [tCa] > 1.95 mmol/L at 1 DIM and > 2.2 mmol/L at 4 DIM, n = 62); transient SCH (tSCH; [tCa] = 1.95 mmol/L at 1 DIM and > 2.2 mmol/L at 4 DIM, n = 29); delayed SCH (dSCH; [tCa] > 1.95 mmol/L at 1 DIM and = 2.2 mmol/L at 4 DIM, n = 22); and persistent SCH (pSCH; [tCa] = 1.95 mmol at 1 DIM and = 2.2 mmol/L at 4 DIM, n = 19). Milk production was recorded daily, and components were analyzed monthly. The PROC MIXED (SAS v. 9.4) with repeated measures was used to analyze differences between subclinical hypocalcemia groups and milk production. In total, 31% of cows were determine to be in dSCH or pSCH groups and 17% to be in the tSCH. Blood tCa prepartum by SCH group was highest in tSCH and lowest in pSCH (tSCH = 2.69 mmol/L vs. pSCH = 2.37 mmol/L; $P = 0.01$). Blood tMg prepartum and postpartum were generally not significant. Energy-corrected milk was lowest in pSCH cows and greatest for tSCH cows in the first 9 wk of lactation (pSCH: 26.9 kg vs. tSCH: 33.1 kg vs. NC 29.3 kg vs. dSCH: 30.2 kg; $P = 0.0002$). Overall, subclinical hypocalcemia is a significant challenge in grazing systems in Colombian dairy herds and may affect the performance of dairy cows.

Key Words: hypocalcemia, transition cow, pasture

2302 Association of subclinical hypocalcemia classification with blood haptoglobin concentrations during the periparturient period. G. Graef*, S. Tompkins, and T. R. Overton, *Department of Animal Science, Cornell University, Ithaca, NY.*

Recent work has established that proper classification of subclinical hypocalcemia (SCH) groups has important implications around cow health and production around the time of calving and early lactation. However, the dynamics of blood calcium status and inflammation are far less understood. The objective was to determine the relationships between SCH dynamics and periparturient circulating haptoglobin concentrations and milk production during the first 9 wk of lactation. Data were collected from multiparous Holstein cows (n = 96) and retrospectively classified into 1 of 4 SCH groups based on blood concentrations of total Ca (tCa) at 1 and 4 d in milk (DIM): normocalcemic (NC; [tCa] > 1.95 mmol/L at 1 DIM and > 2.2 mmol/L at 4 DIM, n = 39); transient SCH (tSCH; [tCa] ≤ 1.95 mmol/L at 1 DIM and > 2.2 mmol/L at 4 DIM, n = 29); delayed SCH (dSCH; [tCa] > 1.95 mmol/L at 1 DIM and ≤ 2.2 mmol/L at 4 DIM, n = 14); and persistent SCH (pSCH; [tCa] ≤ 1.95 mmol at 1 DIM and ≤ 2.2 mmol/L at 4 DIM, n = 14). PROC MIXED (SAS v. 9.4) was used to analyze pre and postpartum blood mineral concentrations, haptoglobin concentrations, milk production, and differences between SCH groups using repeated measures for time. Prepartum blood haptoglobin concentrations were similar by SCH status. Postpartum haptoglobin concentrations by SCH status were highest in the cows considered pSCH (0.50 g/L; $P = 0.01$). Differences between haptoglobin concentrations of nSCH, pSCH, and tSCH were significant as early as 1 DIM with the highest concentrations in pSCH group (pSCH = 0.16 g/L vs. nSCH = 0.19 g/L vs. tSCH = 0.16 g/L vs. dSCH = 0.29 g/L; $P < 0.001$). Cows in the pSCH group tended to make less milk than cows in the other 3 calcium groups (pSCH = 37.09 kg/d, dSCH = 40.3 kg/d, tSCH = 40.6 kg/d, nSCH = 38.01 kg/d; $P = 0.07$). Overall, results suggest that periparturient calcium dynamics and inflammatory responses are tightly associated; however, more work needs to be done examining this relationship with additional markers of inflammation and immunity.

Key Words: subclinical hypocalcemia, inflammation, transition cow

2303 Multiparous Holsteins with early postpartum calcium dysregulation have a blunted anti-inflammatory cytokine response. J. A. Seminara*, A. S. Sipka, C. R. Seely, B. Wagner, and J. A. A. McArt, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Many multiparous cows experience dysregulated blood calcium dynamics in early lactation. This condition, also known as dyscalcemia, is associated with a variety of adverse health and production outcomes. Cytokines are molecules that play essential roles in initiating and resolving systemic inflammation. Our objective in this case-control study was to establish if temporal patterns of cytokines differ between early lactation cows with and without dyscalcemia. We hypothesized that dyscalcemia would be associated with sustained proinflammatory cytokine responses. Multiparous Holstein cows on 2 farms in central New York were enrolled into our study at 1 or 2 d prepartum. Blood was collected at enrollment, for the first 5 DIM, and at 7 and 10 DIM. Samples from 4 DIM were analyzed for total serum calcium concentration (tCa) to classify cows as dyscalcemic (tCa <2.2 mmol/L) or eucalcemic (tCa ≥2.2 mmol/L). Dyscalcemic cows (n = 20) were matched with eucalcemic cows (n = 36) such that the final proportion of dyscalcemic cows in our study would be comparable to the dyscalcemia prevalence on US commercial herds (~35%). Samples from each time point were then analyzed using a bead-based multiplex assay for 3 cytokines: tumor necrosis factor-α, interferon-γ, and IL-10. Temporal patterns were compared between groups using linear mixed effects models including the fixed effects of calcium group, DIM, parity group (2, 3, or ≥4), farm, all relevant 2-way interactions, and the random effect of cow. We adjusted for confounding using a common marker of systemic inflammation measured at 4 DIM. We only detected a group effect on the anti-inflammatory cytokine IL-10 ($P = 0.01$). There was evidence for a group × DIM interaction ($P = 0.09$), whereby dyscalcemic cows had lower blood concentrations of IL-10 than eucalcemic cows at 3 ($P = 0.06$), 4 ($P = 0.07$), 5 ($P = 0.008$), 7 ($P < 0.001$), and 10 DIM ($P = 0.03$). Our study is a preliminary investigation; however, these results suggest that eucalcemic cows may resolve inflammation more effectively than dyscalcemic cows, hinting at a mechanism behind differing outcomes.

Key Words: dyscalcemia, inflammation, interleukin-10

2304 Effect of propylene glycol treatment on hyperketonemia persistence in cows with or without hypoglycemia at hyperketonemia diagnosis. M. R. Dorella*, C. C. Florentino, E. Shepley, M. Ruch, F. N. S. Pereira, J. N. C. Praxedes, A. Bouleau, S. Davis, and L. S. Caixeta, *University of Minnesota, St. Paul, MN.*

Our objective was to investigate the effects of propylene-glycol (PG; 300 g administered for 3 consecutive days) on the persistence of hyperketonemia (HYK; blood BHB ≥1.2 mmol/L) in cows with or without hypoglycemia (glucose ≤2.2 mmol/L) at the time of HYK diagnosis. A randomized-controlled trial was conducted on a dairy farm in Minnesota. Multiparous Holstein cows were screened for HYK using a hand-held device (Precision Xtra; Abbott Laboratories) at 3 ± 1 and 7 ± 2 DIM. Cows identified with HYK were randomly assigned to receive PG (n = 87; TRT) or not (n = 91; CON). To evaluate HYK persistence, cows were sampled at 3 ± 1 d following diagnosis to assess post-treatment BHB levels. The outcome assessed was the risk of persistent HYK after receiving PG or not and difference between BHB levels. A mixed logistic regression model was used to evaluate the effect of PG on the risk of persistent HYK by hypoglycemia status. To evaluate the differences between BHB concentrations by group, a mixed linear regression model was used. Both models included the fixed effects of treatment, hypoglycemia status, parity, DIM at diagnosis, BHB at diagnosis, and the interaction between hypoglycemia and treatment. The risk of normoglycemic cows continuing to have high BHB after treatment was 37.8% for TRT compared with 51.5% for CON (OR 1.75; 95% CI: 0.83, 3.70; $P = 0.14$). For hypoglycemic cows, HYK persisted in 41.1% of TRT cows and 45.3% of CON cows (OR 0.84; 95% CI: 0.26, 4.85; $P = 0.88$). In normoglycemic cows, BHB levels decreased by 0.14 mmol/L in CON and by 0.28 mmol/L in TRT (95% CI: −0.13, 0.42; $P = 0.31$), while in hypoglycemic cows, it decreased by 0.04 mmol/L in CON and 0.24 mmol/L in TRT (95% CI: −0.32, 0.73; $P = 0.45$). Cows in the TRT group, irrespective of hypoglycemia, showed a 0.68 times lower risk of persistent HYK compared with CON (95% CI: 0.38, 1.16; $P = 0.16$). In this study, the effect of PG on both HYK persistence and blood BHB concentrations remained consistent regardless of the cow's glycemic status at the time of diagnosis.

Key Words: hypoglycemia, ketosis, post-partum

2305 Inflammatory and metabolic profiles in cows with postpartum reproductive tract disease: A case-control study. T. C. Bruinjé*[1], O. Bogado Pascottini[2], and S. J. LeBlanc[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Internal Medicine, Reproduction and Population Medicine, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

We investigated associations of early postpartum systemic inflammatory and metabolic markers in Holstein cows with different manifestations of reproductive tract disease. This retrospective case-control study on 2 commercial dairy herds in Ontario, Canada, enrolled a total of 1,641 cows. Postpartum cows were sampled at 2 and 6 ± 2 DIM for serum haptoglobin (Hp), total calcium (Ca), and nonesterified fatty acids (NEFA); at 5, 8, 11, and 15 ± 2 DIM for blood BHB; and at 21 and 35 DIM for serum Hp. Cows with a calving-related or clinical disorder before 35 DIM were excluded and only clinically healthy cows were evaluated ($n = 355$ primiparous and 548 multiparous). Purulent vaginal discharge (PVD) and endometritis (≥6% polymorphonuclear cells in endometrial cytology) were assessed at 35 DIM. Disease status was classified as PVD only (without endometritis; $n = 64$), subclinical endometritis (SCE, without PVD; $n = 147$), clinical endometritis (CE, endometritis with PVD; $n = 73$), or healthy (no PVD and no endometritis; $n = 619$). We used multivariable linear regression models with disease status and time as fixed effects and blood markers as outcomes, and logistic regression models with blood marker (continuous scale) as predictors and disease status (compared with healthy) as outcomes. Tested covariates included parity, season, BCS, milk yield, and herd. Concentrations of Ca were lower in CE than in SCE, PVD, or healthy at 2 and 6 DIM ($P < 0.01$). Concentrations of Hp were greater in CE or SCE than in PVD or healthy at 2 and 6 DIM ($P < 0.01$) but were not different at 21 and 35 DIM. At 6 DIM, Hp concentration was positively associated with greater odds of SCE and CE ($P < 0.01$), and BHB at 15 DIM with greater odds of SCE and PVD ($P < 0.01$). In primiparous cows only, Ca concentration at 2 DIM was negatively associated with the odds of CE ($P = 0.02$). The present data suggests that transition period maladaptation, characterized based on different markers of systemic inflammation or metabolic imbalance, may predispose cows to develop reproductive tract inflammatory disease.

Key Words: acute phase protein, fertility, transition health

2306 Associations among body condition score, body weight, and serum biochemistry in dairy cows. D. B. Sheedy[1,2], H. M. Golder[1,2], S. C. Garcia[1], P. Reddy[3,4], S. J. Rochfort[3,4], J. E. Hemsworth[3], D. E. Vincent[3], J. E. Pryce[3,4], and I. J. Lean*[1,2], [1]*School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, New South Wales, Australia,* [2]*Scibus, Camden-New South Wales, Australia,* [3]*Agriculture Victoria Research, AgriBio, Centre for AgriBioscience, Bundoora, Victoria, Australia,* [4]*School of Applied Systems Biology, La Trobe University, Bundoora, Victoria, Australia.*

We explored the association of dairy cow BCS and BW, indirect measures of tissue reserves, with serum analytes, which reflect cows' metabolic status. Pre-calving (PC; 739 cows, ~27 d prepartum) and peak milk (PM; 691 cows, ~58 DIM) cohorts were selected by stratified (parity: 1, 2, 3, >3) random sampling from 30 farms (15 pasture, 15 TMR). A single serum, BCS, BW, and milk production datum were collected per cow, per cohort between November 2022 and July 2023. Eleven analytes were processed in a ChemWell and standardized within group (cohort/breed/farm). Mixed linear models for BCS and BW were specified, with random effect of group. A 6-point, unordered, categorical body-group metric that combined BCS (>, equal, or < group median; as high, median, or low BCS) and BW (> or < group median; as high or low BW) was analyzed by polytomous logistic regression. Significance was set at $P < 0.05$. The PC BCS was positively associated with albumin, urea, and glucose, and negatively with cholesterol. The PC BW positively associated with albumin and negatively with cholesterol. The PM BCS positively associated with albumin, β-hydroxybutyric acid (BHBA), and glucose. The PM BW positively associated with albumin and negatively with Ca. Increasing BW and decreasing BCS was observed with increasing parity, except in PC second parity cows that had low BCS. The PC polytomous model associated a 1 SD increase in albumin with a 5.3% decreased risk of being low BCS or low BW and 5.9% increased risk of high BCS or high BW. Risk change associated with 1 SD glucose was −5.7% for low BCS or high BW and 3.1% for high BCS or high BW. For the PM cohort, change in risk was associated with albumin for low BCS or low BW (−3.4%), low BCS or high BW (−3.2%), and median BCS or high BW (2.8%). Risk change with 1 SD BHBA was −3.5% for median BCS or low BW and 2.8% for high BCS or high BW. Risk of low BCS or low BW was greatest in second parity cows, while high BCS or high BW was greatest in >third parity PC and third parity PM. There were no interactions between parity and analytes. Albumin was consistently associated with BCS and BW, potentially reflecting innate differences in cow protein metabolism.

Key Words: albumin, body condition, parity

2307 Blood biomarker relationships to energy balance associated metabolic disorders. R. S. Pralle*[1,2], O. E. Adams[1], E. M. Kammann[2], N. S. Jozik[1], and H. M. White[2], [1]*School of Agriculture, University of Wisconsin–Platteville, Platteville, WI,* [2]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Metabolic disorders (MD) in early lactation dairy cows are thought to have shared pathologies related to energy balance. The study objective was to indirectly evaluate MD co-morbidity based on blood biomarker (BM) responses. On 3 dairy farms, 501 multiparous cows had blood plasma BM quantified at 7 and 14 ± 1 DIM: aspartate transaminase to alanine transaminase ratio (AST:ALT), BHB (mmol/L), cholesterol (mg/dL), fatty acids (FA, mEq/L), glucose (mg/dL), and magnesium (MG, mg/dL). Cows were diagnosed with MD cases based on achieving BM thresholds at either DIM: high FA (HFA) when FA ≥0.7 mEq/L, hyperketonemia (HYK) when BHB ≥1.2 mmol/L, and hypoglycemia (HPG) when glucose <40 mg/dL. Data were analyzed by linear mixed models (SAS, v.9.4) with fixed effects of MD, parity group, DIM, and all interactions; a random effect of farm and repeated measures of cow were included too. Effects are reported as mean differences in BM concentration (MD case − respective noncase) ± SE of the difference. Greater AST:ALT was observed for HFA (0.9 ± 0.1 units, $P < 0.01$) and HYK cases (0.9 ± 0.1 units, $P < 0.01$). Although BHB had MD × DIM effects ($P \leq 0.02$) for HFA and HYK, MD cases had greater BHB at both DIM. Also, HPG cases had greater BHB (0.1 ± 0.02 mg/dL, $P < 0.01$). Cholesterol was lower for HYK cases (−8.4 ± 2.3 mg/dL, $P < 0.01$) and HFA cases on 14 DIM (−8.3 ± 2.4 mg/dL, $P < 0.01$, MD × DIM). Glucose was lower for HYK (−3.4 ± 0.9 mg/dL, $P < 0.01$) and HPG cases ($P < 0.01$, MD × DIM), as well as HFA cases on 14 DIM (−3.2 ± 1.1 mg/dL, $P < 0.01$, MD × DIM). Lower MG was observed for HYK cases (−0.1 ± 0.03, $P < 0.01$) and HPG cases on 14 DIM (−0.2 ± 0.04, $P < 0.01$, MD × DIM). Although FA had a MD × DIM effect for HPG ($P = 0.05$), mean differences were not separable ($P > 0.10$). Greater FA was observed for HFA cases across DIM ($P < 0.01$, MD × DIM), as well as HYK cases (0.13 ± 0.02 mEq/L, $P < 0.01$). The observation of BHB, FA, and GLC differences between all MD cases and their respective noncases suggests that the MD are co-morbid. Cholesterol and AST:ALT differences were unique to HFA and HYK cases; this may reflect liver responses related to lipoprotein formation and tissue injury unique to those MD.

Key Words: ketosis, nonesterified fatty acid, transition cow

2308 Effect of calving season on the time and magnitude of the nadir body condition score in Holstein cows under grazing systems.

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The objective was to evaluate the association between calving season (CS; summer, fall, winter, and spring) and the time and magnitude of the nadir BCS (nBCS) in Holstein cows in a pasture-based system. This study used data from 2,184 lactations (548 primiparous [PP] and 1,636 multiparous [MP]) of cows calving from 2021 to 2023 in a commercial dairy in Southern Chile. The BCS were generated by an automated BCS camera system (DeLaval, Sweden) at calving (BCSc) and at nadir (lowest daily BCS after calving). Lactations were categorized based on the cow health status (HS) before nBCS as healthy (no disease events), with one event, or with multiple events. Multivariable linear models were used to examine the data, considering BCSc, CS, and HS as covariables. The PP and MP cows were evaluated separately. Least squares means (SE) DIM at nBCS differed by CS in PP (F = 164 [10] d; W = 148 [7.8] d; SP = 114 [9.5] d; S = 127 [9.2] d; $P < 0.001$) and MP (107 [6.1] d; 130 [4.5] d; 114 [4.7] d; 93 [5.4] d; $P < 0.001$). Moreover, healthy cows reached nBCS earlier than cows with one or multiple health events in PP (105 [4.1] d; 132 [7.5] d; and 178 [17] d; $P = 0.001$) and MP (90 [2.0] d; 101 [5.6] d; and 142 [10] d; $P < 0.0001$). The LSM for BCSc, nBCS, and ΔBCS from calving to nadir are presented in Table 1. Differences in BCSc were identified among CS in both PP and MP, while only MP cows had differences in nBCS among CS. Overall, CS was associated with time at nBCS and BCSc in PP and MP cows, while only MP cows evidenced an association between CS and both nBCS and ΔBCS from calving to nadir.

Key Words: BCS, grazing, health

2309 Association between automated body condition score at dry-off and calving and hyperketonemia in Holstein cows.

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Hyperketonemia is a common metabolic condition in high-producing dairy cows and has been associated with many fresh cow diseases. The objective was to test the association between automated BCS at dry-off and calving and hyperketonemia (HYK) in Holstein cows. This retrospective observational study used data collected from 12,042 lactations in 7,626 Holstein cows calving between April 2019 and January 2022 in a commercial dairy operation in Colorado. Cows were tested for hyperketonemia at 6 ± 3 DIM (PortaCheck Inc.) and were considered positive when blood BHB ≥1.2 mmol/L. Scores generated by an automated BCS camera system (DeLaval, Sweden) at dry-off (BCSd) and calving (BCSc) were selected from multiparous cows (n = 7,681 lactations) and subsequently categorized into quartiles (Q1 = 25% lowest BCS; Q4 = 25% greatest BCS). Changes in BCS (ΔBCS) were calculated from dry-off to calving and assigned into quartile categories considering Q1 as the 25% of cows with greater loss of BCS. Data were examined using logistic regression with calving season included as covariable. The adjusted odds ratios (OR) and 95% CI of HYK by categories of BCSd, BCSc, and ΔBCS are presented in Table 1. Greater BCS at dry-off and greater loss of BCS from dry-off to calving were associated with greater odds of hyperketonemia. Greater BCS at calving was partially associated with the incidence of this metabolic disorder.

Key Words: BCS, calving, hyperketonemia

Table 1 (Abstr. 2308). Least squares means (SE) for BCS at calving and nadir, and ΔBCS from calving to nadir by calving season and parity category

Calving season	Calving BCS	Nadir BCS	Δ BCS
Primiparous			
Fall	3.52 (0.03)^{c}	2.85 (0.03)	−0.48 (0.03)
Spring	3.16 (0.03)^{a}	2.85 (0.02)	−0.48 (0.02)
Summer	3.43 (0.03)^{c}	2.89 (0.02)	−0.45 (0.02)
Winter	3.34 (0.03)^{b}	2.85 (0.02)	−0.49 (0.02)
Multiparous			
Fall	3.10 (0.03)^{a}	2.82 (0.02)^{c}	−0.29 (0.02)^{c}
Spring	3.15 (0.02)^{ab}	2.70 (0.01)^{a}	−0.41 (0.01)^{a}
Summer	3.20 (0.02)^{b}	2.72 (0.02)^{ab}	−0.39 (0.02)^{ab}
Winter	3.12 (0.02)^{a}	2.75 (0.01)^{b}	−0.37 (0.01)^{b}

$^{a–c}$Different superscripts within columns indicate $P < 0.05$.

2310 Metabolic and inflammatory biomarker patterns in high-priority cow groups during the transition period.

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The objective of this study was to assess weekly patterns of metabolic and inflammatory biomarkers in high-priority cow groups (i.e., over-conditioned cows and nulliparous cows) during the transition period (TP). Nulliparous (NUL; n = 38) and parous (PAR; n = 71) cows were grouped by BCS (Over-conditioned [OVERC] = BCS ≥3.75 points, n = 77; optimal conditioned [OPT] = BCS 3–3.5 points, n = 32). Blood samples were collected weekly starting at −21 ± 3 d relative to calving (d.r.c.) until 21 ± 3 d after calving for assessment of nonesterified fatty acids (NEFA), haptoglobin (HP), IL-1β, IL-6, and tumor necrosis factor-α (TNF-α). The data were analyzed using linear regression

Table 1 (Abstr. 2309). Adjusted odds ratios (95% CI) of presentation of hyperketonemia by category of BCS (dry-off and calving) and by ΔBCS from dry-off to calving

BCS quartile[1]	Time of BCS		
	Dry-off	Calving	Dry-off to Calving
Q1	—	—	—
Q2	0.99 (0.75–1.31)	1.07 (0.88–1.30)	0.69 (0.54–0.88)
Q3	1.49 (1.13–1.96)	1.19 (0.98–1.44)	0.63 (0.49–0.81)
Q4	2.21 (1.69–2.88)	1.65 (1.37–1.99)	0.59 (0.45–0.76)

[1] Q1 = smallest BCS (dry-off and calving) or greatest loss of BCS (dry-off to calving).

models with the MIXED procedure of SAS. Parous cows had higher concentrations of NEFA at 7 ± 3, 14 ± 3, and 21 ± 3 DIM compared with NUL cows. On the other hand, NUL cows had a higher concentration of HP at 7 ± 3 DIM ($P = 0.007$; NUL = 159.39 µg/mL, 95% CI = 94.29–241.47; PAR = 58.85 µg/mL, 95% CI = 30.99–95.57), while PAR cows had a higher concentration at 21 ± 3 DIM ($P = 0.03$; NUL = 27.65 µg/mL, 95% CI = 5.34–67.35; PAR = 83.04 µg/mL, 95% CI = 48.94–126.11). Over-conditioned cows had higher concentrations of TNF-α compared with OPT cows during the TP ($P = 0.01$; OVERC = 367.20 pg/mL, 95% CI = 321.41–416.04; OPT = 266.29 pg/mL, 95% CI = 207.61–332.29). Similarly, NUL cows tended ($P = 0.05$) to have higher concentrations of TNF-α compared with PAR cows (NUL = 388.59 pg/mL, 95% CI = 321.66–461.84; PAR = 308.38 pg/mL, 95% CI = 264.86–355.22). Interestingly, PAR cows tended ($P = 0.07$) to have higher concentrations of IL-6 compared with NUL cows during the TP (NUL = 340.58 pg/mL, 95% CI = 303.16–380.17; PAR = 384.93 pg/mL, 95% CI = 356.75–414.18). These results suggest that high-priority cow groups may have altered metabolic and inflammatory status during the TP and that different management approaches should be considered for these groups of animals.

Key Words: over-conditioned cow, nulliparous cow, metabolic and inflammatory biomarker

2311 Subcutaneous adipose tissue sympathetic markers vary with periparturient metabolic changes in dairy cows. B. B. Sparks*[1], A. F. Souza Lima[3], H. R. Ford[1], J. S. Osorio[3], and C. Strieder-Barboza[1,2], *[1]Department of Veterinary Sciences, Texas Tech University, Lubbock, TX, [2]School of Veterinary Medicine, Texas Tech University, Amarillo, TX, [3]School of Animal Sciences, Virginia Tech University, Blacksburg, VA.*

Our objective was to evaluate changes in sympathetic nervous system (SNS) markers in subcutaneous adipose tissue (SAT) and circulating metabolites in periparturient dairy cows. Blood and SAT were collected from 25 (parity ± SD, 3 ± 2) Holstein cows at 8 ± 3 d prepartum (PRE) and 7 d (blood) and 10 d (SAT) postpartum (POST). The SAT expression of uncoupling protein-1 (UCP1), tyrosine hydroxylase (TH), dopamine β-hydroxylase (DBH), myelin protein zero (MPZ), early B-cell factor-2 (EBF2), perilipin-1 (PLIN1), and peroxisome proliferator-activated receptor gamma (PPARG) was quantified by RT-PCR. Blood glucose, insulin, NEFA, BHB, GPx activity, and glucose:insulin ratio (GIR) were evaluated. Insulin and GPx activity were categorized by low and high based on minimum (low) and maximum (high) median quartiles. Statistical analysis was performed using PROC GLM in SAS for period, metabolites, and period-metabolite interaction effects. As expected, blood glucose ($P = 0.0005$) and insulin ($P2.2 <4.0$ mmol/L) compared with hypoglycemic (<2.1 mmol/L). Activity of GPx was not affected by period, but cows with lower GPx, higher insulin, and normoglycemic expressed more MPZ in PRE ($P < 0.05$). Notably, in POST, MPZ was higher in cows with higher GPx activity and hypoglycemia ($P < 0.05$). Cows with lower GPx activity in POST had increased SAT PPARG ($P < 0.05$). These results suggest that SAT of prepartum cows may undergo beiging and increased myelination in anticipation for increased energy demand and lipid mobilization postpartum.

Key Words: peripartum, adipose nervous system, metabolism

2312 Association of plasma oxylipins at dry-off with early-lactation milk yield, health, and removal in Holstein dairy cows. N. Taechachokevivat*, A. Jannasch, C. Ferreira, J. Grantz, K. D'Amico, A. Ueda, A. Hubner, and R. Neves, *Purdue University, West Lafayette, IN.*

Systemic inflammation contributes to unsuccessful transition from gestation to lactation, and some studies suggest it may start as soon as the cessation of lactation. Early identification of cows at risk for exacerbated systemic inflammation in the postpartum is of interest. Oxylipins are oxygenated metabolites of PUFA and important inflammatory mediators. A cohort study was undertaken to evaluate the associations between plasma haptoglobin (Hp) and pre-selected oxylipins at 1 d before dry-off with milk yield, health, and herd removal within 60 DIM. Blood samples were collected from 359 cows 1 d before dry-off. Plasma Hp concentrations were analyzed using a bovine Hp ELISA kit. Liquid chromatography-tandem MS was used to analyze plasma concentrations of 9,10-DiHOME, 12,13-DiHOME, and 9(S)-HOTrE. Over 80% of samples had Hp concentrations below the limit of quantification of 0.002 g/L; thus, only descriptive statistics were carried out for Hp. Linear mixed models were built to evaluate the associations of each oxylipin with milk yield across the first 8 wk of lactation. Multivariable Poisson regression models were built to evaluate the associations of each oxylipin with the risk of retained placenta, metritis, clinical mastitis, and removal. Critical thresholds were derived from receiver operating characteristic curve analysis for significant analytes. Plasma 12,13-DiHOME and 9(S)-HOTrE were associated with milk production (0.27 ± 0.12 and 0.36 ± 0.14 kg increase in daily milk yield for every 1 ng/mL increase in 12,13-DiHOME [$P = 0.02$] and 9(S)-HOTrE [$P = 0.01$], respectively). Plasma 9,10-DiHOME was associated with the risk of metritis; cows with 9,10-DiHOME above 4.13 ng/mL were 2.4 times (95% CI: 1.1–5.2, $P = 0.03$) more likely to develop metritis. Plasma 9,10-DiHOME tended to be associated with the risk of removal; cows with 9,10-DiHOME less than 4.02 ng/mL were 2.6 times (95% CI: 1.3–5.5, $P = 0.008$) more likely to be removed from the herd. The oxylipins evaluated at dry-off had moderate performance in identifying cows at risk of suffering from negative health outcomes.

Key Words: oxylipin, systemic inflammation, dry-off

2313 Endotoxemia alters transcriptome of adipose tissue in dairy cows. M. Chirivi*, L. Worden, J. Parales-Giron, A. Lock, and G. A. Contreras, *Michigan State University, East Lansing, MI.*

The periparturient period is characterized by intense lipolysis in adipose tissues (AT) and inflammation that together increase the risk of disease. Endotoxemia is often associated with periparturient diseases. We aimed to determine the effects of endotoxemia on AT transcriptomic profile. Multiparous Holstein dairy cows (204 [SD = 21.7] DIM) were infused IV with saline solution (SS, 100 mL, n = 4) or LPS (from *Escherichia coli* O55:B5 n = 4) at 1 µg/kg BW (in 100 mL of saline solution). The LPS triggered fever and signs of endotoxemia 4 h after infusion. Subcutaneous AT (SCAT) biopsies were collected (right flank) 24 h after IV infusion. RNA was extracted using the TRIzol method and sequenced on the Illumina platform. After data filtering, clean reads were mapped to the bosTau7 reference genome using HISAT 2.1.0. False discovery rate (FDR) and P-values were adjusted using the Benjamini-Hochberg method. Compared with CON, differentially expressed genes (DEG) analyses showed 515 DEG in LPS. Of these, 316 were upregulated and 196 downregulated. Functional Enrichment Analysis revealed functions affected by LPS were grouped into categories for lipid metabolism, energy production, inflammation, and free radical scavenging (FDR <0.05). The LPS upregulated canonical pathways including macrophage activation, neutrophil degranulation, interferon-gamma, ERK/MAPK, endoplasmic reticulum stress, and unfolded protein response. In contrast, LPS downregulated pathways for fatty-acyl-CoA biosynthesis, IL10 signaling, and cholesterol biosynthesis. Genes such as TNF, TLR4, and LBP were categorized as upstream inflammatory triggers. In contrast, a lower abundance of FASN, ACACA, LPIN1, and ELOVL5 were identified as upstream inhibitors of lipid synthesis. TNFR1, STAT3, and SOCS3 upregulation were defined as links between inflammatory pathways and insulin resistance. Our results provide evidence that endotoxemia activates inflammatory responses in AT and alters metabolic functions including lipolysis and lipogenesis. Future studies will explore the effects of endotoxemia on AT inflammation and macrophage trafficking and its association with metabolic disease in dairy cows.

Key Words: adipose tissue, endotoxemia, RNA-seq

2314 Associations between physical examination findings, blood parameters, and bacteremia in neonatal dairy calves with diarrhea: A case-control study.

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The objective of this case-control study was to investigate the associations of physical examination findings and laboratory analyses with bacteremia in neonatal dairy calves with diarrhea. This study was conducted at a commercial calf-rearing facility with calves <21 d old. Diarrhea incidence was determined through twice-daily scoring, with calves categorized as diarrheic if their fecal consistency was runny or watery. Calves with signs of other diseases were not included. Diarrheic calves underwent physical exams and blood collections at diarrhea onset, 24 h, and 48 h later. Laboratory analyses included blood culture, complete blood cell count, serum biochemistry profile, venous blood gas and electrolyte analysis, and L-lactate measurement. Bacteremia was determined by positive blood culture. Cases comprised diarrheic bacteremic calves, while controls were diarrheic nonbacteremic calves enrolled within ± 4 d of the cases. Forward stepwise multivariable exact conditional logistic regression models were built to identify physical exam findings and blood variables associated with bacteremia. Among the 100 calves with diarrhea, 37% were bacteremic. On the first day the bacteremia was detected, higher pH (for every 0.1 unit increase: OR: 0.01; 95% CI: 1.4e-11 to 0.90; $P = 0.04$) and albumin concentration (for every 1 g/L increase: OR: 0.7; 95% CI: 0.5–0.9; $P = 0.02$) were associated with lower odds of bacteremia, whereas the presence of scleral injection ($P = 0.04$; OR: 7.1; 95% CI: 0.8-inf) was associated with bacteremia. On the day after the onset of bacteremia, higher rectal temperature (for each 1°C increase: OR: 5.2; 95% CI: 0.8–87.0; $P = 0.08$) and absolute neutrophil count (for each 10^9/L unit increase: OR: 1.3; 95% CI: 0.9–1.8; $P = 0.05$) were associated with higher odds of bacteremia. This study suggests that pH, albumin, absolute neutrophil count, rectal temperature, and the presence of scleral injection may aid in identifying diarrheic calves with bacteremia.

Key Words: sepsis, neonatology, scour

2315 Comparison of 2 oral calcium treatments at calving on blood ionized calcium and BHB, plasma total calcium, and milk yield in multiparous Holstein cows.

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Anionic and low calcium (Ca) prepartum diets have long been used as preventive strategy for milk fever. However, subclinical hypocalcemia is still prevalent in dairy cattle, particularly in older cows (≥3 lactations). The supplementation of oral Ca products at parturition has become a common strategy to improve calcium status, health and lactational performance of postpartum (pp) cows. The objective of this study was to compare the effect of 2 oral Ca boluses at calving on the concentrations of blood ionized Ca (iCa), plasma total Ca (tCa), and milk production during the early pp of multiparous Holstein cows. The study was conducted during 2023 at the University of Missouri Foremost Dairy. Multiparous cows with normal parturition were randomly assigned to 1 of 2 treatment groups and matched by parity number and previous lactation 305ME: treatment 1 (T1; n = 23) or treatment 2 (T2; n = 26), and treated within 1 to 6 h pp. Treatment 1 was an oral bolus based on $CaCl_2$/calmin 50 g (RumiLife CAL24 Bolus, Genex) and T2 was an oral bolus based on $CaCl_2$ and 1,25-dihydroxyvitamin D3 glycosides found in the *Solanum glaucophyllum* leaf. Blood samples were taken after calving and before treatment, and at d 1, 2, 3, 4, 7 pp. Milk yield was recorded daily for the first 30 d pp. iCa (mmol/L; Cal-Meter, Horiba, Japan) and total Ca (mg/dL) in plasma were measured. The results were analyzed by mixed models for repeated measures. After correcting by lactation, there was a tendency for the interaction treatment × day for higher iCa (1.12 and 1.06 mmol/L, respectively) and tCa in T2 than T1 (9.05 and 8.55 mg/dL, respectively). For milk yield, there was a tendency ($P = 0.09$) for a higher milk production over time for T2 than T1 (41.9 vs. 40.1 kg/d, respectively). An oral bolus containing *S. glaucophyllum* leaf improved calcemia and milk yield during the early postpartum in Holstein cows when compared with another oral Ca treatment.

Key Words: transition, calcium, milk

2316 Association of transient, persistent, or delayed subclinical hypocalcemia with milk and milk component yields in early lactation Holstein cows.

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Our objective was to evaluate the effect of subclinical hypocalcemia (SCH) dynamics on early lactation milk production and milk composition. Recent research has determined that postpartum dairy cows experience different types of subclinical hypocalcemia: transient, persistent, or delayed. We conducted a retrospective observational study in 300 multiparous (3.1 ± 1.2 lactations) Holstein cows in 1 Wisconsin dairy herd. Data were from an experiment where treatment at calving did not affect Ca status or subsequent milk production. Cows were classified into 1 of 4 groups based on postpartum total plasma Ca concentrations: normocalcemic (NC; Ca >1.77 at 1 DIM and >2.20 mmol/L at 4 DIM, n = 124); transient SCH (tSCH; Ca ≤1.77 at 1 DIM and >2.20 mmol/L at 4 DIM, n = 14); persistent SCH (pSCH; Ca ≤1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM, n = 134); or delayed SCH (dSCH; Ca >1.77 at 1 DIM and ≤2.20 mmol/L at 4 DIM, n = 28). Cows were fed an acidified diet (1.3% Ca, −170 mEq/kg) beginning 21 d before calving. After calving, cows were milked 3× daily with milk yield recorded until 60 DIM and milk composition determined at 16 and 46 DIM. Blood samples were taken 24 to 48 h (1 DIM) and 96 to 120 h (4 DIM) after calving and plasma measured for total Ca by automated analyzer (Beckman DXC700). Data were analyzed using the mixed procedure of SAS with Ca status and lactation group as fixed effects. There was an interaction for milk production by week of lactation where NC (49.1 kg) produced more milk than dSCH (47.3 kg) over the first 6 wk of lactation but were similar thereafter. Milk yield for tSCH (50.2 kg) and pSCH (49.3 kg) did not differ. Other than the first week in milk, milk yield for NC did not differ from pSCH. Milk composition, milk component yield, and

ECM did not differ for Ca status classification. Compared with other Ca status classifications, plasma P was lower at 1 DIM for tSCH (1.08 vs. 1.66 mmol/L) but higher at 4 DIM. Although Ca status classification based on plasma Ca at 1 and 4 DIM affected milk yield through 42 DIM for dSCH, milk fat and protein yield were not altered.

Key Words: subclinical hypocalcemia, calcium, milk yield

2317 Association between hyperketonemia and automated body condition score at nadir in Holstein cows. P. Pinedo*[1], D. Manriquez[1,2], P. Melendez[3], and A. DeVries[4], *[1]Department of Animal Sciences, Colorado State University, Fort Collins, CO, [2]AgNext, Colorado State University, Fort Collins, CO, [3]Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong SAR, [4]Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective was to test the association between hyperketonemia (HYK) and automated BCS at nadir in Holstein cows. This retrospective observational study used data from 12,042 lactations in 7,626 Holstein cows calving between 2019 and 2022 in a commercial dairy in Colorado. Cows were tested for HYK at 6 ± 3 DIM (PortaCheck Inc.) and were considered positive when blood BHB $\geq$1.2 mmol/L. Scores generated after each milking by an automated BCS camera system (DeLaval, Sweden) at calving (BCSc) and at nadir (BCSn; lowest daily BCS from calving to 100 DIM) were selected. Nadir BCS were categorized into 2 groups using the median (low = 50% lowest nBCS), separately for primiparous and multiparous cows. Health events (dystocia, retained fetal membranes, metritis, pyometra, clinical hypocalcemia, left displaced abomasum, lameness, clinical mastitis, digestive problem, injury, and respiratory disease) occurring before BCSn were considered to classify the lactations as affected by one disorder, multiple disorders, or unaffected. Data were examined in primiparous and multiparous cows separately using ANOVA and logistic regression. Multivariable models considered calving season, BCSc, health status, and milk yield during the first 30 DIM as covariables. Least squares means (SE) for nBCS and ΔBCS by presentation of HYK are presented in Table 1. The adjusted odds ratios (95% CI) of being in the low nBCS category for cows with HYK compared with healthy cows were 4.16 (2.72–6.37) and 1.48 (1.27–1.73) in primiparous and multiparous cows, respectively. Presentation of HYK was associated with lower nadir BCS and greater loss of BCS from calving to nadir.

Key Words: BCS, hyperketonemia, nadir

Table 1 (Abstr. 2317). Least squares means (SE) for nBCS and ΔBCS from calving to nadir by hyperketonemia status in primiparous and multiparous cows

	BCS at nadir		ΔBCS from calving to nadir	
Hyperketonemia	Primiparous	Multiparous	Primiparous	Multiparous
No	3.03 (0.002)^a	2.86 (0.003)^a	−0.34 (0.002)^a	−0.45 (0.002)^a
Yes	2.94 (0.01)^b	2.80 (0.006)^b	−0.42 (0.01)^b	−0.50 (0.006)^b

^{a,b}Different superscripts within each column indicate $P < 0.01$.

2318 Effect of subclinical ketosis on the behavior and productivity of dairy cows milked in robotic milking systems. S. Moore*, J. Marques, R. Conceicao, J. Denis-Robichaud, and R. Cerri, *University of British Columbia, Vancouver, BC, Canada.*

The use of robotic milking systems is increasing exponentially worldwide. Cows in these systems milk more often and typically produce higher quantities of milk. The increase in energy required to support this production level has been linked to an increase in BHB concentrations, indictive of subclinical ketosis (SCK). However, many studies investigating SCK are conducted on cows milked in conventional milking systems. Therefore, the objective of this study is to explore BHB concentrations, within robotically milked herds, to further understand the effect on milking characteristics and cow behavior. Multiparous Holstein cows (n = 300) were enrolled from a robotic milking herd in the Fraser Valley of British Columbia, Canada, 1 wk before dry-off and followed until 60 DIM. Behavior outcomes (rumination, eating time) were measured continuously, with production outcomes followed until 60 DIM and blood samples (analyzed for nonesterified fatty acids [NEFA] and BHB) collected once daily across the first 21 DIM. All data analyses were conducted using generalized linear mixed models with cow as a repeated measure. Area under the curve (AUC) for each cow's BHB values throughout the first 21 DIM was calculated. For every 1-unit increase in AUC, milk yield increased by 0.9 kg/d, eating time decreased by 7.2 min/d, and rumination decreased by 7 min/d ($P = 0.01$, $P = 0.02$, $P = 0.05$, respectively). Cows with higher milk yields (above than the group mean) across the first 60 DIM had higher NEFA values ($\geq$0.7 mmol/L) within the first 21 DIM ($P = 0.03$). Cows who tested positive for SCK (BHB $\geq$1.2 mmol/L) in wk 2 (8–14 DIM) tended to produce 2.4 kg/d less of milk across the first 60 DIM compared with non-SCK cows. Positive SCK cows in wk 3 (15–21 DIM) had decreased rumination and eating time of 58 min/d ($P < 0.01$) and 70 min/d ($P < 0.01$) respectively across 0 to 60 DIM. This research further highlights the energy demands associated with greater milk production and provides insight into the importance of time of SCK testing.

Key Words: robotic milking system, subclinical ketosis, BHB

2319 Associations of body condition score and ketosis with pregnancy loss, pregnancy or artificial insemination, mastitis, and leaving the herd. K. C. Krogstad*[1,2] and B. J. Bradford[1], *[1]Department of Animal Science, Michigan State University, East Lansing, MI, [2]Department of Animal Science, The Ohio State University, Wooster, OH.*

Ketosis and excessive body condition loss have been associated with the disease risk but there is little investigation of associations with pregnancy loss (PGL). Our objective was to conduct a retrospective analysis investigating associations between BCS or ketosis with milk production, PGL, mastitis, and risk of leaving the herd. The BCS was recorded for 826 cows prepartum (14 d before calving), 956 cows postpartum (14–21 DIM), and 628 multiparous cows were evaluated for ketosis at 3 to 7 DIM. Records were gathered from herd management software. Univariate associations of BCS, ΔBCS (postpartum − prepartum), and BHB (measured with Nova Max Plus) with pregnancy or AI (P/AI), PGL, mastitis, and risk of leaving the herd were evaluated. Cox Proportional Hazard analyses investigated associations of ketosis with pregnancy, mastitis, and hazard of leaving the herd. We examined associations of subclinical ketosis (SK; BHB $\geq$1.2 mM) with whole-lactation milk yield. Prepartum BCS was inversely associated with risk of leaving the herd (OR 95% CI: 0.32–0.79) and positively associated with PGL (95% CI:

1.11–9.56; $P \le 0.03$) whereas postpartum BCS was inversely associated with risk of leaving the herd (95% CI: 0.16–0.44) and tended to be associated with P/AI (95% CI: 0.95–2.11; $P \le 0.08$). The ΔBCS was inversely associated with risk of leaving the herd (95% CI:0.35–0.99) and tended to be inversely associated with PGL (95% CI: 0.08–1.15; $P \le 0.08$). Blood BHB was positively associated with risk of leaving the herd (95% CI: 1.25–2.25), PGL (95% CI:0.97–5.12), and mastitis (95% CI: 1.01–1.38; $P \le 0.04$). SK tended to be associated with reduced hazard of pregnancy (95% CI: 0.63–1.03), increased hazard of mastitis (95% CI: 1.08–2.01), and increased hazard of leaving the herd (95% CI: 1.36–2.66; $P \le 0.08$). The SK cows within third+ parity had 5.4 ± 1.04 kg/d (difference ± SE of the difference; $P < 0.01$) less milk yield compared with parity-matched cows without SK. These data suggest losing BCS and SK are associated with increased risk of pregnancy loss and removal from the herd.

Key Words: health, disease, culling

2320 Association between subclinical ketosis, body condition score, and pregnancy at first artificial insemination in Holstein cows calving at different seasons.

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The objective was to test the association of BCS at calving and incidence of subclinical ketosis (SCK) with pregnancy at first insemination (PAI1) in Holstein cows calving in different seasons, housed in a large dairy in Mexico. This retrospective observational study used data from 1,904 Holstein cows calving from October 2022 to October 2023 in a commercial dairy in Torreon, Coahuila, Mexico. Cows housed in a dry-lot system were tested for SCK at 5 ± 2 DIM using a hand-held device (FreeStyle Optium Neo, Abbott). Test results were considered positive when blood BHB >1.2 mmol/L. Cows were submitted to AI1 at 77 ± 2 DIM, following a double OvSynch protocol. Cows were scored for body condition at calving (BCS_C; n = 1,820) and at first AI (BCS_AI; n = 614). Subsequently, BCS_C and BCS_AI were categorized into 3 categories (low [<3.25]; medium [3.25–3.75]; and high [>3.75]), and cows were grouped in primiparous and multiparous. Data were examined using ANOVA and logistic regression. Multivariable models considered calving season, BCS_C, BCS_AI, the occurrence of retained fetal membranes and metritis, and milk yield at wk 4 and 8 as covariables. Mean ± SE BHB was 0.86 ± 0.01 mmol/L, and no differences were identified by the BCS_C category. The BHB concentrations were lower in cows calving in winter (0.83 ± 0.02 mmol/L; $P = 0.02$) and fall (0.86 ± 0.02 mmol/L; $P = 0.04$) than in cows calving in spring (0.94 ± 0.03 mmol/L). The adjusted odds (95% CI) of SCK in spring calvings were 2.05 (1.12–3.76) times the odds of SCK in winter calvings. No association between BCS_C or BCS_AI and PAI1 was identified. However, PAI1 was 32.4% versus 51.7% in cows with SCK versus nonaffected cows ($P = 0.02$). It is concluded that the incidence of SCK was associated with calving season, but there was no association between BCS at calving and SCK. Body condition at calving and BCS at first AI were not associated with PAI1, but a greater incidence of SCK resulted in lower fertility at first artificial insemination.

Key Words: BCS, subclinical ketosis, pregnancy

2321 Co-morbidity of energy balance associated metabolic disorders.

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The study objective was to evaluate the interaction between hyperketonemia (HYK) and co-morbid metabolic disorders (MD) on lactation performance, morbidity, and fertility in dairy cows. On 3 dairy farms, 501 multiparous cows had blood plasma analytes quantified at 7 and 14 ± 1 DIM. Cows were diagnosed with MD cases based on analyte thresholds: high fatty acid (HFA) when fatty acids ≥0.7 mEq/L, HYK when BHB ≥1.2 mmol/L, and hypoglycemia (HPG) when glucose <40 mg/dL. The following are the possible co-morbid MD categories (sample size): no cases (173), HFA only (83), HYK only (26), HPG only (77), HFA and HYK (53), HFA and HPG (30), HYK and HPG (14), and all MD (45). Milk production, morbidity, and fertility data were retrieved from farm electronic records. The 3-way interaction of all MD was not tested due to the low sample size of the co-morbid categories. First test-day milk production data were analyzed by linear mixed models (SAS, v. 9.4) with fixed effects of HYK, a second MD, parity group (PG), test-day DIM, and all interactions; a random effect of farm was included. Morbidity data were analyzed by generalized estimating equations with fixed effects of HYK, a second MD, PG, and farm; interactions were tested stepwise for inclusion but were not retained ($P > 0.10$). Effects are reported as mean differences ± SE of the difference or relative risk ratios (RR). Greater HYK risk was observed for HFA ($P < 0.01$, RR = 3.3) and HPG cases ($P = 0.01$, RR = 1.5); likewise, HYK cases had greater risk for HFA ($P < 0.01$, RR = 2.1) and HPG ($P = 0.01$, RR = 1.4). Milk yield had a HYK × HFA effect ($P = 0.03$) where HYK cases with HFA had lower production than those without (43.8 ± 1.3 vs. 49.0 ± 2.6 kg/d, respectively). No other HYK × MD effect was detected for milk energy output, milk fat %, or milk protein % ($P > 0.19$). Models evaluating DA did not converge, as well as HYK × HPG for metritis and pneumonia. Metritis incidence was greater ($P = 0.01$) for HYK cows with HFA than without (19.7 ± 4.6 vs. 6.11 ± 5.9, respectively). Culling incidence had an inseparable HYK × HPG effect ($P = 0.03$). These data suggest that some HYK-associated consequences may be dependent on other metabolic responses.

Key Words: ketosis, nonesterified fatty acid, transition cow

2322 Hyperketonemia prognosis when considering additional blood biomarkers.

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This prospective cohort study's objective was to identify blood biomarkers (BM) that alter the milk production and morbidity responses associated with hyperketonemia (HYK) cases. On 3 dairy farms (WI), 501 multiparous cows had blood plasma BM analyzed by colorimetric assay at 7 and 14 ± 1 DIM: aspartate transaminase:alanine transaminase (AST:ALT), BHB (mmol/L; cow-side meter on 7 DIM), cholesterol (CHOL, mg/dL), fatty acids (FA, mEq/L), glucose (GLC, mg/dL), and magnesium (MG, mg/dL). Cows were diagnosed with HYK when BHB ≥1.2 mmol/L. Cow performance metrics related to production, morbidity, and fertility were extracted from farm records. These metrics were initially associated with fixed effects via stepwise regression (linear or logistic; JMP Pro 17), using corrected Akaike information criterion for model selection. In all stepwise models, HYK status, parity group, farm, and test-day DIM (production metrics) effects were included. Regressors allowed to enter or exit the models were the min BM (CHOL, GLC, and MG) and max BM (AST:ALT, BHB, and FA) concentrations (linear and quadratic), as well as the BM × HYK effects. Optimal models were refit using generalized estimating equations (GEE, Gaussian or Poisson distribution; SAS, v.9.4). Data are reported as the GEE coefficient ± SE. First test-day milk production (kg/d) had associations ($P \le 0.02$) with HYK (−3.7 ± 1.5), GLC (−0.27 ± 0.06), and CHOL (0.14 ± 0.04). First test-day milk energy yield (Mcal/d) had associations ($P \le 0.04$) with HYK (−1.4 ± 1.3), GLC (−0.15 ± 0.05), and CHOL (0.06 ± 0.02); also, a MG × HYK effect was detected ($P = 0.07$) but was not separable. Displaced abomasum, mastitis, and cull risk had HYK effects ($P \le 0.03$), but no additional BM. First service conception rate had associations ($P \le 0.08$) with HYK (−0.31 ± 0.13), GLC (0.01 ± 0.005), and CHOL (−0.005 ± 0.003). Metritis had associations ($P \le 0.02$) with HYK (1.1 ± 0.35), GLC (0.04 ± 0.02), MG (−1.5 ± 0.4), and AST:ALT (0.08 ± 0.03). Pneumonia had associations ($P \le 0.03$) with HYK (1.5 ± 0.61) and MG (−2.68 ± 0.68). Limited BM × HYK evidence suggested BM did not alter HYK prognosis; however, CHOL, GLC, and MG may have unique prognostic value.

Key Words: ketosis, nonesterified fatty acid, health

2323 Assessment of the proinflammatory cytokine response in RAW 264.7 macrophages after lipopolysaccharide stimulation under microfluidic conditions.

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Microfluidic cell culture methods aim to provide a cellular environment closer to physiological conditions for in vitro experiments. Unlike most static culture protocols, these methods offer dynamic nutrient supply and waste removal similar to physiological conditions. Therefore, the objective of this study was to explore the potential of microfluidic technology to characterize the inflammatory activation of RAW 264.7 mouse macrophage cell line under LPS challenge by observing cytokine secretion at high-frequency intervals. RAW 264.7 cells were harvested with 85% confluency by scraping; cells were counted and then transferred to 2 treated microfluidic slides (Ibidi u-Slide 0.2 μm) at 3×10^5 cells/slide and kept overnight in the incubator at 37°C, 5% CO$_2$. Each replicate of this experiment consisted of 1 slide for a control condition (CON; complete media) and 1 slide for an LPS challenge with 100 ng/mL *Escherichia coli* O111:B4 diluted in complete media. The microfluidic flow was set up to run at 50 μL/min range (0.2 dyn. cm^2 shear stress) controlled by a flow sensor. Data were analyzed using the MIXED procedure of SAS, including a total of 4 replicates run on different days. Flow-through media was collected at −6, −3, 0, 1, 2, 3, 4, 5, and 6 h relative to LPS challenge and used to measure TNFα concentration via ELISA. Additionally, TNFα was measured via flow cytometric bead array (FCBA) methods at 0, 3, and 6 h relative to the LPS challenge. Greater ELISA TNFα ($P < 0.05$) was detected in the LPS group compared with CON at 2, 3, 4, 5, and 6 h relative to the LPS challenge. Similarly, greater TNFα ($P < 0.05$) was observed at 3 and 6 h post-LPS challenge by FCBA. The TNFα release from the LPS group increased steadily over time, reaching the highest concentration at 3 h and returning to pre-LPS levels at 6 h (58.63 and 29.45 pg/mL, respectively). This study provides a unique perspective on cytokine release dynamics in macrophage cells under microfluidic conditions.

Key Words: microfluidic, inflammation, on-chip

2324 Examining *Klebsiella pneumoniae* isolates from cows with clinical mastitis for adherence and invasion of Mac-T cells.

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Bovine mastitis is recognized as the most impactful disease affecting dairy farming globally. This condition causes economic losses in dairy and poses health threats. *Klebsiella pneumoniae* plays a significant role in environmental bovine mastitis, and its pathogenicity arises from various virulence factors. This study aimed to evaluate the adhesion and invasion capacity of *K. pneumoniae* using 3 different isolates for each severity degree (mild, moderate, and severe), totaling 9 isolates obtained from cases of bovine mastitis in 10 dairy farms in the State of São Paulo and Minas Gerais, Brazil. The clinical severity scores of the cases were classified as: score 1 (mild), cows with milk abnormalities; score 2 (moderate), cows with milk abnormalities and inflammatory signs in the mammary gland; score 3 (severe), cows with significant systemic signs. Cultivation of bovine mammary epithelial cells (Mac-T) occurred in 24-well microplates. Cultures of the 9 isolates, incubated at 37°C/24 h in BHI broth, were diluted in Opti-MEM supplemented with 2% FBS, yielding a bacterial suspension containing 1.5×10^6 cfu/mL, and added in duplicate to the wells. The plate was incubated at 37°C/3 h at 5% CO$_2$. After washes with PBS, the adhered cells were detached using trypsin. Concurrently, invasion tests were executed with adhesion tests. After 2 h with gentamicin, Triton X-100 0.1% was added to lyse the cells and release the invading bacteria. Analyses were carried out using Kolmogorov-Smirnov, followed by Kruskal-Wallis and Dunn's Multiple Comparison tests. All isolates demonstrated the capacity to adhere to Mac-T cells with recovery rates ranging from 1.5 to 4×10^6 cfu/mL. A significant difference in invasion rates was observed between mild (0.8×10^3 cfu/mL) and severe grade isolates (2.1×10^3 cfu/mL), as well as between moderate (0.9×10^3 cfu/mL) and severe grade isolates (2.1×10^3 cfu/mL), $P < 0.05$. However, no significant difference was observed between mild and moderate grade strains. Preliminary findings suggest that the invasion of host cells does play a role in the severity of mastitis induced by *K. pneumoniae*.

Key Words: bovine mastitis, invasion, *Klebsiella pneumoniae*

2325 *Staphylococcus aureus* leukocidins play a role in clinical mastitis.

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Staphylococcus aureus is one of the main pathogens associated with mastitis, which can be subclinical or manifest clinically, with mild, moderate or severe severity. Leukocidins are virulence factors produced by some isolates and they can destroy leukocytes, affecting the host's immune response. In this study, the leukocidins *lukAB*, *lukED*, and *lukMF* genes were assessed to correlate their presence with subclinical and clinical bovine mastitis. A total of 103 isolates from cows with subclinical mastitis and 103 clinical isolates (73 mild, 28 moderate, and 2 severe cases) were used. *S. aureus* isolates kept frozen at −70°C were seeded on sheep blood agar (5%) to check colony purity, and the DNA was extracted for polymerase chain reaction (PCR) tests, using the MiniSpin kit (GE Healthcare) following the manufacturer's instructions. Taq Green Master Mix (Promega) was used for nucleic acid amplification. Positive (*lukAB* and *lukED*: *S. aureus* USA 100; *lukMF*: *S. aureus* RF 122) and negative controls (*Escherichia coli* ATCC 25.9222) were included in all reactions. Considering clinical mastitis, the *lukMF* gene was present in 97 (94.1%) isolates, *lukED* in 102 (99%), and *lukAB* in 99 (99.1%). In subclinical isolates, these genes were present in lower percentages, 51.5% (53 isolates), 46.6% (48 isolates), and 18.4% (19 isolates), respectively. All differences were statistically significant in the Chi-squared test ($P > 0.0001$). Considering these results, it can be concluded that leukocidins appear to play an important role in the severity of bovine mastitis.

Key Words: virulence factor, severity of mastitis, *Staphylococcus aureus*

2326 Unraveling the mammary epithelial cell response to *Staphylococcus aureus* infection using *TLR4* and *IL10RA* knockout bovine MAC-T cells.

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Staphylococcus aureus (SA) causes subclinical mastitis in dairy cows. The discovery of small colony variants (SCV) has raised concerns about variant survivability within mammary tissues. Previous studies have found associations between SNPs in Toll-like receptor 4 (*TLR4*) and interleukin 10 receptor (*IL10RA*) and susceptibility to mastitis. However, their roles in regulating the host inflammatory response to *SA* infection remain unclear. This study focused on elucidating the roles of TLR4 and IL10RA in *SA* infection by utilizing previously engineered *TLR4* and *IL10RA* knockout (KO) bovine mammary epithelial cells (MAC-T cells). These KO and wild-type (WT) MAC-T cells, were infected with *SA* parent strain and SCV for 3.5 h to identify potential inflammatory genes and assess cytokine production affected by *SA* infection in the presence or absence of *TLR4* and *IL10RA*. Multiplexed immunoassays measured cytokines concentrations in culture supernatants. Total RNA was extracted from infected and uninfected samples, and qPCR was performed. qPCR results indicated a decrease in expressions of *TNF-α*, *IL-6*, and *IL-1α* genes in the *TLR4* KO cells compared with WT MAC-T cells in the SCV-infected group. *IL10RA* KO cells exhibited a significant reduction in the expression of *TNF-α*. Notably, there were no significant changes among the different cell lines infected with the *SA* parent strain. Multiplexing revealed that IL-8, IL-36RA, MCP-1, MIP-1b, and IP-10 significantly increased in *TLR4* KO cells compared with WT MAC-T cells post-SCV infection. In contrast, IL-1α production decreased in *TLR4* KO cells compared with *IL10RA* KO after SCV infection. These results underscore the essential role of *TLR4* in eliciting inflammation in response to *SA* SCV infection. However, the varied responses of *TLR4* KO cells to both parent and SCV strains of *SA* suggest distinct mechanisms of infection and host response, particularly in how these strains interact with TLR4. These novel findings reveal potential divergent roles for TLR4 in *SA* infections, holding implications for the therapeutic control of mastitis in cattle.

Key Words: mastitis, *Staphylococcus aureus*, *TLR4*

2327 Management culture and the prevalence and persistence of mastitis on dairy farms in the United States.

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This study aims at determining the association between management culture, including the management of employees, managers' own beliefs, attitudes, and behaviors on the prevalence of IMI and the occurrence of new IMI. Data were drawn from a 15-mo trial research project of 87 dairy herds in Michigan and Pennsylvania. Statistical methods include descriptive statistics and multilevel models. The model selection process is 2-fold: significant variables at $P < 0.10$ level were combined, and then a forward stepwise selection of the variables was performed. Only variables significant at $P < 0.05$ level were kept in the final model. The association between management culture and IMI was evaluated using a multilevel linear regression model. The association between management culture and high new IMI (1 = high new IMI, i.e., 75th percentile and above; 0 = otherwise) was evaluated using a multilevel logistic regression model. Results show that managers' belief that mastitis was a problem on farm was positively associated with IMI. Herds in which managers set a low SCC goal (<100,000 cells/mL) were negatively associated with IMI. Herds where managers train employees by showing them how to do things, make sure they understand the instructions, and repeat the training were positively associated with IMI compared with those where managers just tell or show them how to do things. Herds where new employees are trained by other milkers were positively associated with IMI compared with herds where new employees are trained by their managers. Results also show that only standard management practices such as teats disinfection after milking, not using systemic antibiotic for mild mastitis, following protocols for cleaning stalls, parlors, and gutters, and perceiving unimportant veterinarian advice for mastitis treatment were negatively related to the odds of high new IMI. These findings highlight the importance of accounting for the overall management culture in addition to standard management practices on dairy farms in preventing IMI and new IMI.

Key Words: management culture, IMI, new IMI

Breeding and Genetics 2

2328 Genetic parameters of milk yield and fertility traits in Moroccan Holsteins. N. Chafai*[1,2], B. Badaoui[1], and R. Rekaya[2], [1]*Laboratory of Biodiversity, Ecology, and Genome, Department of Biology, Faculty of Sciences, Mohammed V University in Rabat, Rabat, Morocco,* [2]*Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

The dairy industry in Morocco is going through a modernization process. Large and medium size farms are rapidly increasing their presence in the Moroccan dairy industry. Importation of purebred dairy heifers and semen has led to an increase in milk production. However, less than optimum farm management procedures and environmental factors are becoming a major challenge, particularly for fertility traits. The negative relationship between milk yield and fertility traits is being exacerbated by the severe heat stress conditions. As the country attempts to locally select their replacement heifers, improvement or at least the avoidance of further deterioration of reproduction performance is a priority. A data set consisting of 4,829 first parity cows was used to assess the genetic correlations between milk yield (MY), number of inseminations per conception (NI), success of first insemination (SF), days open (DO), calving interval (CI). The pedigree file included 9,545 animals. A threshold-linear model that included the fixed effects of herd, calving year, calving month, health status, abortion status, and 2 covariates (age at calving days in milk at first insemination). The random factors consisted of the additive genetic effects and the error terms. Heritability estimates were 0.29, 0.12, 0.13, 0.10, and 0.10 for MY, NI, SF, DO, and CI, respectively. The genetic correlations were positive between MY and all 4 reproductive traits and ranged between 0.07 and 0.38. The positive correlation between MY and SF was not expected and may be due to a potential difference in the persistency between high and low producing cows. Similar trend was observed for the residual correlations between MY and the 4 reproductive traits. The genetic correlations between the reproductive traits ranged between −0.40 (NI and SF) and 0.98 (DO and CI). The residual correlations varied between −0.90 (NI and SF) and 0.99 (DO and CI). These results seem to indicate that DO and CI are highly related and only one of the 2 traits should be used. Overall it seems that there is sufficient genetic variability for selection for the 4 fertility traits used in this study.

Key Words: fertility, genetic parameter, Holstein

2329 Milk fatty acids of Holstein cows compared with Holstein, Jersey, Montbeliarde, Normande, and Viking Red crossbred cows. K. M. Bosley*, L. B. Hansen, and B. J. Heins, *University of Minnesota, St. Paul, MN.*

Improved milk fatty acid (FA) profiles may open marketing opportunities for dairy producers. The objective of the study was to compare FA of Holstein (n = 73), ProCROSS (n = 198), and Grazecross (n = 110) cows at the dairy at the University of Minnesota West Central Research and Outreach Center (Morris, MN). ProCROSS is a 3-breed rotational cross of Holstein, Montbéliarde, and Viking Red breeds, and Grazecross is a 3-breed rotational cross of Jersey, Normande, and Viking Red breeds. Total, de novo, mixed and preformed FA concentrations from test days were provided by Minnesota DHIA (Buffalo, MN) from February 2021 to November 2023. Eight test days were required per cow. For statistical analysis, fixed effects were breed group, parity group (primiparous or multiparous), sire breed nested within breed group, herd (organic or conventional), month, and interaction of herd and month. Random effects were cow nested within breed group and days in milk. Organic cows had lower ($P < 0.05$) de novo (0.98 g/100 g) and higher ($P < 0.05$) preformed (1.51 g/100 g) and total (3.93 g/100 g) FA concentrations than conventional cows (1.02, 1.34, and 3.80 g/100 g, respectively), but had lower total FA. Grazecross cows had higher ($P < 0.05$) total (4.01 g/100 g), de novo (1.05 g/100 g), mixed (1.51 g/100 g), and preformed (1.48 g/100 g) FA compared with Holstein cows (3.78, 0.98, 1.45, and 1.39 g/100 g, respectively) and ProCROSS cows (3.81, 0.98, 1.43, and 1.42 g/100 g, respectively). Holstein cows had higher ($P < 0.05$) total (708 g/d), de novo (185 g/d), mixed (274 g/d), and preformed (249 g/d) FA followed by ProCROSS cows (627, 164, 237, and 227 g/d, respectively) and Grazecross cows (553, 147, 208, and 199 g/d, respectively). Grazecross cows had +6.1% higher total milk FA than Holstein cows and +5.2% higher than ProCROSS cows. Holstein cows had +12.9% more total FA than ProCROSS cows and +28.0% more total FA than Grazecross cows. Differences between breed groups may provide an opportunity for dairy producers to meet future market demands for improved milk FA profiles.

Key Words: milk fatty acid, crossbreeding, organic

2330 Genetic ancestry and admixture of *Bos taurus* and *Bos indicus* cattle for African dairy production systems. S. Gebeyehu*[1], E. S. Kim[2], T. S. Sonstegard[2], and B. J. Heins[1], [1]*University of Minnesota, St. Paul, MN,* [2]*Acceligen, Eagan, MN.*

African dairy production is based on local breeds with poor genetic potential for milk production. This study aimed to determine the genetic diversity and evolutionary relationships of 9 *Bos taurus* and 6 *Bos indicus* breeds composed of 1,100 individuals. The breeds were Gyr, Red Sindhi, Sahiwal, Sheko, Maure, Ankole, Kerry, N'Dama, Muturu, Carora, Brown Swiss, Guernsey, Jersey, Holstein, and Holstein SLICK. *Bos indicus* and *Bos taurus* cattle were genotyped with a high-density 777K SNP BeadChip. Pairwise FST, phylogenic trees, and runs of homozygosity (ROH) were evaluated for all breeds. The pairwise FST estimates differed for degrees of genetic divergence among breed pairs, with lower FST values observed between taurine breeds compared with indicus breeds. The pairwise FST estimate ranged from 0.05 to 0.22 when comparing European and dual-purpose African taurine breeds. The pairwise FST estimate between indicus and African dual-purpose taurine breeds ranged from 0.03 to 0.32. The FST estimates between African dual-purpose breeds (Ankol, Maure, Muturu, and N'Dama) ranged from 0.06 to 0.17. The phylogenetic tree showed that African taurine breeds cluster together with other taurine breeds; at the same time, African indicus breeds form distinct clusters with other indicus breeds. The ROH analysis showed varying patterns of homozygosity across chromosomes. Chromosomes 23, 9, and 17 had the highest ROH by having 17 ROH, 6 ROH, and 6 ROH, respectively. Unique patterns, such as single ROH on chromosomes 4, 6, 7, 19, 22, and 26, reflected distinct selective pressures. The absence of ROH in some chromosomes indicated efforts to maintain genetic diversity or regions under balancing selection. The findings contribute to a better understanding of the African breed genetic landscape and population dynamics, with implications for breeding strategies, conservation efforts, and sustainable management practices.

Key Words: Africa, *Bos indicus*, genetic diversity

2331 Genetic analysis of health disorders in Holstein dairy cattle. H. Mulim*, G. S. Campos, J. Maskal, L. Brito, J. Boerman, and H. Rojas de Oliveira, *Purdue University, West Lafayette, IN.*

Health disorders in dairy animals, such as mastitis, lameness, and metabolic diseases, pose significant economic and welfare challenges to milk production systems. In this study, we aimed to estimate variance components and genetic parameters for mastitis, lameness, and levels of BHB (a biomarker for ketosis and sub-clinical ketosis) in North American Holstein cattle from a dairy cattle farm in the United States. Our data set comprised 36,081 observations from 9,003 animals born between 2012 and 2019. Mastitis cases accounted for 3% of observations (1,226 records from 803 animals), and lameness for 5% (1,741 records from 1,072 animals). The BHB data set included 25,024 observations from 7,776 animals. All animals were genotyped using different SNP panels and imputed to a common marker density of 72,820 SNPs. After quality control, 62,030 SNP markers were used in subsequent analyses. Variance components, heritability, and repeatability were estimated using a threshold model for the binary traits (i.e., mastitis and lameness), and a linear model for BHB. All analyses were performed using a Bayesian approach, implemented in the GIBBSF90+ software. A total of 200,000 interactions, a burn-in of 50,000, and sampling interval of 100 were used in all analyses, as these parameters enabled the convergence of the chains. Heritability estimates were 0.07 ± 0.02 for lameness, 0.09 ± 0.02 for mastitis, and 0.09 ± 0.01 for BHB. Repeatability estimates ranged from 0.19 (BHB) to 0.29 (mastitis). Although the heritability estimates were low, these traits still have substantial additive genetic variation, suggesting potential for selection to improve animal health and welfare. Further investigations on genomic prediction for these traits and using different statistical models are warranted.

Key Words: Holstein, genetic parameter, selection

2332 DNA methylation changes induced by heat stress may affect cattle immune systems: A view on active enhancers. G. Costa Monteiro Moreira*[1,2], M. Joigner[3], A. Chaulot Talmon[1,2], H. Kiefer[1,2], D. Rico[4,5], and H. Jammes[1,2], [1]*BREED, Université Paris Saclay, UVSQ, INRAE, Domaine de Vilvert, Jouy-en-Josas, France,* [2]*BREED, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort, France,* [3]*Institut de l'Elevage, Paris, France,* [4]*Centre de Recherche en Sciences Animales de Deschambault (CRSAD), Deschambault, QC, Canada,* [5]*Département des Sciences Animales, Université Laval, Québec, QC, Canada.*

Epigenetic processes, such as DNA methylation, play roles during development and in response to environmental changes, creating a memory of such events. They drive changes in gene expression modulating phenotypes. In livestock, recent efforts from the FAANG consortium offers a new functional annotation of the bovine genome. Here, we aimed to (1) investigate and characterize enhancer regions highlighted on the blood methylome of cattle by intersecting functional annotation public data and, (2) identify heat-stress induced DNA methylation changes potentially affecting active enhancers (AE) and consequently different phenotypes in cattle. Using reduced representation bisulphite sequencing (RRBS) to perform DNA methylation analysis from blood, a set of 1,471,206 selected CpGs (>10× coverage; no overlap with a list of known SNPs) were retained and annotated: the vast majority in intronic and intergenic regions (25% and 29%, respectively). A credible set of 4,792 AE annotated in cattle was created intersecting publicly available ChIP-Seq and CAGE data for 7 different tissues. Interestingly, selected CpGs were highlighted in 609 AE regions from which 442 (73%) are hypermethylated (80%–100%) and probably inactive, whereas 90 (15%) are hypomethylated (0%–50%) and probably active in blood. Comparing 2 different groups of dairy cows according to heat stress exposure (n = 4 thermoneutral [TN]; n = 4 heat-stress [HS]), differentially methylated cytosines (DMC) were identified using methylKit (Δmeth ≥25%; P_{adj} < 1%). From the identified 866 DMC, 4 were co-located with AE. As an example, HS increased DNA methylation in AE in intron of *LSP1* gene. This gene, expressed in blood cells (lymphocytes, neutrophils, macrophages) and blood or lymphatic vessels (endothelium), regulates neutrophil motility, affecting the host response to infection and immune system homeostasis. Taken together, our preliminary results suggest that heat stress induced DNA methylation variations. Our results also highlight the importance of integrating functional annotation to better understand the functional role of the candidate biomarkers.

Key Words: epigenetic, DNA methylation, enhancer

2333 Selecting core animals in the algorithm for proven and young for genomic evaluations in Holstein cattle. A. Cesarani*[1,2], M. Bermann[2], N. P. P. Macciotta[1], and D. Lourenco[2], [1]*Dipartimento di Agraria, Università di Sassari, Sassari, Italy,* [2]*Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

Single-step GBLUP relies on the inverse of the genomic relationship matrix (G), which has a cubic cost with the number of genotyped animals. Given enough memory, the inverse is feasible for about 150,000 genotyped animals. The current number of genotypes in several populations far exceeds this limit; the US Holstein population has 7.4 million. In such a case, the algorithm for proven and young (APY) can be used to ease computations. However, genotyped animals should be categorized into core and noncore, with genomic relationships for noncore animals based on recursions on core animals. Choosing core animals at random in APY has proved to deliver the same accuracy as using the direct inverse of G. However, a more informed way to choose core animals may be advantageous under certain population structures and may help select new core animals when the core group needs to be updated. This study aimed to test different criteria to select core animals based on genomic contribution (Gcontr). In this method, the eigenvalue decomposition of G is exploited to identify key components in the structure of this matrix. Specifically, the coefficients of the eigenvectors of G are used to calculate the genetic contributions of animals to the genetic population covariance. A US Holstein data set that comprised 4.5 million records for milk, fat, and protein yields was used. From the 1,053,002 animals in the pedigree, 34,506 were genotyped. We compared GEBV for genotyped animals when the core was selected based on Gcontr (lowest or highest) or randomly against GEBV based on the direct inverse of G. Additionally, a validation was done based on the correlation between GEBV estimated for 31 bulls with no daughters in the reduced data set and at least 20 daughters in the complete data set. Using animals with the highest Gcontr values in the core led to the lowest accuracies, and the lowest Gcontr values provided the largest accuracies. Although accuracies from Gcontr and random core were similar when the core size was equal to the number of eigenvalues explaining 98% of the variance in G, Gcontr can still be used when an informed core choice is needed.

Key Words: algorithm for proven and young, genetic contribution, single-step GBLUP

2334 Investigating the potential of genomic selection to decrease abortion risk in Holstein cattle. G. S. Campos*, H. A. Mulim, J. M. Maskal, L. Brito, J. Boerman, and H. Rojas de Oliveira, *Purdue University, West Lafayette, IN.*

Pregnancy loss in dairy cattle leads to significant economic losses for producers. Therefore, this study aimed to investigate the potential of genomic selection to decrease abortion risk in North American Holstein cattle. Phenotypic records were collected between 2014 and 2022 on a commercial dairy cattle farm in Indiana. Abortion was defined as the loss of a confirmed pregnancy between 42 and 260 d of gestation, considered as a binary trait (normal = 1, abortion = 2). A total of 42,962 records were used, sourced from 14,469 cows possessing both genotypic and phenotypic data. Four commercial SNP arrays containing 11k, 17k, 18k, or 30k SNP were imputed to 62k SNP. After imputation and quality control, 57,606 SNPs and 14,469 individuals remained in the analysis. A repeatability animal model was used to estimate variance components and GEBV using a threshold model and the genomic relationship matrix. The statistic model also considered the systematic effects of contemporary group (year and season of conception), lactation number, and age (linear and quadratic). Validation was performed using the linear regression (LR) method, where young animals had no phenotypes in the reduced data set (r) and they had complete records in the whole data set (w). The following statistics were calculated to evaluate the performance of genomic prediction: (1) prediction accuracy (*acc*); (2) regression coefficient (b1) of GEBVw on GEBVr; and (3) bias (μ), calculated as GEBVr-GEBVw. The average incidence of abortions across all years was 4.68%. The h^2 and repeatability estimated for abortion were 0.02 ± 0.006 and 0.13 ± 0.01, respectively. The LR statistics for the validation animals were 0.27 for the *acc*, 0.92 for the b1, and 0.01 for the μ. Although abortion risk is lowly heritable, our results suggest promising opportunity to decrease abortion rates in Holstein cattle through genomic selection. Further investigations on the use of different statistical models and genome-wide association analyses are warranted to deepen our understanding of the genetic basis of abortion in Holstein cattle.

Key Words: genomic prediction, pregnancy loss, reproduction

2335 Optimizing dairy cattle genetics: Can we breed for cows with high milk yield and good fertility?

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The objective of this study was to assess the relationship between genomic daughter pregnancy rate (gDPR), genomic merit for milk production (gMilk), and pregnancy at first AI and pregnancy losses (PL). A total of 6,739 Holstein cows (first lactation: n = 2,636; second lactation: n = 2,057; third lactation: n = 2,046) from one commercial dairy herd were included. Cows were bred following a timed AI protocol for first service at 60 ± 3 d based on estradiol and progesterone. Hair samples were collected from the tail switch, and cows were genotyped using a SNP platform (Clarifide, Zoetis). Pregnancy diagnosis was performed at d 32 and 60 after AI using ultrasonography. Pregnancy loss was defined as a pregnant cow on d 32 that was nonpregnant on d 60. Data were analyzed by using the linear mixed regression models from SAS. Cows were grouped based on their median for gDPR and gMilk into 4 groups: (1) low gDPR and low gMilk (LgDPR_LgMilk); (2) high gDPR and low gMilk (HgDPR_LgMilk); (3) low gDPR and high gMilk (LgDPR_HgMilk); (4) high gDPR and high gMilk (HgDPR_HgMilk). There was a mild-negative correlation between gDPR and gMilk. Pregnancy per AI was affected by a combination of gDPR and gMilk. Pregnancy per AI was 40.6% ± 1.5%, 50.4% ± 1.4%, 43.1% ± 1.4%, and 53.3% ± 1.6% for cows in LgDPR_LgMilk, HgDPR_LgMilk, LgDPR_HgMilk, and HgDPR_HgMilk, respectively. Pregnancy loss was not associated with gMilk, but there was an association with gDPR. Cows with high gDPR have reduced PL compared with low gDPR cows (7.2% ± 0.6% vs. 13.5% ± 1.2%). There was a clear association of gDPR and gMilk on milk production at d 60 with 44.4 ± 0.16, 44.9 ± 0.15, 48.7 ± 0.15, and 48.4 ± 0.17 kg/d for cows in LgDPR_LgMilk, HgDPR_LgMilk, LgDPR_HgMilk, and HgDPR_HgMilk, respectively. Increase in gDPR was associated with greater odds of pregnancy per AI and lower odds of PL. High gDPR and high gMilk suggest that both genotypes could be selected for. These results provide further evidence that gDPR is associated with better reproduction outcomes.

Key Words: daughter pregnancy rate, pregnancy per AI, pregnancy loss

Dairy Foods 2: Cheese

2336 New processing routes for cheese making—The effect of fat content on rheological properties of casein gel. M. Lorenzen*[1], F. W. J. van den Berg[1], S. K. Lillevang[2], and L. Ahrné[1], [1]*Department of Food Science, University of Copenhagen, Copenhagen, Sjælland, Denmark,* [2]*Arla Innovation Centre, Arla Foods Amba, Aarhus, Jylland, Denmark.*

To meet changing consumer demands, improve efficiency, and address sustainability challenges, there is a need to develop innovative processing routes for cheeses. The incorporation of milk fat into skim milk curd (SMC) at different concentrations enables cheesemakers to tailor the rheological properties of cheeses toward specific melting behavior and unique textures. The objective of this study was to evaluate the effect of milk fat on the rheological and structural properties of renneted casein gels made from SMC and butter. A one-factor design of casein gels containing 4 levels of fat (0.01%, 7%, 11%, 18% wt/wt was developed using shear mixing. The viscoelastic behavior of the gels was assessed by small amplitude oscillatory measurements (strain, frequency, and temperature sweep tests) with at least 3 replicates. Milk fat addition (from 0.01%–18% wt/wt) decreased the linear viscoelastic region (from 1.26% ± 0.00% to 0.71% ± 0.12%) and increased the gel strength by approximately 134% as evidenced by an increase in G' and G''. All gels exhibited a weak degree of frequency dependency (<0.20) similar to values reported on mozzarella cheese (0.16–0.36). The gel-sol transition temperature defined as the crossover temperature (G' = G''), was used to estimate the melting behavior of the gels. Increasing fat content led to lower protein and moisture content, respectively, which resulted in an increase in G' = G'' from 65.6°C ± 0.7°C to 71.9°C ± 0.2°C. Confocal laser scanning microscopy images revealed that the addition of milk fat at a level of >11% wt/wt led to a more open gel microstructure with larger fat globules and more serum pockets. Similarly, higher water mobility was observed in casein gels with >11% wt/wt fat by low-field nuclear magnetic resonance. The results provide new opportunities for developing innovative cheese products with specific functionalities by better understanding the rheological behavior of mixtures of SMC and butter in the range of 0% to 20% wt/wt.

Key Words: casein emulsion gel, viscoelastic properties, gel-sol transition temperature

2337 Influence of hydrocolloid gums on the functional properties of processed cheese. K. A. Alsaleem*[1] and A. R. A. Hammam[2], [1]*Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia,* [2]*Dairy Science Department, Faculty of Agriculture, Assiut University, Assiut.*

Processed cheese (PC) is a stable oil-in-water emulsion. It made by blending natural cheese with emulsifying salts and other dairy and nondairy ingredients, then heating and mixing to create a homogeneous product with a long shelf life. Hydrocolloid gums are the most effective carbohydrate-based fat substitutes because they can control the rheology of water-based systems. These additives are used to maintain the structure of dairy products, increase their viscosity, and modify their textural properties. The study aimed to assess the effect of 3 different hydrocolloid gums on the functional properties of PC. The ingredients in PC formulations were Cheddar cheese, butter, water, milk permeate, disodium phosphate (DSP) with the addition of either pectin (T1), cellulose (T2), or xanthan gum (T3) at 0.25%. They were formulated to contain 18% protein, 25% fat, 45% moisture, and 2% salt. PC with 3% DSP was also produced as a control. A 25-g sample of the mixture was cooked in the rapid visco analyzer for 3 min at 95°C with 1,000 rpm for the first 2 min and 160 rpm for the last min. The PC was then transferred into molds and refrigerated till further analyses. The PC was analyzed for TS, pH, cooked viscosity, hardness, melted diameter, and melting temperature. The experiment was repeated 3 times using different batches. No significant differences ($P > 0.05$) were detected in the TS of PC; however, the pH of control and T1 (5.64–5.63) was slightly lower than T2 and T3 (5.7). The cooked viscosity ($P < 0.05$) was 1,879, 2,241, and 1,662 cP for T1, T2, and T3, respectively. However, it was 1,445 cP in the control. No significant differences ($P > 0.05$) were found between T1 and T2 in the cooked viscosity, hardness, and melted diameter. The cooked viscosity and hardness of T2 were slightly higher ($P < 0.05$) and slightly lower in melted diameter than the control and other treatments. The hardness of PC in treatments had higher values ($P > 0.05$) than in the control. No significant differences ($P > 0.05$) were detected in the melting temperature between treatments. We conclude that gums are a powerful tool that could be used to modify PC functionality.

Key Words: processed cheese, gum, functional property

2338 Exploring the influence of aging temperatures on flaking process in Provola dei Nebrodi Protected Designation of Origin cheese. G. A. Calandra-Checco*[1], M. Caccamo[2], S. Ruta[1], R. Petriglieri[2], G. Belvedere[2], C. Caggia[1], G. Marino[2], F. La Terra[2], and G. Licitra[1], [1]*University of Catania, Catania, Italy,* [2]*CoRFiLaC, Ragusa, Italy.*

Provola dei Nebrodi Protected Designation of Origin cheese (PDO) is a traditional pear-shaped Sicilian stretched raw-milk cheese. It is characterized by internal flaking with lamellar cracks of a few millimeters, occurring after 4 to 5 mo of aging. Historically, production has been limited to spring, which represents the optimal environmental conditions (EC). To integrate production also during winter, production needs to be assessed under winter EC. This study aimed to compare the cheese flaking process under 2 conditions (C) representative of winter and spring productions across an aging period (P) of 0, 60, 90, 120, 150, 180, and 240 d, during which quality parameters were assessed. Under winter conditions (W), cheeses were aged at 7°C ± 1°C at 0 to 60 d, and at 15°C ± 1°C at 60 to 240 d. Under spring conditions (S), aging temperatures were 13°C ± 1°C for 0 to 60 d, and 15°C ± 1°C for 60 to 240 d. Specifically, 3 batches of curd were produced in January at weekly intervals, and each batch was divided into fourteen 3-kg-Provola, one for each P in both W and S. Chemical analyses included pH, moisture (M), fat on DM (FDM), total protein on DM (TNDM) as well as proteolytic activity assessed by pH 4.6-soluble N and 12% trichloroacetic acid soluble N fractions. During 8 mo of aging, there was an overall decrease in M (45% ± 0.3% to 34% ± 0.4%), pH (5.1% ± 0.1% to 5.5% ± 0.1%), FDM (45% ± 0.3% to 41% ± 0.2%), TNDM (45% ± 0.2% to 48% ± 0.2%) varied in response to C. Proteolytic activity was calculated for each aging interval, considering increases over the time interval. The statistical model included C, P, and C × P as fixed effects, with significance set at $P < 0.05$. Both flaking and proteolytic activity were accelerated in S compared with W cheeses indicating an increased speed of aging, with cheeses appearing softer and flaking occurring earlier (120 d compared with 180 d). Sensory analyses revealed that S cheeses were spicier and with more intense flavors and rich green-herbaceous, hay, butter, and mushroom notes. In conclusion, extending the production period to include winter production is only feasible with aging rooms equipped with controlled environmental conditioning at 12°C to 14°C.

Key Words: flaking, proteolysis, aging

2498 Milk coagulation induced by milk clotting enzymes (MCE) from traditional Chinese Jiuqu. M. Malkowska-Kowalczyk*, A. Lobacz, and J. Zulewska, *University of Warmia and Mazury in Olsztyn, Olsztyn, Warmia and Mazury, Poland.*

With increasing cheese production there is an increasing demand for milk-clotting enzymes (MCE). Traditional Chinese Jiuqu (cluster of microbials, in which prokaryotes and eukaryotes cohabit, interact, and communicate) used as starter for fermentation of rice to produce traditional rice wine contains microorganisms producing MCE (MCEj). Micellar concentrate casein (MCC), retentate of buttermilk after microfiltration (RMF_B), and retentate of mixture of MCC and permeate of buttermilk after ultrafiltration (RUF_B) were subjected to a coagulation process with MCEj. The aim of the study was to evaluate the implicational potential of MCEj to coagulation in different milk preparations. The protein content of all samples was adjusted to 3.2% using ultrafiltered (UF) milk permeate. The milk coagulation properties of the MCE were studied by dynamic rheological measurement (MCR 102 rheometer, Anton Paar, Graz, Austria). The gels were subjected to microscopic observations using scanning electron microscopy (Quanta 200, Fei Company, Hillsboro, OR). The control sample was conventional milk from a local market (OSM Piatnica, Poland) treated with coagulating agent Naturen (Chr. Hansen, Hørsholm, Denmark). The studies showed the potential of using Jiuqu as milk coagulant. The dosage of Jiuqu to milk was 5% and was calculated so as to obtain the coagulum during 30 min. The coagulation point of milk using Jiuqu occurred at 31.3 min, with $G' = 0.0593$ and $G'' = 0.0580$, and the coagulation point of milk using the conventional coagulant Naturen occurred at 25 min, with $G' = 0.1399$ and $G'' = 0.1439$. The coagulation temperature using Jiuqu was similar (30°C) to that for the commercial enzyme Naturen, widely used in cheese production. There were statistically significant differences ($P \leq 0.05$) in the coagulation time between the same sample treated with different coagulating agents. Longer coagulation time was measured in the case of Jiuqu, that is, 27.8, 19.3 and 21.7 min for MCC, RMF_B, and RUF_B, respectively, in comparison to Naturen, 21.5, 20, and 14.3 min for MCC, RMF_B, and RUF_B, respectively. The microscopic image of the gels differs for these coagulants; the gel resulting from using Jiuqu has a much looser structure.

Key Words: Jiuqu, gel, MCE

Dairy Foods 2: Dairy Products

2339 The effect of dairy-derived *trans* fatty acids on glucose homeostasis in a mouse model of diet-induced obesity. Y. Xu*, C. Prajogo, M. F. Hsu, F. G. Haj, and P. Vahmani, *University of California–Davis, Davis, CA.*

The recent removal of industrial *trans* fatty acids (TFA) from the food supply in developed countries has renewed interest in understanding the human health effects of ruminant TFA. In contrast to industrial TFA, several studies have shown that *trans* vaccenic acid (VA, *trans11*–18:1), the predominant TFA in dairy products, may have health benefits such as reduced risk of type 2 diabetes (T2D). However, direct causative evidence is lacking, and potential underlying mechanisms are poorly understood. Furthermore, there is a lack of studies on other ruminant TFA isomers, such as *trans*-10–18:1 (T10), the second predominant TFA in dairy fats. Thus, we aimed to examine the effect of T10 and T11 on glucose homeostasis in diet-induced obese (DIO) mice. To fulfill this aim, 48 male C57BL/6J mice (7 wk old) were fed either a low-fat diet (LFD, 10% kcal from fat), a high-fat diet (HFD; 45% kcal from fat) enriched with oleic acid (HFD-OA; 6% kcal from pure OA), a HFD enriched with T10 (HFD-T10; 6% kcal from pure T10), or a HFD enriched with VA (HFD-VA; 6% kcal from pure VA) for 19 wk. Body weight and food intake were measured weekly throughout the study. At wk 8, blood sampling was performed in 12-h fasted animals to measure blood glucose, insulin, and C-peptide levels. The homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin. A glucose tolerance test (GTT) was performed during wk 16. Data were analyzed using the mixed models procedure of SAS with a significance level of 0.05. Compared with LFD, feeding HFD resulted in a higher BW gain with the HFD-OA group gaining the most. The fasted blood glucose and area under the curve for GTT were comparable among the 3 HFD groups. Notably, HFD-VA-fed mice showed significantly lower fasted plasma insulin and C-peptide levels and lower HOMA-IR compared with HFD-T10 or HFD-OA-fed mice. In conclusion, VA prevented hyperinsulinemia but not hyperglycemia or glucose intolerance in DIO mice. More cause-and-effect studies are warranted to understand the effect of dairy-derived TFA on the risk of T2D.

Key Words: *trans* fatty acid, type 2 diabetes, biohydrogenation

2340 Southeastern US consumers' willingness to pay for farmstead dairy products. C. Zaring*[1], A. Rihn[2], and E. Eckelkamp[1], [1]*Department of Animal Science, University of Tennessee, Knoxville, TN,* [2]*Department of Agricultural and Resource Economics, University of Tennessee, Knoxville, TN.*

Nationally, the most consumed dairy products are cheese, milk, butter, and ice cream. Cheese, milk, and ice cream are the top produced and consumed Tennessee (TN) farmstead dairy products. Farmstead dairy processors (operations that produce and process raw milk into a salable dairy product) must understand consumer willingness to pay (WTP) for specialty dairy products to be successful. This study used 3 choice experiments to estimate Southeast US WTP for farmstead milk (FSM), farmstead cheddar cheese (FSC), and farmstead ice cream (FSIC). All products had processor source, packaging method, purchase location, production method, and price attributes. Fat content was included in FSM and FSIC, while FSC included aging time. These attributes created 432 FSM, FSC, and 576 FSIC combinations and were reduced to 14 choice sets per product through fractional factorial design. A Qualtrics survey was distributed from October to November 2023 with 1,809 responses ($n_{fsm} = 601$; $n_{fsc} = 605$; $n_{fsic} = 603$). Respondents were TN, North Carolina, and Kentucky residents, ≥18 yr old, and primary food shoppers of dairy-product-consuming households. Choice sets were created using JMP Pro 17 and analyzed in Stata 18 using mixed logit models and nonlinear combinations. The presence of a theoretical farmstead logo on FSM had a high WTP (\$0.93; $P < 0.001$), whereas milk in a glass bottle had a decreased WTP (−\$0.82; $P < 0.001$). State promotional program logos had a premium (\$2.27; $P < 0.001$) compared with products without those logos. The farmstead FSC logos also generated a premium when present (\$2.15; $P < 0.001$). Mild FSC had a reduced WTP (−\$0.72; $P < 0.001$) compared with medium FSC. State promotional logos for FCIS had high WTP (\$2.83; $P < 0.001$). Reduced fat FCIS had reduced WTP (−\$1.10; $P < 0.001$) compared with standard FCIS. Additionally, WTP for grass-fed FCIS was the same as for FCIS made from cows not raised with particular production standards. This study provided insight into consumer-desired FSM, FSC, and FSIC attributes and their associated WTP. Farmstead dairy processors may use this information to refine product production, packaging, and marketing to appeal to Southeast US consumers.

Key Words: value-added, dairy product, willingness to pay

2341 Socio-psychological factors in milk choice at California coffee shops. L. Hoang*, *Chapman University, Orange, CA.*

This study investigated factors influencing consumers in selecting dairy milk and plant-based milk alternatives (PBMA) for their coffee in California coffee shops. Prior research has focused on sensory preferences and socio-demographic factors. In contrast, this study explores the effect of socio-psychological factors and perceptions on consumers' choices of dairy and PBMA in coffee within the United States. The first phase involved interviewing coffee shop consumers, operators, and distributors to develop an online Qualtrics survey for the second phase. After the thesis committee validated the survey, it was distributed through Cloud Research to 300 regular coffee drinkers in California. The participant demographics were representative of the 2020 US Census data. The population was split into 3 groups: dairy milk drinkers (n = 100), PBMA drinkers (n = 100), and flexitarians, who consume both dairy milk and PBMA (n = 100). Using One-Way ANOVA and Tukey HSD with a $P < 0.05$, the findings indicate that taste and texture, as well as health and nutrition, are the primary drivers behind consumers' choice of milk in their coffee, regardless of whether they choose dairy milk or PBMA. The PBMA drinkers also prioritize animal welfare, which is less important to the other groups. Convenience and cost are secondary drivers for all 3 groups. Digestibility was a secondary driver for flexitarians and PBMA drinkers. Among PBMA drinkers, popular options such as almond and oat milk are preferred due to their taste, texture, and perceived health and nutrition benefits, surpassing whole and 2% dairy milk. However, lactose-free (LF) dairy milk is on par with PBMA regarding taste and texture, health and nutrition perception, and digestibility. Notably, 16.3% of PBMA drinkers preferred LF dairy milk when asked to choose between it and PBMA for their coffee. This study sheds light on the factors influencing consumers' choices between dairy milk and PBMA in their coffee. Taste and texture, health and nutrition, animal welfare, convenience, and cost are crucial in these decisions. Furthermore, the study highlights the demand for LF dairy milk within coffee shop environments.

Key Words: socio-psychological driver, milk choice, coffee

2342 Feasibility study of beer production using acid whey: Preliminary results. K. Nathania*[1], P. Freire[1], E. Dormedy[1], W. Whalen[1], and C. Licon[2], [1]*California State University, Fresno, Fresno, CA,* [2]*California Polytechnique State University, San Luis Obispo, CA.*

Utilizing dairy by-product acid whey (AW) is a challenge and has the potential to replace water in beer brewing. This study explores the development of a consumer-acceptable whey beer by standardizing and evaluating production methods and feasibility through physicochemical, microbiological, and sensory analyses. Various yeasts and bacteria cultures (*Kluyveromyces marxianus*, *Brettanomyces claussenii*, *Saccharomyces cerevisiae*, lactic acid bacteria) were used and investigated for their efficiency in fermenting available sugars, of particular interest lactose in acid whey, with addition of lactase enzymes. Cultures were tested for fermentation capacity by colorimetric reaction and CO_2 production after 72 h. Experiments were conducted in flask cultures with whey fortified with dry malt extract. Lactase was added to one of the yeast-bacteria blend treatments. Fermentative capabilities were tracked through specific gravities over 3 wk. Scaled-up production was performed with a gallon batch of whey brewed with grains and hops in triplicate for each inoculum, for a total of 9 fermentations, to collect parameters of specific gravity and temperature until fermentation was completed. Products were cooled to a near-freezing temperature to clarify and bottle-conditioned for sensory evaluation. A 2-way ANOVA was used to determine significant differences between treatments and over time. Results showed that *K. marxianus* performed poorly when used in isolation due to its less favorable environment from the addition of glucose and maltose from malt. Based on the variety of different yeast and bacterial species for metabolizing lactose sugar to ethanol and CO_2, the combination of Cosmic Punch (OYL-402; Omega Yeast Labs LLC) yeast-bacteria blend with lactase enzymes is the most efficient. Results showed significant differences in the specific gravity of the fermentations over time (P <0.001) and for the treatments ($P < 0.001$). In conclusion, beer was successfully produced using acid whey using a combination of yeast and lactic acid bacteria, and further beer analysis will include physicochemical and sensorial characteristics.

Key Words: acid whey, beer production, yeast

2344 Effects of on-farm dietary palmitic acid supplementation and cream ripening conditions on butter manufacture and texture. M. Landry*[1,2], Y. Lebeuf[1,2], M. Blouin[1,2], J. Chamberland[1,2], G. Brisson[1,2], P. Y. Chouinard[1,2], and R. Gervais[1,2], [1]*Université Laval, Québec, QC, Canada,* [2]*Dairy Science and Technology Research Centre (STELA), Québec, QC, Canada.*

Feeding dairy cows a palmitic acid (PA) supplement can increase milk fat yield, but this practice can affect the functional properties of dairy products. We hypothesized that the temperature treatment used for cream ripening can diminish the effect of PA on butter firmness. Four butter-making trials (blocks) were performed with milk collected from pairs of cows randomly allocated a diet containing 0% or 1.8% PA (DM basis). For each trial, after 16 d of diet adaptation, milk from 2 consecutive days was collected in refrigerated bulk tanks and transferred to our pilot plant facilities to be pasteurized and skimmed. Cream was standardized to 41.5% ± 0.1% fat (mean ± SEM) and submitted to 1 of 2 ripening treatments: (1) an isothermal protocol at 10°C for 18 h or (2) a cold-warm-cold protocol (8°C for 2 h, 20°C for 7 h, and 10°C for 9 h), leading to 4 treatments arranged in a 2 × 2 factorial. Then, the cream was churned at 10°C with a Stephan mixer. After draining the buttermilk, the butter grains were salted (2%, wt/wt), and the fat content was adjusted to 80.5%. Data were analyzed as a randomized complete block design. No dietary PA × ripening interaction was observed. There were no effects of treatment on cream fat globule diameter ($D_{4,3}$) before (4.33 ± 0.13 µm; $P \geq 0.28$) or after ripening (4.29 ± 0.15 µm; $P \geq 0.23$). Churning time was increased by dietary PA (+ 69%; $P \leq 0.01$) but decreased by the cold-warm-cold protocol (−15%; $P = 0.01$). Butter yield (49.6% ± 0.2%, wt/wt; $P \geq 0.25$) and buttermilk fat content (1.27% ± 0.11%; $P \geq 0.09$) were not affected by treatment. At 20°C, dietary PA increased butter firmness (+43%; $P = 0.02$) and its spreading resistance (+76%; $P \leq 0.01$) compared with no-PA diets. At 4°C, dietary PA increased butter spreading resistance (+11%; $P = 0.04$) but did not affect its firmness ($P = 0.27$). The cold-warm-cold protocol only reduced butter firmness at 4°C (−16%; $P = 0.03$). In this study, both dietary PA and ripening treatment had an effect on butter manufacture. However, the cold-warm-cold cream ripening could not completely compensate for the increased butter firmness caused by dietary PA.

Key Words: butter, palmitic acid, texture

2345 Sensory characteristics of novel fermented milk drinks based on donkey milk and commercial probiotic bacteria. P. Mousikos[1], P. Papademas[1], G. Marino[2], and M. Caccamo*[2], [1]*Cyprus University of Technology, Limassol, Cyprus,* [2]*Corfilac, Ragusa, Italy.*

In recent years, donkey milk is being considered as a promising non-bovine milk alternative due to its unique composition and its potential functionality and positive effect on human health. In addition, fermentation and added probiotics could further enhance donkey's milk health benefits to the consumer. In this study, a starter culture (*Streptococcus thermophilus*) and 3 probiotic strains (*Lactobacillus rhamnosus* LGG, *Lactobacillus paracasei* ssp. *paracasei* L. CASEI 431, and *Lactobacillus helveticus* R0052) were used to ferment donkey milk, enhance its nutritional value, and prolong its shelf life. Probiotics were used in pure cultures and in cocultures with and without prebiotic (inulin) addition. Fermentation temperature was 37°C. The process was subsequently stopped by cooling milk at 4°C (i.e., when milk had reached pH <5). Sensory profiles of fermented donkey milk for each treatment at d 0 and 21 were defined using a quantitative descriptive analysis with 9 trained panelists. To test consumer acceptability, a check-all-that-apply (CATA) test was applied as a simple and fast sensory profiling tool. Significant differences ($P < 0.05$) were found between the days' attributes of sweetness, astringency, and body. Higher values were recorded at d 0 for sweetness and body while astringency increased with time (d 21). The treatment had a significant effect for body attribute, with higher values for R0052 ($P = 0.01$). The CATA test revealed a higher preference ($P < 0.001$) for R0052 compared with the other treatments.

Key Words: donkey milk, sensory, probiotic

2346 Evaluation of retorted complex nutritional beverages at different pH levels to minimize thermal protein aggregation and optimize emulsion stability. D. C. Strozier* and M. K. Fleshman, *Abbott Nutrition, Columbus, OH.*

When complex nutritional beverages were subjected to retort processing, product developers found it challenging to form stable emulsions. The objective of this study was to determine the effect of pH on a nutritional beverage (54% carbohydrates, 27% fat, 17% protein) with caseinates and soy protein which undergoes retort sterilization (122.8°C for 13.5 min). Increased amounts of KOH were added to samples before retorting. These 10 samples were tested for protein degradation by a reverse phase HPLC method where area under the curve was compared for all 10 samples with pH range 6.31 to 6.77. A smaller area indicated less soluble protein in the sample at 214 nm wavelength. Samples at pH 6.73

and 6.77 show less area and a shift in retention time which indicated loss of protein through degradation. In addition, these samples were tested for protein aggregation testing (grain testing), particle size testing, viscosity, and lumisizer testing (analytical centrifuge with near-infrared light) for emulsion stability. The direct correlation between greater protein degradation (214 nm area) and protein aggregation readings was significant ($R = -0.766$, $P = 0.01$). Observed grain particulates consisted of aggregated proteins and fat globules. Grain ratings for samples with pH 6.31–6.55 exhibited an absence of aggregation. For the 10 samples with pH range 6.31–6.77, pH was significantly correlated with protein aggregation ($R = 0.883$, $P = 0.001$), emulsion instability index 1 h ($R = -0.791$, $P = 0.006$), and emulsion instability index 2 h ($R = -0.766$, $P = 0.01$). Post retort samples at pH 6.73 and pH 6.77 showed signs of protein degradation at 214 nm wavelength (protein absorption from peptide groups). Both samples showed an increase in nonpolar peptides and proteins. The optimal post retort pH range is from 6.31 to 6.55 as supported by the grain readings and lumisizer results. Emulsion instability at the higher pH levels may be due to kappa casein's ability to dissociate from the micelle.

Key Words: nutritional beverage, pH, emulsion stability

2348 Effects of plant-based prebiotics on the physicochemical properties of a probiotic goat milk yogurt.

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Dairy products represent the largest segment of functional foods in the market. These dairy products have been considered good carriers of prebiotics, probiotics, and nutrients. Supplementation of probiotic yogurt with hemp protein concentrate and carrot powder as prebiotics may give sufficient protection to probiotics in the human gut. We investigated the physicochemical stability of probiotic goat milk yogurt containing plant-based ingredients at d 1 of production and at d 28 of storage at 4°C. The basic mix for the probiotic yogurt had 82.85% milk, 10.68% powdered milk, and 6.47% sugar. Additionally, the mix was supplemented with carrot powder (0.6% CP, T1), hemp protein (2.5% HP, T2), and a combination of 0.6% CP and 2.5% HP (T3). A control (T0) yogurt without plant-based ingredients was also prepared. The mix was heated at 80°C for 30 min and then cooled to inoculate with starter culture and 2 probiotics (YF-L812, BB-12, LA-5; Chr Hansen). The inoculated mix was incubated at 42°C for 5 h. The effect of the plant-based supplementation on firmness, color (L^*, a^*, and b^*), water-holding capacity (WHC), pH, titrable acidity (TA), lipid oxidation, total phenolic compounds (TPC), antioxidant capacity, and particle sizes of yogurts were studied at day-1 and day-28. Data were analyzed by a factorial design within a split-plot design using the PROC MIXED model procedure of SAS. The plant-based ingredients affected ($P < 0.05$) some of the physicochemical properties of probiotic yogurt during the 28 d of storage, but they did not adversely affect the WHC and lipid oxidation. Compared with the control, T2 increased the firmness (2.01 Newtons), pH (4.92), and mean particle size diameter (9.63 µm), while T3 increased a^* (0.63) and b^* (16.83) color values, pH (4.90), and TPC (25.57 mg GAE/g) in the probiotic yogurt. The plant-based ingredients had a slight effect on the physicochemical properties of probiotic yogurt. Future studies will focus on optimizing the levels of plant-based ingredients in probiotic yogurt, sensory attributes, and microbiological analyses of supplemented goat milk yogurt.

Key Words: goat yogurt, carrot powder, hemp protein

Dairy Foods 2: Microbiology

2349 ***Leuconostoc* sp. causes slimy defect in chocolate milk.** A. I. Rapacioli*, S. J. Reichler, R. L. Weachock, and N. H. Martin, *Cornell University, Ithaca, NY.*

Leuconostoc are gram-positive lactic acid bacteria that produce gas in blue cheeses to ferment milk (e.g., kefir). A commercial chocolate milk sample was tested microbially and sensorially throughout its shelf life, and developed a slimy, viscous texture 21 d after HTST pasteurization. A bacterial isolate from this sample was determined to be *Leuconostoc* sp. using 16S rDNA sequencing. In this study, we inoculated a chocolate milk sample and an unflavored milk sample with this *Leuconostoc* isolate to confirm that it was the cause of the slimy defect. Samples were inoculated with 4 log cfu/mL of *Leuconostoc* sp. and incubated at 6°C for 5 d. Standard plate count, lactic acid bacteria count, and viscosity were measured on d 0, 3, and 5. On d 5, the lactic acid bacteria counts were 8.8 log cfu/mL and 7.7 log cfu/mL for the chocolate and unflavored milk samples respectively, whereas uninoculated chocolate and unflavored control milk samples both had lactic acid bacteria counts below the detection limit. The viscosity on day initial for the inoculated chocolate milk sample was 46 mPa·s, and by d 5 the viscosity for that sample was above the measurable range and the sample had a noticeable slimy defect. The slimy defect was not observed in the inoculated unflavored milk which was also represented in the viscosity measurements of 6.0 mPa·s on the initial day of testing and 6.4 mPa·s on d 5. The control chocolate and unflavored milks had viscosity readings that remained relatively unchanged throughout the study period. The generation of a slimy defect in chocolate milk, but not in unflavored milk, is likely driven by the higher sugar content in chocolate milk. Unusual cases of visual microbial spoilage in dairy products can be recorded and widely disseminated via social media, damaging a brand or entire product category. Dairy processors should be aware of this spoilage potential for chocolate milk, measures that can be taken to prevent *Leuconostoc* contamination, and methods to detect *Leuconostoc* in their finished product.

Key Words: microbiology, fluid milk, quality

2350 **Sporeforming bacteria in cocoa powder vary in count and proteolytic activity.** F. M. Quintana Pérez, R. L. Weachock*, and N. H. Martin, *Cornell University, Ithaca, NY.*

Cocoa powder is used in dairy applications to create products such as chocolate milk and chocolate ice cream. Chocolate milk has been observed to have a shorter shelf life than unflavored milk, likely due to the higher levels of sporeforming bacteria which can survive pasteurization and cause spoilage. Here, we aim to characterize sporeforming bacteria isolated from commercial cocoa powder and to evaluate the variability of the mesophilic and thermophilic spore counts within 10 individual 50-kg bags of cocoa powder. From each 50-kg bag of cocoa powder, 10 individual 10-g samples were collected from various locations throughout the bag. Mesophilic and thermophilic spore tests were performed on each cocoa powder sample (n = 100). The mean spore count in the 10 cocoa powder bags ranged from 0.65 to 2.69 $\log_{10}$ cfu/g for mesophilic spores and 1.28 to 2.68 $\log_{10}$ cfu/g for thermophilic spores with standard deviations ranging from 0.07 to 0.44 $\log_{10}$ cfu/g and 0.23 to 0.47 $\log_{10}$ cfu/g for mesophilic and thermophilic spores respectfully. Isolates representing unique bacterial morphologies were characterized for proteolytic activity on skim milk agar and identified using rpoB sequencing. Characterization of 127 isolates revealed that approximately 32% were *B. subtilis s.l.*, 31% were *B. licheniformis*, 13% were *B. gibsonii*, 6% were *Thermoactinomyces vulgaris*, and the remaining isolates (~18%) were other sporeforming bacteria found less frequently. Most of the isolates (120/127) were characterized as being proteolytic. Our findings show that cocoa powder may contribute considerable levels of bacterial spores to dairy products. These spores pose a potential threat to the quality and the shelf life of products that incorporate them (e.g., chocolate milk, ice cream mix, and so on) which contributes to monetary losses for the industry and higher rates of food waste. Improving the methods used to detect, eliminate, and safeguard cocoa or dairy products containing cocoa from further spore contamination throughout processing will help preserve their quality and minimize the potential threat of spoilage and subsequent food waste.

Key Words: cocoa powder, sporeforming bacteria, dairy quality

2351 **Characteristics of psychrophilic bacterial communities and associated metabolism pathways in different environments by a metagenomic analysis.** W. Xu[1,2], L. Meng[1], Y. Zhao[1,3], J. Wu[1], H. Liu[1], J. Wang[1], and N. Zheng*[1], [1]*Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*College of Food Science and Engineering, Qingdao Agricultural University, Qingdao, P. R. China,* [3]*Institute of Quality Standards & Testing Technology for Agro-products, Xinjiang Academy of Agricultural Sciences, Urumqi, P. R. China.*

Psychrophilic bacteria are the most important spoilage microorganism in raw milk. In this study, we characterized the psychrophilic bacteria communities in 165 raw milk samples from 4 seasons and 6 regions in China using shotgun metagenomic sequencing. *Pseudomonas* (47.9%), *Stenotrophomonas* (9.75%), *Sphingomonas* (6.73%), *Latilactobacillus* (6.38%), and *Lactococcus* (5.16%) were the dominant genera in the raw milk samples. Unclassified *Pseudomonas*, *Pseudomonas fluorescens*, *Pseudomonas helleri*, *Pseudomonas lundensis*, *Pseudomonas fragi*, and *Pseudomonas putida* were the dominant *Pseudomonas* species. The diversity of psychrophilic bacteria communities in raw milk was affected by seasonal and regional factor, and carbohydrate metabolism and AA metabolism were the main potential metabolic pathways of psychrophilic bacteria. Metagomic sequencing can accurately determine the species and functional information of the community of psychrophilic bacteria in raw milk, which is helpful to understand the community structure of psychrophilic bacteria, and promote the quality and safety monitoring of raw milk.

Key Words: raw milk, psychrophilic bacteria, metagenome

2352 **Culturomics-based discovery of probiotics in raw milk from Holstein dairy cow.** H. Doo*, J. Kwak, E. S. Kim, G. B. Keum, S. Ryu, Y. Choi, J. Kang, H. Kim, Y. Chae, S. Kim, and H. B. Kim, *Department of Animal Biotechnology, Dankook University, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

The microbiological quality of raw milk is pivotal for maintaining dairy farm profitability and enhancing product quality. However, there is a notable scarcity of comprehensive studies focusing on the microbiota of raw milk. This study used the culturomics to meticulously screen the microbiome of raw milk obtained from Korean dairy farms, with a specific focus on isolating and identifying bacteria with potential probiotic properties. The raw milk was directly collected from healthy Holstein cows at a dairy farm in South Korea. We cultured the samples on variety of selective media, including MRS agar, modified RCM agar, M17 agar, and TSA agar, under optimal growth conditions tailored for each medium. The isolated bacterial colonies underwent identification through full-length 16S rRNA gene sequencing, utilizing universal primers (27F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R 5′-TACGGYTACCTTGTTACGACTT-3′). For comprehensive gene sequence analysis, we employed the Molecular Evolutionary Genetics Analysis 11 (MEGA11) software. A total of 81 bacterial colonies were isolated and classified into 2 phyla, 2 classes, 3 orders, 5 families, 7 genera, and 19 species. The identified bacterial species were meticulously categorized based on the GRAS (Generally Recognized as Safe, FDA) and QPS (Qualified Presumption of Safety, EFSA) criteria. This classification revealed the presence of 3 beneficial bacteria: *Lactococcus lactis*, *Streptococcus salivarius*, and *Streptococcus thermophilus* (3.7% of total isolates). *Lactococcus lactis* and *S. thermophilus* have been extensively studied as probiotic starters for the manufacture of fermented dairy products. Additionally, recent studies have focused on *S. salivarius* as a probiotic aimed at enhancing health. Our culturomics data not only provides fundamental insights into the bacterial species present in raw milk from Korean dairy farms but also emphasizes the pivotal role of culturomics in unraveling the complexities of raw milk microbiota. This, in turn, contributes to a broader understanding of dairy microbiology.

Key Words: Holstein cattle, culturomic, beneficial bacteria

2353 Milk fat globule membrane affects lactic acid bacterial secretome and subsequent effect on intestinal cell differentiation.

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Serotonin is a neurotransmitter that is primarily produced in the gut and is produced by lactic acid bacteria (LAB). It plays a crucial role in regulating mood and mental health disorders via the gut-brain axis. Exopolysaccharides (EPS) are complex metabolic end products of probiotic LAB comprised of sugar residues. The EPS contribute health promoting activities such as immunomodulatory and antioxidant among others. Because LAB are naturally present in dairy foods, components of the food matrix such as the milk fat globule membrane (MFGM) can affect the bacterial secretome. The MFGM is a bioactive component of milk that positively influences brain function and the gut microbiota. Therefore, the objectives of this work were to determine if supplementing LAB growth medium with MFGM increases serotonin and EPS production and uncover the effect of the bacterial secretome on intestinal cell differentiation. One hundred thirty-five strains of LAB were screened for serotonin production following subculture with tryptophan supplementation. The cell-free supernatant was collected and filtered using a C_{18} column with methanol elution. Samples were concentrated using vacuum evaporation and measured using a spectrophotometer following reaction with Ehrlich's reagent. The top 10 producing strains received supplementation with MFGM, and their growth curves were measured spectrophotometrically. Samples were collected during the growth phase, early stationary phase, and late stationary phase. The cell free supernatants were evaluated for serotonin as well as EPS using phenol sulfuric and DNS sugar assays. These cell free supernatants were used to treat Caco-2 intestinal cell lines on d 1 and 7 of confluency. RNA was extracted with TRIzol and used for RT-qPCR analysis of differentiation genes. Results were analyzed using a *t*-test, and there was a significant increase ($P < 0.05$) in production of serotonin and EPS with MFGM supplementation as well as faster intestinal cell differentiation. These findings indicate a novel application of MFGM as a functional ingredient in dairy foods, and when combined with LAB has great potential to enhance human health.

Key Words: serotonin, milk fat globule membrane, gut health

2354 Fermentation of whey permeate by *Brettanomyces claussenii* for the production of an alcohol-free acetic acid beverage.

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Valorization via fermentation provides a low-overhead option for dairy processors to upcycle whey waste streams, reducing both the economic and environmental burdens of this byproduct. Prior work in our laboratory group has established that fermentation of whey permeate (WP) by *Brettanomyces claussenii* under aerobic conditions with exogenous β-galactosidase can enable the production of a beverage base rich in galactose and acetic acid, which could appeal to consumers seeking popular functional beverages such as kombucha. This study was carried out to understand the effects of agitation (100–250 rpm), pitch rate (5–8 log cells/mL), time (6–30 d), and temperature (25–35°C) on final fermentate composition after aerobic fermentation of hydrolyzed WP by *B. claussenii*. The WP powder was reconstituted at 20% solids (168 g/L lactose) and treated with a commercial β-galactosidase at 33 BLU/g lactose for 24 h. The 150-mL fermentations were then carried out in biological triplicate for each combination of time, temperature, agitation, and pitch rate in an environmental chamber, where the humidity was maintained at 65%. Due to the limitation of only being able to run one temperature at a time in the environmental chamber, treatments were run in single-temperature blocks. After fermentation, cells were removed by centrifugation and initial mass, final mass, pellet mass, mass of cell-free media, density as measured by a DMA-35, and substrate concentrations quantified by HPLC-RID were used to account for evaporation and calculate the total amount of acetic acid and ethanol produced, as well as galactose consumed and residual glucose. An ANOVA with all 2 factor interactions on the 25°C samples showed that time, agitation, and pitch rate had significant effects ($P < 0.05$) on all 4 substrates of interest (aside from the effect of agitation on galactose consumed, $P = 0.132$). The highest acetic acid production was in the 6 d, 8 log cells/mL, 250 rpm treatment, corresponding to a concentration of 15.7 g/L. Further, the data collected in this study will be used to establish optimal conditions for beverage production.

Key Words: upcycling, whey permeate, fermentation

2355 Valorizing dairy and poultry byproducts: Bioactive protein hydrolysates with enhanced antioxidant activity.

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Low-value waste such as acid whey and byproducts such as mechanically separated meat (MSM) are environmental and economic burdens. This study explored the potential of converting these byproducts into high-value, bioactive protein hydrolysates using lactic acid fermentation with *L. rhamnosus*. Blends of MSM, acid whey, and crude molasses were fermented for 10 d, and changes in pH, proteolytic activity, antioxidant activity, soluble protein content, and molecular weight distribution were monitored. The initial pH of 5.27 ± 0.02 significantly decreased ($P < 0.05$) to 3.70 ± 0.01 by d 2, indicating efficient acidification by *L. rhamnosus*. Notably, the pH remained stable afterward, suggesting a controlled fermentation process. Encouragingly, peptide concentrations demonstrably increased ($P < 0.05$) throughout fermentation, implying effective protein hydrolysis. Interestingly, antioxidant activity measured by DPPH, FRAP, and ABTS assays was significantly enhanced ($P < 0.05$) by d 10, exceeding a 22.8% ± 0.1% inhibition ratio. This suggests the formation of bioactive peptides with potential feed and food applications. Overall, this work demonstrates that judicious selection of a proteolytic LAB and fermentation conditions can increase the value of by-products in the dairy and poultry industries by generating bioactive protein hydrolysates with improved antioxidant activity through fermentation. This sustainable approach not only promotes waste management but also offers promise for developing functional food ingredients with diverse applications in the food industry, contributing to a more circular and sustainable food system.

Key Words: acid whey, fermentation, protein hydrolysates

Dairy Foods 2: Processing

2356 Nisin susceptibility of *Staphylococcus aureus* isolated from artisan cheese farms in Northern Uruguay.

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Small cheese-producing farms mitigate the risk of the growth of pathogenic bacteria by relying on ripening. At the same time, adding products such as nisin is proposed to suppress toxigenic bacteria that may present, such as *Staphylococcus aureus* (*S. aureus*). This study aims to evaluate the antibacterial properties of nisin on *S. aureus* isolated from artisan cheese farms. One hundred forty-four isolates were retrieved from 330 lactating cows from 15 farms across the northern region of Uruguay. Antibacterial activity was assessed using the agar dilution method described on the M07-A10 CLSI standard. Descriptive statistics were used to evaluate the MIC data. The MIC Range, MIC$_{50}$, MIC$_{90}$ (represented as the MIC for 50% and 90% of the tested isolates, respectively) and the Geometric Mean (GM) MIC with 95% CI were calculated. The resulting MIC range was from 3 to >192 ppm, with the MIC$_{50}$ at 24 ppm and the MIC$_{90}$ at 192 ppm and the GM MIC was 28.54 ppm with a 95% CI (19.05 ppm, 38.03 ppm). Less than half of the isolates (42/144) were susceptible to the recommended maximum cheese concentration, 12 ppm. This suggests that while nisin demonstrates antimicrobial activity against *S. aureus* at the permitted maximum concentration, it is unsuitable as a standalone measure. Additional barriers are necessary for comprehensive bacterial control. A small proportion of isolates (8/144) did not exhibit growth inhibition within the tested range. This may be attributed to the presence of resistance mechanisms, suggesting the existence of a nisin-resistant subpopulation. Nisin demonstrates potential as an antimicrobial and is available as a food additive. However, further studies are necessary to establish appropriate susceptibility breakpoints for bacteria of interest.

Key Words: bacteriocin, *Staphylococcus aureus*, artisan cheese

2357 Degree of fractionation of whole milk using microfiltration: Comparison at cold (19°C) and heated (42°C) temperatures.

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Microfiltration (MF) of whole milk (WM) is beneficial because the milk without separation can be fractionated and used in different products. The fat in the cold milk can clog the membrane pores and cause early fouling which leads to processing inefficiency. Much of the fat is in melted condition at and above 40°C. The fat in the melted condition processed through the MF should help with better processing efficiency and less fouling of the membrane. Hence the study was designed to fractionate whole milk using MF in cold (19°C ± 1°C) and heated (42°C ± 1°C) conditions. The trials were conducted using two 0.1-µm flat-sheet polyvinylidene difluoride MF membranes. Pasteurized and unhomogenized WM was procured from the dairy plant. Two treatments selected for MF processing included WM at 19°C (WM-C) and at 42°C (WM-H). The process efficiency was determined using the flux rate throughout the process. The initial, 2×, 3×, final, retentate, and permeate samples were analyzed for proximate composition, particle size, and minerals. Trials were replicated thrice and data were analyzed using Costat. Flux rate of MF in WM-C rapidly decreased throughout the trials compared with the WM-H run, however the 3× concentration (mass) was achieved faster in the WM-H processing run. The WM-H processing took 14.3% less time than WM-C run. Hence all the comparisons were made at 2× concentration. The fat loss in WM-H permeate was 0.70% ± 0.16% which was almost double that of the WM-C sample. At 2× concentration, the WM-H and WM-C retentate samples had similar values for proximate and mineral composition, and the differences were too low to be significant ($P < 0.05$). The WM-C had a significantly ($P < 0.05$) larger particle size at 2× concentration. This study concludes that WM-H can be concentrated up to 3× using MF at a faster rate without fouling compared with WM-C run which took a longer time to achieve 2× concentration with fouling the membrane. This difference is because most of the milk fat is in a melted state and does not stick to the membrane surface. In conclusion, the heating of milk helps in faster MF processing of WM without membrane fouling.

Key Words: whole milk, microfiltration, processing temperature.

2358 Assessing whey quality through synchronous front-face fluorescence: A preliminary study.

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Whey, a by-product of the dairy industry, has gained popularity due to its nutritional value and versatile functional properties. However, whey protein denaturation can vary depending on the processes involved in its production. Therefore, synchronous front-face fluorescence (SFFF) potential was assessed as a rapid and noninvasive technique to predict the content of undenatured whey protein for on or in or at-line estimation. As heat treatment induces whey protein denaturation, heat treatments (90°C for 0–30 min) were applied to reconstituted skim milk to obtain whey with different undenatured whey protein concentrations (UWP). The SFFF of whey, obtained by isoelectric precipitation of caseins (pH 4.6) with HCl 1 *N*, was measured at an excitation of 250 to 550 nm ($\Delta\lambda = 20$ nm). The spectra of SFFF were then analyzed using the R program where peaks were enclosed into an area. The whey spectra were divided into 10 areas and each area was associated with at least one fluorophore by conducting a literature review. Pearson correlation was applied between each area and the UWP concentration. The intensities of areas 2, 3, 6, 8, and 9 showed positive correlations with the UWP concentration ($P \leq 0.001$). Excitation and emission wavelengths, as well as maximum intensities, were collected for 10 areas. These FFF-parameters and their mathematical transformations were used in the maximum R^2 procedure of SAS software to select the predictors and obtain the best models, which were cross-validated. Excellent prediction models with one to 3 variables (R^2 >0.95, RPD >4) were obtained using the information of areas 1 to 6 and 10. These spectral regions seem to contain relevant information to evaluate the state of whey proteins that could be useful in predicting functional properties, with clear potential for in-line application.

Key Words: front-face fluorescence, fluorophores, whey quality

2359 Innovative approaches to beverage stability: Exploring milk whey protein isolate in acidic drinks.

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Whey protein-rich ingredients such as whey protein concentrate (WPC) or whey protein isolate (WPI) are manufactured from cheese whey or directly from skim milk. The ingredient derived directly from skim milk is called milk whey protein isolate (mWPI). High-acid beverages typically have a pH of around 3.5. This study aims to understand acidic beverage characteristics by developing stability diagrams using mWPI as a source of protein. Two lots of mWPI powders were obtained from a commercial manufacturer. A 10% (wt/wt) stock solution was prepared by rehydrating the powders at room temperature for 30 min and refrigerating overnight for full rehydration. Using distilled water, the protein concentration was adjusted to the desired levels (2%, 4%, 6%, 8%, and 10%). Subsequently, the sample was divided into equal parts (300 mL) in different beakers. The pH was then adjusted to 3, 3.5, 4, 4.5, and 7. Subsequently, the samples were divided into smaller portions of 100 mL, and NaCl was added at concentrations of 0, 5, and 10 µ*M*. Low pH samples (≤4.5) were heated to 109°C for 3 s, whereas high pH samples (>4.5) were heated to 141°C for 6 s. After heating, samples were immediately cooled in an ice bath. Acidic drinks were analyzed for turbidity, aggregation index (AI), soluble proteins, viscosity, and protein interactions. Stability diagrams were constructed based on the clarity of the drinks. The beverages had transitioned from clear to turbid at pH 3.5 or higher, regardless of the salt content of the drinks. Samples with pH 3 and protein content up to 8% had the lowest particle size (<150 nm), reflecting the samples' appearance as clear. In addition, regardless of the salt content, those samples had turbidity, AI, and soluble proteins of ≤0.05 ($P < 0.05$), ≤10 ($P < 0.05$), and 93% to 100% ($P < 0.05$), respectively. In conclusion, incorporating mWPI as a protein source in acidic beverages allows dairy manufacturers to produce drinks with a protein content reaching up to 8% without affecting their appearance or physical characteristics. Therefore, the stability diagrams generated in this study can serve as a valuable tool for formulating acidic drinks.

Key Words: whey, acidic drink, stability

2360 Role of mix viscosity on the meltdown behavior of ice cream.

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The meltdown test is an efficient tool widely and commonly used to characterize structural changes in frozen desserts resulting from different ingredients and processing conditions. Several factors influence the meltdown of ice cream, including the fat content, type of hydrocolloids, freezing, overrun, and viscosity. The objective of this work is to evaluate the role of mix viscosity on the meltdown (weight vs. time) and shape retention of ice cream. Ice creams formulated with 0%, 0.3%, and 0.45% of stabilizer blend (guar gum, locust bean gum, carrageenan, polysorbate 80, and mono- and diglycerides) were used to obtain 3 levels of mix viscosity, low-, medium-, and high-viscosity (0.63, 1.25, and 3.35 Pa s at 10 s^{-1} of shear rate, respectively; $P < 0.05$). The meltdown was gravimetrically determined, where 40 to 60 g of ice-cream were placed on a suspended wire mesh (6 holes per cm). The temperature of the ice-cream and the dripped weight were continuously recorded throughout the duration of the test. The meltdown test was conducted at room temperature. The onset of meltdown significantly increased ($P < 0.05$) with increasing mix viscosity, from 1758 ± 88, 1968 ± 98, to 3020 ± 151 s for low-, medium-, and high-viscosity samples, respectively. However, the values of meltdown rate decreased significantly ($P < 0.05$) with the viscosity from 0.0125% ± 0.001%, 0.0096% ± 0.0001%, and 0.0035% ± 0.0001% s^{-1} for low-, medium-, and high-viscosity samples, respectively. The shape retention of all samples exhibited a sigmoidal behavior, where the values were 56.47% ± 1.96%, 68.57% ± 6.22%, and 78.47% ± 6.32% ($P < 0.05$) for low-, medium-, and high-viscosity samples, respectively. The results indicate that the mix viscosity is an important parameter that influences the meltdown behavior and shape retention of ice cream.

Key Words: ice cream, meltdown, mix viscosity

Extension Education 1

2361 Enhancing sustainability in dairy farming through technical support and dietary optimization. M. H. De Oliveira*[1,2], F. F. Cardoso[3], and T. Fernandes[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*State University of São Paulo, Botucatu, SP, Brazil,* [3]*University of Maryland, College Park, MD.*

The precise use of nitrogen (N) remains a major challenge for optimizing the sustainability of dairy farming. Due to the lack of technical assistance for underserved farmers, many producers are overfeeding N in cows' diets, which increases N excretion into the environment. Cows are inefficient in N utilization, up to 60% of dietary N is excreted in manure. Adjusting dietary composition is an effective strategy to improve N efficiency and reduces N environment excretion and feed costs. The market uses N footprint (N inputs and losses in food production and processing systems) to drive consumers' choices, which can negatively affect dairy facilities that are not following those requirements. Thus, we aim to collect data on dairy farmers through a screening online survey, encompassing questions about farmers' profiles, property size, herd, nutritional practices (feed ingredients, diet formulation, orts destination, track of animals' efficiency), and sustainability practices related to manure waste and its destination (storage, use as fertilizer, amount applied, water source and quality). Collecting farm management and farmer profile data provides insights into nitrogen use and producers' unique challenges. A preliminary gathering of information from Virginia farms showed that, out of 364 dairy farmers, 98% are classified as grade "A," indicating that their milk is suitable for industry processing, and 2% are classified as manufactured grade producers (mostly cheesemakers). Additionally, 25% of these producers do not have network access due to religious beliefs or lack of infrastructure. Therefore, we limited our survey to 17 dairy producers, 7 originating from Floyd County and 10 from Montgomery County. Each of these producers holds a grade "A" classification, and none uses automatic milking systems. The application of a preliminary survey enhanced farmer adherence by ensuring practical and customized recommendations for each farm. The lack of information access by a considerable number of dairy farmers highlights the need to support these producers regarding the efficient use of N.

Key Words: N footprint, N excretion, nutritional practice

2362 Implementing a multicomponent extension program on a long-term multidisciplinary project developing a dairy manure bioeconomy. M. E. de Haro Marti*[1], M. Chahine[2], L. Schott[2], and M. A. McGuire[3], [1]*University of Idaho, Gooding, ID,* [2]*University of Idaho, Twin Falls, ID,* [3]*University of Idaho, Moscow, ID.*

Modern dairy production faces significant challenges handling manure nutrients, especially in dairy-dense regions where the use of imported feed and fertilizer concentrates nitrogen, phosphorus, and other nutrients that crops cannot sufficiently use. The University of Idaho, through a USDA-NIFA Sustainable Agriculture Systems grant, has implemented a project known as the Idaho Sustainable Agriculture Initiative for Dairy (ISAID). The 5-yr project, now at its half-life point, involves a multidisciplinary team of 25 faculty and post-doctoral researchers, 9 PhDs, 18 Masters, and dozens of undergraduate students. The project's 3 pillars are research, extension, and education. The research components emphasize the creation of a circular bioeconomy around dairy manure with the objective of developing technically and economically viable ways of recycling, exporting, or using dairy manure as input for new marketable products. The educational section emphasizes the training of graduate and undergraduate students on the different aspects of the system approach to prepare them to work in associated industries or institutions. The extension component of the grant started its activities from the inception of the project. Contrary to other methodologies that trigger extension involvement at the latter stages, extension activities were included from the beginning. It was designed to allow researchers to explain to the public, especially dairy and crop producers, the science and technology of their research and their expectations of results. A multifaceted media campaign using diverse platforms, popular press publications, field days, demo videos, interviews, progress reports, and podcasts are the main strategies used by the extension team to keep the audience engaged and informed on how each research project is advancing, as well as receiving audience's feedback. Extension components are embedded in the education section, requiring all graduate students to generate extension products (bulletins, infographics) as part of their studies.

Key Words: extension, dairy manure, bioeconomy

2363 Evaluating undergraduate students' cow handling knowledge after playing the *Mooving Cows*^{TM} game. J. Van Os*[1], M. Ruiz-Ramos[1], E. Ronk[1], and N. B. Cook[2], [1]*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI,* [2]*Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI.*

Proper dairy cow handling is a common topic in introductory animal science courses. Our objective was to evaluate the effectiveness of using *Mooving Cows*^{TM}, a digital educational game, for undergraduates to learn cow handling practices. Students (n = 101; 72% first years; 82% female; 25% with dairy or beef cattle experience; age: 18.5 ± 0.9, mean ± SD) in an introductory animal science course played the game during a laboratory session. Outside of class, before and after playing the game, students completed online Qualtrics questionnaires to assess knowledge of best handling practices, using 10 multiple choice questions (including an "unsure" option), with the order of questions and response choices scrambled. In the post-class survey, they answered 5-point Likert scale items (1 = not at all, 5 = extremely) on (1) how clearly they understood the in-game feedback and how useful they thought the game would be for (2) those with little cattle experience or (3) those who already had experience. During class, students were subdivided into 9 groups of up to 12 students each. Due to time constraints, they played only the first 5 (of 8) game levels at their own pace on mobile tablets. A paired *t*-test was used to evaluate the change in the number of correct, incorrect, and unsure responses before vs. after playing the game. Descriptive summary statistics are reported for the perceptions questions. After playing the game, compared with before, students answered 1.2 ± 0.14 (mean ± SE) more questions correctly (9.1 vs. 7.9), 0.5 ± 0.10 fewer questions incorrectly, and responded "unsure" to 0.7 ± 0.12 fewer questions ($P < 0.0001$). Students found the in-game feedback very clear (median = 4; interquartile range, IQR = 4–5) and thought the game would be very useful (median = 4; IQR = 3–4) or moderately useful (median = 3; IQR = 2–4) for inexperienced versus experienced cattle handlers, respectively. Based on the evaluation of *Mooving Cows*^{TM} by undergraduate students in an introductory animal science course, our results suggest the game provides an interactive opportunity for students to improve their knowledge of appropriate dairy cow handling practices.

Key Words: animal handling, stockmanship, undergraduate education

2364 Effect of a good milking hygiene practices training on the assessment of somatic cell count in bovine milk from different milking systems. P. A. B. Mac-Lean[1], J. F. Barbosa[1], I. G. M. A. Santos*[2], E. G. Satolo[1], C. P. Cremasco[1], S. G. Gonzales[1], F. M. Rocha[1], I. B. Sanches[1], and S. V. B. Feitosa[1], *[1]São Paulo State University (Unesp), School of Sciences and Engineering, Tupã, São Paulo, Brazil, [2]Purdue University, College of Agriculture, West Lafayette, IN.*

Milk quality is influenced by various factors, with good milking practices playing a crucial role in ensuring a high-quality product and financial gains. This study aimed to evaluate the effectiveness of a training session focused on implementing proper milking practices to reduce SCC in bulk milk tank from farms. Sixteen milk producers from the Alta Paulista region, Brazil, all members of the same association, were randomly selected and divided into 2 groups employing different milking systems: automatic ($n = 8$) and manual ($n = 8$). The SCC values, assumed to have a normal distribution and without transformation, were analyzed 3 times: before the training session, 20 d later, and 50 d later. The training session included demonstrations of udder anatomy, emphasizing the importance of enhancing milk quality, and outlining milking routine procedures to prevent milk tank contamination and ensure quality preservation. Samples were collected in sterile tubes, refrigerated, and analyzed using a hematology analyzer. Data were subjected to ANOVA, with means compared using the Tukey test if significant differences were observed. No significant difference in SCC values was found between the analyzed milking systems. However, when examining the average SCC content, it was noted that before the training session, the mean SCC was 2.7×10^5 cells/mL. After 20 d, there was a decrease to 2.5×10^5 cells/mL, and after 50 d, it further decreased to 2.4×10^5 cells/mL, keeping below the limit allowed by legislation (30×10^5 cells/mL), indicating a reduction of 2.9×10^5 cells/mL in SCC concentration. This reduction highlights the effect of extensionist practices, where producers receive guidance from technically knowledgeable teams to ensure proper hygienic management during milking and enhance milk quality, regardless of the milking process. Furthermore, this research contributes to the food chain by linking management practices with extensionist interventions and food quality, thereby fostering development and financial growth for families engaged in milk production in the region.

Key Words: good milking practice, milk quality, extension action

2365 Evaluating the effectiveness of the United States-Mexico-Canada Agreement on increasing intra-continental dairy foods trade. J. S. Myers*[1], J. Holderieath[2], J. M. Thompson[1], and T. Malone[1], *[1]University of Arkansas, Fayetteville, AR, [2]Northwest Missouri State University, Maryville, MO.*

In 2022, total dairy foods exports from the United States (US) to Canada accounted for ~$531 billion in income to the US dairy industry. However, trade between these 2 countries has been a hotly debated topic. This study aimed to determine the effectiveness of the United States-Mexico-Canada Agreement (USMCA) in increasing dairy foods trade between the US and Canada. Two separate analyses were conducted using annual trade data from the Canadian Dairy Information Center to examine trade in 2020 and 2021. Four dairy foods were analyzed: fluid milk (FM), butter, skim milk powder (SMP), and whey protein concentrate (WPC). All data were analyzed on a metric-ton (MT) basis. Using historical trade values ($n = 30$), (1) an ARIMA model was used to forecast predicted dairy foods trade between the 2 countries and (2) an equilibrium displacement model (EDM) was used to simulate the enactment of the USMCA on trade values. Forecasted and estimated values were then compared with actual trade data to determine the effectiveness of the USMCA. The SMP forecast increased significantly ($P < 0.05$) over historical mean averages (114.8 + 984.0) for 2020 and 2021. Regardless, the forecasted values indicated that US exports to Canada in 2020 should have increased by 39,247.4; 3,9191.4; and 41,500.5 MT for FM, SMP, and WPC, whereas butter decreased by 918.3 MT. In 2021, FM, SMP, and WPC increased by 40,576.8; 4,049.7; and 42,808.7, respectively. Butter decreased by 10,270.2 MT. The estimated values from the EDM indicated that 2020 exports from the US increased by 0.17%, 0.60%, 29.0%, and −36.4% for FM, butter, SMP, and WPC, respectively. In 2021 exports increased by 0.02%, 0.07%, 3.95%, and −4.84% for FM, butter, SMP, and WPC, respectively. When results from the quantitative analysis were compared with actual trade values, FM exports increased by 34.4% and 53.7% in 2020 and 2021, butter increased by 3.1% and 59.6%, SMP increased by 25.6% and 18.1%, and WPC decreased by 2.6% and 2.5%. These results provide valuable policy insights on the effectiveness of current and proposed dairy foods trade agreements with the US.

Key Words: dairy trade, dairy market, dairy solids demand

Forages and Pastures 3

2366 Effects of preservative type and dose on the ruminal in vitro gas production kinetics of high-moisture alfalfa hay. J. Poblete*[1], B. Escudero-Alejos[1], M. Chusho-Guevara[1], D. Zamudio-Ayala[1], A. P. Jimenez[1], K. Nishimwe[1], A. S. Mindiola[1], M. V. Cardoso[1], C. Knight[2], S. Annis[3], J. Garzon[2], and J. J. Romero[1], *[1]Animal and Veterinary Science, University of Maine, Orono ME, [2]University of Maine Cooperative Extension, Orono ME, [3]School of Biology and Ecology, Orono ME.*

This study aimed to determine the effects of preservative type (TY) and dose (DO) on the in vitro gas production kinetics of alfalfa hay baled at high moisture (29.18%). The TY were (1) propionic acid (61% vol/vol; PRP); (2) PRP buffered with 5% vol/vol ammonium hydroxide (AMP); and (3) a commercial mixture of organic acids (incl. propionic and acetic acids) buffered with ammonium hydroxide. These were applied at 0%, 0.25%, and 0.5% (wt/wt, fresh basis) on a volatile organic acid equivalent basis (i.e., propionic plus acetic acids). The alfalfa hay was packed into mini bales (~500 fresh grams; ~239 kg of DM/m^3) using hydraulic presses and stored for 78 d. The ruminal in vitro gas kinetics were recorded for 48 h using the Ankom RF gas production system. Data were analyzed as a randomized complete block design (5 blocks) with a 3 TY × 3 DO factorial arrangement using the PROC GLIMMIX of SAS v.9.4 software. The areas within the alfalfa field served as the blocking factor. Differences were declared at $P < 0.05$. The TY did not affect the asymptotic maximum (M), rate (K), and lag time (L) of gas production of the alfalfa hay. For all TY, a quadratic decrease in the M was observed as DO increases (x = 181.34, 173.90, and 174.65 ± 2.19 mL/incubated DM g for 0%, 0.25%, and 0.5%, respectively). For all TY, a similar quadratic decrease was observed in K as DO increases (x = 7.75%, 7.06%, and 7.12% ± 0.21%/h, respectively). No difference was observed in the L as DO increases (x = 3.83 ± 0.34 h). The TY and increasing DO did not affect the ruminal pH (x = 6.90 ± 0.03) and in vitro OM digestibility (x = 45.95% ± 1.25%). For all TY, increasing DO from 0 to 0.5% improved the in vitro DM digestibility (x = 37.97% to 41.27% ± 1.29%, respectively). In conclusion, TY did not affect in vitro gas production kinetics, but increasing DO decreased M and K while increasing the in vitro DM digestibility of alfalfa hay baled at 29.18% moisture.

Key Words: spoilage, in vitro fermentation, buffered organic acid

2367 Effect of filter bag on dry matter and starch recovery of pure corn starch and dry ground corn. C. Heinzen Jr.* and L. F. Ferraretto, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The objective of this study was to evaluate the effect of filter bags commonly used in starch digestibility assays on DM and starch recovery of pure corn starch and finely-ground corn following a 0 h incubation in rumen fluid. Duplicate samples of pure corn starch and dry ground corn were placed in Dacron polyester cloth bags (DPB; R1020, 10 cm × 20 cm, 50-µm porosity; Ankom Technology) and F57 bags (F57; 25 µm porosity; Ankom Technology). Approximately 5 and 1 g of DM was used for DPB and F57, respectively. Rumen fluid from 2 ruminally-cannulated lactating Holstein cows was collected and filtered. Samples were incubated in vials containing rumen fluid for 0 h. After washout, sample bags were placed in icy water and rinsed. Four independent runs were conducted. Original samples and incubation residues were analyzed for DM and starch concentration to calculate DM and starch recovery. Data were analyzed as a completely randomized design in a 2 × 2 factorial arrangement of treatments using PROC GLIMMIX of SAS with the Random effect of run and Fixed effects of starch source, filter bags, and their interaction. Interactions between filter bag and starch source were detected for DM recovery ($P < 0.001$) and starch recovery ($P < 0.001$). Pure starch incubated in DPB bags had the lowest DM recovery (43% of as fed), followed by dry ground corn incubated in either in DPB or F57 (85.2% of as fed on average), whereas the greatest DM recovery was observed for pure starch incubated in F57 bags (93.3% of as fed). Similarly, pure starch incubated in DPB had 59.1% of starch recovery, followed by dry ground corn in either DPB or F57 (93.6% of incubated starch, on average). Pure starch incubated in F57 bags had no loss of starch (100.0% of incubated starch). In conclusion, greater particulate loss was observed in filter bags traditionally used for ruminal in situ disappearance assays. Our results reinforce the need for standardization of bags' pore size for digestibility assays. Research is warranted to understand if particulate losses of starch using bags of smaller pore sizes impair the calculation of fraction A of starch.

Key Words: starch, starch disappearance, corn

2368 Characterization of the nutritional quality of corn silage from the cool humid region of southern Chile using the Cornell Net Carbohydrate and Protein System model. P. Melendez[1], I. Castro[2], P. Pinedo*[3], and A. Adesogan[4], *[1]City University of Hong Kong, Kowloon, Hong Kong, SAR, [2]Cooprinsem, Osorno, Chile, [3]Department of Animal Sciences, Colorado State University, Fort Collins, CO, [4]Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective was to characterize the quality of dent corn silages (CS) produced on southern Chile (cool humid region) using a data set from a commercial laboratory. Corn silage samples submitted for routine nutritional analysis (n = 690) from 68 dairies in 2021 were analyzed by NIRS. Regression models between nutritional variables to determine potential associations were carried out. Data set was arranged by DM content and divided in half using the median value of the distribution (low and high DM). Then, nutritional values for each subsample were input in a ration formulation software that use the CNCPS model. There was a positive curvilinear association between DM and starch, but no association with its digestibility. There was a moderate positive curvilinear association between DM and ND; but no association with NDF digestibility at 240 h. There was a linear negative association between DM and VFA concentration in silage; but a slight positive association with pH. Maximum milk yields were obtained with DM between 32.5% and 37%. Crude protein, ADF, aNDFom, lignin, soluble fiber and soluble carbohydrates were statistically higher in low DM CS than high DM CS ($P \leq 0.05$); however, starch was statistically greater in high DM CS than low DM CS ($P \leq 0.05$). Digestibility of NDF was similar between silages. Milk yield responses predicted by the CNCPS model in the ration formulation software were higher in high DM CS than low DM CS. Methane production (L/d) was lower for high DM CS than low DM CS. Metabolizable protein produced from rumen bacteria was higher in high DM CS than low DM CS and the opposite occurred with the nitrogen excess produced by the cow. In conclusion, the nutritional composition of CS produced in the cold and humid weather of southern Chile is within the normal values reported by the scientific literature. Data suggest that corn for silage should be harvested with DM between 32 and 38% to maximize starch content and milk yield, with no losses in digestibility.

This led to a predicted greater production of rumen microbial protein, milk yield and lower methane.

Key Words: corn silage, Cornell Net Carbohydrate and Protein System (CNCPS), milk yield

2369 Survey on the occurrence of road hay fires in the United States and their economic impact.

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This study evaluated the occurrence of road hay fires in the US and their economic impact (EI). A search was conducted on Google News, Global Newsstream, and TikTok. A month-by-month search was done on the first, and a search grouped by relevance was done on the second and latter. The key words were "hay fire." Only results reporting a fire involving hay loaded on a vehicle were considered, and results describing the same fire were grouped as one. From the sources listed, we identified 127 reports from 2002 to 2023. After removing reports that did not include information on the status of the vehicle and trailer after the fire, 73 reports were selected for further analysis. Our EI assessment was limited to the costs of firefighter response and the loss of vehicles and trailers, adjusted for inflation (January 2024). Nonparametric tests were used because the data were not normally distributed. Kruskal-Wallis was used for between-variable tests, followed by the Dunn test with Bonferroni adjustment when a difference was found. Chi-squared goodness of fit was used for frequency tests. R 4.3.2 was used to run the tests. Differences were declared at $P \leq 0.05$. It was found that highways have more hay fires (48.5%) compared with interstates (20.6%) but were not different when compared with all other roads grouped (30.9%). However, the EI did not differ by road type (x-barx = \$81,527, SD = 67,665). The EI was more significant when involving a dry freight trailer than a flatbed and gooseneck trailers (\$185,465 vs. x = \$51,680, respectively). Also, the EI was higher when semi-trucks were involved than pickup trucks and trucks (\$123,360.46 vs. x = \$50,638, respectively). When the fires were grouped by climate region according to NOAA, the North-East (21.6%), North-West (18.9%), and South (13.5%) regions were the most affected compared with other regions in the US (x-barx = 6.8%). To conclude, the economic impact of hay fires on roads varies with the type of vehicle and trailer. Also, the most affected regions are the Northeast, North-West, and South compared with other regions in the US, and the roads most involved in hay fires are highways.

Key Words: hay fire, economic impact, road

2370 The effects of homolactic silage inoculant on bacterial community of tropical grass silage.

S. Farooq*[1], K. Arriola[1], F. Amaro[1], P. Eckert[1], S. Anwar[2], C. A. Niño de Guzmán[2], L. Lima[1], B. T. Po[1], K. C. Jeong[1], D. Vyas[1], and A. Adesogan[1], [1]*University Of Florida, Gainesville, FL,* [2]*University of Veterinary and Animal Sciences.*

The objective was to evaluate the effects of homolactic microbial inoculant on bacterial community in bahiagrass (BAH) and bermudagrass silages (BERM). Both grasses were established in 5 individual rows and each row was considered block. Randomly applied treatments were (1) control (CON): BAH and BERM ensiled without inoculant; (2) inoculant (INOC: *Pediococcus pentosaceus*, *Lactiplantibacillus plantarum* + enzymes): BAH (29.9% DM at harvest) and BERM (40.0% DM at harvest) ensiled with recommended doses of inoculant (2 g/ton fresh forage i.e., 10e5 cfu/g). Forages were ensiled for 1, 3, 7, 60, and 90 d in a 20-L plastic bucket silo (4 replications for each ensiling duration) and stored at room temperature (25°C). Alpha diversity (Chao 1 index), β-diversity (weighted UniFrac distance), and relative abundance (RA) of bacteria at different taxonomical levels (from phylum to genus level) were analyzed on d 60 and 90 of silage samples from both grasses. Data were analyzed using the GLIMMIX procedure of SAS and model included fixed effect of treatment, ensiling duration, and their interactions. Significance was declared when $P < 0.05$ whereas tendency was declared when $0.10 > P > 0.05$. Microbial inoculation increased species richness (Chao1) for BAH and BERM, regardless of the ensiling duration ($P < 0.01$). Similarly, β-diversity, a measure of similarity or distance between 2 communities, confirmed the separation in bacterial communities of CON and INOC samples ($P < 0.01$). The majority of sequences obtained with microbiome analysis in BERM and BAH belonged to *Proteobacteria*, *Firmicutes*, and *Bacteroidetes*, regardless of the ensiling duration. The RA of *Protobacteria* decreased whereas Firmicutes RA increased in both BERM and BAH with INOC after 60 and 90 d of ensiling. Microbial inoculation increased the RA of *Pediococcus* and *Lactobacillus* whereas decreased the RA of *Enterobacter* for both BERM and BAH after 60 and 90 d of ensiling. In conclusion, homofermentative inoculant modulated the bacterial community as indicated by β-diversity analysis, and increased species richness for both grass silages.

Key Words: bahiagrass, bermudagrass, homolactic inoculant

2371 The effects of homolactic silage inoculant on fermentation profile, and dry matter recovery of tropical grass silage.

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The objective was to evaluate the effects of homolactic microbial inoculant on pH, fermentative parameters and DM loss in bahiagrass (BAH) and bermudagrass silages (BERM). Both grasses were established in 5 individual rows and each row was considered block. Following treatments were randomly allotted (1) control: BAH and BERM ensiled without inoculant; (2) inoculant (INOC: *Pediococcus pentosaceus*, *Lactiplantibacillus plantarum* BAH and BERM ensiled with recommended doses of inoculant (2 g/t fresh forage, i.e., 10e5 cfu/g). Forages were ensiled for 1, 3, 7, 60, and 90 d in a 20-L plastic bucket silo and stored in dark at room temperature (25°C). A total number of 48 buckets (with 2 grasses × 4 reps × 6 d) per treatment were ensiled. Samples of freshly treated forage were stored at −20°C for chemical analyses. Data were analyzed using the GLIMMIX procedure of SAS and model included fixed effect of treatment, ensiling duration and their interactions. Significance was declared when $P < 0.05$. Microbial inoculation decreased silage pH for BAH (5.72 vs. 5.14; $P < 0.01$) and BERM (5.67 vs. 5.23; $P < 0.01$) and effects were more pronounced for BERM after 7-d ensiling (Treatment × Day; $P < 0.01$). Lactic acid was increased whereas butyric acid was decreased with INOC for both grasses ($P < 0.01$). Ammonia-N concentrations were lower with INOC for BAH and BERM regardless of ensiling duration; however, the concentration was lower at 60-d compared with 90-d for BERM. Microbial inoculation decreased DM loss in BAH ($P = 0.05$) whereas no effects were observed in BERM ($P = 0.63$). Microbial inoculation decreased mold counts ($P < 0.01$) whereas increased lactic acid bacteria counts ($P = 0.01$) for BERM. Yeast counts were lower for INOC with BERM ($P < 0.01$); however, no effects were observed in BAH with INOC ($P = 0.77$) where yeasts decreased between 60 and 90 d. In conclusion, INOC improved the fermentative quality grass silages by reducing pH levels, enhancing lactic acid concentration, and decreasing undesirable microbial counts; however, DM loss was only decreased for BAH suggesting a forage-specific response to inoculation.

Key Words: bahiagrass, bermudagrass, homolactic inoculant

2372 Inoculation with a mixture of *Lentilactobacillus hilgardii*, *Lentilactobacillus buchneri*, and *Pediococcus pentosaceus* improves the starch digestibility of corn silage.

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Improving the starch digestibility (starchD) of silage enhances its energy and nutritional values. Two trials were conducted to evaluate the capacity of an inoculant to improve the starchD of corn ensiled in 7.5 L buckets. Corn silage was harvested at 35.4% DM in trial 1 and at a low (26.2%) or high (39.7%) DM in trial 2. Silage was either not treated (CTR) or treated with an inoculant (INO) containing *Lentilactobacillus hilgardii* CNCM-I-4785 and *Lentilactobacillus buchneri* NCIMB 40788 at 150,000 cfu/g of fresh forage each, *Pediococcus pentosaceus* NCIMB 12455 at 100,000 cfu/g of fresh forage, β-glucanase, and xylanase in replicates of 5. After 90 d of ensiling in trial 1, and 30, 60, 120, and 180 d in trial 2, silages were analyzed for the concentrations of starch, starchD at 7 h, and ammonia-N, with trial 2 silages additionally analyzed for soluble protein and prolamin. Trial 1 data were analyzed as a completely randomized design using Kruskal-Wallis test, and trial 2 data were analyzed as a 2 × 2 factorial design using ANOVA. In trial 1 INO did not affect the starch and ammonia-N concentrations, but it increased the starchD compared with CTR by 21.04% (56.72 vs. 46.86%, $P = 0.028$). In trial 2, the starch concentration was higher in INO than in CTR at 60 ($P = 0.038$) and 180 d ($P < 0.001$). Inoculated silage had a higher starchD at 120 (81.8 vs. 77.1% of DM, $P < 0.001$) and 180 d (80.1 vs. 77.5% of DM, $P = 0.021$) than CTR, whatever the DM content. Compared with CTR, INO tended to have more NH_3-N at 180 d ($P = 0.052$) and had more soluble protein at 120 ($P = 0.016$) and 180 d ($P < 0.050$). In addition, INO had less prolamin than CTR at 60 ($P = 0.003$) and 180 d ($P < 0.001$) but had more prolamin at 120 d ($P < 0.001$). The inoculant improved the starchD of corn silage. This finding was corroborated by the indications that inoculation increased the degradation of the protein-starch matrix (e.g., more ammonia-N and soluble protein and less prolamin in INO than CTR), which could have facilitated the access of microorganisms and enzymes to the starch granules.

Key Words: Lentilactobacillus hilgardii, Lentilactobacillus buchneri, starch digestibility

2373 Effects of silage inoculants on fermentation profile and *in situ* rumen degradability of elephant grass silage.

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The objective of this study was to assess the effect of 2 commercially silage inoculants, applied at 2 doses, as well as the inclusion of ground corn grain (GCG) in low DM (18%) elephant grass cultivar BRS Capiaçu on silage fermentative characteristics, and in situ ruminal degradability. The experimental design was completely randomized, employing a 6 × 3 factorial scheme (6 inoculants × 3 fermentation periods: 30, 60, and 90 d with 3 replications). The treatments were: (1) no inoculant control, (2) 0.5 g/t of a commercial product composed of *Lentilactobacillus buchneri* (LBU), with minimum guarantee of 1.0 × 10^11 cfu/g, at a final inoculation rate of 5.0 × 10^4 cfu/g of forage (LBU0.5); (3) 1 g/t of LBU at a final inoculation rate of 1.0 × 10^5 cfu/g of forage (LBU1); (4) 1 g/t of a commercial product composed of *Lactiplantibacillus plantarum* (LPP) with minimum guarantee of 5.0×10^{10} cfu/g and *Pediococcus acidilactici*, with minimum guarantee of 1.5×10^{10} cfu/g, reaching a final inoculation rate of 6.5×10^4 cfu/g of forage (LPP1); (5) 2 g/t of LPP at a final inoculation rate of 1.3×10^5 cfu/g of forage (LPP2); and (6) 8% of flint GCG with 88% vitreousness ground with a Wiley mill (3-mm screen). The silage was maintained in experimental silos consisting of plastic buckets. At 60 d of fermentation, the silage was subjected to in situ rumen degradability procedures using 3 rumen fistulated lactating Holstein cows, with the following incubation times: 0, 6, 12, 24, 48, 72, 120, and 240 h. Means were separated by Tukey's test, and differences were declared at $P < 0.05$. Regarding the fermentation profile, GCG had the lowest pH, butyric acid, ammoniacal nitrogen, and the greatest lactic acid concentration. Total DM losses did not vary among treatments. The effective degradability of NDF and its fractions "C" and uNDF240 were not influenced by inoculants. In summary, the inclusion of 8% GCG in the silage resulted in an ideal fermentative profile, and it was observed that bacterial strain inoculants did not contribute to the improvement of grass silage quality, particularly when dealing with low DM grass silage.

Key Words: inoculant, fermentation, grass silage

Lactation Biology 1

2374 Relationships between mammary composition and colostrum weight and quality. A. L. Vang*, E. Casella, G. L. Menezes, W. S. Frizzarini, H. H. Webster, T. Cunha, G. J. M. Rosa, J. R. R. Dorea, and L. L. Hernandez, *University of Wisconsin–Madison, Madison, WI.*

Calves are born with no immunity against disease relying on passive immunity acquired through colostrum. The focus of this research was to identify relationships between histological measurements and colostrum production in heifers. Mammary biopsies were performed on 24 heifers at 52 wk of age and QuPath was used to annotate and extract features to quantify and create morphological measurements of tissue microstructure. Colostrum was collected shortly after parturition and the weight as well as brix were measured for each sample. A linear fixed model was used to analyze brix including calving weight, colostrum weight, proportion of ducts, proportion of adipose tissue, birthweight, dietary treatment, and sex of the calf. A linear model was also used to analyze colostrum weight including calving weight as a covariate, proportion of ducts, proportion of adipose tissue, birth weight, dietary treatment, and sex of the calf. Brix value is positively associated with the proportion of ducts and tends to be positively associated with proportion of adipose tissue in the histological images ($P = 0.032$; $P = 0.054$ respectively). Colostrum weight was also positively associated with the proportion of ducts ($P = 0.049$). These results suggest that parenchymal microstructure before gestation and parturition may be indicative of colostrum weight and quality. Increased brix value and colostrum weight may be due to increased total tissue reorganization and differentiation overall in animals with increased proportions of ductal structures, as discontinuity of the mammary epithelium during secretory differentiation allows paracellular passage of molecules between the blood and milk. As ultrasound imaging has been shown to accurately quantify secretory tissue, further development of ultrasound imaging as a quantification tool could prove useful in identification of animals producing high quality colostrum, contributing to overall health and development of calves.

Key Words: colostrum, histology, heifer

2375 Mineral composition of whole milk from mammary glands repeatedly challenged with killed *Staphylococcus aureus*. K. M. Enger, S. Park, A. E. Relling, and B. D. Enger*, *The Ohio State University, Wooster, OH.*

Milk quality is negatively affected during mastitis by changes in milk ion concentrations that coincide with reductions in milk lactose content. The aim of this study was to assess what minerals are affected during acute raises in somatic cell count during a sterile mastitis challenge. Four mid-lactation primiparous Holstein cows were used. Quarters of a randomly selected udder half of each cow were infused with saline (SAL) while the quarters of the opposite udder half were infused with 2 billion cfu of formalin-fixed *Staphylococcus aureus* (FX-STAPH). Intramammary infusions were repeated every 3 d over 12 d. Milk samples were collected at each milking (2×/d). Whole milk mineral composition was determined by inductively coupled plasma spectroscopy. Data were analyzed in SAS using PROC MIXED. Fixed effects included udder half treatment, time, and their interaction; cow was specified as a random effect. The interaction between treatment and cow was included as a repeated measure. Udder half milk somatic cell scores and milk yields were similar pre-challenge ($P > 0.25$). FX-STAPH udder half somatic cell scores increased and were greater than SAL udder halves post-challenge (6.9 vs. 1.9 ± 0.5; $P < 0.01$), while milk yield of FX-STAPH udder halves became lower than SAL udder halves (8.5 vs. 9.0 ± 0.5 kg; $P < 0.01$) indicating mastitis was induced. Whole milk concentrations of Cu, Fe, P, Se, and Zn did not differ between FX-STAPH and SAL udder halves ($P \geq 0.25$). FX-STAPH halves had greater mean concentrations of Ca ($P = 0.02$), Na ($P < 0.01$), Mg ($P < 0.01$), Mo ($P < 0.01$), and S ($P < 0.001$) than SAL udder halves, but K and Mn concentrations were greater in SAL udder halves ($P < 0.01$). Results indicate certain minerals are differently affected during leukocyte infiltration into the mammary gland. That only some ions were increased during leukocyte infiltration indicates that either transporter mechanisms may exist that help retain certain minerals or that the degree of mastitis and damage to the epithelial barrier influences how much ion leakage can occur from the interstitial fluid and blood.

Key Words: mastitis, disease, somatic cell

2376 Amino acids and hormones transactivate casein α in bovine mammary cells. H. Ford*[1], P. Piantoni[2], G. Schroeder[2], and M. Bionaz[3], [1]*Texas Tech University, Lubbock, TX,* [2]*Cargill Animal Nutrition and Health, Innovation Center, Elk River, MN,* [3]*Oregon State University, Corvallis, OR.*

Among hormones, it has been indicated that insulin and prolactin can regulate casein expression. Recent evidence has also revealed the role of amino acids in controlling the expression of casein genes. Thus, AA can serve not only as building blocks of milk protein, but also have nutrigenomic properties with the potential to improve milk protein yield. The objective of this study was to assess the transactivation of casein by hormones and AA in immortalized bovine mammary cells. For this purpose, we designed a plasmid containing a luciferase driven by the promoter of the casein αS1. Using reverse transfection, the casein plasmid was co-transfected in MACT cells with a renilla plasmid, using Lipofectamine 3000 (~30% transfection efficiency), and cells were cultivated in 96 well plates for 24 h, followed by luciferase treatment. Statistical analysis was performed using the GLIMMIX procedure of SAS with the fixed effects of treatment (and dose) and the random effect of replicate ($n = 4$). Initial experiments demonstrated that the sensitivity of the system to detect an effect of AA on casein transactivation was highest when cells were cultivated in media without AA plus 10% dialyzed FBS compared with cells cultivated with AA or 100% bovine serum. A series of tests were performed to assess the response of the casein luciferase plasmid to AA (at 100–1,000 u*M* range of doses) or hormones. Among tested hormones, insulin (5 ug/mL) and hydrocortisone (10 ug/mL) increased approx. 2-fold the transactivation of casein, whereas prolactin (5 ug/mL) did not. This finding underlines the importance of energy level in the transcription of casein genes. Among all 20 amino acids tested, histidine and methionine significantly increased ≥2-fold the transactivation of casein with doses ≥100 u*M*. Leucine increased casein α transactivation >2-fold but at dose >500 u*M*. Overall, results revealed that insulin and hydrocortisone can play a role in the transcription of casein α. More importantly, our data confirmed that some AA, particularly histidine, methionine, and leucine, can have a nutrigenomic effect on bovine mammary cells to improve protein yield.

Key Words: casein, hormone, nutrigenomic

2377 Phenotyping mammary gland of dairy cows through histological image analysis. E. Casella*, A. Vang, G. L. Menezes, G. J.

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Mammary gland growth is an important phenotype due to its correlation with future lactation performance. Currently, biopsies represent the standard procedure to procure histological data to assess mammary gland development. However, analyzing individual microscopic images is a labor-intensive and time-consuming task that can only provide a few visual and manual measurements, limiting the ability to extract meaningful or novel phenotypes. Computer vision has been a powerful tool in medical sciences for real-time identification and segmentation of biological tissues, as well as quantitative analysis on tissue size, and geometric features. Therefore, this study aims to implement a computer vision system for the multilabel semantic segmentation of microscopic images from biopsies of the mammary gland using a U-net model. The segmentation model was trained on over 80,000 samples, as a result of patching 80 high-resolution histological tissue biopsies from 20 dairy calves at 10, 26, 39, and 52 wk of age, and tested on 56 images (56,000 patches from 14 animals). Tissue growth was analyzed with a linear mixed model including diet, week, and their interaction as fixed effects, birthweight as a covariate, and animal as a random effect. The segmentation model achieved an intersection over union of 0.88. The correlations between predicted and observed adipose, stroma, and duct area in the testing set were 0.99, 0.99, and 0.97, respectively. There was a significant effect ($P < 0.03$) of week on adipose and stroma area (% of total issue), ducts and adipose circularity, and ducts and stroma eccentricity. The adipose tissue area (%) increased 5 times from wk 10 to 26 and 1.34 times from wk 26 to 52. This suggests a nonlinear and decelerating growth development of the mammary gland during the period evaluated. Although the association between such rate of growth and lactation performance should be investigated, the use of image-based features can allow for large-scale phenotyping and consequently the study of such biological interactions.

Key Words: mammary gland development, computer vision, histological image analysis

2378 Effect of training milking routine on gene expression related to milk ejection in primiparous Gyr cows.

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Training dairy heifers for routine milking can enhance their behavior during their first lactation. This study aimed to assess the effect of gestation training on the first milking of primiparous Gyr cows and its effect on the expression of genes related to milk ejection. Forty Gyr heifers were subjected to treatments: routine milking training sessions (TR, n = 20), or control receiving no specific training (CT, n = 20). Milk yield, milk quality, residual milk and cow behavior were evaluated at d 0, 15, 30, 45, and 60 of lactation, while gene expression on mammary cells at d 15 lactation. Cow temperament were taken individually by same observer, considering the head and legs movements, kicks, vocalization and rumination during all milking procedures, and each cow was categorized into 5 levels: 1) very calm, 2) calm, 3) adequate, 4) restless, and 5) very restless. Oxytocin (OT) and cortisol (CORT) concentrations in milk were measured by immunoassay analysis. Gene expression of oxytocin and glucocorticoid receptors (*OTR* and *GR*) was analyzed by quantitative PCR on mammary cells (2×10^4 cells/mL) obtained from milk of the morning milking. Data were analyzed using the GLM or MIXED procedure, the treatment effect was considered fixed, and day of lactation and animals were considered random effects. Statistical significance was defined as $P \leq 0.05$. During first milking, CT cows exhibited a higher percentage of restlessness compared with TR cows (20% versus 0%, $P \leq 0.05$). However, TR did not significantly affect subsequent milk yield or quality. The TR cows showed significantly higher OT, lower CORT and residual milk, compared with CT cows ($P \leq 0.05$), suggesting that TR improved the milk ejection during the first milking. Thus, TR led to the upregulation of *OTR* expression and the downregulation of *GR* expression ($P \leq 0.05$), suggesting that TR changed positively the responsiveness of oxytocin and cortisol in mammary gland. These findings suggest that training heifers for routine milking improves milking temperament, reduces residual milk, and affects the expression of OTR and GR genes in Gyr cows.

Key Words: milking, behavior, mammary cell.

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2379 Aromatic amino acid supplementation reduced triglyceride concentration and altered proteome in primary bovine hepatocytes. J. R. Daddam*, C. Collings, M. Sura, and Z. Zhou, *Animal Sciences, Michigan State University, East Lansing, MI.*

During the peripartal period, accumulation of triglycerides (TG) in the liver is associated with decreased liver function and lactation performance. In rat, aromatic AA (AAA) supplementation reduced TG accumulation in the liver but the extent to which AAA supplementation affect hepatic TG concentration in dairy cows remians unknown. Objectives were to determine the effect of AAA supplementation on hepatic TG concentration in primary bovine hepatocytes (PLEH) and the underlying mechanism using proteomics. The PLEH were cultured in customized media mimicking cow plasma AA and fatty acid (FA) profile on d 4 postpartum with (AAA, 150% of physiological circulating AAA concentration) or without AAA (CON). After 72 h, PLEH were harvested for intracellular TG quantification with flow cytometry and iTRAQ-based proteomics with multi-dimensional LC-MS. Treatment effects were determined using PROC MIXED in SAS. Hepatocytes receiving AAA treatment had lower intracellular TG compared with CON ($P = 0.01$; MFI = 6,503 vs. 7,774, SEM 177). Proteomic analysis identified and quantified a total of 963 proteins. Compared with CON, 141 proteins were differentially abundant ($P \leq 0.05$ and fold change > ± 1.5). Pathway analysis revealed that AAA supplementation enriched >20 pathways (FDR <0.05), including various pathways regulating AA metabolism (Biosynthesis of amino acids, Arginine and proline metabolism, Tryptophan metabolism, Branched-chain amino acid degradation, and Lysine degradation), Glycolysis/gluconeogenesis, Pyruvate metabolism, Fatty acid degradation, and, Citrate cycle (TCA cycle). Overall, these results suggest that AAA supplementation affect various aspects of hepatic amino acid, fatty acid, and carbohydrate metabolism, which likely contribute to the observed decrease in intracellular TG concentration. Future work should determine the specific changes in key genes/proteins in these pathways.

Key Words: aromatic AA, triglyceride, primary bovine hepatocyte

2380 Effects of level and duration of acidogenic supplementation in the prepartum diet on the metabolism and health of dairy cows. J. T. R. Carvalho[1], J. H. Carneiro[1], J. C. S. Lourenço[1], L. S. Nogueira[1], R. Zimpel[2], V. B. Carvalho[3], A. Vieira-Neto[4], and R. Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Zoetis, São Paulo, SP, Brazil,* [3]*Phibro Animal Health Corporation, Campinas, SP, Brazil,* [4]*Merck Animal Health, Colorado Springs, CO.*

The objectives were to evaluate the effects of feeding diets with 2 levels of negative dietary cation-anion differences (DCAD) during the last 4 or 3 wk of gestation on the metabolism and health of dairy cows. The experiment had a 2 × 2 factorial arrangement design, with 2 levels of DCAD: −50 or −150 mEq/kg DM, and 2 durations: 21 d (3W) or 28 d (4W) of supplementation during the prepartum period. One hundred seventy-seven Holstein cows from a commercial herd were blocked by parity and expected calving date, and randomly assigned to 1 of 4 treatments: 3W-50 (n = 42), 3W-150 (n = 43), 4W-50 (n = 46), and 4W-150 (n = 46). Blood metabolites data were analyzed using the MIXED procedure of SAS, containing the fixed effects of level, duration, parity, time, and their interactions, and the random effects of block and cow. Disease incidence variables were analyzed using the GLIMMIX procedure, including the fixed effects of duration, level, and their interaction and the random effect of block. Urine pH was lower at −150 level (5.83 vs. 6.96 ± 0.07; $P < 0.01$) and, as expected, acidogenic supplementation duration was less at 3W (25.5 vs. 31.5 ± 0.6 d; $P < 0.01$). The GGT enzyme was lower ($P < 0.01$) for the 3W than 4W duration (27.4 vs. 29.4 ± 0.6 U/L). Reducing the DCAD to −150 level increased ($P = 0.01$) TG (8.0 vs. 8.5 ± 0.1 mg/dL) and decreased ($P = 0.02$) albumin (3.29 vs. 3.19 ± 0.03 g/dL) compared with −50 level. Haptoglobin concentration tended to be lower ($P = 0.07$) at −50 (4.67 vs. 5.45 ± 0.29 mg/dL) than −150 level. Bilirubin, glucose, and NEFA concentrations did not differ ($P > 0.05$) among treatments. Reducing the DCAD to −150 level tended to decrease the incidence of retained placenta (19.9% vs. 10.8%; $P = 0.10$) and subclinical hypocalcemia (83.6% vs. 72.6%; $P = 0.09$) than the −50 level. The treatments did not influence ($P > 0.10$) the incidence of metritis, ketosis, mastitis, and displaced abomasum. Metabolism and health were modestly affected by manipulation of DCAD levels and durations, at least within the recommended range of 4 to 3 wk and −50 to −150 mEq/kg DM level of acidogenic supplementation in the prepartum diet.

Key Words: dietary cation-anion difference, haptoglobin, transition

2381 Relationship between behavior and metabolism in dairy cows during transition. J. A. Ferreyra*[1,2], J. M. Cantet[3,4], D. Sanchez-Otero[5], F. Villamil[6], P. Ricci[2,7], R. Vieyra-Alberto[5], S. Picco[2,8], D. Colombatto[2,9], and R. A. Palladino[1,2], [1]*FCA-UNLZ, Lomas de Zamora, Buenos Aires, Argentina,* [2]*CONICET, Ciudad de Buenos Aires, Argentina,* [3]*FB-UNER, Entre Rios, Argentina,* [4]*Smart Farming, Buenos Aires, Argentina,* [5]*AAMVZ-ICAp-UAEH, Hidalgo, Mexico,* [6]*UNNE, Corrientes, Argentina,* [7]*INTA Balcarce, Balcarce, Buenos Aires, Argentina,* [8]*FCV-UNLP, La Plata, Buenos Aires, Argentina,* [9]*FAUBA, Ciudad de Buenos Aires, Argentina.*

Transition is known to be the most challenged period in dairy cows' life. Cow behavior has been proven to be a good indicator of health. Our aim was to correlate rumination (RT), lying (LT) and consumption (CT) time against some metabolism indicators. Thirty-eight transition dairy cows (FIL, Argentina) were assigned to one of 4 treatments (high and low energy density at prepartum and sunflower seed or linseed pre and postpartum). Cows were equipped with an automated heat and health monitoring system (Datamars Livestock) which allows to record RT, LT, and CT. Blood samples were taken at 1 and 4 DIM. Partial correlation analysis was performed using PROC GLM of SAS, accounting for factors including in the original study. Significant correlation was declared when $P < 0.05$ and trends when $P < 0.10$ and > 0.05. Nonsignificant correlation was found between LT and variables of interest ($P > 0.05$). The CT was correlated with glutamic oxaloacetic transaminase (GOT; r = 0.44; $P = 0.0169$). The RT at 4 DIM (RT4) tended to negatively correlate to GOT (r = −0.37; $P = 0.0512$). Also, the difference between RT4 and 1 DIM (RT4–1) was negatively correlated with GOT (r = −0.43; $P = 0.0204$). The RT4–1 was positively correlated with milk yield (r = 0.41; $P = 0.0269$). In addition, RT4 tended to be negatively correlated with glutamic pyruvic transaminase (GPT; r = −0.37; $P = 0.0512$) and positively with cholesterol (r = 0.33; $P = 0.0785$). Interestingly, RT4 correlated positively to total Ca levels in blood at 4 DIM (r = 0.46; $P = 0.0127$) and tended to correlate with Ca difference between 4 and 1 DIM (r = 0.36; $P = 0.0546$), which means that RT4 may be a good indicator of Ca status at the beginning of lactation. Finally, we calculated the liver health index (LHI) but no correlation was found between LHI and hepatic enzymes (GOT, GPT; $P > 0.05$). However, LHI correlated positively to Ca at 4 DIM (r = 0.65; $P = 0.0001$) and with Ca difference between 4 and 1 DIM ($r = 0.42$; $P = 0.0221$). Glucose and Mg levels at 4 DIM also correlated positively to LHI ($r = 0.52$ and $r = 0.62$ respectively for glucose and Mg; $P < 0.05$). In conclusion, RT4 postpartum seems to be a good indicator of liver health in dairy cows but also Ca blood levels during transition.

Key Words: transition dairy cow, health index, behavior

2382 Oxytocin for colostrum harvest. G. Frederick*[1], S. Mann[1], R. Bruckmaier[2], M. Spellman[1], H. Somula[1], and M. Wieland[1], *[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, [2]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Switzerland.*

The objective was to determine the effect of oxytocin use during colostrum harvest on colostrum yield (CY) and IgG concentration in Holstein dairy cows on a commercial dairy in New York. Animals were enrolled between July and October 2023 using a randomized block design, with day of enrollment as the unit of randomization. A median (range) of 10 (3–19) cows were enrolled/d. Treatments were (1) 40 IU Oxytocin (OXY40), (2) 20 IU Oxytocin (OXY20), and (3) an untreated control group (CNTR). Oxytocin was administered intramuscularly (IM) approximately 45 s before unit attachment in a rotary parlor. Colostrum weight was measured using the colostrum bucket-embedded scale or a platform scale when the yield was less than the smallest bucket scale. The concentration of IgG ([IgG]) in colostrum was determined using radial immunodiffusion (RID). Data were analyzed separately for primiparous and multiparous cows using mixed effects ANOVA controlling for lag time from calving to harvest, enrollment block, and in multiparous cows for parity group, dry period length, calf sex, and milk production at wk 4. Cows in each parity group (1, 2, 3, ≥4) were distributed as 201 (31.6%), 148 (23.2%), 113 (17.8%), and 174 (27.4%) of the 636 enrolled. Primiparous cows were randomized to 35.8% ($n = 72$) OXY40, 32.8% ($n = 66$) OXY20, and 31.3% ($n = 63$) CNTR. Multiparous groups were randomized to 34.7% ($n = 151$) OXY40, 29.7% ($n = 129$) OXY20, and 35.6% ($n = 155$) CNTR. The median (range) CY was 6.0 (0 to 20.6) kg and [IgG] was 98.5 (0.1 to 293.6) g/L in the study population. In primiparous cows, OXY40 had a higher colostrum yield (LSM [95% CI]) of 5.4 (4.9 to 5.9) kg compared with both OXY20 (4.1 [3.5 to 4.7] kg) and CNTR (3.8 [3.3 to 4.3] kg) ($P < 0.001$). In multiparous cows, OXY40, OXY20, and CTNR did not differ in CY (5.9 [5.3 to 6.5], 5.7 [6.3 to 5.1], and 5.4 [6.0 to 4.8] kg, respectively, $P = 0.43$). Treatment did not affect IgG concentration in either primiparous or multiparous cows ($P > 0.56$). In summary, oxytocin use at 40 IU IM in primiparous was associated with a higher CY but not [IgG]. Oxytocin use likely addressed milk ejection disturbance and therefore increased CY in heifers milked for the first time in a rotary parlor.

Key Words: colostrum, oxytocin, IgG

2383 Association of milk de novo fatty acid proportion with productivity and plasma IGF-1 and GLP-1 concentrations in dairy cows at the first postpartum week. R. Fukumori*, J. Shoji, S. Gondaira, and S. Oikawa, *School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido, Japan.*

This study aimed to evaluate the relationship between milk de novo fatty acid (FA) proportions at the first week postpartum and pre- and postcalving blood metabolic markers, milk yield, health, and reproduction performance after calving. Sixty-five Holstein cows (25 primiparous and 40 multiparous) were used in this study and blood samples were collected 3 and 1 wk before the predicted calving date, on the day of calving, and wk 1 to 12 of lactation. Milk samples were also collected from each cow, and blood samples were collected after calving. Using the 25th and 75th percentiles of milk de novo FA proportion in the first week postpartum measured by Fourier-transform infrared, cows were classified into high de novo (HD, $n = 16$), medium de novo (MD, $n = 33$), and low de novo (LD, $n = 16$) in descending order. Milk de novo FA proportions were ≥28.5, 21.3 to 28.4, and ≤21.2 g/100 g FA for HD, MD, and LD, respectively. Statistical analysis was conducted using the fit model and χ square test of JMP. The dystocia score was higher in the LD group than that in the HD group; however, no group differences were observed in milk yield, prevalence of postpartum disease, and removal. For reproductive performance, days open were longer in the LD group than in the HD group in primiparous cows, and fewer cows were pregnant within 150 d in the LD group compared with those in the HD and MD groups. The LD cows showed higher serum concentrations of NEFA and BHBA and lower concentrations of glucose, Mg, and GLP-1 than the HD cows after calving. LD cows showed a decrease in plasma IGF-1 concentration from one week before the predicted calving and were lower than that of the HD cows throughout lactation. These results indicate that milk de novo FA at the first postpartum stage is a noninvasive tool that can be used as an indicator of the nutritional status of transitional dairy cows. Furthermore, IGF-1 may be an earlier predictor than NEFA or BHBA, and GLP-1 may be another useful and novel marker.

Key Words: IGF-1, GLP-1, milk fatty acid profile

2384 The effects of dietary rumen-protected choline on mammary DNA methylation patterns. A. Celemin Sarmiento*[1], B. J. Bradford[2], L. K. Mamedova[2], K. A. Estes[3], and T. H. Swartz[1,2], *[1]Department of Dairy and Food Science, South Dakota State University, Brookings, SD, [2]Department of Animal Science, Michigan State University, East Lansing, MI, [3]Balchem Corporation, Montvale, NJ.*

Choline is a methyl donor, which may influence DNA methylation, gene expression, and cellular processes. Past studies have found an increase in milk yield when periparturient dairy cows were supplemented with dietary rumen-protected choline (RPC); however, the mechanism behind this response is unknown. Therefore, the objective of this study was to assess the effects of dietary RPC supplementation on mammary epigenome-wide DNA methylation. Parous Holstein cows were blocked by calving month and then randomly assigned within block to receive either 30 g/d of RPC (13.6 g/d of choline ions; CHOL30, $n = 21$) or no RPC (CON, $n = 19$) as a top-dress, starting 24 d before expected calving until 21 d postpartum. Mammary tissue was collected at d 17 postpartum and DNA was isolated. A subset of samples ($n = 6$ from each group) were randomly selected and submitted for whole-genome bisulfite sequencing. Differentially methylated cytosines, regions, and genes (DMG) were determined using the “genomation” R package. The cut-off values were set at $q < 0.05$ and absolute methylation difference >10%. DMG-enriched pathways were analyzed using “ReactomePA” and “clusterProfiler” R packages. We found 29 and 34 differentially hyper- and hypomethylated genes, respectively. Reactome pathway enrichment analysis identified 29 and 10 hyper- and hypomethylated pathways, respectively. Hypermethylated Reactome pathways were associated with DNA replication and telomere maintenance/synthesis. Hypomethylated Reactome pathways were affiliated with inflammasomes, metabolic processes, antioxidant defenses, and DNA repair. KEGG pathway enrichment analysis identified 7 and 12 hyper- and hypomethylated pathways, respectively. Hypermethylated KEGG pathways were associated with DNA replication, immune function, fatty acid synthesis/elongation, and AA metabolism. Conversely, hypomethylated KEGG pathways were associated with energy metabolism. In conclusion, dietary RPC supplementation alters mammary DNA methylation patterns, potentially providing a mechanism behind the enhanced lactational performance commonly identified with this peripartum feeding strategy.

Key Words: DNA methylation, mammary gland, methyl donor

2385 Effect of controlled ionized calcium status during the first 24 hours postpartum on milk production and dry matter intake in multiparous Holstein cows.

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The objective was to assess the effects of ionized calcium (iCa) status in the first 24 h postpartum on milk production and DMI in the first 30 DIM. Multiparous Holstein cows (n = 48) were fed a negative DCAD diet (−56.2 mEq/kg) for 21 d before parturition. Urine pH (LSM ± SE) was monitored daily (6.2 ± 0.9). At calving, cows were randomly assigned to receive one of 3 treatments (tx) administered by an infusion pump during the first 24 h postpartum (1) saline (CON; n = 16), (2) 5% calcium gluconate to achieve normocalcemia (iCa 1.0–1.1 mmol/L, CALC; n = 16), or (3) 5% egtazic acid to induce subclinical hypocalcemia (iCa 0.8–0.9 mmol/L, EGTA; n = 16). Whole blood [iCa] was measured at 0, 6, 12, 18, and 24 h relative to infusion start using an iStat portable analyzer, and daily DMI (kg) and milk yield (kg/d) were recorded. Data were analyzed using PROC MIXED in SAS with the fixed effects of tx and parity, the repeated effect of time (h: iCa, infusion rate; d: milk yield, DMI), and the interaction of tx × time and tx × parity. Prepartum DMI was analyzed separately without the effect of tx. As designed, [iCa; mmol/L] was highest in CALC during the infusion, with intermediate [iCa] in CON and lesser [iCa] in EGTA ($P < 0.01$; LSM ± SE 1.17 ± 0.02, 1.08 ± 0.02, and 0.92 ± 0.02, respectively). The CON and EGTA had the greatest infusion rate (mL/h) whereas CALC had the lowest ($P < 0.01$; 185.4 ± 15.9, 167.6 ± 15.4, and 33.3 ± 16.2, respectively). Milk yield was not affected by tx ($P = 0.31$; 35.1 ± 1.1, 34.6 ± 1.1, and 36.8 ± 1.0 for CALC, CON, and EGTA, respectively). CON and CALC tended to eat the most compared with EGTA d 1 postpartum ($P = 0.07$; 12.5 ± 1.1, 11.4 ± 1.3, and 9.1 ± 1.3, respectively), and EGTA and CON tended to eat the most compared with CALC on d 21 postpartum ($P = 0.07$; 23.8 ± 1.1, 22.6 ± 1.1, 20.7 ± 1.1, respectively). In conclusion, tx did not affect milk yield in the first 30 DIM, but EGTA tended to reduce DMI at d 0 and increase DMI at d 21 postpartum, indicating that iCa status 24 h postpartum affects DMI of early lactation Holstein cows.

Key Words: transition period, milk production, calcium infusion

2386 Pre-dry-off milk yield and its association with future metabolism and productivity.

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Objectives were to evaluate the relationship between milk yield (MY) before dry-off (DO) and fecal pH, metabolism, and performance after DO and in the following transition period (TP). Seventy-one Holstein cows from d −12 to +14 relative to DO and −21 to +63 d relative to subsequent calving were enrolled in the study. Based on MY in the 7 d before DO, a subset of cows was retrospectively classified into 1 of 2 groups: (1) High MY (HMY; top 1/5 of cows; >28.0 kg/d; $\mu = 30.7 \pm 1.5$ kg/d; n = 15) or (2) Low MY (LMY; bottom one-fifth of cows; <15.5 kg/d; $\mu = 7.4 \pm 1.3$ kg/d; n = 15). Data were analyzed as repeated measures using the MIXED procedure of SAS with parity and DIM at DO accounted for. The HMY cows consumed more DMI (4.6 kg/d; $P < 0.01$) than LMY before and after DO. Before and following DO, HMY had decreased circulating glucose compared with LMY cows (6%; $P < 0.01$) and tended to have decreased NEFA after DO than LMY cows (21%; $P = 0.06$). Post DO vaginal temperature decreased (0.2°C) and fecal pH increased (0.6 units) similarly between treatments. HMY cows had increased DMI during the TP (3.3 kg/d; $P = 0.01$), but MY did not differ by treatment during the first 9 wk of lactation (47.1 vs. 43.6 kg/d for HMY and LMY, respectfully; $P = 0.27$). Fecal pH decreased (0.8 units) post-calving similarly between treatments. There was a post-calving treatment by time interaction ($P = 0.05$) on feed efficiency as it was higher in LMY during wk 1 to 2 than HMY (23%; $P < 0.01$); furthermore, there was a treatment by time interaction on BCS ($P < 0.03$) as LMY cows lost more condition as lactation progressed than HMY cows. The TP milk somatic cell count did not differ by treatments. Following calving, BHB and NEFA were decreased in HMY relative to LMY cows (33 and 55%, respectively; $P < 0.01$), but circulating glucose was similar among treatments. In summary, high producing cows at DO have increased DMI and reduced adipose tissue mobilization (NEFA and BCS loss) following the subsequent parturition. The idea that high MY before DO is detrimental to future production is not supported herein.

Key Words: periparturient, health, dry cow

2387 Net accretion of skeletal muscle occurs during late lactation in multiparous dairy cattle.

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The objective of this study was to measure *longissimus dorsi* muscle depth (LDD) from 0 to 300 DIM to evaluate changes across lactation. Forty multiparous cows were classified as high muscle (HM; LDD >5.0 cm; n = 18) or low muscle (LM; LDD ≤5.0 cm; n = 22) based on LDD measurements collected after parturition. Body weights and ultrasound scans to measure LDD and back fat depth (BFD) were collected monthly until 300 DIM. Ultrasound scans were analyzed using ImageJ software. Blood samples were taken at 7, 150, and 300 DIM and plasma was analyzed for nonesterified fatty acids (NEFA), creatinine, and 3-methylhistidine (3-MH). Milk yield was recorded daily using AfiMilk with milk components analyzed monthly. Data were analyzed using SAS; models included the fixed effect of muscle group, DIM, their interaction, and the random effect of cow with repeated measures using a first-order autoregressive covariance structure. Muscle group did not affect BW and BFD ($P > 0.05$). A DIM effect was observed for LDD, BFD, and BW ($P < 0.0001$). Cows lost BW from 0 to 60 DIM and gained weight from 60 to 300 DIM ($P < 0.0001$). BFD demonstrated similar effects with cows mobilizing fat from 0 to 90 DIM and gaining BFD after 90 DIM until 300 DIM ($P < 0.0001$). A muscle group by DIM interaction was observed for LDD ($P = 0.0003$). The HM cows had more muscle at 0 DIM, indicative of treatment assignment (1.34 cm more; $P < 0.0001$) and 300 DIM (0.78 cm more; $P = 0.01$) and tended to have more muscle at 60 DIM (0.66 cm; $P = 0.10$) compared with LM. No differences between groups were found for any other time points ($P > 0.05$). After 240 DIM, both muscle groups began net accretion of muscle reserves until 300 DIM. No differences were observed for blood metabolites measured based on muscle group; however, there were significant time effects for creatinine, 3-MH, and NEFA, which supported the observations from ultrasound scans. No muscle group effect was observed for milk yield measured as cumulative milk production ($P = 0.40$). Together data indicate muscle reserves are depleted in early lactation, and accreted in late lactation, whereas BW and BFD started to increase by 90 DIM.

Key Words: longissimus dorsi, tissue reserve, muscle accretion

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2388 Dose-dependent effects of tank-grown red seaweed on enteric methane emissions and production performance of dairy cows. S. E. Omale*, S. L. Rigert, P. Hartoonian, and J. A. D. R. N. Appuhamy, *Department of Animal Science, Iowa State University, Ames, IA.*

The study objective was to investigate enteric methane (CH_4) emission reductions and production performance of dairy cows fed a tank-grown red seaweed (*Asparagopsis taxiformis*) variety (SW) at increasing (1.0×, 1.5×, and 2.0×) dietary inclusion rates (IR) relative to a standard IR (SIR, 0.3% of DM). Forty-eight late-lactating Holstein cows were assigned to 0% (CTL), 0.30% (SIR), 0.45% (1.5SIR), and 0.60% of DM (2.0SIR) in a TMR. After a 10 d baseline measurement period, the desired IR were introduced gradually to the diet in a feed pellet containing 20% SW over 28 d. The DMI, milk production, and BW change (BWc) of cows exposed to full IR were collected over the next 63 d. The CH_4 emissions were measured by using a Greenfeed unit. Treatment effects were analyzed in the MIXED procedure of SAS with the REPEATED option and orthogonal contrasts. Compared with CTL, CH_4 production and yield decreased by 53% and 44%, 71% and 58%, and 75% and 53% in response to SIR, 1.5SIR, and 2SIR, respectively ($P < 0.01$, Table 1). Despite the DMI decrease, SIR and 1.5SIR did not modify milk yield, milk fat and protein %, and BWc compared with CTL ($P > 0.15$). The data demonstrated the potential of SW to decrease CH_4 production and yield by >50% without affecting milk production. Determining the CH_4 emission and milk production responses at different lactation stages will provide an improved understanding of SW as a promising CH_4 mitigation strategy for dairy cows.

Key Words: *Asparagopsis taxiformis*, feed intake, methane yield

2394 Effect of the timing of ruminal infusion of sodium bicarbonate on enteric methane emissions, rumen fermentation, and milk production and composition in dairy cows. M. Arif*, A. N. Hristov, L. Martins, and K. Harvatine, *Pennsylvania State University, University Park, PA.*

Rumination contributes to optimal rumen function including particle size reduction, rumen motility, and the addition of salivary buffers. The relationship between rumination and enteric CH_4 emission has not been well investigated. Our objective was to investigate the effect of the timing of buffer addition to the rumen on fermentation, methane production, and milk synthesis to mechanistically understand the effect of buffers. We hypothesized that $NaHCO_3$ would increase milk fat yield and decrease CH_4 per unit ECM by modifying rumen fermentation. Ten cannulated Holstein cows (203 ± 46 DIM; mean ± SD) were used in a 4 × 4 Latin square design with 16 d rumen infusion periods and a 5 d washout between periods. All cows were fed the same TMR and ruminally infused with 10 L water or 10 L of a solution containing 500 g $NaHCO_3$.Treatments were $NaHCO_3$ infused either from 0800 to 1700 h (DAY), 2000 to 0500 h (NGT), or both day and night 0800 to 1700 h and 2000 to 0500 h (DN) or water infused during both day and night (CON). Data were analyzed using a mixed model that included the random effect of cow and period and the fixed effect of treatment and repeated measures used for variables observed over the day. There was no effect of treatments on DMI, milk yield, and milk fat and protein concentration and yield ($P > 0.05$). All $NaHCO_3$ treatments decreased milk MUN by more than 14% compared with CON ($P < 0.05$). Rumen pH was increased during the day and daily enteric CH_4 emission was increased 75 g/d by DAY compared with CON. However, CH_4 yield (g/kg DMI) and intensity (g/kg milk yield and ECM) were not modified by treatment as observed with the Greenfeed system. Rumination time as quantified by AfiCollars was decreased in all $NaHCO_3$ treatments ($P < 0.001$). Rumen molar concentrations of acetate and propionate were not affected by treatment. Overall, $NaHCO_3$ decreased MUN and rumination but did not change milk yield regardless of the timing of infusion. Infusion during the day increased rumen pH and enteric methane emission.

Key Words: rumination, circadian, diurnal

2395 Effects of a combination of phytogenic compounds on milking performance and enteric methane emissions in dairy cows fed hay to concentrate diet. H. Khelil Arfa*[1], A. Blanchard[1], D. Yáñez Ruiz[2], G. Elcoso[3], and A. Bach[3], *[1]ADM Archer-Daniels-Midland International Sàrl, Rolle, Switzerland, [2]Estación Experimental del Zaidín, CSIC, Granada, Spain, [3]ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.*

The objective of this study was to evaluate milking performance and enteric CH_4 emissions in dairy cows fed diet with no silage, supplemented with a blend of essential oils containing cinnamaldehyde, eugenol, and *capsicum* oleoresin (CEC). Forty Holstein-Friesian cows (32% primiparous) averaging 712 kg of BW, 163 DIM, and 38.0 kg/d of milk yield (MY), were blocked based on parity, DIM, and MY, and

Table 1 (Abstr. 2388). Effects of feeding red seaweed at 0% (CTL), 0.30% (SIR), 0.45% (1.5SIR), and 0.60% of DM (2.0SIR) on the performance and enteric CH_4 emissions of dairy cows[1]

	LSM					*P*-value		
Variable	CTL	SIR	1.5SIR	2.0SIR	SEM	Linear	Quad.	Cubic
CH_4, g/cow per d	325^{a}	153^{b}	94^{c}	82^{c}	12.8	<0.01	<0.01	0.53
CH_4, g/DMI kg	12.9^{a}	7.3^{b}	5.4^{b}	6.0^{b}	0.7	<0.01	<0.01	0.43
DMI, kg/d	26.5^{a}	22.5^{b}	19.6^{b}	15.4^{c}	1.1	<0.01	0.19	0.86
Milk yield, kg/d	20.1^{a}	20.7^{a}	16.3^{ab}	14.7^{b}	1.4	<0.01	0.09	0.19
Fat, %	5.2^{a}	4.9^{ab}	4.8^{ab}	4.6^{b}	0.1	<0.01	0.99	0.88
Protein, %	3.80	3.75	3.83	3.81	0.7	0.79	0.73	0.44
BW change,[2] kg	27.7	32.7	33.2	5.9	12.8	0.38	0.17	0.55

[1] CTL = control; SIR = standard inclusion rate of 0.30% of DM; 1.5SIR = standard inclusion rate of 0.45% of DM; 2.0SIR = standard inclusion rate of 0.60% of DM; Quad. = quadratic.

[2] Body weight change from baseline to the last week of the study.

randomly assigned to 1 of 2 treatments: a basal diet (CON) or a basal diet supplemented with 1.2 g/cow/d of (CEC). The basal diet included 18.0% rye grass hay, 14.4% fescue hay, and 7.6% alfalfa hay, and contained 16.1% crude protein and 34.5% neutral-detergent fiber, on a DM basis. The experiment consisted of a pretreatment period of 10 d in which all cows were fed the CON diet followed by an experimental period of 56 d. Methane emissions were recorded using sniffers at the exit of the milking parlor. Data were analyzed using a mixed-effects model accounting for the random effects of the animal and block, and the fixed effects of treatment; week of study, and their 2-way interaction. Dry matter intake was increased ($P < 0.01$) by CEC (24.8 vs. 25.7 ± 0.12 kg/d, for CON and CEC, respectively) and eating rate (means 115 vs. 121 g/min, for CON and CEC, respectively). Milk yield and composition, ECM and BW were not affected by treatment, except for a treatment by week interaction for milk urea content and BW ($P < 0.01$). Feed efficiency (ECM/DMI) decreased with CEC compared with CON (1.61 vs. 1.55 ± 0.02, $P < 0.05$). Daily CH_4 emission was not affected by treatment (636 vs. 551 ± 29.5 L/d, for CON and CEC, respectively). However, CEC supplement deceased CH_4 yield by 16.4% (21.4 and 25.6 ± 1.22 L/kg DMI, $P = 0.02$) and tended to decrease CH_4 intensity per unit of MY by 14.0% (14.7 and 17.1 ± 0.79 L/kg MY, $P = 0.08$). In conclusion, supplementing CEC blend to a hay concentrate diet has a potential to reduce methane emissions yield and intensity, and increase feed intake of dairy cows without impacting milking yield and thus reducing feed efficiency.

Key Words: feed intake, methane, phytogenic compound

2396 Assessing and developing methane emission prediction models for cattle: A focus on India. S. Alam*[1,2], E. Schlecht[1], and C. A. Bateki[1], [1]*Animal Husbandry in the Tropics and Subtropics, University of Kassel and Georg-August-Universität Göttingen, Witzenhausen, Hessen, Germany,* [2]*Department of Dairy and Poultry Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh.*

India is home to over 525 million ruminants, which are major contributors to global warming via enteric methane (EntCH4) emissions. Various strategies exist to reduce EntCH4 emissions but accurate emission estimates are needed to predict the potential of each mitigation strategy. Measuring EntCH4 emissions is expensive and unrealistic on a large scale, so there is an urgent need for accurate EntCH4 prediction models. The present study evaluated the accuracy of various published models and developed a simplified model for predicting EntCH4 emissions from cattle across the Indian subcontinent. Six EntCH4 prediction models based on either DMI or gross energy intake (GEI) were identified as applicable to India. DMI- and GEI-based models were taken from IPCC (2019; $IPCC_{DMI}$ and $IPCC_{GEI}$), Ribeiro et al. (2020; Rib_{DMI} and Rib_{GEI}), and Patra (2017; Pat_{DMI} and Pat_{GEI}). These were evaluated using 2 independent literature databases containing information on 386 lactating cattle from 15 studies and 122 nonlactating cattle from 13 studies under different management practices across 13 Indian states. In addition, both data sets were combined to develop an empirical EntCH4 prediction model. Data were divided with a 70:30 ratio for model training and testing, respectively. The relative prediction error (RPE) and concordance correlation coefficient (CCC) were used to evaluate model accuracy. A model's prediction was considered acceptable when RPE <20%. Of the 6 models evaluated, the most accurate models for lactating cows were Rib_{DMI} (RPE = 18.6%, CCC = 0.695) and Pat_{DMI} (RPE = 18.4, CCC = 0.690), while the other models yielded estimates with RPE >20%. None of the 6 models predicted EntCH4 for nonlactating cattle with RPE <20% or CCC >0.20. The linear model developed (EntCH4 (g/d per cattle) = 2.82 + 17.53 × DMI (kg/d), $R^2 = 0.78$) predicted EntCH4 with a lower RPE (9.9%) than the 6 models evaluated. In conclusion, Rib_{DMI} and Pat_{DMI} are the best performing models for EntCH4 in India, and the proposed simplified model has good predictive accuracy, until new nationwide models based on larger data sets have been developed.

Key Words: emission, model, mitigation

2397 Investigating the anti-methanogenic potential of seaweed varieties in manipulating the rumen fermentation and methane production. K. Narayan*[1], N. Indugu[1], A. Castaneda[1], A. Johnson[1], A. Post[1], T. Webb[1], and D. Pitta[1], [1]*University of Pennsylvania, Kennett Square, PA,* [2]*Avon Grove Charter School, West Grove, PA.*

Several seaweed varieties have been shown to inhibit enteric methane (CH_4) emissions while improving animal productivity. However, the adaptation of seaweed-containing halogenated biomolecules to rumen fermentation and their effect on individual methanogens is largely unknown. The objective of this study was to examine the effect of 4 varieties of seaweed procured from Ocean Harvest Technology (Reigate, UK) on ruminal fermentation, CH_4 production, and individual methanogens in vitro and in vivo. We conducted several preliminary in vitro experiments using $ANKOM^{RF}$ Gas Production System to evaluate the dose effect (7.5 and 15 mg/g of dry TMR) of seaweeds and the anti-methanogenic potential of seaweed varieties on CH_4 production and effect on individual methanogens. The in vivo experiment was aimed to investigate the effect of selected seaweeds on CH_4 production, rumen fermentation, and production. Twenty-four Holstein cows were enrolled and allocated (n = 8) into 3 age groups (2 to 7 years), parity (1 to 6 lactations), and days in milk (14 to 79 DIM). An ANOVA test was applied using R package (R Core Team, 2020) for statistical analysis. The in vitro study revealed that 2 seaweed varieties (Blue and Bovine) remarkably reduced CH_4 production at the 15mg/g of TMR dose which were selected for the in vivo study. *M. stadtmanae* was significantly ($P < 0.001$) inhibited, whereas *M. ruminantium* remained lower up to 18 h and then increased. *M. archaeon* ISO4-H5 remained low in number throughout the experiment. In the in vivo study CH_4 intensity was significantly ($P = 0.03$) lower in Red compared with Bovine and control cows. Milk yield ($P = 0.01$) and production efficiency ($P = 0.02$) were significantly higher in Red cows compared with Bovine and control cows. In summary, the Red seaweed variety showed the higher reduction in CH_4 production and productive performance; thus, more investigations are required to understand the impact of the Red seaweed on fermentation and the rumen microbiome.

Key Words: in vivo study, methanogenic inhibitor, methane production

2398 Assessing the validation and reliability of methods to measure enteric methane emissions in dairy cattle. A. B. P. Fontoura*[1], P. K. Rosenstein[2], M. E. Van Amburgh[1], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Environmental Defense Fund, New York, NY.*

Methane (CH_4) is a major contributor to global warming. Dairy cattle play a significant role due to enteric CH_4 emissions. Respiration chambers (RC) are the gold standard for enteric CH_4 measurement, however, other methods such as GreenFeed (GF), tracer gas technique (SF_6), and sniffer systems (SNF) may be employed. Our objectives were to evaluate the predictions of GF, SF_6, and SNF, relative to the RC, and to assess reliability of these methodologies to measure enteric CH_4 emissions. Evaluation of GF, SF_6, and SNF emissions was performed using a meta-analytic approach using original research articles and comparative studies. Analysis of reliability was performed using raw data from studies that used the different methodologies and had repeated measurements with no methane-inhibiting strategy. Statistical analysis was performed using R (v. 4.2.1); the comparison between GF, SF_6, and SNF with RC data were assessed with Forrest plots, and the random effect model correlation coefficients using the metafor package. Internal consistency of RC, GF, and SF_6 was assessed with intraclass correlation coefficients (ICC) using the psych package. A total of 14 studies met the criteria for inclusion in the meta-analysis. Moderate agreement was observed for total CH_4 production between GF and RC (r = 0.61 [95% confidence interval [CI]: 0.40, 0.76]). The SF_6 and SNF methods showed stronger agreement with RC (r = 0.82 [95% CI: 0.67, 0.90] and r = 0.81 [95% CI: 0.67, 0.90], respectively). We also observed that RC possess excellent reliability (ICC = 0.98 [95% CI: 0.94, 1.00]), whereas GF and SF_6 possess moderate reliability (ICC = 0.77 [95% CI: 0.39, 0.93], and ICC = 0.53 [95% CI: −0.06, 0.98], respectively). Overall, our meta-analytic approach revealed inconsistencies in CH_4 production metrics among the measurement approaches. There are advantages and limitations of each methodology, emphasizing the importance of standardized procedures and meticulous calibration for accurate measurements and reliable data. Standardized, reliable methodologies are crucial for demonstrating reductions in enteric CH_4 and conducting transparent product comparisons.

Key Words: methane, methodology, validation

2399 Effects of feed additives on intake, milk yield, and methane emission in lactating Holstein cows.

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The objective of this study was to evaluate the effects of sodium monensin or condensed tannin on intake, milk yield, and methane emission in lactating Holstein cows. All procedures were approved by the Ethics Committee for the Use of Production Animals of the UFV, Brazil (protocol 020/2022). Nine Holstein cows were used in a triplicated 3 × 3 Latin Square design, consisting of 3 rumen-fistulated cows and 6 nonfistulated cows. Animals were subjected to 3 treatments: control (CON), monensin (MON; 12 mg/kg of DM), and condensed tannins (TAN; 2 g/kg of DM). The experimental period consisted of 84 d, subdivided into 3 periods of 28 d, with 14 d of adaption. From 15th to 17th day, milk yield and intake were measured. Between the 24th and 28th days, methane was measured using the sulfur hexafluoride (SF_6) tracer gas technique. The animals were equipped with a halter, a capillary tube system, and an evacuated PVC yoke through a vacuum pump with a recorded initial pressure. The concentration of CH_4 in the yoke was determined by gas chromatography equipped with a flame ionization detector. Meanwhile, the concentration of SF_6 in the yoke was determined by a gas chromatograph equipped with an electron capture detector. The data were analyzed using the function lmer of the package lme4 of R, following a replicated Latin Square design, considering $P < 0.05$ as significant and 0.050.05). The NDF intake was higher for animals in TAN (8.81 kg/d) treatment compared with MON treatment (8.26 kg/d; $P = 0.015$). Milk yield did not differ among the treatments ($P > 0.05$), averaging 36.7, 38.3, and 38.1, for CON, MON, and TAN, respectively. The total methane emission did not differ among treatments ($P > 0.05$). However, methane emission per kg of organic matter intake tended to be lower for groups that received additive (MON or TAN; 10.8 g/kg) than CON (12.5 g/kg $P = 0.059$). It can be concluded that the use of feed additives did not improve intake or milk yield but tended to decrease methane emission.

Key Words: additive, methane, nutrition

2400 Methane emissions, production, and rumination of certified-organic dairy cows supplemented with *Asparagopsis taxiformis*.

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The objective of the study was to evaluate methane emissions, production, and rumination of certified-organic Holstein and crossbred cows that were supplemented with *Asparagopsis taxiformis*. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN, organic dairy. Forty Holstein and crossbred certified organic cows were supplemented with *Asparagopsis taxiformis* (SeaGraze, Symbrosia Inc., Kailua-Kona, HI) from July 2023 to January 2024. The study was as a randomized complete block design with 2 treatment levels and cows had mean days in milk of 122 d. Cows were paired by parity, breed, and milk production and randomly assigned to either the control (CON; 0% SeaGraze) or treatment group (SGR; 0.1% SeaGraze per cow/d on a DM basis). Cows grazed fresh pasture during August and September and were fed TMR during October to January. Cows were supplemented with 3.6 kg/head per day of corn grain and minerals. The SeaGraze was mixed with the corn grain. Methane was collected with a GreenFeed System (C-Lock Inc., Rapid City, SD) and was rotated between treatment groups every 2 wk during the 6-mo study. Independent variables for statistical analysis were the fixed effects of breed group, parity group, period (2 wk), time of day, treatment group, and the interaction of treatment group and period. Methane was lower ($P < 0.05$) during the morning (306 g/d) compared with the evening (333 g/d). Across the 6-mo study, SGR cows had lower methane (273 g/d) compared with CON cows (367 g/d). The interaction of period and treatment was significant ($P < 0.05$) and during the final period of the 6-mo study, the SGR cows (225 g/d) had lower ($P < 0.01$) methane compared with the CON cows (385 g/d). Milk, fat, and protein production was not different ($P > 0.10$) for the SGR cows (12.2 kg/d, 4.1%, 3.2%, respectively) compared with the CON cows (12.0 kg/d, 4.0%, 3.2%, respectively). Rumination was not different for SGR and CON cows (448 min/d vs. 431 min/d). Feeding *Asparagopsis taxiformis* to certified organic dairy cattle may reduce methane emissions from 20% to 40%.

Key Words: organic, methane, *Asparagopsis taxiformis*

2401 Biochemical methane potential to evaluate potential trade-offs between methane emissions from enteric and manure fermentation when enteric methane emissions of dairy cows are mitigated.

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The aim of this study was to assess biochemical methane potential of manure from dairy cows subjected to dietary supplementation of 3-nitrooxypropanol (3-NOP) or whole cottonseed (WCS). In particular, the effect of diet was evaluated in 2 types of manure treatments: manure (feces + urine) and feces alone. Fresh feces and urine were separately collected from 16 lactating dairy cows from 4 diets: basal TMR (CON), diets containing 3-NOP, WCS, and 3-NOP + WCS. Feces (5.0 ± 0.05 g) were suspended in 75 mL distilled H_2O and incubated either with or without urine (1–3 mL) in 125 mL serum bottles in shaking incubator at 100 rpm and 39°C. Gas production and composition were determined at 24h, 48h, and weekly thereafter, until the CH_4 production at the last measurement was below 1% of cumulative CH_4 production. The amount of TS and volatile solids (VS), along with NH_3 concentration and pH, were determined in digested samples at the end point. Data were analyzed in R using mixed model with diet by manure treatment interactions. No interaction was observed between diets and manure treatments for any of the variables. There was little difference in TS and VS content between diets or between manure treatments. There were no dietary effects for any of the parameters except for H_2 yield from the 3-NOP treated cow excreta being greater (1.15 mL/g VS; $P < 0.01$) compared with the excreta from other diets (CON: 0.613, WCS: 0.538, 3-NOP + WCS: 0.659). Further, manure incubation had greater ($P < 0.01$) CH_4 yield (49.3 vs. 40.3 mL/g VS) and CO_2 yield (83.2 vs. 55.9 mL/g VS) than incubation with feces alone. Additionally, the incubations of manure and feces differed ($P < 0.01$) in pH (7.8 vs. 6.1), N content (0.031%, 0.022%), and NH_3 concentration (10.9% vs. 2.5%). Overall, the mitigation of enteric CH_4 achieved at cow level by 3-NOP did not lead to an increase in CH_4 from manure fermentation. However, fecal CH_4, CO_2, and NH_3 emissions increased when in contact with urine, indicating that separate storage of feces and urine may reduce CH_4 emissions from manure storage.

Key Words: methane, dairy cow, manure

2402 Comparing different machine learning methods to predict the likelihood of conception in organic dairy cows. C. Niño de Guzmán*[1], N. Bliznyuk[1], P. Pinedo[2], and A. De Vries[1], [1]*University of Florida, Gainesville, FL,* [2]*Colorado State University, Fort Collins, CO.*

This study aimed to compare machine learning methods to predict the likelihood of pregnancy in organic dairy cows. Our final data set contained 68,504 breeding events and their outcomes (pregnant or nonpregnant) from 13,558 lactations. Breedings were based on estrous activity only and performed by artificial insemination. Parity, days in milk when breeding, breeding number, breeding year, breeding season, estrus intensity measured as the relative activity increase, time since last illness event, and number of illnesses simultaneously were the predictors in the data set. We compared the machine learning methods of logistic regression, bagging, and random forest. Training and test sets were created using the “caret” package in R. We used the validation set approach to authenticate the models before testing their predictive accuracy. A correlation analysis was performed to select the final predictors to include in the models. We found a strong correlation (0.89) between the time since the last illness event and the number of illnesses simultaneously. The final models included parity, season, estrus intensity, number of illnesses simultaneously, breeding number, breeding year, and days in milk when breeding. The logistic regression showed an area under the receiver operating characteristic curve of 0.60. The bagging model had an area under the curve of 0.52. Lastly, the random forest model had an area under the curve of 0.54. The bagging model reported that days in milking when breeding was the most important predictor, followed by breeding number and season when building the trees, and the random forest model showed that days in milk when breeding was the most important predictor, followed by season and estrus intensity. In conclusion, logistic regression had a greater predictive potential of likelihood of conception than the other machine learning methods. Further refinement of the methods is needed.

Key Words: machine learning, reproduction, organic

2403 Relationships between circulating glucose concentrations and productivity in heat-stressed dairy cattle. T. Baber*, S. Newman, K. Joyce, and M. L. Rhoads, *Virginia Tech, Blacksburg, VA.*

Dairy cattle exposed to summer heat stress (HS) typically exhibit altered metabolic characteristics, including hyperinsulinemia concurrent with hypoglycemia. The reasons for this change in glycemic status and its consequences are currently unknown. This project aimed to examine the relationship between summer HS, blood glucose concentrations, and milk production in primiparous (n = 26) and multiparous (n = 27) Holstein cows. Glucose was measured in coccygeal vein blood twice weekly using a hand-held glucometer (Abbott Precision Xtra) from May through July. Rectal temperatures were also collected. The days surrounding sample collections were categorized as having no HS (0 h above 68 Thermal Humidity Index [THI]), moderate HS (>0 to 8 h above 68 THI) or severe HS (>8 h above 68 THI). A subset of cows with ≥ 5 blood glucose measurements spanning different THI categories were categorized as hypoglycemic (n = 8 primiparous; n = 9 multiparous) or nonhypoglycemic (n = 6 primiparous; n = 5 multiparous) based upon blood glucose concentrations on no-HS days and severe-HS days (hypoglycemic if sustained reduction on severe-HS days). When analyzed together, milk production did not differ between those categorized as hypoglycemic and nonhypoglycemic. When separated based upon parity, there was a tendency for primiparous hypoglycemic cows to produce more milk than their nonhypoglycemic counterparts ($P < 0.15$). Variability in milk production measured as standard deviation across no-, moderate- and severe-HS days did not differ based upon glycemic status but was greater in multiparous than primiparous cows ($P < 0.01$). Rectal temperatures were greater on severe-HS days than on no- or moderate-HS days ($P < 0.01$) and were positively correlated with the number of h of HS in a day (r = 0.45; $P < 0.01$). Neither milk nor glucose differed based upon the category of HS severity. Interestingly, however, glucose was negatively correlated with milk production on the day of measurement (r = −0.24; $P < 0.01$) and day after measurement (r = −0.23; $P < 0.01$). These results are an initial indication that hypoglycemia during HS is related to milk production, particularly in primiparous cows.

Key Words: heat stress, glucose, milk

2404 Effects of dietary starch concentration on milk production, nutrient digestibility, and methane emissions in mid-lactation dairy cows. R. L. Culbertson*[1], F. A. Gutiérrez-Oveido[1], P. Uzun[1,2], N. Seneviratne[1], A. B. P. Fontoura[1], B. K. Yau[1], J. L. Judge[1], A. N. Davis[3], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Isparta University of Applied Sciences, Isparta, Türkiye,* [3]*State University of New York at Cortland, Cortland, NY.*

Dietary starch enhances ruminal propionate synthesis, which may lower methane production. To define the effects of dietary starch on milk production, nutrient digestibility and methane emissions, we enrolled 30 mid-lactation Holstein cows (2.5 ± 1.8 lactations; 38 ± 9.1 kg milk/d) in a study with a completely randomized design. After acclimation, cows were assigned to 1 of 2 treatments (n = 15/treatment): a high NDF and low starch diet (LS; 37% NDF, 20% starch) or a low NDF and high starch diet (HS; 32% NDF, 25% starch) for an 8-wk experimental period. Cows were fed a diet of corn silage, triticale silage, and concentrates, and milked thrice daily. Milk samples (9 milkings/wk) were collected at wk −1, 2, 4, 6, and 8. Methane (CH_4), carbon dioxide (CO_2), and hydrogen (H_2) measurements were collected 3X/d for 3 d (9 samples/cow) each week. Blood and feces were collected at wk −1 and 8. The statistical model included fixed effects of diet, week, and diet × week and random effect of cow. Dry matter intakes (24 vs. 29 kg/d) and milk yields (39 vs. 41 kg/d) were greater in HS cows, relative to LS cows (diet, $P < 0.01$).

Energy-corrected milk yields (43 vs. 45 kg/d), protein contents (3.3 vs. 3.5%), protein yields (1.2 vs. 1.4 kg/d), and lactose yields (1.9 vs. 2.0 kg/d) were greater for HS cows (diet, $P < 0.04$). Apparent total-tract DM and OM digestibilities were greater for HS cows (69% vs. 74% and 71% vs. 75%, respectively; diet, $P < 0.01$). Digestibility of NDF and starch were not modified by treatment. Plasma glucose concentrations were not modified by treatment. Plasma insulin concentrations tended to be greater in HS cows (1.3 vs. 1.6 ng/mL; diet, $P = 0.12$). Methane production tended to be higher for HS cows (386 vs. 411 g/d; diet, $P = 0.08$). Methane yields were lower for HS cows (16 vs. 15 g CH_4/kg DMI; 18 vs. 16 g CH_4/kg OM intake; diet, $P = 0.03$). Methane intensity (g/kg of ECM), CO_2 production, and H_2 production were not modified. We conclude that a HS diet reduces methane yield with improved DM digestibility; however, improvements in ECM did not develop with improvements in methane intensity.

Key Words: starch, milk, methane

2405 Investigating the antimethanogenic effect of ethyl nitropropionate on methane production and methanogenic archaea in vitro.

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Ethyl nitro-propionate (ENP) is a novel short-chain nitro-compound that exerts a potent suppressing effect on methanogenesis. Previous studies have explored the anti-methanogenic effect of ENP on methane (CH_4) production, total gas, and VFA. However, no studies have reported a dose-by-hour interaction of ENP on CH_4 production and methanogenic archaea. This study assessed a dose-by-hour interaction of ENP on CH_4 production and methanogenic archaea in vitro. A preliminary experiment was performed to establish doses and sampling hours using the ANKOM^{RF} Gas Production System following the methodology described by Camacho et al. (2019) for gas production. ENA was tested at 0, 8, and 16 m*M* doses. Gas and liquid samples were collected at 6, 12, and 24 h post incubation in a 3x3 factorial design. All dose-by-hour combinations were tested in triplicate. CH_4 production was analyzed by gas chromatography. The copy number of the 16S rRNA gene of methanogenic archaea was quantified using real-time PCR. The data were analyzed using the PROC GLM procedure of the SAS software using a fixed effects model. A post hoc analysis was conducted for the dose-by-hour interaction using the lsmeans procedure and the Scheffé adjustment for pairwise comparisons. A dose-by-hour interaction ($P < 0.0001$) was detected in CH_4 production and total gas, whereas the pH was affected by dose ($P < 0.0001$) and hour ($P < 0.0001$). The lowest CH_4 production (5.60 ± 1.52 mL) was detected with the 16 mM dose at 24 h, a reduction of more than 91% compared with the control. These findings are consistent with the copy numbers obtained through rt-PCR, which were significantly lower ($P = 0.02$) than the control. Overall, these findings show that dose and hour have an additive effect on CH_4 production and total gas and a suppressing effect on methanogenic archaea. Further studies to understand ENP's mechanism of action and antimethanogenic effect on other fermenting microbes and VFA are required to optimize the dose without negatively affecting rumen fermentation.

Key Words: methane production, nitro-propionate, rumen microbiome

2406 Evaluation of the potential interaction between a rumen modifier and nitrate on methane production in vitro.

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Reducing enteric methane emissions is an important goal for the dairy industry that could be partially achieved with the use of rumen modifiers. The interaction between additives is not well understood yet. We evaluated the supplementation of a rumen modifier based on a mixture of tannins and spice extracts (Valido Pro, Cargill Inc.), a nitrate-based product that has shown to reduce methane production (SilvAir, Cargill Inc.), and their combination in an in vitro system. Twelve continuous culture fermenters were used in a randomized block design experiment with 2 10-d periods. Treatments were: Control (no additives), Valido Pro (0.20 g/L), with or without Silvair supplementation (2.5 g/L) in a 2 × 2 factorial arrangement. Fermenters (~2 L capacity) were fed (~100 g/d) a grass hay and corn silage based lactating cow TMR diet once a day. Methane in the headspace was monitored continuously in all fermenters using a Micro-Oxymax Respirometer (Columbus Instrument Inc., Columbus OH). Data were analyzed with the lmer package in R with a model that contained the fixed effects of diet, treatment, and their interaction, and the random effects of period and fermenter. There was no interaction between the treatments on methane production ($P = 0.20$). Silvair reduced (−35%; $P < 0.05$) methane production rate between 2 and 21 h post-feeding and average gas production (main effect of Silvair was 16.0 vs. 24.5 mmol/d, SE = 4.7). There was no effect ($P = 0.37$) of Valido Pro on methane production (main effect of Valido Pro was 19.6 vs. 21.0 mmol/d; SE = 4.7). Nutrient degradation, total VFA production, or bacterial flows were not affected by treatments. Silvair increased ($P = 0.01$) butyrate proportion (main effect of Silvair was 13.0 vs. 11.4 g/100 g total VFA). No other VFA proportion was affected by treatment. This study validates that nitrate is effective in reducing methane production in vitro and there were no additional benefits in combining with Valido Pro for methane reduction.

Key Words: methane, nitrate, sustainability

2407 Validation of a model to predict methane production based on the nutritional composition of the diet in lactating dairy cows.

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The goal of this study was to evaluate a model predicting methane production based on the nutritional composition of the diet. Data were collected from 82 peer-reviewed articles published from 2008 to 2022 that measured methane production in lactating cows under a wide range of dietary conditions and reported diet composition on an ingredient basis. The database had a total of 359 treatment observations, with a mean (±SD) milk yield of 30.0 kg (±7.0 kg), DMI of 20.9 kg/d (±3.5 kg) and methane production of 393 g/d (±89 g/d and range: 149–743 g/d). Treatments that included additives to reduce methane production were not included. Each ingredient reported in the article, their nutritional analysis (if provided), and their inclusion in the diet were entered into dairy MAX formulation software (Cargill Inc.). The methane estimation was obtained from the model and recorded in the database. Using lm function in R, predicted values were modeled against observed values reported in the article. The intercept of the observed versus predicted equation for CH_4/kg DMI was 0.72 (SE = 2.46). The slope of the equation was 0.97 (SE = 0.12). The intercept was not different from 0 ($P = 0.77$) and slope was not different from 1 ($P = 0.86$) which means this equation is statistically equivalent to the identity (1:1) line. The R^2 value of the observed vs. predicted equation is 0.22. When the predicted values were multiplied by the DMI observed, R^2 value of the observed vs.

predicted equation increased to 0.44 suggesting some of this variation was explained by DMI. To explain the rest of the variation, the same model was fitted to the database using lmer function in R and including a random effect of study. The concordance correlation coefficient of the mixed model was 0.91, suggesting most of the variation not explained by the current model could be due to different measurement techniques explained by a study effect. This evaluation evidenced that the model used in dairy MAX System is an accurate predictor of methane and more effort is needed to assess the variation in measurements systems across farms and studies when fitting models.

Key Words: CH_4, model, nutrition

2408 Increased pulsation ratio in rear quarters reduces machine-on time and milking on empty teats. P. Fürst[1], M. Affentranger[1], K. Kakoulis[2], C. O. Paulrud[2], and R. M. Bruckmaier*[1], *[1]Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland, [2]DeLaval SA, Tumba, Sweden.*

Dairy cows produce mostly more milk in rear than in front quarters. Thus, at conventional machine milking the milk flow ceases earlier in front than in rear quarters, resulting in milking on empty teats in front quarters. We tested the hypothesis that an increased pulsation ratio (PR) in rear teat cups reduces machine-on time and the time of milking on empty teats in front quarters without an increased effect on teat tissue thickness (TTT). Ten Holstein cows were milked at 14-h (morning) and 10-h (afternoon) milking intervals. Each treatment was performed twice at both milking intervals. The system vacuum was set at 42 kPa. Front quarter PR was 65:35, whereas PR in rear quarters was 65:35, 70:30, 75:25, or 80:20. Whole udder milk flow was recorded, and the time of individual quarter milk flow (tQMF) was determined by characteristic changes of the quarter mouthpiece chamber vacuum. The TTT of all 4 teats was measured with a cutimeter at 5 min after cluster detachment. Statistical evaluations were performed by ANOVA and Tukey's *t*-test. Total milk yield as well as tQMF of front quarters did not differ among treatments and was 21.1 ± 0.3 and 15.6 ± 0.2 kg, and 4.9 ± 0.1 and 3.8 ± 0.1 min at 14-h and 10-h milking intervals, respectively. In contrast, tQMF in rear quarters and consequently the interval of first to last quarter milk flow cessation decreased gradually with increasing PR from 65:35 to 80:20 from 6.8 ± 0.5 to 6.0 ± 0.4 min ($P < 0.05$) and 5.3 ± 0.3 to 4.7 ± 0.3 min ($P < 0.05$), and from 2.7 ± 0.3 to 1.8 ± 0.2 min ($P < 0.05$) and 2.1 ± 0.2 to 1.6 ± 0.2 min ($P < 0.05$) at 14-h and 10-h milking intervals, resp.. Because of immediate cluster detachment at cessation of milk flow machine-on time was reduced from 7.6 ± 0.4 to 6.9 ± 0.4 min ($P < 0.05$) and from 6.2 ± 0.3 to 5.5 ± 0.3 min ($P < 0.05$) at 14-h and 10-h milking interval, respectively. Independent of PR the TTT after milking was increased compared with pre-milking values only in front quarters ($P < 0.05$), but not in rear quarters. In conclusion, increased PR in rear quarters increases the milking performance, and reduces milking on empty teats without increased effect on teat tissue.

Key Words: pulsation ratio, front and rear quarter, empty teat

2409 Assessing the warming effect of methane from dairy cattle industry in distinct world regions by comparing GWP_{100} and GWP* metrics. F. Correddu*, M. F. Lunesu, M. F. Caratzu, A. Nudda, G. Battacone, and G. Pulina, *Dipartimento di Agraria, University of Sassari, Sassari, Italy.*

The 100-year Global Warming Potentials (GWP_{100}) is the standard metric used to assess the effect of GHG on climate change. However, this metric fails to convert emission pathways of short-lived climate pollutants emissions (e.g., methane, CH_4) to their real effect on temperature. In this work we apply and compare GWP_{100} and the new metric GWP* for calculating annual and cumulative CO_2 warming-equivalent (*we*) related to the CH_4 emissions from the dairy cattle sector in different world's regions over the 1980–2020. Data of CH_4 emissions (from enteric fermentation and manure management) for different world regions (Table 1) from 1961 to 2020 were retrieved from FAOSTAT. GWP_{100} and GWP* were assessed as follows: i) $CO_2e = E_{CH4}(t) \times 28$; ii) $CO_2we = 28 \times [4.53 \times E_{CH4}(t)-4.25 \times E_{CH4}(t\text{-}20)]$, where E_{CH4} is the annual CH_4 emission for a considered year (t), or relative to the previous 20 years (t-20). As expected, regions with decreasing CH_4 emissions showed annual GWP* values always lower than GWP_{100} (below or close to zero) and vice versa. Considering the cumulative values (Table 1), overall, the greatest re-dimensioned effect was found for Europe, whereases China showed the worst situation followed by Africa, Asia, India, and South America. World regions also show a different contribution in terms of effect arising from enteric fermentations or manure management, e.g., North America ranks among the last for effect from enteric emissions but second for emissions from manure management, with both values being negative when assessed by GWP*. In conclusion, GWP* allows to better clarify the role of dairy cattle sector of different world's regions in the global warming, in relationship to their CH_4 emissions.

Table 1 (Abstr. 2409). Cumulative (1981–2020) enteric and manure management CH_4 emissions (Gt) for different world regions calculated by GWP_{100} and GWP*

	Enteric fermentation		Manure management	
Word region	CO_2e	CO_2we	CO_2e	CO_2we
Africa	2.3	4.9	0.1	0.1
Australia and New Zealand	0.5	0.5	0.1	0.1
EU-(URSS)	4.0	−4.7	0.7	−0.8
North America	1.5	−1.1	0.6	−0.4
South America	2.5	3.2	0.0	0.0
China	0.5	1.8	0.1	0.2
India	2.3	3.6	0.2	0.3
Asia-(China+India)	2.3	4.5	0.3	0.6
World	19.1	8.2	2.4	−0.3

Key Words: methane, GWP*, dairy cattle

2410 Milking performance of dairy cows when managed in full- or part-time cow-calf contact systems and separated gradually or abruptly from their calves. M. Durrenwachter*[1,2], E. H. Jensen[1], M. B. Jensen[1], and H. W. Neave[1,3], *[1]Department of Animal and Veterinary Sciences, Aarhus University, Tjele, Denmark, [2]Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT, [3]Department of Animal Sciences, Purdue University, West Lafayette, IN.*

The aim of this study was to evaluate milking performance of dairy cows in 4 types of cow-calf systems, and whether milk yield recovers to the level of conventionally managed cows after separation from their calf. 84 Danish Holstein cows and their calves (7 blocks of 12 cow-calf pairs) were assigned to 1 of 3 dam-contact treatments at birth, in groups of 4: full-time (24 h/d) or part-time (10 h/d-daytime) dam contact, or separation at birth. From wk 8 to 10, dam-contact treatment cows were assigned to either gradual weaning (calf contact reduced to 50% then 25% of original time by fence line) or abrupt weaning (no change in contact time). At wk 10, dam-contact treatment calves were separated from their dams. Cows were milked twice daily in a double 12 parallel milking parlor. Daily milk yield between treatments were analyzed in linear mixed regression models for the rearing period, weaning period, and up to 3 wk post-separation. Full-time cows yielded less milk than part-time and separated cows during the rearing period (13.4 ± 0.8 L/d; 24.0 ± 0.8 L/d; 36.0 ± 0.6L/d, respectively; $P < 0.01$). Gradual weaning by reducing dam-calf contact did not increase milk yield (15.9 and 22.5 ± 1.5 L/d; full- and part-time, respectively). However, over the 3 wk post-separation, milk yield depended on both contact and weaning treatments. Full-abrupt cows had the lowest yield (25.6 ± 1.4 L/d) compared with full-gradual, part-abrupt and part-gradual cows (30.3 ± 1.5 L/d; 32.9 ± 1.6 L/d; 33.0 ± 1.5 L/d; $P < 0.01$), and no dam-contact treatment reached a similar milk yield as separated cows (36.3 ± 1.4 L/d; $P < 0.08$) over 3 wk post-separation. These results suggest that cows in full-time contact treatment should be gradually weaned from their calves to improve milk yield after separation, but overall, part-time contact (vs. full-time) returns higher milk yields. However, further investigation is required to understand the longer-term effects of cow-calf contact systems on a 305 d lactation curve.

Key Words: dam-rearing, milk yield, gradual weaning

2411 Enhancing dairy farm simulation: An approach to modeling farm-specific lactation curves with readily available data. Y. Gong*[1], H. Hu[2], K. F. Reed[2], and V. E. Cabrera[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Cornell University, Ithaca, NY.*

Accurate estimation of annual herd milk production (AHMP, reported by the farm) using a lactation model is a cornerstone of dairy farm systems simulation models. Our research introduces a 2-step method for defining parity-specific parameters of the Wood's lactation curve with the objective of improving model accuracy by including observed farm production and the parity composition of the herd as inputs. This method involves: (1) adjusting the model parameters using previously reported parameter estimates and farm contextual metadata (i.e., temporal, geographical, and management factors), and (2) fitting the parity-specific scale parameters to match parity-specific 305-d MY derived from reported AHMP and the herd parity composition. We leveraged the Ruminant Farm Systems (RuFaS) model, a comprehensive dairy farm system model, as our simulation platform to evaluate our method against 2 literature-based methods: (1) US averages; and (2) previously reported values adjusted for contextual metadata alone. The evaluation was conducted on 3 commercial Holstein farms located in WI (58 cows), VT (117 cows), and NY (1,002 cows). Preliminary results demonstrated a marked improvement in the accuracy of estimated AHMP. Overestimation of AHMP was reduced from 175%, 109%, and 116% (method 1); and 164%, 105%, and 113% (method 2) to 114, 102, and 108% (our 2-step method) for WI, VT, and NY farms, respectively. Large improvements relate to disparities between the farms' reported AHMP and local or national average productions that informed parameter estimates reported in the literature. For example, in WI, the 305-d MY (kg/cow per year; 8,545) substantially deviates from the US average (12,802 ± 4,518) or nearby farms (13,218 ± 4,391). By integrating additional data (AHMP) that is readily accessible to farmers, our method provides a practical and efficient approach to improve the accuracy of lactation curve parameter estimates, thereby elevating dairy farm simulation models to a higher standard of reliability. Future evaluations will expand to include more farms across regions.

Key Words: Wood's lactation model, annual herd milk production, farm-specific lactation model

2412 Effects of heat stress on animal physiology, intake, nutrient digestibility, and performance of dairy calves. L. F. M. Neves[1], S. G. Coelho[1], M. B. Gomes[1], J. P. Campolina[1], and M. M. Campos*[2], [1]*Departamento de Zootecnia, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil,* [2]*Empresa Brasileira de Pesquisa Agropecuária—Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil.*

In the tropical environment, characterized by high temperatures and humidity, the rearing phase of calves has progressively become more challenging due to the detrimental effects of heat stress on physiology, performance, and health. Thus, this study aimed to evaluate the physiology, intake, performance, and digestibility of dairy calves subjected to high temperatures in a climate chamber, with controlled temperature and humidity. Thirty-five Holstein calves were divided into 2 treatments: Control (CON, THI of 66, n = 17) and Heat-Stress (HS, THI of 82 for 9 h + THI 66 for 15 h, n = 18). The experimental period ranged from 1 to 28 d of age. Physiological parameters such as respiratory rate (RR), heart rate (HR), and rectal temperature (RT), intake, and health were evaluated daily. Performance and blood parameters (triglycerides, glucose, creatinine, insulin, and cortisol) were assessed weekly, whereas rumen parameters were assessed every other week. Digestibility trials were conducted at 9–12 and 23–26 d of age. Data were analyzed using R software with ANOVA and a 95% confidence in a completely randomized design, with treatment was the main plot and week was the subplot. The HS group showed higher RR and RT compared with the CON group ($P < 0.001$). Intake was reduced in the HS group ($P = 0.007$), whereas water intake was greater ($P = 0.003$) than in the CON group. Differences in intake led to lower concentrations of ruminal ammonia, acetate, and propionate in the HS group ($P < 0.05$). Additionally, HS-calves exhibited lower digestibility of dry matter, organic matter, ether extract, gross energy, as well as higher urinary nitrogen ($P < 0.05$) in the first digestibility trial, and lower ether extract digestibility in the second digestibility trial ($P = 0.02$) compared with CON calves. However, these differences did not affect final weight, average daily gain ($P > 0.05$). There was no effect of HS on blood parameters ($P > 0.05$). Thus, heat stress negatively affected animal physiology, intake, and nutrient digestibility.

Key Words: climate, preweaning, temperature

Reproduction 3

2413 Estrus expression preceding timed artificial insemination is positively associated with fertility in dairy cattle. M. E. Schultz*, J. S. Stevenson, and V. E. Gomez-Leon, *Kansas State University, Manhattan, KS.*

Aim was to assess the association of estrus expression with pregnancy per insemination (P/AI) and pregnancy loss (PL) of heifers and lactating dairy cows submitted to timed AI (TAI). Our hypothesis was that estrus expression preceding TAI correlates with increased P/AI in heifers but decreased P/AI in lactating cows. Further, we hypothesized that estrus is associated with PL. This study was based on retrospective data obtained from the Kansas State University Dairy Teaching and Research Center, current 365-d rolling herd production of milk = 13,948 kg (3.5% fat). Only first services of Holstein cows at 75 ± 5 d in milk (n = 767) and of nulliparous Holstein heifers at 408 ± 10 d of age (n = 252) were used. Protocols used comprised PG3-G on cows and a combination of 5-d CIDR (n = 27) and 6-d CIDR (n = 225) synch on heifers. Records of 1,019 breeding, 293 estrus events (measured by an automated activity monitor), pregnancy diagnoses at 36 ± 3 d post-AI, and calvings from January 2021 to February 2024 were analyzed with Glimmix procedure (SAS v9.4). Incidence of estrus expression was not different between primiparous and multiparous cows (21.6%) but was less than in nulliparous heifers (50.4%; $P < 0.01$). Estrus expression was associated with increased P/AI ($P = 0.004$) regardless of parity, with a respective rise of 18.5%, 16.3%, and 30.2% in nulliparous (54% vs. 64%), primiparous (49% vs. 57%), and multiparous (43% vs. 56%). In terms of odds ratio, nulliparous Holstein heifers and lactating Holstein cows that exhibited estrus had 79% greater odds of P/AI than herd mates with no estrus expression. Finally, animals expressing estrus tended ($P = 0.07$) to have fewer PL (least square probability of calving = 0.98 [0.91; 0.99]) than animals not expressing estrus (0.90 [0.80; 0.95]). In conclusion, despite a significantly lower incidence of estrus in lactating Holstein cows compared with nulliparous Holstein heifers, both groups exhibited improved P/AI and fewer PL when estrus was expressed. These findings suggest the potential for TAI protocol improvement by promoting estrus expression.

Key Words: estrus, pregnancy per insemination, pregnancy loss

2414 Qualitative and quantitative spermatological parameters evaluation of slick hair and wild type Holstein bulls during warmer months. K. S. Luquis-Gonzalez*, E. A. Ralat-Vargas, J. S. Henao-Beltran, L. Santiago-Perez, W. D. Feliciano, E. Jimenez, and V. M. Negron-Perez, *University of Puerto Rico, Mayaguez, Puerto Rico.*

Heat stress affects spermatozoa development creating detrimental effects on bovine reproduction. As a result, dairy sector revenues are negatively affected. A genetic mutation in the prolactin receptor (InDel in p.L462*) causes carrier animals to have short hair and confers greater thermotolerance. The aim of this study was to compare sperm quality between slick hair (SL) and wild type (WT) bulls during warmer months, hypothesizing that SL bulls will have greater sperm quality. A total of 53 ejaculates were collected from n = 11 Holstein bulls (n = 8 SL; n = 3 WT) of 3–4 years of age, by manual stimulation followed by electroejaculation. Samples were collected at the Agricultural Experiment Station at Gurabo, UPR, throughout May–October of years 2021–2023. All animals were subjected to the same procedure; number of ejaculates per bull were 2–13. Semen volume of each ejaculate was measured, and the AndroVision CASA Software was used to determine sperm concentration, progressive motility, and total motility. Data were statistically analyzed using SAS University Edition, 2018. Averages of ejaculate variables were calculated per bull and used to compare by genotype. Bull ID and age were considered in the analysis. Significant differences were found between genotypes for ejaculate volume (WT = 4.44 ± 0.69 mL, SL = 6.26 ± 0.41 mL, $P = 0.03$) and a tendency was found for sperm concentration (WT = 371.04 ± 240.22 10^6/mL, SL = 890.72 ± 134.99 10^6/mL, $P = 0.07$), but none were identified for sperm total motility nor progressive motility. Although age had no significant effect, bull ID significantly affected all variables ($P < 0.05$) regardless of genotype. Results indicate SL bulls ejaculated semen in greater volumes and sperm concentration, but equal motility compared with WT bulls. We conclude SL bulls seem to have reproductive advantages in comparison to WT bulls during warmer months. Thermotolerance can be a key factor for improved quality of bovine sperm in dairy farms in the tropics. Further research should include sperm viability, morphology, and kinetics to extend the characterization of reproductive traits of SL bulls.

Key Words: heat-stress, Holstein bull, sperm quality

2415 Association between estrus characteristics and milk production in lactating dairy cows. V. A. B. Primo*[1], T. D. Gonzalez[1], M. B. Ugarte Marin[1], C. J. Middleton[1], R. C. Chebel[1,2], and R. S. Bisinotto[1], [1]*Department of Large Animal Clinical Sciences, D. H. Barron Reproductive and Perinatal Biology Research Program, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Objectives were to evaluate the association between estrus characteristics and milk production around the time of estrus in lactating dairy cows. Estrus behavior was characterized between 80 and 225 DIM in lactating Holstein cows (n = 548) using collar-mounted activity and rumination monitoring devices. A total of 960 estrus events were included in the analyses, with the number of estrus per cow during the follow up period ranging from 1 to 9. Cows were milked thrice daily during the study and milk yield for individual shifts was recorded using on-farm milk meters. Estrus intensity (heat index; HI) was calculated using a proprietary algorithm (DataFlow II, Allflex Livestock Intelligence) and each estrus event was categorized as high (HI = 80) or low intensity (HI <80). Continuous outcomes were analyzed using ANOVA for repeated measures. Statistical models included the fixed effects of DIM at onset of estrus, estrus intensity category (high vs. low), milking shift relative to onset of estrus (−10 to +10; onset of estrus defined as shift 0), parity (primiparous vs. multiparous), and the 2-way interaction between estrus intensity category and milking shift relative to onset estrus, whereas estrus event was included as a random effect. Estrus intensity category (high intensity = 15.7 vs. low intensity = 15.7 kg/shift; $P = 0.89$) and the interaction between estrus intensity category and milking shift relative to onset of estrus ($P = 0.88$) were not associated with milk yield. Shift relative to onset of estrus was associated ($P = 0.04$) with milk production, as consecutive declines in milk production were observed between shifts −2 (16.2 kg) to −1 (15.9 kg) and between shifts −1 and 0 (14.9 kg). To conclude, a decline in milk production was observed in the 2 shifts before estrus, however estrus intensity was not associated with milk yield.

Key Words: estrus behavior, milk production, reproduction

2416 Effect of treatment with GnRH seven days after artificial insemination on interval to reinsemination and pregnancy per artificial insemination in lactating dairy cows.

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Objectives were to evaluate interval to return to estrus, estrus characteristics using collar-mounted activity/rumination monitoring devices, and pregnancy in lactating dairy cows treated with GnRH 7 d after AI. Daily cohorts of cows were blocked by parity and AI number on d 7 after previous AI (PreAI = d 0) and randomly assigned to receive 172 µg of gonadorelin on d 7 (GnRH7; n = 1,076) or to remain as untreated controls (CON; n = 1,087). Ovaries were scanned on d 7 and 14 to determine ovulation in a subset of cows (GnRH7, n = 139; CON, n = 143). Cows detected in estrus after PreAI (ED; n = 515) were reinseminated (PostAI) on the same day whereas cows not detected in estrus received timed AI (TAI; n = 501) following nonpregnancy diagnosis 33 ± 3 after PreAI. Binary outcomes were analyzed using logistic regression, time dependent variables using Cox-proportional hazard models and Kaplan Meier survival curves, and continuous variables using ANOVA. Treatment with GnRH increased ($P < 0.001$) the proportion of cows that ovulated between d 7 and 14 (79.1 vs. 1.4%). For PreAI, treatment did not affect ($P = 0.43$) P/AI on d 33 (GnRH7 = 49.1 vs. CON = 50.8%) or pregnancy loss between d 33 and 67 (GnRH7 = 5.2 vs. CON = 6.1%). The interaction between PreAI type (ED vs. TAI) and treatment tended to affect ($P = 0.09$) return to estrus, as treatment with GnRH reduced hazard of estrus in cows that received PreAI upon ED (AHR = 0.76; 95%CI = 0.60–0.97) but not TAI (AHR = 0.99; 95%CI = 0.83–1.19). Treatment did not affect ($P = 0.38$) the proportion of cows detected in estrus between d 8 and 17 (GnRH7 = 7.9 vs. CON = 8.0%), estrus duration (GnRH7 = 16.5 vs. CON = 16.3 h), or the proportion of cows with activity peak = 90 (GnRH7 = 84.4 vs. CON = 81.9%). For PostAI, GnRH treatment tended ($P = 0.10$) to increase P/AI on d 33 in cows reinseminated following ED (GnRH7 = 48.3 vs. CON = 42.0%), but not TAI (GnRH7 = 47.3 vs. CON = 46.3%). Treatment with GnRH did not affect ($P = 0.72$) pregnancy loss between d 33 and 67 after PostAI (GnRH7 = 3.7 vs. CON = 4.5%). Treatment with GnRH after AI did not affect return to estrus, but increased P/AI in cows reinseminated following ED.

Key Words: reproduction, ovulation, fertility

2417 Using drinking behavior as a tool to detect estrus in tie-stall-housed dairy cattle.

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The objectives of this study were to determine if drinking behavior changes at estrus and to investigate mount patches (MP) for estrus detection on tie-stall cows with daily exercise. Lactating cows (n = 31; 9 primiparous; 22 multiparous) were synchronized with a modified G7G Ovsynch (omitting the final GnRH) starting at 25 ± 3 DIM, then receiving 2 PG injections (Lutalyse, Zoetis, Canada) 2 and 4 wk after, resulting in 3 estrus events. Each cow had an individual water bowl and water meter. At each PG, a MP (ScratchE, Beacon Automation, Australia) was applied. Cows were let out to an exercise lot each morning for 2.5 h. The MP was scored daily: 1 = not scratched; 2 = <50% scratched; 3 = ≥50% scratched. Ovaries were scanned at PG, MP activation and 7 d later; if no MP activation, ovaries were scanned 5 d post-PG. Ovarian dynamics were used to determine ovulation and estrus. We assessed 3 water intake behaviors: daily intake, mean intake per hour, max intake per hour. Relative change (RC) to cow's baseline was calculated; baseline was created using 7 d prior. Data were assessed using linear mixed models and frequency tables in SAS. Of the 76 synchronized estrus events, 72 resulted in ovulation. Cows in estrus (irrespective of MP score) had lower daily water intake (108.5 vs. 115.7 L/d) but similar maximum water intake per hour (18.8 vs. 20.6 L/h) and mean water intake per hour (4.5 vs. 4.8 L/h). Cows had a RC in maximum daily intake of −7.15%, mean intake per hour of −7.14%, and maximum intake per hour of 12.3%, suggesting that cows do have reduced water intakes at estrus. Cows with MP activation had greater changes in behavior. Cows with MP score ≥2 had greater RC in maximum daily intake (−9.68% vs. −1.61%) and mean intake per hour (−9.86% vs. −1.27%), and smaller RC in maximum intake per hour (11.12% vs. 15.21%). 73% of estrus events were alerted using the MP, with 75% of ovulated events being alerted. Of ovulated events, 25.0% had a MP score of 1, 14.7% a score of 2 and 60.3% a score of 3. Changes in water intake behavior has potential to be used for estrus detection in dairy cattle. Additionally, MP have potential to be an estrus detection aid for tie-stall farms where cows have daily exercise.

Key Words: estrus detection, behavior, water

2418 Reproductive performance of lactating dairy cows managed with a targeted reproductive program aimed at increasing artificial insemination in estrus after synchronization of ovulation.

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We evaluated the effect on reproductive performance of a targeted reproductive management (TRM) program to increase AI in estrus (AIE) after ovulation synchronization. Holstein cows were randomly assigned to the G80 (n = 1,032) and TRM (n = 1,030) treatments (Trt). For first AI (1AI), cows in G80 received Double-Ovsynch with delayed induction of ovulation at the second PGF_{2a} (PG) of the Breeding-Ovsynch (B-Ov) part [GnRH(G)-7d-PG-3d-G-7d-G-7d-PG-1d-PG-2d-G-16h-AI]. Cows with automated estrus alerts were AIE. Cows in TRM were AIE for 7 d after the first PG of B-Ov and if not AIE received Ovsynch plus progesterone (P4-Ov; G+P4in-7d-PG+P4out-1d-PG-32h-G-16h-AI). For subsequent AI (2+AI) cows were AIE if had an estrus alert or received TAI based on ovarian structures if open. Cows with a CL = 15 mm and follicle = 10 mm received a Short-Resynch protocol as follows: G80: PG-1d-PG-56h-G-16h-AI and TRM:PG-1d-PG-6d AIE-P4-Ov if not AIE. Cows with No-CL received P4-Ov. Data were analyzed by logistic regression, ANOVA and Cox regression. The hazard ratio [HR: 0.9(0.8–1.0)] for time to pregnancy was greater for TRM than G80 ($P = 0.08$). Median and mean d to pregnancy were 96 and 113 for G80, and 96 and 109 for TRM. There was no Trt effect (G80 = 84.5% vs. TRM = 86.6%; $P = 0.17$) for percent cows pregnant by 200 DIM but cows in TRM (1.85 ± 0.05) had fewer ($P < 0.01$) AI than in G80 (2.03 ± 0.05). There was a Trt by parity interaction ($P = 0.04$) for 1AI pregnancy per AI (P/AI) at 29–35 d after AI as primiparous (Primi) cows in TRM (64.7%) had greater P/AI than all the other groups (Primi G80 [52.2%]; Multiparous [Multi] G80 [45.6%]; Multi TRM [48.2%]). For 2+AI, there was an interaction ($P = 0.02$) between trt and parity as Multi in G80 (37.8%) had fewer P/AI than Primi in G80 (53.4%) and TRM (48.9%), and Multi in TRM (46.7%). ATRM aimed at increasing AIE after ovulation synchronization had greater 1AI P/AI for primiparous cows and tended to have a greater pregnancy rate but did not increase the percentage of cows pregnant by 200 DIM compared with a fertility program with delayed induction of ovulation.

Key Words: targeted, estrus, synchronization

2419 Effect of different luteolytic strategies at the end of the breeding-ovsynch on fertility of lactating dairy cows submitted to first and subsequent artificial insemination.

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We aimed to determine the effect of doubling the cloprostenol (PGF) dose administered once or delaying the second PGF treatment at the end of Ovsynch on fertility of lactating dairy cows. Lactating Holstein cows (n = 1,223) submitted to a Breeding-Ovsynch [GnRH(G1) –7d– PGF(PGF1) –56h– GnRH(G2) –16h– AI] for first or ≥ second AI were randomly assigned to receive either CON (n = 405), DPG (n = 406), or PG56 (n = 412). Cows enrolled in CON received 2 PGF treatments (0.5 mg) 1 d apart (PGF1 –1d– PGF2), while cows in PG56 received a second PGF at the same time as G2 (PGF1 –56h– PGF2+G2). Cows in DPG were treated with a double dose of PGF (1 mg) only at PGF1. Cows were AI using either Holstein sexed (n = 473) or Angus conventional (n = 742) semen. Inseminations after tail paint estrous detection post-treatment were performed once daily. Pregnancy diagnoses (PD) were conducted with ultrasound at 32 and 67 d post-AI. Interbreeding intervals (IBI) between AI post-treatment and subsequent AI were classified as short (≤17 d), standard (≤18 d and ≥ 25 d), long (≤32 d and ≥ 26 d), and post-PD (≥32 d). Variables were analyzed using logistic regression. The proportion of cows bred 1 or 2 d before TAI did not differ among treatments ($P = 0.75$; CON: 2.2%, DPG: 2.7%, PG56: 2.0%). Treatment did not affect P/AI on d 32 ($P = 0.33$; CON: 52.9%; DPG: 50.7%; and PG56: 47.4%), but CON cows tended to have greater d 67 P/AI than PG56 cows ($P = 0.07$; CON: 51.4% vs. PG56: 44.7%). Cows in DPG had similar d 67 P/AI to the other groups ($P > 0.33$; 48.0%). Pregnancy loss from d 32 to 67 was not different ($P = 0.29$; CON: 2.8%; DPG: 5.4%; and PG56: 5.7%). Finally, treatment did not affect the proportion of cows in each category of IBI ($P = 0.66$; short: CON: 4.6%, DPG: 6.3%, PG56: 5.5%; medium: CON: 28.4%, DPG: 29.7%, PG56: 27.4%; long: CON: 3.4%, DPG: 3.7%, PG56: 7.0%; and post-PD: CON: 63.6%, DPG: 60.4%, PG56: 60.2%). In summary, prolonging the interval between PGF treatments to 56 h tended to decrease 67 d P/AI compared with a 24-h interval. Yet, the fertility of cows treated with a single double PGF dose at PGF1 did not differ from the other treatments.

Key Words: luteolysis, prostaglandin $F_{2\alpha}$, fertility

2420 Insemination dynamics and fertility of lactating dairy cows resynchronized with or without gonadotropin-releasing hormone treatment 32 days after first timed artificial insemination.

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We hypothesized that resynchronization of lactating dairy cows with GnRH 32 d after the first timed AI (TAI) would decrease the proportion of nonpregnant cows artificially inseminated (AI) after a detected estrus and increase pregnancies per AI (P/AI) for cows completing a Resynch protocol. Lactating Holstein-Jersey crossbred cows (n = 1,787) were submitted to a Double-Ovsynch protocol for first TAI (d 0) and were randomly assigned (odd vs. even ear tag) within parity to receive GnRH 32 d after TAI (G32, n = 892) or to serve as untreated controls (NG32, n = 895). Nonpregnant cows (n = 912; G32, n = 461; NG32, n = 451) were twice-daily detected for estrus and inseminated (d 16 to 39) or were diagnosed nonpregnant (d 39) with a corpus luteum to complete a Resynch protocol (d 39 and 40 $PGF_{2\alpha}$; 32 h GnRH [d 41]; 16 h TAI [d 42]). All cows were inseminated with sexed, conventional, or beef semen according to the genetic plan of the farm, and semen type was allocated evenly between treatments. Binomial variables were analyzed by logistic regression using the GLM function of R with the final model including the fixed effects of treatment and parity. Overall, P/AI was 48.9% at first service and did not differ ($P = 0.56$) between treatments, and primiparous cows had more ($P < 0.01$) P/AI than multiparous cows (55.1 vs. 46.8%, respectively). Fewer ($P < 0.001$) G32 cows were detected in estrus and inseminated after first TAI than NG32 cows (73.1 vs. 82.7%, respectively) and fewer ($P < 0.001$) multiparous than primiparous cows were inseminated (75.7 vs. 85.4%, respectively). For cows completing the Resynch protocol, P/AI did not differ between treatments (39.2 vs. 40.6% for G32 vs. NG32 cows, respectively) or parity (30.4 vs. 38.9% for primiparous vs. multiparous cows, respectively). In conclusion, treatment with GnRH 32 d after first TAI decreased the proportion of nonpregnant cows inseminated after a detected estrus, whereas treatment did not affect P/AI for cows completing the Resynch protocol. *Supported by NIFA USDA CARE project 2021–68008–34105.*

Key Words: Resynch, pregnancies per AI, estrus

2421 Effect of prioritizing artificial insemination in estrus or timed artificial insemination and milk yield on cash flow of primiparous cows.

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Our objective was to evaluate the effect of reproductive program and milk yield (MY) during first lactation on cash flow (CF). Holsteins at 6 farms were randomly assigned to a treatment (Trt) that favored timed AI (TAI) and had an extended voluntary waiting period (VWP; P-TAI n = 1,278) or a program that favored AI in estrus (AIE) and used TAI for cows not AIE (P-AIE n = 1,387). Cows in P-AIE were AIE for 21 d after a PGF2α at 53 DIM and if not AIE were TAI after Ovsynch with progesterone (P4-Ov). Cows were re-AI by AIE for 32 d post-AI. If not AIE, were TAI after P4-Ov at 42 d post-AI. Cows in P-TAI were TAI at 84 DIM after Double-Ovsynch. Cows were re-AI by AIE for up to 32 d post-AI. If open at 32 d post-AI and had a corpus luteum, cows completed Resynch25 (TAI 35 d post-AI). Cows open with no corpus luteum received TAI after P4-Ov (TAI 42 d post-AI). The CF per slot generated for 28-mo after first calving was the aggregation of income over feed costs (IOFC), calf value (CV), and replacement (RC), reproductive (RPC), and operating costs. Data were analyzed by ANOVA with farm as random, and Trt, MY group based on 305 d MY (H-MY, M-MY, L-MY), and their interaction as fixed effects. Genomic DPR (gDPR) group (Hi, Med, Low) was a covariate. Data presented are LSM ± SEM ($/slot). No Trt effects ($P > 0.10$) were observed for IOFC (P-TAI 5,047 ± 82, P-AIE 5,039 ± 82), RC (P-TAI 786 ± 50; P-AIE 790 ± 50) or CV (P-TAI 138 ± 2; P-AIE 143 ± 2). The RPC tended to differ ($P = 0.07$; P-TAI 150 ± 3; P-AIE 146 ± 3) but CF did not differ by Trt ($P = 0.57$; P-TAI 1,477 ± 82; P-AIE 1,474 ± 82). Milk yield affected ($P < 0.01$) IOFC (H-MY 5,693 ± 83; M-MY 4,985 ± 83; L-MY 4,452 ± 83), CV (H-MY143 ± 2; M-MY 144 ± 2; L-MY 134 ± 2), RC (H-MY

702 ± 53; M-MY 693 ± 53; Lo-MY 969 ± 54), and CF (H-MY 2,211 ± 86; M-MY 1,516 ± 86; L-MY 699 ± 86) but not RPC. There was a Trt by MY interaction ($P = 0.02$), as the H-MY group in P-TAI (2,309 ± 97) had more CF than in P-AIE (2,112 ± 96). The Hi gDPR group had less ($P < 0.01$) IOFC, RC, and RPC and more CV than the other groups. Overall, MY and gDPR were associated with most outcomes. The observed CF differences may support reproductive management programs that prioritize TAI with longer VWP for primiparous cows with high MY.

Key Words: reproduction, profitability, cow

2422 Timing of nadir weight attainment and its implications for fertility and milk production in dairy cows.

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Understanding the variability in postpartum body weight loss among dairy cows is crucial for effective herd management. This study aimed to investigate the correlation between the timing of nadir weight attainment, pregnancy at first AI, and pregnancy losses at 60 d after AI. We analyzed daily body weight data from 610 cows using the AfiFarm system until 249 DIM. Cows were categorized into early (≤57 DIM; n = 454) and late (>57 DIM; n = 156) nadir weight attainment groups. Receiver operating characteristic (ROC) curve analysis established a cut-off of 57.5 DIM (sensitivity: 79.8%; specificity: 34.63%; AUC: 0.56; $P < 0.02$), indicating the potential utility of this metric in understanding postpartum body weight dynamics and fertility outcomes. Cows reaching nadir weight at first AI achieved it at 44.9 d if pregnant and 54.9 d if nonpregnant. Mixed model analysis revealed a significant difference in milk production between late (9,122.63 kg) and early (7,879.57 kg) groups ($P < 0.01$), as well as between primiparous (7,383.22 kg) and multiparous cows (9,250.48 kg; $P < 0.001$). However, the Group and Parity interaction did not achieve statistical significance ($P = 0.08$). Comparisons considering milk yield indicated differences between late high (LH) to late low (LL) and early low (EL; $P < 0.05$), but not between early high (EH) and LH. The EL differed from LH and was equivalent to LL, with LH showing no significant difference from LL. Furthermore, chi-squared testing revealed a higher pregnancy rate at d 60, as well as higher pregnancy losses at 30 to 60 d ($P < 0.05$) in the Early group. The interaction between parity and group underscored their dependence, emphasizing their role in influencing pregnancy outcomes. These data suggest the potential use of nadir weight in predicting conception chances at the first service and pregnancy losses at 60 d after AI, with implications for milk production and reproductive success.

Key Words: conception, pregnancy, negative energy balance

Ruminant Nutrition 3: Calves and Heifers

2423 Effects of trace mineral source on growth and performance of Holstein calves raised in automated milk feeder. T. B. Burrell* and S. H. Ward, *North Carolina State University, Raleigh, NC.*

The objective was to evaluate the effect of trace mineral source fed in milk replacer on the growth and performance of Holstein calves raised in an automated milk feeder (AMF; DeLaval CF1000+, DeLaval, Sweden). At birth, calves were assigned to either treatment 1) Control, (CON, n = 20) 28% Fat, 20% Protein milk replacer with an inorganic trace mineral blend or 2) Glycine chelated mineral (GLY, n = 20) the same milk replacer with an organic trace mineral. Milk replacer was blended to provide 50 ppm of Mn and Zn, 10 ppm of Cu, and 100 ppm of Fe. Calves were placed in hutches and fed 3.4 L of colostrum in 2 feedings within 20 h of birth. They remained in hutches for 7 d then transferred into respective treatment pens until weaning (56 d ± 1.2 d). Daily intake of starter feed and milk replacer were recorded by the AMF. Growth parameters were measured bi-weekly until weaning and then weekly for 3 measurements. Blood was collected bi-weekly from birth until the final day on the trial and was analyzed for trace mineral concentrations. The MIXED procedure of SAS (Cary, NC) was used to evaluate the fixed effects of treatment, time (day or week), sex and interactions on daily milk and feed intake, growth parameters and blood trace mineral concentrations. The GLM procedure of SAS (Cary, NC) was used to evaluate the fixed effect of treatment and sex and interactions on average daily gain and feed efficiency. Significance was declared at $P < 0.05$. Means were separated by LSD. Daily DMI was greater in GLY fed calves compared with CON fed calves, 1.81 ± 0.013 kg/d and 1.41 ± 0.012 kg/d, respectively ($P < 0.0001$). CON calves had a greater post-wean ADG, 1.18 ± 0.09 kg/d, than those fed GLY, 0.98 ± 0.06 kg/d ($P = 0.0243$), causing calves fed CON to have a greater feed efficiency (feed:gain) than calves fed GLY, 2.00 ± 0.09 and 2.36 ± 0.06, respectively ($P = 0.0152$). Blood mineral concentrations and growth parameters were similar between the 2 treatments. In conclusion, calves raised on AMF fed GLY had similar growth and performance data as calves fed CON, with the exception of a greater overall feed efficiency in CON calves.

Key Words: calf, nutrition, trace mineral

2424 Effect of oral administration of *Megasphaera elsdenii* for young calves. R. D. F. Barboza[1], A. M. Cezar[1], I. M. Nascimento[1], N. A. Carpinelli[2], O. A. Sousa[2], J. Prado[3], and C. M. M. Bittar*[1], [1]*Dept. Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil,* [2]*Nutricorp, Araras, São Paulo, Brazil,* [3]*Axiota Animal Health, Fort Collins, CO.*

Previous studies have demonstrated that preweaning oral administration of *Megasphaera elsdenii* can increase starter intake and improve calves' growth performance. Fifty newborn colostrum-fed heifers were blocked according to birth weight and randomly assigned to 2 treatments: Placebo or Lactipro NXT (*Megasphaera elsdenii* NCIMB 41125, 10 mL, 5x10^9 cfu/animal, MS Biotec, Wamego, KS) in a dose of 10mL at 14 ± 1 d of age. Calves were fed starter feed and water free choice from day one and hay at the 9th week, just after weaning. During the first 20 d, calves were fed 6L/d of nonsaleable milk, from d 21 to 56 calves were fed milk replacer at a rate of 8L/d and then gradually weaned from 56 to 63 d. Data were analyzed as repeated measures using PROC MIXED from SAS 9.4. *Megasphaera elsdenii* oral administration at 14 d, when calves were consuming about 25 ± 51.1 g DM of starter, did not affect growth performance or feed intake in Holstein calves fed 8 L/d of liquid diet (Table 1). Results suggest that *Megasphaera elsdenii* supplementation in such young age for calves fed 8 L/d of liquid diet and presenting low starter intake does not benefit performance.

Key Words: probiotic, rumen microbiota, feed intake

2425 Effects of differential average daily gain in pregnant Holstein × Gyr heifers on digestibility and microbial nitrogen synthesis. A. P. Oliveira Neto[1], K. R. Oliveira*[1], A. L. Silva[1], M. I. Marcondes[2], S. E. F. Guimaraes[1], L. H. R. Silva[1], and P. P. Rotta[1], [1]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil,* [2]*Washington State University, Pullman, WA.*

The aim of this study was to evaluate 2 different ADG on digestibility and microbial N-synthesis during gestation in dairy heifers. Twenty heifers (5/8 Holstein × Gyr), aged 15 ± 2 mo, with a BW of 320 ± 15.0 kg were included in the study. The heifers received sexed female

Table 1 (Abstr. 2424). Performance and feed intake of calves supplemented with a culture of *Megasphaera elsdenii* at 14 d of age

	Treatment			*P*-value[1]		
Item	Placebo	Lactipro	SEM	T	A	T × A
BW, kg						
Average	62.7	62.7	2.23	0.9953	<0.0001	0.7156
Initial	36.9	36.6	2.12	—	—	—
8th week	72.3	72.0	2.33	—	—	—
Final	94.9	94.5	2.72	—	—	—
ADG, kg	0.694	0.702	0.0217	0.7714	<0.0001	0.4063
Feed intake, g of DM/d						
Starter feed	697	711	46.4	0.8067	<0.0001	0.9611
Liquid diet	658	655	5.2	0.6396	<0.0001	0.9363
Hay	82	80	3.9	0.7347	0.0283	0.3520
Total	1387	1404	45.3	0.7546	<0.0001	0.9538
Gain:feed	0.5	0.5	0.01	0.8387	<0.0001	0.7004

[1] T = treatment effect; A = age effect; T × A = interaction between treatment and age.

embryos 3/4 Holstein × Gyr. On the 70th day of gestation, heifers were randomly assigned to 2 treatment groups: moderate ADG (MOD, 0.37 kg) or high ADG (HIG, 0.72 kg). Body condition score (BCS), weight measurements, intake, and digestibility assessments for each heifer were conducted every 30 d. The feed amount offered was adjusted to achieve the specified ADG. Data analysis was conducted using the MIXED procedure of SAS (SAS Institute Inc., 2008). In cases where necessary, means were compared using the Tukey test at a significance level of 0.05, and trends were acknowledged at $0.05 < P \leq 0.10$. Heifers had similar BW ($P = 0.42$) at 70 d. As expected, HIG heifers exhibited the greatest ADG (0.72 *vs*. 0.37; $P < 0.01$), leading to a significant BW difference at 270 d of gestation (602.8 vs. 543.4; $P = 0.02$). They showed similar BCS at 70 d ($P = 0.67$), but HIG heifers had a greater BCS at parturition (3.70 vs. 3.23; $P < 0.01$). The intakes of dry matter, organic matter, crude protein, starch, and neutral detergent fiber increased ($P < 0.01$) throughout the months of pregnancy. Additionally, there was a difference ($P < 0.01$) in these intakes when comparing MOD and HIG heifers, as anticipated. No difference ($P > 0.05$) was observed in nutrient digestibility when comparing the MOD and HIG strategies. However, an interaction was observed ($P < 0.01$) for N of microbial (N-mic) origin produced daily. In mo 4 and 5 of gestation, N-mic was greater for heifers receiving the MOD diet. Nevertheless, no difference in the efficiency of microbial N-synthesis ($P = 0.32$) was observed between HIG and MOD heifers. The diverse ADG evaluated did not influence the digestibility and microbial N synthesis in 5/8 Holstein × Gyr dairy heifers.

Key Words: gestation, heifer, performance

2426 Performance and health of post-weaned Holstein heifers offered a low-moisture block self-fed nutritional supplement with or without menthol. J. Stypinski*[1], E. Dufour[2], A. Plumski[3], M. Robbins[4], J. Peine[4], A. Golombeski[2], B. Ziegler[2], and I. J. Salfer[1], [1]*University of Minnesota-Twin Cities, Saint Paul, MN,* [2]*Hubbard Feeds, Mankato, MN,* [3]*University of Minnesota Southern Research and Outreach Center, Waseca, MN,* [4]*Ridley Block Operations, Mankato, MN.*

Feeding antibiotics is known to increase calf growth, however public concerns about antibiotic resistance make the use of them unsustainable. Feeding natural products such as essential oils and their active compounds might be an alternative for reducing health incidences and promoting growth in calves. The objective of this study was to evaluate calf supplementation with menthol via a low-moisture salt lick block (LMB) on average daily gain, feed intake, and feed efficiency. Twelve pens of 7 post-weaned Holstein calves (60 ± 2.9 d old; mean ± SD; 83 ± 0.4 = 644 kg on d 1) were randomly assigned to 1 of 3 treatments: CTRL (no LMB), LMB (LMB without menthol), or LMBM (LMB with menthol). Targeted menthol intake was 2.5 oz per head per d. Intake of LMB was measured by subtracting final weight from initial weight of LMB weekly. Calves were administered treatments for 84 d. Feed intake was measured daily and body weight was measured on d 1, 28, 56, and 84. Data were analyzed using a mixed model with the fixed effect of treatment and random effect of pen, which was the experimental unit. No differences were observed for body weight ($P = 0.11$). Average daily gain was also similar ($P = 0.27$) for each of these time points. Menthol supplementation did not affect feed intake ($P = 0.12$), except for a tendency for lower ($P = 0.07$) overall grain intake from d 1 to 28 in LMBM (482 kg in LMBM vs. 486 kg in CTRL and LMB). No differences ($P = 0.30$) in feed efficiency were observed during any of the time periods of the trial. Furthermore, treatment did not affect incidence of treatment for disease ($P = 0.45$), despite a 50% numerical reduction in LMBM compared with CTRL and LMBM. Results suggest menthol supplementation of post-weaned calves was not successful in increasing energy partitioning toward growth at the expense of other energetic sinks.

Key Words: menthol, dairy calves, salt lick

2427 Health and performance of Holstein dairy calves fed titrated levels of a cheese ingredient in milk replacer. M. Suazo*[1], A. Plumski[3], D. Ziegler[2], B. Hansen[2], M. Scott[2], and I. J. Salfer[1], [1]*University of Minnesota, Department of Animal Science, Saint Paul, MN,* [2]*Milk Specialties Global, Eden Prairie, MN,* [3]*University of Minnesota Southern Research and Outreach Center, Waseca, MN.*

The objective of this study was to determine effects of increasing concentrations of dried cheese (DC) byproduct as a protein source within MR on health and performance of Holstein dairy calves from birth to weaning. One hundred Holstein heifer calves (age 2 to 5 d) with BW between 34.1 and 45.4 kg were randomly assigned to one of 4 treatments in a randomized block design: control MR with no DC (DC0), MR with 7% of diet DM as DC (DC7), MR with 14% DC (DC14), and MR with 21% DC (DC21). Dried cheese replaced whey protein within MR. The basal MR (24% CP 20% fat [tallow, lard, cheese, coconut]) was fed at 340.2 g (12.5% wt/vol in water) 2×/d from d1 to 42, and 1×/d from d 43 to 49. Calves were offered calf starter (CS; Elite 18 Calf Starter, Hubbard Feeds) and water ad libitum starting on d 1. Body weight was determined on d 1, 14, 28, 42, 49, and 56. Intake of MR and CS were determined daily and summarized biweekly; hip height was recorded on d 1, 49, and 56. Fecal scores were recorded daily. Data were analyzed using a linear mixed model with the fixed effect of treatment and random effects of source herd and nursery room. Initial BW was included as a covariate for BW, ADG, and feed intake. Daily MR intake from d 15 to 28 was decreased by DC14 and DC21 compared with DC0 (0.65 vs. 0.66 kg/d; SE = 0.002; $P = 0.02$) but did not differ across the entire preweaning period (0.59, 0.60, 0.60, 0.60 kg/d for DC0, DC7, DC14 and DC21 respectively; SE = 0.004; $P = 0.63$). Calf starter intake was not affected by treatment ($\mu = 1.1$ kg/d; SE = 1.00; $P = 0.87$). Average daily gain did not differ pre-weaning ($\mu = 0.58$ kg/d; SE = 0.04; $P = 0.88$), post-weaning ($\mu = 0.58$ kg/d; SE = 0.08; $P = 0.83$), or over the entire trial period ($\mu = 0.64$ kg/d; SE = 0.05; $P = 0.90$). Similarly, HH gain ($\mu = 11.6$ cm; SE = 0.27; $P = 0.81$), gain:feed ($\mu = 0.56$ kg/kg; SE = 0.023; $P = 0.73$), and average fecal score ($\mu = 1.32$; SE = 0.033; $P = 0.33$) did not differ among treatments; for DC0, DC7, DC14 and DC21 respectively. Results suggest that DC can replace other protein sources within MR without having negative effects on calf performance.

Key Words: milk replacer, dried cheese, calf performance

2428 High-quality forage as solid feed for unweaned dairy calves on accelerated rearing. N. Amaro*[1], V. Campbel[2], J. Dayuto[1], G. Antunez[3], and C. Cajarville[1], [1]*Departamento de Producción Animal y Salud de los Sistemas Productivos, Facultad de Veterinaria, Universidad de la República, Libertad, San José, Uruguay,* [2]*Independent practice, Montevideo, Uruguay,* [3]*Departamento de Ciencias Veterinarias y Agrarias-CENUR-LN, Facultad de Veterinaria, Universidad de la República, EEMAC, Paysandú, Uruguay.*

In an accelerated calf feeding system, milk, or milk replacer (MR) primarily supports high growth performance, while solid feed is necessary for digestive development. The aim of the study was to test whether high quality forage instead of concentrate fed enables similar nutrient intake, digestion, and body growth on Holstein calves reared in an accelerated feeding. Twenty calves (40 ± 3.4 kg of BW) with adequate passive transfer of immunity were enrolled in the experiment. Calves

Table 1 (Abstr. 2428). Intake and diet digestibility of dairy calves receiving concentrate (CON) or alfalfa hay (ALF) as a solid feed[1]

	Treatment			*P*-value		
Item	CON	ALF	SEM	T	W	T × W
Solid feed intake, g DM	610	450	47.3	<0.01	<0.01	<0.01
Total DM intake, g	1286	1113	48.6	<0.01	<0.01	<0.01
CP intake, g	253	219	8.5	<0.01	<0.01	<0.01
NDF intake, g	142	147	7.9	0.64	<0.01	0.93
ADF intake, g	87	126	12.0	<0.01	<0.01	<0.01
5th week OM dig, %	94.6	93.2	0.45	0.05		
10th week OM dig, %	74.0	66.3	4.64	0.24		

[1] dig = digestibility; T = treatment; W = week; T × W = treatment × week.

were housed individually and received 1000 g of MR DM/d (20% of initial BW) and were weaned during the 8th week of the study. Calves were blocked by birthdate and randomly assigned to ad libitum access to chopped alfalfa hay (ALF) or concentrate fed (CON) as solid feed. Feed, OM, CP, NDF, and ADF intake were measured daily. BW, hip height (HH), and ADG of BW and HH (HHg) were recorded weekly. Variables were analyzed as repeated measurements by PROC GLIMMIX of SAS, considering treatments, weeks, and blocks as fixed, and calves as a random effect. Calves in CON had greater solid feed ($P < 0.01$), total DM ($P < 0.01$), and total CP ingestion ($P < 0.01$) from wk 6 to 9 ($P < 0.01$), and less ADF intake ($P < 0.01$) on wk 8th of the study. We did not find differences in BW (61 ± 1.1 kg), HH (94 ± 0.6 cm), ADG (660 ± 44.5 g/d) or HHg (0.18 ± 0.019 cm/d) among treatments. Calves in CON had higher diet digestibility than ALF on wk 5 ($P = 0.045$), and no differences were detected on wk 10 (Table 1). Therefore, supplying only alfalfa hay as a solid feed before weaning reduced feed and nutrient intake and diet digestibility without differences in performance.

Key Words: dairy calves, chopped hay, digestibility

2429 Effect of a high-protein corn co-product as a replacement for soybean meal in calf starter grains.

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Inclusion of distiller's grains (DG) in calf starter grain (SG) limits growth, feed efficiency and digestibility. Fractionation technologies and addition of yeast bodies produce high-protein corn coproducts (HPCC) with high protein (50% CP) and less fiber content than traditional DG and may be a suitable alternative to soybean meal (SBM) as a protein source in calf SG. Our objective was to assess the value of a novel HPCC as a substitute for SBM in dairy calf SG. Forty-two Holstein heifers (n = 21) and steers (n = 21) were blocked by birthdate and sex and randomly assigned to a SG consisting of 0 (0CC), 50 (50CC) or 100% (100CC) replacement of SBM with HPCC (Protomax, ICM Inc.). Treatments were 16.6% of diet DM, and SG were supplemented with rumen-protected Lys and Met to meet AA requirements for a 110 kg calf. Calves were fed the assigned SG from 2 wk of age. Whole milk was fed according to birthweight until stepdown weaning at 6 wk of age. During the collection period from 8 to 12 wk of age calves were only fed SG. Dry matter intake was determined daily, and body weight, wither height, hip height, and girth circumference were obtained biweekly. Data were analyzed to assess treatment, sex, and week effects using PROC MIXED in SAS (v 9.4) and included orthogonal contrasts (linear; 0CC vs. 50CC and 100CC). An interaction of trt, sex, and time ($P = 0.03$) revealed that male 100CC increased DMI more over time compared with the other treatments. Contrasts showed that inclusion of HPCC tended to linearly increase DMI ($P = 0.08$). Increasing inclusion of HPCC linearly increased body weight ($P = 0.05$) and ADG ($P = 0.05$), and 50CC and 100CC tended to increase body weight ($P = 0.06$) and ADG ($P = 0.08$) compared with 0CC. There was an interaction of treatment and sex for gain:feed ($P = 0.04$) whereby males had greater feed efficiency than females for 100CC, but there was no evidence of difference between other treatments. Overall, increasing HPCC in calf SG increased body weight and ADG but did not affect feed efficiency in the postweaning period. These results suggest HPCC balanced for AA is a suitable replacement for SBM in SG.

Key Words: byproduct, calf nutrition, distiller grain

2430 Influence of average daily gain in pregnant heifers on subsequent milk production and composition.

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A pregnant heifer has specific nutritional demands compared with other categories. In addition to maintenance and gestation requirements, heifers are in a growth phase. A nutritional deficit in a heifer can result in losses that hinder the expression of its genetic potential, affecting productivity throughout its life. The objective of this study was to evaluate the effect of 2 ADG rates in pregnant heifers on their future milk production and composition. On the d 70 of gestation, 20 5/8 Holstein × Gyr heifers were placed in tie-stall and randomly assigned to one of the 2 ADG treatment groups: (1) moderate (MOD; 0.37 kg), and (2) high (HIG; 0.72 kg). After calving, a same diet was provided to all animals and they underwent 3 daily milking sessions, with their weekly MY recorded. Monthly assessments of milk composition were also conducted. Data analysis was conducted using the MIXED procedure of SAS (SAS Institute Inc., 2008). In cases where necessary, means were compared using the Tukey test at a significance level of 0.05, and trends were acknowledged at $0.05 < P \leq 0.10$. In terms of MY for a 305-d lactation period, no difference was observed between MOD and HIG groups ($P = 0.96$). Similarly, the MY for a 305-d corrected for 4% fat also showed no difference ($P = 0.49$). In terms of milk composition, MOD heifers exhibited a greater percentage of fat ($P < 0.05$), with no effect for protein percentage ($P = 0.43$). Total solids showed a tendency to be greater in the MOD group ($P = 0.07$). The HIG group exhibited a trend toward greater MUN ($P = 0.06$). No interaction ($P > 0.10$) was observed between treatments and the time of lactation for globulin, albumin, total proteins, glucose, thyroxine, and IGF-1. However, triiodothyronine exhibits an interaction ($P < 0.01$) between treatment during gestation and time of lactation, with greater values observed for the HIG group at 4 and 9 mo. Moderate ADG during gestation for 5/8 Holstein × Gyr heifers did not affect milk production, yet it led to higher fat percentage in the milk. This finding suggests that MOD strategy could be beneficial for crossbred dairy heifers.

Key Words: gestation, heifer, lactation

2431 Female dairy calves fed with a rumen-protected B-vitamin blend around weaning may have better odds of reaching first lactation and improving milk production. R. Roszkos[1], D. Bouchut[3], V. Asselstine[3], O. AlZahal[2], and V. Brisson*[3], [1]*ADEXGO Kft., Balatonfüred, Hungary,* [2]*Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada,* [3]*AlZahal Innovation & Nutrition, Kitchener, ON, Canada.*

This analysis aimed to follow up on a previous dairy-calf weaning study (Roszkos et al., 2022; JDS, 104[Suppl. 1:346]) to quantify the animals that reached their first lactation and milk production. In the previous study, 80 female dairy calves were allocated based on their date of birth into 4 consecutive batches containing 20 calves each. The batches were then assigned in an alternating sequence to receive either a 3g/d blend of rumen-protected B vitamins (RPBV; B1, B5, B6, B8, and B9) or no RPBV (control). The calves received their dams' milk in the first 4 d of age. Between 5 and 67 d of age, calves received 6 L/d (170 g/L DM) of milk replacer in 2 feedings. Then, from 68 to 75 d of age, they received 3 L/d of the milk replacer in 2 feedings. Solid feeds were given ad libitum to the calves. A calf concentrate was given from 10 to 30 d of age. From 31 until 75 d of age (weaning), a mash feed was offered. After weaning, calves received a dry TMR (refer to Roszkos et al., 2022, for dietary details). The RPBV was included in the mash diet for 21 d before weaning and the TMR for 21 d post-weaning (total of 42). In the previous study, calves that received RPBV gained 26% more than the control calves (+ 8.5 ± 1.4 kg, $P < 0.001$) during the 42-d duration of the trial and had better BW. The weaned calves remained on the same farm and were managed as one group of replacement heifers according to the farm standards (above TMR without B vitamins). We aimed to evaluate the carry-over effect of previous treatment on calves' ability to reach first calving and milk yield (up to 97 DIM)). Culling data were analyzed using Chi-squared, and milk data were analyzed using ANOVA with repeated measures using SAS Studio. Calves receiving RPBV during weaning were more likely to reach first lactation (90 vs. 75%, $P = 0.078$) and tended to produce more milk (36.9 vs. 35.1 ± 0.82, $P = 0.12$). In a previous study, calves receiving RPBV around weaning performed better. However, this follow-up study showed that those weaned calves had a better chance of reaching their first lactation and improving their milk yield.

Key Words: B-vitamin, calving, lactation

Ruminant Nutrition 3: Carbohydrates and Lipids

2433 Ensiled or finely-ground alpha amylase-enhanced corn grain for lactating dairy cows. W. R. Silva[1], A. J. C. Silva[1], M. A. Tiengo[1], J. P. A. Rezende[1], R. B. Silva[2], R. A. N. Pereira[3], T. J. DeVries*[4], and M. N. Pereira[1], *[1]Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, [2]Better Nature Research Center, Lavras, Minas Gerais, Brazil, [3]Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, Minas Gerais, Brazil, [4]Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.*

The objective of this experiment was to evaluate the effect of α amylase-enhanced corn (AAC. Enogen) as finely-ground (GCR) or rehydrated and ensiled (REC) kernels on lactational performance, ruminal fermentation, digestibility and chewing of dairy cows. Twenty-four individually-housed Holstein cows, arranged in 4 × 4 Latin squares (21-d periods), were exposed to each of 4 treatments in a 2 × 2 factorial combination of corn processing (P) and type (T): GRC vs. REC and AAC (48.8% vitreousness) vs. isogenic control (CTL. 51.1% vitreousness). Kernels were ground each week, hydrated (61.7 ± 0.6% DM) and ensiled for 28 ± 3 d. Diets had 13.0% corn and 22.4% starch on DM basis. Cows were fed once a day. The statistical model included the effects of square, cow(square), period, P, T, and P × T interaction. Milk yield (36.1 ± 1.02 kg/d) and DMI (22.4 ± 0.55 kg/d) did not differ ($P \geq 0.22$). Milk TS concentration decreased (12.55 vs. 12.61 ± 0.159%, $P = 0.05$) and milk fat concentration tended to decrease (3.68 vs. 3.74 ± 0.101%, $P = 0.06$) with REC relative to GCR. Cows fed REC tended to have higher milk/DMI than cows fed GCR (1.63 vs. 1.61 ± 0.037; $P = 0.06$). The GCR-CTL diet had higher meal size than REC-AAC and REC-CTL (2.5 vs. 2.3 and 2.2 ± 0.09 kg DM/meal, $P = 0.03$ P × T). Total-tract digestibility of nonNDF OM was higher on REC-AAC than GCR-AAC (83.4 vs. 81.3 ± 0.50%, $P = 0.05$ P × T). Cows fed AAC tended ($P \leq 0.07$) to have higher starch digestibility (93.7 vs. 92.9 ± 0.47%), and lower fecal starch concentration (4.31 vs. 4.78 ± 0.29%) than CTL. Ruminal microbial yield did not differ ($P \geq 0.42$). Molar proportion of isovalerate was higher on REC-AAC than REC-CTL and GCR-AAC (1.84 vs. 1.68, and 1.71 ± 0.064%, $P = 0.05$ P × T). Ruminal pH was higher on GCR than REC (6.83 vs. 6.73 ± 0.055, $P < 0.01$), and tended to be higher on CTL than AAC (6.82 vs. 6.74 ± 0.055, $P = 0.06$). The few interactions of P × T detected suggests that the effect of the amylase on digestion and lactational performance was not dependent of ensiling. Both REC and AAC reduced ruminal pH, but there was no major effects of P and T on ruminal fermentation. The AAC tended to increase starch digestibility and reduce fecal starch concentration.

Key Words: alpha amylase, corn processing, starch

2434 Impact of dried distillers grains levels in conjunction with low and high forage fiber contents on intake, digestibility, and sorting behavior of dairy cows. V. S. S. Viana[1], J. M. V. Pereira[2], M. I. Marcondes[3], S. C. Valadares Filho[1], E. Detmann[1], D. F. Quirino[1], and P. P. Rotta*[1], *[1]Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, [2]University of Vermont, Burlington, VT, [3]Washington State University, Pullman, WA.*

The aim of this study was to assess the influence of incremental levels of dried distillers grains (DDG) in diets combined with a high and low forage NDF (fNDF) on the intake, digestibility, and sorting behavior of dairy cows. Twelve Holstein cows (DIM 99 ± 24.5, BW 502 ± 27.9 kg, and MY 30.5 ± 1.36 kg/d) were allocated to replicated 6 × 6 Latin squares with a 3 × 2 factorial arrangement of treatments. Five experimental periods of 25 d each, with the first 14 d for cow adaptation and 11 d for data and sample collection were conducted. Diets were formulated with 0%, 20%, and 40% DDG on a DM basis, coupled with low forage (21% fNDF) or high forage (31% fNDF). All data were analyzed by SAS® software. For all analyses, multiple comparisons between treatments were performed using the "t" test, and differences were declared when $P < 0.05$. No significant interaction was observed between DDG level and fNDF level for DM, OM, and NDF intakes. However, an interaction was detected for CP intake between DDG and fNDF levels. Cows fed 0% or 20% DDG in low fNDF diets exhibited greater CP intake than those fed 0% or 20% DDG in high fNDF diets. Interestingly, no difference was observed for the 40% DDG level, regardless of the fNDF level. Concerning total-tract digestibility, an interaction between DDG and fNDF level was observed only for NDF. When cows were provided with low forage fiber diets, NDF digestibility exhibited a linear increase. Conversely, for cows on high forage fiber diets, the effect was the opposite, showing a linear decrease. No significant interaction between fNDF and DDG levels was noted for ruminal digestibility of DM, OM, CP, and NDF. A significant interaction effect was observed when evaluating sorting behavior through the particle separator of 19 mm. Cows fed low fNDF diets displayed more sorting tendencies when provided with 0% or 20% DDG compared with 40% DDG. However, for high fNDF diets, no significant effect was observed for varying amounts of DDG. This study emphasizes the importance of considering the fNDF levels in diets when working with DDG in dairy cows diet.

Key Words: byproduct, fiber, sorting

2435 Effect of partially replacing dietary corn grain starch with a rumen-protected lactose in lactating dairy cows. M. A. Taverna[1], R. B. Paez[1], M. E. Mainez[1], A. Smulovitz[1], J. A. Di Rienzo[2], J. P. Russi[3], and A. R. Castillo*[4], *[1]EEA Rafaela INTA, Rafaela, Argentina, [2]Universidad Nacional de Cordoba, Cordoba, Argentina, [3]RUPCA LLC, Merced, CA, [4]University of California Cooperative Extension, Merced, CA.*

The objective was to assess the response to replacing corn grain (CON) with rumen-protected whey permeate lactose (RPL [patent US10799519B2]) fed in pellets to 53 multiparous and 13 primiparous Holstein cows in an automatic milking system (AMS). The trial lasted for 120 d. The first trial day cows were paired based on lactation number, milk yield, and DIM and assigned to the treatments. New calvings were enrolled by lactation number. Pellets were fed by the AMS (70%) and in individual boxes (30%), comprising 64.8% corn grain, 14.2% soy hulls, 14.2% wheat bran, 7.5% mineral premix, and 0.02% monensin. The RPL treatment replaced 50% of corn grain in the CON pellet. All cows had daily access to alfalfa pasture (mean offered 4.5 kg/cow/d) and a partially mixed ration (average 16.9 kg/cow/d), consisting of 32.4% corn silage, 23.1% alfalfa hay, 11.9% cottonseed, 15.7% corn, 10.6% expeller soybean meal, 5% bypass soybean meal, and 1.2% bypass fat. Milk yield was assessed daily and milk composition weekly. Treatments were analyzed according to DIM divided into 3 periods from d 15 to 100 (P1), 101 to 200 (P2), and >201 (P3). The CON–RPL cows sampled in each period were 31–29, 25–25, and 15–14 for P1, P2, and P3. Milking frequency and BCS were recorded by the AMS. The BCS was estimated from daily 3D images of the cows' lower backs, with comparisons made in 15-d periods from 0 to 139 DIM. Data were analyzed by fitting a linear mixed model with treatments, DIM periods, and their interaction as fixed terms effects, and pairs, and cows within pairs

Table 1 (Abstr. 2435).

Diet (kg/d)	CON	RPL	$P <$
Pellets DMI			
P1	7.79	6.43	0.05
P2	5.99	7.32	0.05
P3	3.57	4.52	0.05
Overall	5.78	6.09	NS
FCM3.5%			
P1	40.17	43.90	NS
P2	35.80	43.19	0.05
P3	30.47	37.19	0.05
Overall	35.48	41.43	0.05

as random effects. Significance was declared at $P < 0.05$. The milking frequency increased ($P < 0.05$) from 2.35 (CON) to 2.47 (RPL). Overall pellet DMI and milk protein yield did not differ among treatments. Differences in BCS were nonsignificant, averaging CON 3.28 and RPL 3.19. The FCM3.5% increased when dietary corn grain was partially replaced with RPL (Table 1).

Key Words: dairy cow, whey permeate, bypass lactose

2436 Effect of rumen-protected sugar supplementation on milk performance and subclinical ketosis of dairy cows in the transition period.

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In the transition period, dairy cows are challenged by negative energy balance as well as negative glucose balance, which results in increased risk of subclinical ketosis. The objective of the present study was to determine the effects of supplementation of rumen-protected sugar (RPS) to improve milk performance and to lower the risk of subclinical ketosis in early lactation. In a herd of 638 Holstein cows, weekly data of milk yield, milk components, and DMI per pen were collected for 11 wk. In addition, blood BHB levels up to 35 DIM of animals that differed from the average milk yield were taken. After this initial 11 wk for collecting control data, all transition cows from 3 wk before until 3 wk after calving received the RPS product and data were collected for the subsequent 11 wk for treatment group. Cows were fed 150 g/cow/day RPS (LipoAktiv Glu 60, Berg + Schmidt GmbH & Co. KG, Hamburg, Germany) 3 wk before expected calving and 200 g/cow/d 3 wk after calving. The significance between means per treatment were determined using *t*-tests, and $P < 0.05$ was considered significant using R version 4.1.1. Milk performance of fresh cows (0–20 DIM) supplemented with RPS was significantly greater compared with control cows (40.6 vs. 38.0 kg/h/d, $P = 0.002$). There was no difference in DMI between treatments (25.5 vs. 24.8 kg/h/d, $P = 0.28$) or feed conversion rate (1.60 vs. 1.53, $P = 0.14$) in fresh cows. Supplementation of RPS had no effect on ECM yield (40.0 vs. 37.3 kg/h/d, $P = 0.3$) or milk solids (2.94 vs. 2.66 kg/h/d, $P = 0.34$) in lactating cows compared with control cows (each group averaged 165 DIM). There was, on average, less incidence of cows with blood BHB levels above 1.2 mM in the treated group compared with cows on the control diet, but the effect was not statistically significant (0.45 vs, 0.52, $P = 0.61$). In conclusion, feeding transition cows RPS improves milk yield in fresh cows, but had no significant effect on reduction of cases of subclinical ketosis.

Key Words: rumen-protected sugar, milk performance, subclinical ketosis

2437 The effects of fat supplementation on fecal bile acid concentrations in lactating dairy cows.

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Bile acids (BA) are emulsifying compounds that aid in lipid absorption in the intestine. Although bovine BA have been characterized from bile and plasma, data are limited on fecal BA concentrations. The objective of this study was to investigate fecal BA concentrations of cows fed fat supplements that differ in fatty acid profile. Eight primiparous and 8 multiparous Holstein cows (>70 DIM) were blocked by parity in a 4 × 4 Latin square design with 21 d periods. Treatments were (1) low-fat control (CON), (2) high-fat control (HF), (3) high oleic supplement (HO), and (4) high palmitic supplement (HP). Fecal samples were collected at 2000 h on d 20 of each period. Samples were snap-frozen in liquid N, stored at −80°C, thawed, diluted in methanol containing 70 internal BA standards, homogenized, snap-frozen and rethawed, incubated, and centrifuged to remove particles. The supernatant was analyzed via UHPLC-MS/MS on an ACQUITY BEH C8 column using 2 solvents (A = 10% acetonitrile in H_2O with 1mM ammonium acetate adjusted to pH 4.15 with acetic acid, B = acetonitrile and iso-propanol 1:1) in a gradient elution. Mass spectrometry was analyzed in negative ion mode and used electrospray ionization. Multiple reaction monitoring was optimized for each bile acid in bovine fecal samples before analysis. Peak integration was performed with TargetLynx 4.1. Data were analyzed in JMP Pro 17 and the model included the random effects of cow and period and the fixed effects of treatment, parity, and their interaction. Preplanned contrasts tested the effect of the addition of fat (CON vs. HF), increasing oleic acid (HF vs. HO), and increasing palmitic acid (HF vs. HP). In total, 27 BA were quantified. Increasing palmitic acid levels increased the fecal concentrations of 8 BA, including taurodeoxycholic acid, 3-dehydrocholic acid, and 12-dehydrocholic acid ($P < 0.01$). Increasing oleic acid level increased taurolithocholic acid and 3a, 12a, 23-nordeoxycholic acid ($P < 0.05$). Overall, fecal BA concentrations were quantifiable and some are modified by dietary fatty acid profile.

Key Words: cholic acid, palmitic acid, oleic acid

2438 The short-term effect of increasing doses of palmitic and stearic acid on milk fatty acid profile and milk fat thermal properties in Holstein cows.

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Palmitic and stearic acid are commonly fed to dairy cows, but there is limited data on their effects on thermal properties of milk fat, especially at different dose levels. Our hypothesis was that increasing palmitic acid intake would linearly increase palmitic acid in milk fat and increase percent solids of butter oil at 20°C, whereas increasing stearic acid intake would increase both stearic and oleic acid in milk fat and not change the percent solids of butter oil at 20°C. Twelve Holstein cows (106 ± 31 DIM) were arranged in a replicated 3x3 Latin square design with a dose escalation design within period and a ≥10-d washout between periods. Treatments included a no-supplement control (CON), a fatty acid (FA) supplement high in palmitic acid (PA; >80% palmitic), and a FA supplement high in stearic acid (SA; 80% stearic and 10% oleic). The FA supplements were fed at increasing doses every 4 d targeting 150, 300, 500, and 750 g/d. Milk samples were collected on d 3 and 4 of each dose, composited, and butter oil extracted from fat cake by centrifugation. Fatty acid profile was analyzed using GLC and melting properties using direct scanning calorimetry. Data were analyzed by ANOVA with preplanned contrasts testing CON vs. PA and CON vs. SA at each dose level. Palmitic acid treatment progressively increased

palmitic acid in milk fat, and SA treatment progressively increased both stearic and oleic acid ($P < 0.05$). At 750 g, PA increased milk palmitic acid 5.7 percentage units compared with CON (36.8% vs. 30.1%; $P < 0.001$), and SA increased milk stearic acid 2.4 percentage units and oleic acid 3.0 percentage units compared with CON (11.8% vs. 9.4% and 20.2% vs 17.2%, respectively; $P < 0.001$). The percent solids of butter oil at 20°C was linearly increased by PA but was decreased by SA. At the 750-g dose PA increased percent solids by 4.9 percentage units and SA decreased it by 3.4 percentage units compared with CON ($P < 0.001$). In conclusion, increasing palmitic acid intake increases percent solids of butter oil at room temperature and increasing stearic acid modestly decreases it, with differences likely due to differences in mammary desaturase enzyme activity.

Key Words: nutrition, lipid, butter

2439 Dietary starch content and fatty acid supplementation impact milk fatty acid yields and plasma metabolites and hormones during the immediate postpartum in dairy cows.

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We evaluated the effects of increasing dietary starch content and fatty acid (FA) supplementation on milk FA and plasma metabolites and hormones of early-lactation cows. Fifty-two multiparous cows were used in a randomized complete block design with a 2 × 2 factorial arrangement of treatments. Treatment diets were fed from 1 to 24 DIM and contained 22% or 28% DM starch (LS and HS) and 0% or 2% DM supplemental FA (NF and HF). The FA supplement was a Ca-salt containing 70% palmitic and 20% oleic acid. Treatment diets were formulated to contain 17% CP and 22% forage NDF. The statistical model included the random effect of block, cow within block and treatment, Julian date, and the fixed effects of starch content, FA supplementation, time, and their interactions. Results are presented in the following order: LSNF, HSNF, LSHF and HSHF. Higher dietary starch increased the yield (g/d) of t10 C18:1 (4.62, 5.55, 4.96, 5.81; $P = 0.07$) but had no effect on de novo, mixed, or preformed FA ($P > 0.18$). FA supplementation increased the yields of mixed FA (592, 625, 700, 710; $P < 0.01$), C4:0 (65.8, 69.2, 72.8, 71.9; $P = 0.04$), and C16:0 (550, 579, 657, 669; $P < 0.01$), decreased de novo FA (396, 428, 367, 376; $P < 0.01$), and had no effect on preformed FA ($P = 0.67$). Higher dietary starch increased plasma BHB (9.74, 7.76, 12.1, 9.60 mg/dL; $P = 0.02$), tended to increase insulin (4.64, 3.83, 3.26, 3.10 µIU; $P = 0.09$), but had no effect on glucose and NEFA ($P > 0.11$). FA supplementation decreased plasma glucose (57.0, 57.7, 53.8, 56.2 mg/dL; $P = 0.02$) and insulin ($P < 0.01$), and increased NEFA (0.52, 0.52, 0.66, 0.66 mmol/L; $P < 0.01$) and BHB ($P = 0.03$). There were no interactions between dietary starch content and FA supplementation for milk FA yields or plasma variables. In conclusion, higher dietary starch content increased the yield of t10 C18:1 in milk, increased plasma BHB, and tended to increase plasma insulin. Fatty acid supplementation increased the yield of mixed FA in milk, plasma NEFA, and BHB, and decreased plasma glucose and insulin.

Key Words: starch, early-lactation, fatty acid

2440 Effect of abomasal infusion of docosahexaenoic acid on adipose tissue in lactating dairy cows.

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Excessive lipolysis during the transition period induces a remodeling process in adipose tissue (AT) characterized by an inflammatory response. Docosahexaenoic acid (C22:6 n-3; DHA)is an anti-inflammatory n-3 fatty acid in humans and animals. However, it is unknown if DHA modulates AT function and inflammatory responses in dairy cows. Our objective was to evaluate the effects of DHA on AT insulin sensitivity and macrophage abundance in midlactation dairy cows. Eight ruminally cannulated multiparous Holstein cows (97 ± 37 DIM, 49.2 ± 3.3 kg/d milk) were blocked by milk yield and assigned to treatment sequence in a 4 × 4 Latin square design. Cows were infused abomasally with 0, 2, 4, or 6 g/d DHA for 11 d, with 10-d washouts between infusion periods. Subcutaneous AT was collected on d 11 of each period. Isoproterenol (ISO, 1 µ*M*) stimulated lipolysis and insulin (1 µg/L) inhibition of ISO were determined using an ex vivo explant culture by measuring glycerol release in media (µ*M*/mg AT) using Glycerol-Glo assay. Macrophage abundance was evaluated by immunohistochemistry using CD172a antibody and images were analyzed in ImageJ to quantify CD172a signal intensity (total area, µm^2). The statistical model included the fixed effects of infusion, ex vivo treatments, and their interaction, and the random effect of cow, square, and block. Isoproterenol induced lipolysis (4.82 ± 0.12 vs. 1.00 ± 0.09 unstimulated) in all AT explants ($P < 0.001$). Additionally, insulin decreased (2.38 ± 0.11; $P < 0.001$) this ISO-stimulated lipolysis, which was not affected by DHA treatment, indicating no effect of DHA on insulin sensitivity. Increasing DHA dose (0, 2, 4, 6 g/d), linearly decreased AT macrophage abundance (0.17, 0.11, 0.10, 0.06 ± 0.03, respectively; $P = 0.01$). Our results suggest that DHA did not alter lipolytic and insulin responses in AT in midlactation cows. However, it decreased macrophage trafficking into AT. Studies on the DHA effect during times of AT inflammation and insulin resistance, such as the transition period, are warranted.

Key Words: omega-3, adipose tissue macrophage, insulin sensitivity

2441 Effect of dietary inclusion of high oleic acid soybeans on de novo and preformed milk fatty acids in high-producing dairy cows.

A. M. Bales* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of increasing dietary inclusion of roasted and ground high oleic acid soybeans (HOSB) on milk fatty acid (FA) yields of high-producing dairy cows. Twenty-four multiparous Holstein cows (50.7 ± 4.5 kg/d of milk; 122 ± 57 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were increasing doses of HOSB at 0, 8, 16, and 24% of diet DM. High oleic acid soybeans replaced conventional soybean meal and soyhulls to maintain diet nutrient composition (% of DM) of 28% NDF, 20% forage NDF, 27% starch, and 16% CP. Total FA content of each treatment was 1.7%, 3.1%, 4.5%, and 5.8% of diet DM, respectively. The statistical model included the random effect of cow within square and the fixed effects of treatment and period. Preplanned contrasts included the linear (L), quadratic (Q), and cubic (C) effects of increasing HOSB. Results are presented in the following order: 0%, 8%, 16%, and 24% HOSB. Increasing HOSB decreased yields (g/d) of de novo (464, 446, 412, 368; Q $P = 0.05$) and mixed milk FA (605, 510, 443, 386; Q $P < 0.01$) but increased preformed milk FA (519, 702, 835, 978; Q $P = 0.04$). Increasing HOSB increased yields (g/d) of C4:0 (42.3, 45.3, 46.7, 47.6; L $P < 0.01$), C18:0 (105, 143, 169, 188; Q $P = 0.02$), *trans*-10 (10.4, 13.9, 18.1, 18.3; Q $P = 0.04$), and *cis*-9 C18:1 (241, 371, 471, 562; Q $P < 0.01$) but decreased C16:0 (569, 484, 418, 364; Q $P = 0.01$). Increasing dietary HOSB increased 18-carbon delta-9 desaturase index (DI; 0.71, 0.73, 0.74, 0.74; Q $P < 0.01$) but decreased 16-carbon DI (0.059, 0.059, 0.058, 0.056; Q $P < 0.01$) and tended to decrease 14-carbon DI (0.092, 0.088, 0.089, 0.086; L $P = 0.06$). The transfer efficiency of dietary 18-carbon FA incorporated into preformed milk FA was 43%, 38%, and 37% and for absorbed 18-carbon FA was 62%, 60%, and 62%, for the 8%, 16%, and 24% treatments, respectively. In summary, increasing HOSB up to 24% of DM decreased yields of de novo and mixed milk FA, but increased preformed milk FA yield ~2-fold. Further research is needed to investigate nutritional strategies to avoid substitution of de novo for preformed milk FA when feeding HOSB.

Key Words: oleic acid, soybean, milk fat

2442 Increasing dietary inclusion of high oleic acid soybeans affects nutrient intake and digestibility of high-producing dairy cows.

A. M. Bales* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effect of increasing dietary inclusion of roasted and ground high oleic acid soybeans (HOSB) on nutrient digestibility of high-producing dairy cows. Twenty-four multiparous Holstein cows (50.7 ± 4.45 kg/d of milk; 122 ± 57 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were increasing doses of HOSB at 0%, 8%, 16%, and 24% of DM. High oleic acid soybeans replaced conventional soybean meal and hulls to maintain diet nutrient composition (% of DM) of 28% NDF, 20% forage NDF, 27% starch, and 16% CP. Total fatty acid (FA) content of each treatment was 1.7%, 3.1%, 4.5%, and 5.8% of DM, respectively. The statistical model included the random effect of cow within square and the fixed effects of treatment and period. Preplanned contrasts included the linear (L), quadratic (Q), and cubic (C) effects of increasing HOSB. Results are presented in the following order: 0%, 8%, 16%, and 24% HOSB. Increasing HOSB decreased intakes of DM (31.2, 31.3, 30.8, 30.5 kg/d; L $P = 0.01$) and NDF (9.29, 9.05, 8.75, 8.41 kg/d; L $P < 0.001$) and digestibility of DM (68.0%, 67.1%, 67.0%, 66.8%; L $P < 0.01$) and NDF (47.1%, 45.2%, 45.3%, 44.6%; L $P < 0.01$). Increasing HOSB increased intakes (g/d; all L $P < 0.001$) of 16-carbon (77.8, 96.9, 113, 131), 18-carbon (445, 875, 1,278, 1,609) and total FA (532, 990, 1,419, 1,858) but decreased digestibility of 16-carbon (72.6%, 68.5%, 66.5%, 64.6%; L $P < 0.001$), 18-carbon (78.0%, 71.3%, 67.4%, 64,1%; Q $P = 0.03$), and total FA (76.0%, 70.4%, 67.0%, 63.9;% L $P < 0.001$). Increasing HOSB increased absorbed 16-carbon (55.5, 66.2, 75.4, 83.9; g/d L $P < 0.001$), 18-carbon (330, 624, 861, 1,070 g/d; Q $P < 0.01$), and total FA (386, 698, 950, 1,173 g/d; Q $P < 0.01$). Oleic acid content in plasma lipids increased by 4, 10, and 14 g/100 g FA for the 8%, 16%, and 24% treatments, respectively. In summary, increasing HOSB up to 24% of DM decreased intake and digestibility of DM and NDF and increased FA intake. Despite the decrease in total FA digestibility, absorbed total FA increased by 312, 564, and 787 g/d, for the 8%, 16%, and 24% HOSB treatments, respectively.

Key Words: oleic acid, soybean, digestibility

Ruminant Nutrition 3: General

2443 Polyphenol-based sugarcane extract supplement as a strategy to mitigate methane in lactating Holstein cows. E. D. Marino[1], A. F. Toledo[1], L. Castelani[2], R. Marchetto[2], W. V. B. Soares[2], L. C. Roma Jr[2], and C. M. M. Bittar*[1], *[1]Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil, [2]Animal Science Institute, Secretary of Agriculture and Supply of the State of São Paulo, Nova Odessa, SP, Brazil.*

Polyphenols are used as ruminal fermentation modifiers to reduce methane emissions in dairy cows. This study aimed to evaluate the influence of a polyphenol-rich sugar cane extract on the production of short-chain fatty acids (SCFA) in relation to methane emissions. Sixteen multiparous Holstein cows, 8 per treatment, were blocked according to their average milk production (20 kg/d ± 4.1), DIM (120 ± 23 d), BW (547 ± 17.4 kg), and fed with TMR daily. A product based on polyphenol (treatment; TPM, Australia) or distilled water (control) was administered orally for 42 d at a dose of 50 mL. On d 0, 21, and 42, samples were collected 2 h after the morning feeding. The ruminal fluid to quantify SCFA was collected using an esophageal probe 2h after feeding. Methane emissions were measured using the (SF6) tracer and open-circuit respiration chamber techniques, with 7 d of apparatus adaptations and 6 d of data collection. Data were analyzed using PROC MIXED from SAS 9.4, considering significance when $P < 0.05$ and trend when $0.05 \geq P \leq 0.10$. There was a trend for a higher proportion of propionate (18.89 vs. 18.13 m*M*) and total SCFA (66.44 vs. 58.44 m*M*) for supplemented cows. Other SFCA did not have significant differences and methane emission between control and treatment was not different at 348.24 and 350.24 g/d. Given the results, it was possible to observe that although supplemented cows present a trend to fermentation profile differences, methane emissions were not changed, requiring further studies to understand the dose and period of supplementation effects.

Key Words: propionate, sustainability, ruminal fermentation

2444 Lactation performance and enteric methane emissions of dairy cows fed *Gracilaria parvispora* macroalga. D. J. Nelson*[1,2], K. F. Kalscheur[2], E. A. French[2], S. Gunter[3], A. Salmi[4], S. Cunningham[4], E. Simpson[4], M. Graham[4], S. Hamilton[4], and L. Gardner[4,5], *[1]University of Wisconsin–Madison, Madison, WI, [2]US Dairy Forage Research Center, USDA-ARS, Madison, WI, [3]Oklahoma and Central Plains Agricultural Research Center, USDA-ARS, El Reno, OK, [4]Moss Landing Marine Laboratories, San Jose State University, Moss Landing, CA, [5]California Sea Grant, Scripps Institute of Oceanography, University of California San Diego, La Jolla, CA.*

The use of macroalgae as a feed additive has emerged as a viable strategy to reduce enteric methane emissions from dairy cattle. The objective of this experiment was to investigate the inclusion of the temperate-grown macroalga *Gracilaria parvispora* (GP) on lactation performance and enteric emissions of lactating dairy cows. Twenty-four midlactation multiparous Holstein cows (mean ± SD; 100 ± 33 DIM) were assigned to one of 2 diets using a randomized complete-block design with a 2-wk covariate period followed by a 6-wk experimental period. Diets contained 36% corn silage, 24% alfalfa silage, and 40% concentrate on a DM basis. The treatments were control (CON; basal diet) or basal diet supplemented with 0.75% GP on a DM basis (TRT). Diets were formulated for an average of 16.8% CP, 29.0% NDF, and 27.0% starch. Emissions were measured using the GreenFeed System (C-Lock, Inc., Rapid City, SD). Data were analyzed using the MIXED procedures of SAS. There was a trend for DMI to be lower for cows supplemented with GP compared with CON (29.8 vs. 30.4 kg/d; $P = 0.09$.) Milk production averaged 47.1 kg/d and was not affected by treatment. Energy corrected milk (ECM) was similar for both CON and TRT (51.5 kg/d). Feed efficiency (ECM/DMI) tended be greater for cows supplemented with GP compared with CON (1.73 vs. 1.68; $P = 0.06$). Percentage of milk fat and protein were similar for both groups (4.21% and 3.09%, respectively). Compared with CON, cows supplemented with GP excreted lower levels of MUN (11.6 vs. 12.1 mg/dL; $P = 0.05$). Cows entered the study at an average of 676.9 kg BW and gained 26.6 kg by wk 6. Production of methane (CH_4) and carbon dioxide (CO_2) was comparable between both groups (469 and 1,4981 g/d, respectively). Similarly, there were no differences in methane intensity (CH_4/ECM) or yield (CH_4/DMI; 9.15 and 293 g/kg, respectively). These results suggest that supplementing GP at 0.75% of DM tends to reduce voluntary feed intake without adversely affecting milk production and does not effectively mitigate enteric CH_4 or CO_2 emissions.

Key Words: methane, seaweed, production

2445 Effects of brown macroalgae on enteric methane emissions and lactational performance of dairy cows. D. E. Wasson*, C. V. Almeida, S. F. Cueva, C. J. Eifert, S. Issabekova, L. F. Martins, A. Richards, N. Stepanchenko, and A. N. Hristov, *Pennsylvania State University, University Park, PA.*

This study investigated the effects of 3 brown macroalgae on productive performance and enteric emissions in lactating Holstein cows. Forty-eight cows averaging (±SD) 104 ± 57 DIM and 41.5 ± 9.2 kg/d milk yield (MY) were used in a randomized complete block design consisting of a 2-wk covariate and a 6-wk experimental periods. Basal diet fed to the cows consisted of 45% (dry matter basis) corn silage, 15% alfalfa haylage, and 40% concentrates. Treatments included control (basal diet, CON) or CON + 1.0% (feed dry matter basis) inclusion of *Alaria esculenta* (AE), *Laminaria digitata* (LD), or *Saccharina lattisima* (SL), replacing alfalfa haylage. Dry matter intake was recorded daily using the Calan broad-bent feeding system. Milk yield was recorded daily, and milk composition was analyzed weekly. Enteric gas emission was measured throughout the trial on a voluntary basis using the GreenFeed system. Data were analyzed using the MIXED procedure of SAS with treatment, week, parity and interactions in the model, and block and cow within block as random effects. Body weight, DMI, feed efficiency, MY and composition, and gas emissions were analyzed as repeated measures. There was no effect of treatment on DMI (26.9 ± 0.57 kg/d; mean ± SEM) or MY (42.3 ± 0.95 kg/d). However, milk true protein concentration was decreased ($P = 0.02$) 7% by LD in primiparous cows compared with their CON counterparts, without affecting milk protein yield. Additionally, milk urea N was decreased ($P \leq 0.02$) by AE and LD compared with both CON and SL, by 10% and 12%, respectively. Body weight and BW change were also not affected by treatment. There was no effect of treatment on daily CH_4 (433 ± 8.8 g/d), CH_4 yield (16.3 ± 0.40 g/kg of DMI) and intensity, CO_2, or H_2 emissions. Data suggest that low dietary inclusion of AE, LD, and SL is not detrimental, or beneficial, to cow performance and does not affect enteric gas emissions.

Key Words: macroalga, animal production, enteric methane

2446 Near-infrared reflectance spectroscopy models trained by novel neural network methods can predict apparent digestibility from diet and fecal spectra. J. Goeser*[1,3], D. Meyer[1], M. Rakhshanfar[2], R. Johnston[2], and J. Karlen[1], *[1]Rock River Laboratory Inc., Watertown, WI, [2]Fermentrics (Orugen), Arnprior, ON, Canada, [3]University of Wisconsin–Madison, Madison, WI.*

Machine learning analyzes data with numerous features and requires a multitude of sources and computations. Dimensionality reduction techniques improve efficiency by converting a high-dimensional data set into a lower-dimensional one while preserving the key data aspects. This technique reduces the number of variables in a training data set, improving model development. By projecting high-dimensional data into a lower-dimensional space, the original data set essential variation is maintained while checking the dimensionality of the data. The objective of this effort was to determine whether a hybrid approach to reduce dimensionality of combined spectra sources, TMR and manure, removing 50% of less informative spectra points (nm) using contribution coefficients in the final prediction and then applying a secondary orthogonal transform to further reduce the data for a deep learning model can successfully improve variance explained by models built to predict dairy cattle apparent digestibility coefficients. A total of 3,023 TMR sample spectra were collected, for all samples analyzed with chemistry methods for apparent digestibility, including nutrient and indigestible NDF as an internal marker, since 2018. The InfraSoft International Select function was used to identify 1,085 diverse TMR NIR spectra from this population. The NIR spectra for manure samples collected and submitted with TMR samples were linked together for this deep modeling effort. Following the reduced data dimensionality and subsequent deep learning model training, models trained with spectral data from both TMR and manure outperformed models trained with either TMR or manure data, with greater r^2 and lesser SE following cross validation. The r^2 following cross validation for apparent OM, CP, fat, NDF, and starch digestibility on average improved from roughly 30% with models trained using either TMR or manure data, to 42.6% for the hybrid approach. This effort demonstrates that a novel approach to reduce dimensionality in combined data sources can improve machine learning trained model performance.

Key Words: machine learning, apparent digestibility, data science

2447 Rumen methane production and whole animal energy utilization in lactating dairy cows fed Alga Bio 3.0. S. C. Sherwood*, A. L. Carroll, and P. J. Kononoff, *University of Nebraska–Lincoln, Lincoln, NE.*

Methane is an anthropogenic greenhouse gas and approximately 100 Tg is produced from beef and dairy cattle annually. Alga Bio 3.0 is a proprietary feed supplement containing stabilized bioactive bromoform which is known to reduce methane production in the rumen of cattle. The objective was to evaluate the effects of increasing inclusion of Alga Bio 3.0 on DMI, energy utilization, and milk production in lactating Jersey cows. Twelve midlactation multiparous Jersey cows were used in a quadruplicated 3 × 3 Latin square design consisting of 3 28-d periods using headbox-style indirect calorimetry. Treatments were structured so cows consumed increasing concentrations (DM basis) of Alga Bio 3.0: 0% (0CTRL), 0.46% (LOW), and 0.93% (HIGH). Data were analyzed with the GLIMMIX procedure in SAS (ver. 9.4) with fixed effects of treatment, square, and period nested in square and random effect of cow nested in square. No differences were observed in DMI, milk yield, or ECM, averaging 18.9 ± 0.56 kg, 28.4 ± 0.69 kg, and 35.1 ± 0.84 kg, respectively. Milk fat percentage linearly decreased with increasing Alga Bio 3.0 inclusion (5.22%, 5.04%, 4.92% ± 0.136%) and no difference was observed in milk fat yield (average 1.42 ± 0.048 kg). No difference was observed in DM, NDF, CP, starch, and fat digestibility, averaging 65.6% ± 0.50%, 43.8% ± 0.99%, 67.2% ± 0.64%, 94.3% ± 0.34%, and 74.0% ± 1.87%. Increasing Alga Bio 3.0 inclusion linearly reduced methane production in L/d and L/kg DMI (430, 392, 345 ± 22.0 L/d and 22.8, 20.6, 18.6 ± 1.04 L/kg DMI), and increased hydrogen gas production (none detected, 15.6, 62.4 ± 22.2 L/d). Increasing inclusion tended to linearly increase the concentration of metabolizable energy (ME; 2.49, 2.50, 2.55 ± 0.022 Mcal/kg). The efficiency of converting digestible energy to ME linearly increased (0.88, 0.89, and 0.89 ± 0.003 Mcal/Mcal). Using GC-MS, milk samples were analyzed for presence of bromoform residues, and none was detected. Results suggest that Alga Bio 3.0 can reduce methane production by up to 19.6% in lactating dairy cows, leading to increased efficiency of energy consumed.

Key Words: methane, bromoform, energy

2448 Evaluating the efficacy of plant extract supplementation on lactational performance and enteric methane emission in dairy cows based on corn and legume silages. A. T. Richards*[1], S. F. Cueva[1], C. J. Eifert[1], L. F. Martins[1], N. Stepanchencko[1], C. Vidal de Almeida[1], D. E. Wasson[1], H. Khelil-Arfa[2], and A. N. Hristov[1], *[1]Pennsylvania State University, State College, PA, [2]ADM, La Pièce, Rolle, Switzerland.*

The objective was to evaluate lactational performance and enteric CH_4 emissions of dairy cows fed a diet supplemented with a plant extract containing cinnamaldehyde, eugenol, and *Capsicum* oleoresin. Following 3-wk covariate period, 26 primi- and 22 multiparous Holstein cows averaging 106 ± 46 DIM and 44 kg/d milk yield (MY) were enrolled in a 15-wk randomized complete block design experiment. Cows were blocked in pairs based on parity, DIM, and MY during the covariate period, and randomly assigned to 1 of 2 treatments: (1) basal diet (CON); (2) basal diet supplemented with 47 mg/kg of DM of plant extract (CEC). The basal diet (NDF, 30%) was 58% forage (corn silage, 43% and alfalfa haylage, 15%) and 42% concentrate (ground corn grain, canola meal, soybean products, whole cottonseed). Enteric gas emission data were measured using 2 GreenFeed units. Data were analyzed using the MIXED procedure of SAS, fixed effects of treatment, week, and interactions. Block and block × treatment were random effects. Compared with CON, CEC had no effect ($P \geq 0.16$) on DMI (means 26.8 vs. 26.0 kg/d, for CON and CEC, respectively), MY (37.7 vs. 38.1 kg/d, CON and CEC, respectively), feed efficiency, BW, and BW change. Concentrations of milk fat, protein, lactose, and MUN were also not different ($P \geq 0.11$) between CON and CEC. However, milk fat yield was decreased ($P = 0.04$) 7% by CEC. Yields of other components were not affected ($P \geq 0.32$) by treatment. Cows receiving CEC tended to have lower ($P = 0.07$) ECM yield compared with CON cows. Daily CH_4 emission (440 and 436 g/d), CH_4 yield (16.4 and17.0 g/kg of DMI), and intensity per unit of MY were not different ($P \geq 0.16$) between CON and CEC. Methane emission intensity expressed on an ECM basis tended to be increased ($P = 0.08$) by CEC, compared with CON. In conclusion, CEC did not affect enteric CH_4 emission metrics and production variables in dairy cows, except it decreased milk fat yield. Further studies are needed to investigate effects of CEC in various dietary regimens.

Key Words: milk production, methane, essential oil

2449 Enteric methane production and milk production are not affected by feeding a mixture of essential oils to Holstein cows. C.

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This study examined the effect of feeding a mixture of essential oils (MEO) on milk production and enteric methane emissions of Holstein cows. Thirty-two multiparous lactating cows (DIM = 95 ± 15.4; milk yield = 47.7 ± 4.51 kg) were used in a completely randomized design. Cows were blocked (2 in each block) by DIM and milk yield, and fed a basal diet (i.e., TMR) consisting of 69.3% forages (corn silage, alfalfa silage, hay) and 30.7% concentrates, on a DM basis. Within each block, one cow was fed only the basal diet (i.e., control) and one cow received 1g/day the MEO (Agolin 101; Agolin SA, Bière, Switzerland). The feed additive was mixed thoroughly twice daily with a portion of the basal diet (i.e., carrier) and the mix was offered (i.e., 500 mg/meal) to the animal before delivering the basal diet. The experimental period consisted of 12 wk preceded with 3 wk of covariate. Intake and milk production were recorded daily, and milk was sampled weekly over 8 consecutive milkings to determine milk composition. Enteric CH$_4$ was determined over 4 consecutive days using respiration chambers at wk 1, 4, 8, and 12 after the covariate period. Data were analyzed (MIXED Procedure, SAS) using the repeated statement and adjusted for the covariate. Significant effect of MEO or MEO × sampling week interaction was declared at $P = 0.05$. Interaction between MEO and sampling week was not significant for any response. Intake averaged 27.5 (±0.17) kg of DM/d and was not affected by feeding MEO. Milk yield (43.8 kg/d), fat (3.88%), protein (3.25%), and lactose (4.57%) were unaffected by feeding MEO. Consequently, production efficiency was not affected by MEO and averaged 1.68 kg ECM/kg of DMI. Daily CH$_4$ emissions, CH$_4$ yield, and CH$_4$ intensity averaged 500 ± 7.0 g/d, 18.4 ± 0.20 g/ kg of DMI, and 10.9 ± 0.20 g/kg of ECM, and were unchanged by MEO. Results from this study show that this specific blend of essential oils fed at 1 g/d to dairy cows failed to affect milk production, milk composition, feed efficiency, and emissions, yield, and intensity of enteric CH$_4$.

Key Words: enteric methane, milk production, essential oil

2450 Replacing dietary ingredients with dried distillers grains with solubles in diets fed to lactating dairy cattle. I. Energy utilization, milk production, and methane production.

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When including dried distillers grains with solubles (DDGS) in a dairy diet, some studies observe increases in energy supply and milk production, and reductions in methane production. The objective of this experiment was to examine the effects of feeding DDGS in place of ground corn (GC), alfalfa hay (AH), or a combination of the 2 on energy utilization with indirect calorimetry, milk, and methane production. Twelve multiparous Jersey cows (98 ± 6.5 DIM and weighing 441 ± 26.9 kg) were arranged in a triplicated 4 × 4 Latin square design consisting of 4 periods of 28 d. Cows were randomly assigned to one of 4 treatments: CON (0% DDGS); R-Alf (13% DDGS with AH inclusion reduced from 16.6% to 8.36% of the diet); R-Gc (13% DDGS with GC inclusion reduced from 19% to 9.53% of the diet); R-GcAlf (6.5% DDGS with AH inclusion reduced from 16.6% to 13.9% of the diet and GC inclusion reduced from 19% to 16.3% of the diet). Diets were balanced to be isonitrogenous. Diet aNDFom was 28.6%, 26.9%, 31.9%, 29.2% and starch was 28.2%, 29.7%, 22.8%, 27.2% of DM for CON, R-Alf, R-Gc, and R-GcAlf respectively. Compared with CON, DMI increased when cows consumed R-Alf (18.2 vs. 19.4 ± 0.60 kg/d for CON and R-Alf, respectively), but cows consuming R-Gc or R-GcAlf (average 18.6 ± 0.60 kg/d) were similar to CON. Energy corrected milk yield increased with R-Alf (37.4 vs. 39.7 ± 1.07 kg/d for CON and R-Alf, respectively), but R-Gc or R-GcAlf (average 37.5 ± 1.07 kg/d) were similar to CON. Cows consuming R-Alf or R-GcAlf produced more milk protein than CON cows (1.01, 1.13, 1.06 ± 0.038 kg/d for CON, R-Alf, and R-GcAlf ,respectively), but milk protein yield in cows consuming R-Gc was similar (1.04 ± 0.038 kg/d). Treatments did not affect milk fat yield (average 1.55 ± 0.067 kg/d) or methane production (average 396.6 ± 19.96 L/d). No differences were observed for digestible energy, metabolizable energy, or NE$_L$ intake (54.3 ± 1.75, 48.3 ± 1.60, 34.2 ± 1.20 Mcal/d respectively). Results indicate that feeding DDGS in place of AH, GC, or a combination of the two does not affect energy supply or methane production, but milk production may be increased by replacing AH.

Key Words: DDGS, energy, milk production

2451 Replacing dietary ingredients with dried distillers grains with solubles in diets fed to lactating dairy cattle. II. Manure output and resulting biogas and methane production in an anaerobic digestor.

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Diet has an effect on how much enteric methane a dairy cow produces, but little is known about how it affects methane production when manure is used in an anaerobic digester. The objective of this experiment was to examine how dietary factors affect manure output and its subsequent methane production from anaerobic digestion. Manure samples were collected from a feeding trial with 12 lactating dairy cows in a triplicated 4 × 4 Latin square. Treatments were as follows: CON (0% dried distillers grains with solubles; DDGS); R-Alf (13% DDGS with alfalfa hay [AH] inclusion reduced from 16.6% to 8.36% of the diet); R-Gc (13% DDGS with ground corn [GC] inclusion reduced from 19% to 9.53% of the diet); R-GcAlf (6.5% DDGS with AH inclusion reduced from 16.6% to 13.9% of the diet and GC inclusion reduced from 19% to 16.3% of the diet). Diets were balanced to be isonitrogenous. Diet aNDFom was 31.9%, 29.2%, 28.6%, 26.9% of DM for R-Gc, R-GcAlf, CON, and R-Alf respectively. A biochemical methane potential test was conducted over 2 runs averaging 32 d. Observed methane production was corrected for the inoculum methane production. Compared with CON, manure output increased when cows consumed R-Gc (59.1 vs. 63.2 ± 1.89 kg/d for CON and R-Gc, respectively), but no difference was seen for R-Alf or R-GcAlf (average 60.2 ± 1.89 kg/d). Manure volatile solids (VS) output increased when feeding R-Alf (5.35 vs. 5.70 ± 0.169 kg/d for CON and R-Alf, respectively), but no difference was seen when feeding R-Gc or R-GcAlf (average 5.46 ± 0.169 kg/d). Treatment did not affect manure methane (average 279.7 ± 10.76 mL/g VS) or biogas production (average 444.2 ± 19.96 mL/g VS). Total manure methane potential increased when cows consumed R-Alf or R-Gc (1,464.4, 1,642.0, 1,571.7 ± 48.11 L/d for CON, R-Alf, and R-Gc, respectively), but no difference was observed for R-GcAlf (1466.1 ± 48.11 L/d). Results of this study indicate that feeding DDGS in place of other dietary ingredients has no effect on manure methane production in an anaerobic digester, but it may influence manure VS output.

Key Words: anaerobic digester, biochemical methane potential, manure

2452 Development of a dynamic energy-partitioning model for enteric methane emissions and milk production in cows using

data from indirect calorimetry studies. F. Díaz[1], M. Sánchez*[1], A. Sanna[2], and C. Fernández[2], [1]*Dellait Research Center, Brookings, SD,* [2]*Universitat Politècnica de Valencia, Valencia, 46022, Spain.*

This study addresses the challenge of large-scale measurement of enteric methane (CH_4) in dairy cows, a process known for its complexity, time-consuming nature, and high cost. Leveraging models as a cost-effective alternative, the primary aim was to develop a dynamic model quantifying energy in milk and losses in CH_4 and heat. Drawing data from 18 indirect calorimetry studies comprising 74 individual observations in dairy cows, the model incorporates DMI, NDF, and lipid content as explanatory variables. External evaluation, which included 124 individual observations from 33 nutrition experiments with CH_4 determinations, assessed the model's performance. The results indicate satisfactory predictive accuracy, with root mean square percentage error at 13% for energy in milk (E-Milk), 17% for heat production (HP), and 29% for CH_4 emissions (E-CH_4). Mean bias approximates zero for E-Milk, HP, and E-CH_4, and slope bias is negligible for E-Milk (7%), it is higher for HP (28%) and E-CH_4 (34%). Random bias exceeds 90% for E-Milk, suggesting nonsystematic errors, and the sum of mean and slope biases for HP and E-CH_4 is less than 50%, indicating proper representation of model mechanisms. The model demonstrates accuracy and precision in predicting E-Milk and E-CH_4 within specified energy ranges, underscoring its utility in varying nutritional conditions. Notably, the model underpredicted HP when energy was below 60 MJ/d. External evaluation revealed variations in CH_4 predictions across studies, influenced by methane measurement devices (SF6, Green Feed unit, environmental chambers, air flow chambers). This model represents a preliminary step toward dynamically describing CH_4 emissions in diverse nutritional contexts and offers potential applicability as a predictive tool in smart farming.

Key Words: methane, indirect calorimetry, dairy cow

2453 A comparison of equations used to estimate heat production in cattle using indirect calorimetry; integration of hydrogen production. A. L. Carroll* and P. J. Kononoff, *University of Nebraska–Lincoln, Lincoln, NE.*

Most studies that employ indirect calorimetry to study ruminant energetics estimate heat production (HP) using an equation published by E. Brouwer in 1965. Inputs into this equation include oxygen (O_2) consumption, carbon dioxide (CO_2) and methane (CH_4) production, as well urinary nitrogen losses (UN). In most cases, little-to-no hydrogen (H_2) is eructated by cattle, but this may increase when cattle consume feed supplements that reduce ruminal CH_4 production and, as a result, should be considered when HP is indirectly estimated. Using 10 observations from 3 published studies, a data set was compiled that included O_2 consumption as well as CO_2, CH_4, H_2, and urinary N (g/d) production at 5,958 ± 685.5 L/d, 6,649 ± 719.2 L/d, 473 ± 89.2 L/d, 62 ± 76.4 L/d, and 197 ± 42.0 g/d, respectively .Then HP was estimated using both equations. The first equation used to estimate HP was that proposed by Brouwer, and the second equation was modified to include the enthalpy and constants of H_2 oxidation (HP [kcal/d] = 3.866 O_2 [L/d] + 1.200 CO_2 [L/d] − 1.431 urinary N excretion −0.518 CH4 [L/d] − 1.087 H_2 [L/d]). Based on the derived total HP using both equations a paired sample *t*-test was used to determine whether differences were observed. As expected, volumes of H_2 produced were low in all studied, results indicated small but significant differences in HP summing to 304.9 ± 3.53 Mcal/d and 304.2 ± 3.52 Mcal/d for the 2 equations, respectively. More specifically, mean differences for HP (0.07 ± 0.086. Mcal/d) were significantly different and ranged from 0.005 to 0.238 Mcal/d. Furthermore, using the Bouwer equation HP was 0.23% lower than for the new equation. Differences indicate that not representing H_2 in equations to estimate HP are small but that in cases in which H_2 is increased, estimation of HP using the modified equation should be considered.

Key Words: energy utilization, methane, hydrogen

Ruminant Nutrition 3: Gut Physiology, Fermentation, and Digestion

2455 Combined mineral (copper, zinc, and manganese) effects on 48-hour in vitro fermentation and CH_4 production. C. B. Peterson*[1], G. M. Boerboom[2], J. S. Heldt[1], K. E. Griswold[1], and J. Johnston[3], *[1]Selko® USA, Indianapolis, IN, [2]Selko Feed Additives, Nutreco Nederland BV, Amersfoort, Utrecht, the Netherlands, [3]Fermentrics Technologies Inc., Arnprior, ON, Canada.*

The study objective was to examine the effects of combining Cu, Zn, and Mn from various sources on 48 h in vitro fermentation and CH_4 production. Supplemental trace mineral (STM) levels were calculated to be 125, 750, and 500 mg/d for Cu, Zn, and Mn, respectively, for a lactating dairy cow consuming 25 kg of DM with a 120 L rumen volume. The STM were scaled to fit a 250-mL fermentation vessel containing an 80:20 mix of KSU buffer and rumen fluid with 400 mg of 6-mm ground TMR inside 50-µm Ankom bags. Vessels were incubated for 48 h at 39.5°C. Due to restrictions on sampling number per run, treatments were split into 2 runs, with 6 replicates per treatment, as follows: Run 1: control (C1, no trace mineral), sulfate (SO_4, 1.04 mg Cu, 4.40 mg Zn, 5.04 mg Mn), Selko® IntelliBond (IB, 0.48 mg Cu, 2.84 mg Zn, 3.55 mg Mn), Nutrilock® Chemlock (NC, 0.45 mg Cu, 2.69 mg Zn, 3.55 mg Mn), Phibro Vistore (PV, 0.45 mg Cu, 2.65 mg Zn, 2.89 mg Mn). Run 2: control (C2, no trace mineral) and SAM Nutrition (SN, 0.45 mg Cu, 2.69 mg Zn, 3.19 mg Mn). Data were analyzed as a complete randomized design with PROC MIXED in SAS (SAS Institute Inc., Cary, NC). Significance was set at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.20$. In Run 1, NC ($P = 0.014$) resulted in higher apparent organic matter disappearance (aOMD) than the control. Whereas other treatments were not significantly different (NSD). The NC ($P = 0.049$) and PV ($P = 0.029$) treatments resulted in significantly lower apparent microbial biomass production (aMBP) than the control. In contrast, the IB treatment tended to increase aMBP ($P = 0.10$). Fast pool (FP) results increased with IB and NC ($P < 0.0001$) compared with the control, whereas the other treatments were NSD. For CH_4/OMD, IB, PV, and NC generated lower levels compared with control ($P = 0.0011$; $P = 0.0073$; $P = 0.0256$, respectively). In Run 2, SN produced significantly less aMBP than the control ($P < 0.001$). All other measurements were NSD. Overall, the effects of STM mix on fermentation varied greatly by source, with the IntelliBond mix preventing the negative effects of disassociated metals because of its lower reactivity.

Key Words: hydroxy trace mineral, fermentation, methane

2456 The effect of zinc source on 48-hour in vitro fermentation. C. B. Peterson*[1], G. M. Boerboom[2], J. S. Heldt[1], K. E. Griswold[1], and J. Johnston[3], *[1]Selko® USA, Indianapolis, IN, [2]Selko Feed Additives, Nutreco Nederland BV, Amersfoort, Utrecht, the Netherlands, [3]Fermentrics Technologies Inc., Arnprior, ON, Canada.*

The present study was designed to understand the effect of Zn source on 48-h in vitro fermentation parameters. The experimental unit (vessel) was incubated for 48 h in a 39.5°C insulated water bath. Due to restrictions on sampling number, treatments were split into 2 runs with 6 replicates per treatment, as follows: Run 1: control (C1, no trace mineral), sulfate Zn (SZ, 4.40 mg Zn), Zn oxide (ZO, 2.17 mg Zn), high oxide (HO, 0.32 mg Zn), and low oxide (LO, 0.75 mg Zn). Run 2: control (C2, no trace mineral), IntelliBond® Zn (IBZ, 2.84 mg), low oxide Chemlock® Zn (LCZ, 2.69 mg), and high oxide Chemlock Zn (HCZ, 2.69 mg). The supplemental trace mineral (STM) levels were scaled to fit a 250-mL fermentation vessel containing a mix of KSU buffer (80%) and rumen fluid (20%) with 400 mg of 6-mm ground TMR inside 50 ± 10-µm Ankom bags. Supplemental trace mineral levels were calculated to be 750 mg/d Zn (unless otherwise specified), for a lactating dairy cow consuming 25 kg of DM with a 120-L rumen volume. The LCZ and the HCZ were calculated to deliver 750 mg/d Zn as specified on the SDS as a 58% metal content. The HO and LO treatments were calculated to deliver Zn at the same rate as the oxide found in the LCZ and HCZ treatments. Data were analyzed as a complete randomized design with PROC MIXED in SAS (SAS Institute Inc., Cary, NC). In Run 1, there were no significant differences (NSD) for apparent organic matter disappearance (aOMD). All treatments (SZ $P = 0.002$, ZO $P = 0.017$, HO $P = 0.001$, LO $P = 0.020$) resulted in significantly lower apparent microbial biomass production (aMBP) than the control. In Run 2, there was a trend for higher aOMD with HCZ compared with the control ($P = 0.074$), and all other treatments were NSD. For aMPB, the IBZ treatment tended to be higher than the control ($P = 0.07$), and all other treatments were NSD. For CH4/OMD, HO showed significantly higher numbers than the control; all other treatments were NSD. These results suggest that Zn source may affect in vitro fermentation.

Key Words: zinc, hydroxy trace minerals, fermentation

2457 An in vitro rumen fermentation method to measure gas production while controlling gas concentrations. A. Tiwari* and R. A. Kohn, *University of Maryland, College Park, MD.*

Fermentation gas concentrations drive thermodynamic and kinetic control of fermentation. Previous research showed that infusing hydrogen gas into fermentation vessels increased methane production linearly and volatile fatty acid production nonlinearly in an apparently saturable process. However, it can be difficult to compare physiological changes in gases, particularly H_2, over longer-term experiments because fermentation produces and uses gases so quickly. We constructed an apparatus using solenoid valves on timers to perfuse gases in vitro intermittently and to collect produced gases between infusions. The rumen fluid from dairy cattle was collected, processed, and added (100 mL) into the flask containing ground orchard hay (1 mm; 1.5 g) after mixing (50:50) with in vitro media under CO_2. The flasks were kept in the incubator for 36 h at 39°C. The system was initially tested with 3 compositions of perfused gases with 3 replicates of each treatment. Every half hour from the start of the fermentation, fermentation gases were replaced with one of 3 infused gas compositions of CO_2 with 0%, 0.5%, and 1% H_2. The infusion was carried out automatically for each incubation flask using an automated process with solenoid valves on a timer that applied a vacuum to each flask and replacement gas. Gases produced between infusions were collected in mylar balloons connected directly to each flask with a one-way valve (to prevent mixing of infused gases), and samples were collected from produced gas during 8 repeated measurements (0, 2, 4, 6, 8, 12, 24, and 36 h). Gas was analyzed in gas chromatography, and data were analyzed in JMP pro 15 (SAS Institute Inc.). Increasing H_2 perfusion increased H_2 disappearance ($P < 0.05$) and CH_4 production ($P < 0.02$) at 4, 6, and 8 h. For example at 4 h, 0% H_2 infusion produced H_2 (0.01 mL, SE = 0.032 mL), and 0.5 and 1% H_2 infusion consumed H_2 (−0.08 and −0.14 mL, SE = 0.038 mL). Higher H_2 perfusion increased ($P < 0.02$) methane production at 4 h (0.42, 0.38, and 0.70, SE = 0.587 mL). The results show that intermittent infusion of H_2 increases the activity of methanogens.

Key Words: fermentation, headspace, gas infusion

2458 Higher initial hydrogen concentration accelerated methane production in vitro.

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Enteric fermentation in the rumen is key to providing substrate to produce several other end products of fermentation. During fermentation, bacteria, fungi, protozoa, and archaea determine the fate of feeds. Despite being the lowest number, archaea have the highest potential to utilize substrate hydrogen that produces methane and causes a loss of feed energy value. This study aims to understand the effect of initial higher hydrogen concentration on archaea and bacteria's biochemical pathways. The treatments were arranged in a completely randomized design in a factorial of 2 (2 levels of headspace 0% and 5% of H_2) × 5 replications. Wheaton bottles (150 mL) were anaerobically filled with rumen fluid (30 mL) and timothy hay (0.75 g). Initial H_2 gas was perfused into the respective bottles and kept in the incubator for 48 h at 39°C. Gas samples were collected at 10 min and then at 1-, 2-, 4-, 8-, 24-, and 48-h intervals. Gas samples were analyzed in a gas chromatograph, and data were analyzed in JMP pro 15 (SAS Institute Inc.). The hydrogen remained higher in the bottle with 5% headspace H_2 until 2 h of infusion time, and methane was higher at time point 10 min (0.0067 ± 0.0011 vs. 0.0024 ± 0.00102 mM, $P < 0.013$), 1 h (0.044 ± 0.0017 vs. 0.024 ± 0.0016 mM, $P < 0.001$), and 2 h (0.082 ± 0.0088 vs. 0.044 ± 0.0082 mM, $P < 0.009$) than 0% headspace H_2. Results showed that the higher initial H_2 was used entirely and reduced to a physiological level within 4 h of infusion time.

Key Words: hydrogen, methane, methanogen

2459 Effects of sprouted barley inclusion levels in dairy cow diets on ruminal fermentation in a dual-flow continuous culture system.

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This study aimed to evaluate the effects of 4 inclusion levels of hydroponic sprouted barley in dairy cow diets on in vitro ruminal fermentation. Eight dual-flow continuous culture fermenters were used in a replicated 4 × 4 Latin square. Treatments were a control diet with no hydroponic sprouted barley (CON), and partial replacement of ingredients (corn silage, alfalfa hay, grass hay, corn meal, soybean meal) with hydroponic sprouted barley at 10% (B10), 20% (B20), and 30% (B30) on a DM basis. Experimental diets were formulated to have similar nutrient composition: 16.0% CP; 30.5% NDF; 42.0% NFC; 26.2% starch; 8.0% water-soluble carbohydrates; and 1.7 Mcal/kg NE_L on a DM basis. Fermenters were provided with 106 g/DM each day divided into 2 feedings at 0800 and 1800 h. Experimental periods consisted of 10 d (7 d adaptation and 3 d sample collection). Composite samples of daily effluent were collected for analyses of VFA, NH_3–N, and lactate, nutrient digestibility, and N metabolism. Samples were collected from each fermenter at 0, 1, 2, 4, 6, and 8 h after feeding to determine kinetics of pH, NH_3–N, lactate, and VFA. Data were analyzed using the mixed procedure of SAS. The statistical model included the fixed effect of treatment, and random effects of fermenter(square), square, period, and day. Effects of time and treatment × time were evaluated for kinetics analyses as repeated measures. Increasing levels of hydroponic barley sprouts were evaluated using linear and quadratic orthogonal contrasts. No treatment effects were observed for pH ($P = 0.15$) and NH_3–N kinetics ($P = 0.54$), but a time effect was observed for both ($P < 0.01$), following typical microbial fermentation kinetics. There was a treatment × time interaction for pH kinetics ($P < 0.01$), with a greater pH for B30 compared with B0 (7.38 and 7.00 ± 0.08, respectively) only at 0 h after feeding. Our results demonstrate that including sprouted barley up to 30% of DM does not affect pH and NH_3–N kinetics. Further analyses on VFA, N metabolism, and nutrient degradability will improve our understanding of hydroponic barley fermentation characteristics.

Key Words: hydroponics, fermentation, degradability

2460 In vitro evaluation of barley, wheat, and triticale hydroponic sprouts on methane production, rumen fermentation, and nutrient degradability.

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This study evaluated the effects of hydroponics on CH_4 production and rumen fermentation in vitro. A batch culture experiment was conducted as a randomized complete block design with 3 fermentation runs (blocks). Treatments were corn silage as control, and hydroponic sprouts of barley, wheat, and triticale. Treatment nutrient compositions were as follows: corn silage (32.3% NDF, 8.4% CP, and 41.6% starch, 1.3% water-soluble carbohydrates [WSC]), barley (34.3% NDF, 17.1% CP, and 4.2% starch, 32.9% WSC;), wheat (23.7% NDF, 23.3% CP, and 8.5% starch, 32.7% WSC), and triticale (20.0% NDF, 16.2% CP, and 21.7% starch, 33.2% WSC) on a DM basis. Rumen content was collected from 2 rumen-cannulated lactating Holstein cows, filtered, and mixed in a 1:2 ratio with Van Soest medium. Two incubation setups were carried out in triplicate for 24 and 48 h at 39°C using serum bottles with 52 mL solution and 0.5 g DM treatment (within Ankom bags). Headspace pressure was measured at 0, 2, 4, 8, 16, 24, 36 and 48 h for CH_4 and total gas at 24 and 48 h. At 24 and 48 h, bottles were opened to measure pH and dry matter degradability (DMD). Data were analyzed with the mixed procedure of SAS, with hydroponic source as fixed effect, and run and bottle as random effects. Treatment effects were evaluated with Tukey-Kramer multiple comparisons. Significance was declared at $P \leq 0.05$. Table 1 shows treatment effects on pH, DMD, CH_4, and CH_4 intensity at 24 and 48 h. Our results show that wheat and triticale have greater digestibility at 24 and 48 h. Further analysis on VFA will provide a better understanding of the fermentation characteristics of hydroponic sprouts.

Key Words: hydroponics, methane, fermentation

2461 The effects of increasing inclusion of a fat-protein matrix on methane production, ruminal fermentation parameters, and nutrient degradation in ruminal batch culture.

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Omega-3 fatty acids (FA) have been demonstrated to be anticarcinogenic and prevent cardiovascular disease in humans. An added benefit may be the reduction of methane produced within the rumen due to the increased levels of PUFA. Therefore, the objective of this study was to determine the effects of feeding LinPRO-R, a rumen-protected matrix of fat and protein from flaxseed and peas (LIN; approximately 21% fat, 24% CP, and 5.4% omega-3 FA; % of DM), using in vitro batch culture incubation. Treatments were: 0% (0P), 2.5% (2.5P), 5% (5P), and 7.5% (7.5P) of DM inclusion of LIN, with LIN replacing portions of dried distillers grains and Nurisol in the diet to maintain similar levels of CP and ether extract (EE). Three runs with 4 replicates per treatment were used. A 0.5-g sample of each TMR (16% CP, 33% NDF, 28% starch,

Table 1 (Abstr. 2460). In vitro ruminal fermentation parameters

Item	Treatment				SEM	*P*-value
	Corn silage	Barley	Triticale	Wheat		
24 h						
pH	6.40^{a}	6.36^{ab}	6.29^{c}	6.32^{bc}	0.03	<0.01
DMD, %	55.4^{b}	55.5^{b}	70.2^{a}	71.5^{a}	1.72	<0.01
Gas, mL	257^{c}	347^{b}	350^{b}	409^{a}	33.7	<0.01
CH_4, mg	9.7^{b}	19.4^{a}	17.8^{a}	21.9^{a}	2.86	<0.01
CH, mg/g DMD	0.031^{a}	0.015^{b}	0.025^{ab}	0.017^{b}	0.005	<0.01
48 h						
pH	6.29^{bc}	6.35^{a}	6.27^{c}	6.33^{ab}	0.04	<0.01
DMD, %	63.4^{c}	65.1^{c}	74.6^{b}	77.6^{a}	2.46	<0.01
Gas, mL	321^{c}	388^{b}	412^{b}	448^{a}	26.4	<0.01
CH_4, mg	15.1^{b}	41.8^{a}	37.0^{a}	33.8^{a}	5.58	<0.01
CH, mg/g DMD	0.023^{a}	0.009^{b}	0.010^{a}	0.011^{b}	0.003	<0.01

$^{a–c}$Within a row means with different superscripts differ; $P \leq 0.05$.

4.2% EE; as % of DM) was incubated in buffered ruminal fluid for 24 and 48 h in glass serum bottles. Gas pressure was measured at 0, 3, 6, 9, 12, 24, and 48 h to estimate methane production and VFA and lactate concentrations and OM and NDF degradation were measured at 24 and 48 h after bottles were opened. Degradation of CP was measured for the 24 h time point only. Data were analyzed using PROC GLIMMIX of SAS, where treatment was fixed and run was random. With increasing LIN, methane production decreased ($P < 0.01$; 1.86, 1.63, 1.53, and 1.37 ± 0.38 mg, respectively) with 5P and 7.5P, whereas lactate concentration ($P < 0.01$; 0.16, 0.13, 0.16, and 0.25 ± 0.03 m*M*, respectively) and OM degradability increased ($P < 0.01$; 48.5%, 47.0%, 48.7%, and 53.7% ± 1.23%, respectively) with 7.5P compared with other inclusion levels. There was no effect ($P \geq 0.14$) of LIN inclusion level on any VFA proportion or NDF or CP degradability. Thus, in conclusion, inclusion of LIN at 5% or 7.5% of diet DM reduced methane production without altering the VFA profile and improving OM degradation. However, further investigation is warranted to determine if omega-3 FA are included in milk fat production.

Key Words: flaxseed, organic matter degradation, omega-3

2462 ***In vitro* screening of plant-derived feed additives and algae for their effects on rumen fermentation and enteric methane emission.**

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Plant-derived feed additives (PF) have shown varying degrees of efficacy in decreasing methane (CH_4) emissions, and additional studies are needed to develop strategies to achieve both CH_4 mitigation and improved animal performance. We hypothesized that PF with specific modes of action exhibit different effects on rumen fermentation, depending on the applied dosage. Hence, we evaluated the effect of 42 PF, each added at zero (i.e., control) and 3 additional percentages of the substrate, on *in vitro* rumen fermentation. In vitro 24-h batch cultures were conducted using rumen fluid from 4 individual cows. Ruminal fluid and buffer (1:2 ratio) was inoculated in 120-mL bottles containing a 50:50 hay and concentrate substrate. Gas production was recorded at 2, 4, 6, and 24 h after inoculation, and a sample of gas for the determination of CH_4 concentration was collected at 6 and 24 h. Concentration of volatile fatty acids (VFA) and inoculum pH were determined at 24 h after inoculation. Data were analyzed by randomized block ANOVA, with treatment (percentage of each PF) as fixed effect and individual cows as block effect. Inclusion of a tannin-rich chestnut extract at 4% and 8% decreased CH_4 production (mL/g of DM incubated) by 19% and 30%, respectively ($P = 0.001$) yet decreased VFA concentration by 2% and 11%, respectively ($P = 0.041$) compared with the control. A 4% addition of a tannin-rich commercial product decreased CH_4 by 20% ($P < 0.001$) but also decreased total VFA concentration by 8% ($P < 0.001$) compared with the control. Some algae and their combinations decreased CH_4 by 2%–17%, but only numerically; a tendency ($P = 0.088$) to decrease CH_4 (12%–17% reduction at 3%–9% inclusion level) was observed with *Fucus* compared with the control. Our results suggest that tannin-rich products may need to be added at lower levels in the diet of ruminants to maintain their antimethanogenic effect while avoiding impairment of ruminal fermentation. Potential synergistic effects obtained by combining different additives also warrants further investigation.

Key Words: feed additive, fermentation, methane

2463 **Effects of *Porphyra* spp. supplementation on *in vitro* nutrient digestibility, fermentation characteristic, and greenhouse gas emission in the rumen.**

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This study aimed to investigate the effect of *Porphyra* spp. (PO) supplementation on in vitro nutrient digestibility, fermentation characteristics, and greenhouse gas emissions in the rumen. The PO was harvested from Busan, South Korea, and blanched with hot water (60°C) for 0.5 min. A commercial diet of Hanwoo steers in the growing period was used as a basal diet. The blanched PO and a basal diet were dried and ground with a cutting meal to pass a 1-mm screen for the chemical compositions and in vitro rumen incubation. For the experimental diets, the ground PO was supplemented into the basal diet at 0%, 0.5%, 1%, and 2%, respectively. Rumen fluid was collected from 2 cannulated Hanwoo heifers and mixed with Van Soest medium at a 1:2 ratio to prepare the rumen buffer. The rumen buffer (40 mL) was placed into a glass bottle (125 mL) and incubated with the experimental diets (0.5 g) at 39°C for 48 h in 4 replications with 2 blanks. After 48 h of incubation, total gas was measured and collected for CO_2 and CH_4 analyses. The rumen buffer was centrifuged to separate the residue and supernatant for the nutrient digestibility and rumen fermentation characteristics, respectively. All data were analyzed using PROC MIXED with polynomial contrasts (linear and quadratic effects) to generate the effects of supplementation levels. In vitro digestibility of dry matter (IVDMD; $P = 0.973$) and neutral detergent fiber (IVNDFD; $P = 0.933$) were not affected by PO supplementation levels. However, rumen pH ($P < 0.001$) and propionate ($P = 0.004$) were increased linearly by increasing PO supplementation, but total VFA ($P = 0.058$), acetate ($P = 0.002$), and acetate-to-propionate ratio ($P = 0.035$) decreased. By increasing PO supplementation, total gas and CO_2 emissions based on DM, IVDMD, and IVNDFD decreased ($P = 0.012$) linearly, and CH_4 emission decreased ($P < 0.001$) only in IVNDFD. Therefore, this study could conclude that PO supplementation had beneficial effects on total gas and CO_2 mitigations without adverse effects on in vitro nutrient digestibility.

Key Words: greenhouse gases, *Porphyra* spp., rumen fermentation characteristics

2464 Effects of dietary rumen-derived microbial product supplementation on rumen fermentation in lactating dairy cows.

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Rumen-derived microbes are microorganisms that have been selected and cultured from rumen content with the purpose of altering the rumen microbiome to improve performance. Our objective was to evaluate the effect of a rumen-derived microbial product (MP) composed of 4 species: *Clostridium beijerinckii* at 2×10^6 cfu/g, *Pichia kudriavzevii* at 2×10^7 cfu/g, *Ruminococcus bovis* at 2×10^7 cfu/g, and *Butyrivibrio fibrisolvens* at 2×10^7 cfu/g on rumen fermentation and metabolism in lactating dairy cows. Thirty-four Holstein cows were blocked by parity (2.3 ± 1.1), DIM (98 ± 37), and milk yield (43 ± 13 kg/d) in a randomized complete block design. Cows were assigned one of 2 treatments: control (CON, TMR + 150 g/d of ground corn; n = 17) or treatment group (MP, TMR + 150 g/d of ground corn + 5 g/d of MP; n = 17), top-dressed once a day. The study included a 2-wk covariate period and 16 wk of treatment supplementation. Rumen fluid and blood samples were collected at the end of the covariate period and at wk 16, 3 h after feeding. Rumen fluid samples were analyzed for volatile fatty acids (VFA) and ammonia-N, and plasma samples were analyzed for insulin and glucose. Data were analyzed using linear mixed models (PROC GLIMMIX) with covariate, treatment, and their interaction as fixed effects and block as random effect. The covariate total VFA concentration interacted with treatment ($P = 0.01$). When covariate total VFA was minimal (<30th percentile), MP cows had greater total VFA than CON cowa. Cows supplemented MP tended to increase butyrate molar proportion (12.9 vs. 11.6 ± 0.6 mol/100 mol, $P = 0.09$). Molar proportions of acetate, propionate, valerate, iso-valerate, and iso-butyrate were unaffected by treatment (all $P \geq 0.12$). Compared with CON cows, MP cows had greater insulin concentration (0.23 vs. 0.20 ± 0.01 mg/L, $P < 0.05$). Conversely, MP cows had lower glucose concentration (80.5 vs. 94.5 ± 3.2 mg/dL, $P = 0.02$). Overall, our results suggest that MP supplementation has potential benefits on the rumen environment by increasing total VFA concentration; however, these effects are dependent on initial total VFA concentration in the rumen.

Key Words: feed additive, metabolism, rumen environment

2465 The effect of adding different levels of bromoform on enteric methane and rumen fermentation under in vitro conditions.

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Ten concentration levels of bromoform were tested for their effects on enteric CH_4 emissions and rumen fermentation using a series of 24-h rumen batch cultures to determine the optimal inclusion dose of bromoform. Levels administered for treatments were as follows: 0.02, 0.05, 0.10, 0.20, 0.41, 0.83, 1.67, 3.35, 6.7, and 13.4 µL of bromoform/L of rumen fluid. All treatments were tested using the ANKOM batch culture system. One-way ANOVA was used in JMP Pro (SAS Institute). Student's *t*-test and Dunnett's model were used to analyze the *P*-values for all possible pairwise comparisons of the least squares means (LSMEANS). Results show that increasing concentrations of bromoform from 0.02 to 13.4 µL/L of rumen fluid linearly reduced CH_4 production ($P < 0.001$) with a total mitigation of CH_4 at 0.20 to 13.4 µL/L of rumen fluid ($P < 0.01$), and adding bromoform at 0.02, 0.05, 0.10 µL, was able to reduce CH_4 ($P < 0.001$) by 4%, 8%, and 23%, respectively, compared with the control. Concentrations of total VFA were affected in a quadratic fashion in response to treatment. A reduction in the total VFA was observed when bromoform was added at high doses (1.67–13.4 µL/L of rumen fluid), and there was no significant effect at the low doses (0.10, 0.05, and 0.02 µL/L of rumen fluid). Volatile fatty acids were maximally reduced at 13.4 µL/L of rumen fluid ($P < 0.001$). The reduction in total VFA was accompanied by reductions in the molar proportions of acetate and propionate and an increase in the acetate:propionate ratio at the high doses ($P < 0.001$); however, at the low doses, the acetate:propionate ratio decreased ($P < 0.001$). The molar proportions of butyrate increased with all doses ($P < 0.01$). In conclusion, bromoform can be used at low doses to reduce enteric CH_4 from ruminants without negatively affecting rumen fermentation.

Key Words: seaweed, methane, bromoform

2466 Comparing the rumen and feces microbiota composition of high-producing dairy cows before and after removal of monensin from the diet.

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Monensin modifies the rumen fermentation patterns and decreases DMI to improve feed efficiency in lactating dairy cows. A previous study showed it took 3 wk for the monensin-induced feed efficiency to dissipate. The study objective was to investigate the rumen and fecal microbiota composition changes of dairy cows after removing monensin from the diet (−R). Twelve Holstein cows (81 ± 7 DIM) fed a TMR diet with monensin at 16 mg/kg were enrolled in the study. Following a 7-d baseline DMI and milk yield measurements, monensin was removed from the diet over the next 28 d. Rumen fluid samples were collected with a stomach tube and feces samples were collected directly from the rectum on d 1 and 22 after −R. Milk yield and DMI were recorded from d 22 to 28. Rumen fluid and feces DNA was sequenced using 16S rRNA gene-amplicon sequencing on an Illumina MiSeq system. Differences in ruminal and fecal microbiota composition between 2 sampling time points and corresponding production performance were analyzed by using the MIXED procedure of SAS with the REPEATED option. Compared with the baseline, DMI increased (4%, $P = 0.05$), and milk yield (9%) and feed efficiency (13%) decreased ($P < 0.01$) during d 22 and 28 after −R. The α-diversity indexes remained unchanged, except the ruminal species richness (Chao1), which decreased ($P = 0.03$), and the observed operational taxonomic units (OTU) in the rumen tended to decrease 21 d after −R ($P = 0.06$). The ratio of *Firmicutes* to *Bacteroidetes* in both rumen fluid and feces did not change ($P > 0.85$). Of the 50 most abundant genera in the rumen, the relative abundance of unclassified *Lachnospiraceae* decreased, and that of *Lachnospiraceae_ge* increased ($P < 0.05$). Of the 50 most abundant genera in feces, the relative abundance of *Rikenellaceae_RC9_gut_group* decreased and that of *Prevotella* increased ($P < 0.05$). The present data support the notion that a 21-d monensin removal could dissipate its efficiency-enhancement effect and demonstrate the associated changes in gut microbiota composition to be marginal.

Key Words: feed efficiency, monensin, rumen microbiota

Ruminant Nutrition 3: Protein and Amino Acids

2467 Effect of rumen-protected methionine supplementation on vaginal temperature and respiration rate in lactating cows during summer. C. R. Monteiro[1], V. A. Oliveira[1], G. M. Souza[1], R. Schmith[1], T. L. Resende[2], J. P. A. Rezende[1], and M. A. C. Danes*[1], *[1]University of Lavras, Lavras, MG, Brazil, [2]Evonik Industries, São Paulo, SP, Brazil.*

Rumen-protected methionine (RPM) may help cows to deal with the negative effects of heat stress. We evaluated the effect of RPM supplementation on intravaginal temperature (IT) and respiratory rate (RR) in lactating cows during the summer in the southeast of Brazil. Thirty primiparous Holstein cows (45.0 ± 3.2 kg/d of milk and 189 ± 40 DIM) were blocked by milk yield and distributed on a diet without or with 0.75 g/kg of RPM (Mepron®, Evonik Industries, Essen, Germany) in the TMR to meet methionine recommendation for 9 wk. Cows were in a compost barn. In wk 3 (W3), 6 (W6), and 9 (W9), IT was measured every 5 min for 4 d with a Thermochron iButton® and RR was measured at 1000 h and 1700 h by counting flank movements for 30 s for 2 d. Air temperature and humidity were recorded at 10-min intervals and the temperature humidity index (THI) was calculated. Results were analyzed with a mixed model including treatment, week, and treatment × week as fixed effects and block and cow as random effects. For IT, data were summarized by hour, and hour was the repeated effect for repeated measures. Autoregressive covariance structures were used. Least squares means were compared using Tukey's test. Significance was declared when $P \leq 0.05$ and tendency with $0.05 < P \leq 0.10$. The THI was greater than 68 for 13.5, 18.4, and 16.4 h/d during W3, W6, and W9, respectively. Mean IT was not affected by treatment (38.9°C; $P = 0.29$). However, contrary to our hypothesis, RPM tended to increase time above 39°C (9.6 vs. 7.8 h/d, SEM 1.1, $P = 0.09$) and increased mean IT at 2 p.m., 3 p.m. and 4 p.m. in all weeks ($P < 0.01$). Additionally, RR tended to be greater for RPM at 10 a.m. (51.9 vs. 48.4 breaths/min, SEM 0.8, $P < 0.10$) and was greater for RPM at 5 p.m. (58.5 vs. 51.9 breaths/min, SEM 1.3, $P < 0.01$). The observed responses in temperature and RR are likely a consequence of the increase in milk yield with RPM (45.5 vs. 43.8 kg/d, SEM 0.6, $P = 0.03$), which might have resulted in higher metabolic rate. The RPM did not reduce IT and RR of lactating cows during the summer; however, it resulted in greater milk production.

Key Words: amino acid, heat stress, iButton

2468 Effects of feeding controlled-energy and high-energy diets with rumen-protected lysine and methionine prepartum on uterine health of Holstein cows. E. O'Meara*[1], D. del Olmo[2], J. Aguado[2], J. Drackley[1], and F. Cardoso[1], *[1]University of Illinois, Urbana, IL, [2]Kemin Industries, Inc., Des Moines, IA.*

This study aimed to determine the effects of rumen-protected lysine (RPL) and methionine (RPM) fed at the same AA-to-metabolizable energy ratio in prepartum diets with different net energy of lactation (NE_L) concentrations on uterine health of Holstein cows. Sixty-two multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, and BCS during the far-off dry period, were assigned to 1 of 3 dietary treatments. Prepartum (−21 d to expected calving), animals were fed a controlled-energy diet (CEAA; 1.45 NE_L, Mcal/kg of DM) with rumen-protected lysine (RPL; Kemin Industries Inc., Des Moines, IA) and rumen-protected methionine (RPM; Kemin Industries Inc., Des Moines, IA) at 0.15% and 0.09% of DMI, respectively; a controlled-energy diet (control, CENAA) without RPL and RPM; or a high-energy diet (HEAA; 1.71 NE_L, Mcal/kg of DM) with RPL and RPM at 0.22% and 0.12% of DMI, respectively. Postpartum, cows received the same lactation TMR (1.73 NE_L, Mcal/kg of DM) without RPL and RPM (CENAA, n = 19) or with RPL and RPM at 0.38% and 0.15% of DMI, respectively (CEAA, n = 21; HEAA, n = 21) until 70 d relative to calving (DRC). Vaginal discharge was evaluated using a Metricheck® device on 4, 7, 10, 13, 15, and 17 DRC, and at 15 and 30 DRC polymorphonuclear (PMN) leukocytes were evaluated, using a cytology brush, as a percentage of the epithelial cells. Statistical analyses were performed using the MIXED procedure of SAS; odds ratio (OR) was used to compare the likelihood of metritis and endometritis; and frequency analysis was performed using the FREQ procedure of SAS considering the binary response variable of metritis and endometritis. Two treatment contrasts CENAA vs. CEAA and CEAA vs. HEAA were compared. On DRC 4 (OR = 5.18) and 13 (OR = 10.5) cows in the CEAA group were more likely ($P = 0.04$) to be classified as having metritis than cows in the HEAA group. Cows in the CENAA group were less likely ($P = 0.02$) to be classified as having metritis (OR = 0.06) than cows in the CEAA group on DRC 13. Cows in the HEAA group (30.4% ± 3.0%) had a greater ($P < 0.01$) percentage of PMN than cows in the CEAA group (16.2% ± 3.0%). In conclusion, cows in the CEAA group had higher scores for vaginal discharge than cows in the CENAA group but less uterine inflammation (PMN) than cows in the HEAA group.

Key Words: AA, PMN, vaginal discharge

2469 A meta-analysis to identify factors affecting milk component responses and intake of dairy cows to metabolizable methionine fortification evaluated based on INRA 2007. L. Bahloul*[1], S. Lemosquet[2], and C. Loncke[3], *[1]Adisseo, Commentry, France, [2]INRAE, Saint-Gilles, France, [3]AgroParisTech, Paris, France.*

The objective is to establish response equations on milk fat contents (MFC) and yield (MFY), milk yield (MY), and DMI to rumen-protected methionine (RPM; Adisseo Inc., France) supplementation and to determine factors affecting the responses. Meta-analysis was used on peer-reviewed data (25 publications) with a variance–covariance generalized mixed model (GLM) (Minitab V18). Dairy Holstein cows in early- and midlactation consumed 20.3 ± 4.1kg of DM, 1,643 ± 70 MJ NE_L/kg of DM, and 2.35% ± 0.34% digestible (DI) methionine (MetDI). Within-study models predicted MFC, MFY, MY, and DMI with R^2_{adj} = 0.91, 097, 0.98, 0.98, respectively, and RMSE = 0.10%, 52.1 g/d, 0.99 kg/d, and 0.57 kg/d, respectively, to RPM based on MetDi expressed as percent of protein digestible in the intestine (PDI). Milk fat content and MFY models suggested an increase of 0.7% MetDI induced an increase of 0.12% and 84 g/d, respectively. Within-study, slopes were negatively affected by fermentable organic matter (FOM, $P < 0.01$), starch degradability and digestible isoleucine (IleDI) ($P < 0.1$) and positively by lactation stage, DMI, and fat content ($P < 0.05$) for the MFY model and were positively affected by DMI and NE_L ($P < 0.1$) for the MFC model. For a same MetDI, MFC increased in the early lactation period, with DMI, starch, and decreased with NDF, starch degradability, FOM, ThrDI, ValDI, IleDI, and GlyDI contents ($P < 0.01$). Milk yield and DMI models indicated an increase of 0.48 kg/d and 0.49 kg/d, respectively, by increasing 0.7% MetDI. Within-study slopes were positively affected by lactation stage ($P < 0.05$) and negatively by DMI ($P < 0.1$) and FOM ($P < 0.01$) in the MY model and positively affected by the percent of concentrate and ProDI content ($P < 0.1$) in the DMI model. For the same MetDI, MY increased with DMI, fat content ($P < 0.01$) and decreased with NDF, starch degradability, FOM, ThrDI, ValDI, IleDI,

Table 1 (Abstr. 2470). Plasma sulfur AA concentrations (µ*M*)

Item	CON	INF	MSG1	MSG2	SM	SEM	*P*-value
Methionine	23.6^{b}	46.8^{a}	42.4^{a}	41.8^{a}	42.8^{a}	2.56	<0.01
Cystine	21.7	23.1	23.5	22.3	22.9	0.50	0.07
Cystathionine	4.04^{c}	8.31^{a}	7.49^{ab}	6.81^{b}	7.54^{ab}	0.538	<0.01
Taurine	48.2^{b}	60.8^{a}	56.7^{a}	56.6^{a}	58.7^{a}	2.32	<0.01
TSAA	101^{c}	142^{a}	134^{ab}	131^{b}	135^{ab}	4.7	<0.01
RBV[1]	—	—	77.7 ± 2.2	76.9 ± 2.2	80.3 ± 1.4	—	—

[a–c]Means within rows differ at $P < 0.05$.

[1]Calculated as [(slope of RP − Met/slope for infusion) ×100].

PheDI, and GlyDI contents ($P < 0.01$). For the same MetDI, DMI was increased by NE_L ($P < 0.01$) and decreased by starch degradability and FOM ($P < 0.01$). In summary, it is possible to predict the responses of lactating dairy cows as a function of RPM and determine factors that could affect these responses. These results provide deeper insight to biology processes and may help with improving dietary formulation.

Key Words: amino acid, meta-analysis, ruminant

2470 Further evaluation of RPMet™ using the plasma amino acid dose-response method. N. L. Whitehouse*[1], A. E. Deshaies[1], J. J. Albrecht[2], O. R. Drehmel[2], P. S. Yoder[2], and M. F. Scott[2], *[1]University of New Hampshire, Durham, NH, [2]Milk Specialties Global, Eden Prairie, MN.*

The plasma-free AA dose-response technique was used to determine the relative bioavailability (RBV) of methionine in rumen-protected methionine supplements. Our objective was to measure the relative Met bioavailability and determine the metabolizable MET of 2 different lots of RPMet™ and Smartamine®M. Ten multiparous Holstein cows (144 ± 38 DIM) fitted with ruminal cannulas were used in a replicated 5 × 5 Latin square with 7-d periods. Treatments were (g/d): (1) 0 (CON), (2) 12 Met abomasally infused (INF), (3) 24 Met from RPMet-early manufactured (MSG1), (4) 24 Met from RPMet-later manufactured (MSG2), and (5) 24 Met from Smartamine (SM). The rumen-protected Met was fed in 3 daily aliquots at 0500, 1300, and 2100 h, with the infusion being continuously into the abomasum via the ruminal cannulas. Intake, milk yield, and components were collected during the last 3 d of each period. Blood was collected from the tail vein the last 3 d of each period at 2, 4, 6 and 8 h after the morning feeding (0500 h). Data were analyzed using PROC MIXED to generate means. For the REG procedure total sulfur AA TSAA % of (total AA-TSAA) was regressed against 0 and 24 g of infused or fed Met to generate the linear regression variables. Significance was declared at $P \leq 0.05$. Average milk yield was 40.2 ± 0.67 kg/d, DMI was 26.1 ± 0.45 kg/d, and milk protein was 3.11% ± 0.03%. For all treatments plasma Met, cystathionine, Tau, and TSAA concentrations were different from CON (Table 1). RPMet-early manufactured and MSG2 had lower RBV than SM, but higher metabolizable Met due to more Met content (MSG = 80% min; SM = 75% min). The metabolizable Met (g/kg) was calculated to be 622 for MSG1, 615 for MSG2, and 614 for SM. RPMet is a highly bioavailable and metabolizable rumen protected Met.

Key Words: bioavailability, methionine, amino acid

Small Ruminants 1

2471 The effect of prepartum high-starch diet on colostrum yield and serum biochemical profile in dairy goats. M. González-Cabrera*[1], A. Morales-delaNuez[1], A. Torres[2], A. Argüello[1], N. Castro[1], and L. E. Hernández-Castellano[1], *[1]IUSA-ONEHEALTH 4. Animal Production and Biotechnology, Institute of Animal Health and Food Safety, Universidad de Las Palmas de Gran Canaria, Arucas, Spain, [2]Unit of Animal Production, Pasture, and Forage in Arid and Subtropical Areas, Canary Islands Institute for Agricultural Research, La Laguna, Spain.*

This study hypothesizes that feeding a high-starch (HS) diet during the last month of gestation can enhance the metabolic status of goats as well as colostrum yield and composition. Fourteen multiparous *Majorera* dairy goats were randomly assigned to a prepartum dietary treatment (control vs. HS) at wk −4 relative to expected parturition. Goats were fed either a control (n = 8; 100% of starch requirements) or HS (n = 6; 150% of starch requirements) diet during the last month of gestation according to INRA (2018) guidelines. Blood samples were collected on wk −5, −4, −3, −2, and −1 relative to parturition and immediately after parturition. Yield, SCC, and BRIX values on colostrum, as well as blood serum metabolites, were determined. Data were analyzed using the MIXED procedure of SAS (SAS 9.4). The model included the prepartum diet (PD), time (T), and the interaction between both as fixed effects. The statistical significance was set as $P \leq 0.05$. No differences were observed in colostrum yield, SCC, and BRIX values between groups ($P > 0.137$). An interaction (PD × T) was observed for BHB ($P_{\text{PDxT}} = 0.017$) and free fatty acids (FFA; $P_{\text{PDxT}} < 0.001$) concentrations. The prepartum HS group showed lower BHB concentration on week −4, which increased progressively until parturition, whereas FFA concentrations remained constant until parturition. In contrast, BHB and FFA concentrations in the control group decreased from week −4 to −2 and peaked at parturition. In addition, the HS group showed higher glucose (46.8 [41.5–52.7] vs. 40.3 [36.4–44.6] mg/dL; $P_{PD} = 0.028$) and lower albumin (2.9 ± 0.05 vs. 3.2 ± 0.05 g/dL; $P_{PD} = 0.001$), lactate dehydrogenase (322.3 ± 24.56 vs. 406.2 ± 21.30 U/L; $P_{PD} = 0.022$), total protein (5.8 ± 0.21 vs. 6.9 ± 0.18 g/dL; $P_{PD} = 0.001$), and urea (24.1 [21.9–26.6] vs. 31.7 [29.1–34.5] mg/dL; $P_{PD} < 0.001$) concentrations than the control group. The present results indicate that prepartum HS diet does not increase either colostrum yield or composition but promotes a smoother transition from late pregnancy to early lactation in dairy goats.

Key Words: physiology, gestation, energy

2472 Effect of slow-releasing urea encapsulated by microspheres lipidic of low-*trans* vegetable fat on intake, digestibility, and nitrogen metabolism of lambs. L. R. Bezerra*[1,2], P. H. S. Mazza[3], K. H. D. O. S. de Lucena[1], A. M. Barbosa[3], M. A. Fonseca[4], and R. L. Oliveira[3], *[1]Federal University of Campina Grande, Postgraduate Program in Animal Science and Health, Patos, Paraiba, Brazil, [2]CNPq-Brazil Researcher, Brasilia, Distrito Federal, Brazil, [3]Federal University of Bahia, Salvador, Bahia, Brazil, [4]University of Nevada, Reno, Reno, NV.*

This study produced a slow-release urea (SRU) coated from low-trans vegetable fat (LTVF) lipid matrix and to compare it to free urea (U) in the diet of lambs. Three urea:LTVF formulations, 30:70 (SRU$_{30}$), 40:60 (SRU$_{40}$), and 50:50 (SRU$_{50}$) ,were produced by the melt-emulsification method and evaluated. The SRU$_{40}$ showed higher ($P < 0.05$) encapsulation and yield (82%), efficiency (85.5%), and CP content (115% in DM) than the other formulations. The SRU$_{40}$ formulation was then tested in the diet of 32 Santa Ines male lambs (Animal Ethics Approval number 059/2021) with an average age of 6 mo and BW of 17.9 ± 2.01 kg from a randomized design into 4 treatments and 8 replicates: 3 SRU$_{40}$ levels at 1.25% (SRU$_{1.25}$); 2.0% (SRU$_{2}$), and 3.0% (SRU$_{3}$) compared with a control diet with 0.5% free urea (U$_{0.5\%}$) as total dry matter-DM basis, evaluating intake, digestibility, and nitrogen (N) metabolism. Data means were compared using orthogonal contrasts (PROC IML, SAS). Differences were considered significant when $P < 0.05$. The inclusion of SRU linearly increased ether extract, NFC, and total digestible nutrients (TDN) intake ($P < 0.05$) and did not change DM, CP, and NDF intake. Slow-release urea dietary inclusion linearly increased CP digestibility. In contrast, including SRU linearly reduced N-urinary and N-fecal excretion and linearly increased N retention ($P < 0.05$). Blood urea nitrogen (BUN) and rumen pH decreased linearly with the inclusion of SRU, but ammonia nitrogen (NH_3-N) concentration increased linearly ($P < 0.05$). Nitrogen retained and N-microbial production linearly increased ($P = 0.014$) due to the inclusion of SRU in the lambs' diet. Lambs fed free U$_{0.5\%}$ presented the highest ($P < 0.05$) BUN concentrations postfeeding. The BUN concentration linearly decreased in lambs fed SRU at 4 and 6 h postfeeding ($P < 0.05$). Low-trans vegetable fat was efficient in encapsulating urea, and SRU$_{40}$ is recommended in sheep's diets up to 3% (in DM total) because it improves the use of rumen N, reducing N excretion and increasing CP digestibility and N-retention. We thank FAPESq-PB, CNPq, and CAPES (Brazil).

Key Words: hydrogenated fat, rumen, urea

2473 Improving ruminant diets: Controlled urea release through calcium pectinate microparticles. L. R. Bezerra*[1], A. L. Silva[1], M. K. Melo[1], M. A. Fonseca[2], and E. C. Silva Filho[3], *[1]Federal University of Campina Grande, Patos, Paraiba, Brazil, [2]University of Nevada, Reno, Reno, NV, [3]Federal University of Piauí, Teresina, Piaui, Brazil.*

Managing ammonia release in the rumen is pivotal for optimizing ruminant nutrition, enhancing the conversion of dietary nitrogen into microbial protein, and mitigating animal intoxication risks. Our study aimed to explore calcium pectinate as a microencapsulation agent for achieving efficient and gradual urea release (SRU), thereby enabling higher urea supplementation in sheep diets without compromising the microflora or ruminal environment. We developed 3 microencapsulated formulations: 10% urea (MPec1), 20% urea (MPec2), and 30% urea (MPec3), in dry matter total in ratio of calcium pectinate as the shell and urea as the core, employing external ionic gelation/extrusion techniques based on citrus pectin solution mass. These SRU systems were characterized and compared with free urea (U) in 5 fistulated male Santa Ines sheep (Ethics Approval 116/2018-CEUA, University of São Paulo, São Paulo, Brazil). Microscopic analysis revealed that all 3 SRU systems exhibited uniform urea distribution without porosity or cracks, with MPec1 and MPec2 showing a more regular distribution. Incorporating urea into calcium pectinate microencapsulated systems increased microencapsulation yield (92.2%, 93.3%, and 97.1% for MPec1, MPec2, and MPec3, respectively). However, microencapsulation efficiency decreased with urea addition, resulting in values of 262%, 218%, and 264%, and actual urea retention rates of 25.2%, 28.4%, and 31.1% for MPec1, MPec2, and MPec3, respectively. The MPec1 and MPec2 systems presented higher ($P < 0.05$) blood serum concentrations of albumin, urea nitrogen (BUN), creatinine, and total cholesterol and did not affect ($P > 0.05$) the other blood metabolites. The MPec2 systems are recommended because they consist of microspheres with more ($P < 0.05$) controlled core release, delaying the peak of urea released in the rumen and BUN without affecting ($P < 0.05$) ruminal pH and temperature. Calcium pectinate microencapsulation improved urea utilization without altering ruminal pH and temperature, thereby reducing ruminant intoxication risk. We are grateful to FAPESq (Paraiba) and CNPq and CAPES Brazil for the financial support.

Key Words: ammonia, citrus pectin, rumen

2474 Effect of cooling on milk fatty acid profile of lactating Saanen goats during the summer season. M. F. Lunesu*, S. Carta, R. Rubattu, G. Battacone, and A. Nudda, *Dipartimento di Agraria, University of Sassari, Sassari, Italy.*

The aim of this study was to evaluate the effect of heat stress on milk production and milk fatty acid profile in lactating Saanen goats. A total of 20 Saanen dairy goats at 120 DIM were allocated to 2 groups and simultaneously exposed to cooling (COO; n = 10 goats; temperature humidity index: THI < 80) or to heat stress (HS; n = 10 goats; THI > 80) conditions for 1 mo. Both groups were fed a diet containing 60.4% DM, 21.2% CP, 36.7% NDF, 21.7% ADF, 6.7% ADL, and 9.3% ash. Data on environmental temperature and humidity were recorded throughout the experiment by 2 data loggers. Individual milk production was recorded once a week. Individual milk samples were collected weekly and analyzed for chemical components and milk fatty acid (FA) profile. Water and feed intake were recorded daily. Data were analyzed by using the PROC MIXED procedure of SAS with repeated measurements. A principal component analysis (PCA) was used to summarize the complex correlation pattern of the milk FA profile by extracting a reduced number of new variables. Experimental results showed that heat stress slightly reduced dry matter (2.24 vs. 2.34 ± 0.02 kg/d) and NDF intake (0.78 vs. 082 ± 0.02 kg/d) and tended to increase water consumption (6.14 vs. 5.52 ± 0.35 kg/d; $P = 0.08$). Milk production (3.15 vs. 3.43 ± 0.23 kg/d; HS and COO, respectively) and composition were not affected by the treatment ($P > 0.05$). The FA profile of milk fat was not affected by heat stress except for C18:2n6, C18:3n-6, polyunsaturated FAn6 (PUFAn6) and branched-chain FA (BCFA), which were lower in the HS than in the COO groups. Results from the PCA evidenced that only 5 principal components explaining 93% of the total variance, with the first 2 components being associated with fiber intake and heat stress. In conclusion, even in a “normal” THI risk threshold (THI < 80), the effect of short-term cooling slightly alleviated heat stress, without negatively influencing DMI and milk production, and increasing some FA indicators of rumen microbial fermentation. However, a longer treatment period may be useful to evaluate the effect on milk FA profile.

Key Words: heat stress, goat, milk fatty acid profile

2475 Effect of feeding whole pumpkin seeds on parasite load in sheep. M. Tacuri Vera*, B. Campbell, and B. Wenner, *Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Parasite management is a major concern for small ruminants—especially pasture-based—due to treatment cost and growing risk of parasite resistance to anthelmintic drugs. Fall agritourism in the Midwestern United States leaves a large volume of waste pumpkins after the season ends. Pumpkin seeds (PS) have demonstrated anthelmintic properties in vitro. Therefore, our objective was to observe changes in parasite load due to PS supplementation, estimated by measuring sheep BW, fecal egg count (FEC), FAMACHA score (FAM), and blood packed cell volume (PCV). We hypothesized that the supplementation of PS would decrease parasite load. Each treatment group had 5 pens (indoor, slatted) of 2 sheep each. All sheep were fed 1.14 kg/d of a common pelleted diet with chopped hay. The control group (CON) was supplemented with 0.211 kg/head (hd) per day of whole shelled corn and the PS treatment group was supplemented at 0.455 kg/hd per day. Treatments were isocaloric and applied for 4 wk. Seeds were obtained from 2 crops of *Cucurbitis pepo*, mixed, frozen, and thawed the week of feeding. Nutrient analysis of PS indicated 33.7% DM, 32.4% CP, 22.2% NDF, 19.0% ADF, 9.6% lignin, and 34.4% ether extract. Analysis of data collected from sheep included a fixed effect of pen and treatment, random effect of sheep, and repeated week. The FAM (1.08 and 1.02; $P = 0.17$) and log(FEC) (2.94 and 2.96; $P = 0.90$) did not differ between treatments. However, PCV was greater ($P < 0.01$) for PS (38.7%) than CON (36.4%), and BW was decreased by PS (40.3 kg) compared with CON (42.5 kg; $P < 0.01$). However, BW measured one month after the trial evidenced numerical compensatory gain; the daily weight gain of sheep supplemented with PS was (0.254 kg/d) compared with the CON sheep (0.191 kg/d; $P = 0.14$). These data indicate PS failed to decrease parasite load, but the lack of effect may be due to the decreased feed intake of PS and pen-to-pen variation in acceptance of PS. More work is needed to find a preferred method of PS supplementation to improve feed intake and treatment efficacy. Furthermore, the variability of FEC within animal—the average SD of log(FEC) was 3.08—urges experiments to include more regular collection intervals for FEC.

Key Words: parasite, fecal egg count, pumpkin seed

2476 Identification of variables influencing perfluorooctane sulfonic acid (PFOS) serum levels in sheep and cattle through feature selection data mining methods. K. Nishimwe*, A. Jimenez, L. Nowak, S. Benner, A. Mindiola, G. Pereira, and J. Romero, *University of Maine, Orono, ME.*

We aimed to identify variables influencing perfluorooctane sulfonic acid (PFOS) serum levels based on published data. In Web-of-Science, we searched for articles measuring per- and polyfluoroalkyl substances (PFAS) in cattle and sheep. From 10 publications, we extracted the following variables, where available: publication ID, sex, species, BW, exposure duration to PFAS, treatments (animals categorized as clean animals exposed to PFAS, contaminated animals exposed to PFAS, and contaminated animals not exposed to PFAS), and PFOS liver and serum levels. The dataset was arbitrarily divided into training (70%) and test (30%) subsets, with the former used for developing the model and the latter to report the accuracy of it. Least absolute shrinkage and selection operator (LASSO) and ridge algorithms were used on the training subset with a 10-fold cross-validation approach. Stepwise multiple regression was also used with Akaike information criterion (AIC) as the selection criterion. R ver. 4.3.2 was used for the analysis. Although errors were not normally distributed for stepwise, this assumption does not apply to LASSO. The root mean square error (RMSE) obtained with the test subset were 2.61, 2.18, 3.30, 3.28, and 2.24 (µg/mL of plasma PFOS), and the number of variables in the model (exclusive of the intercept) was 6, 7, 8, 8, and 5 for LASSO set to lambda.1se (l1se) and lambda.min (lmin), Ridge set to (l1se) and (lmin), and stepwise (respectively). LASSO (l1se) and stepwise methods resulted in among the lowest test RMSE with the fewest independent variables required. No differences in the median of the test absolute errors were observed between the latter two methods ($P = 0.289$). The LASSO (l1se) method identified a positive relationship between PFOS plasma levels and exposure duration, PFOS liver levels, and being female when the effect of publication was included in the model (adjusted $R^2 = 0.86$). Similarly, the stepwise method identified a positive relationship with exposure duration and being female when the effect of publication was included in the model (adjusted $R^2 = 0.89$). In conclusion, LASSO indicates that key variables influencing PFOS plasma levels in cattle and sheep include sex, exposure duration, and PFOS liver levels.

Key Words: PFAS, cattle, sheep

2477 Digesta passage rate in the gastrointestinal tract of lambs fed whole-plant sesame silage.

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The digesta passage rate (kp) in ruminants is a very complex and dynamic process and related to many factors that depend on animal and diet. Thus, the objective of this study was to evaluate the effect of the replacement of whole-plant corn silage by whole-plant sesame silage (WPSS) in diets on gastrointestinal (GIT) kp of particles and solutes in growing lambs using the slaughter technique. A total of 32 castrated crossbred lambs with an initial BW of 21.1 ± 1.89 kg were distributed in a randomized block design with 4 replacement levels: control (no sesame) and 333, 670, and 1,000 g/kg of DM in 8 replications. The feed was offered as TMR using a forage:concentrate ratio of 40:60. For estimating kp we considered the intake during the 5-d period before slaughter day. The kp of solutes was determined by administering co-EDTA, and indigestible NDF (iNDF) was used to determine the kp of particles. The digesta kp in different GIT segments was determined by the flux/compartmental pool method. The data were analyzed in a randomized complete block design, and orthogonal polynomial contrasts were used to determine linear and quadratic effects of substitution levels when it was significant ($P \leq 0.05$). No influence was observed on kp of particles in the reticulorumen (RR), omasum, small intestine, cecum, colon–rectum segments, and total GIT ($P > 0.05$). Moreover, abomasum kp of particles was quadratically changed as WPSS increased in the diet, with the highest values (40.2%/h) observed at the level of 670 g/kg of DM ($P = 0.03$). The kp of solute in the RR ($P = 0.01$) and total GIT ($P = 0.05$) linearly decreased; however, it linearly increased in the abomasum ($P = 0.001$) with increasing WPSS in the diet. Furthermore, cecum kp of solute changed quadratically ($P = 0.01$) as the WPSS in the diet increased and it reached the highest values (185%/h) at the level of 333 g/kg of DM. No influence was observed on kp of solute in the omasum, small intestine, and colon–rectum segments ($P > 0,05$). The results showed that kp of particles and solutes were not constant in the different TGI segments and that the inclusion of WPSS decreased the kp of solute in the RR and total TGI.

Key Words: Co-EDTA, iNDF, *Sesamum indicum*

2478 A survey describing the early rearing environments of dairy goat kids in the U.S.

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Annual dairy goat inventory reports estimate that nearly 300,000 kids are born yearly in the United States. Previous research on goat kid rearing is focused on milk feeding/weaning and general management, with a lack of knowledge on their early physical and social environments. Therefore, the objective of this study is to describe US dairy goat kid housing environments and exposure to environmental enrichment (EE) from birth to weaning via a descriptive online questionnaire. To participate, respondents had to be 18 years of age or older, operate a farm located in the United States, have and breed 5 or more adult dairy goats as a source of income, and raise their own kids on-farm from birth to weaning. A minimum sample size of 381 respondents was calculated using a 95% confidence level and 5% margin of error. Participants were recruited by emailing national and state level goat associations and US university small ruminant extension staff, and by attending the American Dairy Goat Association Convention and UC Davis Goat Day. The questionnaire consists of (1) participant demographics, (2) farm characteristics and management, (3) kid rearing strategy (separated immediately from their dams: SEP; kept with their dams for any amount of time: DAM), (4) details of each SEP/DAM physical housing environment (PHE; the number a typical kid goes through until weaning), (5) general husbandry, and (6) optional entry for incentive raffle (n = 34–114 total questions depending on number of PHE and EE provision). Preliminary descriptive statistics show 192 responses fulfill inclusion criteria. The largest percentages of respondents operate a farm in California (28%, 46/165), use the LaMancha breed (35%, 59/168), and produce an average of 20–49 kids per year (40%, 67/167). About half of respondents rear kids with SEP (51%, 86/168) versus DAM (49%, 82/168) strategies. During the first PHE, hiding EE is more frequently provided for DAM kids (63%, 47/75) than SEP kids (13%, 11/84). Describing farm/housing characteristics of SEP versus DAM operations will be used to inform future research to assesses rearing environments that optimize dairy goat kid health and welfare.

Key Words: kid rearing strategy, housing, environmental enrichment

Animal Behavior and Well-Being 3

2479 Calf-rearing practices in pasture-based and organic dairy farms in the Midwest United States.

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There is an increased societal demand for improved conditions in calf-rearing management. Calf-rearing practices in organic and pasture-based dairy farms in the Midwest can be very diverse but are not well documented. This ongoing study aims to describe calf-management practices of organic and pasture-based dairy farms in the Midwest. Pasture-based (at least 120 grazing days per year) dairy farmers in the Midwest received an invitation (emails, social media, farming events, and phone calls) to participate in an in-person survey. Thirteen (certified organic n = 7; certified organic and grass-fed n = 4; transitioning to organic certification n = 1; pasture-based noncertified n = 1) farmers agreed to the in-person interviews, performed with a standard questionnaire with open-ended questions. Due to the diversity of the systems and the low number of farms, only descriptive data analyses are shown. The median herd size was 75.5 cows (34–321 cows). Most farms (67%) separated cows and calves 1 to 48 h after birth. A smaller portion of farms (33%) allowed more than 3 d of full or partial cow-calf contact with the dam or foster cows and reported reduced labor and improved animal health from this practice. Calves separated from cows received 2.4 L to 16 L of milk daily, with 66% of farms feeding 6 L of milk or less. Milk was offered using one or more of the following methods: buckets with nipples (11%), bottles (33%), buckets without nipples (33%), or mob feeders (66%). Step-down weaning strategies were used on 92% of farms. Calves housed in groups (75%) were the most predominant housing type and were used in combination or as an alternative to individual, cow-calf, or pair housing. Only 36% of organic farmers agreed they could treat diseases in calves using organic-approved options, and 82% reported pneumonia as the most challenging disease to cure. Also, 33% of farms used a vaccination program. This survey demonstrates a high diversity of practices allowing dairy calves to have social contact.

Key Words: calves, organic, welfare

2480 A survey of preweaned calf transportation practices on US dairies: Colostrum management and age at transport for replacement heifers, beef-dairy crossbreds, and dairy bull calves.

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Transportation is stressful for cattle, but little research is available describing transportation practices of preweaned dairy calves in the United States. The objective of this study was to describe current industry transportation practices for 3 preweaned calf groups: dairy replacement heifers (RH), beef-dairy crossbreds (BD), and dairy bull calves (BUL). Individuals that make calf transport decisions on dairies were recruited through digital advertisement, email, and extension agents to complete an online survey in 2023. Respondents had <80 (n = 4), 80–200 (n = 6), 201–500 (n = 18), 501–1,000 (n = 5) to >1,000 lactating cows (n = 29) in the Midwest (51%; n = 37), West (17%; n = 12), Northeast (8%; n = 6), and Southern (6%; n = 4) US regions. Frequency tables were used to determine the proportion of operations for each question, by calf group. Respondents (n = 72) transported a total of 284,702 RH, 263,119 BD, and 33,178 BUL calves. Fifty-eight percent (n = 42), 82% (n = 59), and 81% (n = 58) reported transporting RH, BD, and BUL, respectively. All respondents reported providing colostrum to calves before transport off of the facility. Most respondents provided colostrum to RH (86%; n = 36), BD (80%; n = 48), and BUL (76%; n = 44) within 2 h after birth. Sixty-eight percent (n = 28), 50% (n = 30), and 44% (n = 25) of participants provided colostrum with a BRIX reading ≥22 (IgG > 50g/L) to RH, BD, and BUL, respectively. Respondents most frequently indicated they transported calves at 4 to7 d for RH (24%; n = 10), BD (29%; n = 17), and BUL (35%; n = 20). Less than 24 h was the second most frequently reported age at transport; 24% (n = 10), 19% (n = 11), and 19% (n = 11) of respondents transported RH, BD, and BUL at this age, respectively. Most respondents report following industry best practices for timing of colostrum feeding; however a lower proportion of respondents indicate feeding BD and BUL colostrum with a BRIX score ≥22 compared with RH. Most respondents transported calves at ≤7 d of age, highlighting the need for management strategies that mitigate transportation stress on young calves.

Key Words: pretransport, surplus calves, welfare

2481 Characterizing management practices of dry and early lactation cows in Wisconsin and Minnesota.

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Management of cows leading up to and shortly after calving can affect dairy cow welfare and productivity. An understanding of management practices provides an opportunity to optimize how cows are managed for improved outcomes. The objective of this study was to characterize reported housing and management from 60 d before and 30 d after calving on dairy farms in Wisconsin and Minnesota. A total of 1,503 surveys were mailed and 466 surveys were returned completed (31% response rate). Data were descriptively summarized to describe the prevalence of management practices. More than half of the producers used a designated pen(s) for nonlactating cows or cows within 3 wk of calving (56.2%). Dry cow housing varied, including designated far dry only (1.1%), close up only (12%), a single pen for far-dry and close-up cows (12.3%), pasture (3.9%), any combination of 2 or more of the previously mentioned pens (65.8%), and other (4.9%). A calving area was reportedly used on 72% of farms; and movement into a calving pen occurred most frequently from 1 to 6 d before calving (30.3%) with the remaining producers moving cows into a calving pen more than 21 d (13.1%), 15 to 21 d (11.9%), 7 to 14 d (18.1%), and a few hours to minutes before calving (26.7%). Producers who used a hospital or colostrum pen before movement into a fresh or lactating pen (20.6%) kept the cows in that pen for 3 or more milkings (63.3%). Fresh pen use was reported by 63.2% of producers; any combination of 2 or more fresh pen types was most commonly used (37.7%), including freestall, tiestall, bedded pack (compost or noncompost), stanchion, and pasture. Most farms kept cows in a fresh pen for 1 to 6 d after calving before being moved into a lactating pen (29.8%), with the remaining producers keeping cows in a fresh pen for less than 1 d (18.3%), 7 to 14 d (8.4%), 15 to 21 d (11.5%), 22 to 28 d (12.2%), and depending on individual cow needs (19.8%). These results help characterize what management practices for transition cows are being used in Wisconsin and Minnesota, aiding in future research to improve transition cow performance and productivity.

Key Words: transition period, management practices, regrouping

2482 Understanding the connection between dairy farmer mental well-being and cattle health in Western Canada and Ontario.

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With stress and mental health of farmers becoming of greater concern, understanding the connection between farmer well-being and animal welfare is crucial. We developed a survey aimed at primary or secondary decision makers on dairy farms in Western Canada and Ontario. Farmers' mental health was evaluated using validated psychometric scales to assess stress, anxiety, depression, and resilience. Respondents were also asked about farm management, calf mortality, and mastitis. On-farm scoring (n = 66) was conducted to assess lameness, body condition, and knee, neck, and hock lesions of a representative sample of each herd's lactating cows (30% to a maximum of 69 cows). Cows with a lameness score of ≥3 or 4 out of 5 in loose housing and ≥2 or 3 of 4 behavioral indicators in tiestalls were categorized as clinical and severe lameness, respectively. The prevalence of clinical lameness was 7.9% ± 7.7%, severe lameness was 2.3% ± 2.9%, and mastitis was 16.8% ± 17%. The prevalence of BCS outside the target range (2.75–3.25) was 16.2% ± 11.7%, with overconditioned cows more prevalent (13.8% ± 11.8%) than underconditioned cows (2.4% ± 4.2%). Using a linear model, we found that lower anxiety scores were associated ($P = 0.02$) with having more overconditioned cows (BCS ≥ 3.5). Using *t*-tests, farmers with reported mastitis rates <10% had lower stress scores ($P = 0.02$) compared with those with ≥10%. Additionally, farmers with a clinical lameness prevalence of ≥5% tended to have lower stress ($P = 0.07$) and lower anxiety scores ($P = 0.06$), but higher resilience scores ($P = 0.10$) than farmers with <5% lame cows. Using chi-squared tests with Bonferroni corrections, more farmers with high stress scores had <5% clinical lameness compared with those scoring low ($P = 0.009$) and moderate ($P = 0.008$) stress. In this case, farmer and animal well-being had limited associations; however, animal scores were better than in previous studies. If farmer well-being and animal welfare are interrelated, it is important to approach circumstances where either are at risk to support more vulnerable farmers and their animals.

Key Words: welfare, stress, resilience

2483 Public knowledge and perceptions of dairy cattle welfare at a dairy farm open house.

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The objective of this study was to examine public perception of dairy cattle welfare and knowledge of the industry before and after attending a family-oriented dairy open house. Responses from a presurvey and postsurvey were collected from participants (n = 146) via digital and paper surveys, asking questions about prior experience with the dairy industry and opinions on dairy cattle welfare before and after touring the farm. Both surveys included a short quiz to assess participants' knowledge of the dairy industry and asked them to assess the importance of 5 factors related to animal welfare: outdoor access, nutrition, health care, freedom from pain, and ability to express natural behaviors. Responses were analyzed using nonparametric methods (Fisher's exact test). Perception of the importance of factors related to animal welfare did not differ between the pre- and postsurvey or between groups of respondents who had previously visited a dairy (65% of respondents; $P > 0.8$). Respondents who had never previously visited a dairy considered themselves less knowledgeable about dairy production (28% vs. 63% selected "somewhat" or "very" knowledgeable; $P < 0.001$) and had lower scores on the prequiz ($P = 0.035$) but not the postquiz ($P = 0.63$), compared with respondents who had visited a dairy farm previously. In the postsurvey, 50% of participants claimed that their perception of the dairy industry was either "changed" or "somewhat changed," and 88% classified this change as positive. The perceived importance of aspects of welfare was unrelated to knowledge gain; for example, improved quiz scores indicating knowledge gain regarding how frequently dairy cows access pasture were not related to changing perception of the importance of outdoor access for dairy cattle ($P = 0.6$). These results suggest that attending a dairy farm open house event may positively affect perception of dairy farming, but values surrounding animal welfare were unrelated to knowledge gain or the experience of touring the farm.

Key Words: survey, management, extension

Animal Health 4

2484 Accuracy and precision of diets fed to close-up cows on dairy farms and their association with early lactation metabolic markers. L. S. Gheller[1,2], C. Wagemann-Fluxá[1], and T. J. DeVries*[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Animal Science, College of Animal Science and Food Engineering, University of Sao Paulo, Pirassununga, SP, Brazil.*

A consistent ration is critical for lactating cow performance, but this is unknown for dry cows. We aimed to: (1) characterize close-up dry cow diets fed on Ontario, Canada farms, (2) determine the accuracy and precision of the nutrient composition of those diets, and (3) associate that accuracy and precision with fresh cow blood parameters. Forty dairy farms (median milking cows = 137, range = 60–550) were visited once every 4 wk (6 visits/farm). At each visit, the close-up diet was sampled for nutrient composition using wet chemistry. Close-up diet formulations were obtained from farm nutritionists. At each visit, fresh cows (n = 1,540) within 14 DIM (7.1 ± 4.3 d; mean ± SD) were blood sampled for BHB, albumin, cholesterol, and bilirubin concentrations; the latter 3 were used to calculate a liver health index (LHI). Multivariable linear regression models were used to associate close-up diet nutrient variability (CV; in relation to the formulated diet and across time) and metabolic markers. In the dry cow diets, corn silage (67.6% of farms) and straw (24.3% of farms) were the predominant primary forage sources, and soybean meal (37.8% of farms) and canola meal (18.9% of farms) were primary concentrate sources. Fed diets did not accurately represent formulations, with nutrient CV > 5%, except for NE_L. The average fed diet exceeded diet formulation for NE_L, K, fat, NDF, ADF, and NFC, and underfed CP, starch, Na, Mg, P, and Ca. Each percentage point (p.p) decrease in NFC variability (CV% = 11.7 ± 8.9) between fed and formulated diets was associated ($P = 0.02$) with a 0.034-unit higher (better) LHI (mean = −0.23 ± 1.44). For visit-to-visit variability, each p.p. increase in fat variability (CV% = 15.7 ± 10.6) was associated ($P = 0.01$) with a 0.005 mmol/L increase in BHB concentration (mean = 0.74 ± 0.14 mmol/L). Each p.p. increase in NFC variability (CV% = 8.6 ± 4.5) was associated ($P = 0.005$) with 0.013 mmol/L less BHB, possibly due to farms, on average, feeding diets higher in NFC (+4.3 p.p.) than formulated. The results highlight the importance of consistency in close-up diets, relative to formulation and across time, to optimize fresh cow health.

Key Words: diet accuracy, close-up, fresh cow health

2485 Metabolomic analysis of essential oil blends: Implications for rumen pathway enrichment in large ruminants. D. O. Okedoyin*, J. O. Alabi, and U. Y. Anele, *North Carolina A&T State University, Greensboro, NC.*

This study investigated the effect of direct infusion of essential oil blends comprising anise, clove, oregano, and peppermint in different ratios on rumen metabolites in Black Angus beef cattle. Using a 6 × 6 Latin square experimental design over a 90-d study period, 6 ruminally cannulated Black Angus beef cattle were subjected to 6 15-d cycles involving infusion, sampling, and rest periods. The treatments were 5 essential oil blends (EOB1, EOB2, EOB3, EOB4, and EOB5) and a control (no essential oil). Rumen fluid was collected from the cannulated animals during the sampling period, and the rumen fluid samples underwent quantitative untargeted metabolome analysis using chemical isotope labeling coupled with liquid chromatography–mass spectrometry. Volcano plot analyses revealed diverse differentially abundant metabolites between the control and the essential oil blends. To determine the metabolic pathways that were enriched in each EOB group, a pathway enrichment analysis using the Kyoto Encyclopedia of Genes and Genomes (KEGG) was performed, and this analysis revealed significant alterations in ruminal metabolite profiles following essential oil infusion. Differential abundance of specific metabolites was observed across the EOB treatments compared with the control, with EOB5 exhibiting the highest abundance. Twenty-six metabolites, including elevated valine and reduced gallic acid, affecting pathways such as selenocompound metabolism; valine, leucine, and isoleucine biosynthesis; valine, leucine, and isoleucine degradation; steroid hormone biosynthesis; arginine biosynthesis; glycolysis/gluconeogenesis; pyruvate metabolism; starch and sucrose metabolism; and fructose and mannose metabolism pathways. These findings highlight the potential for essential oils to modulate metabolic pathways in cattle, providing valuable insights for optimizing rumen function and enhancing animal productivity.

Key Words: metabolome, essential oil, ruminant

2486 Effects of omega-3 fatty acids supplementation on metabolism and performance of early lactation dairy cows. G. Madureira*[1], C. Van Dorp[1], I. Witelus[1], B. Van Winters[1], M. G. S. Santos[1], I. I. Avalos-Rosario[1], F. S. Schenkel[1], D. W. L. Ma[2], S. J. LeBlanc[3], and E. S. Ribeiro[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON, Canada, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Our objective was to evaluate the effects of omega-3 fatty acids (n3 FA) supplementation on circulating metabolites and lactation performance. In a randomized controlled trial, cows (n = 104) were blocked by parity and BCS, and assigned randomly to 1 of 4 dietary treatments from 2 to 90 DIM: (1) UNSUP: unsupplemented control; (2) CaPO: calcium salts of palm oil (EnerGII; Virtus Nutrition) through 90 DIM; (3) CaFO35: calcium salts of fish oil (Strata; Virtus Nutrition) through 35 DIM; and (4) CaFO90: CaFO through 90 DIM. Fat was supplemented at 1% of DM. Cows were milked twice a day and BW was measured after each milking. Milk composition was evaluated weekly, and plasma and milk samples were collected on d 14, 35, and 71 for FA composition. Serum metabolites were evaluated on d 2, 7, 10, 14, 21, 35, 50, and 71. For statistical analyses, data were divided into 2 periods: P1 (2–35 DIM) and P2 (36–90 DIM). The linear models included the fixed effects of treatment, parity, time, their interactions, and the random effect of cow. Supplementing CaFO increased eicosapentaenoic acid (EPA)+docosahexaenoic acid (DHA) in plasma (UNSUP: 13.3 ± 1.5^{b}; CaPO: 15.6 ± 1.4^{b}; CaFO: 38.5 ± 1.0^{a} μg/mL; $P < 0.01$) and milk (UNSUP: 19.0 ± 4.3^{b}; CaPO: 27.8 ± 4.3^{b}; CaFO: 61.4 ± 3.4^{a} μg/mL; $P < 0.01$) in P1, which remained higher in CaFO90 during P2. No differences were observed in BW, DMI, milk yield, milk fat, and ECM. Milk protein was similar among groups in P1, but greater in CaFO35 than CaFO90 and CaPO during P2 (UNSUP: 3.09 ± 0.04^{ab}; CaPO: 3.01 ± 0.04^{b}; CaFO35: 3.14 ± 0.04^{a}; CaFO90: 3.02 ± 0.04^{b} %; $P = 0.04$). Milk BHB was lower ($P < 0.01$) in CaFO groups during P1 and P2. In P1, serum glutamate dehydrogenase (GLDH) and gamma-glutamyl transferase (GGT) tended ($P \leq 0.1$) to be higher in CaFO. In P2, CaFO90 had higher GLDH (UNSUP: 28.1 ± 0.06^{b}; CaPO: 39.7 ± 0.05^{ab}; CaFO35: 31.7 ± 0.06^{b}; CaFO90: 51.2 ±

0.05^{a} U/L; $P < 0.01$) and GGT (UNSUP: 20.4 ± 0.03^{b}; CaPO: 22.2 ± 0.03^{b}; CaFO35: 20.8 ± 0.03^{b}; CaFO90: 27.1 ± 0.03^{a} U/L; $P < 0.01$). In conclusion, supplementation of CaFO enriched n3 FA in circulation, had a minor effect on liver function, but did not affect performance. Different superscript letters (a,b) denote statistical difference ($P < 0.05$).

Key Words: metabolism, PUFA, nutraceutical

2487 Evaluation of the feed compounds for inflammatory modulation in immune and epithelial cells.

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Bioactive feed components can alter tissue physiology in numerous ways, including by activating receptors and downstream signaling cascades. One transcription factor involved in gut inflammation is nuclear factor κ-light-chain enhancer of activated B cells (NFκB). To evaluate the anti-inflammatory effects of bioactive compounds, RAW 264.7 murine macrophages and the human HCT116 colorectal carcinoma cell line were transfected with a vector driving alkaline phosphatase (AP) expression upon activation of NFκB. Cells were treated with onion extract (OE), grape seed extract (GSE), or elemental Mn at concentrations of 0%, 0.062%, 0.125%, 0.25%, 0.5%, and 1% (wt/vol) in cell culture media with or without lipopolysaccharide (LPS; *E. coli* 0127:B8, 100 ng/mL) stimulation in RAW cells, and with and without high molecular weight poly(I:C) (10 µg/mL) in HCT cells. After treatment, metabolic activity and AP activity were measured. Data were analyzed by mixed models with treatment as a fixed effect and plate as a random effect (n = 4–8 per dose), with significance declared at $P < 0.05$. Compared with negative controls, Mn had no effect on metabolic activity, whereas GSE decreased it in both cell types at all concentrations, indicating toxicity. Onion extract increased RAW cell metabolic activity but decreased it at 0.5% and greater concentrations in HCT cells, suggesting potential for immune cell activation but epithelial toxicity at higher concentrations. Onion extract increased AP activity in RAW cells at the 1% dose with or without LPS stimulation but decreased it in HCT cells at all concentrations with or without poly(I:C) stimulation, perhaps due toxicity in these cells. Grape seed extract dramatically increased AP activity in RAW cells in all treatments, despite loss of cell viability, but decreased it in HCT cells in all treatments. Manganese decreased AP activity at all doses relative to relevant controls, pointing to consistent anti-inflammatory effects. Inflammatory effects of feed compounds can differ dramatically between macrophage and epithelial cell models, and accounting for toxicity is essential for unbiased assessment of anti-inflammatory effects.

Key Words: macrophages, inflammation, bioactive compounds

2488 Effects of source of trace minerals on health and survival of dairy cows.

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Objectives were to evaluate the effects of replacing sulfate sources (STM) with hydroxychloride sources (HTM) of Zn, Cu, and Mn on health in dairy cows. One hundred forty-one Holstein cows were blocked by parity group (lactation 0 vs. lactation >0) and assigned to a randomized complete block design (STM, 30 nulliparous and 40 parous cows; HTM 31 nulliparous and 40 parous cows). Supplemental Zn, Cu, and Mn were incorporated at, respectively, 30, 7, and 22 mg/kg of diet DM prepartum, and at 44, 8, and 36 mg/kg of diet DM postpartum. The pre- and postpartum diets contained, respectively, 64 and 77 mg/kg of Zn, 15 and 19 mg of Cu, and 58 and 65 mg of Mn. Treatments started at 245 d of gestation and stopped at 105 d postpartum. Cows were monitored daily for clinical diseases in the first 105 d postpartum and survival was evaluated until 305 DIM. Risk of diseases was analyzed by logistic regression with generalized mixed-effects models fitting a binomial distribution. Days to morbidity and days to leaving the herd were analyzed by Cox's proportional hazard regression. Replacing STM with HTM reduced the risk of retained placenta and tended to reduce the risk of clinical and subclinical endometritis (Table 1). Cows fed HTM had lower risk and tended ($P = 0.07$) to have a slower rate (adjusted hazard ratio, AHR = 0.58; 95% CI = 0.33–104) of morbidity than those fed STM. The rate of leaving the herd by 305 d postpartum tended ($P = 0.07$) to be slower for HTM than STM (AHR = 0.45; 95% CI = 0.19–1.06), resulting in a tendency for greater proportion of HTM than STM cows surviving in the herd by 305 d postpartum. Feeding HTM benefited health and survival of dairy cows.

Table 1 (Abstr. 2488). Effect of source of trace minerals on health and survival of dairy cows (percent of cows, unless otherwise stated)

Item	STM (n=70)	HTM[1] (n=71)	SEM	*P*-value
Milk fever	1.1	1.2	1.3	0.93
Retained placenta	11.5	3.8	4.3	0.01
Metritis	34.5	26.4	8.7	0.43
Clinical endometritis	16.4	4.0	6.2	0.09
Subclinical endometritis	29.8	16.4	7.4	0.09
Endometrial PMNL[2] (% of cells)	3.9	4.5	1.2	0.61
Ketosis	6.4	5.7	2.8	0.86
Morbidity	52.0	34.2	8.1	0.05
Multiple diseases	11.7	10.9	5.5	0.90
Survived by 305 d	77.1	88.8	4.5	0.08

[1] IntelliBond® Z, C, M; Selko USA, Indianapolis, IN.

[2] Polymorphonuclear leukocytes.

Key Words: health, hydroxychloride, trace minerals

2489 Effect of postbiotic supplementation on proinflammatory cytokines in Holstein-Friesian steers inoculated with digital dermatitis.

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Digital dermatitis (DD), a leading cause of lameness in the cattle industry, causes economic losses and decreased welfare. Antimicrobial resistance has pushed for alternative treatments and prevention. Recent studies demonstrated the positive effect postbiotic *Saccharomyces cerevisiae* fermentation products (SCFP) have on the innate immunity of cattle. The objective of this study was to evaluate the effect of SCFP (NutriTek®, Diamond V) supplementation on proinflammatory cytokines (PIC) in steers that have been experimentally inoculated with DD. Lightweight Holstein-Friesian steers (n = 120), aged 6 to 7 mo and weighing 160 to 200 kg, were randomly assigned to SCFP or control groups. The SCFP steers received NutriTek® (12 g/d) and controls received a grain mix (12 g/d) supplementation mixed with TMR. At 42 d after starting the diets, subsets of steers (n = 25 per group) were randomly inoculated with a homogenate of DD-affected skin biopsies. Blood samples were collected as follows: before starting diets (n = 43), before inoculation (n = 40), 3-wk post inoculation (n = 49), and 6-wk post inoculation (n = 36). Interleukin-1β and IL-6 PIC levels were measured using commercial ELISA assays with the following stimulants: none, PAM3CSK4, Poly(I:C), and LPS. Generalized linear mixed models were used to quantify the association between PIC level and predictors. An error level of $\alpha \leq 0.05$ was used. Supplementation with SCFP was associated with reduced IL-1β and IL-6 concentrations following toll-like receptor stimulation when compared with controls ($P \leq 0.05$). Steers with stage M4 and M1 lesions that were supplemented with SCFP had increased IL-1β and IL-6 concentrations in stimulated cells of whole blood ($P \leq 0.01$). Steers with M4.1 lesions that were supplemented with SCFP had decreased IL-1β and IL-6 concentrations ($P \leq 0.05$). Based on these findings in dairy steers, SCFP supplementation had modulatory effects of releasing PIC IL-1β and IL-6 to provide a potential increase of immune support during the development of DD lesions.

Key Words: digital dermatitis, postbiotics, inflammatory markers

2490 Peripartal supplementation of postbiotics on performance in transition dairy cows.

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The increased consumer concern about food safety and environmental issues has led to increased interest in feeding natural additives to livestock animals. Postbiotics are inanimate microorganisms or their components that confer a health benefit on the host. The effects of postbiotics on dairy cows are still not well understood. Therefore, the objective of this study was to evaluate the effects of postbiotic supplementation (Diamond V, Cedar Rapids, IA) on lactation in dairy cows during the transition period until 60 DIM. Sixty multiparous Holstein dairy cows were enrolled in a randomized complete block design from −35 to 60 DIM and blocked according to expected calving day, parity, and previous lactation ECM. At −35 DIM, cows were assigned to either a basal diet plus 100 g/d of ground corn and 25 g/d of placebo (CTL; n = 30) or a basal diet plus 100 g/d of ground corn and 25 g/d of postbiotic (PB; n = 30). Cows were milked twice a day and fed TMR individually once a day, and treatments were top-dressed immediately after morning feeding. From −35 to −22 DIM, cows were fed a far-off diet; from −21d until calving, cows received a close-up diet. From calving to 30 DIM, cows received a fresh cow diet, and from 31 to 60 DIM, cows were fed a postfresh diet. Milk composition, BW, and BCS were collected weekly. Data were analyzed using linear mixed models in PROC MIXED of SAS. Significance was declared at $P \leq 0.05$ and trends at $P < 0.10$. A significant difference in BW prepartum ($P < 0.01$) was observed, so initial BW was used as a covariate to adjust all the other parameters. A trend ($P = 0.10$) was observed for greater milk yield (47.4 vs. 50 kg/d) in PB cows compared with CTL. Moreover, a trend ($P = 0.10$) for greater protein yield (1.43 vs. 1.50 kg/d) was observed in PB cows compared with CTL. Effects for greater postpartal DMI ($P = 0.20$) and ECM ($P = 0.12$) in PB cows compared with CTL did not reach statistical significance. The results suggest that supplementation of postbiotics can help to increase milk production; however, more research is needed to clarify the effects of this prototype on transition dairy cows' performance.

Key Words: rumen modifier, additive, peripartal period

2491 The effect of quorum-sensing science-based products on the health of preweaned calves.

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Biofilm formation, often orchestrated by quorum sensing (QS), has been linked to the etiology of bovine respiratory diseases (BRD) and diarrhea in calves. The objective of this study was to evaluate the effect of supplementation with QS science-based products (QSP; containing propyl-propane thiosulfonate and propyl-propane-thiosulfinate) on the health and growth performance of pre-weaned Holstein (n = 60, all male) and beef-on-dairy calves (33 female and 27 male). In this blocked randomized controlled trial, a total of 120 newborn calves were transported from a calf ranch to an experimental facility and were randomly allocated to either control (CON; no supplementation) or treatment (TRT; receiving 70 and 40 g of QSP supplemented in milk replacer daily within the first 14 and between 15 and 28 d of life, respectively). Calves were bottle fed with 6 L of milk replacer (24% CP and 20% fat all-milk) divided into 2 feedings and had libitum access to a texturized calf starter and water. Severe diarrhea was defined as presence of watery feces and severe dehydration, and BRD was based on the presence of clinical signs such as cough, eye discharge, abnormal respiration, nasal discharge, ear droop or head tilt, and rectal temperature ≥39.2°C. Body weight was measured on d 0, 28, 56 and 70. Survival analyses and linear regression models were fitted to the data. Treatment did not influence the likelihood of BRD ($P = 0.16$), diarrhea ($P = 0.40$), and mortality ($P = 0.38$). The hazard ratio (95% CI) of BRD, diarrhea, and mortality was 0.55 (0.22–1.37), 0.71 (0.23–2.21), and 1.51 (0.48–4.80) when comparing TRT with CON calves. The average daily gain (95% CI) for CON and TRT calves was 0.38 (0.35–0.41) and 0.42 (0.39–0.45) kg/d within the first 28 d of life ($P = 0.04$), 0.55 (0.52–0.58) and 0.58 (0.55–0.61) kg/d within the first 56 d of life ($P = 0.09$), and 0.64 (0.61–0.68) and 0.68 (0.64–0.75) within the first 70 d of life ($P = 0.06$), respectively. Findings demonstrated that QSP supplementation improved growth performance of calves, but no differences in the incidence of BRD, severe diarrhea, and mortality were observed.

Key Words: quorum-sensing, calves, health

2492 *Bacillus*-based direct-fed microbial improved performance and health status in early postpartum dairy cows.

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Direct-fed microbials (DFM), a source of live viable microorganisms, have been shown to improve health status and productivity of livestock animals. This study's objective was to evaluate the effects of a *Bacillus subtilis* and *Bacillus pumilus*-based DFM (Strateris® ECL; United Animal Health, IN) in early postpartum dairy cows. Late-lactation cows sourced from the Gharb region of Morocco were allocated into 1 of 2 dietary treatments (TRT) from calving up to 180 DIM: (1) DFM (15 g/hd per day of DFM, 7.4×10^9 cfu/d; n = 24) and (2) control (CON; n = 48). Milk samples from a subset of 30 cows (15/TRT) were analyzed every 15 d up to 65 DIM for yield (MY), fat, protein, ECM, FCM, and SCC. Metritis, ketosis, mastitis, gastrointestinal (GI) disease incidences, and fecal score (FS) were evaluated for all 72 animals throughout the study. Body condition score (1–5) was recorded at calving and at first service (1stAI). Production data were analyzed using PROC GLM in SAS 9.4 with DIM and TRT as fixed effects. Health events and reproduction were evaluated with NPAR1WAY or PROC FREQ with ChiSq. Due to small N, $P > 0.05$ and $P < 0.15$ were considered tendencies. The DFM did not affect MY ($P = 0.75$); however, ECM (+4.2 ± 1.5 kg, $P = 0.01$) and

FCM tended (+3.8 ± 1.4 kg, $P = 0.06$) to increase in DFM-fed cows. Direct-fed microbials also increased milk fat yield (+0.23 ± 0.05 kg, $P = 0.01$), fat percent (+0.49% ± 0.09%, $P = 0.01$), and protein percent (+0.13 ± 0.04, $P = 0.03$). No differences were noted for SCC ($P = 0.22$). Fecal scores were increased in DFM cows (+1.04 ± 0.11, $P = 0.01$), indicating a decrease in scours. Cows fed DFM tended to have lower cases of GI disease ($P = 0.06$) and clinical mastitis ($P = 0.14$), and had lower ($P = 0.03$) overall health events compared with CON cows, though culled rate was not affected ($P = 1.00$). The BCS at 1stAI was higher for DFM ($P = 0.03$; 2.6 vs. 2.4), and DFM tended to have more cows pregnant at 180 DIM ($P = 0.10$; 78.6% vs. 50%) compared with CON. Overall, supplementation of DFM led to an increase in performance and improvement of health outcomes, indicating its suitability as an early postpartum nutritional supplement.

Key Words: probiotic, multi-strain, feed additive

2493 Effects of synbiotic supplementation on postpartum performance and markers of inflammation in transition dairy cows.

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Synbiotics are used as feed additives in animal production to minimize the use of antibiotics on farms and promote animal health and production. The objective of this study was to evaluate the effects of a synbiotic supplement on performance, inflammation markers, and blood metabolites in transition cows. Multiparous Holstein cows (n = 62) housed in a freestall barn with Calan gates were assigned to 2 treatments (trt) in a completely randomized design from 28 d before expected parturition to 21 d postpartum. Cows were randomly assigned to trt based on previous 305-d milk yield and expected calving date, and all animals were followed until 60 DIM. Cows were fed once per day with a prepartum (−28 to calving) and 2 postpartum TMR (0–21 and 22–60 DIM). Treatments mixed into TMR consisted of a control (CON, 454 g/d concentrate on a DM basis) or a Synbiotic group (SYN, 440 g/d concentrate plus 14 g/d of *Bacillus subtilis* spores and yeast products on a DM basis, Amulet, Purina, St. Louis, MO). Blood inflammatory markers (n = 11/trt) were assessed on days −3, 0, 2, 3, 5, and 10 relative to calving. Dry matter intake, BW, and milk yield (MY) were measured daily, and milk samples were collected twice a week. Data were analyzed using the MIXED procedure (SAS, version 9.4) for repeated measures. The model included fixed effects of trt, time, their interactions, and the random effect of cow within trt. Dry matter intakes were similar between trt ($P > 0.05$). The synbiotic increased ($P < 0.05$) ECM and 3.5%FCM by 4.3 and 4.6 kg/d during the first 3 wk postpartum, respectively. There were interactions of trt with time ($P < 0.05$) for MY, ECM, and BW. After trt removal at 21 DIM, no differences ($P > 0.05$) were found for MY and FCM. Serum haptoglobin decreased in SYN relative to CON cows (0.085 vs. 0.204 g/L, $P = 0.03$). Cows fed SYN had higher postpartum serum albumin (3.57 vs. 3.42 g/dL, $P < 0.01$) than CON cows, with similar serum BHB ($P > 0.10$). Overall, the inclusion of SYN in the diet resulted in less haptoglobin, greater albumin, and improved milk yield in transition dairy cows.

Key Words: synbiotic, milk yield, inflammation

2494 Effects of calf gut-originated probiotics and weaning pace on health measures, hematology, and productivity in Holstein dairy calves.

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Weaning is a stressful event for a young calf and can lead to increased morbidity. With rising concerns about antimicrobial usage and antimicrobial resistance, alternative methods of disease treatment and prevention are needed. This study examined the effects of probiotic supplementation using a calf gut-originated direct-fed *Lactobacillus agili, L. delbrueckii, L. mucosae, and L. rueteri* 1×10^9 cfu/d (control vs. supplemented) and weaning pace (abrupt vs. gradual) on feed intake, growth, vitals (TPR), and hematological values in dairy calves in a 2 × 2 factorial arrangement of treatments. Holstein bull calves (n = 38) were blocked by birth weight and randomly assigned to one of 4 treatments: abrupt-control (AC; n = 10), abrupt-supplemented (AP; n = 10), gradual-control (GC; n = 9), and gradual-supplemented (GP; n = 9). The calves were fed 3.4L of milk replacer (22% CP, 22% fat) twice daily. Water, calf starter (18% CP), and chopped alfalfa were provided ad libitum, with intake of milk replacer, calf starter, and alfalfa recorded daily. The probiotic was supplemented in milk replacer once daily for 7 d, beginning 4 d before weaning. Abrupt weaning occurred over d 54–57 in 3 incremental step-downs of 1.13 L. Gradual weaning occurred over d 49–63 in 7 incremental step-downs of 0.49 L. Health parameters and BW were measured weekly, and blood hematology was measured at d 3 and 7 of age, onset of treatment, and the day after completion of weaning. Data were analyzed using a mixed model, with pace and supplementation as fixed effects. No differences in red blood cells, white blood cells, and platelets were detected by pace or supplementation. No differences in intakes were detected. During the treatment period (abrupt d 50–58, gradual d 45–64) ADG was larger in abrupt-weaned calves than in gradual-weaned calves (1.25 vs. 0.83 kg/d, $P = 0.009$). No differences in vitals were detected. Overall, the abrupt pace influenced ADG, but hematological and vital parameters were not significantly affected by pace, supplementation, or their interactions.

Key Words: probiotics, weaning pace, antimicrobial resistance

2495 Improving antibody titers in milk replacers with IgY.

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Protein sources in milk replacers (MR) contain varying amounts of IgG that can help reduce enteric pathogens. The objective of this study was to determine the IgG specificity profile of different MR and bovine plasma, and then see whether this profile could be improved with the addition of IgY, the avian equivalent to IgG. Five commercial MR without plasma were analyzed along with a 5% bovine plasma sample (to approximate a MR with plasma; Table 1). The IgY was purified from hyperimmunized egg powder generated by vaccinating laying hens with inactivated pathogens that cause scours. The specific product tested was EggTek®-C. The MR were obtained and randomized along with a bovine plasma sample. The IgG was purified from each by first precipitating with ammonium sulfate and then cleanup through a Protein A column. The IgY was purified from whole egg powder by removing the lipid fraction using dilute acidification followed by ammonium sulfate precipitation. The purified antibodies were conjugated to a horseradish peroxidase (HRP) and used in direct customized ELISA. The amount of IgG loaded was equal to 10 oz. of MR or 5% wt/vol plasma. The amount of IgY loaded was equivalent to a 2-g dose. Antibodies were mixed together and assayed against the following pathogens: bovine rota and corona viruses, *Cryptosporidium parvum*, and *Escherichia coli* K99. Specificity results are presented in A450 values (absorbance at 450 nm). This provides a measure of the amount of antibody bound to the pathogen of interest.

Key Words: calves, IgG, IgY

Table 1 (Abstr. 2495).

	Specificity (A450 value)							
	Rotavirus		Coronavirus		*Cryptosporidium parvum*		*Escherichia coli* K99	
Sample	IgG	IgG + IgY	IgG	IgG + IgY	IgG	IgG + IgY	IgG	IgG + IgY
MR A	0.136	0.321*	0.075	0.231*	0.084	0.354*	0.131	0.401*
MR B	0.009	0.171*	0.004	0.127*	0.006	0.155*	0.012	0.212*
MR C	0.031	0.265*	0.024	0.218*	0.022	0.271*	0.058	0.208*
MR D	0.128	0.261*	0.117	0.245*	0.116	0.275*	0.261	0.324*
MR E	0.301	0.376*	0.277	0.342*	0.305	0.402*	0.494	0.643*
5% Plasma	0.678	0.922*	0.696	0.933*	0.714	1.059*	0.221	0.353*

**t*-test, $P \leq 0.05$. The addition of the egg powder significantly improved the specific titers for all milk replacers (MR) and the 5% plasma sample.

2496 On-field application of near-infrared portable spectrometer for cow colostrum quality assessment.

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Besides IgG, which is crucial for passive immunity transfer, bovine colostrum (BC) contains essential bioactive factors that contribute to the growth and health of calves. In most cases, determination of composition traits is, however, costly and time-consuming. We tested the predictive ability of a pocket near-infrared spectrometer (SCiO, Consumer Physics) for BC traits to evaluate its suitability for in-field IgG and quality assessment. For this purpose, we (1) developed prediction equations for IgG (g/L) and some composition traits and (2) then tested the SCiO's discrimination ability between good and poor quality BC (IgG ≥ or <50 g/L). Seven hundred and nine BC samples from Holstein cows were analyzed. Each spectrum was then paired with reference values of IgG, fat, protein, lactose, essential AA, and minerals (Ca, P, S, K, Na, Mg, Zn, and Fe) determined via the respective gold standard, that is, radial immunodiffusion, VDLUFA, Kjeldahl method, HPLC, and ICP-OES. The dataset was randomly split into a testing set (75%) and a validation set (25%). The first set was for prediction models development via partial least square regression using 5-fold cross-validation in WinISI 4.10 software and the second set was for models validation. In addition, a discriminant analysis was carried out to assess SCiO's classification ability based on the spectral wavelengths. SCiO showed promising predictive performance for IgG, fat, and AA with $R^2_{CV} \geq 0.75$ and excellent prediction accuracy for protein and S ($R^2_{CV} \geq 0.93$). External validation confirmed the strong prediction ability for these traits in masked samples, with all $R^2_V > 0.81$. The device correctly identified the 61.3% of the poor-quality samples (n = 75). For the good-quality samples (n = 572), SCiO succeeded in 74.5% of cases. The area under the curve for samples in external validation was 0.98, highlighting that classification of BC quality (IgG) is thereby robust. SCiO provides accurate punctual determination of various BC components, including IgG. This allows farmers and veterinarians to make informed decision about calves' immunity and precision feeding and enables large-scale BC phenotypes collection.

Key Words: colostrum, calf health, spectroscopy

2497 Oral temperature as an indicator of fever in preweaned dairy calves.

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Diarrheal and respiratory diseases pose significant threats to preweaned dairy calves. Early detection affords prompt intervention, thereby minimizing disease spread and severity and improving welfare and performance. Change in core body temperature can be an early indicator of illness. In calves, this is typically measured via rectal temperature (RT), which is time intensive, stressful to the calf, and invasive. Our study explored the potential of measuring oral temperature (OT) as an alternative indicator of fever in dairy calves with a goal of informing the design of novel health-monitoring sensors. Our prospective cohort study was conducted at a single dairy farm in central New York where calves were housed in indoor group pens of 20 with free access to an automated milk feeder rationing up to 11 L/d of whole milk. Female Holstein calves (n = 150) were enrolled at birth and followed through 28 d of life. Ambient temperature and wind speed were recorded daily to account for environmental conditions (TC-3, ITC, Seneca Dairy Systems). Health scores, OT, and RT were measured at 1, 2, 4, 6, 8, 10, 12, 14, 18, 22, and 28 d. Daily milk intake records were available from the automated milk feeder starting at 5 d. Probe thermometers were used to measure OT (4" probe) and RT (2" probe) in Peak Hold temperature mode until stabilized. Statistics were generated in R. Preliminary findings (n = 121) showed an overall mean OT of 38.6°C ± 0.6 and RT of 39.0°C ± 0.5, with 15.2% of calves having a fever (RT ≥39.5°C). There was a strong positive correlation (r = 0.77) between OT and RT. Calves with fever had a mean OT of 39.5°C ± 0.4, and a mean RT of 40.0°C ± 0.4. A receiver operating characteristic curve aimed at identifying a fever via OT yielded an area under the curve of 0.96, with a sensitivity of 94% and a specificity of 92% at an OT threshold of ≥39.1°C. Our results demonstrate the capability to detect a fever via OT. These findings indicate that OT might be useful in monitoring health of preweaned dairy calves. Future work should assess the associations of OT with disease and inflammation.

Key Words: body temperature estimates, automated health monitoring systems, calf health

Dairy Foods 3: Cheese

2499 Making mozzarella cheese from high-casein milk: Challenges with coagulation and moisture/fat losses. G. J. Cassidy*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], Y. Lu[2], M. E. Johnson[2], and J. A. Lucey[1,2], [1]*Department of Food Science, University of Wisconsin–Madison, Madison, WI,* [2]*Center for Dairy Research, University of Wisconsin–Madison, Madison, WI.*

There is growing interest in using microfiltration (MF) to concentrate cheese milk, which helps increase cheese yield and removes whey proteins before cheesemaking. However, milks with casein (CN) levels >4% present significant challenges, including rapid coagulation and high fat and moisture losses during cutting. Milk was concentrated by MF to 9% CN. Cheese milks containing 2.5%, 5%, 7%, and 9% CN were prepared by blending retentate, permeate, and cream to a CN:fat ratio of ~1.15. Low moisture part-skim (LMPS) mozzarella was made from these milks (n = 3). We explored the effect of lowering the coagulation temperature from 33.3°C in the control (2.5% CN) to 27.8, 28.9, and 30°C for the 5%, 7%, and 9% CN milks, respectively. Rennet concentration was also lowered from 97 to 48.5 g/kg milk for the 3 high-CN milks. Rheological properties during the coagulation process were monitored by small amplitude oscillatory rheology. Multiple comparison ($\alpha = 0.05$, Duncan test) were used for statistical analyses. In preliminary studies, moisture was higher in control (48.2%) than in treatment cheeses (43.8%–45.3%), indicating moisture loss, so changes were made to discourage excessive syneresis. For the high-CN milks, curds were left to rest for half an hour after cutting before being cooked, stirred, or manipulated. After draining, matted curds were cut into large chunks by hand instead of being machine milled. These changes increased the moisture content (>48%), but extensive fat losses still occurred. Gelation time, defined as the time after rennet addition at which a milk gel reached a storage modulus of >0.25 Pa, was 11.2 ± 0.7, 18.5 ± 2.6, 17.5 ± 1.6, and 20.4 ± 0.1 min for 2.5%, 5%, 7%, and 9% CN milks, respectively. Despite the longer gelation times for the higher CN milks, curd firming rates at cutting were still higher than the control (1.6 ± 0.6 Pa/min) at 6.0 ± 1.1, 15.7 ± 0.1, and 23.0 ± 10.3 Pa/min, respectively. The fracture stress of milk gels increased with increasing CN content, from 25 Pa in the control to 118 Pa in the 9% CN milk. We are exploring additional approaches to help reduce fat losses in these high-CN milks.

Key Words: low-moisture part-skim (LMPS) mozzarella, coagulation, high casein milk

2500 The chemical and physical properties of cheese produced from milk of cows fed different dietary starch concentrations. P. Uzun[1,2], R. L. Culbertson*[1], N. Senevirathne[1], E. Tumino-Van Amburgh[1], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Isparta University of Applied Sciences, Isparta, Türkiye.*

The diet of the cow has the potential to influence the chemical and physical properties of cheese. We examined the effects of dietary starch fed to cows on the chemical and physical properties of cheese. We enrolled 30 midlactation Holstein cows (2.5 ± 1.8 lactations; 38 ± 9.1 kg milk/d) in a study with a completely randomized design. Cows were assigned to 1 of 2 diets (n = 15/diet): a high ash-free neutral detergent fiber (NDF) and low starch diet (LS; 37% NDF, 20% starch) or a low NDF and high starch diet (HS; 32% NDF, 25% starch) for an 8-wk period. Cows were fed a diet of corn silage, triticale silage, and concentrates, and milked thrice daily. For each treatment group, milk samples (9 milkings/wk) were collected at wk 8. Milk fat percent was lower and milk protein percent greater in cows fed HS diets ($P < 0.10$). Milk C16:0% was lower and C18:2 *cis*-9, *cis*-12% was greater in cows fed HS diets ($P < 0.01$). Collected milk samples were pasteurized and treated with starter culture and rennet to prepare Toma cheese. The cheese samples ripened for 0, 45, and 90 d at 10°C. Data of chemical composition, fatty acids (FA), and instrumental color and texture of cheeses were analyzed by 2-way ANOVA (GLM procedure) to determine the fixed effects of diet, ripening, and their interaction. Moisture content was comparable for LS and HS cheese (~37 for d 0; $P = 0.42$). For d 0, protein percent was higher for LS cheese (35.6% and 31.4% for LS and HS, respectively; $P < 0.05$). Although cheese fat percent was not modified by diet, diet modified the FA profile of cheese (e.g., HS cheese had higher unsaturated FA percent; $P < 0.05$). The yellowness of cheese samples at d 0 was not markedly affected by treatment but increased during the ripening, and the interaction between diet and ripening was significant ($P < 0.05$). Hardness and chewiness values of the cheese samples were significantly higher for LS cheese for all ripening periods ($P < 0.01$). Modifying the dietary starch content did not modify adhesiveness, cohesiveness, or springiness of cheese. Modifying the dietary starch content of Holstein cows modified the chemical and physical properties of Toma cheese.

Key Words: cheese, cow, diet

2501 Understanding the effects of different starter cultures on the pH of early aged Cheddar cheese. S. Clarke*, T. Oberg, and P. Sharma, *Utah State University, Logan, UT.*

During Cheddar cheese production, starter lactic acid bacteria (SLAB) lower the curd pH by acid production of lactose fermentation. As the pH decreases, acid is buffered by calcium phosphate, which solubilizes in the casein micelle, allowing constant buffering during cheese make and storage, enough to cause quality defects such as off-flavors, texture, and food safety. Typically, mesophilic *Lactococcus* species are used as SLAB, but recently thermophilic *S. thermophilus* strains are being used in SLAB blends to speed up the rate of acid production. These blends have different acidification rates during cheese make, with faster acid production potentially lowering pH faster than calcium phosphate solubilization, delaying buffering until the cheese is in storage. Four cheese SLAB blends (100% *L. lactis*, 100% *S. thermophilus*, 75% *L. lactis* and 25% *S. thermophilus*, and 75% *S. thermophilus* and 25% *L. lactis*) were used to study acidification profiles in a simulated cheese make under controlled conditions in 1-L bioreactors using standardized milk with temperature profiles similar to commercial makes. A 4.5% salt solution was added at 4 h. The pH profiles show the 3 blends containing *S. thermophilus* had delayed buffering around 4 h as shown by the plateau in pH. The 75% and 100% *S. thermophilus* blends had a longer period of buffering compared with the blend with only 25% *S. thermophilus*. Data show a decrease in buffering in all blends containing *S. thermophilus* when 4.5% (wt/vol) of salt was added. This was not observed in the 100% *L. lactis* blend, which showed delayed buffering when salt was added. With salt addition, we observed similar acidification rates but at a higher pH compared with cultures without salt addition in all the blends except the 75% *L. lactis* and 25% *S. thermophilus.* The 100% *L. lactis* blend with salt addition showed slower acid development than without salt addition, an indication that salt has a greater effect on *L. lactis* acid production compared with *S. thermophilus*. These results show that acidification rate is affected by the SLAB cultures and that acidification rates could affect buffering in Cheddar cheese during early storage.

Key Words: Cheddar cheese, buffering, calcium phosphate

Dairy Foods 3: Dairy Products

2502 Meltdown rate of various sugars in ice cream. L. Peck*, J. Waite-Cusic, and Z. Atamer, *Oregon State University, Corvallis, OR.*

Rare sugars are "label friendly" alternative sweeteners that are gaining popularity in the food industry, particularly those with high sweetness and low glycemic index. These sweeteners support the formulation of products for diabetics as well as those following low-carbohydrate diets (e.g., keto). Sugars affect product formulations beyond added sweetness; they can significantly affect texture and product performance. The objective of this study was to evaluate how different sugars, particularly tagatose, affect the hardness and melting rate of ice cream. A basic ice cream recipe (240 g whole milk, 720 g butterfat cream [36% fat], and 159 g sugar) was made in a Whynter 2.1 quart ice cream maker. Ice cream samples were produced using different types of sugar in each production. Sugars included were sucrose, dextrose, fructose, lactose, and tagatose. The hardness of ice cream samples was measured using a McCormick pocket penetrometer. Meltdown measurements of ice cream samples were determined at 22°C for a cylindrical cut of ice cream (55 ± 2 g; 6.4-cm diameter, 3-cm height). The hardness of the ice cream varied, with fructose being the softest (1.8 kg), followed closely by tagatose (2 kg), dextrose (3 kg), and sucrose (4.6 kg). The ice cream formulated with lactose was so hard that it could not be measured by the hand-held penetrometer (>13 kg). The results of melting down followed an identical pattern. After the initial thawing period, thawing rates ranged from 0.09 $\mathrm{mL\ min^{-1}}$ (fructose) to 0.05 $\mathrm{mL\ min^{-1}}$ (sucrose). The meltdown of tagatose and fructose was significantly faster than that of sucrose (P-value < 0.05). The lactose-formulated ice cream did not demonstrate any measurable melting until the end of the test (3 h). This study solidifies that bench-top ice cream makers allow for the evaluation of hardness and meltdown in ice cream with various sugars. The rare sugar, tagatose, made a viable alternative, with its properties being softer and faster melting than sucrose.

Key Words: formulations, texture, tagatose

2503 Effects of stabilizers (conventional versus unconventional) on the qualities of yogurt. G. Ibhaze* and D. Olatunji, *Federal University of Technology, Akure, Ondo State, Nigeria.*

Yogurt is a healthy dairy product. During production, conventional stabilizers such as gelatin are used as thickeners. Due to the high cost and lack of ready availability of gelatin, there is the need for a low-cost, readily available alternative source. This study therefore examined the effect of cassava, corn, and sweet potato flours as unconventional stabilizers on yogurt qualities. A 20 g sample of each flour was dissolved in 200 mL of water (1:10 wt/vol) and boiled to form a paste. Yogurt was manufactured following the standard manufacturing procedure using 20 g of paste of each of the stabilizers. Samples were refrigerated at 4°C for 14 d. The gelatin served as the positive control and a stabilizer-free yogurt served as the negative control. The experiment was a 5 × 3 factorial arrangement in a completely randomized design. All data obtained were subjected to ANOVA, and means were compared by the methods of Duncan's Multiple Range Test using SAS 2010 version 9.3. Analyses were done at 24 h, and 7 and 14 d. Storage effects showed that lactose and vitamin C concentrations were significantly ($P < 0.001$) at their peak at 24 h of storage, and the pH concentration (4.77) and peroxide value (5.85 Meq/kg) were highest at the 14th day of storage. Mineral concentrations were at their peak at 24 h of storage and depreciated most at the 14th day. Treatment effect revealed that Vitamin C (20.59 mg/100g) and lactose (8.42 mg/mL) values were highest in yogurt stabilized with cassava flour, and yogurt stabilized with gelatin had the highest potassium (3,449.44 mg/100 g), calcium (2,211.18 mg/100 g), magnesium (8,734.87 mg/100 g), zinc (48.25 mg/100 g), and manganese (0.68 mg/100g) concentrations. However, iron was significantly ($P < 0.001$) highest (16.06 mg/100 g) in yogurt stabilized with cassava flour. Conclusively, this study revealed that these unconventional stabilizers compared favorably with the conventional stabilizer in the quality of yogurt produced. However, cassava flour demonstrated better potential among the flours explored in comparison with gelatin.

Key Words: yogurt, stabilizers, quality

2504 Odd- and branched-chain fatty acid from dairy farms with different levels of pasture in Holstein cows' diet. L. Grille*[1], I. Vieitez[2], M. Romero[2], A. Garay[3], S. Jorcín[3], E. Krall[4], B. Irigaray[2], M. N. Méndez[1], E. Bejarano[3], A. Casal[5], and T. López[3], *[1]Departamento de Ciencias Veterinarias y Agrarias, Cenur Litoral Norte, Universidad de la República (UdelaR), Paysandú, Uruguay, [2]Departamento de Ciencia y Tecnología de Alimentos, Facultad de Química, Universidad de la República (UdelaR), Montevideo, Uruguay, [3]Unidad Tecnológica de Lácteos, Instituto Tecnológico Regional Suroeste, Universidad Tecnológica del Uruguay (UTEC), Colonia, Uruguay, [4]Carrera Tecnólogo Químico, Instituto Tecnológico Regional Suroeste, Universidad Tecnológica del Uruguay (UTEC), Paysandú, Uruguay, [5]Departamento de Producción Animal y Pasturas, Estación Experimental Mario A. Cassinoni (EEMAC), Facultad de Agronomía, Universidad de la República (UdelaR), Paysandú, Uruguay.*

Ruminant milk contains lipids that are abundant in bioactive components. More recently, odd-chain branched fatty acids (OBCFAs) have been shown to have human health benefits. Specifically, C15:0 and C17:0 iso and anteiso have been found to improve the fluidity of cell membranes. The concentration and composition of milk fat can be easily altered by dietary adjustments. This study aims to evaluate the variation in milk of OBCFAs in dairy farms with differing levels of pasture in cow diets during spring. Two treatments were carried out according to the proportion of pasture in the diet: high pasture (HP, > 65%) and low pasture (LP, < 35%; n = 6). Milk, pasture, and supplement (roughage+concentrate) were sampled weekly from each farm. The samples were transported for fatty acid (FA) analysis to the chemistry faculty at Universidad de la República. High-pasture cows consumed in average 74% pasture and 26% supplement, and LP cows consumed 10% pasture and 90% supplement. The FA (g/100 g of fat) were analyzed with an ANOVA for repeated measures using the GLIMMIX procedure of SAS. High-pasture treatment had higher OBCFA total than LP (2.57% vs. 2.03%, respectively; $P < 0.01$). The C15:0 iso (0.36% vs. 0.28%; $P < 0.05$), anteiso (0.69% vs. 0.48%; $P < 0.01$), C17:0 iso (0.43% vs. 0.34%; $P < 0.01$) were higher in HP than LP, respectively. However, C17:0 anteiso was not different between treatments. There was no period effect nor interaction between treatment and period. The higher forage:concentrate ratio in HP could have increased the cellulolytic bacteria in the rumen, which are related to a higher proportion of iso FA in milk, compared with LP. Therefore, this work reaffirms the importance of including pasture in the cows' diets and the resulting benefits on milk fat quality, because HP achieved greater bioactive capacities toward human health.

Key Words: grass, milk fat, human health

2506 Hyaluronic acid addition in ice cream manufacturing as a novel method to enhance its characteristics. M. Hamouda* and P. Salunke, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Maintaining ice cream's stability to prevent melting and undesirable texture changes and controlling the formation of large ice crystals during freezing are critical challenges for manufacturers. These issues can decrease the desired smooth and creamy consistency and affect the quality of ice cream. In some countries, hyaluronic acid (HA) has been allowed for incorporation in some food products as a novel ingredient to provide health benefits. Its unique ability to retain water also offers potential improvements in various foods' texture and moisture content, enhancing their appeal and nutritional profile. This study was designed to evaluate the effect of HA with varying molecular weights (MW) on enhancing ice cream characteristics. The ice cream formulation consisted of 33.92% cream, 41.80% skim milk, 5.89% nonfat dry matter, 14.85% sugar, and 3.29% dry corn syrup, totaling 99.75%. The remaining 0.25% comprised ice cream stabilizer in the control sample (C), HA with a MW of 980 kDa (T1), and HA with a MW of 2,500 kDa (T2). The ice cream mix was blended, aged 6 h, and manufactured in a laboratory-scale freezer. Ice cream samples were evaluated for hardness, rheological properties (storage modulus G′, loss modulus G″), melting rate, and ice crystal size. The data were statistically analyzed using ANOVA, mean comparison was done using Tukey, and the experiment was replicated thrice. Results indicated that adding HA led to a significant ($P < 0.05$) decrease in hardness values from 3,279 g in sample C to 2,067 g in T2. Additionally, compared with the control sample, HA addition significantly ($P < 0.05$) increased elastic modulus (G′) and viscous modulus (G″) and significantly ($P < 0.05$) decreased melting rate and ice crystal size in ice cream samples (T1 and T2). Moreover, the study showed that the high MW of HA (2,500 K) in T2 exhibited a more significant effect on decreasing the hardness, melting rate, and ice crystals and increasing (G′) and (G″). In conclusion, adding HA significantly improved ice cream characteristics, and as MW increased, the properties changed.

Key Words: hyaluronic acid, ice cream characteristics, rheological properties

2507 Milk powder flowability: Effects of water activity, particle size distribution, and storage conditions. M. S. Elfaruk*[1], A. R. A. Hammam[2], K. A. Alsaleem[3], and M. E. A. Hamouda[2,4], *[1]Valley Queen Cheese Factory Inc., Milbank, SD, [2]Dairy Science Department, Faculty of Agriculture, Assiut University, Assiut, Egypt, [3]Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, Buraydah, Saudi Arabia, [4]Dairy and Foods Science Department, South Dakota State University, Brookings, SD.*

The shelf life of milk is short, due to its high water content, which makes it a suitable medium for microorganisms and enzymatic reactions and requires preservation techniques such as spray drying. However, skim milk powder (SMP) encounters some challenges during storage because of the changes in functionality, such as particle size distribution (PSD), density, flowability, and water activity (a_w), which can be affected by the raw milk, drying process, relative humidity, storage temperature, and time. Therefore, the objective of this study was to examine the effect of a_w, PSD, and storage temperature (4, 25, and 37°C) for 7 d on the flowability of SMP. Samples from 3 distinct spray-drying plants in South Dakota and Minnesota were obtained and subjected to compositional analysis for moisture and lactose content determination. Additionally, a_w, PSD, and flowability were assessed. The extent of powder caking was evaluated by examining the alterations in flowability that were measured by the Evolution Powder Tester. The experiment was repeated 3 times, and all measurements were performed in triplicate. Statistical analysis was performed using R software. Significant differences ($P < 0.05$) were observed in moisture content among samples (ranging from 1.22% to 4.20%), but the lactose content exhibited no significant effect (ranging from 54.5% to 56.1%). Notably, a_w values ranged from 0.11 to 0.36 and exhibited significant differences ($P < 0.05$) at different storage temperatures. Although PSD exhibited relatively narrow distributions ($P > 0.05$), it significantly influenced powder flowability ($P < 0.05$). The values of flowability ranged from passable to poor (19.7% to 29.4%), and they were significantly affected by the storage temperature ($P < 0.05$). These findings emphasize the critical role of a_w and storage conditions in determining the quality of SMP during production and subsequent use as a food ingredient. Moreover, these results provide insights into optimizing storage conditions to maintain desired flowability characteristics, thereby enhancing the overall quality and usability of milk powder products.

Key Words: milk powder, flowability, water activity

2508 Elaboration and characterization of dulce de leche with reduced sugar content. V. F. Moebus[1,2], T. R. Cunha[1], F. B. N. Köptcke[1], L. A. Pinto[3], and L. A. M. Keller*[1], *[1]Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil, [2]Universidade Castelo Branco, Rio de Janeiro, Rio de Janeiro, Brazil, [3]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.*

Dulce de leche (DL) is a South American traditional dairy product with a high sugar content. As such, it is unsafe for consumers with special conditions, such as diabetes. With the intent to develop a light dulce de leche (LDL) formulation for those consumers while keeping the sensory characteristics, the present study used sweeteners to substitute for sucrose and used gums as thickening and stabilizing agents. Physical-chemical analytics were realized according to the AOAC methodologies for both formulations, with DL being used as the control group. Statistical analyses were carried out by comparing means and frequencies employing the Tukey tests and ANOVA at a significance level of 5%. The standards and average of centesimal composition of both products are presented in Table 1 within the parameters according to MERCOSUL legislation. It was observed that DL stayed within legislation in moisture and ashes, and the LDL surpassed those parameters. Both formulations presented fat content below the standard, with the LDL presenting a higher content. The protein content of both formulations was above the minimum required, with LDL being more proteinic. Rheological analyses showed that both samples had excellent adhesiveness and firmness. The results showed that the replacement of sugar

Table 1 (Abstr. 2508). Centesimal composition

Item	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)
MERCOSUL legislation (identity and quality standard)	<30.00	<2.00	6.00–9.00	>5.00
Traditional dulce de leche (DL)	29.88 ± 0.93	1.42 ± 0.11	2.65 ± 0.23	7.54 ± 0.55
Light dulce de leche (LDL)	65.63 ± 1.60	2.88 ± 0.02	3.50 ± 0.33	12.07 ± 0.19

with other ingredients can offer the necessary characteristics of DL. A limited reduction in energy value was observed, with LDL presenting 263.46 Kcal and DL 288.05 Kcal, which was occasioned by the addition of compounds to substitute sucrose. Even though the energy value required for the product be considered light by Brazilian legislation was not achieved, the light formulation presented good stability. Thus, studies to improve a methodology that has proven promising for the development of a product with functional characteristics for consumers with restrictions are favorable.

Key Words: nutraceuticals, light foods, food safety

2509 Comparison of instrumental and sensory texture evaluations of different types of refrigerated butter. R. Choriego*, B. Patel, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Butter is a dairy product that can be eaten as a spread. Cold spreadability is an attribute desired by consumers. One of the main factors determining butter spreadability is the proportion of unsaturated fatty acids in the milk used in manufacturing, but other factors can also play an important role. For example, prior findings from a survey of commercial butters have suggested that salt content in butter could decrease its spreadability. Similarly, culturing cream in butter manufacture could also produce a softer butter. The objectives of this study were to evaluate the texture of different types of butter and to assess how different analysis techniques compare with each other, aiming particularly to explore the results from instrumental and sensory evaluations. Six types of butter were made for the experiment, varying in salting, cattle feeding practices, and cream fermentation. Additionally, a blend of butter with sunflower oil was used as positive control, and another blend with coconut oil acted as negative control due to their high and low unsaturated fatty acids profiles, respectively. Texture was instrumentally evaluated in cold samples by cone compression, forward extrusion, and backward extrusion with a with a TA.XT.plusConnect texture analyzer and by rheology. For the nonoral sensory evaluation, 20 panelists were asked to rate samples' spreadability using a discrete 9-point scale and to rank the samples by perceived spreadability. Other butter parameters evaluated were total moisture, total fat, and solid fat content. Scalar results were analyzed with an ANOVA and Tukey's HSD as post hoc test. Significant differences between butters ($P < 0.05$) were observed in the instrumental assessments. A higher force was required for forward extruding nongrassfed unsalted butter (138.74 ± 9.64 N) compared with nongrassfed unsalted cultured butter (110.16 ± 5.59 N). This shows that cultured cream tended to result in a more spreadable butter. Moreover, the forward extrusion approach allowed for a higher throughput of samples with an easier and cost-effective sample preparation method.

Key Words: butter, rheology, texture profile analysis

2510 Fatty acids profile and lipid indices of ghee (butter oil) manufactured from bovine colostrum and sweet cream. Z. Modi, K. Dubey*, and P. Salunke, *South Dakota State University, Brookings, SD.*

Bovine colostrum is a secretion from the mammary gland formed in the first days after parturition. Large-scale milk production yields a significant byproduct called colostrum. Colostrum cream is the byproduct of the industry where the colostrum cream is separated, and the colostrum milk is processed and converted into a colostrum formula, immunoglobulin colostrum powder. Colostrum cream composition differs significantly from milk fat and can be a valuable source of fatty acids in the human diet. Knowing the fatty acids and lipid indices of colostrum ghee (butter oil; BO) and milk fat is crucial because they can significantly influence health. The study was planned to see the effect of fatty acids profile and health lipid indices of colostrum cream and BO samples made from bovine colostrum and sweet cream. One sweet cream sample from a dairy plant and 2 colostrum cream samples were collected from a private company. The experiment was replicated thrice. All butter oil samples were made in an atmospheric condition using a standard protocol. The cream (sweet and colostrum) and BO samples were analyzed for chemical composition and the fatty acids profile using GC/MS combined as an analytical method. The atherogenicity index (AI) and thrombogenicity index (TI) were determined using reference equations. The data were statistically analyzed using Costat. The monounsaturated fatty acids (MUFA) of sweet cream was significantly ($P < 0.05$) lower, and trans-fat values were significantly ($P < 0.05$) higher than the colostrum cream. Sweet cream's AI and TI values were significantly ($P < 0.05$) higher than those of the colostrum cream samples. The proximate composition and triglyceride level of the BO sample were similar. The saturated and trans fatty acids of sweet cream BO were significantly ($P < 0.05$) higher, and MUFA were significantly ($P < 0.05$) lower than colostrum BO. The AI and TI values of colostrum BO samples were significantly ($P < 0.05$) lower than the sweet cream BO. In conclusion, colostrum cream and its products, such as BO, can be used as an essential source of fatty acids in the human diet.

Key Words: colostrum cream, butter oil, fatty acids

2766 Flavor mitigation of hemp extract–infused ice cream. H. Shi*[1], K. Freeman[2], E. Kawka[3], M. McHenry[4], and M. Guo[1], [1]*Department of Nutrition and Food Sciences, University of Vermont, Burlington, VT,* [2]*Departments of Emergency Medicine and Pharmacology, University of Vermont, Burlington, VT,* [3]*Cattis Scientific, Hardwick, VT,* [4]*Department of Pharmacology and Plant Biology, University of Vermont, Burlington, VT.*

Hemp extract (HE)-infused dairy products are blooming in the United States, but the bitterness and astringent "hempy" flavor may limit consumer acceptance. Beta-cyclodextrin (β-CYD) and polymerized whey protein (PWP) have been applied to improve unwanted flavors in some products. However, studies on improving the flavor of HE-infused dairy products and their consumer acceptance are very limited. This study aimed to investigate the use of β-CYD and PWP to mitigate the hempy flavor of HE-infused ice cream and explore the overall consumer acceptance. Hemp extract-infused ice cream was formulated with 4.26% protein, 22.87% carbohydrates, 12.39% fat, 0.55% mixture of stabilizers and emulsifiers, and 0.27% hemp extract oil. All of the samples contained 200 mg cannabidiol (CBD) per serving (236 mL). Combinations of β-CYD and PWP were added into the ice cream mix at different mass ratios (0:100, 50:50, 25:75, 100:0), and compared with the control group of HE-infused ice cream without any flavor masking agent. Sensory evaluation from 19 participants was conducted to measure flavor attributes and overall acceptance of all ice cream samples using a 5-point hedonic scale test and check-all-that-apply test. Results showed that compared with the control group, the β-CYD solely group greatly reduced the hempy flavor scores from 3.94 ± 1.30 to 2.72 ± 1.23 ($P = 0.043$); the other groups did not show significant reduction in hempy flavor. There was no significant difference in consumer acceptance among the 5 groups ($P > 0.05$). The PWP-only group had the highest scores of hempy flavor (4.61 ± 0.85), but the texture (gumminess) of the ice cream was improved by PWP. No panelist reported gumminess in the ice cream samples with a higher portion of PWP fortification. The combination of β-CYD and PWP at a ratio of 25:75 had a better effect on reducing hempy flavor than the PWP-only group (3.53 ± 1.36, $P = 0.046$) and showed a similar overall acceptance (3.17 ± 1.50) as β-CYD only group (3.36 ± 1.53). In conclusion, β-CYD and PWP may be useful flavor-masking agents to mitigate the hempy flavor of the HE-infused ice cream.

Key Words: hemp extract-infused dairy food, flavor mitigation, polymerized whey protein

Dairy Foods 3: Microbiology

2512 Microbiological evaluation of dulce de leche with reduced sugar content under storage conditions. T. R. Cunha[1], V. F. Moebus*[1,2], F. B. N. Köptcke[1], L. A. Pinto[3], C. E. R. Coutinho[1], and L. A. M. Keller[1], [1]*Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil,* [2]*Universidade Castelo Branco, Rio de Janeiro, Rio de Janeiro, Brazil,* [3]*Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil.*

In South America one of the traditional products is dulce the leche (DL), a dairy product with a high sugar content. The present study aims to evaluate the microbiological stability of a light dulce de leche (LDL) formulation developed using sweeteners replacing sucrose and gums as stabilizing and thickening agents. For the study, 2 formulations, LDL and DL, were produced. The formulations were prepared by heating and stirring continuously until the desired point after adding the ingredients. Microbiological analysis was performed according the Compendium of Methods for the Microbiological Examination of Foods (APHA) for microorganisms presented in Table 1 following the MERCOSUL legislation. The analyzes revealed that both final products are safe to consume. The microbial counts of raw material were in compliance with established standards, which is important to ensure quality of the products. As observed in Table 1, the DL and LDL present lower values in microbiological counts, confirming the efficacy of heat treatment and sanitation of the manufacturing process. Sugar content of the final products was 69.7 and 56.6 °Brix for DL and LDL, respectively. High sugar content also has antimicrobial properties and with the heat treatment, which reduces water in product (A_w = 0.81 and 0.72 for DL and LDL, respectively), contribute to the food preservation. The use of sweeteners associated with DL processing can guarantee the safety of the final product. The methodologies proved to be promising in the development of a quality product, noting that microbiological parameters were adequate and contributed to a product with good stability, serving as basis to improve development of dulce the leche with functional properties.

Key Words: nutraceutical, light food, food safety

2513 Yeast and mold represent the most common group of microbial contaminants in ice cream. W.-Y. Chueh*, R. L. Weachock, M. Wiedmann, and N. H. Martin, *Cornell University, Ithaca, NY.*

Microbial contamination in ice cream may originate from raw materials, inadequate equipment cleaning, environmental exposure during manufacturing, and contaminants present in ingredients added after pasteurization. Despite the perceived safety of frozen products, such as ice cream, recent foodborne outbreaks have shown that pathogen contamination of ice cream is a food safety concern, and microbial contamination should be carefully examined. To that end, our goal was to determine the primary groups of microbial contaminants in contemporary manufactured ice cream supplies in the United States. A total of 347 ice cream samples were collected, 56 of which had inclusions (i.e., chocolate chip, butter pecan, and so on). Samples were evaluated using standard plate count (SPC) for total bacterial count, crystal violet tetrazolium agar (CVTA) for total gram-negative bacteria, 3M Coliform Petrifilm for coliforms, and 3M YM Petrifilm for yeast and mold count. Our results showed that of the ice cream samples tested here 70% had detectable total bacteria counts, and had a mean SPC of 2.54 $\log_{10}$ (cfu/g). A total of 15%, 22%, and 44% of the samples showed evidence of contamination with coliforms, gram-negative bacteria, and yeast and mold, respectively. Of the samples with evidence of contamination from each of these groups, the mean coliform count was 1.48 $\log_{10}$ (cfu/g), the mean CVTA was 2.92 $\log_{10}$ (cfu/g), and the mean yeast and mold (YM) was 1.60 $\log_{10}$ (cfu/g). Our results indicate that there are diverse microbial contaminants in contemporary ice cream products and suggest that there is a need for improved hygienic handling of ice cream to maintain the desired safety and quality parameters. Our results also suggest that fungal contaminants (i.e., yeast and mold), which were found in approximately half of the products tested, may provide a better indicator of hygienic conditions than coliforms, which are the current indicator commonly used by the industry and were only detected in 15% of the products tested. Overall, these results can be used by the ice cream industry as a starter point to enhance the microbial quality and safety of their products.

Key Words: ice cream, coliform, microbial contamination

2514 Characterizing the microbial ecosystem within an Ontario on-farm washed-rind cheese production facility. T. Wilson*, M. Siddiqi, Y. Xi, and G. LaPointe, *University of Guelph, Guelph, ON, Canada.*

Good farm management practices of the dairy farm environment, proper sanitation of the cheese processing facilities, and pasteurization help to decrease bacteria found in milk and cheese. Well water is an influential component of the 2 facilities that could be contributing to the consortium of bacteria in the environment and final product. The aim of this study was to investigate the microbial diversity of a dairy production facility and artisanal cheese manufacturing plant of an on-farm commercial operation. Over a 6-mo period, milk, water, and swab samples were collected after sanitation from 40 sites on the farm (n = 14) and in the cheese-making plant (n = 26). Extracted DNA was subjected to 16S rRNA gene amplicon (V3-V4 region) sequencing for amplicon sequence variants (ASV) analysis using R Studio. In total, the combined production and processing facilities (n = 100) revealed that milk (n = 19) contained the least number of ASV (279), showing the lowest diversity compared with swabs (n = 69; 8,747 ASV), and

Table 1 (Abstr. 2512). Microbiological evaluation after refrigerated storage[1]

Microorganism	MERCOSUL legislation	Dulce de leche (DL)	Light dulce de leche (LDL)
Coagulase-positive *Staphylococcus*	2.0	<1.0	<1.0
Total coliforms	2.0	<1.0	<1.0
Thermotolerant coliforms	2.0	<1.0	<1.0
Filamentous fungi and yeast	2.0	<1.0	<1.0
Salmonella spp.	Absent	Absent	Absent

[1] CFU counts are expressed in $\log_{10}$cfu g^{-1}; limit of detection = <1.0 $\log_{10}$cfu g^{-1}

water (n = 12; 3,444 ASV) at ≥0.1% relative abundance, unfiltered for prevalence. When filtering sequence reads to ≥20% prevalence, 7 genera were revealed in milk, 8 in swabs, and 12 in water samples. Of these, 2 out of 7 genera in milk were shared with swabs and water. Three genera unique in ≥20% swabs were identified as *Delftia*, *Pseudomonas*, and *Stenotrophomonas*, and 7/12 genera in water were also found in either milk or swabs. Genera which were consistently found across all 3 sample types included *Brevibacterium* spp. and *Yaniella* spp. In total, 171 *Brevibacterium* spp. ASV and 26 *Yaniella* spp. ASV were seen across milk, water, and swabs, highlighting the intragenus diversity. In water, *Brevibacterium* spp. was the most abundant genus (32%), and it was the second most abundant genus in swab samples (18%). *Stenotrophomonas* spp. was the most abundant genus in swabs (38.5%). These naturally occurring *Brevibacterium* spp. could contribute beneficially to the cheese rind ecosystem.

Key Words: milk, ecology, ASV

2515 Behavior of *Listeria monocytogenes* in commercial Hispanic-style cheeses.

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Hispanic-style cheeses (HSC) are a growing market segment for the dairy industry. The microbial safety of these cheeses continues to be a concern due to recent outbreaks and recalls associated with *Listeria monocytogenes* contamination. Hispanic-style cheeses have a low-to-neutral pH and high moisture content, providing suitable conditions for the survival and growth of *L. monocytogenes.* This study compared the ability of commercial HSC products to support the growth of *L. monocytogenes* during refrigerated shelf life and identify product characteristics that could be contributing to growth inhibition. Seven commercial HSC products representing 4 cheese types were purchased at retail: queso blanco (QB1, QB2), queso fresco (QF5, QF6), queso cotija (QC3), and queso oaxaca (QO1, QO2). Parameters (pH, water activity [a_w], moisture content) and background microbial composition (standard plate count, *Enterobacteriaceae,* yeast and mold, and lactic acid bacteria) of each cheese were determined. Cheeses were divided into 20-g samples and inoculated with a 5-strain cocktail of *Listeria monocytogenes* at an initial cell density of 3 log colony-forming units (cfu)/g. Inoculated cheeses were vacuum sealed, stored at 7°C and analyzed every 4 d for up to 20 d. *L. monocytogenes* cell density was determined by serial dilution and spread plating on Harlequin *Listeria* Chromogenic agar with incubation at 37°C for 48 h. HSC varied in pH (4.91–5.79), a_w (0.915–1.00), and moisture content (35–49%). QC3 had the lowest value of all 3 parameters, with relatively stable *L. monocytogenes* counts and a slight decrease by the end of the 20-d storage period. Most of the Hispanic-style cheeses did not support significant (>0.5 log cfu/g) increases in *L. monocytogenes* within 20 d, but QO1 supported significant growth of *L. monocytogenes* (>3 log cfu/g increase within 12 d). These findings highlight the variance in HSC ability to support the growth of *L. monocytogenes.* Behavior of *L. monocytogenes* in commercial HSC will continue to be explored, to identify additions that can protect HSC against *L. monocytogenes* growth.

Key Words: *Listeria monocytogenes*, Hispanic-style cheeses, food microbiology

2516 Antibody-sugar oxidase conjugates as antimicrobial tools against food pathogens.

M. Valdiviezo*, T. DeMarsh, and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Sugar oxidases such as glucose and lactose oxidase have been proven effective as growth inhibitors against different food pathogens and spore-forming bacteria, by release of hydrogen peroxide subsequent to sugar oxidation. However, the application of these enzymes has some limitations, such as nonspecificity and changes in sensory attributes. Combining these oxidases with specific antimicrobials such as antibodies could address this limitation by targeting the location of hydrogen peroxide production and therefore minimizing secondary effects. The objective of this study was to synthesize antibody-oxidase conjugates and to assess their inhibition potential against target pathogens. For this aim, a sugar oxidase was conjugated to an antibody specific for *Listeria monocytogenes* (IgOx), and was added to inocula ranging from 2 to 9 log colonoy forming units (cfu)/mL. Solutions of unconjugated enzyme (Ox), unconjugated antibody (Ig), or PBS (P) were applied as positive and negative controls, respectively. All 4 treatments were incubated at room temperature with agitation for 30 min, and were subsequently plated on 2.5% glucose/lactose-enriched BHI medium for enumeration. Additional treatments were also subjected to filtration to remove any unconjugated enzyme before plating. Differences between control and conjugate treatments were analyzed using 2-way ANOVA ($P < 0.05$). When performing the test without separation of unconjugated material, treatments with conjugates (IgOx) and treatments with unconjugated enzyme (Ox) both exhibited the same effectiveness at inhibiting the outgrowth of *L. monocytogenes*, but Ig and PBS showed no inhibition. When incorporating a filtration step to remove excess enzyme not bound to the cell wall, both IgOx and Ox still demonstrated similar inhibitory effect against the target pathogen. Further work will investigate the differences between conjugated and unconjugated enzyme ability to inhibit *L. monocytogenes*. These tools could provide novel methods to control *L. monocytogenes* as well as other target pathogens in dairy environments with minimal secondary effects.

Key Words: sugar-oxidase, antimicrobial, *Listeria monocytogenes*

2517 *Paucilactobacillus wasatchensis* gas production through symbiotic amino acid metabolism with *Lactococcus lactis* starter cultures.

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Paucilactobacillus wasatchensis WDC04 is a nonstarter lactic acid bacteria linked to unwanted gas production in Cheddar cheese. It has the capability of producing gas from 6-carbon sugars; however, WDC04 can produce splits and cracks in cheese in the absence of these sugars. In cheese production trials, more CO_2 was released than can be accounted for by added carbon substrates. One possible source of gas production could be the decarboxylation of free amino acids with the formation of biogenic amines. Previous research has shown that *Pa. wasatchensis* can produce gas from the decarboxylation of ornithine, and to a lesser degree lysine, but not from arginine. Cheddar cheese has traditionally been made using *Lactococcus lactis* starter cultures, which have the ability to utilize arginine for energy production with the generation of ornithine during carbohydrate starvation via the arginine deiminase (ADI) pathway. To determine if *Pa. wasatchensis* WDC04 can produce gas in a symbiotic relationship with *L. lactis*, strains of *L. lactis* were grown in M17 broth, M17 + 1% lactose, and M17+0.2% lactose and 80 m*M* arginine for 72 h. As a negative culture control, strains of *L. cremoris*, which lack the ADI pathway, were also grown under the same conditions. After incubation, the cultures were centrifuged and filtered through a 0.2-μm filter to produce cell-free fermentates, which were then lyophilized. To test for gas production, 100 mg of the lyophilized fermentates were added to 10 mL of MRS+1% ribose broth tubes containing a Durham tube for gas collection. Negative control tubes, which contained no fermentate, were also made with MRS+1% ribose. These tubes were inoculated with *Pa. wasatchensis* WDC04 and incubated at 25°C for 7 d. Gas production was only observed in the tubes containing the fermentate of *L. lactis* grown on M17+0.2% lactose and 80 m*M* arginine. These results show that *Pa. wasatchensis* has the ability to produce gas from only the fermentation byproducts of *L. lactis* and indicates another potential risk factor for late gas production in Cheddar cheese.

Key Words: Cheddar cheese, gas defect, amino acid decarboxylation

2518 The cloning and characterization of *Brettanomyces claussenii*'s beta-galactosidase.

K. Kennedy*, T. DeMarsh, and S. Alcaine, *Cornell University, Ithaca, NY.*

This study details the cloning and characterization of *Brettanomyces claussenii*'s β-galactosidase, also known as lactase. The β-galactosidase gene from *B. claussenii* was inserted into a pET28a vector and transformed into *Escherichia coli* Bl21 (DE3). The protein was expressed and then purified using his-tag purification. *Brettanomyces claussenii*'s β-galactosidase was tested for optimum activity while using ONPG (4 mg/mL) and lactose (100 m*M*) as substrates. Lactase activity was tested under various conditions of pH and temperature, and in the presence of divalent cations (1 m*M*), with activity measured as a percentage of relative activity compared with the condition with the highest activity. Additionally, the enzyme's kinetic parameters were determined by way of a Lineweaver-Burk plot. *Brettanomyces claussenii*'s β-galactosidase exhibits an optimum pH of 7 with lactose as a substrate and pH of 8 with ONPG as a substrate. An optimum temperature of 50°C was experienced for both lactose and ONPG, with notable activity in a temperature range of 40–60°C and a pH range of 5–8. This lactase experiences increased activity in the presence of Mn, Mg, Co, and Fe. The kinetic profile of this lactase, when lactose was a substrate, indicated a Km of 4.65 m*M*, Vmax of 13 μ*M* min^{-1}, and a specific activity of 1.231 ± 0.48 units mg^{-1}. The enzyme's thermophilic and alkaline-tolerant nature suggests its suitability for processing dairy products such as milk or whey for pre- and postpasteurized products.

Key Words: lactase, cloning, characterization

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2519 Turning weeds into feed: Ensiling *Calotropis gigantea* reduces its toxicity potentially for ruminants. A. Ayemele Kenfack Gnetegha*[1,2], L. L. Maffo Donfack Gnetegha[3], L. Ma[4], D. Bu[4], and J. Xu[1], [1]*Honghe Center for Mountain Futures, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, China,* [2]*University of Bamenda, Bambili, Nord-West, Cameroon,* [3]*University of Dschang, Faculty of Sciences, Department of Animal Biology, Dschang, West Cameroon,* [4]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, Hebei, China.*

Calotropis gigantea (giant milkweed, GM) has the potential to be a new feed additive for ruminants; however, the presence of unpalatable or toxic compounds decreases animal feed intake. This study aimed to valorize GM as a new feed resource through the chemical and microbial biotransformation of toxic compounds that reduced cows' feed intake. After collection of 2,000 kg of wild GM and sun-drying (DM 40%), leaves and stems were mechanically chopped to a maximum size of 2 cm. Then, SiloSolve inoculant was added at 2 g/ton. Finally, 12 bales of 50 kg each were anaerobically filled and compacted with an automated baling machine. The bales were stored at a room temperature varying from 17 to 27°C. After 60 d, the bales were opened and sampled for nutritional and UHPLC-MS/MS analysis. Giant milkweed (collected 2 d before opening the bales, at the same physiological stage with the previous collection, DM 40%), GM silage, and SiloSolve inoculant were the samples assigned in a complete randomized design to understand the metabolomic biotransformation after ensiling. Illumina Miseq of the 16S rRNA genes and ITS1 were used to describe the microbial population colonizing GM silage. Data were analyzed using PROC MIXED of SAS 9.1 and significance declared at $P < 0.05$. After ensiling, crude proteins, fat, NDF, ADF, and ash were maintained ($P > 0.05$) and pH decreased ($P < 0.05$). In addition, 23 flavonoids and 47 amino acids and derivatives increased ($P < 0.05$), but the other 14 flavonoids and 9 amino acids and derivatives decreased ($P < 0.05$). *Lactobacillus buchneri*, *Bacteroides ovatus*, and *Megasphaera elsdenii* were specific to ensiled GM and correlated with functional plant metabolites ($P < 0.05$), and *Sphingomonas paucimobilis* and *Staphylococcus saprophyticus* were specific to nonensiled GM and correlated with the toxic metabolite 5-hydroxymethylfurfural ($P < 0.05$). "Xenobiotics biodegradation and metabolism" were the highly expressed microbial Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways in nonensiled GM. This study provides essential information for valorizing GM as a potential new fodder.

Key Words: *Calotropis gigantea*, chemical biotransformation, toxicity

2520 Effect of nitrogen fertilization on yield and nutritive value of fall-stockpiled tall fescue, meadow fescue, or orchardgrass. M. A. Akins*[1], J. S. Cavadini[2], K. G. Wells[2], D. M. Pizarro[3], M. A. Wattiaux[3], and V. D. Picasso[4], [1]*USDA-ARS Dairy Forage Research Center, Marshfield, WI,* [2]*Division of Extension, University of Wisconsin–Madison, Madison, WI,* [3]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [4]*Department of Plant and Agroecosystem Sciences, University of Wisconsin–Madison, Madison, WI.*

Extending the grazing season using stockpiled forages can reduce costs, but minimal information on N fertilizer rates for stockpiled forage is available. This study evaluated stockpiled forage yield and nutritive value of 3 grass species (tall fescue, TF; meadow fescue, MF; and orchardgrass, OG) when fertilized with 0, 34, 67, or 100 kg N/ha as urea and harvested at 3 times in a split-split-plot design. The study was replicated over 2 years (2020, 2021) with 4 replicate blocks. Urea was applied in early August after a forage harvest, then forage allowed to accumulate until harvested in fall (mid-October, early November, late November). Data were analyzed with the Proc Mixed procedure of SAS. Yield was affected by a species × N-rate interaction ($P < 0.01$) with N increasing yield for all species; however, TF was more responsive (Table 1). Harvests in mid-October (4,077 kg of DM/ha) or early November (4,255 kg of DM/ha) had similar yields, but by late November yield decreased (3,531 kg of DM/ha). Forage CP increased ($P < 0.01$) slightly with N (9.6%, 9.5%, 9.8%, and 10.2% CP for 0, 34, 67, and 100 kg of N/ha, respectively), but was more affected by species with MF (12.7%) having higher CP ($P < 0.01$) than TF (7.8%) or OG (8.7%). Grass species had a large effect on NDF (54.8%, 58.5%, and 59.7% NDF for MF, TF, and OG, respectively; $P < 0.01$), and N fertilizer only slightly increased NDF by 1 to 2 percentage units ($P < 0.01$). In vitro 48-h NDFD was unaffected by N rate ($P = 0.30$), but species had a greater effect (58.9%, 63.0%, and 65.9% NDFD for MF, OG, and TF, respectively; $P < 0.01$). Also, TF (11.7%) had greater ($P < 0.01$) water-soluble carbohydrates (WSC) than MF (8.8%) and OG (9.3%), but harvest date had a large effect ($P < 0.01$) on WSC, with higher values in early and late November due to frost and plant hardening. Overall, N fertilizer is key to higher stockpiled forage yield but had limited effects on nutritive value.

Key Words: stockpile forage, nitrogen, grass species

Table 1 (Abstr. 2520). Yield (kg of DM/ha) of grass species with different N fertilization rates[1]

	Grass species		
N rate, kg/ha	Tall fescue	Meadow fescue	Orchardgrass
0	3,912^{d}	2,137^{b}	3,319^{c}
34	4,925^{c}	2,444^{b}	4,077^{b}
67	5,596^{b}	2,800^{ab}	4,276^{b}
100	6,198^{a}	3,001^{a}	4,772^{a}

$^{a–d}$Within species, N rates with different letters differ ($P \leq 0.05$).

[1]SEM = 526 kg/ha.

2521 Effects of fertilization strategy on triticale forage quality and dairy cow performance. A. M. Grev*, J. W. Semler, and S. B. Potts, *University of Maryland, Keedysville, MD.*

Triticale silage has become a popular forage choice for many dairy producers to increase forage supply. The objectives of this study were to (1) investigate the effect of increasing nitrogen (N) fertility rates with and without sulfur (S) on triticale yield and quality and (2) evaluate production implications when incorporating the forage into dairy cow diets. Triticale was established at 2 locations in Maryland in September 2020–2022. Fertility treatments (Table 1) were applied in March in a randomized complete block design with 4 replicates at each location. Plots were mechanically harvested when forage reached the boot stage in April. Harvested forage was weighed and subsampled for DM yield determination and forage analysis. In 2020–2021, the NLOW, NMED, and NHIGH treatments were also applied to 3 2-ha fields to provide triticale for lactation studies that used 28 cows in a replicated 4 × 4 Latin

Table 1 (Abstr. 2521).

Treatment	Nitrogen (kg/ha)	Sulfur (kg/ha)
CON	0	0
SUL	0	17
NLOW	56	0
NSLOW	56	17
NMED	112	0
NSMED	112	17
NHIGH	168	0
NSHIGH	168	17

square design (21-d period; data collection final 3 d). The standard (ALF) diet contained 60% forage (48% corn silage, 22% alfalfa silage) and 40% concentrate (DM basis). The LOW, MED, and HIGH diets were formulated by replacing alfalfa silage with NLOW, NMED, or NHIGH triticale silage at a rate of 18%–20% of diet DM. Both field and feeding studies were analyzed using mixed model analysis (significance at $P \leq 0.05$). Application of N increased forage yields in 3 out of 6 site-years irrespective of S inclusion ($P < 0.01$). In 2021, crude protein (CP) increased linearly with N application rate from 87 to 180 g/kg irrespective of S inclusion ($P < 0.01$); however, this same effect was not seen in 2022–2023. Neutral detergent fiber and net energy did not differ ($P > 0.05$). Feed intake, milk yield, milk fat, and milk protein production averaged 23.3 kg of DM/d, 32.8 kg/d, 1.4 kg/d, and 1.0 kg/d, respectively, and were similar for the LOW, MED, and HIGH diets relative to ALF ($P > 0.05$). Results indicate that increasing N application rates can influence forage yield and CP content, and that this forage can be included in the ration without affecting milk production.

Key Words: protein, triticale, nitrogen

2522 Evaluating the influence of forage candidates for smart foodscapes on rumen fermentation using continuous-culture fermenters.

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Animal production on North American rangelands is mainly centered around ruminant livestock. Nevertheless, the seasonal variations in forage production and quality have a detrimental impact on the production system. Introducing smart foodscapes, which involve creating islands of deep-rooted perennial legumes and forbs with high nutrient content and functional biochemicals, may offer a solution. These strategically placed islands across the landscape can be cultivated and stockpiled to serve as cost-effective supplements for ruminants, thereby improving productivity. Here, our objective was to evaluate the effect of potential forage candidates for the islands on rumen fermentation. The study was conducted as a 5 × 5 Latin square using continuous-culture fermenters. All treatments contained 75% of crested wheatgrass plus (1) 25% alfalfa, (2) 25% sainfoin, (3) 25% small burnet, (4) 12.5% sainfoin + 12.5% small burnet, and (5) 8.3% sainfoin + 8.3% small burnet + 8.3% birdsfoot trefoil. The diets (60 g DM/day) were fed twice daily. The periods were 10 d long with 6 d of adaptation and 4 d of sampling. Data were analyzed using a mixed model including the fixed effect of treatment and the random effects of period and fermenter. The combination of sainfoin + small burnet and sainfoin + small burnet + birsdsfoot trefoil increased fiber digestibility and total short-chain fatty acids compared with the other treatments ($P < 0.05$). No treatment effect was observed for rumen pH. Our initial findings suggest that various forage combinations have distinct effects on rumen fermentation. These results will guide the selection of the most promising plants for implementing smart foodscapes.

Key Words: forbs, functional biochemicals, legumes

2523 Cropping methodology and ensiling time influence total rumen disappearance of corn silage.

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This study assessed potential effects of cropping methodology and ensiling time on total in situ rumen disappearance of corn silage. Chopped, whole-plant corn samples were collected from 4 corn test plots, each exposed to different cropping methods to include no tillage and no cover crop (NTNC), tillage and no cover crop (TNC), no tillage and a cover crop (NTC), and tillage and a cover crop (TC). Chopped corn samples (~900 g) from each corn plot were placed in individual Cryovac® bags, vacuum sealed, and ensiled for 30, 60, 90, or 150 d (10 replicate bags per ensiling time–cropping method combination, 160 total bags). At the end of each ensiling time, samples were frozen at −2°C. All silage samples were thawed, composited by ensiling time–cropping method, and dried at 60°C for 24 h. Dried, composited, unground silage samples were used to determine in situ rumen disappearance using 2 ruminally fistulated steers as individual experimental units. Samples were incubated at 0, 6, 12, 24, 48, and 72 h using the common nylon bag protocol. Data were analyzed using the GLIMMIX procedure of SAS, with model factors of cropping method, ensiling time, incubation time, and animal. Not surprisingly, ensiling time did affect total disappearance. Ensiling for 90 and 150 d resulted in higher disappearance than 30 d, but not 60 d ($P < 0.05$). There were no differences in rumen disappearance between 30 and 60 d of ensiling ($P > 0.05$). Interestingly, an effect of cropping method on disappearance was detected, with NTC substantially higher (53.3%) than TNC (48.5%), TC (47.7%), and NTNC (45.6%; $P < 0.05$). There was no interaction between ensiling time and cropping method ($P > 0.05$), but an incubation time and cropping method interaction did exist ($P < 0.05$). Noticeably, total lactic acid levels of analyzed silage were highest and DM lowest from corn produced with NTC cropping methodology. That could explain, in part, the rumen disappearance effect. However, the connection between cropping method and these results needs elucidation. Based on the results of this study, corn production using cover crops and no till establishment may improve silage rumen disappearance.

Key Words: corn silage, ruminant, degradability

2524 Microbial characterization of corn forage silage (*Zea mays*) with banana peel inclusion (*Musa paradisiaca*).

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This research aimed to carry out the microbial and fermentative characterization of corn forage silage (*Zea mays*) with the inclusion of banana peel (*Musa paradisiaca*). Corn forage harvested at 90 d and banana peel residue, chopped into 1-cm pieces, were used, and stored together in 40-kg-capacity plastic bags. After 30 d of fermentation upon opening the silos, crucial variables such as the presence of molds, yeasts, lactic acid bacteria, and total bacteria, were analyzed. A completely randomized experimental design with 5 treatments and 5 replications was implemented, detailed as follows: T1: 100% corn silage; T2: 75% corn silage + 25% banana peel; T3: 50% corn silage + 50% banana peel; T4: 25% corn silage + 75% banana peel; and T5: 100% banana peel. To assess differences between treatments, ANOVA and Tukey's test were conducted at a significance level of 5%. The focus was on key variables of silage microbial composition, such as molds, yeasts, lactic acid bacteria, and total bacteria. Significant differences ($P < 0.05$) were observed among treatments in the variables of molds and yeasts. Treatment 4 exhibited the highest average for molds (4.41 colony-forming units [cfu]/mL), and T1 recorded the lowest average (1.87 cfu/mL). Regarding yeasts, T5 showed the highest value ($P < 0.05$; 3.08 cfu/mL), and T1 had the lowest value (2.01 cfu/mL). The lactic acid bacteria variable did not show significant differences among treatments ($P > 0.05$), with values of 8.60, 8.43, 8.43, 8.50, and 8.52 cfu/mL for T1, T2, T3, T4, and T5, respectively. As for total bacteria, no significant differences ($P > 0.05$) were observed among treatments, with values of 8.81, 8.69, 8.78, and 8.78 cfu/mL for T1, T2, T3, and T4, respectively. The research revealed that the inclusion of banana peel in corn forage silage significantly affects the microbial composition, especially the presence of molds and yeasts. Although lactic acid bacteria and total bacteria remained stable, these results emphasize the importance of considering additional ingredients in silage production.

Key Words: fermentation, residues, population

2525 Chemical composition of corn silage and palm effluent for tropical ovine: Effect of different inclusion levels. I. F. Espinoza*[1], A. E. Barrera[1], A. R. Sánchez[1], M. L. Medina[1], E. D. Torres[1], R. L. Bástidas[1], G. J. Muñoz[1], and A. García[2], [1]*Universidad Técnica Estatal de Quevedo, Quevedo, Los Ríos, Ecuador,* [2]*Universidad de Córdoba, Córdoba, España.*

The research assessed the chemical composition of corn silage and palm effluent at 5 different levels. Variables such as DM, OM, inorganic matter (IM), CP, NDF, and ADF were analyzed. A completely randomized experimental design was employed with 5 treatments and 5 replications: T1 (100% corn silage + 0% palm effluent), T2 (90% corn silage + 10% palm effluent), T3 (80% corn silage + 20% palm effluent), T4 (70% corn silage + 30% palm effluent), and T5 (60% corn silage + 40% palm effluent). The evaluated variables were subjected to ANOVA and the Tukey test at a 5% probability level. The results for DM in treatments T1, T2, T3, T4, and T5 (37.48%, 36.64%, 35.26%, 34.80%, 34.50%, respectively) did not show significant differences ($P > 0.05$). Organic matter was different ($P < 0.05$) in treatments T1 and T2 (89.20% and 88.98%) compared with T3 and T4 (88.04% and 87.95%). The IM content was higher ($P < 0.05$) in T4 and T5 (11.97% and 12.04%) when compared with T1 and T2 (10.79% and 22.02%, respectively). Crude protein was higher ($P < 0.05$) in T5 (8.60%) compared with T1, T2, and T3 (7.38%, 7.32%, and 7.67%, respectively). The fat extract content in T5 (11.13%) was different ($P < 0.05$) when compared with T1, T2, T3, and T4 (2.10%, 2.21%, 3.52%, 4.23%, respectively). The NDF and ADF contents were lower ($P < 0.05$) in treatment T1 (70.22% and 20.60%, respectively), and the treatment with a higher palm effluent content showed the highest values, T5 (75.84% and 25.40%, respectively). In conclusion, this study reveals that the inclusion of palm effluent in corn silage significantly influences its chemical composition, with notable changes in organic matter, protein, fat, and fiber fractions. These results are relevant for understanding how to adjust ovines feeding in tropical environments by varying the proportions of these ingredients in silage, emphasizing the importance of considering chemical composition in animal diet formulation.

Key Words: proximal analysis, animal diets, tropical environment

Table 1 (Abstr. 2526). HL predictions of OMD and CP (g/kg of dry matter) contents at weeks relative to main harvest (week 0)

	Week							
Field	−2	−1	0	+1	+2	RMSEP	R^2	RPD
RG 1st cut								
OMD	775^{a}	760^{a}	740^{a}	729^{a}	670^{b}	6.8	0.409	1.30
CP	161^{a}	128^{b}	124^{bc}	108^{c}	106^{c}	26.5	0.561	1.51
RG 2nd cut								
OMD	790^{a}	718^{b}	669^{c}	563^{d}	577^{d}	7.4	0.977	6.57
CP	178^{a}	138^{b}	117^{c}	91^{d}	101^{e}	13.0	0.914	3.42
PG								
OMD	758^{a}	727^{a}	689^{b}	681^{b}	605^{c}	4.9	0.713	1.87
CP	166^{a}	132^{b}	126^{bc}	116^{cd}	106^{d}	20.3	0.785	2.16

[a–e]Different letters in a row indicate significant (p<0.05) differences.

2526 Using portable near-infrared spectroscopy devices to determine ideal harvest time in grass based on organic matter digestibility and crude protein predictions. B. Ollertz-Mertens*[1,2], H. Volden[2], E. Prestløkken[2], M. F. Digman[3], and B. Büchner[1], [1]*John Deere GmbH & Co.KG, Kaiserslautern, RP, Germany,* [2]*Norwegian University of Life Sciences, Ås, Viken, Norway,* [3]*University of Wisconsin–Madison, Madison, WI.*

Timing of grass harvest is a trade-off between yield and quality. The objective of this study was to evaluate if a John Deere HarvestLab 3000 (HL) NIRS device can predict changes in organic matter digestibility (OMD) and crude protein (CP) content and therefore give an indication of planned harvest time. Strips in permanent grassland (PG) and hybrid ryegrass (RG) (*Lolium hybridum*) fields were cut weekly, 2 wk before main harvest and continued for 4 consecutive weeks. Hand-cut samples of 0.5 m by 0.5 m were taken at 10 cm cutting height and OMD and CP were predicted using 3 HL devices. A near-infrared spectroscopy (NIRS) laboratory reference sample of the same material was conducted for all samples. The RPD (ratio of standard deviation of reference analysis to standard error of prediction of HL) and R^2 were used to evaluate the NIRS predictions. The results consist of data from a 1st (RG1) and 2nd (RG2) cut in RG and a 1st cut in PG. Day 0 of sampling was determined to be the day of the farm's main cut for the respective field. The HL results showed decreasing OMD and CP values with increased grass maturity (Table 1). The RPD and R^2 values were good in RG2 but need improvement in RG1. In PG the R^2 showed reliable results. The root mean square error of prediction (RMSEP) showed a trend to be higher at higher values of OMD and CP. Although especially high CP and OMD samples were sometimes underestimated, the HL measurements mostly showed good correlations with laboratory reference values. In most cases weekly changes in constituents were higher than RMSEP, therefore the trends are visible. In summary, HL can indicate the changes in OMD and CP to predict the targeted harvest timing.

Key Words: NIRS, grass harvest, digestibility

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2527 Branched-chain amino acids and branched-chain keto-acids treatment altered transcriptome in primary bovine hepatocytes. J. R. Daddam*, C. Collings, M. Sura, and Z. Zhou, *Animal Sciences, Michigan State University, East Lansing, MI.*

Previous in vivo results from our group demonstrated that branched-chain amino acids (BCAA) supplementation improved lactation performance whereas branched-chain keto acids (BCKA) supplementation decreased liver triglycerides in the fresh period. The objective of this study was to determine the transcriptomic changes in response to BCAA and BCKA supplementation in primary bovine hepatocytes. Primary bovine hepatocytes (PLEH) were cultured in customized media mimicking cow plasma AA and FA profile on d 4 postpartum with BCAA (133% of physiological circulating BCAA concentration), BCKA (equal molar amount as BCAA), or CON (100% of physiological BCAA). After 72h, PLEH were harvested for RNA sequencing. Differential gene expression (log2 fold change > 1.5) analysis was performed with DESeq2. Functional enrichment analyses of the differential gene expression (DEG) were carried out using the Reactome database. Compared with CON, a total of 568 genes were differentially expressed in BCAA cows, whereas in BCKA cows, a total of 1,484 genes were differentially expressed compared with CON. Pathway analysis revealed that BCAA supplementation upregulated RHOJ GTPase cycle, EPHB-mediated forward signaling, RHO GTPases Activate WASP and WAVE, and downregulated interferon gamma signaling, formation of WDR5-containing histone-modifying complexes, and formation of a pool of free 40S subunits pathways compared with CON. In contrast, BCKA supplementation upregulated DNA double strand break response and ephrin signaling and downregulated inhibition of replication initiation of damaged DNA by RB1/E2F1, and E2F mediated regulation of DNA replication pathways compared with CON. Overall, these results suggest that BCAA and BCKA supplementation altered various aspects of the hepatic transcriptome of PLEH cells.

Key Words: branched-chain amino acids, branched-chain keto-acids, transcriptomics

2528 Comparisons of glucose concentration measurements using different methods and glucose stability over time and after freeze-thaw cycles. A. D. Ravelo*[1], M. Ruch[1], I. J. Salfer[2], and L. S. Caixeta[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN,* [2]*Department of Animal Science, University of Minnesota, Saint Paul, MN.*

There are several different approaches for measuring blood glucose concentrations of dairy cows. Additionally, samples may need to be frozen and thawed several times during analysis. Thus, our first objective was to investigate the agreement between 2 methods, a chemistry analyzer (CA) or a peroxidase-glucose plate method (PM), for glucose measurement. Our secondary objective was to investigate the stability of the glucose in samples frozen and thawed several times. Blood samples were collected into sodium fluoride tubes from 166 dairy cows, across different lactation stages, on 3 farms over a 2-d interval. Average days carrying calf for 49 dry cows was 261 ± 28 d (mean ± SD), DIM for 94 fresh cows was 6 ± 3 d, and DIM for 23 midlactation cows was 49 ± 16 d. Plasma samples were separated into 3 aliquots. All aliquots were analyzed using CA and PM. One aliquot was used for the serial freeze-thaw cycles comparison and the other 2 were used to investigate weekly glucose degradation in frozen samples. Bland–Altman plots, Pearson correlations, and paired *t*-tests were used to compare CA and PM. A linear mixed model with the fixed effect of week and the random effect of cow was used to test the effects for freeze-thaw cycles and time frozen on measured glucose concentrations. In samples analyzed 2 wk after collection, the mean difference between PM and CA was 10.52 mg/dL (95% CI: 9.24, 11.79; $P < 0.01$; r = 0.64). In samples analyzed 3 wk after collection the mean difference between PM and CA was 10.43 mg/dL (95% CI: 9.17, 11.68; $P < 0.01$; r = 0.65). In samples analyzed 4 wk after collection, the mean difference between PM and CA was 7.12 mg/dL (95% CI: 5.75, 8.49; $P < 0.01$; r = 0.64). Glucose concentrations varied inconsistently based on time frozen ($P < 0.01$) and serial thawing ($P < 0.01$) for both methods. Finally, both methods differed in the glucose concentrations reported; however, the concentrations measured by both methods were positively correlated. Moreover, storage time and freezing and thawing of the samples did not follow expected patterns of decrease.

Key Words: sodium fluoride, chemistry analyzer, peroxidase-glucose

2529 Identification of variables influencing perfluorooctane sulfonic acid (PFOS) serum levels in rodents through feature selection data mining methods. L. Nowak*, K. Nishimwe, A. Mendoza, A. Jimenez, S. Benner, G. Pereira, and J. Romero, *School of Food and Agriculture, University of Maine, Orono, ME.*

This study aimed to identify variables influencing perfluorooctane sulfonic acid (PFOS) serum levels in rodents based on data published in research articles. In the Web-of-Science database, we searched for articles measuring per- and polyfluoroalkyl substances (PFAS) in rodents. From 12 publications, we extracted the following variables, where available: publication ID, sex, species, BW, exposure duration to PFAS, treatments (animals categorized as clean animals exposed to PFAS, contaminated animals exposed to PFAS, and contaminated animals not exposed to PFAS), daily PFAS exposure extent, and PFOS liver and serum levels. The dataset was arbitrarily divided into training (70%) and test (30%) subsets, with the former used for developing the model and the latter to report the accuracy of it. Least absolute shrinkage and selection operator (LASSO) and ridge algorithms were used on the training subset with a 10-fold cross-validation approach. Similarly, stepwise multiple regression was also used with Akaike information criterion (AIC) as the selection criterion; R 4.3.2 was used for the analysis. The root mean squared error (RMSE) obtained when analyzing the test subset were 27.9, 28.5, 36.0, 28.4, and 28.0 (µg/mL of plasma PFOS), and the number of variables in the model (exclusive of the intercept) was 5, 14, 16, 16, and 14 for LASSO set to lambda.1se and lambda.min, Ridge set to lambda.1se and lambda.min, and stepwise (respectively). The LASSO (lambda.1se) and stepwise methods resulted in among the lowest test RMSE with the fewest independent variables required. The median of the absolute errors for the test subset was lower for stepwise versus LASSO (lambda.1se; $P = 0.05$). The LASSO (lambda.1se) method identified a positive relationship between PFOS plasma levels and exposure duration (d) and extent (µg/kg of BW/d) when the effect of publication was included in the model. Similarly, stepwise identified a positive relationship with exposure duration and extent when the effect of publication was included in the model (adjusted $R^2 = 0.93$). In conclusion, the key variables influencing PFOS plasma levels in rodents are exposure duration and extent in the dataset analyzed.

Key Words: PFAS, rodent, serum

2530 Assessing the coefficient of creatinine excretion rate in lactating dairy cows.

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Creatinine concentration in urine is often used to estimate the urine volume of cows where 29 mg/kg BW/d of the creatinine excretion rate has been often used as the coefficient. However, unreasonable N excretion in urine (10%–55% as proportion of N intake) was observed in our previous studies when 29 mg/kg BW/d of the coefficient was used to estimate urine volume. To validate the coefficient, we conducted 2 experiments. Experiment 1 used 8 cows (214 ± 41 DIM) in a replicated Latin square design with 4 treatments (50% corn silage, 6% alfalfa silage, and 44% concentrate; 16% CP on a DM basis). Experiment 2 used 4 cows (161 ± 63 DIM) in a Latin square design with 4 treatments (38% corn silage, 19% alfalfa silage, and 43% concentrate; 16.6% CP;). Total urine was collected for multiple days in each period and subsamples of urine were composited by cow and period to determine creatinine concentration. For statistical analysis, dietary treatment and period effects were first determined within trial using the mixed procedure of SAS where they did not affect BW, DMI, urine volume, creatinine concentration, and coefficient. Data from 2 experiments were analyzed using the mixed procedure of SAS where cow within trial was a random effect and trial, period, and their interaction were fixed effects. Although BW was different (673 vs. 755 kg; $P = 0.04$), urine volume and DMI were not different between trials. Creatinine concentration in urine (429 vs. 281 mg/L; $P < 0.01$) and coefficients (19.1 vs. 11.8 mg/kg BW/d; $P < 0.01$) were largely different. Interestingly, the coefficients obtained from 2 experiments were much lower than 29 mg/kg BW/d. An analysis for correlation between BW and creatinine excretion (mg/d) indicated that they were not correlated ($R^2 = 0.002$; creatinine [g/d] = −0.002 × BW + 12.8]. In conclusion, no difference in coefficient among periods within trial indicates the creatinine excretion rate was likely constant. However, the large difference in coefficient between trials and no correlation between creatinine excretion and BW indicates that the estimation of urine volume with the fixed coefficient of 29 mg/kg BW/d may need to be revisited.

Key Words: creatinine, excretion, urine

2532 Ethanol metabolism in dairy cows fed sucrose- and ethanol-rich diets.

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The aim was to examine ethanol metabolism in cows fed sucrose- or ethanol-rich diets by including either fresh or ensiled sugar beets in the diets. Sugar beets were washed, crushed, and stored either fresh (frozen at −20°C) or ensiled (barrels) in daily portions. Four rumen-cannulated Danish Holstein cows were used in a replicated (one complete and one incomplete) 3 × 3 Latin square design with 21-d periods. The 3 diets were fed ad libitum as TMR. The control diet (CON) was based on grass/clover silage, corn silage, barley, soybean meal, dried beet pulp, urea, and minerals. In the sucrose- (SUC) and ethanol- (ETH) rich diets, 20% of DM from CON was replaced with either fresh or ensiled beets. Each period comprised 10-d adaptation and 11-d of collection. To determine whole-body irreversible loss rate (ILR) and mammary extraction of ethanol by isotope enrichment, bilateral jugular catheters were placed for either [^{13}C]-labeled ethanol infusion or blood sampling, and mammary vein blood was sampled by venipuncture. Data were analyzed using linear mixed models with treatment and period as fixed effects and cow as random effect. For blood concentrations, time and treatment × time interaction were included as fixed effects considering time as repeated measure. The DMI ($P = 0.37$; mean ± SEM; 22.3 ± 0.99 kg/d) and ECM yield ($P = 0.13$; 29.2 ± 2.90 kg/d) did not differ among diets. Ethanol intake was higher for ETH than for SUC and CON ($P < 0.01$; 0.35 vs. 0.05 ± 0.02 kg/d). Jugular whole blood ethanol concentrations were higher for ETH than for SUC and CON 1 h after feeding (treatment × time $P < 0.01$; 0.52 vs. 0.06 ± 0.07 mmol/L). Whole-body ILR of ethanol ($P = 0.24$; 21.4 ± 7.66 mmol/h) and mammary extraction of ethanol ($P = 0.19$; 0.47 ± 0.26) were unaffected by diet. Mammary net uptake of ethanol ($P = 0.87$; 12.4 ± 8.41 mmol/h), ethanol excreted in milk ($P = 0.50$; 0.21 ± 0.09 mmol/h), and ethanol concentration in milk ($P = 0.40$; 0.09 ± 0.03 mmol/L) were unaffected by diet. In conclusion, feeding 0.35 kg/d of ethanol did not increase whole-body ILR and the mammary gland appeared to be responsible for 50% of the whole-body ILR of ethanol, with a minor part being secreted into milk.

Key Words: beets, ILR, stable isotope

2533 Effects of exposure to white light-emitting diode (LED) or electrodeless induction lamp on milk production performance and blood compositions of dairy cows.

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Electrodeless induction lamp (EIL) provides lower blue light as compared with white LED (WLED) and are now widely used in various facilities. The objective of this study was to evaluate the response of milk production and physiological parameters to exposure to WLED or EIL in Holstein cows. Ten Holstein lactating cows (225 ± 32.5 DIM, 710 ± 24.6 kg initial BW, 2.56 ± 1.59 parity) were managed under the long-day photoperiod (16:8 h light-dark cycle) and were assigned to 2 treatments for 3 wk each in a 2 by 2 crossover design as follows: exposure to WLED (453 nm peak wavelength, 231 Lux) or EIL (550 nm peak wavelength, 237 Lux) during daytime. The dark period (2100 h to 0500 h) for all treatments was no illumination (0.0 lx). All cows were fed same TMR ad libitum throughout the experiment. Milk samples were collected every week to measure milk compositions. Serial blood sampling was performed on the last day of each treatment to measure metabolite and hormone concentrations in plasma. Data were analyzed by ANOVA using the Fit Model procedure of JMP® 17 pro (SAS Institute Inc., Cary, NC). Dry matter intake, BW, milk yield and composition, rumination time, and plasma metabolite concentrations were not affected by light treatment. Contrary to our hypothesis, plasma concentrations of melatonin and prolactin were not different between cows exposed to WLED or EIL. However, plasma cortisol concentration was lower ($P < 0.01$) for the EIL group (6.85 ± 4.49 ng/mL) than for the WLED group (23.9 ± 4.49 ng/mL) at the dark period. Our results suggest that EIL exposure reduces stress experienced by cows as indicated by decreased cortisol concentration but did not affect lactational performance and plasma concentrations of melatonin and prolactin.

Key Words: lactating dairy cows, blue light, cortisol

2534 Effects of heat stress and nutritional plane on metabolism, inflammation, and fecal biomarkers in lactating dairy cows.

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Study objectives were to investigate the effects of heat stress (HS) and nutritional state on metabolism, inflammation, and fecal biomarkers in lactating Holstein cows. Primiparous cows (604 ± 3 kg BW; 156 ± 5 DIM; n = 18) were enrolled in a study consisting of 2 experimental periods (P). During P1 (10 d), cows were ad libitum-fed and baseline data were obtained. During P2 (7 d), cows were assigned to 1 of 3 treatments (Trt): (1) thermoneutral (TN) ad libitum-fed (AL; n = 6), (2) TN and pair-fed (PF; n = 6) to their heat-stressed counterparts, or (3) HS (via an electric heat blanket; Thermotex Therapy Systems Ltd.; n = 6) and ad libitum fed. Blood and fecal samples were obtained on d 10 of P1 and d 1, 3, 5, and 7 of P2. Data were analyzed using PROC MIXED with repeated measures in SAS 9.4. The main effects of Trt, time, and Trt × time were evaluated, with time as repeated and cow as random. Overall, HS increased rectal temperature and respiration rate (2.2°C and 73 breaths/min, respectively; $P < 0.01$) and decreased DMI (38%) relative to AL cows. Milk yield was reduced in HS (38%) and PF (15%) compared with AL cows ($P < 0.01$). In P2, basal insulin was increased in HS (24%) and decreased in PF (67%) relative to P1 ($P < 0.01$). Circulating nonesterified fatty acids (NEFA) increased over time (1.9-fold; $P = 0.02$) in PF compared with HS and AL cows, BHB (0.95 m*M*) and glucose (75 mg/dL) did not differ among treatments ($P > 0.28$) in P2. Heat stress markedly increased lipopolysaccharide-binding protein (LBP) levels on d 1 (59%) relative to PF and AL cows, but LBP progressively decreased with time ($P = 0.03$) in the HS cows. Alkaline phosphatase progressively decreased ($P < 0.05$) during P2 in the HS cows. There was an interaction ($P < 0.01$) in fecal pH as it gradually increased during HS but decreased by PF. Heat stress acutely increased fecal cortisol metabolites (4.8-fold) compared with PF and AL cows ($P < 0.01$). In summary, reduced feed intake only accounted for ~50% of the decrease in milk yield during HS. Hyperthermia induced an acute phase response and markedly increased fecal cortisol metabolites when compared with TN feed-restricted or ad libitum-fed cows.

Key Words: hyperthermia, feed restriction, gut health

2535 Development and application of an analytical method for the measurement of polyethylene glycol 400 and 1,500 in assessing gut permeability in dairy cows. S. Jantzi[1], J. Yu*[1], J. Renaud[2], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Agriculture and Agri-Food Canada, London, ON, Canada.*

During the transition period, inflammation is thought to be associated with increased gut permeability, heightening the risk of disease. The number of markers specifically for transcellular gut permeability, such as polyethylene glycols (PEG), are limited. The aim of this study was to develop a liquid chromatography, high resolution mass spectrometry (LC-HRMS) method to quantify low mass (Mn 400 Da; $\alpha = H$, $\omega = OH$) and higher mass (Mn 1,500 Da; $\alpha = H$, $\omega = OH$) PEG oligomers as an indigestible marker in dairy cows. The sample preparation method used acetonitrile extraction with ammonium and involved a protein-precipitation step. The different oligomers, ranging from a 3-mer to a 45-mer in length, were distinguished by their *m/z* and were resolved within a 7-min method. As both the low- and high-mass PEG contains multiple oligomers, methyl terminated PEG ($\alpha = H$, $\omega = OCH_3$) of equivalent sizes were used as internal standards because they differ in mass from the dosed PEG by 14.02 Da. The effectiveness of this method was demonstrated by the analysis of serum samples collected from 11 multiparous cows. Gut permeability was evaluated by oral dosage of PEG 400 and 1,500 (0.11g/kg of BW), on d −30, 7, and 70 relative to parturition, followed by blood sampling. In humans, increased gut permeability has been observed through the concentrations of low Mw PEG (seen at a larger concentration than high Mw PEG) and high Mw PEG excreted in urine. Similarly, we observed a higher concentration of PEG 400 than PEG 1,500 in the serum of cows at d 7 and notably observed a similar pattern at d 70 but with lower concentrations for both PEG 400 and 1,500. Thus, using LC-MS/MS is a reliable method for the detection of PEG 400 and 1,500 in serum to assess gut permeability in cows. The liquid chromatography, high resolution mass spectrometry analysis used in this study can be applied to even higher Mw PEG, which is important for further understanding on transcellular gut permeability in cows.

Key Words: gastrointestinal permeability, nontarget analysis, PEG oligomers

2537 Abomasal infusion of branched-chain amino acids or branched-chain ketoacids altered glucose tolerance in fresh cows. G. Ahmad*, J. R. Daddam, K. Gallagher, I. Bernstein, C. Collings, M. Sura, M. Vandehaar, and Z. Zhou, *Animal Sciences, Michigan State University, East Lansing, MI.*

The objective of this study was to determine the effect of branched-chain amino acids (BCAA) or branched-chain ketoacids (BCKA) infusion on insulin sensitivity in fresh cows. Thirty-six multiparous Holstein cows were enrolled in a randomized block design experiment. Cows were abomasally infused for the first 21 d postpartum with solutions of (1) saline (CON, n = 12); (2) BCAA (67 g valine, 50 g leucine, and 34 g isoleucine, n = 12); and (3) BCKA (77 g 2-ketovaline calcium salt, 57 g 2-ketoleucine calcium salt, and 39 g 2-ketoisoleucine calcium salt, n = 12). All cows received the same diet. Intravenous glucose tolerance tests (IVGTT) with administration of 0.25 g of glucose/kg of BW were performed at 10 and 20 d after calving. Blood was sampled at −1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, 100, and 120 min relative to glucose infusion. Total area under the curve (AUC) for plasma glucose, insulin, and nonesterified fatty acids (NEFA) were calculated using a trapezoidal method. Treatment effects were determined using PROC GLIMMIX in SAS. The BCAA or BCKA infusion did not alter baseline glucose ($P = 0.12$) or insulin concentration ($P = 0.13$); however, BCKA cows had lower baseline NEFA concentration compared with BCAA cows ($P = 0.04$). After glucose infusion, BCKA cows had higher AUC (mg; 120 min/dL) for glucose compared with CON ($P = 0.04$, 7,137.2 vs. 6,568.2, SEM 185), suggesting a decrease in glucose tolerance. In contrast, AUC (µIU; 120 min/mL) of insulin, at d 10 was lower in cows receiving BCAA compared with CON ($P = 0.05$, 16,303 vs. 21,272, SEM 1,727), suggesting better insulin sensitivity in these cows. Additionally, cows receiving BCKA had a lower AUC (mEq; 120 min/L) for NEFA ($P = 0.05$, 47,103 vs. 63,192, SEM 4,508.2) compared with BCAA. Compared with CON, a tendency toward lower NEFA AUC was also observed in BCKA cows on d 20 ($P = 0.10$ 41,119 vs. 52,081, SEM 4,611.1). Overall, these results suggest that BCAA and BCKA altered glucose tolerance in fresh cows. Future work should determine the underlying mechanism for these changes, especially for the divergent response from BCAA and BCKA infusion.

Key Words: branched-chain amino acids, branched-chain ketoacids, glucose tolerance test

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2538 Milk yield and composition differences in A1 and A2 milk.

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Milk protein polymorphism has long been studied for its significant role in determining milk productivity and technological traits. Recently, there has been a growing focus on the A2 β-casein (β-CN) protein, driven by its positive effect on human health. However, the increasing emphasis on breeding for A2 cows without thorough studies raises concerns about its potential effect on overall milk quality. This study aimed to evaluate the effect of A1 and A2 β-CN variants on milk yield and composition (protein, fat, and SCC) in Holstein Friesian cows, considering their κ-CN (AA, AB, AE, BB, BE, and EE) and β-lactoglobulin (β-LG; AA, AB, and BB) protein genotypes. For analytical purposes, A1A1 and A1A2 cows were grouped under A1 variant, and A2A2 cows were designated as A2 variant. The E variant of κ-CN was never present along with β-CN genotype A2A2. Data on milk samples from monthly controls were retrieved from the databases of commercial farms located in northeastern Spain. The dataset consisted of 23,088 observations from 1,233 cows across 6 farms between the years 2016 and 2022. Outliers for milk yield, protein, fat, and SCC were defined as values falling beyond the mean ± 3 SD and were disregarded in the analysis. Fixed effects considered in the mixed linear model were β-CN, κ-CN, and β-LG genotypes, DIM, parity, calving year, and calving season. Animal nested within farm was considered as a random effect. Results showed that farm, parity, DIM, calving season, and calving year were all significant ($P \leq 0.05$). The β-CN genotypes did not have any significant effect on milk traits. Nevertheless, κ-CN genotypes influenced protein content, where AA genotype (3.36% ± 0.01%) exhibited lower ($P < 0.05$) protein content compared with AB (3.42% ± 0.01%) and BB (3.44% ± 0.01%) genotypes. Moreover, β-LG genotype affected fat content, with AA genotype (3.60% ± 0.04%) demonstrating lower ($P < 0.05$) fat content than AB (3.67% ± 0.04%) and BB (3.68% ± 0.04%) genotypes. In conclusion, increasing the frequency of A2 allele does not alter milk yield or composition in dairy cows.

Key Words: β-casein, κ-casein, β-lactoglobulin

2539 Use of automated milking systems on Wisconsin dairy farms.

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Automated milking systems (AMS) can affect farm productivity, animal welfare, and farmer quality of life, but assessments of their use and effects in the United States are limited. This study evaluates adoption patterns and the perceived challenges and benefits of AMS on Wisconsin dairy farms. A survey mailed to more than 2,000 randomly-selected Wisconsin dairy farms in January 2023 returned 673 responses from both users and nonusers of AMS. Approximately 8% of respondents currently use AMS and an additional 18% are considering use. Users of AMS account for about 11% of the total acres, 13% of total cows, and 14% of total milk production reported by respondents. Adopters of AMS have larger herd sizes, own and operate more land, and have higher rolling herd averages than respondents considering AMS use or not considering use (Table 1). The number of respondents adopting AMS in each year since 2012 is similar, and a majority (66%) purchased either 2 or 4 units when they began AMS use. Most respondents using AMS perceive that its use improves their outlook for the farm's future viability (72%) and allows increased time to be spent with family (67%). About one-third (38%) of AMS users perceive that its use reduces the stress associated with dairy farming. Nonusers of AMS indicate that the principal reasons for nonuse are cost-related. Nearly 60% of nonusers report that AMS are "too expensive to purchase and install," whereas more than one-third of nonusers indicate AMS are "too costly to maintain." Two-thirds of AMS users reported an initial cost >$150,000 per AMS unit, but 54% report that maintenance and repair costs in 2022 were <$10,000. Other reasons reported for nonuse of AMS include farm size, current configuration of milking systems, and access to hired labor. Our results suggest both perceived benefits and potential limitations of AMS technology.

Key Words: automatic milking systems, management, economics

Table 1 (Abstr. 2539). Mean farm characteristics by AMS use status

Characteristic	Current AMS use	Considering AMS use	Not using or considering
Number of respondents	50	120	483
Milking and dry cows	335^{a}	248^{a}	191^{b}
Rolling herd average, kg/cow/year	12,127^{a}	11,116^{b}	10,171^{c}
Land owned and rented, hectares	333^{a}	233^{b}	229^{b}

$^{a–c}$Indicate statistically significant differences.

2540 Evaluation of the effect of a hydrological Keyline system design in *Megathyrsus maximus* pastures.

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The Keyline system uses principles and techniques to regenerate agroecosystems through water resource optimization and restitution of soil depth and fertility. A Keyline design was implemented in a 4-ha paddock at the Heifer Rearing Unit, Pan-American Agricultural School, Honduras. The objective of this study was to determine the initial effects of the Keyline design on the growth dynamics of *Megathyrsus maximus* pasture, as well as on topsoil and subsoil hydraulic variations. Two of the 4 paddocks were plowed at 30-cm depth and 1.5 m between chisel plow shanks, and the other 2 were not. Soils were characterized using augers and soil pits. Comparative analysis of paddock biomass production and its nutritional content was conducted every 29 d. Differences in hydraulic tension between paddocks were determined using tensiometers at 30 and 60 cm in depth. Plowed pastures had higher ($P = 0.0367$) biomass production (1.3 kg/m^2 ± 4.5) than those not plowed (1.04 kg/m^2 ± 4.1). Greater differences in biomass production ($P < 0.0001$) were found during the period of drought (1.23 kg/m^2 ± 0.3 vs. 0.36 kg/m^2 ± 0.07). Plowed pasture soils resulted in lower hydraulic tension

levels ($P = 0.0006$) in plowed soils (15.4 kPa ± 16.3 kPa) compared with unplowed (24.4 kPa ± 24.5 kPa) soils. Keyline pastures produced higher densities of biomass and dry matter throughout the rainy season, particularly during the period of drought. Plowed soils tend to retain more moisture at the topsoil and to slow drying rates at 30 and 60 cm soil depths. Keyline systems favor degraded pasture regeneration and strengthen their resilience toward climate variability.

Key Words: climate resilience, degraded pasture, regenerative livestock farming

2541 Exploration of factors affecting the mineral profiles of drinking water in commercial dairy operations.

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In dairy farming, significant emphasis is placed on ensuring that the diets of dairy cows contain adequate and balanced mineral content to promote their overall welfare and maximize productivity. Yet, minimal consideration is typically given to the mineral composition of water and its potential influencing factors. This study aimed to first characterize the mineral profile of drinking water on commercial dairy farms and to explore the factors that may affect it. To achieve these aims, drinking water samples were collected from 50 dairy farms located in 6 different regions of the province of Québec (Eastern Canada) during both winter and summer seasons. Within 24 h, the mineral profile, including calcium, sulfate, chlorine, sodium, phosphorus, iron, copper, and magnesium, among others, was analyzed in a certified laboratory by atomic absorption spectrometry. Data regarding the water system and its maintenance, the presence or absence of water treatment systems, as well as information on feed rations and herd management, were collected. To evaluate the seasonal effect on mineral profiles, we conducted a *t*-test comparing samples collected at different times. To further explore factors affecting water quality on dairy operations, a principal component analysis (PCA) was conducted. Our results indicated that increased levels of iron, sulfate, chloride, and calcium were the primary factors associated with water quality concerns. Overall, 22% of the enrolled farms had at least one water quality issues, with most issues observed over the summer. Several significant differences ($P < 0,05$) were observed between samples collected during winter and summer seasons, indicating that season may affect water mineral quality. Finally, the results of the PCA analysis identified distinct clusters in the mineral composition of drinking water across different dairy farms, with mineral composition clustering by type of water system, presence of treatment system, and season. Water contributes to mineral intake in dairy cows, and this intake varies depending on the water system and the season.

Key Words: water, quality, mineral composition

2542 Influence of temperature-humidity index on individual lying behavior in lactating dairy cattle in Canada.

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The objective of this study was to explore the interaction between in-barn temperature humidity index (THI) and lying behavior in lactating dairy cattle across different provinces of Canada. A total of 5 commercial dairy farms (ON42, AB53, AB50, NS45, ON45) in 3 Canadian provinces were enrolled in this study. Data were collected for 3 years on each farm, during the spring, summer, and fall. A total of 272 animals were enrolled (ON42 = 54; AB53 = 62; AB50 = 46; NS45 = 30; and ON45 = 80). All animals were housed in freestall dairy barn systems. Each barn was equipped with a temperature and humidity sensor that was used to calculate the THI. Multiparous cows were selected in equal proportions, ensuring that all 5 farms had animals enrolled with comparable DIM. At the time of enrollment animals were equipped with a leg-mounted sensor (IceRobotics, Scotland), that measured steps, lying time, and number of bouts every 15 min. Data were analyzed using general linear mixed models from SAS, where cows were nested within farm. The slope for the relationship between the daily lying and the THI was calculated. The THI was classified as low THI (≤66) and high THI (≥72). Cows that were exposed to lower THI were more likely to be lying down longer compared with cows exposed to higher THI (11.4 ± 2.2 vs. 10.3 ± 2.1, 11.7 ± 2.3 vs. 10.8 ± 3.2, 12.9 ± 5.7 vs. 10.2 ± 6.0, 11.3 ± 3.9 vs. 10.1 ± 5.1, and 12.8 ± 2.5 vs. 10.8 ± 2.5 h, for ON42, AB53, AB50, NS45, ON45, respectively). The same effect was observed in number of bouts. There was great individual variation, on all farms, where there were cows that did not change their lying behavior as THI level changed (mean ± SD [range] slope; −0.09 ± 0.4 [−1.4 to 0.6], −0.10 ± 0.08 [−0.3 to 0.09], −0.02 ± 0.08 [−0.2 to 0.2], −0.05 ± 0.2 [−0.4 to 0.3] and, −0.07 ± 0.1 [−0.4 to 0.5] for ON42, AB53, AB50, NS45, ON45, respectively). Individual cows showed varying responses to different THI levels in terms of their lying behavior, suggesting that certain cows may be more susceptible to changes in the ambient barn THI.

Key Words: lying time, heat stress, temperature-humidity index

2543 Perception of agroecological performance of dairy farms by smallholders in Mexico and Peru.

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The Tool for Agroecology Performance Evaluation (TAPE) is a novel FAO tool developed to collect data on how the 10 Elements of Agroecology (EA) can contribute to sustainable food and agricultural systems. One of the steps of TAPE involves a participatory analysis of survey results with participant farmers during a workshop. Our goal was to assess the likelihood of change in agroecological practices and to determine the perception of workshop participants regarding key actors responsible for these changes. We conducted a 2-h workshop in Aculco, Mexico (n = 12 participants), and in San Martin, Peru (n = 13). Using a Likert-scale from −2 (very unlikely) to 2 (Very likely), farmers rated the likelihood of changing practices related to the 10 EA. To identify key actors responsible for these changes, farmers were tasked with allocating 10 points among 5 options: individual, community, or government at the municipal, regional, or central level. Median values were analyzed to compare countries with the Mann–Whitney U test. The likelihood that farmers would change practices to strengthen *Synergies* was higher in Peru than in Mexico (1.0 vs. 0.5, $P < 0.01$), whereas the likelihood of changes related to *Resilience* was higher in Mexico than in Peru (1.0 vs. 0.0, $P < 0.05$). Farmers from both countries overwhelmingly agreed

that they are the main actors responsible for implementing changes. Community as an agent of change was scored greater by farmers in Mexico than in Peru for *Resilience, Culture & Food Traditions*, and *Co-creation & Sharing of Knowledge* ($P < 0.05$). However, the regional government as an agent of change was scored greater by farmers in Peru than in Mexico for *Recycling, Resilience,* and *Co-creation & Sharing of Knowledge* ($P < 0.05$). There were no differences between the 2 countries for the 5 remaining EA (*Diversity, Efficiency, Human & Social Values, Circular & Solidarity Economy,* and *Responsible Governance*). In evaluating the workshop, farmers showed a high degree of interest in learning more about agroecological practices. This study provides a case study on giving smallholder farming communities a voice to chart their own future.

Key Words: transdisciplinary, agroecology, sustainability

2544 Agroecological performance of smallholder dairy cattle systems in Aculco, Mexico.

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There is a growing interest in conducting agroecological assessments of farming practices, yet there is limited data on the agroecological transition of smallholder dairy systems (SDS). Our study aimed to characterize and determine the level of agroecological transition of SDS. The study included 60 randomly selected SDS farms from the rural area of Aculco, State of Mexico, Mexico. Data were collected using the Tool for Agroecology Performance Evaluation, a survey tool of the FAO, during the spring of 2023. The SDS farms were grouped through a factor and cluster analysis based on the scores from the 10 Elements of Agroecology (EA: *Diversity*, *Synergies*, *Efficiency*, *Recycling*, *Resilience*, *Culture & Food Traditions*, *Circular & Solidarity Economy*, *Human & Social Values*, *Co-creation & Sharing of Knowledge*, and *Responsible Governance*) The Characterization of Agroecological Transition (CAET) score was calculated as the overall mean of the scores of the 10 EA on a scale of 0 to 100. The factor analysis identified 3 factors explaining 64.8% of the total variance, supported by a Kaiser Meyer Olkin coefficient of 0.72, indicating sampling adequacy. The Bartlett value was significant ($P < 0.001$), confirming the analysis's reliability. Differences between groups were identified through ANOVA analysis and the Games–Howell posthoc test ($P < 0.05$). Factor 1 highlighted the positive association among *Efficiency*, *Diversity*, *Synergies*, and *Recycling*. Factor 2 showed the positive association between *Culture & Food Traditions* and *Circular & Solidarity Economy*. Factor 3 included the positive association among *Human & Social Values*, *Resilience*, *Co-creation & Sharing of Knowledge*, and *Responsible Governance*. The cluster analysis identified 4 different groups ($P < 0.05$) with the highest CAET score for Group 1 (55.9, n = 12), the second highest for Group 3 (43.5, n = 22), and the lowest for Group 2 (34.5, n = 16) and Group 4 (37.8, n = 10). This study allowed us to understand the relationships among the EA and to create a typology of SDS farms for targeted interventions aiming to promote sustainability through the implementation of agroecological practices.

Key Words: agroecology, clustering, livestock

2545 Effects of wildfire smoke on production, health, and mortality of lactating dairy cows in the Pacific Northwest.

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From 2020 to 2022, wildfires burned over 2.1 million hectares in the Pacific Northwest (PNW). Our group previously found that wildfire fine particulate matter ($PM_{2.5}$) decreased milk yield (MY) in a limited number of cows over one summer. However, the impact of wildfire smoke on dairy cattle has not been assessed across multiple years with varying wildfire conditions. The objective of this retrospective study was to assess milk production, health, and mortality of lactating cows in the PNW in relation to wildfire-$PM_{2.5}$ exposure. Data from the University of Idaho DairyComp305 system, and daily average $PM_{2.5}$ and temperature-humidity index (THI) from the Idaho Department of Environmental Quality were collated over a 3-yr period (2020–2022). HYSPLIT modeling was used to track $PM_{2.5}$ to wildfires. Data within 7 d of 24-h average $PM_{2.5} > 35$ μmg/m^3 were considered exposures to wildfire-$PM_{2.5}$. Animal data included treatments for mastitis, pneumonia, and lameness (TRT, n = 79), all-cause mortality (n = 35), and daily MY (n = 40). Weekly milk composition (MC) was also assessed in 2020–2021. Individual cow MY and MC were evaluated as continuous variables for a 3-mo period (Jul. 1-Oct. 7), coinciding with wildfire season, and health and mortality events were evaluated as count data annually. All data were analyzed in SAS. Treatments and mortality were analyzed with PROC FREQ, with TRT count and wildire-$PM_{2.5}$ exposure as factors. Milk yield and milk composition were analyzed using PROC MIXED, with fixed effects of $PM_{2.5}$, THI, year, and their interactions, and cow as a random effect. Mixed models included lags of up to 7 d to assess delayed or persistent effects of $PM_{2.5}$ and THI, on MY and MC. There was no detectable association found between wildfire-$PM_{2.5}$ and TRT ($P > 0.15$) or mortality ($P = 0.30$); $PM_{2.5}$ and THI interactions reduced MY on lag d 7 ($P = 0.04$) and tended to reduce MY on lag d 6 ($P = 0.06$). Together $PM_{2.5}$ and THI reduced milk fat percent on lag d 2, 3, and 5 ($P \leq 0.01$), and protein percent on lag d 1 through 5 ($P \leq 0.032$). These data suggest that year after year milk production and components are affected by wildfire-$PM_{2.5}$.

Key Words: air quality, milk yield, morbidity

2546 Air quality dynamics in tunnel-ventilated barns: The role of routine farm activities.

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Dairy farms have been identified as sources of air pollutants that might affect the overall sustainability of the dairy industry. It was hypothesized that farming operations might produce transient changes in indoor air quality (IAQ). This research aimed to evaluate the effect of different routine farm activities on the dynamics of air components. Air components, including ammonia, carbon monoxide, carbon dioxide, methane, nitrogen dioxide, ozone, and particulate matter 2.5, were measured at one commercial dairy farm located in Northern Colorado and milking 6,000 Holstein cows. One automated sensor (IEQMax, CO) was installed 4 m from the ground in a tunnel-ventilated barn (freestalls bedded with sand). This sensor collected real-time air component measurements at 1-min intervals. Data collection occurred between August 8 and October 19, 2023. In addition, the start and finish times of routine farm activities, including movement of cows to the parlor, vehicles and equipment traffic, and manure removal were recorded. When no farm operations were observed, the period was classified as nonactivity. These observations were conducted between 8 a.m. and 10 a.m. MST. Descriptive statistics and mixed model analyses were conducted in R, including the activity variable (active operation vs. no activity) and date as covariates. The mean CO_2 concentration was significantly higher during farm activities compared with nonactivity periods (575 ± 1.9 vs. 570 ± 1.5 ppm; $P = 0.04$). In addition, an increase in NH_3 concentration was observed during

Table 1 (Abstr. 2547).

Item	Cool	Warm	Hot	SE	*P*-value
No fans, 2019					
n	41	26	25		
RT, °C	39.03^{c}	39.23^{b}	39.72^{a}	0.04	<0.01
Lying, min/d	713^{a}	642^{b}	570^{c}	8	<0.01
Lying bouts/d	9.1^{b}	9.1^{b}	9.5^{a}	0.1	0.04
Fans, 2023					
n	49	27	11		
RT, °C	39.12^{c}	39.33^{b}	39.72^{a}	0.02	<0.01
Lying, min/d	704^{a}	671^{b}	633^{c}	6	<0.01
Lying bouts/d	9.1^{b}	9.4^{b}	10.8^{a}	0.1	<0.01

$^{a–c}$Different letters within a row are significantly different ($P < 0.05$).

farm activities relative to nonactivity periods (8.0 ± 0.1 vs. 7.7 ± 0.08 ppm; $P = 0.02$). Conversely, no significant differences in concentration were observed for other air components evaluated in this study. This research highlights the necessity of mitigation strategies to address air pollutant emissions during farm activities because CO_2 and NH_3 adversely affect human and animal health. Moreover, future research studies are essential to better understand the underlying factors driving IAQ dynamics in dairy farms.

Key Words: air quality, management, emissions

2547 Effect of episodic heat events on body temperature, lying time, and lameness of dairy cows before and after fan installation for heat abatement in Northern New York.

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A retrospective study was conducted from July through September of 2019 and 2023 on a Holstein herd in Northern New York to evaluate animal well-being before (2019) and after (2023) installation of fans. A sand-bedded 6-row freestall with no mechanical ventilation in 2019 was equipped with 6 130-cm variable-speed fans positioned over the center 2 rows of stalls. Thirty multiparous cows with lameness scores ≤2 served as focal groups averaging 55 and 48 kg milk/d in 2019 and 2023, respectively. Temperature and relative humidity in the pen were recorded and temperature-humidity index (THI) was calculated every 10 min. Wind speed (m/sec) was measured weekly at standing and lying positions throughout pen. Lameness was scored at the beginning and end of the study year (scale 1–5). Body temperature (smaXtec) and lying behavior (HOBO Pendant G) were measured continuously. Lameness (≤2 vs. >2) from the beginning to the end of the study was analyzed by Chi-squared analysis using Proc Freq (SAS 9.4). Retrospectively, days were classified as cool (THI < 68), warm (68 ≤ THI < 72) and hot (THI ≥ 72). Lying behavior (time, bouts) and median reticular temperature (RT, °C) were averaged for all cows daily then analyzed by year using the Mixed procedure (SAS 9.4) with fixed effect of THI level adjusted for unequal variances. Average windspeed was increased from 0.3 m/sec to 1.2 m/sec with the installation of fans. There was a 26.7% increase in lameness during study period with no fans in the housing area ($P < 0.05$) and only a 6.7% increase with fans present ($P = 0.15$). Even with the installation of fans, cows were affected by heat events as indicated by increased RT and reduced lying time (Table 1). Increased windspeed appeared to have mitigated some heat stress because lying time was reduced by 10% compared with 20% without fans.

Key Words: heat stress, lying time, body temperature

2548 Linking farm management to dairy farmer mental and physical well-being.

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Management practices, on-farm duties, and unpredictable stressors can all affect dairy farmers' well-being and overall quality of life. This study explored how farm management and farm-level factors are associated with farmer well-being. Dairy farmers (n = 86) across Western Canada and Ontario completed an online survey that included questions about farm responsibilities, work–life balance, management, social environment and support, finances, animal health, and validated psychometric scales to assess stress, anxiety, depression, and resilience. Of the 86 farmers surveyed, 87% and 13% reported moderate and high stress levels, 31% and 22% of farmers had mild and severe anxiety scores, and 39% and 20% of farmers had mild and severe depression scores, respectively. Univariable analyses using *t*-tests, chi-squared tests, and linear models showed that mental well-being scores did not differ based on gender, milking system, housing type, personal finances, time working alone, or animal welfare concerns. Farmers who were most concerned about farm finances, feed costs, milk prices, workload, and how much consumers valued them had higher levels of anxiety and stress ($P < 0.05$). Concerns about farm finances, feed costs, and workload were associated with the highest depression scores ($P < 0.05$). It was found that farmers who demonstrated greater resilience were less stressed by their workload. As for physical well-being, the number of work-related injuries and health problems was greater among men than women. Overall, 84% of farmers experienced work-related injuries and health problems. There were no differences in physical well-being based on milking system, housing type, or time spent working alone. Dairy farmers appear to have comparable well-being in different management systems, but financial constraints, including high interest rates and inflation, are key stressors. We identified a greater risk of poor mental well-being for farmers who worry about finances and workload. Financial support or other support networks may be needed for farmers to understand and manage work stressors, reduce mental health stigma, and instill compassion in consumers.

Key Words: mental health, well-being, farm stress

Reproduction 4

2549 Fertility of nulliparous heifers inseminated with sexed semen after twice-daily detection of estrus or timed AI after a 5-day PRID-synchronization protocol. W. M. Brown*[1], M. R. Lauber[1], A. Valenza[2], and P. M. Fricke[1], *[1]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI, [2]Ceva Salute Animale SpA, Milano, Italy.*

Heifers inseminated with sexed semen after once-daily detection of estrus had fewer pregnancies per AI (P/AI) than heifers submitted to timed artificial insemination (TAI) after a 5-d controlled internal drug release (CIDR)-Synchronization (Synch) protocol (Lauber et al., 2023; J. Dairy Sci. 104:12953). We hypothesized that heifers inseminated with sexed semen after twice-daily detection of estrus would have similar P/AI to heifers submitted to a 5-d progesterone-releasing intravaginal device (PRID) synchronization protocol for 1st TAI. Holstein heifers from 2 Italian dairy herds (n = 598; Farm 1, n = 328; Farm 2, n = 270) were randomized within farm to either: (1) treatment with PGF$_{2\alpha}$ (d 5) and twice-daily (a.m./p.m.) detection of estrus and AI (Estrus; n = 298), or (2) TAI after a 5-d PRID-Synch protocol (n = 300; d 0 GnRH, d 5 and 6 PGF$_{2\alpha}$, d 8 GnRH^{+}TAI). Farm 1 detected estrus using an accelerometer system, whereas Farm 2 used visual observation of rubbed tail chalk. Variables were analyzed by logistic or linear regression (R studio 4.3.2), including fixed effects of treatment and farm. Heifers received their 1st PGF$_{2\alpha}$ treatment at 405.1 ± 14.13 (Farm 1) or 447.22 ± 25.78 (Farm 2) days of age. There was a farm × treatment interaction ($P < 0.01$) for days to first AI in which Farm 1 averaged 4.4 ± 5.2 d for Estrus compared with 2.7 ± 0.5 d for PRID heifers, whereas Farm 2 averaged 8.4 ± 8.9 d for Estrus compared with 3.0 ± 0.0 d for PRID heifers. In agreement with our hypothesis, P/AI did not differ between PRID vs. Estrus heifers at 38 (59.0% vs. 63.4%, respectively; $P = 0.79$) or 68 (57.7% vs. 60.7%, respectively; $P = 0.93$) d after AI. Pregnancy loss from 38 to 68 d after AI did not differ ($P = 0.53$) between PRID vs. Estrus heifers (2.3% vs. 3.2%, respectively). By contrast, mean (±SEM) d to pregnancy differed ($P < 0.05$) between farms (22.5 ± 30.4 vs. 29.3 ± 37.7 d for Farm 1 vs. 2, respectively). Twice-daily detection of estrus and AI is more optimal than once-daily detection and yielded similar fertility to TAI because insemination with sexed semen occurred at a more optimal time relative to ovulation in Estrus heifers. *Supported by WI Experiment Station Hatch project WIS04012.*

Key Words: dairy heifer, sexed semen, synchronization

2550 Differences in reproductive and productive performance, and culling, associated with time to clinical cure in dairy cows treated for metritis. F. N. S. Pereira*[1], V. R. Merenda[2], E. B. de Oliveira[3], F. S. Lima[3], R. C. Chebel[4], K. N. Galvão[4], J. E. P. Santos[4], R. S. Bisinotto[4], and C. C. Figueiredo[1], *[1]Washington State University, Pullman, WA, [2]North Carolina State University, Raleigh, NC, [3]University of California, Davis, Davis, CA, [4]University of Florida, Gainesville, FL.*

The objective was to assess differences in reproductive and productive performance, and culling associated with time to clinical cure in dairy cows treated for metritis. This retrospective cohort study included data from 3 studies conducted in 5 dairies in Florida. Metritis was characterized by the presence of fetid, watery, reddish-brownish vaginal discharge (VD) within 12 DIM (study d 0). Cows with metritis were treated with ampicillin (AMP) or ceftiofur (CEF). Clinical cure (absence of fetid, watery, reddish-brownish VD) was assessed on d 5 and 12 postdiagnosis, resulting in 3 study groups (CureD5, n = 705; CureD12,n = 406; NoCure, n = 299). Risk of pregnancy and culling by 300 DIM were analyzed using logistic regression, hazard of pregnancy by 300 DIM was analyzed using the Cox's proportional hazard regression, and milk production within 90 DIM was analyzed using ANOVA with repeated measures. Orthogonal contrasts were set to assess the effects of cure (CureD5 + CureD12 vs. NoCure) and time to cure (CureD5 vs. CureD12; Table 1). Risk of pregnancy was higher for cured cows than for NoCure cows. CureD5 had higher risk of pregnancy than CureD12. Risk of culling was lower for cured cows than for NoCure cows. CureD5 had lower risk of culling than CureD12. Cured cows got pregnant faster than NoCure cows, but no differences were observed by time to cure. An interaction between cure status, parity, and month postpartum was associated with milk production ($P < 0.001$). In primiparous cows, milk production was similar among the 3 cure groups. In multiparous cows, milk production was lower in NoCure than in CureD5 and CureD12. No differences in milk production were observed by time to cure. Hastened time to clinical cure of metritis after antimicrobial therapy was associated with improved reproductive performance and reduced culling in dairy cows.

Key Words: cure, antibiotic, uterus

2551 Leukemia inhibitory factor, insulin-like growth factor 1, fibroblast growth factor 2, and epidermal growth factor (LIFE) supplementation during *in vitro* maturation does not improve bovine embryo development. K. J. Alward*, M. A. Oliver, and A. D. Ealy, *Virginia Polytechnic Institute & State University, Blacksburg, VA.*

The in vitro production (IVP) of bovine embryos is one of the fastest growing sectors of assisted reproductive technologies in the cattle industry, despite low efficiency of embryo production from harvested oocytes. We hypothesized that supplementing oocyte medium with a mixture of agents (leukemia inhibitory factor [LIF], insulin-like growth factor 1

Table 1 (Abstr. 2550).

	Group			*P*-value	
Item	CureD5	CureD12	NoCure	Cure	Time to cure
Risk of pregnancy, %	69.6^{a}	62.9^{b}	56.4^{b}	< 0.01	0.03
Culling, %	15.5^{a}	23.7^{b}	26.7^{b}	< 0.01	< 0.01
Median days to pregnancy, d	133^{a}	136^{a}	163^{b}	0.03	0.43
Milk production, kg/d					
Primiparous	34.6^{a}	34.6^{a}	34.4^{a}	0.03	0.99
Multiparous	45.9^{a}	45.5^{a}	42.7^{b}		

^{a,b}Within a row, different letters indicate differences ($P \leq 0.05$).

[IGF1], fibroblast growth factor 2 [FGF2] and epidermal growth factor [EGF], collectively known as LIFE), which are proposed to influence oocyte quality will improve IVP embryo development. Cumulus-oocyte complexes (COC) were collected from abattoir ovaries (Brown Packing; Gaffney, SC) and good quality COC underwent in vitro maturation (IVM) either in standard COC medium (control) with 1% BSA carrier or in the standard COC medium supplemented with 20 ng/mL LIF, 20 ng/mL IGF1, 0.5 ng/mL FGF2, and 10 ng/mL EGF (LIFE) in 1% BSA (25 COC/drop; 4 drops/treatment per replicate; 8 replicates). Cumulus-oocyte complexes were matured and fertilized using pooled Holstein semen. Cleavage was evaluated on d 2 postfertilization and blastocyst development was evaluated on d 7.5. A portion of blastocysts (25 embryos/treatment) were fixed and stained for immunofluorescent detection with 4′,6-diamidino-2-phenylindole (DAPI) and caudal type homeobox x (CDX2) to quantify total cell number, trophectoderm (TE) cells, and inner cell mass (ICM) cells. Data were analyzed with PROC GLM of SAS 9.4 with fixed effects of treatment and replicate. Supplementation with LIFE did not affect cleavage rate; however, a reduction in blastocyst rate was detected both based on total number of zygotes ($P = 0.02$; 20.2% ± 1.8% vs. 25.6% ± 1.8% for controls) and based only on cleaved embryos ($P = 0.01$; 26.5% ± 1.9% vs. 34.0% ± 2.1% for controls). The LIFE supplement did not affect total, TE, ICM, or ICM:TE cell numbers. In summary, this data does not support our hypothesis that providing LIFE supplement during COC maturation improves IVP embryo production. There may, however, be alternative benefits to LIFE supplementation regarding embryo quality that have not yet been evaluated because similar mixtures improve the cryoprotective capacity of IVP blastocysts. *This work was supported by USDA NIFA grant 2021–67015–34485.*

Key Words: bovine, embryo, in vitro

2552 Challenges in development of a consistent, rapid timed artificial insemination rebreeding program for lactating dairy cows.

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Our laboratory has developed a consistent, rapid rebreeding program for heifers for timed AI (TAI) every 21 d, ReBreed21, but this has not been optimized for lactating dairy cows. Experiment one tested whether luteolysis could be synchronized using oxytocin (50 IU i.m.) treatments. Cows after previous TAI were randomized to receive 3 (3Oxt; d 17, d 18, d 19; n = 129), 2 (2Oxt; d 18, d 19; n = 130), 1 (1Oxt; d 18; n = 129), or 0 (CON; n = 129) oxytocin treatments. Pregnancy diagnoses (PD) were done on d 25 (PAG) and d 32 (ultrasound) and luteolysis (P4 < 1ng/mL) by P4 radioimmunoassay. There was a similar pregnancy/AI (P/AI) to the first AI ($P = 0.89$) but the percentage of nonpregnant cows (d 25 PD) with luteolysis by d 20 was greater ($P = 0.01$) in cows treated with 2Oxt (76.4%) than CON (53.8%) and intermediate for 3Oxt (72.4%) and 1Oxt (64.4%). Thus, 2Oxt synchronized luteolysis without reducing P/AI. Experiment 2 used the 2Oxt in a rapid ReBreed21 program for multiparous lactating dairy cows. After a first TAI with DoubleOvsynch, cows were assigned to one of 3 rebreeding programs: Resynch25 (R25 [n = 150]; gonadotropin releasing hormone [GnRH] d 25, PD d 32, NP PGF [d 32 and d 33], GnRH d 34, and AI d 35), ReBreed21 (RB21 [n = 150]; Oxt on d 18 and d 19, MilkP4 rapid test on d 20, LowP4 GnRH on d 20 p.m., AI d 21 a.m.), and ReBreed21G (RB21G [n = 150]; similar to RB21 but with GnRH treatments on d 5 and d 11]). The P/AI for first AI was greater ($P = 0.03$) for R25 (54%) than RB21 (37.3%), and intermediate for RB21G (44%). The percentage of cows with luteolysis by d 20 was greater ($P < 0.01$) for RB21 (75.7%) than for R25 (54.5%) and RB21G (52.2%). The P/AI during rebreeding was greater ($P < 0.01$) for R25 (42.3% [82/194]) than RB21 (27.6% [45/163]) and RB21G (24.4% [30/123]). Pregnancies at 180 DIM were greater ($P = 0.03$) for R25 (86.1%) than RB21 (73.5%) and intermediate for RB21G (79.3%) with an earlier time to pregnancy ($P < 0.01$) for R25 (116) than RB21 (131), and RB21G (127). In summary, the 2Oxt ReBreed21 program synchronized luteolysis by d 20, but, unexpectedly, decreased fertility to first TAI and rebreed TAI. Thus, ReBreed21 allows an early timed rebreeding, but the program still needs optimization for dairy cows.

Key Words: oxytocin, resynch, ReBreed21

2553 Effect of large (= 10 mm) follicle number on fertility of the short-term timed artificial insemination protocol in lactating dairy cows.

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Short-term ovulation synchronization (SS) is a protocol that starts with the administration of prostaglandin $F_{2\alpha}$ (PGF) for cows that have a functional corpus luteum (CL). Because this protocol does not induce the emergence of a new follicular wave, fertility of the protocol is dependent on the follicle developmental stage at the onset of the program. Fertility of the first wave dominant follicle (1WDF) is lower than that of second one (2WDF; Denicol et al., 2012). In our previous study, we determined that large (= 10 mm) follicle (LF) number can be used to determine the follicular wave, such that one LF (1F) is indicative of 1WDF whereas 2 or more LF (2F) is indicative of 2WDF (Miura et al., 2023). We hypothesized, therefore, that fertility of the SS is lower in cows with 1F rather than 2F. The objective was to compare the fertility of the SS among 1F and 2F cows using 2 types of ovulation inducer; estradiol benzoate (EB) and gonadotropin releasing hormone (GnRH). Holstein cows (n = 704, DIM = 164.0 ± 2.9, parity = 2.6 ± 0.1; mean ± SEM) were examined using ultrasonography to determine presence of a functional CL (= 20 mm) and number of LF (1F: n = 463, 2F: n = 241). Cows were administered PGF and randomly assigned to receive 100 µg of GnRH (1F: n = 272, 2F: n = 143) 48 to 56 h after PGF (d0), or 2 mg of EB (1F: n = 191, 2F: n = 98) 24 h after PGF. Artificial insemination was performed 16–20 h after GnRH treatment, or 24–28 h after EB treatment, using conventional or sexed frozen semen. Pregnancy diagnosis was performed 25–90 d after AI. Pregnancy per AI (P/AI) was evaluated using logistic regression. Follicle number, ovulation inducer, and their interaction were all significant ($P < 0.01$). Pregnancy per AI was lower in GnRH-1F group than in the other 3 groups (GnRH-1F: 26.8%, GnRH-2F: 50.3%, EB-1F: 40.3%, EB-2F: 44.9%, $P < 0.01$). There was no difference in P/AI among the GnRH-2F, EB-1F, and EB-2F groups. In conclusion, the fertility of the SS in the cow presumed to be 1WDF stage is lower than 2WDF if GnRH is administered, but the use of EB did not affect fertility of 1WDF or 2WDF cows.

Key Words: estradiol, prostaglandin $F_{2\alpha}$, timed AI

Ruminant Nutrition 4: Calves and Heifers

2554 Intake of colostrum from cows with high or low somatic cell count at dry-off: Effects on the development of calves during the preweaning period.

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This study aimed to evaluate the effects of supplying maternal colostrum from cows with high or low somatic cell count (SCC) at dry-off on the transfer of passive immunity (TPI), performance, and metabolism of dairy calves during the preweaning period. Twenty-two Holstein calves, daughters of cows that had low SCC (<200,000 cfu/mL) or high SCC (≥300,000 cfu/mL) at dry-off, were distributed in randomized blocks according to weight and date of birth. Newborns were fed their mother's colostrum (≥30% Brix) in a volume of 10% of BW, within the first 2 h and a further 5% within 8 h after birth. After the colostrum feeding protocol, all calves remained under the same conditions, receiving 6 L/d of milk replacer and ad libitum access to water and initial concentrate until 56 d, when the experimental period ended. Blood collections were carried out at 24, 48, and 72 h of life and weekly to determine selected metabolites. Fecal score (FS) was evaluated daily using the scale 0 to 3. Data were analyzed as repeated measures using Proc Mixed from SAS 9.4. Both treatments showed excellent TPI, with no differences when evaluated with a protein refractometer (7.9 ± 0.38 g/dL; $P > 0.84$) or Brix (11.6% ± 0.49%; $P > 0.70$). Weaning weight was similar between groups ($P > 0.16$). However, there was a trend toward higher ADG in calves in the low SCC group (443 vs. 357 g/d; SEM: 0.05; $P = 0.06$). Conversely, we found a tendency ($P = 0.07$) for a higher FS (more fluid feces) in calves that received colostrum from cows with high SCC at dry-off (1.44 vs. 1.29; SEM: 0.12). There was also a trend ($P = 0.07$) of higher hematocrit values in the same group (30.9% vs. 28.9%; SEM: 0.76). The treatments did not affect preweaning starter DM intake (145 ± 41.6; $P > 0.27$) or the blood concentrations of glucose (105.1 ± 4.66 mg/dL; $P > 0.41$), protein (7.99 ± 0.287 g/dL; $P > 0.38$), and lactate (24.7 ± 2.474 mg/dL; $P > 0.82$). The results indicate that feeding colostrum from cows with a high SCC at dry-off may increase intestinal challenge but not to a point to interfere with the development or blood metabolites concentration of preweaning calves.

Key Words: metabolism, performance, TPI

2555 Multicarbohydrase enzyme complex in texturized calf starter feed affects calf growth and health.

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The objectives were to evaluate the effects of a multicarbohydrase enzyme complex (ENSP; Enspira®+; United Animal Health, IN) added to calf starter feed on calf growth and health. Two 71-d trials (T) were conducted using 128 crossbred (Holstein × Angus) 2 to 10 d old calves (42.7 ± 3.95 kg) randomly assigned to receive 1 of 3 dietary treatments (TRT): (1) negative control (CON; no ENSP; n = 39), (2) ENSPLo (ENSP 125 ppm; n = 38), and (3) ENSPHi (ENSP 500 ppm; n = 38). Three different premixes containing the TRT were added to a commercial texturized starter feed (17% CP, 3% Fat; Belstra Milling Co., IN) and fed ad libitum to calves from each corresponding TRT from d 1 until the end of the trial (d 71). All calves received the same milk replacer (MR; 24% CP, 20% fat) schedule (d 1–42: 2×/day at 14.1% to 13.2% solids [S]; d 43–49: 1×/day at 13.2% S; weaned on d5 0), and had free access to water. Calves were housed individually and had MR intake, fecal scores (FS), and health checks recorded daily. Body weights and starter feed intakes (sFI) were taken weekly. Prewean (PREW; d 1–49), postwean (POSTW; d 50–71), and overall (ALL; d 1–71) data were evaluated using PROC MIXED in SAS 9.4, with fixed effects of TRT and T, and random effect of calf. Orthogonal contrasts were used to compare CON versus both ENSP TRT together. Health metrics (FS, medications counts) were analyzed using PROC FREQ with chisq. Body weight and ADG did not differ ($P = 0.23$) among the 3 individual TRT at any time point. Calves receiving ENSP, independently of dose, had increased (contrast $P < 0.05$) POSTW BW gain (25.27 vs. 23.81 ± 0.6) and ADG (1.20 vs. 1.13 ± 0.03), and tended (contrast $P < 0.09$) to have higher PREW BW gain (35.27 vs. 33.08 ± 1.07) and ADG (0.83 vs. 0.78 ± 0.02), as well as ALL (BW gain 60.03 vs. 56.89 ± 1.41; ADG 0.95 vs. 0.90 ± 0.02; contrast $P < 0.07$), compared with CON. CON calves received 3 times more ($P = 0.01$) anti-inflammatories than ENSPLo and ENSPHi (15 vs. 4 and 5 doses, respectively), and ENSPLo had fewer days with scours POSTW ($P = 0.001$; −50%) and ALL ($P = 0.02$; −26%) compared with CON and ENSPHi. Because both ENSP doses enhanced calf health and growth compared with CON, ENSPLo may be more cost-effective.

Key Words: feed efficiency, NSP, digestibility

2556 Effect of probiotic fed in milk and starter feed on growth and prevention of diarrhea in preweaned Holstein × beef cross calves.

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Administering probiotics can lower the incidence and severity of diarrhea in preweaned calves. This study evaluates the effectiveness of feeding a *Lactobacillus* probiotic supplement (PS: 1×10^9 cfu) in milk and starter grain 1×/d on growth, fecal scores, and fecal shedding of *Salmonella*. On a commercial calf ranch in California, preweaned Holstein × Angus calves age <48 h with serum total proteins >5.2 g/dL were randomly enrolled into 2 treatment groups: (1) control (CON: n = 106), and (2) probiotic (PRO: n = 102). Fifty mg/calf per day of PS were added to individual milk bottles in the morning feeding, and 50 mg/calf per day were top dressed in starter grain until weaning at 60 d of age. Calves were housed individually in wooden slatted hutches. Starter grain was provided beginning at 14 d of age and 2 L of milk replacer was fed twice daily. Fecal consistency was evaluated daily using a 1–3 scale, with 1 representing normal, and 3 indicating loose. Fecal samples were collected from approximately 30 calves per treatment at 7, 14, 21, and 42 d via digital stimulation to assess *Salmonella*. Results were analyzed using general linear models (SAS v. 9.4, 2024) with backward elimination of covariates. Model covariates included treatment, initial BW (41 kg), hutch, number of fecal score 2 (FS2; n = 8 per treatment), and number of fecal score 3 (FS3). Four models were constructed, including gain, ADG, FS2, FS3, and number of dead calves. Comparisons with $P < 0.05$ were statistically different. Treatment did not affect growth (gain = 26 kg) or prevention of diarrhea (FS2 = 8 d, FS3 = 7 d), and the number of dead calves was not different by treatment (n = 1 per treatment). Fecal shedding of *Salmonella* was not detected. Although treatment did not affect FS2 or FS3, overall FS2 and FS3 affected ADG. This suggests that calves with diarrhea gained less during the prewean period of 60 d.

Key Words: fecal score, gain, fecal pathogen shedding

2557 Effects of synbiotic supplementation to late gestation cows on offspring growth and health.

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Maternal nutrition and health status during pregnancy can affect offspring outcomes. The objective of this study was to evaluate the effects of a synbiotic (SYN) fed to late-gestation cows on offspring intake, growth, and health. Multiparous cows (n = 62) were randomly assigned to 1 of 2 treatments: (1) control (CON, 454 g/d concentrate on a DM basis) or (2) SYN (440 g/d concentrate plus 14 g/d of *Bacillus subtilis* spores and yeast products on a DM basis; Amulet, Purina, St. Louis, MO) 28 d before expected calving in a completely randomized design, and their female offspring were assessed. All heifers (n = 24) were removed from their dams at calving and housed in individual hutches bedded with straw. All calves were offered 0.82 kg of DM/d of milk replacer (MR, 27% CP, 20% fat; Land O'Lakes Inc., Arden Hills, MN) from d 1 to 8, 1.13 kg of DM/d from d 7 to 47, 0.75 kg of DM/d from d 48 to 54, and 0.38 kg of DM/d from d 55 until weaning at d 61. Calves were offered a phase feeding starter program (28.5% CP starter from d 2 to d 28 and 25% CP from d 29 to d 87 [AMPLI-CALF® Starter, Land O'Lakes Inc., Arden Hills, MN] or 25% CP starter from d 2 to d 87 of age) *ad libitum*. Milk replacer and starter intake (SI) were measured daily, and body measurements were taken weekly. Data were analyzed using SAS (9.4) including the fixed effects of dams' treatment, time, their interactions, and the random effect of heifer within treatment. The effect of starter program did not affect calf performance ($P > 0.60$). No differences in birth BW (39 vs. 41 kg) were observed between groups. There was a trend ($P = 0.07$) for increased SI over time in calves from dams fed SYN. The final body weight (87 d) was 8 kg greater for heifers from cows fed SYN than for CON (123 vs. 115 kg, $P = 0.04$), resulting in a trend toward greater ADG (+72 g/d, $P = 0.09$). Calf scours, hip height gain, and heart girth gain were similar between groups ($P > 0.05$). Heifers from SYN dams had greater ($P < 0.05$) final body length (+ 5.9 cm) and gain (+ 4.6 cm). In conclusion, the use of a synbiotic to late gestation cows had beneficial effects on the growth of their female offspring.

Key Words: synbiotic, offspring, growth

2558 The effect of probiotic supplementation on serum tumor necrosis factor α, lipopolysaccharide binding protein, and immunoglobulin concentrations in young Holstein heifers.

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Young dairy calves are exposed to a multitude of environmental stressors that can result in dysbiosis of the gastrointestinal tract (GIT) microflora. This can trigger an inflammatory response that results in the partitioning of nutrients away from productive functions. Probiotics have the potential to mitigate this dysbiosis and thus increase the efficiency of nutrient utilization for production. We evaluated the effects of probiotics on the serum concentration of tumor necrosis factor α (TNFα), lipopolysaccharide binding protein (LBP), and immunoglobulins (IgG, IgM). Newborn Holstein heifer calves (n = 112) from a commercial dairy were placed into 2 treatment groups: (1) control and (2) *B. subtilis* 810, *B. licheniformis* 809, *L. animalis* PTA-6750, and *P. freudenreichii* PTA-6752 probiotic (Bovamine Dairy Plus, Chr. Hansen, Milwaukee, WI). Control calves received 0.5 g lactose in milk once daily until weaning and 0.75 g daily in grain thereafter. Treatment calves received 0.5g (1.1×10^{10} cfu/g) probiotic in milk once daily until weaning and 0.75 g (1.65×10^{10} cfu/g) probiotic in grain thereafter. Calves were weaned at 60 d of age and blood was collected at 45, 63, and 180 d. Serum concentrations of TNFα and LBP at 63 d were determined; treatment effects were evaluated by ANOVA. Serum concentration of IgG and IgM were determined at 45, 63, and 180 d from a subset of calves (n = 10/treatment); data were analyzed as a mixed linear model to determine the effect of treatment, day (as a repeated factor), and the interaction of treatment with day (trt × day). Probiotics decreased TNFα ($P < 0.001$) but increased LBP ($P < 0.02$). For IgG, there was no treatment effect ($P = 0.80$), yet there was an effect of day ($P < 0.001$) and trt × day ($P = 0.013$). IgG was greater ($P < 0.001$) at 63 d and greater for probiotics versus control at 180 d ($P = 0.005$). IgM increased linearly with day ($P < 0.001$), but there was no effect ($P \geq 0.16$) due to either treatment or trt × day. These data indicate that probiotics may be beneficial in decreasing inflammation as indicated by significant reduction in the proinflammatory marker TNFα and may improve immunity.

Key Words: probiotics, calves, TNFα

2559 Supplementation of naturally-sourced caffeine, from green tea extract, on early-life weight gain of Holstein heifer calves.

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The objective of this study was to determine the effect of green tea extract (a natural caffeine source), and its dosage, on newborn dairy calf weight gain in the first 4 wk of life. Calves were supplemented once at birth and followed for 4 wk. Holstein heifer calves were randomly assigned to 1 of 3 treatment groups: (1) single (SNGL; n = 42) 15-mL dose of green tea extract (327.6 mg caffeine; Calf Perk, TechMix, LLC), (2) 30-mL double dose (DBL; n = 39; 655.2 mg caffeine), and (3) control (CON; n = 39; 15-mL placebo). At birth, calves were scored for dystocia (yes/no), weighed, and given treatment orally before their meal of colostrum (within 2 h of life, 4 L of colostrum replacer containing 300 g of IgG). Body weight was recorded weekly for 4 wk using a scale. Serum total protein (STP) was measured at 24 h using a refractometer. Data were analyzed using mixed-effect, repeated measures, linear regression models, including the covariates of birth BW (40.8 ± 0.66 kg), STP (5.79 ± 0.77 g/dL), and dystocia (n = 18%). Average birth BW did not vary by treatment group ($P = 0.40$). Dystocia affected ADG ($P = 0.01$); however, a dystocia by treatment interaction was not detected. A treatment by week interaction was detected ($P = 0.05$) for ADG. No differences in ADG were detected in wk 1 (0.54 ± 0.05 kg/d) and 3 (0.49 ± 0.05 kg/d); however, in wk 2 SNGL calves (0.46 ± 0.05 kg/d) tended ($P = 0.06$) to gain more than CON calves (0.33 ± 0.05 kg/d). The SNGL calves (0.98 ± 0.05 kg/d) also tended ($P = 0.09$) to gain more than CON calves (0.87 ± 0.05 kg/d) in wk 4. The DBL calves did not differ in ADG from CON calves in wk 2 (0.37 ± 0.05 kg/d) or wk 4 (0.81 ± 0.05 kg/d). A treatment by week interaction for BW was also detected ($P = 0.005$). No differences in BW were detected between treatments in wk 1 (44.8 ± 0.27 kg), 2 (47.7 ± 0.27 kg), or 3 (51.3 ± 0.28 kg); however, in wk 4 SNGL calves (58.9 ± 0.47 kg) were heavier ($P = 0.03$) than CON calves (57.3 ± 0.5 kg), and DBL calves (57.3 ± 0.48 kg) did not differ ($P = 0.94$) from CON calves. The results indicate that supplementing newborn dairy calves with naturally-derived caffeine may positively affect calf weight gain during the first 4 wk of life.

Key Words: dystocia, caffeine, weight gain

Ruminant Nutrition 4: General

2560 Survey of bacterial populations in various feed types from farms across the United States. F. R. Mazza*, J. S. Thompson, B. A. Saylor, and A. H. Smith, *Arm & Hammer Animal and Food Production, Waukesha, WI.*

Clostridium, coliforms, *Escherichia coli* and *Salmonella* are frequently found on farms. Ingesting these bacteria in high quantities can disrupt the natural balance of a cow's gastrointestinal microbiome and lead to metabolic disorders such as bloat, diarrhea, and septicemia. *C. perfringens* is also suspected to play a key role in hemorrhagic bowel syndrome. If feed is not stored or ensiled properly, these bacteria may also decrease feed quality by degrading available carbohydrates and proteins. The objective of this survey was to determine bacterial levels and prevalence in various feed types from dairy and beef farms across the United States. From February 2016 to January 2024, samples from 3 feed categories, TMR (n = 5,899), fermented feeds (FF; n = 4,216), and other feedstuffs (cottonseed [CS], distillers grain, flake corn, dry hay, protein mix, ground corn, soybean meal, water and whey; n = 1,315) were collected from 998 farms across 36 states. *E. coli* and other coliforms were enumerated on CHROMagar, clostridia were enumerated on tryptose sulfite cycloserine agar, and samples were enriched for *Salmonella*. Statistical relationships were determined using a one-way ANOVA. Clostridia, *C. perfringens*, and *E. coli* were detected at higher levels in TMR compared with other feed types ($P < 0.05$). *Salmonella* was detected more often in TMR (9.0%) than FF (alfalfa haylage [1.5%], corn silage [1.1%], small grain silage [1.2%]; $P < 0.05$), but no other differences were detected in *Salmonella* prevalence between other feed types. Coliforms were also detected at higher levels in CS and dry hay compared with other feed types ($P < 0.05$). These data highlight that TMR has higher bacterial loads or prevalence compared with other feed types. Once TMR or other feedstuffs are contaminated, bacteria may begin to proliferate, especially in a hot and humid environment. This illustrates the importance of minimizing the contamination of clean feed, especially at the time of gathering feed ingredients and mixing the TMR. These data can be used in future research to identify novel methods to prevent the growth of undesirable bacteria in TMR and other feed types.

Key Words: TMR, feed hygiene, pathogens

2561 Potential use of hydrated sodium-calcium-aluminosilicate for reduction of multiple mycotoxins in rumen fluid of dairy cows. R. D. Pires[1], A. M. Borowsky[1], R. C. Pires[1], R. E. Rosim[2], F. G. Tonin[2], C. A. Oliveira[2], and C. H. Corassin*[2,1], [1]*University of São Paulo, Luiz de Queiroz College of Agriculture, Piracicaba, São Paulo, Brazil,* [2]*University of São Paulo, School of Animal Science and Food Engineering, Pirassununga, São Paulo, Brazil.*

Aflatoxin, fumonisin, deoxynivalenol, and zearalenone are the main mycotoxins that can contaminate feed. The problems associated with the presence of these toxins in ruminant diets can range from reduced feed intake to health problems, reproductive inefficiency, and reduced profitability. The use of mycotoxin adsorbents in feed has been suggested as an option by several studies. There are many adsorbent products currently on the market, but not all of them show results that prove their protective efficacy, so they need to be carefully evaluated. This experiment has been conducted to investigate the ability of hydrated sodium-calcium-aluminosilicate (HSCAS) to adsorb multiple mycotoxins in the rumen fluid of dairy cattle. After 7 d of adaptation to a TMR either without (CON, n = 3) or with (HSCAS, n = 3) 30 g HSCAS added to the basal diet, rumen fluid samples were collected 2 h after feeding artificially-contaminated TMR with aflatoxin (0.8 μg/kg of BW), fumonisin (15 μg/kg of BW), deoxynivalenol (8 μg/kg of BW), and zearalenone (3 μg/kg of BW) using an esophageal probe. Mycotoxins in rumen fluid were determined using a high-performance liquid chromatography system coupled to a mass spectrometer. Statistical analyses were performed with SAS general linear models with Tukey's test for significance at $P < 0.05$. In the evaluation of various mycotoxins in rumen fluid, only the quantification of aflatoxin showed a statistically significant difference between cows treated with HSCAS and CON cows. The HSCAS group had aflatoxin levels lower than the method's quantification limit. For the other mycotoxins, the CON and HSCAS treatments did not differ statistically. The results indicate that HSCAS was only able to adsorb some of the aflatoxins consumed by the animals and was not able to adsorb fumonisins, deoxynivalenol, and zearalenone in the rumen fluid of cows.

Key Words: mycotoxins, rumen, adsorbent

2562 A sodium and calcium aluminosilicate-containing bentonite adsorbent (AB20) decreased mycotoxin absorption in dairy cattle. A. O. Oyebade*, M. Garcia, S. Bascom, K. Saddoris-Clemons, J. D. Chapman, and B. D. Humphrey, *Phibro Animal Health Corporation, Teaneck, NJ.*

Deoxynivalenol (DON) and zearalenone (ZEA) are among mycotoxins most frequently found in grains and silages for dairy cattle. This study used urine mycotoxin concentrations to evaluate the adsorptive efficacy of AB20 nutritional specialty product (Phibro Animal Health), a bentonite adsorbent containing hydrated sodium and calcium aluminosilicate, in dairy heifers fed mycotoxin-contaminated corn. Holstein heifers (n = 9) were used in a before-and-after study. Cows were fed a high mycotoxin diet (4.32 ppm DON and 575 ppb ZEA) for 21 d, followed by a 5-d experimental period where 0.5% AB20 was added to the concentrate portion of the diet. Concentrate was completely consumed before offering fescue hay. Urine samples collected on d 0 (prefeeding) and d 5 were analyzed for ZEA, ZEA derivatives (α-ZOL and β-ZOL), DON, and a DON derivative (DOM-1). Count of heifers positive or negative for mycotoxins were compared between d 0 and d 5 using Fisher's exact test, and urine mycotoxin levels were compared using the Mann–Whitney test on R v.4.0. Prefeeding, α-ZOL and β-ZOL were undetectable in urine, but 8 out of 9 heifers were ZEA positive. Post-AB20 feeding, the number of heifers with detectable levels of ZEA decreased to 1 out of 9 ($P = 0.003$; odds ratio [OR] = 0.03; 95% CI = 0.0003 to 0.41). The counts positive for β-ZOL numerically increased from 0 to 2 cows ($P = 0.4706$; OR = ∞; 95% CI = 0.1915 to ∞). Concentration of urinary ZEA was reduced ($P = 0.003$) from 9.1 to 1 ng/mg; creatinine and β-ZOL numerically increased ($P = 0.17$) from undetectable to 13.2 ng/mg creatinine, pre-and post-AB20 feeding, respectively. All heifers were positive for both DON and DOM-1 pre- and post-AB20 feeding (DON, $P = 1.0$, OR = ∞, CI = 0.03 to ∞; and DOM-1, $P = 1.0$, OR = 0, CI = 0 to ∞). Prefeeding urinary DON and DOM-1 concentrations increased ($P = 0.008$ and $P = 0.005$) from 72.5 and 461 ng/mg creatinine to 242.4 and 1,982 ng/mg creatinine post-AB20 feeding. These results suggest that feeding AB20 could reduce absorption of ZEA as indicated by decreased urinary concentrations of ZEA posttreatment relative to pretreatment, but had no effect on DON absorption.

Key Words: bentonite, urine mycotoxins, dairy cows

2563 Effects of supplementing a *Bacillus*-based probiotic on milking performance of lactating dairy cows. A. Bach*[1,2], M. Escartín[3], N. Rialp[3], and B. Cappellozza[4], [1]*Marlex Recerca i Educació, Barcelona, Spain,* [2]*Institució de Recerca i Estudis Avançats (ICREA), Barcelona, Spain,* [3]*Blanca from the Pyrenees, Hostalets de Tost, Spain,* [4]*Chr. Hansen A/S, Hørsholm, Denmark.*

Strains of *Bacillus subtilis* and *B. licheniformis* have been shown to exert beneficial effects in young ruminants and beef cattle. However, scarce information is available on dairy cattle. This study aimed to assess potential effects of supplementing a *Bacillus*-based probiotic on milking performance. Sixty Holstein cows (43% primiparous, 61 ± 22.5 DIM, producing 39.3 ± 9.4 kg/d and weighing 538 ± 68 kg) were allocated to 2 treatments, following a randomized design, for a duration of 90 d. Cows were fed a TMR (16.0% CP, 1.67 Mcal of NE_L/kg; 34.0% NDF; DM basis). Half of the cows (n = 30) received no supplementation (CON), and the other half were fed 3 g/d of a *Bacillus*-based probiotic (DFM; 9.6×10^9 cfu /head per day of Bovacillus, Chr. Hansen, DK). Cows were housed in a single pen equipped with electronic feed bins to monitor daily individual feed intake. Milk yield and its protein and fat contents were determined individually at each milking. Body weight was monitored daily using an electronic scale. Data were analyzed using a mixed-effects model with treatment, week, and their interaction as fixed effects, and cow as a random effect. Dry matter intake decreased ($P < 0.05$) after about 50 d of study in DFM versus CON cows and, overall DMI was lower ($P < 0.05$) in DFM cows. Body weight did not differ between treatments, but CON cows lost more BW than DFM after 7 wk of study. Milk yield tended ($P = 0.10$) to increase in DFM vs. CON cows after 7 d of the study, but overall milk yield was not affected by treatments. Milk fat content decreased ($P < 0.05$) in DFM compared with CON cows soon after 7 d of study, but milk fat yield was unaffected. Feed efficiency (kg of milk/kg of DMI) was greater ($P < 0.05$) in DFM (1.73 ± 0.03) than in CON (1.52 ± 0.03) cows, with this difference becoming apparent after 4 wk of study. Efficiency of protein utilization (EPU) was greater in DFM cows after 56 d of study, and overall EPU tended ($P = 0.06$) to be greater for DFM (34.9% ± 1.03%) vs. CON cows (32.1% ± 1.03%). Supplementing a TMR with Bovacillus reduces DMI, but maintains BW of cows and increases or tends to increase milk yield, feed efficiency, and EPU over time.

Key Words: bacteria, efficiency, rumen

2564 Effects of increasing dietary levels of condensed tannin on milk composition and feed efficiency in dairy cows. B. de Magalhães Ceron[1], N. T. Scognamiglio Grigoletto[1], N. Pereira Martins[1], G. Poletti[1], D. J. Cavalli Vieira[1], A. C. Silvério Victor[1], J. Reis da Silva[1], N. Terassi da Silva[1], D. Dias Brutti[2], V. M. Silva[2], G. S. Maysonnave[3], C. S. Takiya[1,4], and F. P. Rennó*[1], [1]*University of São Paulo, Pirassununga, SP, Brazil,* [2]*SETA S.A., Estancia Velha, RS, Brazil,* [3]*Federal University of Rio de Janeiro, Rio de Janeiro, Brazil,* [4]*Federal University of Technology–Paraná, Pato Branco, PR, Brazil.*

Condensed tannins (CT) are polymerized compounds that can be extracted from *Accacia mearnsii*. This study aimed to evaluate the effect of a CT supplement on performance in dairy cows. Twenty-four Holstein cows (33.8 ± 1.16 milk yield [MY], 171 ± 26 DIM, 8 primiparous and 16 multiparous) were blocked according to MY and parity, and enrolled in a 4 × 4 Latin square design experiment consisting of 21-d periods with sampling conducted during the last 7 d. Cows within blocks were randomly assigned to treatment sequences, with: (1) control (CON), no CT; (2) condensed tannin 1 (CT1), CT at 0.06% of DM; (3) CT2, CT at 0.12% of DM; and (4) CT3, CT at 0.18% of DM. Treatments targeted CT doses of 0, 15, 30, and 45 g/d, for CON, CT1, CT2, and CT3, respectively. The CT supplement (NutreSet PNT; Seta S.A.) was offered mixed into the concentrate. Cows were fed a TMR twice daily, targeting refusals between 5% and 10%. Samples of refusals and feed ingredients were collected during the sampling period for chemical analyses. Cows were milked twice daily. Milk samples were collected from 6 consecutive milkings during the sampling period for fat, protein, and lactose analyses using a mid-infrared method (Lactoscan, Entelbra). Data were analyzed using the Mixed procedure of SAS, modeling the fixed effects of treatment, period, block, and their interaction, as well as the random effect of cow within block. Dry matter intake showed a negative quadratic response ($P = 0.02$) to increasing doses of CT (27.0, 26.7, 26.1, and 27 kg/d for CON, CT1, CT2, and CT3, respectively). Milk yield had a linear increase ($P = 0.02$) with CT supplementation (32.6, 32.8, 32.8, and 33.6 kg/d, for CON, CT1, CT2, and CT3, respectively). The yields of 3.5% FCM, fat, protein, and lactose exhibited a linear increase ($P \leq 0.02$) with CT. Milk fat concentration had a linear increase ($P = 0.02$) with CT supplementation (3.77%, 3.78%, 3.98%, and 3.89%, for CON, CT1, CT2, and CT3, respectively). Feed efficiency, calculated either as MY/DMI or 3.5% FCM/DMI, showed a linear increase ($P \leq 0.02$) with diet CT. Increasing doses of CT improved productive performance of dairy cows.

Key Words: *Accacia mearnsii*, milk yield, productive efficiency

2565 Effects of a botanical preparation on lactation performance of dairy cows. K. Griswold*[1], E. H. Wall[2], and A. N. Hristov[3], [1]*Selko USA, Indianapolis, IN,* [2]*Nutreco Exploration, Nutreco, Amersfoort, The Netherlands,* [3]*Department of Animal Science, The Pennsylvania State University, State College, PA.*

Research has demonstrated host-mediated effects of botanicals in ruminants, but analyses across trials testing a common formula is lacking. We analyzed 4 university studies (3 randomized complete block, one 4 × 4 Latin Square) to assess the effect of a blend of capsaicin and clove essential oil (CapClo) on lactation performance of dairy cows. Holstein cows (parity 1+ in 2 studies; and 2+ in 2 studies) were supplemented with CapClo and performance responses were measured; one study tested 3 feeding levels (150, 300, and 600 mg/cow per day), whereas the other studies tested 1 level (300 mg/cow per day). In 2 studies, cows were in the first 100 DIM and supplemented for 4 or 10 wk, and the other studies supplemented cows from −21 to +42 DIM. For each outcome, data were analyzed using a MIXED model procedure, with treatment as a fixed effect and trial as a random effect. For all variables there was an effect of trial ($P < 0.001$). Dry matter intake and milk yield were not affected by CapClo (Table 1). Milk fat percent and yield were increased, whereas milk protein was not affected. Consistent with the increase in milk fat,

Table 1 (Abstr. 2565).

	Least squares means				
Item	Control	CapClo	Difference	SED	*P*-value
DMI, kg	24.1	24.4	0.30	0.35	0.50
Milk yield, kg/d	42.7	42.9	0.20	0.45	0.70
Milk fat, %	4.24	4.36	0.12	0.04	0.05
Milk fat, kg/d	1.82	1.87	0.05	0.008	0.003
Milk protein, %	3.15	3.14	−0.01	0.02	0.70
Milk protein, kg/d	1.37	1.36	−0.01	0.01	0.80
ECM, kg/d	44.9	45.6	0.70	0.30	0.08
ECM:DMI	1.91	1.93	0.02	0.005	0.09
BW, kg	647	662	15	6.6	0.08
BW change, kg/d	−0.15	0.38	0.53	0.20	0.12

ECM and ECM ÷ DMI tended to increase with CapClo. There was a tendency for BW to increase with CapClo. In conclusion, supplementation with CapClo increased milk fat percent and yield, with a tendency for increased ECM, ECM efficiency, and BW. This, combined with no effect on DMI or milk yield, suggests a shift in energy partitioning or improved nutrient availability. Therefore, across multiple settings and stages of lactation, CapClo appears to improve milk composition and efficiency in dairy cows.

Key Words: capsicum, clove, milk fat

2566 Effect of monensin and essential oil blend supplementation on the lactation performance, rumen fermentation, apparent total-tract digestibility, and feeding behavior of dairy cows. E. C. Diepersloot*[1], M. R. Pupo[1], C. Heinzen Jr.[1], M. S. Souza[1,2], and L. F. Ferraretto[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Animal Sciences, Federal University of Amazônia, Belém, PA, Brazil.*

The objective of this experiment was to determine the effect of dietary supplementation with monensin and essential oil blend on lactation performance, rumen fermentation, apparent total-tract digestibility, and feeding behavior of dairy cows. Sixty-four multiparous Holstein cows (89 ± 21 DIM) were randomly assigned to 1 of 4 treatments with a 2 × 2 factorial arrangement for a 10-wk treatment period, after a 2-wk acclimation period and 2-wk covariate period. Treatments consisted of both (MON-EOB) monensin (16 g/ton) and essential oil blend (5g/hd per day; carvacrol, eugenol, thymol, and capsaicin), only monensin (MON-CON; 16 g/ton), only essential oil blend (CON-EOB; 5g/hd per day; carvacrol, eugenol, thymol, and capsaicin), or neither (CON-CON). Data were analyzed as a completely randomized design with data from the covariate period included as a covariate using a mixed model with week of treatment as repeated measures. The model included MON, EOB, week, and their 2- and 3-way interactions as fixed effects, and cow (treatment) was a random effect. Monensin tended to increase ($P = 0.06$ and $P = 0.07$, respectively) milk and lactose yields by 1.3 kg/d and 0.07 kg/d, respectively. Additionally, monensin decreased ($P = 0.04$) milk fat concentration by 0.2 percentage units. Essential oil blend did not affect production ($P > 0.05$) but decreased ($P = 0.03$) BW 7 kg and tended to decrease ($P = 0.08$) BW change 0.14 kg/d. Treatments had no effects ($P > 0.10$) on rumen pH or VFA, except for EOB increasing ($P = 0.05$) maximum rumen pH by 0.12 units. Interactions between monensin and the essential oil blend ($P = 0.001$ and $P = 0.001$, respectively) were observed for DM and OM digestibility. For DM and OM digestibility, CON-CON was greatest, followed by CON-EOB, then MON-EOB, and MON-CON had the lowest digestibility. These results suggest that monensin may improve milk production at the expense of milk fat concentration, although milk fat yield was unaffected. Additionally, the feed additives tested in this experiment appear to have slightly decreased digestibility.

Key Words: monensin, essential oil, milk production

2567 Comparative analysis of NRC (2001) and NASEM (2021) models for predicting feed intake, milk production, and enteric methane emission in lactating dairy cows. A. T. Richards*, L. F. Martins, and A. N. Hristov, *The Pennsylvania State University, State College, PA.*

The objectives were to evaluate accuracy and precision of NRC (2001) and NASEM (2021) models to predict DMI, milk yield (MY), milk true protein (TP) and fat yields, and enteric CH_4 emission (NASEM only) in dairy cows. Data from 17 randomized complete block design experiments with lactating Holstein cows (n = 682) conducted between 2016 and 2022, in some of which enteric CH_4 emission (n = 486) was measured using the GreenFeed system, were used for the analysis. Models were evaluated for accuracy and precision based on root mean squared error (RMSE) and Lin's concordance correlation coefficient (CCC) calculated from predicted and observed values using RStudio. Compared with measured data (mean ± SD; 25.8 ± 4.2 kg/d), DMI was closely predicted by NRC (mean, RMSE, and CCC; 26.0 kg/d; 3.18 kg/d; 0.65) and underestimated by NASEM's animal-based (24.9 kg/d; 3.03 kg/d; 0.65) and feed-based models (25.1 kg/d, 3.50 kg/d, and 0.46). Milk yield (40.0 ± 8.7 kg/d) was overestimated by NRC NE_L allowable MY (41.8 kg/d, 7.38 kg/d; 0.64) and NASEM NE_L allowable MY (44.1 kg/d; 8.66 kg/d; 0.62). However, both NRC MP allowable MY (39.5 kg/d; 7.06 kg/d; 0.70) and NASEM MP allowable MY (40.7 kg/d; 6.57 kg/d; 0.69, respectively) closely predicted MY. Milk TP (1,218 ± 226 g/d) and fat (1,533 ± 376 g/d) yields were underestimated by NASEM prediction models (RMSE = 182 and 417 g/d; CCC = 0.58 and 0.27, respectively). Compared with measured data (412 ± 87 g/d), enteric CH_4 emission was not accurately predicted by NASEM (476 g/d; 123.7 g/d; 0.23, respectively). Overall, predictions for DMI were similar for both NRC and NASEM animal-based models. Both NRC and NASEM MP allowable MY models predicted MY well, with differences of 18% and 16%, respectively, when compared with the actual MY. NASEM did not accurately predict milk fat yield and daily CH_4 emission from lactating dairy cows in the current data set.

Key Words: nutritional model, production, methane

Ruminant Nutrition 4: Gut Physiology, Fermentation, and Digestion

2252 In vitro compatibility of different cattle direct-fed microbials with monensin sodium in a rumen-containing medium. R. Gresse[1,2], E. Forano[2], O. C. M. Queiroz*[1], B. I. Cappelloza[1], and G. Copani[1], *[1]Novonesis, Animal and Plant Health & Nutrition, Hørsholm, Denmark, [2]UCA INRAE UMR 454 MEDIS, Saint-Genès-Champanelle, France.*

Antibiotics are still extensively administered to livestock animals for a wide range of applications, such as for coccidiosis prevention, adverse health event treatments, increased feedlot growth rate, and feed efficiency. Orally ingested, antibiotics are known to disturb rumen microbiota, and they can negatively affect the survival and growth of direct-fed microbials (DFM). Direct-fed microbials contain beneficial live bacteria supporting the health of the host, also leading to increased growth rates, feed efficiency, lower occurrence of adverse events, establishment of the commensal GIT microbiota as well as development of rumen functions. In this study we investigated the compatibility of *Bacillus licheniformis* 809 and *Bacillus subtilis* 810 (BOVACILLUS®); *Lactobacillus animalis* 506 and *Propionibacterium freudenreichii* 507 (Bovamine® Dairy); *B. licheniformis* 809, *B. subtilis* 810, *L. animalis* 506, and *P. freudenreichii* 507 (BOVAMINE® Dairy Plus); and *Enterococcus faecium* 669 (LACTIFERM®; Novonesis, Denmark) with monensin sodium, a widely used antibiotic in ruminants, in a complex medium rumen containing 40% of rumen content. The DFM incubated with monensin sodium at 0.535 µg/mL of rumen content was compared with a control with DFM only. Samples were performed in independent triplicates, and growth curves were evaluated over a 24-h period. All data were analyzed with SAS software (version 9.4). Regardless of the DFM, monensin incubation did not affect mean growth rates ($P \geq 0.16$). Treatment × hour interactions were observed on growth rates of BOVACILLUS® and BOVAMINE® Dairy Plus ($P < 0.001$), as lag phase was extended by 1 h in the monensin conditions ($P < 0.001$). However, growth curves resumed as expected at the start of the exponential phase ($P > 0.01$). Overall, this study indicates that the tested bacterial-based DFM BOVACILLUS®, BOVAMINE® Dairy, BOVAMINE® Dairy Plus, and LACTIFERM® are compatible with monensin sodium when added to a rumen-fluid-based media.

Key Words: direct-fed microbials, antibiotics, ruminants

2568 The effect of mineral-vitamin free choice blocks on transition cows' rumen health. G. Esposito*[1,3], M. Simoni[1], T. Danese[1], G. Krabbe[2], G. Mantovani[1], R. Pitino[1], and F. Righi[1], *[1]Department of Veterinary Medicine, University of Parma, Parma, Italy, [2]Crystalyx Products GmbH, Münster, Germany, [3]Department of Animal Science, Faculty of AgriSciences, Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

The transition period is an extremely difficult phase of the lactation cycle. The cows face the challenge of NEB, massive mineral utilization, and perturbed immune function. Furthermore, in high-forage diets, minerals may be insufficient. The study tested the effect of different molasses-based mineral-vitamin free choice blocks (FC-BLOCK) as alternatives to conventional TMR mineral-vitamin additives on rumen health in transition dairy cows. Multiparous Holstein cows (n = 42), blocked by BCS, parity, and previous lactation, were fed a TMR at 75% of the mineral requirements during the dry period, followed, postpartum, by a lactating TMR at 75% of the mineral requirements plus concentrates fed via robotic milking system. The treatments were: control (CTL; n = 16) receiving a top-dressed mineral supplementation to reach 100% of the requirement; mineral-vitamin (MINVIT; n = 14) receiving, in the dry period, an FC-BLOCK followed by an FC-BLOCK containing propylene glycol (FC-BLOCKg) postpartum; linseed (LIN; n = 12) receiving, in the dry period, an FC-BLOCK with linseed oil and yeast, followed by an FC-BLOCKg postpartum. Intake, eating, rumination time, and milk yield were recorded daily; rumen fluid was collected as farm monitoring practice via stomach tube at 20 and 60 DIM to evaluate rumen pH, protozoa count, and enzymes activity. Data were analyzed with GLM procedures. The FC-BLOCK intake was significantly higher in LIN compared with MINVIT ($P < 0.01$). However, none of the treatments affected TMR intake in the dry period, but CTL and LIN had a significantly higher intake compared with MINVIT ($P < 0.05$) in summer. Postpartum TMR intake in spring and summer was the highest in MINVIT ($P < 0.05$). In the first 60 DIM, CTL had the highest milk yield, thus receiving more concentrate ($P < 0.05$) compared with the other groups. No differences in protozoa count and enzymatic activity were observed. In conclusion, the FC-BLOCK may be a valid approach to ensure satisfaction of mineral requirements while keeping ruminal health, showing possible applications under free-range conditions. Furthermore, the MINVIT proved to sustain intake in potential heath stress season.

Key Words: mineral-vitamin blocks, rumen health, transition cow

2569 Effects of time of rumen collection and dietary NDF on rumen fermentation *in vitro*. A. O. Oyebade*, E. Hass, M. Garcia, J. D. Chapman, and B. D. Humphrey, *Phibro Animal Health Corporation, Teaneck, NJ.*

In vitro batch culture is commonly used to test the effects of feed additives on rumen fermentation, but various factors can influence test results. Two experiments (EXP) were conducted to study the effects of the rumen fluid collection time and dietary NDF on in vitro fermentation, using rumen cannulated Jersey heifers. In EXP1, 6 heifers were fed a low NDF diet (28%), and on collection days, feed was withheld from 3 heifers and offered to 3 heifers, and rumen fluid was collected 2 h post feeding. In EXP2, 2 heifers were fed a low NDF diet (28%) and 2 heifers were fed a high NDF diet (35%), and rumen fluid was collected before feeding. Rumen fluid was composited and buffered with artificial saliva. In vitro treatments were control (CON, no additive), monensin (MON), and phytochemical-*Bacillus* blend (PBB), with 4 replicates and 2 experimental runs used in EXP1, and 3 replicates and 3 experimental runs in EXP2, all incubated for 24 h at 39°C, with corresponding substrate. After 24 h, pH, DMD, NH_3-N, and VFA were measured. Data were analyzed using treatment, collection relative to feeding, and their interaction as fixed effects in EXP1, and treatment, diet, and their interaction as fixed effects for EXP2. In EXP1, samples collected post-feeding were lower in pH and isobutyrate (%), and higher in total VFA, acetate (m*M*, %), propionate (m*M*), butyrate (m*M*), and acetate:propionate (A:P) ratio, versus pre-feeding samples. In EXP2, high NDF increased pH, percentage of acetate, and A:P ratio, but reduced DMD, m*M* of propionate, butyrate, and isovalerate, versus low NDF. In EXP1 and EXP2, MON increased m*M* of propionate and decreased m*M* of butyrate and isobutyrate, percentage of isobutyrate, and A:P ratio versus CON and PBB. MON also increased acetate (m*M*, %) in EXP1 and reduced pH in EXP2. For EXP1, MON increased m*M* of isovalerate and valerate in pre-fed but not post-fed samples, whereas PBB increased the percentage of butyrate in pre-fed but not post-fed samples. In EXP2, the reduced propionate (%) and valerate (m*M*, %)

due to MON were limited to the low NDF dietary only. In summary, rumen fluid collection time and diet NDF produced markedly distinct fermentation parameter responses to the rumen modifiers.

Key Words: batch culture, feeding time, NDF

2570 Effect of *Komagataeibacter*-based microbial symbiotic complex on methane mitigation and dairy rumen microbiome using multi-omics analysis. M. G Kang[1], J. Park[1], M. Kwak[1], J. N. Kim*[2], and Y. Kim[1], [1]*Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul, Korea,* [2]*Department of Food Science and Nutrition, Dongseo University, Busan, Korea.*

Mitigating significant emissions of greenhouse gases, including methane, is a key component of addressing the growing climate change challenge. Recently, direct-fed microbials (DFM), constituted by either a single or mixed culture of viable microorganisms, have aimed at regulating metabolic fermentation, such as propionogenesis and acetogenesis, and reshaping the dynamics of microbial composition in the rumen, resulting in reduction of methane emission. In our study, we indicated that specific probiotic bacteria and their metabolites, including organic acids in traditional fermented foods, could influence on enteric fermentation in the dairy rumen fluids using high-performance liquid chromatography (HPLC) analysis. Interestingly, among them, based on gas chromatography (GC) results, we isolated the methane-reducing bacterium, *Komagataeibacter* spp. SLAM NK1B from various fermented foods. Interestingly, *Komagataeibacter* spp. SLAM NK1B strongly produced volatile fatty acids (VFA) including acetic acid, as well as bacterial cellulose; hence, we developed *Komagataeibacter*-based synbiotics (KBS; containing bacteria and fermented products) and assessed their potential for methane mitigation using in vitro dairy rumen fermentation. Our additional HPLC for VFA analysis and GC methods for methane and hydrogen concentration revealed that KBS treatment (concentration of 2% in rumen fluids) significantly reduced methane emissions by 19.28 ± 0.01% and increased VFA concentrations by 7.42 ± 1.07%, indicating its efficacy in reducing methane and enhancing beneficial VFAs during enteric fermentation ($P < 0.05$; group comparisons were conducted using an unpaired t-test with Welch's correction). Furthermore, next generation sequencing (NGS)-based rumen metagenome analysis revealed that KBS selectively induces a decrease in ruminal fluid methanogens and an increase in propionate-producing bacteria. Taken together, this study demonstrates that KBS shows promise as a potential methane reducer as well as probiotic DFM, offering a novel insight into microbial strategies for methane mitigation in dairy cows.

Key Words: methane mitigation, *Komagataeibacter*, microbial symbiotic complex

2571 Enhanced methane reduction by combination with *Escherichia coli* Nissle 1917 and biochar in dairy rumen fermentation and homeostasis. J. Park[1], M. G. Kang[1], M. Kwak[1], J. N. Kim[2], and Y. Kim*[1], [1]*Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Science, Seoul National University, Seoul, Korea,* [2]*Department of Food Science and Nutrition, Dongseo University, Busan, Korea.*

In this study, using in vitro dairy rumen fermentation, we observed on the methane reduction by supplementing *Escherichia coli* Nissle 1917 (EcN) combined with biochar that activated carbon produced with pyrolysis of biomass. Our high-performance liquid chromatography (HPLC)- and gas chromatography (GC)-based analysis revealed that in anaerobic cultures of EcN treated with biochar, a significant increase in acetate and hydrogen was observed compared with the control group. Additionally, RNA-seq analysis revealed an upregulation in the expression of acetate synthesis genes, namely *pta*, *poxB*, and *ackA*. Subsequently, treatment of rumen fluid with EcN and biochar significantly decreased methane (7.4 mL/g to 6.1 mL/g) and increased acetate (from 698.9 to 763.3 mg/L) and propionate (340.5 to 403.5 mg/L) production, as observed through the analysis of short-chain fatty acids, and these effects could accelerate with additional supplementation of biochar ($P < 0.05$; statistical analysis was conducted using the one-way ANOVA tool available in GraphPad Prism 10). The observation of changes in the microbial composition of rumen fluid following treatment with EcN and biochar was made through rumen metagenomic analysis and RT-qPCR. The results revealed an increase in acetogen and propionate producing bacteria abundance and a decrease in methanogen abundance. Based on these findings, the CRISPR/Cas9 system was employed to elucidate the molecular mechanism of the methane-reducing capability of EcN. Gene deletion was performed targeting the genes *poxB*, *ackA*, and *pta* as key factors in acetate pathway in *E. coli*. The knockout of *poxB*, *ackA*,and *pta* could lead to the elimination of the methane-reduction of EcN, as well as acetate-producing abilities ($P < 0.05$). Taken together, the methane reduction ability of EcN in rumen fluid is associated with its acetate production capability, and the addition of biochar significantly enhances methane mitigation capability of EcN.

Key Words: methane reduction, probiotics, biochar

2572 Volatile fatty acid prediction from ruminally sensed temperature, pH, dissolved oxygen, and oxidation reduction potential. R. K. Wright* and R. R. White, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Existing methods for measuring ruminal volatile fatty acid (VFA) concentrations are expensive and sensitive. This study evaluated the viability of a sensor suite in predicting VFA concentrations in situ. The sensor included a DF Robot Bluno microcontroller, SD card reader, and battery in a waterproof junction box with wired, waterproof probes for monitoring aqueous temperature, oxidation reduction potential (ORP), dissolved oxygen (DO), and pH. Probes were extended into the rumen through the cannula plug. A crossover experimental design using 4 ruminally cannulated cows and 2 diets was used to collect data over two 3-d periods. Cows were fed at 0600 h and 1800 h daily. Diets were a corn silage and grass hay base and formulated to vary in forage percent (75% or 100%). Hourly from 0600 h to 1800 h, rumen fluid samples were collected and stored at −20 C until later analysis for VFA concentrations via gas chromatography. The sensor suite was in place for the duration of each period. Sensor measurements outside the physiological range were omitted, and remaining data were aligned with VFA concentrations based on collection time. Features used in analysis included raw sensor data along with rolling averages, rolling standard deviations, and leading and lagging values. Data were split into 3 parts with 15% for hyperparameter tuning, 55% for model training, and 30% for independent evaluation. Random forest regressions were fit using the measured and engineered features to predict each VFA concentration. Lin's concordance correlation coefficient (CCC) was calculated from model predictions and evaluation data. The CCC of all individual or of total VFA were greater than 0.99. However, we also explored splitting data for derivation and evaluation by animal (leaving out one animal at a time) or by diet (fitting against only one diet and comparing to the other). These alternative derivation strategies yielded very poor CCC (<0.10).

Although this system successfully predicted VFA concentrations, these differences in fit by derivation approach highlight the importance of evaluation manner aligned with anticipated tool use.

Key Words: sensing, VFA, feature engineering

2573 Effects of corn grain on methane production and ruminal fermentation in vitro.

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Decreasing the forage-to-concentrate ratio (F:C) has been shown to decrease CH_4 emissions in dairy cows in vivo. With the in vitro gas production method (IVGP) being widely used to study rumen methanogenesis and CH_4-mitigating strategies, it is important to validate that responses to treatments in vitro represent responses in vivo. The objective of the study was to investigate the effects of changes in F:C on rumen fermentation and methanogenesis in vitro. Two ruminally cannulated lactating Holstein cows fed a standard 52% forage and 48% concentrate diet were used as inoculum donors. Repeated incubations were carried out for 48 h at 39°C using an automated IVGP system. Fermentation substrates (1% wt/vol) were dried and ground corn grain (CG), corn silage (CS), alfalfa haylage (AH), and grass silage (GS). Corn grain was included replacing 0%, 25%, 50%, 75%, and 100% of CS, AH, or GS. Total gas (TG) production was continuously monitored. Headspace samples were collected at 12, 24 and 48 h and analyzed for CH_4 concentration. Incubation medium samples collected at 48 h were analyzed for volatile fatty acids (VFA). Data were analyzed using the MIXED procedure of SAS with the fixed effects of forages, F:C, and the interaction. Polynomial orthogonal contrasts were used to evaluate linear and quadratic effects of F:C. Compared with AH and GS, CS increased ($P < 0.001$) CH_4 production and concentration in TG. Increasing CG inclusion linearly decreased ($P < 0.001$) medium pH and increased ($P < 0.001$) TG and CH_4 production and CH_4 concentration in TG, regardless of the forage source. For all forages, total VFA concentration increased linearly ($P < 0.001$), and acetate-to-propionate ratio decreased ($P < 0.001$, quadratic effect) with increasing CG. In summary, decreasing F:C decreased acetate-to-propionate ratio and increased total VFA concentration as well as CH_4 production and concentration in TG in vitro, in contrast to observed decreases in CH_4 emissions in ruminants fed high-grain diets in vivo. Further research is needed to elucidate the reason for the discrepancy in the effect of starch on methanogenesis between in vitro and in vivo systems.

Key Words: concentrate inclusion, enteric methane, in vitro

2574 *In vitro* effects of starch with or without buffers on dairy cattle fecal microbiome.

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Lactating cows are often fed diets high in fermentable carbohydrates, resulting in the risk of ruminal and hindgut acidosis. We sought to determine whether buffers could alleviate the effects of hindgut acidosis on the fecal microbiome. Anaerobic fecal samples (n = 4) were cultured (1% feces, 99% media) with 6 dietary conditions in triplicate (FECC = no additive; STAR = 1% food grade corn starch product; SBA = 1% starch and 0.05% buffer prototype 1 [50% $CaCO_3$, 25% MgO, and 25% crushed oyster shell]; SBB = 1% starch and 0.05% buffer prototype 2 (100% MgO); BA = 0.05% buffer prototype 1; BB = 0.05% buffer prototype 2). Samples were collected at 0, 3, 6, 12, 18, and 24 h to determine fermentation products, and 0, 6, and 24 h for microbial populations. Fermentation products were analyzed in JMP Pro using the Fit Model procedure for standard least squares, with the main factors being treatment, time, and their interaction. We extracted DNA from pooled pellets, and the V4–V5 region of the 16S rRNA gene was amplified and sequenced using Illumina MiSeq. Microbiome analysis occurred using QIIME2 and RStudio. Buffer inclusion increased pH, acetate, and propionate, whereas starch inclusion decreased pH and increased lactate, butyrate, and endotoxin. Microbial α-diversity was affected by treatment based on observed species ($P = 0.01$), but treatment did not affect the Shannon index ($P > 0.05$). We found that BA increased observed species compared with STAR ($P < 0.01$), whereas BB increased observed species as compared with FECC ($P = 0.02$). β-Diversity Unweighted UniFrac was affected by treatment (PERMANOVA, $P = 0.02$; betadisper, $P < 0.01$), with BA and BB being different than STAR, SBA, and SBB at 24 h ($P < 0.05$). At the 24 h time point, analysis of compositions of microbiomes and Spearman correlations identified *Desulfovibrionales* and *Clostridiales* increases in BB (r = 0.68 and 0.52, respectively), and *Betaproteobacteriales* and *Bacteroidales* decreases in STAR (r = −0.53 and −0.52, respectively). These data suggest that starch addition favored lactate production and reduced microbial diversity, typical conditions of acidosis. Buffers promoted bacterial growth by increasing diversity and preventing lactate buildup or enhancing lactate utilization.

Key Words: hindgut, buffers, microbiome

Ruminant Nutrition 4: Protein and Amino Acids

2576 A novel comprehensive analysis of amino acid profile in rumen microbial protein and its implications in feed efficiency. H. F. Monteiro*[1], R. Profeta[1], B. C. Weimer[1], M. Van Heule[1], J. E. P. Santos[2], R. S. Bisinotto[3], E. S. Ribeiro[4], F. Penagaricano[5], A. P. Faciola[2], M. I. Marcondes[6], and F. S. Lima[1], *[1]Department of Population Health and Reproduction, University of California, Davis, Davis, CA, [2]Department of Animal Sciences, University of Florida, Gainesville, FL, [3]Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL, [4]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [5]Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI, [6]Department of Veterinary Clinical Sciences, Washington State University,, Pullman, WA.*

Despite efforts in characterizing the AA profile in rumen microbial protein (MP), previous techniques only account for bacteria and protozoa that can be successfully isolated. However, findings from recent metatranscriptomics studies indicate a vaster (up to 62,418 microbial genomes) and broader (64.2% bacteria, 34.9% protozoa and fungi, 0.43% archaea, and 0.50% viruses) rumen microbiome. The aim of this study was to leverage de novo protein characterization to assess the microbial AA profile in the rumen of dairy cows. Considering that MP varies depending on microbial species, it was hypothesized that a comprehensive summary of MP would differ from previously published MP and affect feed efficiency. The rumen content of 24 lactating dairy cows from the extremes of residual feed intake (RFI) but not differing in any other measured traits (efficiency: least = 12 and most = 12, out of 454), had their total RNA extracted, ribo-depleted, and analyzed using an ultra-deep dual-RNA sequencing approach. Host-derived reads were removed by aligning them against the bovine reference genome. De novo gene assembly was performed in *Trinity*, and their abundance was calculated using *Salmon*. A peptide translation prediction was performed in *Transdecoder*, given minimum length criteria to minimize false positives. The AA sequences of 4.6 million rumen MP and isoforms were found. The AA profile in MP from this study was correlated ($r = 0.92$ and $R^2 = 0.84$) with NASEM (2021), where major differences were in non-essential AA, but the limiting AA Lys was lower (7.06% ± 0.39 SD). Interestingly, fungal proteins exhibited an unusually high Met content, up to 25%. The least efficient cows had no RFI correlation with any AA from MP, whereas the most efficient cows' RFI was mostly correlated with AA in MP, especially serine and arginine ($r = -0.78$ and $r = -0.63$, respectively). The de novo protein approach had a strong correlation with previous methods, suggesting its robust evaluation in omasal samples may help tackle the diversity of the rumen microbiome and their AA contribution to the protein requirements of dairy cows.

Key Words: metabolizable protein, nutrient requirements, residual feed intake (RFI)

2577 Effect of rumen-protected methionine supplementation on milk fatty acids profile of Holsteins lactating cows during the summer. C. R. Monteiro[1], V. A. de Oliveira*[1], G. M. de Souza[1], J. P. A. Rezende[1], R. Schmith[1], J. Petrini[3], T. L. Resende[2], and M. A. C. Danes[1], *[1]Department of Animal Science, Federal University of Lavras, Lavras, MG, Brazil, [2]Evonik Industries, Sao Paulo, SP, Brazil, [3]Instituto Clínica do Leite, Piracicaba, SP, Brazil.*

Lipid metabolism, which involves several metabolic pathways, can be altered during heat stress (HS). We aimed to evaluate the effect of rumen-protected Met (RPM) supplementation on milk fatty acids (FA) composition in mid-lactation dairy cows during the summer. A total of 80 primiparous Holstein cows (43.7 ± 4.0 kg/d of milk and 182 ± 63 DIM) were blocked by milk yield and distributed into a diet either without or with 0.75 g/kg of RPM (Mepron®, Evonik Industries) in the TMR for 9 weeks. Metabolizable Met was 100% of required for CON and 125% for RPM (Lys:Met = 2.8:1 in MPR). In wk 3 (W3), 6 (W6), and 9 (W9), milk yield and composition were assessed. The model included treatment, week, and the interaction treatment × week as fixed effects, and block and cow as random effects. Least squares means were compared using Tukey's test. Significance was declared when $P \leq 0.05$ and tendency with $0.05 < P \leq 0.10$. The temperature humidity index was higher in W9 (76) than in W6 (72) and W3 (69). Higher milk production recorded for RPM (44.9 vs. 42.9 kg/d, $P < 0.01$) and RPM did not affect the total fat yield (1316 vs. 1269 g/d, $P = 0.36$) and FA profile for C14:0 (0.28 vs. 0.27%, $P = 0.24$), C16:0 (0.85 vs. 0.80%, $P = 0.30$), C18 (0.22 vs. 0.22%, $P = 0.85$) and C18:1 (0.70 vs. 0.71%, $P = 0.87$). De novo FA yield was not affected (271 vs. 259 g/d, $P = 0.54$), as well as mixed FA (357 vs. 348 g/d, $P = 0.59$) and preformed FA (586 vs. 590 g/d, $P = 0.81$). There was an interaction between treatment and week for total fat composition ($P = 0.02$), with the RPM fat content being higher in W9 than in W3, whereas for the control, it remained constant. A similar interaction between treatment and week was observed in preformed FA ($P = 0.02$) and a tendency in mixed FA ($P = 0.08$). For RPM, preformed and mixed FA was greater in W9 compared with W3 (1.44 vs. 1.36% and 0.87 vs. 0.79%, respectively; $P = 0.02$), whereas for the control, preformed and mixed FA was unaffected (1.40 vs. 1.42% and 0.83 vs. 0.80%, respectively). The RPM supplementation did not alter milk FA composition, although the interactions observed raised possibilities for future studies.

Key Words: fat composition, heat stress, primiparous

2578 Alteration in diet RUP proportion affects N metabolism and alters N excretion. M. H. De Oliveira*[1,2], T. Fernandes[1], A. Hruby-Weston[1], J. Klendworth[1], M. Meador[1], and M. D. Hanigan[1], *[1]Virginia Polytechnic Institute and State University, Blacksburg, VA, [2]State University of São Paulo, Botucatu, SP, Brazil.*

This work aimed to evaluate the influence of different amounts of RUP on N balance, N metabolism, and plasma AA concentrations of Holstein heifers. A total of 6 rumen-cannulated heifers (398 ± 28 kg BW) were randomly allocated in a replicated 3 × 3 Latin square design. Treatments were 3 dietary RUP proportions related to a fixed total dietary protein (15.25% CP), (1) 29% of RUP (29RUP; 9.77% MP; 95.2 g/d Lys; 26.3 g/d Met), (2) 39% of RUP (39RUP; 10.72% of MP; 95.0 g/d Lys; 26.2 g/d Met), and (3) 50% of RUP (50RUP; 11.70% of MP; 91.5 g/d Lys; 24.8 g/d Met), across 6 periods. For the first 10 d of each 14-d period, heifers were fed once per d; for the last 4 d of each period, heifers were fed 12 times/d at 95% to establish a steady state. In the last 4 d, TMR and refusal samples were collected to evaluate N, and spot samples (every 6h) of blood, saliva, feces, and urine were collected to evaluate ammonia, urea, and plasma AA concentrations. Total urine and feces collection occurred on d 14 of each period to quantify excretion. Data were analyzed using the lme4 package of R. We found that DMI was not affected by treatment (10.9; $P = 0.76$). The DM apparent digestibility tended to decrease ($P = 0.08$) from 39RUP to 50RUP (75 vs. 70%). The RUP amounts did not affect ($P > 0.05$) N intake, excretion in feces, or retention (263, 82, 162, and 53 g/d). However, it decreased urine output

(76.0 g/d; $P < 0.01$) along with BUN ($P < 0.01$) from 19.8 mg/dL to 17.7, and 14.8 mg/dL, and saliva urea N ($P = 0.02$) from 14.3 mg/dL to 13.6, and 11.4 mg/dL for 29RUP, 39RUP, and 50RUP, respectively. Lesser RUP tended to reduce plasma ammonia ($P = 0.06$). Additionally, increased RUP (29RUP, 39RUP, and 50RUP) decreased Met (38.2, 36.2, and 34.9 μmol; $P = 0.03$) and Thr (29.4, 26.1, and 22.0 μmol; $P = 0.03$) and tended to decrease Ala ($P = 0.09$) in plasma. Pro was greater ($P = 0.05$) for 29RUP (80.7 μmol) and less for 39RUP and 50RUP (71.2 and 72.6 μmol), which may reflect alterations in amino acid metabolism and absorption across treatments. Therefore, our results indicated that alteration in RUP and RDP proportions in the diet affects N metabolism, blood AA, and N excretion.

Key Words: amino acid, nitrogen balance, RDP

2579 Evaluating the TMR stability of rumen protected lysine products. K. A. Estes*[1,2], M. G. Zenobi[1], C. A. Zimmerman[1], and M. D. Hanigan[2], [1]*Balchem Corporation, Montvale, NJ,* [2]*Virginia Tech, Blacksburg, VA.*

The TMR stability of rumen-protected lysine (RPL) products has been previously investigated, albeit without a positive control, potentially introducing bias. The objective of this experiment was to evaluate the TMR stability of 6 RPL products using a modified technique that used an unprotected Lys treatment as a positive control. Triplicate Ziploc bags containing RPL (equivalent of 1 g of Lys), no RPL (negative control) or unprotected Lys-HCl (positive control; 1 g of Lys) mixed with 200 g of TMR (36% DM) were incubated from 0 to 24 h. At each time point, bag contents were transferred to strainer bags and soaked in 1 L of distilled water for 1 min to solubilize any free Lys released from the products. A sample of solution was filtered (0.45 μm) and stored frozen (−20°C) until analysis for free Lys content via cation exchange HPLC. The Lys content for samples containing RPL or unprotected Lys were corrected for background Lys measured in the negative control samples. Recovery of unprotected Lys was influenced by TMR exposure time and was not complete (85%, 54%, 50%, and 44% at 0, 6, 12, and 24 h, respectively). Thus, a correction factor was applied to samples containing RPL to account for unrecoverable Lys released from the products. Total Lys release was calculated for RPL. Data were analyzed using PROC MIXED of SAS. Means within an incubation time were separated using the Tukey adjustment. Products B and E were the least stable from 6 to 24 h of incubation. Products D and F lost the least amount of Lys at 6 and 12 h of exposure. Product F was the most stable at 24 h (Table 1). These findings highlight significant differences in TMR stability among RPL products and underscores the importance of using unprotected lysine as a positive control to account for incomplete recovery of released lysine.

Key Words: rumen-protected lysine, TMR, lysine

Table 1 (Abstr. 2579). RPL release (%) at different TMR exposure times

TMR exposure time (h)	RPL product						SEM
	A	B	C	D	E	F	
0	1.5^a	1.8^a	0.4^b	0.1^b	1.4^a	0.1^b	0.1
6	9.4^a	74.4^b	10.6^a	0.6^c	74.9^b	0.6^c	1.3
12	17.8^a	80.1^b	19.0^a	5.7^c	75.1^b	1.6^c	1.2
24	22.6^a	87.2^b	29.6^{ac}	34.5^c	80.0^b	9.1^d	1.9

$^{a-d}$Within a time point, values with different superscripts differ significantly ($P < 0.05$).

2580 Duodenal N kinetics of ^{15}N-labeled canola meal and soybean meal in dairy cows. F. Nadon*[1,2], E. Charbonneau[1], S. Binggeli[1], H. Lapierre[2], and D. R. Ouellet[2], [1]*Université Laval, Québec, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

In dairy rations, substitution of soybean meal (SBM) by canola meal (CM) predicts a lower MP supply. The aim of this project was to compare duodenal N kinetics of CM and SBM. We used 4 cows fitted with duodenal cannulas in a crossover design (28-d/period). Cows were fed TMR formulated to meet NE_L and MP requirements (NRC, 2001); CM substituted SBM on a CP basis with 10.8% of SBM in the SBM-diet and 16.7% of CM in the CM-diet. After 14 d of adaptation, cows received in the rumen a pulse-dose of 355 mg of ^{15}N from labeled SBM or CM and 18 duodenal samples were collected on the 3 following days. Samples were divided in fractions using differential centrifugations and ^{15}N isotopic enrichments (IE) were measured using an isotope ratio mass spectrometer. Statistical analyses for IE kinetics were performed with R using the *gnls function* of the *nlme* package and a nonlinear multilevel model. The function used to describe kinetics was based on a modified Erlang function with a good fit ($R^2 > 0.84$). Area under the curve (AUC) and fractional outflow rate (Lambda) are presented in Table 1. Small particles-N AUC, particle associated bacterial-N (PAB-N) AUC and fluid associated bacterial-N (FAB-N) AUC were larger for CM than for SBM ($P \leq 0.01$). The fractional outflow rate of FAB-N and PAB-N were higher for CM than for SBM ($P \leq 0.04$). In conclusion, these results suggest that the in sacco method used to evaluate the degradable N fraction of protein supplements is not appropriate for CM. They imply that CM probably contains a significant amount of small particles that leave the bags during rumen incubation but, contrary to actual assumptions, they are not degraded in the rumen and contribute intact to MP supply.

Key Words: canola meal, N kinetics, ^{15}N-labeled feed

Table 1 (Abstr. 2580). Coefficients of the modified Erlang function of ^{15}N IE of duodenal samples in cows pulsed with ^{15}N-labeled CM or SBM

Fraction	Coefficient	Diet		SEM	P
		CM	SBM		
Small particles-N	AUC, atom% × h	0.062	0.043	0.004	< 0.01
	Lambda, 1/h	0.176	0.159	0.008	0.15
PAB-N	AUC, atom% × h	0.066	0.042	0.005	< 0.01
	Lambda, 1/h	0.178	0.151	0.008	0.04
FAB-N	AUC, atom% × h	0.076	0.054	0.006	0.01
	Lambda, 1/h	0.185	0.149	0.008	< 0.01

2581 Amino acid analysis using multiple time hydrolysis and non-linear regression for determination of amino acid profiles and 21-hour correction factor of standards, dairy cattle milk, tissue, rumen microbes, and various feeds. A. Ortega*, J. Marumo, R. Coombe, A. Benoit, and M. Van Amburgh, *Cornell University, Ithaca, NY.*

Recent data has demonstrated the AA content of milk and other substrates related to nutrient requirements and supply in dairy cattle was not correctly described using 21 to 24 h hydrolysis and that longer or shorter hydrolysis times might be needed for certain AA. Thus, the objective of this study was to quantify the AA composition of ruminant tissue, milk, microbes, and feeds using multiple time hydrolysis and nonlinear regression to determine optimal AA profiles and develop correction factors (CF) for single time point hydrolysis. The AA were analyzed on 16 feeds (5 forages and 11 concentrates), 9 cattle tissue samples (carcass, head/hide/feet/tail, and blood/organs), 8 milk samples (early, first lactation milk and bulk tank milk), and 6 ruminal microbial samples (liquid associated bacteria, solid associated bacteria, and protozoa) for a wide variety of protein sources. Samples were analyzed by HPLC-UV or hydrophilic interaction liquid chromatography–triple quadrupole mass spectrometry following hydrolysis at 110°C for 10 to 14 different time points ranging from 2 to 360 h using acid and alkaline hydrolysis. Following hydrolysis, least-squares nonlinear regression was used to determine the true AA content and CF. In all substrates, most AA, except Ser and Thr, continued to be released after 24 h and were all observed to be declining by 360 h. Due to incomplete recovery, Leu, Val, and Ile (branched chain AA) and Trp had the greatest CF, averaging 1.04, 1.10, 1.11, and 1.08, respectively. The average CF for Ser was of 1.07 because of significant hydrolytic loss by 21 h. This leads to the conclusion that there is currently a misestimation of the AA content in substrates related to diet formulation in dairy cattle. Among different protein sources, CF were different ($P < 0.10$) for Ala, Arg, Asp, Glu, Trp, and Val, which implies that a substrate-specific CF could be needed for an appropriate and balanced correction. Updating AA profiles and correcting hydrolytic measurements should help improve AA formulation for relevant predictions of nutrient supply and requirements.

Key Words: amino acid, hydrolysis, correction factor

2582 Effects of varying isoleucine, leucine, lysine, and methionine concentrations on milk fat synthesis in mammary epithelial cells.

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Previous studies indicated that essential amino acids (EAA) can increase milk fat synthesis, attributed to the EAA regulatory effect on the mTORC1 pathway, which is linked to SREBP-1, a transcription factor involved in lipid synthesis. Understanding the interplay of EAA in regulating milk fat synthesis is key for predicting milk fat production. This study aimed to investigate the effects of varying extracellular concentrations of isoleucine (Ile), leucine (Leu), lysine (Lys), and methionine (Met) on milk fat synthesis in primary mammary epithelial cells. Mammary cells within 3 passages of isolation from 3 lactating cows were subjected to 30 treatments in a replicated central composite design with 5 levels of the 4 EAA (Ile, Leu, Lys, and Met) with 3 center points. This design enabled the estimation of all 2-way interactions among AA. Treatments (n = 30) were centered on mean AA plasma concentrations of lactating dairy cows (0.108 m*M* Ile, 0.185 m*M* Leu, 0.042 m*M* Lys, and 0.017 m*M* Met) with EAA levels set at 3%, 55%, 100%, 145%, and 197% of the mean. Each treatment featured a unique combination of these 5 levels of the 4 EAA, enabling the creation of a response surface. De novo milk fat synthesis was measured using labeled acetate as a tracer. Statistical analyses were conducted using R studio with AA level as a fixed effect and cow as a random effect. Among the EAA tested in this experiment, Met (slope = 10.283 ± 3.578) and Ile (slope = 0.318 ± 0.149) significantly influenced myristic acid synthesis. Met had quadratic effects on the myristic acid synthesis. Palmitic acid synthesis was mainly regulated by Ile (slope = −0.497 ± 0.220), Met (slope = 2.3087 ± 1.099), and the interaction Lys × Met. Higher levels of Ile were associated with an increase in palmitic acid synthesis. This effect seemed to be more evident at higher levels of Met or lower levels of Leu. Conversely, lower levels of Ile were associated with stearic fatty acid synthesis, especially when Met and Leu levels were also lower. In summary, EAA has a combined effect on de novo milk fat synthesis, with Met and Ile showing the most impact.

Key Words: amino acid, de novo synthesis, milk fat

Table 1 (Abstr. 2583). Bioavailability calculation using changes in plasma Lys percentage of total AA

Item	SML	LYSE
Slope	0.01628 ± 0.002	0.01012 ± 0.001
Bioavailability of LYSE[1]	—	49.8 ± 2.6

[1]Calculated as (slope of LYSE/slope Smartamine ML) × 0.80, assuming 80% bioavailability for SML.

2583 Determining the bioavailability of LysiGEM Extend using Smartamine ML instead of infusion using the *in vivo* plasma dose response method.

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The bioavailability of LysiGEM Extend (49 ± 3.0) and Smartamine ML (80 ± 3.5) have been determined using the plasma dose response method with the positive control being infusion of lysine with the use of ruminally-cannulated cows. The objective of this trial was to determine if bioavailability of LysiGEM Extend (Kemin Industries, Des Moines, IA) could be replicated when the positive control was Smartamine ML (Adisseo Inc., France). We used 6 multiparous Holstein cows (120 ± 18 DIM) in a replicated 3 × 3 Latin square with 7-d periods. Treatments were: (1) 0 g additional lysine (CON), (2) 60 g lysine from LysiGEM Extend (LYSE), and (3) 60 g lysine from Smartamine ML (SML). The lysine products were fed in 3 daily aliquots at 0500, 1300, and 2100 h with the basal diet being deficient in Lys. Intake, milk yield, and components were collected the last 3 d of each period. Blood was collected from the tail vein the last 3 d of each period at 2, 4, 6, and 8 h after the morning feeding (0500 h). Amino acids in plasma were determined using HPLC. Data were analyzed using PROC MIXED to generate means for the Lys percentage of total AA, which were then used in the REG procedures of SAS to generate the linear regression variables. Significance was declared at $P \leq 0.05$. Average milk yield was 42.4 kg/d ± 1.4 and DMI was 27.2 ± 0.7 kg/d. The relative bioavailability for LysiGEM Extend was 49.8 (CI = 2.6; Table 1), which is similar to what was determined when the positive control was infused with lysine. Evaluating bioavailability of rumen protected lysine can be done relative to a reference rumen protected lysine with a known bioavailability.

Key Words: lysine, bioavailability, plasma

Animal Behavior and Well-Being 4

2600 Effect of aeronautical noise on the productive and reproductive performance of dairy herds in the municipality of Funza, Colombia. D. C. Mogollon[1], R. D. Castañeda-Serrano[2], J. A. Pardo[2], A. Mejia[1], and O. Barreto-Cruz*[1], *[1]University Cooperative of Colombia, Ibagué, Tolima, Colombia, [2]University of Tolima, Ibagué, Tolima, Colombia.*

The objective was to evaluate the effect of aeronautical noise on productivity in dairy herds in the municipality of Funza. The study was carried out in the rural area of the international airport, in the municipality of Funza, at an altitude of 2,548 m above sea level. There were 18 dairy farms stratified into 3 groups according to exposure levels (n = 6 farms per group): low (0%–33.3%), half (33.3%–66.6%), and high (66.6%–100%), corresponding to low, average, and high levels of noise exposure, respectively. The variables evaluated were reproductive, productive, and acoustic measurement over 5 mo. The treatments evaluated were exposure levels; data were analyzed by ANOVA, and means were compared using Tukey's test ($P < 0.005$; Table 1). Greater grazing noise (dB) was observed in the medium and high treatments ($P < 0.05$). Meanwhile, noise in the milking facilities was greater in the medium treatment ($P < 0.05$), which can be attributed to the type of milking carried out in different productions. Milk production was higher at the low and high exposure levels ($P < 0.05$). The low and high treatments had a higher fertility rate and fewer services compared with the medium treatment ($P < 0.05$). It is concluded that exposure to noise during milking can generate a decrease in production, fertility, and services in the dairy herd.

Key Words: fertility, milk production, acoustic pressure

2601 Effect of different genres of music on behavior and milking parameters of dairy cows. M. Pinkerton*[1], Z. England[1], B. A. Wenner[1], and J. A. Pempek[2], *[1]The Ohio State University, Columbus, OH, [2]USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN.*

The aim of this study was to explore the effect of music played in the milking parlor on behavior and milking parameters of dairy cows. A total of 91 lactating Jersey cows were introduced to 1 of 5 music treatments (no music, classical, country, Latin, rock) in the milking parlor over 4 periods. Music treatment sequence was randomized within each 5-d period, and one music treatment was applied per day (afternoon milking only). The amount of milk harvested from the afternoon milking, milking duration, and average milk flow rate were recorded daily for all cows. Cow behavior at milking cluster attachment was video recorded and later assessed for a subset of cows (n = 38) by scoring flinch, step, and kick responses using a 4-point system based on the direction and height of hind leg movements. Behavior was analyzed using an ordinal logistic regression model (PROC GLIMMIX, SAS), and milking parameters were analyzed using a linear mixed-effects model (PROC MIXED, SAS). All models included the fixed effects of treatment, sequence, period, parity, days in milk, and temperature-humidity index, and the random effect of cow within sequence. The amount of milk harvested significantly increased ($P < 0.01$) by 0.5, 0.3, 0.4, and 0.2 kg per afternoon milking when classical music was played compared with no music, country, Latin, and rock, respectively. The amount of milk harvested also significantly increased ($P < 0.01$) by 0.3 kg per afternoon milking when country and rock versus no music was played in the parlor. There was no evidence for a significant effect of treatment on milking duration ($P = 0.23$), average milk flow rate ($P = 0.15$), or cows' flinch, step, kick responses ($P = 0.60$). This study indicates certain genres of music influenced afternoon milk yield, with classical music positively affecting the amount of milk harvested. Future research is needed to explore the possible influence of parlor music on human behavior. Other aspects of animal behavior (e.g., temperament, total number of flinches, step, kick responses) should also be considered in future studies, as well as cows' response to music in other farm environments.

Table 1 (Abstr. 2600). Effect of aeronautical noise on temperature, milk production, fertility, and services per conception in dairy herds in the municipality of Funza, Colombia

	Mean exposure level				
Variable	Low	Half	High	SEM	*P*-value
Milking noise, dB	75.30^{a}	78.67^{b}	75.33^{a}	1.11	0.0246
Grazing noise, dB	65.52^{a}	68.47^{b}	69.20^{b}	0.81	<0.0001
Temperature, °C	11.50^{a}	12.80^{b}	11.33^{a}	0.41	0.0078
Milk yield, L^{-1}	15.7^{b}	10.30^{a}	17.09^{b}	1.29	<0.0001
Fertility, %	61.67^{b}	66.67^{b}	56.69^{a}	2.19	0.0003
Services per conception	2.13^{b}	2.28^{b}	1.81^{a}	0.16	0.0141

^{a,b}Within rows, means with common letter are not significantly different ($P > 0.05$).

Key Words: behavior, dairy cow, music

2602 Determining feed bunk preference and its association with performance in dairy cows. M. R. Pupo*, J. R. R. Dorea, and L. F. Ferraretto, *University of Wisconsin–Madison, Madison, WI.*

Little is known about feed bunk preference by lactating dairy cows and its relationship with productive performance and feed efficiency. This study aimed to assess feed bunk preference among high-producing dairy cows. A data set from 10 feeding trials conducted at the University of Wisconsin–Madison from 2019 to 2023, with a total of 629 lactating cows was used. Each study had 64 cows with free access to each of 32 electronic gate feeders, and a minimum of 6 weeks of data collection. We used entropy measurement to classify cows based on their feed bunk pattern, from very random to not random, where entropy quantifies the uncertainty or disorder in a data set. For each cow, the probability distribution of bunk visits was determined and used to calculate the entropy. Entropy values were normalized to create an index ranging from 0 to 1, where 0 indicates the least random (more predictable) behavior and 1 indicates the most random (less predictable) behavior. Cows were divided into quartiles by normalized entropy values (Q1 = 0.0–0.5; Q2 = 0.5–0.7; Q3 = 0.7–0.9; Q4 = 0.9–1.0). Data were analyzed with fixed effect of quartile and random effect of trial. Means were determined using the LSMEANS statement, and quartile means were compared using the Bonferroni *t*-test option after a significant overall *F*-test. Some dairy cows appeared to prefer a specific feed bunk, with about 25% of lactating dairy cows showing predictable bunk preference, supported by entropy values equal to or lower than 0.5. However, most dairy cows in this data set did not show a bunk preference, as evidenced by entropy values greater than 0.5. Milk yield (kg/d) was greater ($P = 0.03$) for Q3 (49.1 kg/d) than Q1 and Q4 (46.6 kg/d, on average). Intake of DM (kg/d) was greater ($P = 0.02$) for Q2 and Q3 (28.7 kg/d, on average) than Q1 (27.6 kg/d). Both the concentration of fat ($P = 0.01$; 3.78 vs. 3.54, on average) and protein ($P = 0.001$; 3.29 vs. 3.16, on average) were greater for Q4 than other treatments, respectively. Although some cows have a preferred spot to eat, various animal characteristics may affect feeding behavior and performance of dairy cows.

Key Words: freestall, nutrition, entropy

2603 Effect of milking permission and concentrate allowance on motivation of lactating primiparous cows to visit an automated milking system.

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Compared with early studies investigating motivation to visit an automated milking system (AMS), dairy cows today produce more milk and, therefore, may be more motivated to visit the AMS by udder pressure compared with receiving supplemental AMS feed. The study objective was to determine the effects of milking permission and concentrate allowance on the motivation of cows to visit an AMS. A total of 31 primiparous Holstein dairy cows (92.2 ± 18.6 DIM) that had been milked on a free-traffic AMS for at least 14 d were enrolled in the study. Cows were exposed to each of 2 treatments in a crossover design, with exposure order balanced for DIM and milk production. Treatments consisted of: (1) minimum milking interval increased from 6 to 9 h (INT) and (2) AMS concentrate allowance decreased from 6 to 0 kg/d DM (CONC). Milking activity data were collected in 4 periods of 6 d each; baseline (6 h milk interval, 6 kg/d DM concentrate), period 1 (INT or CONC), period 2 (CONC or INT), and baseline (6 h milk interval, 6 kg/d DM concentrate). Data were analyzed using mixed-effect, repeated measures linear regression models. Cows on INT, as compared with CONC, tended to make more total AMS visits (7.1 vs. 5.0 visits/d; SE = 0.81; $P = 0.06$), more voluntary AMS visits (7.0 vs. 4.8 visits/d; SE = 0.84; $P = 0.07$), and had more AMS rejections (5.0 vs. 2.5 rejections/d; SE = 0.73; $P = 0.02$). There was a treatment × period interaction ($P = 0.05$), whereby in period 1, cows who were on INT yielded more milk than cows who were on CONC (35.9 vs. 32.2 kg/d; SE = 1.73; $P = 0.04$), and cows produced more milk if they were on INT in period 1 compared with period 2 (35.9 vs. 32.4 kg/d; SE = 1.72; $P = 0.05$). Within each period, regardless of treatment, cows made more total and voluntary AMS visits ($P \leq 0.04$) on d 1 (7.2 total and voluntary visits/d) compared with d 4 (no difference in total visits, 5.6 voluntary visits/d), d 5 (5.6 total and 5.4 voluntary visits/d), and d 6 (5.6 total and 5.5 voluntary visits/d). The results demonstrate that removal of AMS concentrate reduces motivation for voluntary AMS visits to a greater extent than altering milking permissions.

Key Words: motivation, robotic milking, behavior

2604 Milking behavior dynamics within the first 4 weeks of lactation in primiparous and multiparous cows in an automated batch milking system.

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The objective was to compare the dynamics of milking behavior between primiparous and multiparous cows starting their lactation in an automatic milking system in a semi-voluntary batch milking design. This retrospective study included information from milking events in 2,138 cows from May to December 2023 in an organic-certified herd in Dublin, TX. Milking behavior information for the first 4 weeks of lactation included the percentage of incomplete milkings, percentage of kick-offs, percentage of teat cleaning failure, and milking duration (time in the milking box per milking visit) collected from DelPro software (DeLaval, Sweden). Parity and calving data were extracted from PCDART software (DRMS, NC). Data were analyzed by logistic regression and univariable linear regression to assess the differences in milking behavior between primiparous and multiparous cows in weekly intervals. After edits, 344,395 milking records were analyzed in 2,138 cows (27% primiparous, 73% multiparous). Milking behavior differed between parity categories for all the variables during all the weekly periods (Table 1). Milking duration was greater in primiparous during wk 1 (+0.40 min, $P < 0.001$) and wk 2 (+0.20 min, $P < 0.001$), but no differences were observed during wk 3 and 4. These results highlight the differences in milking behavior during the early lactation of primiparous vs. multiparous cows. As indicated by the odds ratio (OR), the magnitude of the differences between parity groups decreased as the lactation progressed, which may be associated with the process of habituation to milking in primiparous cows.

Key Words: milking, behavior, primiparous

Table 1 (Abstr. 2604). Odds ratios and 95% CI for milking behavior events within the first 4 weeks of lactation in primiparous vs. multiparous cows; multiparous cows were considered as the reference group

	Milking behavior variable								
	Incompletes			Kick-offs			Teat cleaning fail		
Week	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
1	4.76	2.70-10.0	<0.001	4.17	2.78-6.66	<0.001	2.94	1.75-5.00	<0.001
2	4.00	3.22-5.00	<0.001	2.44	2.00-2.94	<0.001	3.13	2.44-4.00	<0.001
3	2.17	1.75-2.50	<0.001	1.96	1.66-2.38	<0.001	1.59	1.19-2.08	0.001
4	1.96	1.56-2.50	<0.001	1.49	1.23-1.78	<0.001	1.47	1.03-2.00	0.017

2605 Robot selection preference in cows under voluntary milking in a batch milking system.

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The efficiency of automatic milking systems (AMS) depends on the continuous flow of cows. The objective was to analyze the robot selection behavior of cows in an AMS using a semi-voluntary batch milking design. The study used data from 812,712 milking events in 2,682 Holstein (HO, n = 1,062), Jersey (JE, n = 1,356), and crossbred (XB, n = 264) cows from 2023 to 2024 in a commercial organic grass-fed dairy in Texas. Cows were moved to the milking center twice per day, where they selected their milking visits among 22 robot units (DeLaval, Sweden). Robots were classified by location in the barn (east or west) and arm configuration (left or right). Visit information was collected to

Table 1 (Abstr. 2605). Cow distribution by the top choice of the milking robot, location within the milking barn, and robot arm configuration by breed group

	Percentage of cows			
Value	HO	JE	XB	Herd
Top choice category (% of visits)[1]				
<10	5.3	21.1	12.9	14.0
10–15	31.4	39.7	37.1	36.1
15–20	26.3	18.6	21.6	21.9
20–25	14.7	8.8	9.1	11.1
25–30	7.6	5.3	7.6	6.5
30–35	5.5	2.2	3.4	3.6
35–40	3.2	1.4	1.1	2.1
40–45	2.3	1.0	4.5	1.8
45–50	0.9	0.6	1.1	0.8
50–55	1.1	0.4	0.4	0.7
>55	1.8	1.0	1.1	1.3
East vs. west[2] (%)				
50–60	22.2	37.5	25.8	30.3
61–70	19.8	26.2	22.3	23.3
71–80	21.7	16.4	18.6	18.7
81–90	17.3	12.5	15.9	14.7
91–100	19.0	7.5	17.4	13.0
Left vs. right arm[2] (%)				
50–60	48.7	66.6	58.0	58.7
61–70	25.1	24.6	28.8	25.2
71–80	12.1	6.3	7.6	8.7
81–90	6.6	1.2	2.7	3.5
91–100	7.4	1.4	3.0	4.0

[1] E.g., the most frequently visited robot was between 10% and 15% of total visits in 31.4% of Holstein cows.

[2] 50 = no preference; 100 = high preference

determine the frequency of specific robot usage per cow during the study period. Subsequently, the most visited robot was identified for each cow and the frequency of visits was calculated for every animal (top choice frequency, %). A chi-squared test of independence was used to establish if robot selection differed from random (4.54%/robot) and to compare usage distributions among breed groups. Results describing robot selection preference are presented in Table 1. Top choice percentages were different from those expected by random selection ($P < 0.0001$), and HO showed more consistent selection preference behavior than JE for AMS unit, location, and configuration ($P < 0.0001$).

Key Words: robot, selection, cow

2606 Impact of a sensory feed additive on performance of dairy cows in automated milking system. Q. Tiqui[1], M. G. Fabry[2], and G. Desrousseaux*[1], *[1]Phodé, Terssac, France, [2]Gex & Cow, Fillinges, France.*

The objective of this study was to determine if feeding a sensory feed additive (SFA; derived from aniseed) improves the number of visits of lactating dairy cows in a robotic milking system. It was hypothesized that cows supplemented with SFA would demonstrate a greater willingness to be milked and a greater milk production. A total of 339 multiparous mid-lactation Montbeliarde dairy cows (153 ± 23 DIM) were enrolled in this study and housed in commercial facilities where they were assigned to 1 of 2 treatment diets: (1) control partial mixed ration (PMR) + robot pelleted compound feed (control; n = 339), or (2) control PMR with 2 kg of aniseed extract per ton of robot pelleted compound feed (Cristalfeed, Phodé, Terssac, France; SFA, n = 339). Cows were fed the control experimental diet for 30 d (P1), then the SFA experimental diet for a period of 30 d (P2), and then again fed the control experimental diets for 30 d (P3). Parameters were recorded daily and registered by the milking robot software. Data were analyzed using ANOVA with DIM and type of diet as covariates to test whether the number of visits, milk yield, milk solids, and rumination time varied in P2, from their P1 and P3 average. The SFA cows had been milked 2.54 ± 0.02 times/day, whereas control cows had been milked 2.48 ± 0.01 times/day ($P < 0.001$). For control cows, energy-corrected milk (ECM) was 27.8 ± 0.1 kg/cow per d, whereas for SFA cows, ECM was 28.4 ± 0.1 kg/cow per d ($P < 0.001$). No interaction between treatment and type of diet on ECM was shown. Milk fat increased in P2 (control 40.1 ± 0.01 g/kg vs. SFA 40.9 ± 0.02 g/kg; $P < 0.001$). Fat: protein ratio was higher in P2 (control: 1.21 ± 0.01 vs, SFA: 1.25 ± 0.01; $P < 0.001$). In P2, SFA cows ruminated 513 ± 3 min/d, wheras control cows ruminated 503 ± 3 min/d ($P < 0.05$). The results indicate that feeding an aniseed extract in the robot compound feed to mid-lactation dairy cows may encourage visits of the milking robot and promote greater milk performance. Further research is required with multiple group studies.

Key Words: sensory feed additive, milking robot, performance

2607 Machine learning models to predict sleep state and stages of dairy cows using noseband sensor parameters and respiratory rate. X. Zhao, M. Wang*, A. M. Serviento, S. E. Räisänen, R. Peng, Z. Zeng, and M. Niu, *Department of Environmental Systems Science, Institute of Agricultural Sciences, ETH Zürich, Zürich, Switzerland.*

Sleep is essential to all animals and is crucial to ensuring their health and welfare. However, monitoring sleep pattern using polysomnography in farm animals, such as dairy cows, is challenging and impractical. The objective of this study was to develop and evaluate prediction models for sleep and sleep stages of dairy cows using data from non-invasive and wearable sensors. Electrophysiological recordings of brain, eye, muscle activities, and respiratory rate (256 Hz), as well as jaw and head movements (10 Hz) were collected from 9 cows housed in a tiestall barn for 24 h in using RemLogic devices, and respiration belts, and RumiWatch noseband sensors, respectively. After removing all missing data, the final data set had a total of 156.1 h, including 12.9 h of sleep and 143.2 h of awake state. Different sleep stages were annotated in 30-s intervals including awake, rumination, drowsing, rapid eye movement (REM) sleep, and non-REM sleep. Two different machine learning models based on random forest (RF) and gradient boosting (GB) were used for classification. A 5-fold cross validation was conducted to 2 models, and data from one animal was either in a training or testing set. For classification of awake and sleep states, the sensitivity, specificity, and area under curve were 76.8%, 75.3%, and 76.1% for RF, and 62.0%, 61.8%, 61.9% for GB, respectively. The sensitivity, specificity, and area under curve for classification of sleep stages were 30.4%, 65.2%, 50.6% for RF, and 28.2%, 64.1%, 49.7% for GB, respectively. The performance of RF was better than the GB model in predicting sleep vs. awake states and in differentiating the sleep stages. However, both RF and GB models underperformed in predicting different sleep stages, possibly due to the limited data set. This result suggests that machine learning techniques have the potential to predict sleep behavior of dairy cows using the data from noseband sensors and respiration belts. More data are being collected to improve prediction accuracy and assess if sleep stages can be predicted.

Key Words: dairy cow, sleep prediction, wearable sensor

2608 Using computer vision as a tool to analyze social interactions in dairy cows.

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Precision livestock farming (PLF) technologies have been able to help with these increasing demands in dairy farms. Computer vision is one of these PLF technologies that has been used to generate health, behavior, and welfare information. The objective of this study was to create a computer-vision model using deep learning that can accurately recognize and predict the active (aggressor) and passive (receiver) animals during social interactions of 31 multiparous dairy cows at the feed bunk. Video data were collected and sorted based on 5 classes of observed behaviors including body butting, head butting, licking, rubbing stall, and throwing feed. The video frames were then sorted into groupings of 7 frames and segmented into active and passive cows using polygon segmentation. Segmented masks were generated for both active and passive animals independently. A custom Python code was run to generate a synthetic frame using the masked frame distance difference for each behavior type. Frame step distances one through 6 were tested for each behavior. These data were used to determine which masked frame differences would create the best overall model to run the 2 active and passive classes on each of the original 5 behavior types. A YOLOv8 behavior classification model was able to be created to identify all 5 behaviors with a 4-step mask frame difference with an overall performance at 60.00%. The results for each of the identification of the active and passive animal or object for each behavior follow: body butting at 69.79%, head butting at 66.67%, licking at 76.79%, rubbing stall at 84.21%, throwing feed at 100.00%. This type of computer vision analysis allows for a behavior-based type of farm management strategy to be implemented.

Key Words: computer vision, social behavior, precision livestock farming

2609 An objective and repeatable measure of cow back shape.

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Body condition scoring (BCS) is a widely used method for assessing cow health, but it is subjective and prone to inconsistency between assessors. This study evaluates a novel measure of cow shape based on analysis of backline dimensions and a body condition index (BCI) derived from these. The objectives of this study were to develop a new method for assessing cow condition that is objective, repeatable, and independent of sensor technology, and to validate this method by comparing it to traditional BCS assessment. We measured backline contours using daily 3D imaging in a herd of 1,750 Holstein cows at various stages of lactation, extracting 6 key values from these contours. These values are derived from the upper convex hull of lines between the hook bones, between the pin bones, along the left/right side of the cow, the cow height at the center between the hook bones, and the cow width between the hook bones. These can be measured manually by taking physical measurements of the cow, or automatically with the use of 3D imaging. We propose a linear combination of these 6 values to create a single consolidated value to provide the BCI. This index demonstrated high repeatability (interquartile range of 1.8%) and a significant correlation ($R^2 = 0.74$) with the mean of multiple manual BCS values provided human veterinary experts for the same cows. This suggests that BCI is a reliable and valid measure of cow shape that can be used to improve ongoing monitoring of an animal's condition. This automated approach eliminates subjectivity and facilitates standardized comparisons within and across herds, potentially improving dairy cow management and welfare.

Key Words: body condition, automated, health

2610 Semi-supervised machine learning techniques to identify ingestive behavior of dairy cows.

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Using predictive models to predict animal behavior is a reality. The most common models require labeled data. However, in semi-supervised learning models, only part of the data needs to be labeled. Thus, our objective was to compare different types of machine learning models to identify feeding behavior of Holstein cows housed in a compost barn in Brazil. Three-axis accelerometers and gyroscopes were attached to the neck of 3 cows, collecting data every second during 36 h (12 h per cow). Visual observations were carried out in the same period, and the activities were classified as "feeding" and "not-feeding." A supervised machine learning model (random forest [RF]), was compared with 5 semi-supervised models: label propagation (LP), graph-based methods (GM), Yarowsky algorithm (YA), co-training (CT), and tri-training (TT). The ratio of training/validation data was 80:20. The performance of the models was compared in terms of accuracy, precision, and sensitivity. As shown in Table 1, the RF, LP, GM, and CT predictive models obtained similar results. The TT predictive model presented the worst results when compared with the other predictive models. On the other hand, the YA model presented the best results. Supervised models, such as RF, require an evaluator to label data based on the behavior expressed by the animals, taking time and money and exposing the algorithm to human error. In contrast, semi-supervised algorithms require that only part of the data be labeled, reducing these demands and minimizing the chances of human error. This is extremely useful when considering research that collects data over a long period of time, where the researcher can label the data for a short period. We concluded that the semi-supervised models YA, LP, and GM, performed at least as well as the supervised RF model, and can be used to anticipate the ingestive behavior of cattle when only 2 classes of behavior are predicted.

Key Words: algorithm, precision livestock, sensor

Table 1 (Abstr. 2610). Performance of different predictive models

Predictive model	Accuracy (%)	Precision (%)	Sensitivity (%)
RF	89.3	89.5	89.3
LP	88.6	84.8	85.9
GM	90.1	87.5	86.8
YA	95.7	92.8	96.3
CT	91.1	86.9	90.9
TT	85.3	81.6	80.1

2611 Water intake increases prior to calving may be more informative for multiparous cows.

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Drinking behavior can be indicative of an animal's internal state, including the onset of parturition. The objective of this study was to characterize the changes over time in the water intake of lactating Holstein dairy cows (n = 157) between 14 d before calving (d −14) and day of calving (d 0). Retrospective data from January 2024 was collected from a freestall dairy in central Texas that uses rumen boluses to monitor cows' daily water consumption (L/d). To evaluate the impact of time, lactation number, and their interaction on water intake before parturition, daily water consumption was analyzed using a generalized linear mixed model (PROC GLIMMIX) in SAS 9.4. The model included the fixed effects of lactation number (1, 2, 3, and 4), day (−14 to 0), and their interaction, as well as a random effect of cow within lactation number × day interaction. Water intake differed among lactation numbers ($P \leq 0.001$) and increased over time ($P \leq 0.001$). The interaction between lactation number and day did not affect daily water consumption ($P = 0.15$). Average daily water intake (L/d) was the least on d −14 (40.11 ± 1.42) and most on d 0 (calving; 64.53 ± 1.42) for all cows. Average daily water intake was lower ($P < 0.003$) for 1 lactation cows (n = 39; 48.96 ± 0.71) compared with 2 (n = 59; 52.41 ± 0.57), 3 (n = 31; 54.21 ± 0.79), and 4 (n = 31; 52.79 ± 0.83) lactation cows. Primiparous cows drank less than multiparous cows (2+) on d −6 ($P = 0.02$), d −4 ($P = 0.007$), d −2 ($P = 0.006$), d −1 ($P = 0.03$), and on the day of calving ($P = 0.01$). Age and body size may have influenced the differences observed in water intake between multiparous and primiparous cows. Including average daily water intake, monitored by rumen boluses, may help to provide a more accurate estimation of calving time for lactating dairy cows; however, this strategy may need to be adjusted for primiparous cows.

Key Words: rumen bolus, water intake, calving

2612 Effect of transdermal non-steroidal anti-inflammatory on behavioral responses of Holstein cows challenged with *Escherichia coli.*

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The objectives were to determine the effects of a transdermal non-steroidal anti-inflammatory drug (flunixin transdermal) on behavioral responses of Holstein cows challenged intramammarily with *Escherichia coli*. A total of 88 cows (272 ± 89.2 DIM) free of clinical mastitis, and negative for gram-negative bacterial growth in milk samples from individual quarters were enrolled in the experiment. Cows were fitted with automated monitoring devices at 67 ± 53 d before challenge for the establishment of a baseline. At enrollment (d −11; d 0 = day of challenge), cows were blocked by parity and within block were assigned randomly to: PCON (one quarter infused with sterile PBS, n = 30), NCON (one quarter challenged with *E. coli* + one quarter infused with sterile PBS; n = 29), and TFM (one quarter challenged with *E. coli* + one quarter infused with sterile PBS+ flunixin transdermal 12 h after the challenge, n = 29). The bacterial strain used in this experiment (*E. coli* P4, USDA) resulted in mild/moderate cases of clinical mastitis. Cow-state data generated was decoded to calculate minutes of rumination, feeding, activity, and idle within the hour. Data were analyzed by ANOVA. Treatment with flunixin transdermal affected ($P = 0.02$) daily rumination but the effect was dependent on time relative to challenge. Treatment did not ($P = 0.26$) affect daily activity (NCON = 391 ± 4.1, PCON = 400.4 ± 4.0, TFM = 393.6 ± 4.3 min/day). On d 1, cows in the NCON treatment had ($P < 0.01$) reduced eating time compared with TFM and PCON cows. On d 3, cows in the NCON treatment tended to ($P = 0.06$) have reduced eating time compared with PCON cows, whereas TFM cows did not differ from either treatment. On d 1, cows in the NCON treatment had ($P < 0.01$) greater idle time when compared with cows in the PCON and TFM treatments, which did not differ. On d 2, NCON cows had ($P < 0.01$) greater idle time compared with PCON cows, whereas TFM cows did not differ from either treatment. Flunixin transdermal had a positive effect on welfare of Holstein cows challenged intramammarily with *E. coli* by improving feeding and rumination time in the days immediately following the challenge.

Key Words: mastitis, *Escherichia coli*, behavioral response

2613 Influence of previously training multiparous Holsteins on an automated head chamber system versus none on heart rate, enteric methane, milk production and composition.

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Introductions of novel procedures to dairy cattle have previously demonstrated altered animal behaviors questioning measurement reliability. A study was conducted to determine if previous training (TRN) of animals to a tiestall GreenFeed system (GFS) allowed easier adaptation to GFS training in a freestall setting. The TRN cows were compared with cows with no previous GFS exposure (UNT). A total of 49 multiparous Holsteins (mean ± SD; 271 ± 40 DIM) were enrolled and split into 2 groups to have a maximum of 25 cows on the GFS. The TRN cows had been enrolled in a tiestall experiment 2 mo before the start of the experiment. Group 1 had 12 TRN and 13 UNT cows. Group 2 had 10 TRN and 14 UNT. Animals were in the pen 8 d before training to GFS over a 2-d period (PRE). Each animal was individually brought to the GFS unit and remained for 10 min. After PRE, cows had free access to the system until the end of the experimental period (d 2–14; POST). Within each group, 10 TRN and 10 UNT cows were fitted with POLAR heart rate (HRT) monitors on day −2 relative to training (day = 0) until d 2. Monitors were removed on d 4 and replaced d 6 to 9 ± 1 d. Data were analyzed with R-studio using the fixed effects of treatment, day as a repeated measure, and interaction of treatment by day. Cow within group was considered a random effect. Preplanned contrasts were PRE versus POST for TRN versus UNT for HRT, CH_4, CO_2, and milk. The pretraining period was compared between UNT and TRN cows. Average POST visits to GFS were similar (23.7/cow). Average HRT and daily CO_2 production increased POST ($P < 0.01$) for TRN animals where heart rate trended ($P = 0.07$) upwards POST for UNT. No difference was observed in daily CH_4 (403 ± 10 g/d). Milk protein concentration was greater POST for TRN and UNT cows ($P < 0.01$). Results indicate the TRN group was more active based on increased CO_2 output and HRT although no increase in GFS visits. Previous training did not affect CH_4 measurements but suggest animal behavior is influenced based on GFS exposure and could affect reliability and repeatability of measurements.

Key Words: methane, behavior, training

2614 The effect of tilling depth on pack conditions and lying behavior in Ontario compost bedded pack dairy farms.

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Managing moisture in compost bedded pack (CBP) barns is a challenge for Canadian dairy producers, specifically in the fall and winter months. The objective of this study was to determine the effect of shallow or deep tilling depth on pack conditions, cow cleanliness, and lying behavior in Ontario CBP dairy herds. Treatments were deep (D), where the pack was tilled to a depth of 35 to 41 cm, or shallow (S), with the pack tilled to 20 to 25 cm. Producers tilled the pack twice daily. Commercial CBP farms (n = 8) in Ontario, Canada, were assigned to one of 2 treatment sequences (D-S-D or S-D-S) in a switchback design. Sequence was balanced for equipment type (either cultivator or rototiller). Farms were visited every 28 d, at the beginning and end of each treatment period, from September to December 2023. Each treatment period (D or S) in the sequence lasted 4 weeks. The CBP samples taken at every visit were analyzed for depth temperature and moisture content. Cleanliness was scored live during visits on the same 25 cows per farm, 6 areas per cow were scored on a scale of 1 to 4 (1 and 2 indicating clean) and averaged to provide a full body score. Lying behavior was collected via data loggers on 8 cows per farm, during the last 7 d of treatment. All outcome variables are reported on a herd level, with the farm as the experimental unit. Data were analyzed using PROC GLIMMIX. Tilling depth did not affect any of the outcome variables. Depth temperatures at 20 cm and 37 cm averaged 34.0°C (SE = 2.55) and 34.1°C (SE = 2.38), respectively. Surface and 20 cm CBP moisture were on average 61.9% (SE = 1.46) and 61.8% (SE = 1.50), respectively. Average herd cleanliness scores were 1.5 (SE = 0.04). Cows lay down on average 11.1 h/d (SE = 0.13) and performed 9.9 bouts/d (SE = 0.39). The results suggest that a short-term tilling depth change did not affect CBP moisture content and depth temperature, or cow cleanliness or lying behavior. Further research is needed to identify which management practices dairy producers can address to reduce CBP moisture.

Key Words: cow comfort, housing, welfare

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2615 Identification of functional SNPs and INDELs within novel alternative splicing associated with mastitis in Holstein dairy cows. V. Asselstine*[1], J. F. Medrano[2], C. F. Baes[1], P. Stothard[3], F. Miglior[1], F. S. Schenkel[1], and A. Cánovas[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Animal Science, University of California-Davis, Davis, CA, [3]Department of Agricultural, Food and Nutritional Science/Livestock Gentec, University of Alberta, Edmonton, AB, Canada.*

Bovine mastitis is one of the most common diseases in lactating dairy cows, resulting in milk yield reduction, discarded milk, and early culling. Although mastitis has been extensively studied, it remains one of the most challenging diseases for dairy producers. Integrating data from high-throughput transcriptomic and genomic sequencing technologies offers a new opportunity to characterize the biological basis underlying complex traits, such as mastitis. With the advent of RNA-Sequencing (RNA-Seq), the host transcriptome can be studied at a high-throughput level to identify key differences between healthy and mastitic animals. In this study, RNA-Seq was performed on 6 cows using somatic cell (SC) RNA from milk samples from 2 separate quarters; one quarter classified as healthy (n = 6) and one as mastitic (n = 6), based on having a SC count of >100,000 cells/mL. The goal was to identify novel alternative splicing isoforms that are differentially expressed (DE) between healthy and mastitic samples (FDR < 0.05; FC > ± 2). From the RNA-Seq analysis, 3 DE candidate alternatively spliced isoforms were identified, and each isoforms were from genes previously shown to have key roles in host immune response. Whole-genome sequencing (WGS) from a unique population of 7 healthy and 7 mastitic Holstein dairy cows was then used to identify variants, including single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs), that were fixed and uniquely present in all the healthy or all the mastitic cows within the DE candidate alternatively spliced isoforms. The WGS analysis identified 58 variants (26 SNPs and 32 INDELs) in the DE candidate alternatively spliced isoforms. Further research will aim to identify if these variants may affect the expression or functionality of the alternatively spliced isoforms. In conclusion, this research is integrating multi-OMICs approaches to better understand genetic resistance to mastitis in Holstein dairy cattle.

Key Words: mastitis, -OMICs technology, alternative splicing

2616 Supplementing cell culture media with essential fatty acids alters the response of MAC-T cells to inflammatory mediators in triacylglyceride synthesis. C. Lalonde*, J. Kraft, and F. Q. Zhao, *The University of Vermont, Department of Animal and Veterinary Sciences, Burlington, VT.*

Mastitis is known to lower milk fat yield, but the mechanisms have not yet been elucidated. However, cells cultured with common culture media have an unnatural fatty acid composition, and mammalian cells cannot produce essential fatty acids (EFA). We hypothesized that supplementing cell culture media with EFA would provide a better model to study lipid metabolism during mastitis. To test this hypothesis, we cultured mammary epithelial cells (MAC-T) with and without EFA and inflammatory mediators and assessed their triacylglyceride (TAG) synthesis. MAC-T cells were grown in Dulbecco minimal essential medium with 10% fetal bovine serum, 10 μg/mL insulin, 10 units/mL penicillin, 10 μg/mL streptomycin, and 25 ng/mL amphotericin B. Cells were seeded in 96-well plates at a density of 6,000 cells per well. After 24 h, cells received either 1 mL PBS with 37 m*M* linoleic acid, 10 m*M* α-linolenic acid, and 0.2% ethanol (EFA+) or 1 mL PBS with 0.2% ethanol (EFA−). Subsequently, cells from both groups were treated with 1 mL of PBS containing either no inflammatory mediator (PBS), 3 mg/mL TNF-α, 3 mg/mL of IL-6, 100 mg/mL *Escherichia coli* lipopolysaccharide (LPS), or 1 mg/mL prostaglandin E2 (PGE_2). Supernatant was collected after 24 h. The TAG concentration was measured from the supernatants using a colorimetric enzymatic assay. Data were analyzed by ANOVA with Tukey's correction for multiple comparison in Prism 10. Supernatant from EFA+ cells had 32% more TAG than that from EFA− cells (139.77 vs. 106.06 mM, $P < 0.01$). Treatment with inflammatory mediators had no impact on TAG concentration from EFA− cells. EFA+ cells treated with PBS (31.66 ± 4.98%, $P < 0.001$), TNF-α (28.37 ± 4.19%, $P < 0.001$), IL-6 (23.88 ± 4.19%, $P < 0.001$), and LPS (21.09 ± 7.82%, $P < 0.001$) also had increased TAG concentrations compared with EFA− cells. However, EFA+ cells had no increase in TAG concentration compared with EFA− cells when treated with PGE_2 ($P = 0.14$). In summary, MAC-T cells grown in media enriched with linoleic acid and α-linolenic acid produced more TAG than cells grown without, but this increase was mitigated by PGE_2 treatment.

Key Words: prostaglandin E2 (PGE2), triacylglyceride (TAG), essential fatty acids (EFA)

2617 The association between bovine leukemia virus infection and metritis in dairy cows. M. Hernandez*[1], S. Casaro[1], F. Cunha[1], M. B. Ugarte Marin[1], R. Bisinotto[1], F. Maunsell[1], C. Figueiredo[2], and K. Galvão[1], *[1]University of Florida, Gainesville, FL, [2]Washington State University, Pullman, WA.*

Enzootic bovine leukosis is a cattle disease induced by the bovine leukemia virus (BLV). BLV infection is associated with reduced longevity and reduced milk yield in dairy cows, which leads to economic losses of approximately $500 million annually. The innate and adaptive immune cells of BLV-infected cattle have abnormal phenotypes and infected cattle have been shown to have an increased risk of mastitis, digestive problems, and pneumonia. Metritis is a highly prevalent disease in dairy cows and is associated with neutrophil and monocyte dysfunction. Therefore, we hypothesized that dairy cows infected with BLV have an increased risk of developing metritis. The objectives were to evaluate the association between BLV infection and the risk of metritis, and the association between BLV infection and the risk of leaving the herd across lactations. Holstein heifers with BLV tests from 2012 to 2019 (n = 6,121) were followed until the end of lactation 3. A single agar gel immunodiffusion test was used to diagnose BLV at 382 ± 16 d of age. Individual electronic farm records were reviewed for the main events of metritis diagnosis and leaving the herd. Metritis was diagnosed by examining the uterine discharge expressed after palpation per rectum at 4, 6, and 10 d after parturition, and defined as reddish-brownish, fetid, watery vaginal discharge. Logistic regression was used to analyze the odds of metritis in lactations 1, 2, and 3. The Cox proportional hazards model was used to evaluate the risk of leaving the herd by the end of lactation 3. The odds of metritis in lactation 1 were greater for BLV-positive than for BLV-negative cows (19.3 vs. 15.4%; OR = 1.31; 95% CI = 0.99–1.74; $P = 0.05$). No differences in the odds of metritis were observed in lactations 2 ($P = 0.76$) and 3 ($P = 0.66$). The hazard of leaving the herd by the end of lactation 3 was greater for BLV-positive than for BLV-negative cows (HR = 1.33; 95% CI = 1.20–1.49; $P < 0.01$). In conclusion, BLV infection was associated with increased odds of metritis in lactation 1 and an increased hazard of leaving the herd by the end of lactation 3.

Key Words: bovine leukemia virus, metritis, survival in the herd

2618 The influence of service sire breed type, whether dairy versus beef, on the incidence of health events in fresh cows.

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Fresh cow health is a critical focus for researchers and dairy producers. With the increased use of beef semen in dairy operations, it is worth exploring service sire breed type and its potential impact on fresh cow health events (FCHE). This study aimed to examine whether service sire breed type, beef vs. dairy, affected the probability of having a fresh cow health event. Dairy records provided by Dairy Records Management Systems (DRMS) included records from 201 herds from 2020 through 2022 with an average herd size of 284 ± 631. Individual cows were categorized by service sire breed type (dairy vs. beef), production quartile, and FCHE. A FCHE was defined as any adverse health event within the first 30 DIM. The incidence of FCHE was 14.42%, and there was no relationship between herd size and FCHE. The data were imbalanced due to the number of herds and incidence of FCHE. Weights were then calculated and assigned to the data using R. Then the data were analyzed using the "survey" and "ordinal" packages in R. A chi-squared test was used to determine whether there was a difference between service sire breed types and having a FCHE. In addition, an ordinal model was fitted to the data to examine the relative risk ratio for beef and dairy service sires. There was a significant ($P < 0.05$) difference between dairy and beef service sires and the incidence of a FCHE. Based on the ordinal model, the relative risk of cows bred to beef service sires having a FCHE was 32.07% greater than that of cows bred to dairy service sires. The results of this study support further investigation into the effects of service sire type (dairy vs. beef) on dairy cow transition health and subsequent lactation parameters.

Key Words: dairy, beef, fresh cow health event

2619 Hub regulatory genes and their roles in bovine *Staphylococcus chromogenes* subclinical mastitis.

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Staphylococcus chromogenes (SC) is among highly prevalent coagulase negative staphylococcal pathogens found on Canadian dairy farms. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are post-transcriptional regulators of gene expression in response to disease pathogens. The aim of this study was to identify hub miRNAs and lncRNAs involved in the host response to SC sub-clinical mastitis (SCM). Transcriptome sequencing (smRNA Seq and RNA sequencing [RNA Seq]) was performed for milk somatic cells from 3 cows with SCM (high SCC of ≥350,000 cells/mL for ≥3 mo consecutively and positive to SC only) and 4 healthy cows (low SCC <100,000 cells/mL for ≥3 mo consecutively and negative for mastitis pathogens). We used RNASeq and smRNASeq nf-core bioinformatics pipelines to process the data. Competing endogenous networks of co-expressed differentially expressed mRNAs (DEGs), differentially expressed miRNAs (DEM), and differentially expressed lncRNAs (DEL) were constructed and CytoHubba (a Cytoscape plugin) was used to identify the top hub genes (novelLncRNA_2117, *LOC112448783*, bta-miR-320a, *H2B_2*, *HIST1H2AH*, bta-miR-141, and so on) involved in SCM. Downregulated bta-miR-141 and upregulated novelLncRNA_2117 were the main hubs that potentially regulated many DEGs (e.g., CXCL8, NFKB1, ICAM1, JAK2, FCGR3A) during SCM. Functional analysis of their target genes revealed enrichment in several Kyoto Encyclopedia of Genes and Genomes pathways (e.g., TNF signaling pathway, NF-κB signaling pathway and cytokine-cytokine receptor interaction pathway) and biological process gene ontology terms (e.g., leukocyte activation, inflammatory response, immune system development) involved in triggering inflammatory and immune responses. Overexpression of miR-141 was reported to reduce the levels of inflammatory cytokines involved in the development of neonatal sepsis in humans, suggesting roles during SCM. Thus, the hub DEMs and DELs and in particular bta-miR-141 and novelLncRNA_2117 may be key regulators of immune related genes and can serve as biomarker targets for the development of therapeutic and diagnosis tools for the management of SCM.

Key Words: *Staphylococcus chromogenes*, dairy cow, microRNA (miRNA) and long non-coding RNA (lncRNA)

2620 MicroRNA expression signature in cecal Peyer's patch and implication in host response to Johne's disease.

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Mycobacterium avium ssp. *paratuberculosis* (MAP) is the causative agent of Johne's disease (JD), a globally distributed contagious granulomatous gastroenteritis. Despite research efforts in recent years, the regulatory mechanisms governing host responses to JD remain unclear. The microRNA (miRNA) expression in cecal Peyer's patches (CPP) and their potential roles in the pathogenesis of JD was investigated. Canadian Holstein cows (mid-late lactation, culled/nonlactating at euthanasia) with 3 distinct MAP infection conditions were studied: 4 MAP-infected (MAPI; MAP+/+), 4 MAP-resistant (MAPR; MAP+/−), and 4 healthy control (HC; MAP−/−) cows. The CPP tissues were collected for miRNA transcriptome sequencing, differential expression analysis, and functional exploration with standard bioinformatics tools. A total of 311 miRNAs (includes 60 novel miRNAs) were identified in CPP, including highly expressed bta-miR-143, bta-miR-145, bta-miR-378, and bta-miR-100. Comparative analysis (MAPI vs. HC, MAPR vs. HC, and MAPI vs. MAPR) revealed 39, 21, and 11 differentially expressed ($P < 0.05$) miRNAs (DEMs), respectively. Among the DEMs, bta-miR-2422 (log2FC = −5.82), bta-miR-2285u (log2FC = −4.90) and novel miR-chr8_75928628 (log2FC = −6.24) were the most downregulated DEMs in the 3 comparisons, respectively. Bta-miR-200a was the most upregulated DEM in MAPI versus HC (log2FC = 5.01) and MAPR versus HC (log2FC = 5.27) comparisons, whereas bta-miR-150 showed the most upregulated expression in MAPI compared with MAPR group. Enrichment analysis of target genes of DEMs revealed involvement in numerous Gene Ontology terms and Kyoto Encyclopedia of Genes and Genomes pathways related to the immune response (e.g., immune response, regulation of immune system processes, leukocyte activation, and defense response), and response to absorption and metabolism disorders (e.g., biosynthetic, metabolic, and developmental processes), suggesting regulatory roles of the DEMs in CPP in response to MAP infection. In summary, the miRNA expression profile of CPP revealed distinct DEMs during JD, suggesting their potential involvement in the regulation of host responses to MAP infection.

Key Words: paratuberculosis, Holstein, miRNA and immune functions

2621 Forecasting early-lactation diseases in Holstein dairy cows using milk spectra and machine learning.

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Fourier-transform infrared (FTIR) spectroscopy offers a non-invasive and cost-effective method for analyzing milk composition, but its potential in forecasting early-lactation diseases has yet to be fully explored. We aimed to uncover the ability of milk FTIR spectra to forecast postpartum diseases in 1,114 Holstein cows from a dairy farm in New York. We collected proportional milk samples once daily on all early lactation cows and stored milk at 4°C until analysis via FTIR. Cows were followed through 30 DIM and classified as healthy (n = 825; no adverse health events) or diseased (n = 289; diagnosis of clinical ketosis, metritis, displaced abomasum, or mastitis). We constructed predictive models for 8 distinct time periods before disease diagnosis (>14 d, 14–11 d, 10–8 d, 7–6 d, 5–4 d, 3 d, 2 d, and 1 d) by employing machine and deep learning techniques and incorporating milk spectra and cow-level variables including milk yield, somatic cell count, and parity. Model performance was evaluated based on accuracy (Ac), sensitivity (Se), and specificity (Sp) under a combined scheme of multiple downsampling and 10-fold cross-validation. As disease progressed, critical spectral regions related to the absorbance of fat, protein, and lactose exhibited progressive changes. This enhanced the average Ac of 0.52, Se of 0.49, and Sp of 0.55 at >14 d before disease diagnosis to an Ac of 0.73, Se of 0.70, and Sp of 0.77 at 1 d before disease diagnosis. The inclusion of cow-level variables into the spectra-based models resulted in an average increase of 7.4%, 9.0%, and 5.9% in Ac, Se, and Sp. Deep learning models demonstrated their superiority with an average Ac of 0.76, Se of 0.75, and Sp of 0.77 across the 8 distinct time periods, outperforming the baseline partial least squares discriminant analyses which averaged an Ac of 0.60, Se of 0.52, and Sp of 0.68. These results highlight the opportunity to use milk FTIR spectra and cow-level variables to forecast health conditions and enable timely management interventions and improve the overall efficiency of modern dairy farms.

Key Words: Fourier-transform infrared spectroscopy, machine learning, deep learning

2622 Increased serum prostaglandin E2 concentrations during inflammation correlate with plasma triacylglycerides Δ9 desaturase index and milk fatty acid chain length.

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The objective of this study was to investigate the link between inflammatory lipid mediators and lipids in plasma and milk in dairy cows. Prostaglandin E2 (PGE_2) is a potent inflammatory mediator induced during mastitis and derived from polyunsaturated fatty acids (PUFA). Fatty acid desaturase enzymes are responsible for the biosynthesis of most PUFA, and the Δ9 desaturase index (Δ9 DI) is a measure of stearyl-CoA desaturase activity and biomarker of metabolic diseases. We hypothesized that Δ9 DI in plasma triacylglycerides (TAG) would influence serum PGE_2 and milk fatty acid composition during mastitis. A total of 10 Holstein or Holstein × Jersey crossbreed cows were placed in 2 groups. One group received an intramammary infusion of *Escherichia coli* O55:B55LPS (50 μg/quarter) in one udder half and saline in the other half. The other group received a saline infusion in one udder half. Blood (tail vessel) and milk were collected at 0, 3, 6, 12, and 24 h. Serum PGE_2 concentration was determined by ELISA. Plasma TAG were extracted from total lipids by solid-phase extraction. Plasma TAG and milk fatty acid composition was determined by gas-liquid chromatography. Pearson's r and mixed-model ANOVA (time [fixed] × group [fixed] × cow [random]) were computed using JMP pro 16. The LPS-infused cows had increased serum PGE_2 concentrations compared with control (68.4 ± 7.1 pg/mL vs. 45.7 ± 6.9 pg/mL, $P = 0.02$). Serum PGE_2 concentration correlated with Δ9 DI of plasma TAG for 14:1 9*c*/14:0 (0.45, $P < 0.001$), 18:1 9*c*/18:0 (0.52, $P < 0.001$), and 18:2 9*c*,11*t*/18:1 11*t* (0.43, $P = 0.009$). Proportion of milk fatty acids with chain length >16 carbons correlated with serum PGE_2 (0.39, $P < 0.01$) regardless of if milk was from LPS- or saline-infused quarter. However, plasma TAG Δ9 DI did not correlate with milk fatty acids chain length. In summary, serum PGE_2 concentrations were moderately associated with plasma TAG Δ9 DI and proportion of milk fatty acids >16 carbons. This indicates that systemic PGE_2 may be implicated in milk lipid changes during mastitis through a mechanism independent from systemic Δ9 desaturase activity.

Key Words: prostaglandin E2 (PGE2), triacylglyceride (TAG), SCD

2623 The effect of treating hyperketonemic cows with different rumination behavior with propylene glycol on milk yield.

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The objective of this randomized clinical trial was to evaluate the effect of propylene glycol treatment on milk yield during the first 18 wk of lactation in cows with hyperketonemia (HYK), along with accounting for differences in their rumination behavior during the first week of lactation. We hypothesized that, depending on the rumination activity of the cows, the propylene glycol would have a different effect on milk yield. Multiparous Holstein cows (n = 573) from a single commercial farm in Colorado were screened for HYK (blood BHB ≥1.2 mmol/L) at 5 d in milk (DIM) between February and April of 2023. Cows with HYK (n = 106; 18.5% of all cows) were randomly assigned to receive (TRT; n = 62) or not (CON; n = 44) propylene glycol (PG; 300 g administered orally for 3 consecutive days). Enrolled cows were blocked by parity (2, 3+) and rumination behavior (SenseHub Dairy, Merck Animal Health) was retrospectively calculated based on the average daily rumination time (RT) during the first wk postpartum. Rumination behavior was further dichotomized based on the median (447 min/d and 426 min/d for second and >third, respectively) RT into low (LRT), and high (HRT) RT. The effect of treatment on milk yield over the first 18 wk of lactation was assessed using a mixed-effect model accounting for repeated measures within each cow. The final model included treatment group ($P = 0.16$), rumination group ($P < 0.01$), wk postpartum ($P < 0.01$), parity ($P = 0.28$), BHB concentration at enrollment ($P = 0.18$) and an interaction between treatment and rumination group ($P = 0.39$) as fixed effects. In the LRT group milk yield was 2.2 kg/d lower ($P = 0.16$) for the untreated cows (TRT = 49.9 kg/d; 95%CI: 47.9, 51.8 vs. CON = 47.7 kg/d; 95%CI: 45.3, 50.1). No evidence of a difference ($P = 0.96$) was observed in the HRT (TRT = 52.2 kg/d; 95%CI: 49.4, 54.7 vs. CON = 52.1 kg/d; 95%CI: 48.9, 55.1). The findings from this exploratory

study suggest that hyperketonemic cows with LRT during the first wk postpartum benefit the most from PG treatment, whereas administering PG to hyperketonemic cows with HRT in the first wk postpartum did not result in greater yield.

Key Words: ketosis, BHB, rumination time

2624 Prevention of digital dermatitis using bacterial complex-based solution. R. Guatteo[1], R. Chinon-Goron[1], G. Scimia[2], M. Galais*[2], and A. Brame[2], *[1]Oniris, INRAE, BIOEPAR, Nantes, France, [2]Mixscience, Bruz, France.*

Lameness, especially digital dermatitis (DD), is a major threat in dairy farms worldwide. The objective of this study was to assess the effect of a bacterial complex-based solution applied directly to the feet of cows compared with no treatment in preventing the occurrence or reducing the persistence of active DD lesions. This trial was carried out in 5 dairy herds for a total of 640 dairy cows. The statistical unit of interest was the foot. To reduce the sample size required and to limit the herd effect, it was decided to consider the cow as its own control. In each farm, the left foot of the animals received an application of the bacterial complex once a week (without pre-washing the foot) for 6 mo, and the right foot was left untreated and served as a control. Every month, the presence of any DD lesions (based on the M5 scoring system) was checked in the milking parlor using a swiveling mirror. The effect on the occurrence or persistence of DD lesions was assessed using survival analysis methods and the Cox model. In the model, the farm, the presence of a contralateral lesion, and the stage of lactation are included as covariates. The mean initial prevalence of DD lesions was 32.6% and 32%, respectively, for the right and left feet. First, our findings demonstrated that treated feet had a significantly lower incidence (HR = 0.77, 95% CI = 0.62–0.96) than untreated feet. Besides the application of the bacterial complex, the presence of a contralateral lesion and the stage of lactation (>150 DIM) also significantly increased the risk of a new DD lesion. The study showed that the application of the bacterial complex significantly delayed the occurrence of DD lesions ($P > 0.05$). Indeed, 50% of healthy feet at the start of the study developed a DD lesion after 90 d for untreated feet and 150 d for feet treated with the bacterial complex. Furthermore, no effect on the persistence of DD lesions was found. To our knowledge, this is the first study investigating and reporting a significant preventive effect on the occurrence of digital dermatitis lesions in dairy cows based on the use of a complex of bacteria directly applied to the feet.

Key Words: digital dermatitis, bacteria, dairy cow

2625 Associations of transition period stressors with inflammation, liver health, and milk production in early-lactation dairy cows. C. Wagemann-Fluxá[1], S. J. Leblanc[2], E. S. Ribeiro[1], and T. J. DeVries*[1], *[1]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, [2]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

This study identified associations between stressors during the transition period and inflammation, liver health, and milk production in early-lactation cows on commercial dairy farms in Ontario, Canada. From April 2022 to October 2022, 40 farms (190 ± 137 milking cows, mean ± SD) were visited 6 times (once/4 wk). Management practices were surveyed. On each visit, blood was collected from up to 12 fresh cows (n = 1,540) within 14 DIM (7.1 ± 4.3 d) to measure haptoglobin (HAP) and calculate a liver health index (LHI) based on albumin, cholesterol, and total bilirubin concentrations. Dairy herd information milk production data were collected for each enrolled cow up to 120 DIM. Herd-level averages of these parameters were calculated for each farm and visit. Mixed-effect linear regression models were used to identify associations of stressors with HAP (0.74 ± 0.49 g/L), LHI (−0.17 ± 1.60), milk yield (43.4 ± 6.81 kg/d), milk fat content (4.08 ± 0.53%) and yield (1.8 ± 0.32 kg/d), and milk protein content (3.14 ± 0.21%) and yield (1.4 ± 0.21 kg/d). Housing prepartum multiparous and primiparous cows separately as compared with together was associated with higher milk fat yield (+0.1 kg/d; $P = 0.02$) and tended to be associated with higher milk yield (+1.9 kg/d; $P = 0.07$), lower HAP (−0.10 g/L; $P = 0.05$), and a better LHI (+0.28; $P = 0.09$). A better LHI was also associated (+0.31; $P = 0.01$) with housing cows in 1 pen during the first 14 DIM as compared with 2 pens. Regrouping prepartum cows multiple times per week as compared with every 2 wk or less was associated ($P < 0.001$) with higher HAP (+0.27 g/L) and lower milk (−4.9 kg/d) and milk fat (−0.2 kg/d) yield. A higher milk fat content was associated with moving cows in groups into the prepartum pen as compared with individually (+0.30 percentage points; $P < 0.001$). Housing cows in a single dry cow pen as compared with having a far-off and close-up pen was associated with a lower milk protein yield (−0.2 kg/d; $P < 0.001$) and tended to be associated with a lower milk protein content (−0.07 percentage points; $P = 0.08$). To conclude, minimizing housing and management stressors for transition cows may improve health and production in early lactation.

Key Words: stress, metabolic marker, health

2626 Impact of automatic milking system on prevalence of subclinical mastitis-causing pathogens. K. F. Nogara[1], M. Busanello[2], L. de Moura Pereira*[1,3], L. L. Baréa[4], J. Lovatel[4], J. Eidt[4], and M. Zopollatto[4], *[1]Federal University of Paraná, Curitiba, Parana, Brazil, [2]Integrated University, Frederico Westphalen, Brazil, [3]University of Idaho, Moscow, ID, [4]Lácteos Vacaria Farm, Vacaria, Rio Grande do Sul, Brazil.*

We aimed to study the association between subclinical mastitis (SCM)-causing pathogens and automatic milking systems (AMS) from Lely and DeLaval. Over 4 years (2020–2023), 1,502 SCM cases were recorded on a farm in Rio Grande do Sul State, Brazil, which had both Lely (n = 4) and DeLaval (n = 4) AMS. Each set of 4 AMS from each brand was placed in a compost barn, with ~58 cows/AMS. Microbiological culture was done following AMS udder-health alerts. The microbiological data on SCM-causing pathogens comprised negative culture (42%), *Staphylococcus chromogenes* (13%), *Staphylococcus aureus* (9%), *Staphylococcus haemolyticus* (7%), *Staphylococcus warneri* (6%), other non-*aureus* staphylococci (NAS) (6%), other prevalent pathogens (6%), *Escherichia coli* (4%), *Klebsiella* spp. (4%), and *Corynebacterium bovis* (3%). The AMS-pathogen associations were assessed via chi-squared analysis at a 5% significance level (SAS PROC FREQ). A significant association between the SCM-causing pathogens and AMS was found ($P < 0.0001$). Results indicated 545 SCM cases for Lely and 325 for DeLaval AMS. The AMS from Lely had higher cases of *C. bovis* (83%), *S. aureu*s (81%), and *E. coli* (78%), whereas higher cases of NAS (62%) were found in AMS from DeLaval. *Corynebacterium bovis*, NAS, and *S. aureus* are mainly spread during milking. *Staphylococcus aureus* is a major cause of contagious mastitis in Brazil, leading to a great decrease in milk yield. AMS-milked cows face heightened teat exposure to equipment with increased milking frequency, posing a risk for contagious pathogens. The AMS differ in teat cleaning methods: Lely's AMS uses brushes for teat cleaning, removing foremilk in the milking flow cup. DeLaval's AMS employs circular teat cleaning with warm water, using an exclusive disinfection cup for foremilk removal. The higher prevalence of contagious pathogens in Lely AMS could possibly be linked to inadequate pre-milking teat disinfection with brushes. The NAS group was more prevalent in DeLaval AMS, though not all species within it are considered opportunistic. Our study suggests a possible link between the AMS cleaning system and the spread of pathogens causing SCM within the herd.

Key Words: inflammation, robotic dairy, udder health

2627 Inclusion of oxalic acid in enzyme-based BHB assays mitigates signal interference from lactate dehydrogenase activity. A. E. Mark*[1], B. L. Hiltz[1], A. R. Bujold[3], and A. H. Laarman[1,2], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*University of Idaho, Moscow, ID,* [3]*Public Health Agency of Canada, Winnipeg, MB, Canada.*

In ruminant science, blood β hydroxybutyrate (BHB) is considered the gold-standard test for ketosis. In these gold-standard tests, blood BHB concentration is often quantified with enzymatic assays which detect NADH directly or an NADH-based indicator as proxy for BHB. Other blood-borne enzymes, such as lactate dehydrogenase (LDH), also produce NADH, leading to potential signal interference with BHB-produced NADH. This study aimed to explore the inclusion of oxalic acid (a competitive LDH inhibitor) in blood BHB assays using NADH- (N), NADH-based dye (Nd), and thio-NADH- (tN) based detection. Two pooled plasma samples of 10 cows, collected either 1 week pre-calving (PRE) or 1 to 3 weeks post-calving (POST), were analyzed by commercial N, tN, and Nd-based assays and one in-house N (IH-N) assay. All methods were performed in biological triplicate; data were analyzed in PROC GLIMMIX using method, replicate, and inhibitor as fixed effects. Inclusion of oxalic acid at 100 m*M* in IH-N assay buffer did not affect the linearity of dilution ($P = 0.87$). Across all assay types and samples, oxalic acid decreased reported BHB ($P < 0.01$). Unexpectedly, assay type and an assay type × sample interaction ($P < 0.01$ and <0.01) led to different reported BHB values from the same PRE and POST samples ($P < 0.01$). For POST samples, all assay types reported hyperketonemia (>1.2 m*M* BHB); for PRE samples, 2 assay types were above this threshold and 2 were below. When a combination of PRE and POST plasma was spiked with 6.25 or 12.5 m*M* lactate, the addition of oxalic acid prevented NADH signal increase ($P = 0.01$ and <0.01, respectively) and restored reported BHB to the same level as the unspiked sample ($P = 0.99$) in the IH-N assay. Overall, these data show that the inclusion of oxalic acid in BHB assays is effective against interference by LDH. Inconsistencies among assay types highlight a need for further validation of BHB assays.

Key Words: ketosis, BHB, assay development

2628 Exploring leukocyte diversity through single-cell RNA sequencing analysis in peripartal dairy cows. J. Halfen*[1], A. F. S. Lima[1], G. P. Schettini[1], H. Ford[2], C. Strieder-Barboza[2], and J. S. Osorio[1], [1]*School of Animal Science, Virginia Tech University, Blacksburg, VA,* [2]*School of Veterinary Medicine, Texas Tech University, Amarillo, TX.*

This study aimed to uncover differences in cellular diversity of leukocytes in dairy cows transitioning from late pregnancy to lactation using single-cell RNA sequencing technology. Four multiparous Holstein dairy cows were selected for this study, and they were subjected to the same management practices, diet, and facilities. Blood samples were collected from the coccygeal vein in the morning before the feeding at −10 ± 3.2 (PRE) and 7 (POS) days relative to parturition. The immune cell isolation, cryopreservation, and resuspension were performed following the 10x Genomics Single Cell RNA Sequencing (scRNA-Seq) protocol for peripheral blood mononuclear cells. Cells were subjected to Chromium Next GEM Single Cell 3′, targeting 10,000 cells recovery. Gene expression libraries for each sample were performed using NovaSeq 6000. Fastq files were used to generate count matrices using the CellRanger Pipeline 10X Genomics. Data were analyzed in RStudio (v12.1) with scRNA-Seq package Seurat v3.1.4. Clusters were identified based on 4 samples from PRE and 3 from POS, resulting in 14 clusters from 22,346 cells. Out of those cells, 10,219 were from PRE and 12,127 from POS. Expression of signature genes was used to recluster cells according to specific cell types, including B cells, CD4 T, and CD14+Mono. Overall, the analysis for CD14+Mono revealed 231 genes differentially expressed between PRE and POS (*FDR* ≤0.05, log2FC >1), where 187 were upregulated PRE and 43 POS. Cluster analysis on CD14+Mono cells revealed 7 subclusters, with clusters 3 and 6 being more prominent in POS than PRE. The 3 most expressed genes in cluster 3 POS were *GPN1*, *CUL4B*, and *TOR1A*, which are involved in cell cycle regulation, DNA damage repair, and transcriptional regulation. Cluster 6 POS top genes were *NELFE*, *GLRX3*, and *ACAP3*, which are related to transcriptional regulation, cellular redox homeostasis, and membrane trafficking. Immunosuppressed conditions such as early lactation in dairy cows may lead to variations in leukocyte diversity. Our initial scRNA-seq analysis showed alterations in monocyte populations, which may represent a coping mechanism in peripartal dairy cows.

Key Words: single-cell, gene expression, immunity

2629 Epidemiology of energy balance associated metabolic disorders. R. S. Pralle*[1,2], O. E. Adams[1], E. M. Kammann[2], N. S. Jozik[1], and H. M. White[2], [1]*School of Agriculture, University of Wisconsin–Platteville, Platteville, WI,* [2]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The objective of this epidemiology study was to assess the impacts of metabolic disorder (MD) on cow performance. On 3 dairy farms, 501 multiparous Holstein cows had blood plasma analytes quantified at 7 and 14 ± 1 DIM. Cows were diagnosed with MD cases based on analyte thresholds: high fatty acid (HFA) when fatty acids ≥0.7 mEq/L, hyperketonemia (HYK) when β-hydroxybutyrate ≥1.2 mmol/L, and hypoglycemia (HPG) when glucose <40 mg/dL. Treatment of HYK and other illnesses were at the farm's discretion. Milk production, morbidity, and reproduction data were retrieved from farm electronic records. The milk production data were analyzed by linear mixed models (SAS, v.9.4) with fixed effects of MD, parity group (PG), test-day (TD), and all interactions; a random effect of farm and repeated measures of cow were included. Morbidity data were analyzed by generalized estimating equations with fixed effects of MD, PG, and farm; interactions were tested stepwise for inclusion, but none were retained ($P > 0.10$). Effects are reported as mean differences ± SE of the difference or relative risk ratios (RR). Incidence of HFA, HYK, and HPG was 42%, 28%, and 33%, respectively. First TD milk yield was lower for HYK vs. non-HYK cows (−4.2 ± 1.1 kg/d, $P < 0.01$, MD × TD). Second parity cows with HFA had greater milk yield than second parity non-HFA cows (3.2 ± 1.3 kg/d, $P = 0.05$, MD × PG), but no other PG had differences ($P > 0.10$). Greater milk energy output (1.4 ± 0.7 Mcal/d) was observed for HPG vs. non-HPG cows ($P = 0.04$). Displaced abomasum risk was greater for HFA ($P = 0.02$, RR = 10) and HYK cases ($P = 0.01$, RR = 23). Metritis risk was greater for HYK cows ($P < 0.01$, RR = 3.7) and lower for HPG cows ($P = 0.01$, RR = 0.31). Cow survival to 30 DIM was lower for HYK cows ($P = 0.04$, RR = 0.95). First service conception rate (FSCR) was lower for HYK ($P = 0.01$, RR = 0.74) and HPG cows ($P = 0.07$, RR = 0.82). The count of economically adverse associations was more for HYK (n = 5) than HFA (n = 1) and HPG (n = 1), whereas HPG had more favorable associations (n = 2) than HFA (n = 1) and HYK (n = 0). Paradoxically, HPG cases had greater production but lower FSCR. In this data, HYK cases had more detrimental outcome associations than HFA and HPG.

Key Words: ketosis, NEFA, transition cow

2630 Effect of heat stress and electrolyte treatment on immune status of lactating dairy cows.

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The objective was to test the effect of heat stress and dietary electrolytes on the immune status of lactating dairy cows. A total of 28 multiparous and 24 primiparous early lactation cows were blocked by parity and milk yield and assigned to a pen with cooling (CL, n = 26) provided by fans and soakers or no cooling (HT, n = 26). Within each pen, cows were randomly assigned to dietary treatment with Bovine BlueLite® (BL; TechMix LLC, Stewart, MN) or no BL (CON), resulting in 4 treatments (n = 13/treatment). Cows were housed in a sand-bedded freestall barn equipped with Calan gates for individual feed intake. Cows were fed a total mixed ration, and 113 g of BlueLite was mixed in the ration daily for BL cows. Treatments were applied for 28 d. The average THI was 77.2 ± 1.3. Blood was sampled at 0, 14, and 28 d for leukocyte profiles and neutrophil function by flow cytometry. Continuous data were analyzed using mixed models with cows as the experimental unit to examine the effects of heat stress (HT vs. CL), diet (CON vs. BL), and their interaction. A significant interaction between cooling and day ($P < 0.01$) was observed for multiple leukocyte populations. The concentration of neutrophils in blood increased from d 14 to d 28 for HT cows (d 14 = 1,261 vs. d 28 = 1,381 ± 72 cells/mL) but decreased by day for CL cows (d 14 = 1,322 vs. d 28 = 1,176 ± 74 cells/mL). In contrast, the concentration of lymphocytes in blood decreased from d 14 to d 28 for HT cows (d 14 = 2,712 vs. d 28 = 2,150 ± 320 cells/mL), but not for CL cows (d 14 = 2,597 vs. d 28 = 2,719 ± 327 cells/mL). Neutrophils, lymphocytes, and monocytes of HT cows expressed more ($P < 0.01$) L-selectin (CD62L) compared with CL cows. Neutrophils of HT cows had greater ($P < 0.01$) oxidative burst intensity compared with CL cows. The BL treatment increased ($P = 0.02$) the concentration of neutrophils in blood (CON = 256 vs. d 28 = 307 ± 11 cells/mL) but decreased ($P = 0.01$) the intensity of the oxidative burst of neutrophils compared with CON. In conclusion, heat stress and electrolytes altered peripheral blood leukocyte function.

Key Words: heat stress, electrolyte, immunity

2631 Using supervised machine learning algorithms to predict bovine leukemia virus seropositivity in dairy cattle in Florida: A 10-year retrospective study.

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Supervised machine-learning (SML) algorithms are potentially powerful tools that may be used for screening cows for infectious diseases such as bovine leukemia virus (BLV) infection. Here, we compared 6 different SML algorithms to identify the most important risk factors for predicting BLV seropositivity in dairy cattle in Florida. We used a data set of 1,279 dairy blood sample records from the Bronson Animal Disease Diagnostic Laboratory that were submitted for BLV antibody testing from 2012 to 2022. The SML algorithms that we used were logistic regression (LR), decision tree (DT), gradient boosting (GB), random forest (RF), neural network (NN), and support vector machine (SVM). A total of 312 serum samples were positive for BLV with corrected seroprevalence of 26.0%. The RF model was the best model for predicting BLV seropositivity in dairy cattle indicated by the highest Kolmogorov-Smirnov (KS) statistic of 0.75, area under the receiver operating characteristic (AUROC) of 0.93, gain of 2.6; and lowest misclassification rate of 0.10. The results indicate a high accuracy of prediction. The RF model showed that age and region were the most important predictors for BLV seropositivity. More specifically, dairy cows aged ≥5 yr raised in southern Florida were more likely to test positive for BLV. We conclude that SML algorithms hold promise for predicting BLV seropositivity in dairy cattle, which could allow for the development of screening tools for BLV infection.

Key Words: supervised machine learning algorithm, bovine leukemia virus, dairy cattle

2632 Comparison of antimicrobial usage using online benchmarking software or inventory of discarded drug packaging.

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Antimicrobial usage (AMU) measurements are useful for setting goals for animal health but requires the ability to summarize data recorded using diverse dairy management programs. The aim of this study is to compare AMU estimated using benchmarking software to an inventory of discarded drug packaging. Dairy farms (MI = 12; OH = 9) recording >90% of antibiotic treatments in dairy management software were enrolled for 4 mo. In the first and fourth months, researchers estimated AMU using online benchmarking software (https://dairyantibioticbenchmar.msu.edu). Containers were provided for workers to discard drug packaging during the 4 mo and researchers visited regularly to inventory discarded packaging. Estimates of AMU were expressed as defined daily doses/cow per yr (DDD). The Kruskal-Wallis test was performed to determine if DDD varied by month or method and to determine if the proportion of primiparous cows or bulk tank SCC (BTSCC) were associated with AMU. Herds contained a median of 1,321 cows (range:

Table 1 (Abstr. 2632). Defined daily doses/cow per year estimated by the benchmarking software and inventory of drug packaging (n = 21 herds)[1]

	Total		IMM lactation		IMM dry-off		Injectable	
Method	Mean	SE	Mean	SE	Mean	SE	Mean	SE
SM1	4.42	0.62	0.84	0.33	2.14	0.24	1.44	0.32
SM4	4.34	0.55	0.81	0.25	2.14	0.23	1.39	0.29
Inventory	4.86	0.78	0.93	0.31	2.27	0.31	1.66	0.44
P-value	0.99		0.96		0.95		0.99	

[1]IMM = intramammary; SM1 = estimate from software for mo 1; SM4 = estimate from software for mo 4; inventory = drug packaging inventory.

246–8,389), ~36% of cows were primiparous (range: 24–48%), and the average BTSCC was 160,000 (± 11,435). Total DDD did not vary between the first and fourth months ($P = 0.74$) nor between estimates from the benchmark software and the inventory ($P = 0.33$). Intramammary treatments at dry off (73%) and for clinical mastitis (23%) accounted for most DDD. The number of DDD decreased as the proportion of primiparous cows increased ($R^2 = 0.30$; $P = 0.006$) but was not associated with BTSCC ($P = 0.92$). Estimates of AMU from the software were consistent over 4 mo and did not vary from estimates generated by the inventory (Table 1). Increased proportions of primiparous cows are associated with less AMU on dairy farms.

Key Words: defined daily dose, antimicrobial, cow

2633 Effect of administration of mineral supplement tablets on postpartum beta-hydroxybutyrate levels in dairy cows.

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Elevated blood β-hydroxybutyrate (BHB) levels has been associated with impaired health and performance of dairy cows. Aiming to investigate improvements in transition cow performance, a randomized controlled trial was conducted in 2 commercial dairy farms to assess the effect administering a mineral supplement tablet on postpartum BHB levels. Holstein cows were enrolled at 14 ± 3 d before the expected calving date and randomized into 2 groups: the treatment group (TRT; n = 165) received a single mineral supplement tablet (AHV Booster, AHV International) at enrollment followed by another during the first week of lactation (6 ± 3 DIM), and the control group (CON; n = 160) had no tablet administration. The TRT and CON groups were composed, respectively, of 32% and 34% primiparous cows, 31% and 28% second lactation cows, and 37% and 38% third or greater lactations cows. Blood samples were collected at 6 ± 3 DIM (wk 1) and 14 ± 3 DIM (wk 2) to measure blood BHB levels using a hand-held device (Precision Xtra, Abbott Laboratories). Cows with BHB ≥1.2 mmol/L were considered to have hyperketonemia (HYK+), otherwise non-hyperketonemic (HYK−). The outcomes observed were BHB levels and HYK status in the first 2 weeks postpartum, with the association between treatment and the outcomes assessed using mixed-effect linear regression and logistic regression, respectively. Mean BHB concentrations were 0.70 mmol/L (95% CI: 0.64, 0.76) for TRT cows and 0.77 mmol/L (95% CI: 0.70, 0.82) for CON cows ($P = 0.11$) in wk 1. By wk 2, the mean BHB concentration was not significantly different ($P = 0.59$) for TRT (0.74 mmol/L; 95% CI: 0.66, 0.81) and CON (0.78 mmol/L; 95% CI: 0.69, 0.87) cows. In wk 1, 7.4% and 9.6% of cows were HYK+ in the TRT and CON groups, respectively (risk ratio: 0.79; 95% CI: 0.40, 1.49; $P = 0.47$). In wk 2, 7.3% and 11.3% of TRT and CON cows were HYK+, respectively (risk ratio: 0.63; 95% CI: 0.27, 1.41; $P = 0.26$). Despite numerical differences, no statistically significant differences were observed in BHB levels or occurrence of HYK+ between treatments in the first 2 weeks postpartum.

Key Words: ketosis, transition cow management, bolus administration

2634 Associations of blood metabolites and postpartum disorders with failed resolution of uterine inflammation in dairy cows.

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This retrospective observational study aimed to identify factors associated with resolved or persistent endometritis. We used data from Holstein cows without dystocia or retained placenta (n = 240) from a larger observational study in 2 dairy herds in Ontario, Canada. Serum calcium (Ca) and haptoglobin (Hp) were measured at d 2 and 6 (±2). Metritis was diagnosed at d 3, 6, 10, and 14 (±2), and purulent vaginal discharge (PVD) at d 25 (±3) postpartum, using a Metricheck device. Metritis and PVD were defined as brown or reddish fetid discharge with or without fever and >50% pus (score 3) at Metricheck examination, respectively. A cytobrush technique was used to assess the proportion of polymorphonuclear cells (PMN) and determine uterine inflammation at d 25 and 45 (±3). Cows were classified into 3 groups: no endometritis, PMN <6% at 25 d and <4% at 45 d (NE; n = 111); resolved endometritis, PMN ≥6% at 25 d and <4% at 45 d (RE; n = 70); and persistent endometritis, PMN ≥6% at 25 d and ≥4% at 45 d (PE; n = 59). Differences in serum metabolites between groups were tested with univariable linear regression models. Data were log-transformed and presented as back-transformed means with 95% CI. Associations of categorical predictors with uterine inflammation groups were assessed with the Pearson chi-squared test and reported as odds ratio (OR) with 95% CI. Serum Hp in wk 1 tended to be greater in PE than RE (0.40 [0.36–0.46] vs. 0.31 [0.27–0.38] g/L, $P = 0.06$) and was greater than NE (0.29 [0.26–0.31] g/L, $P < 0.01$). Serum Ca in wk 1 tended to be lesser in PE than NE (2.11 [1.99–2.18] vs. 2.19 [2.14–2.24] mmol/L, $P = 0.07$). Cows had greater odds of PE than NE when they had metritis (OR: 6.6 [2.1–18.7], $P < 0.01$) or PVD (OR: 3.5 [1.6–7.8], $P < 0.01$), but these were not associated with RE vs. PE. These results show that cows with greater Hp postpartum despite uncomplicated calving are less likely to resolve uterine inflammation, and that metritis or PVD are associated with PE. Additional investigation of initiation and resolution of endometritis will inform prevention and treatment.

Key Words: endometritis, inflammation, resolution

2635 Relationship between blood β-hydroxybutyrate and blood markers of liver and kidney function, previous and current milk production variables in multiparous Holstein cows in the first 21 days in milk.

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Blood glucose (GL), current fresh period milk yield, and previous lactation milk yield have been suggested to predict blood BHB. The objective of this prospective observational study was to examine if blood GL, albumin (ALB), alkaline phosphatase, aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total proteins (TP), globulins (GLB), BUN, creatinine kinase (CK), phosphorus, parity, DIM, previous DIM (pDIM), previous days dry, previous days in closeup, previous days to conception (pDTC), current 305 d mature equivalent milk production (ME305), previous lactation 305 d mature equivalent milk production, previous lactation total milk (pTMilk), previous lactation total protein (pTPro), previous lactation total fat (pTFat), and total fresh milk were associated with BHB in the first 21 d of lactation. Blood was collected via the coccygeal vein from 108 Holstein multiparous cows at a commercial dairy in Tulare, CA, once a week for 3 weeks from June 23, 2011, to August 20, 2011, and analyzed for whole blood GL and BHB using Precision Extra meters. Milk data were harvested from the dairy's herd management software. Blood variables were estimated using an Abaxis VetScan Large Animal Profile rotor. Data were analyzed using general linear models (SAS v. 9.4, SAS Institute 2024) with BHB as the dependent variable and repeated measures listed previously. Residuals were normally distributed. Independent variables with $P > 0.05$ were eliminated. The final model included the independent variables GL, parity, ME305, pTMilk, pTPRO, pDTC, ALB, AST, GGT, TP, GLB, and CK, with $R^2 = 0.54$. If only variables that were easily available from farm records were considered, $R^2 = 0.43$, and GL, parity, pDIM, ME305, pTFat, and pTPro were included in the final model. These results indicate that GL, ME305, and pTPro could be important predictors of BHB, liver and kidney function are associated with BHB levels and dairy farm production records particularly in the previous lactation could be used to predict BHB.

Key Words: blood glucose, milk yield, milk protein

2636 Prepartum acetylsalicylic acid in over-conditioned and nulliparous cows: Effects on reproductive performance and milk components.

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The objective of this study was to assess the effects of prepartum administration of acetylsalicylic acid (NSAID) on the reproductive performance and milk components in high-priority cow groups (i.e., over-conditioned cows [OVERC, BCS ≥3.75 pts.]; nulliparous cows [NUL]). At −21 ± 3 d relative to calving (d.r.c), cows were blocked by parity (NUL, n = 159; parous [PAR], n = 400) and body condition score (OVERC, n = 403; optimal conditioned [OPT], BCS 3–3.5 pts., n = 156) and randomly allocated to one of 2 treatment groups: (1) ASA (n = 283) in which cows received one oral administration with 4 boluses of acetylsalicylic acid (200mg/kg) at −14 ± 3 d.r.c.; or (2) PLC (n = 276) in which cows received one oral treatment with 4 gelatin capsules filled with water at −14 ± 3 d.r.c. Reproductive performance for 300 DIM and milk components from 10 DHIA tests were collected from on-farm computer records. The data were analyzed using MIXED and LIFETEST procedures of SAS as a randomized complete block design. Although cows treated with ASA tended ($P = 0.09$) to require fewer days to become pregnant compared with PLC cows, there was a treatment × parity interaction ($P = 0.03$), where NUL animals treated with ASA required fewer days to become pregnant compared with PLC-NUL cows (ASA-NUL = 116.7 ± 4.55 d; PLC-NUL = 133.37 ± 5.19 d). Moreover, the ASA-NUL group tended to have a higher percentage of cows that became pregnant at first service than the PLC-NUL group ($P = 0.10$; ASA-NUL = 62.5 ± 5.72%; PLC-NUL = 49.32 ± 5.88%). There was no difference between treatment groups in DHIA fat-corrected milk yield, milk fat percentage, and milk protein percentage. Interestingly, ASA cows tended to have higher SCC on the first DHIA test compared with PLC cows ($P = 0.10$; ASA = 57,960 cells, 95% CI = 49,602–67,173; PLC = 44,381 cells, 95% CI = 38,018–51,808). The results of this study suggest that prepartum ASA may be beneficial for improving the reproductive performance of high-priority dairy cows, without affecting milk yield or components.

Key Words: prepartum acetylsalicylic acid, reproductive performance, high-priority cow group

2637 Effects of postpartum acetylsalicylic acid administration and calcium supplementation on production, reproductive performance, and culling in Holstein dairy cows.

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This study aimed to assess the effects of postpartum acetylsalicylic acid (ASA) administration and calcium supplementation on production, reproductive performance, and culling in multiparous cows. Within 12 h after calving, cows were randomly allocated to one of 4 treatment groups: (1) ASA (n = 155), in which cows received 2 oral administrations with ASA (125 g/cow per d; four 480-grain aspirin boluses) 24 h apart; (2) ASACAL (n = 164), in which cows received 2 oral administrations with ASA and calcium (Ca; 43 g/cow per d; 2 Ca boluses) 24 h apart; (3) CAL (n = 171), in which cows received 2 oral administrations with Ca 24 h apart; and (4) UNT (n = 156), in which cows remained untreated. On-farm records were used to assess DHIA test data, reproductive performance, and culling for 300 DIM. The data were analyzed using MIXED, GLIMMIX, and LIFETEST procedures of SAS. Regarding fat-corrected milk, there was a test × treatment × parity interaction ($P = 0.03$). Second-lactation ASACAL cows produced 1.54 kg, 3.4 kg, 1.45 kg, and 1.27 kg less fat-corrected milk in DHIA tests 1, 2, 4, and 5, respectively, compared with second-lactation UNT cows. Conversely, third+ lactation ASACAL cows produced 3.42 kg and 3.53 kg more fat-corrected milk in DHIA tests 2 and 3, respectively, compared with third+ lactation UNT cows. Similarly, second-lactation ASACAL cows tended to produce 537 kg less ME305 milk compared with second-lactation UNT cows, whereas third+ lactation ASACAL cows tended to produce 443 kg more ME305 milk than third+ lactation UNT cows. Cows treated with ASACAL cows tended to require fewer days ($P = 0.10$; ASACAL = 101.77 ± 4.18 d; PLC = 111.45 ± 4.13 d) and fewer services ($P = 0.05$; ASACAL = 1.91 ± 0.13 services; PLC = 2.27 ± 0.13 services) to become pregnant compared with UNT cows. There was no difference in culling rate between treatment groups. These results suggest that postpartum acetylsalicylic acid combined with Ca may improve milk yield in third+ lactation cows, whereas it may be detrimental for second-lactation cows. Moreover, ASACAL treatment might positively affect fertility in multiparous cows.

Key Words: postpartum acetylsalicylic acid and calcium, fat-corrected milk, reproductive performance

2638 Differential effects of prepartum acetylsalicylic acid administration on microbiome composition and volatile fatty acid production in nulliparous and parous cows.

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Excessive systemic inflammation in cattle during the transition period is associated with an increased risk of metabolic and infectious diseases. Researchers have explored anti-inflammatory treatments, such as acetylsalicylic acid (ASA), showing consistent improvements in post-calving cow performance. However, research into mechanisms has focused on host metabolic and inflammatory physiology. We hypothesized that the impact of ASA on inflammation correlates with variations in rumen microbial composition, potentially identifying candidate bacteria as biomarkers of periparturient inflammation. Cows (nulliparous [NUL; n = 75]; parous [PAR; n = 130]) were randomly allocated to one of 2 treatment groups administered once orally at 14 ± 3 d relative to calving: (1) ASA: 4 boluses of ASA (200 mg/kg); or (2) placebo (PLC): 4 gelatin capsules filled with water. Rumen solids and liquids were collected before treatment, 28 h after treatment, and weekly for 3 weeks for microbial analysis from a random subset of cows (ASA, n = 41; PLC, n = 40). Samples of DNA were extracted from 17 NUL and 23 PAR cows and analyzed for bacterial diversity, and fatty acid (FA) levels were assessed using high-performance liquid chromatography. At the community level, all cows showed differences in bacterial community composition by sampling day ($P < 0.001$); however, ASA effects were noted only in NUL cows ($P = 0.013$ weighted s; $P = 0.002$ unweighted analyses). Although notable taxonomic differences, including *Prevotellaceae*, *Ruminococcaceae*, and *Succiniclasticum*, were observed between parity groups, ASA-NUL cows demonstrated a differential abundance of S*taphylococcus*. Firmicutes lineages were maintained, and ASA cows had reduced lactate concentrations (−2.533 ± 1.199 m*M*, $P = 0.037$) compared with controls. Other FA varied by sampling day. These findings suggest a complex interaction between prepartum ASA treatment, parity, and rumen microbiome composition, highlighting the potential for interventions to mitigate inflammation in high-priority cattle during the periparturient period.

Key Words: prepartum acetylsalicylic acid, rumen microbiome, rumen fatty acid

2639 Effects of an automated health monitoring versus visual observation on dairy cow profitability. C. Rial*[1], M. L. Stangaferro[2], M. J. Thomas[2], and J. O. Giordano[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Dairy Health and Management Services, Lowville, NY.*

We evaluated the effect of health monitoring strategies on cash flow (CF) of cows. Nulliparous and parous Holsteins with a neck-attached rumination and activity sensor (SenseHub; Merck Animal Health) were randomly assigned to an automated health monitoring (AHM; n = 607) or a visual observation (VO; n = 597) treatment for monitoring health from 3 to 21 DIM. In VO, cows were selected for a clinical exam (CEx) by a veterinarian (C.R.) based on VO of clinical signs of health disorders (HD). Cows in AHM were selected for a CEx if had a Health Index Score <86 points, rumination <250 min/d, or a daily milk yield reduction >20%. The CEx, conducted by C. R., was the same for all cows. Cows with clinical HD were treated by farm personnel using veterinarian-defined protocols. Health monitoring (HMC) and treatment costs (TrtC) were estimated until 21 DIM and income over feed cost (IOFC) and replacement costs (ReplC) were estimated up to 100 DIM. Values in US$ per slot were aggerated from 2 to 21 or 2 to 100 DIM. Slots (i.e., stalls) occupied by cows sold or dead were re-filled by a randomly selected heifer from the same treatment. Data for HMC, TrtC, ReplC, and CF were analyzed with the Wilcoxon test and IOFC by ANOVA. A CEx was conducted in 62% and 29% of cows in AHM and VO and 35% and 21% of cows were diagnosed with at ≥1 HD in AHM and VO, respectively. From 2 to 21 DIM, AHM had more IOFC ($P = 0.05$; VO: 144 ± 3, AHM: 150 ± 3), HMC ($P < 0.01$; VO: 2.4 ± 0.1, AHM: 5.6 ± 3.4), and TrtC ($P = 0.01$; VO: 4.7 ± 0.5, AHM: 6.4 ± 0.6), whereas ReplC did not differ ($P = 0.61$; VO: 40 ± 10, AHM: 51 ± 13). From 2 to 100 DIM, IOFC ($P = 0.12$; VO: 1,087 ± 16, AHM: 1,109 ± 15) and ReplC ($P = 0.65$; VO: 125 ± 18, AHM: 117 ± 18) did not differ. Cash flow per slot from 2 to 21 ($P = 0.13$; VO: 110 ± 5, AHM: 118 ± 5) or 2 to 100 DIM ($P = 0.23$; VO: 856 ± 38, AHM: 898 ± 37) did not differ. We concluded that despite greater HMC and TrtC, a health monitoring program based exclusively on automated monitoring alerts resulted in more IOFC compared with a program based on visual observation of signs of disease. Although of potential value for farms, the overall CF differences were insufficient to generate statistically significant differences.

Key Words: health, sensor, economics

2640 Quantitative evaluation of whey protein concentration in first lactation cows. M. O. Akindoyin*, N. Price, P. Pande, M. R. Uzzaman, M. Worku, and S. Ibrahim, *North Carolina Agricultural and Technical State University, Greensboro, NC.*

Whey protein is essential in milk for its nutritional benefits and bioactive components. The somatic cell count (SCC) is also a vital indicator of milk quality and udder health. This study aims to determine the quantitative changes in whey protein concentration and SCC in milk at the early lactation phase of dairy cows. We collected 50-mL milk samples biweekly from 4 lactating cows on d 5, 68, 162, and 163 of their lactation at North Carolina Agricultural and Technical State University farm in January 2024. Whey separation from milk and protein concentration measurement was conducted using the Pierce Bicinchoninic Acid Protein Assay Kit (Thermo Scientific, Waltham, MA). An automatic cell counter was used to count the milk cells. Individual variation was observed in whey protein concentration and SCC. The results show that in 3 of 4 cows, whey protein increased from the first to the second week, with a decrease in the fourth cow, indicating metabolic shifts in the mammary gland. A paired sample *t*-test was computed for protein concentration and SCC at a 0.05 level of significance. The protein concentration result shows that there is no significant difference ($P = 0.1$). The SCC data varied among the cows over the 2 weeks, showing diverse levels of udder health and inflammation. The statistical analysis of SCC variations over 2 weeks also resulted in a non-significant outcome ($P = 0.7$) suggesting that the differences in SCC are not statistically significant ($P > 0.05$), implying the changes might be due to random variation rather than consistent trends. Although raw SCC data indicate variations in udder health, these changes are not statistically significant. The study reveals valuable quantitative insights into whey protein concentration at the first lactation stage, offering potential indicators of lactation performance and health status in dairy herds. Findings show that the evaluation of whey protein levels and somatic cells over 2 weeks are not statistically different ($P > 0.05$) longer periods and increased sample size will be used.

Key Words: whey, protein concentration, somatic cell count

2641 Peripheral leukocyte transcriptomic changes in preweaning Holstein dairy calves with varying stages of bovine respiratory disease. L. C. Richmond*, J. A. Velasco, S. P. Ficklin, N. C. Stegelmeier, H. R. Hinnant, C. D. Fisher, C. T. Cauchy, L. M. Parrish, and C. S. McConnel, *Washington State University, Pullman, WA.*

Bovine respiratory disease (BRD) is a key contributor to calf morbidity and mortality. Historically, BRD diagnosis has relied on clinical assessments and clinical respiratory scoring charts (CRSC), which may underrepresent BRD prevalence and subclinical pathology. Thoracic ultrasonography has helped overcome CRSC limitations. This study aimed to identify (1) peripheral leukocyte transcriptomic changes in preweaning Holstein heifer calves with varying stages of lobar consolidation diagnosed via thoracic ultrasonography, and (2) potential molecular biomarkers of preweaning BRD. Calves born May 2021 and May 2022 on 2 conventional dairies in Washington were enrolled at birth and followed for the first 12 wk of life. Weekly physical examinations and thoracic ultrasonography were performed alongside biweekly jugular venipuncture and blood chemistry. Representative calves were retrospectively categorized based on the presence or absence (onset vs. healthy), and duration (healthy vs. chronic or resolved) of lobar consolidation (≥1 lobe) between 5 to 9 weeks of age: healthy (2021: n = 8; 2022: n = 15), onset (2021: n = 16; 2022: n = 15), chronic (2021: n = 8; 2022: n = 9) or resolved (2021: n = 7; 2022: n = 11). Leukocyte isolation of whole blood samples from each representative calf health state was conducted via modified Ficoll-Paque separation followed by RNA extraction (miRNeasy kit, Qiagen) and sequencing (Novogene Corporation). Differentially expressed genes (DEG) and predictive features were identified by EdgeR and random forest classification modeling. The analysis identified 536 DEG for healthy versus onset, 152 DEG for healthy versus chronic, and 99 DEG for healthy versus resolved, of which predictive features related to each health state were identified among known enrichment pathways. Enrichment pathways related to immunological functions such as positive regulation of natural killer cell mediated cytotoxicity, c-type lectin receptor signaling pathways, and immunoglobulin regulation were observed. Identification of potential biomarkers underlying disease susceptibility and progression may help guide calf management

Key Words: bovine respiratory disease (BRD), dairy, calf health

2642 Potential bacterial pathogens increase in the feces of dairy cows after calving. M. N. de Jesus*, J. S. Thompson, F. R. Mazza, E. Walker, and A. H. Smith, *Arm & Hammer Animal and Food Production, Waukesha, WI.*

The transition period of dairy cattle is defined as 3 wk before and 3 wk after parturition. During this time, cattle experience reduced feed intake, negative energy balance and a decrease in immune function, which may lead to increased infectious disease susceptibility. After calving, bacterial contamination of the uterus by feces is possible and could lead to reproductive diseases such as metritis. The objective of this research was to assess and identify if the levels of potential pathogenic bacteria increase in feces of dairy cattle during the transition period. For this study, 2 separate research trials were included. The first trial was conducted at the University of Illinois at Urbana-Champaign and included 65 multiparous cows. Fecal samples were collected from each cow once weekly, from 4 wk before expected calving through 10 wk after calving. The second trial was conducted at Pond Hill Dairy (Fort Atkinson, WI) and included 46 multiparous cows. Fecal samples were collected on week −3, −2, and −1 before expected calving, the day of calving (day 0), day 1, and week 6 after calving. Fecal samples were serially diluted for enumeration of clostridia on TSC (Neogen) and incubated anaerobically overnight at 37°C. Fecal samples were additionally enumerated for *Escherichia coli* on CHROMagar™ *E. coli* in the second trial, which was incubated overnight at 37°C. Data for each trial was compared separately from the other trial, all using one-way ANOVA. *Clostridia* and *Clostridium perfringens* levels on day 1 and week 1 after calving were significantly increased compared with week −2 before calving and week 6 after calving in both trials ($P < 0.05$). The *E. coli* levels on day 1 were significantly increased compared with week −2 and week 6 in the second trial ($P < 0.05$) and tended to be higher on day 1 compared with day 0 ($P < 0.1$). These data indicate that calving may increase the levels of potential pathogens within the gastrointestinal tract. Shedding of these pathogens in feces could contribute to uterine contamination and lead to health events after calving. This data can be used in future research to identify improved methods of pathogen control during stress events.

Key Words: transition period, bacterial pathogen, *Clostridium perfringens*

2643 Evaluation of chitosan hydrogel containing antimicrobial peptide for subclinical mastitis therapy in dairy cows. A. J. S. Moreira[1,2], A. H. Peres Assumpção*[1], K. D. V. Camargo[3], F. L. V. Umaña[2], K. C. A. Domingues[1], and H. Mantovani[2], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*University of Wisconsin–Madison, Madison, WI,* [3]*Universidade Estadual Paulista–UNESP, Jaboticabal, SP, Brazil.*

Bovine mastitis is the most prevalent and costliest disease in dairy farming and the main cause of antibiotics usage in the dairy sector, which raises concerns about antimicrobial resistance. Alternative therapies, such as antimicrobial peptides (AMP), are being explored to control udder infections. This study aimed to evaluate a chitosan hydrogel (CTH) containing antimicrobial peptide as intramammary (IMM) treatment of multiparous Holstein cows with somatic cell counts (SCC) > 400,000. Cows were paired based on SCC and randomly assigned to either treatment group (n = 13), receiving an IMM formulation containing AMP at a concentration of 2.24 mg/mL diluted in 2.5% (wt/vol) CTH, or control group (n = 13) treated with the commercial AMOXI-MAST® (6.25 mg/mL amoxicillin). Treatments were administered post-milking, over 4 milkings excluding the third, following farm protocols for mastitis treatment. Milk samples were collected at baseline (D 0) and before each milking on d 1, 2, 5, 8, and 15 post-treatment for SCC and total bacterial count (TBC). Data were analyzed by a mixed-effects model (MEM) followed by Bonferroni's test. Results are presented in Table 1. No significant differences (MEM, $P > 0.05$) were observed between treatments. Time effect was only significant (MEM, $P < 0.05$) for SCC parameter, as well as time × treatment interaction. During the trial, one cow treated with ATB developed clinical mastitis. Ongoing

Table 1 (Abstr. 2643). Effects of AMOXI-MAST® (AMX) and antimicrobial peptide-infused chitosan hydrogel (CTH) treatments in somatic cell count and total bacterial count of Holstein cows[1]

	Somatic cell count				Total bacterial count			
	Treatment ($\times 10^6$)				Treatment ($\times 10^3$)			
Day	AMX	CTH	SE ($\times 10^6$)	P-value	AMX	CTH	SE ($\times 10^3$)	P-value
0	1.2	1.5	1.03	0.99	2.6	2.3	1.3	0.99
1	1.6	5.1	1.02	<0.05	—	—	—	—
2	0.9	3.2	1.02	0.16	2.5	2.9	1.3	0.99
5	1.2	1.5	1.02	0.99	1.0	2.8	1.3	0.99
8	1.4	1.0	1.03	0.99	2.3	2.1	1.3	0.99
15	2.1	1.7	1.03	0.99	0.9	1.2	1.3	0.99

[1] Treatment values expressed as LSM. SE = standard error of the difference between means. P-values obtained from Bonferroni's test.

experiments aim to assess changes in the milk microbial community of these cows.

Key Words: mastitis, alternative treatment, SCC

2644 Feeding spent hemp biomass inhibits drug metabolism and clearance in cattle. A. Irawan*[1], D. Hasan[1], K. Lin[2], G.-X. Lu[2], S. Valliere[1], J. Cruickshank[1], and M. Bionaz[1], [1]*Oregon State University, Corvallis, OR,* [2]*Asia University, Taichung, Taiwan.*

Spent hemp biomass (SHB) is an extracted byproduct from industrial hemp (*Cannabis sativa*) posing excellent nutritional profile for ruminants. Prior data indicated that feeding SHB with >1% cannabidiolic acid (CBDA) and cannabidiol (CBD) to lambs or dairy cows increases bilirubinemia, suggesting a possible reduction of liver drug clearance capacity. The observed inhibition of drug clearance by CBD in monogastric species partly supports this finding. To assess if feeding SHB can inhibit drug clearance in cattle, we enrolled 8-mo-old Angus steers (n = 24) that were stratified (blocked) according to BW and BCS and randomly assigned to either a control diet (CON; alfalfa hay + 2% BW corn + alfalfa meal) or the same diet but with SHB replacing the alfalfa meal (TRT). Animals were fed the 2 diets for 28 d before a pharmacokinetic study was carried out by administering a single oral dose of meloxicam (1 mg/kg BW). Blood was collected via jugular vein from all steers immediately before (0 h) and at 6, 12, 24, 48, and 60 h after administration of meloxicam. Plasma meloxicam and cannabinoid concentrations were measured using HPLC and UHPLC-MS/MS, respectively. Non-compartmental pharmacokinetic analysis was performed using “NonCompart” and “ncar” packages in R. The steers fed SHB had higher ($P < 0.001$) plasma meloxicam peak concentration (C_{max}): 2.53 μg/mL (2.36–2.70 μg/mL) compared with 2.05 μg/mL (1.88–2.22 μg/mL) in the CON group. The TRT steers (vs. CON) also exhibited higher ($P < 0.01$) mean of time at C_{max} (T_{max}; 22 vs. 14.5 h vs. 22 h), greater area under the curve extrapolated to infinite time ($AUC_{0\text{-}\infty}$; 149 vs. 90.5 h × μg/mL), greater AUC to last nonzero concentration ($AUC_{0\text{-last}}$; 102 vs. 77.1 h × μg/mL), and lower clearance per fraction of the dose absorbed (*CL*; 2.35 vs. 3.59 L/h). However, the terminal half-life ($T_{1/2\lambda z}$) was not statistically different ($P > 0.05$) between the groups. Overall, our pharmacokinetics data confirmed that SHB partly inhibits clearance of meloxicam in ruminants. We cannot extrapolate this to all drugs, but our data suggest that drug withdrawal time can be affected by feeding SHB.

Key Words: cannabinoid, meloxicam, cattle

Breeding and Genetics 3

2645 Genetic relationship among colostrum traits, feed efficiency, and growth traits in Canadian Holstein calves. A. G. R. Phillips*[1], R. E. Jahnel[1], B. O. Makanjuola[1], F. Miglior[1,2], F. S. Schenkel[1], and C. F. Baes[1,3], *[1]Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, [2]Lactanet, Guelph, ON, Canada, [3]Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland.*

Genetic improvement of calf feed efficiency has recently gained research interest. Previously estimated genetic parameters for preweaning calf feed efficiency suggest that residual metabolizable energy intake (RMEI) shows potential as a trait for selection. However, the relationship with other important traits, such as with those relating to colostrum, is unclear. The objective of this study is to estimate the genetic relationship between colostrum intake and quality, feed efficiency, and growth traits in 471 preweaning Canadian Holstein calves. Phenotypic and pedigree records of calves between 0 and 65 d of age born between 2015 and 2021 were provided by the Ontario Dairy Research Centre (Elora, Ontario, Canada). Genetic relationships between colostrum intake and feed efficiency traits were estimated using multivariate animal models implemented in ASREML 4.1. Average colostrum intake for calves (6–12 h of life) was 3.09 L with an average 74.01 g of IgG. Calves had an average daily gain of 0.89 kg/d in the first month, and 0.91 kg/d in the second month. The average RMEI was 0.08 Mcal for the first month and −0.69 Mcal for the second month. Further investigation into the role of colostrum traits in calf feed efficiency could allow for the incorporation of colostrum quality as indicator traits for selection.

Key Words: residual metabolizable energy, milk colostrum quality, calf growth

2646 Variation in a Y-linked single copy gene, *AMELY*, was shared among modern and lost male Holstein lineages. W. Yousaf*[1], W.-S. Liu[1], G. E. Liu[2], H. B. Blackburn[3], and C. D. Dechow[1], *[1]Department of Animal Science, Pennsylvania State University, University Park, PA, [2]Animal Genomics and Improvement Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD, [3]USDA-ARS National Animal Germplasm Program, Fort Collins, CO.*

Modern US Holstein sires trace their Y-chromosome lineage to Round Oak Rag Apple Elevation (Elevation), Pawnee Farm Arlinda Chief (Chief), or Osborndale Ivanhoe (Ivanhoe). Recently, Y lineages lost since 1960 were reconstituted using semen from U-of-Minnesota W Caliban Cuthbert (Cuthbert) and Zimmerman Alstar Pilot (Pilot), which was available through the National Animal Germplasm Program. The Y-chromosome is complex and includes features such as a pseudoautosomal region, a pseudoautosomal boundary (PAB) with a male-specific region that contains repetitive gene families, and X-degenerated (X-D) regions. The objective of this study was to compare Y-chromosome sequence alignment of various Holstein Y-lineages to the ARS-UCD2.0 reference sequence for a single copy gene from the PAB (*AMELY*; Amelogenin, Y-linked) and a highly conserved single copy gene (*SRY*; Sex determining region Y) on the distal end of the Y. The reference chrY sequence is a Wagyu bull. Whole genome Illumina pair-ended reads from Elevation and one son, Chief and one son, Ivanhoe, one Cuthbert son, and one Pilot son were aligned to the reference sequence using Bowtie2 (v 2.4.2). Following alignment, we retained SNP with a read depth ≥10 and a base call accuracy of ≥99.8% (Phred quality score ≥27). All bulls shared a G to A substitution at bp 6,936,835 in the *AMELY* gene; at bp 6,937,734, the initial analysis indicated an A to G substitution for all bulls except Elevation and his son. However, visual inspection of the region revealed that Elevation and son had the same SNP, but the alignments did not meet our depth requirement. No variants were observed for any bull in the *SRY* gene. The pseudoautosomal region and highly repetitive nature of the Y-chromosome make sequence alignments more difficult than alignment to autosomal sequence. Nevertheless, the analysis identified 2 potential SNPs in the *AMELY* gene that appeared to be shared across Holstein Y lineages and indicated that there was no variation of the *SRY* gene.

Key Words: Y-chromosome, Holstein, genetic conservation

2647 In utero heat stress affects genome-wide DNA methylation in blood cells at birth in Holstein heifers. A. Boucher*[1], P. Dufour[2], H. Martin[1,2], J. Laporta[3], M.-A. Sirard[1], and V. Ouellet[1], *[1]Department of Animal Sciences, Université Laval, Québec, QC, Canada, [2]Department of Molecular Medicine, Université Laval, Québec, QC, Canada, [3]Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Dairy calves exposed to in utero heat stress (IU-HS) exhibit a distinctive phenotype throughout their life, such as impaired immunity, reduced milk production across multiple lactations, as well as reduced productive life and longevity. Recent research links these postnatal outcomes to altered methylation profile, yet our understanding of birth-related epigenetic alterations remains limited. We conducted an epigenome-wide study to identify the consequences of IU-HS on DNA methylation status at birth. Blood from Holstein heifers born to dams exposed to active cooling (IU-CL; n = 5; provided fans, soakers and shade) or heat stress (IU-HS; n = 3; only provided shade of the barn) during late gestation (~54 d before calving) were used. Blood samples collected at birth underwent total DNA extraction, followed by whole-genome enzymatic methyl sequencing to identify differentially methylated regions (DMR). Methylation was analyzed using sliding window analysis (window size = 500 bp; step = 250 bp). We analyzed a total of 6,422,432 regions of the whole genome. Analysis focused on the promoter region only. We considered a DMR as a region with a q-value <0.01, a methylation difference >15%, and only if all regions in the same promoter have a change in similar direction signal. The clustering including all heifers showed a clear separation between both in utero treatments. A total of 1,716 DMR were identified in the promoter region, involving 1,061 genes. Of those, 548 were hypermethylated (playing a role in processes such as inflammation and immune response), and 513 were hypomethylated (playing a role in processes such as cell signaling and development) promoters in IU-HS treatment compared with IU-CL treatment. In utero heat-stressed heifers display a unique methylation profile indicating a significant epigenetic adjustment that might contribute to suboptimal traits, potentially affecting their productivity and lifespan. These characteristic traits could be used as selection criteria in newborn calves, thus avoiding raising heifers that may not express their production potential.

Key Words: epigenetic, hyperthermia, DNA methylation

2648 Methane emissions of crossbreds of Holstein with Jersey, Montbéliarde, Normande, and Viking Red compared to pure Holsteins in a pasture production system. B. J. Heins*[1,2], I. J. Salfer[1],

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The objective of this preliminary study was to compare methane emissions of Holstein (HO) and crossbred cows, as well as an exploratory genome-wide association study (GWAS) on emissions. Holstein cows (n = 5) were compared with Grazecross crossbred cows (n = 24) composed of the Normande, Jersey, and Viking Red breeds and ProCross crossbred cows (n = 12) composed of the Montbéliarde, Viking Red, and HO breeds using 7,905 emissions records. Methane records were from a much larger dairy cow nutrition study. Cows were housed at the University of Minnesota West Central Research and Outreach Center (Morris, MN), and data were collected from July 2023 to January 2024. Methane was collected with a GreenFeed System (C-Lock Inc., Rapid City, SD). Cattle were genotyped with either a 50K, 65K, or 150K SNP chip from Neogen (Lincoln, NE) and imputed to 65K. Quality control and association analysis was with the SNP and Variation Suite v8.10.0 (Golden Helix Inc., Bozeman, MT). For significance testing, a *P*-value of 4×10^{-6} was the significance threshold. Independent variables for statistical analysis were fixed effects of genetic group (HO vs. Grazecross crossbred vs. ProCross crossbred), lactation number, time of day of methane measurement, and sire breed group nested within breed group. The Grazecross (305 g/d) and ProCross (314 g/d) cows emitted less ($P < 0.05$) methane than Holsteins (369 g/d). However, methane intensity (methane/kg milk) was similar ($P > 0.30$) for Holstein (12.6), Grazecross (12.2), and ProCross (10.8) cows. ProCross cows (0.597) produced less ($P < 0.05$) methane per kg of body weight than Holstein (0.656) and Grazecross (0.636) cows. Preliminary GWAS analysis identified 2 SNP on chromosome 1 with a strong signal. The top variant (BTA-00030962; $P = 4.51 \times 10^{-10}$) was mapped to a G > A and the second the top variant (Hapmap42177-BTA-31679; $P = 3.733 \times 10^{-10}$) was mapped to a A > G. The GWAS results should be interpreted with caution because of small numbers. Results suggest that ProCross cows have some benefits for reduced methane emissions in grazing systems.

Key Words: crossbreeding, pasture, methane

2649 Relationships between novel breeding values for heat tolerance in dairy cattle with rectal temperature during heat stress in lactating Holstein cows.

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Heat stress can compromise production and health in dairy cattle; the magnitude of these deleterious effects will be exacerbated by global climate change. The resistance of cows to heat stress is under genetic control. Recently, Zoetis has developed 2 novel breeding values for heat tolerance in lactating Holsteins based upon the change in milk yield (MILKTHI) or cow conception rate (CCRTHI) with temperature-humidity index. The goal here was to test whether variation in predicted transmitting ability (PTA) for heat tolerance is associated with cow regulation of body temperature during heat stress. Data on afternoon (1400–1700 h) rectal temperatures during the hot months of the year (May–August) were collected from lactating cows on farms in Florida (n = 3), California (n = 1), and Turkey (n = 1) over a period spanning 2007 to 2023. A total of 4,678 values of rectal temperature from 3,620 cows were analyzed by the GLIMMIX procedure of SAS. Models included parity, stage of lactation, year-farm and with cow as a random variable. Breeding values were analyzed 2 ways: by comparing rectal temperatures of cows above and below a PTA of 100 (94.3 ± 4.3 vs. 104.4 ± 3.5 for CCRTHI and 96.3 ± 2.9 vs. 104.3 ± 3.4 for MILKTHI), and by comparing cows in each quartile of the data set. There was no significant relationship between MILKTHI and rectal temperature. Cows with CCRTHI >100 had lower rectal temperature ($P < 0.0001$) than cows with CCRTHI <100 (38.70 ± 0.01 vs. 38.62 ± 0.01°C). Similar results were obtained when evaluating CCRTHI quartiles. Rectal temperature was 38.67 ± 0.06°C (PTA <96.49), 38.63 ± 0.06°C (PTA >96.49 and <100.33), 38.63 ± 0.06°C (PTA >100.33 and <103.92) and 38.60 ± 0.06°C (PTA >103.92; $P = 0.044$). The percentage of cows with high rectal temperature (>39°C) was also lower ($P = 0.0062$) in cows in quartile 4 (17.9%) than in quartile 1 (22.6%). Results demonstrate that CCRTHI is related to body temperature during heat stress. The fact that CCRTHI but not MILKTHI was related to rectal temperature is indicative to the more direct relationship between body temperature and fertility than body temperature and milk yield.

Key Words: heat stress, resiliency, breeding value

2650 Runs of homozygosity in modern Holsteins and crosses with lost Y-lineage bulls.

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The modern Holstein (HO) breed has been traced to 2 Y-chromosome lineages from the 1880s which have been propagated by Chief and Elevation since the 1960s. This study aimed to determine the effect of reintroducing lost genetic lineages into the modern HO population on homozygosity. Lost Y-lineages were reconstituted by mating 2 bulls (Cuthbert and Pilot) with semen procured from the National Animal Germplasm Program (NAGP) to modern dams. Semen from 5 first generation (G1) sons (2 Cuthbert and 3 Pilot) was distributed to cooperating farms to generate a second generation (G2) composed of 91 heifers and 6 bulls. These were genotyped (Illumina BovineSNP777k BeadChip) along with 157 modern HO herdmates and 4 G1 Pilot daughters. After quality control, 758,443 SNPs were evaluated with principal component analysis (PCA) to determine genetic distance and analyzed for runs of homozygosity (ROH) using Plink1.9 for modern, Cuthbert, and Pilot G1 and G2 lineages. The ROH segments were required to be a minimum of 2,000 kb and 60 SNPs in length with allowance for 1 heterozygous SNP. The first (PC1) and second (PC2) principal components explained 10.5% and 6.75% of the variance, respectively, with distinct clusters for modern, Pilot, and Cuthbert lineages. An ANOVA was conducted for ROH in R (Rstudio v. 4.2.2) with genetic lineage as the independent effect. On average, modern HO had a total ROH of 435,334 kb, corresponding to 17% of the 2.6 Gb covered by the genotyping array. Cuthbert G1 offspring had 99,543 kb (4%), Cuthbert G2 had 269,422 kb (10%), Pilot G1 had 84,553 kb (3%), and Pilot G2 had 249,608 kb (10%). All lineages were different ($P < 0.001$) from modern HO and each other. In conclusion, the reintroduction of genetic lineages from the NAGP repository resulted in distinct genetic clusters and substantial reductions of within-breed homozygosity.

Key Words: Y-lineage, runs of homozygosity, genetic conservation

2651 Investigating the genetic background of bovine spastic syndrome (crampy) in Canadian Holsteins.

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Bovine spastic syndrome (crampy) is a neuromuscular disorder causing concern in the Canadian dairy industry due to its adverse effects on the health and welfare of cattle. Crampy is characterized by the involuntary bilateral spasm of the affected hind limb when attempting movement, occasionally accompanied by kyphosis, and is predominantly diagnosed in cattle older than 2 years. The objective of this study was to identify candidate genes associated with the disorder through a genome-wide association study (GWAS) to better understand the genetic background of crampy in Canadian Holstein dairy cattle. For this, data from 678 dairy herds with at least one recorded case of crampy (totaling 1,952 cases) were analyzed. Quality control measures, implemented using PLINK software, resulted in 43,493 autosomal SNPs for analysis. Through a GWAS using GCTA software, a total of 9 significant SNPs were found to be significantly associated (genome-wise Bonferroni correction threshold of 0.05, based on the number of independent genomic segments) with crampy on BTA8, BTA10, BTA16, BTA20, and BTA23. The initial functional analysis revealed 2 genes, *ZC2HC1C* and *TMED10* on BTA10, that have been previously associated with traits such as hip and rump width in Simmental cattle in Ireland. This study is a critical step toward understanding and addressing the challenges posed by crampy in the Canadian dairy industry. By identifying the genetic factors contributing to crampy, this study provides a foundation for potential genomic selection strategies, offering a pathway to improve cattle well-being and mitigate the economic impact on dairy producers.

Key Words: crampy, neuromuscular disorder, quantitative genetics

2652 Potential dairy goat for tropical conditions. M. Moaeen-ud-Din*[1], J. Reecy[2], and M. Khan[3], [1]*PMAS-Arid Agriculture University, Rawalpindi, Rawalpindi, Punjab, Pakistan,* [2]*Iowa State University, Ames, IA,* [3]*Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Punjab, Pakistan.*

A survey study was carried out among 383 farmers of 29 goat breeds in various districts of Pakistan. Data were collected on productive and reproductive traits performance (age at first kidding in days and lactation yield in kilograms). Data were arranged and analyzed using Mixed Procedure by restricted maximum likelihood methodology in SAS University Edition to obtain mean and standard deviation for the surveyed traits of 29 breeds. The index value of a potential dairy goat breed was calculated for each breed by using the index I = 3.1(X2 − X1), where X1 and X2 were the breed estimated effects on age at first kidding. We ranked 29 breeds based on this index, where Kamori was first with an index value of 1,676.85, followed by Bugi-Tori (897), Nachi (727.92), Jattal (615.06), Pateri (515.01), Beetal (500.7729), Buchi (435.12), Daira Din Panah (424.5), Barbari (345.15) and Jattan (334.2), whereas the lowest breed in term of index value was Thari (−324.54). The performance of Kamori is definitely far lesser than that of Saanen goats (2,661.6); however, Saanen goat performance was found comparable to Kamori when performance was recorded in a relatively challenging climate (Brazil) outside the comfort zone (1,624.25). Thus, it may be inferred that Kamori has potential to be reared as a dairy animal; thus, it is needed to exploit the production potential along with a concrete genetic improvement program. Moreover, at the same time, Bugi-Tori, Nachi, Jattal, Patteri, and Beetal need to be exploited for their genetic potential under optimum feeding and management conditions as potential dairy breeds in countries under warm climatic conditions.

Key Words: dairy goat, Kamori, Pakistan

2653 Initial investigation of detailed hoof health phenotypes for genetic evaluation. K. L. Parker Gaddis*[1], J. M. Layton[1], H. D. Norman[1], J. F. Burchard[1], D. Swartz[2], and G. Cramer[2], [1]*Council on Dairy Cattle Breeding, Bowie, MD,* [2]*College of Veterinary Medicine, University of Minnesota, St. Paul, MN.*

Hoof health and mobility remain a leading concern for both producers and consumers. Genetic improvement of hoof-related traits based on producer-recorded lameness is difficult due to low heritability estimates resulting from limited, noisy data. The objective of this research is to identify new phenotypes developed from more precise data collected from multiple sources. Data were available for the current analyses from 3 participating large herds across the United States from 2021 to 2024. Common hoof lesions (digital dermatitis, ulcer, white line disease, foot rot, thin sole and toe ulcers, corkscrew claw, injury, and other) were reported by specifically trained hoof trimmers. Autonomously collected mobility scores on all lactating cows were also available through a video analytics platform (CattleEye Ltd., Belfast, United Kingdom). Pipelines of editing constraints and cross-reference with the National Dairy Cooperator database were developed to ensure data validity. The overall proportion of at least one reported hoof lesion across the herds was 7.4%, ranging from 5.0% to 11.5% in each herd. A total of 3.5 million mobility scores were provided on a scale from 0 to 100 with values >50 indicating a possible mobility problem. The mean mobility score in the data was 36.9 ± 12.2, with the average number of mobility scores per cow-lactation being 103. Preliminary genetic analyses were completed to obtain initial heritability estimates for possible traits of interest. Data were aggregated on a lactation basis resulting in 13,962 records from 10,868 cows and included potential phenotypes such as a binary indicator if a cow exceeded the threshold of 50 throughout lactation, the log-variance of mobility score throughout lactation, and the lactational mean mobility score. Heritability estimates from univariate analyses ranged from 0.02 for hoof lesions up to 0.09 for the lactational variance of mobility score. Initial heritability estimates are promising as data continue to be collected and additional herds participate.

Key Words: hoof health, mobility, heritability

2654 Genome-wide association study on origin groups of milk fatty acids at early stages in the first lactation for Holsteins. Y. Masuda* and N. Yamamoto, *Rakuno Gakuen University, Ebetsu, Hokkaido, Japan.*

Milk fatty acids (FA) are grouped by origin and can be used to monitor the health condition of milking cows. A group of shorter-chain FAs originates from de novo synthesis in the mammary gland. Longer-chain FA originate from preformed dietary fats and body fat. The genetic control of each FA group is expected to differ by lactation stage. This study aimed to perform a genome-wide association study (GWAS) on FA groups at different stages of early lactation. Data included 1,448,373 test-day records from 211,462 Holstein cows in their first lactation, calving in Hokkaido, Japan, between April 2021 and October 2022. The content of groups of de novo and preformed FA was predicted by Fourier-transform infrared spectroscopy for a milk sample. The de novo FA percentage (between C4 and C14; DnF%) and preformed FA percentage (≥C18; PrF%) were calculated as the ratio of the predicted FA content to the total FA content for each origin group. The data set was divided by lactation stage, defined as every 30 DIM in each lactation, i.e., DIM 6 to 35 as stage 1 and up to stage 4. A total of 21,013 genotyped animals with 45,724 SNP markers were used after quality control. We performed GWAS in each lactation stage using the method based on single-step GBLUP with a nonlinear function. For DnF%, the 85.2 to 89.0 MB region on chromosome 6 had significant markers only at stage 1. This region was reported to be associated with clinical mastitis, somatic cell score, net merit, and length of productive life.

Another region (50.4–51.4 MB on chromosome 19) was also significant across all stages and was known to be associated with various FA in milk. For PrF%, the same regions as DnF% were significant. Two additional regions on chromosomes 14 and 27 were significant and were associated with DGAT1 and milk FAs, respectively. At stage 2 or later, as with DnF%, the region on chromosome 6 was not significant. The results agree with our previous study, showing that DnF% and PrF% had higher genetic correlations with fertility and somatic cell score at stage 1 compared with the later stages.

Key Words: test-day record, milk fatty acid, dairy cattle

2655 Improved tracking of recent mutations within common haplotypes causing cholesterol deficiency, muscle weakness, and BLIRD. D. J. Null*[1], P. M. VanRaden[1], A. Al-Khudhair[1], and E. L. Nicolazzi[2], [1]*USDA Animal Genomics and Improvement Laboratory, Beltsville, MD,* [2]*Council on Dairy Cattle Breeding, Bowie, MD.*

This research improved the methods to report harmful new mutations within haplotypes for cholesterol deficiency (HCD), muscle weakness (HMW), and bovine lymphocyte intestinal retention defect (BLIRD) in 5.6 million Holsteins. Some were gene tested for HCD and HMW, but none for BLIRD. Gene tests were available for HCD and HMW but not yet for BLIRD. Concordances of gene tests with haplotype status were improved by processing pedigree from earliest to latest and including gene tests of ancestors to confirm true carriers and document connections to the carrier ancestors. Without including gene tests, the previous code correctly identified 37.2% of the carriers (code 1), 7.6% as probable carriers (code 3), and 55.2% were labeled non-carriers, whereas the new code correctly identified 58.8% as HCD carriers (code 1), 32.0% as probable carriers, and only 9.1% were still labeled non-carriers. Directly imputing gene tests sometimes gave unstable results. Recessive effects of BLIRD homozygotes from US data are consistent with the French research. Heifer livability was 97.6% for normal calves but averaged 88.8% for 178 homozygotes (code 2) and 94.1% for 2,029 probable homozygotes (code 4) with corresponding estimates of −8.6% and −3.3% from an animal model. Yield trait effects for 412 code 2 homozygotes were −1,799 kg of milk, −63 kg of fat, and −55 kg of protein with a cost of −$1,206 using lifetime net merit values; other traits not yet studied may increase that cost. Mating a BLIRD carrier randomly to a population with 8.9% allele frequency would cause economic loss of $1,206 × 0.089/2 = $54. Those losses should already be reflected in evaluations that average the merit across normal, carrier, and homozygous daughters. Genomic predictions do not fully track those losses because new mutations are poorly correlated with nearby markers. However, US adjustments for future inbreeding automatically reduce evaluations of popular ancestors by more than the cost of these individual defects. Gene tests are needed for new mutations within common haplotypes because tracking can be difficult even with accurate pedigrees.

Key Words: haplotype, gene test, recessive mutation

2656 Genomic evaluation for oocyte and embryo production in Holstein cattle. G. Gouveia*, F. Campagnari, S. Westberry, V. Ribeiro, P. Khanal, P. Ross, and N. Deeb, *STgenetics, Navasota, TX.*

Biotechnologies such as in vitro fertilization (IVF) and multiple ovulation and embryo transfer (MOET) allow to increase selection intensity and decrease generation intervals, both factors contributing to the efficiency of breeding programs. An ideal donor has superior genetics for economically important traits and should also have the potential to produce many progenies per reproductive cycle. Both IVF and MOET programs have been implemented by STgenetics in 4 different farms since 2012, where the numbers of oocytes and viable embryos produced per aspiration were continuously recorded. Furthermore, all donors are genotyped, allowing the development of genomic predictions for oocyte and embryo counts. The number of oocytes aspirated during OPU (OOC) and number of viable embryos produced through IVF (VEI) were recorded for 3,056 and 2,622 purebred Holstein donors, respectively. Additionally, 2,674 donors were superovulated, and the number of viable embryos flushed 7 d after insemination (VEA) was recorded. The phenotypic averages (median) of OOC in the study were 14.1 (12.0), 3.8 (2.0) for VEI, and 4.5 (3.00) for VEA. Variance components for the different traits were estimated using a 10-generation pedigree in LMT software, and the estimated heritabilities ± SE were 0.39 ± 0.05 for OOC, 0.11 ± 0.03 for VEI, and 0.13 ± 0.05 for VEA, suggesting opportunities for implementing these traits in genetic selection. As collections were realized multiple times in each donor, repeatabilities ± SE of OOC, VEI, and VEA were 0.58 ± 0.02, 0.26 ± 0.04, and 0.22 ± 0.03, respectively. Moreover, GEBV were predicted for the donors with reliability ranging from 50% to 85% for OOC, 21% to 67% for VEI, and 15% to 59% for VEA. Genomic predictions were extended to animals with genotypes but no phenotypic records as well as for animals at a younger age and from both sexes. This is the first genomic prediction available commercially for oocyte and embryo production. In sequence, genetic correlations with other important phenotypes will be explored in order to better understand the impact of including these traits into dairy cattle breeding programs.

Key Words: female biotechnology, genomic prediction, Holstein donor

2657 Estimation of the scope of milk prediction equations for new breeds by selection of representative spectra and comparison of spectral variability. H. Wilmot*[1,2], S. Franceschini[2], N. Gengler[2], C. Nickmilder[2], and H. Soyeurt[2], [1]*National Fund for Scientific Research (F.R.S.-FNRS), Brussels, Belgium,* [2]*Gembloux Agro-Bio Tech, ULiège, Gembloux, Belgium.*

The objective of this study was to estimate if the use of milk prediction equations based on mid-infrared (MIR) spectrum could be extended to other breeds than Holstein (HOL). Most of the time, MIR-based equations are developed on HOL data sets, which can prevent their use for other breeds. However, if the milk spectral variability of other breeds is included in the HOL calibration data set, the spectral extrapolation is limited. We compared spectral variability between Walloon (Southern Belgium), HOL (n = 6,867,134 spectra), and 2 other Belgian breeds: the Dual-Purpose Blue (DPB; n = 292,106 spectra) and the East Belgian Red and White (EBRW; n = 42,418 spectra), with records from 2007 to 2023. To achieve this purpose, for each breed, we projected the standardized spectra on the first 3 principal components (PC) estimated from a sample of one million Walloon spectra. Localization index of each spectrum, computed from the scores of the first 3 PC, were used to select representative spectra: n = 165,507 for HOL, n = 53,561, for DPB and n = 21,752 for EBRW. Finally, to estimate if the spectral variability of the DPB and the EBRW was included in the spectral variability of HOL, we used PC scores to compute the barycenter of selected HOL spectra weighted by their density. We then computed the GH distance of each DPB and EBRW selected spectrum to this HOL barycenter. If the GH distance of the DPB or EBRW spectrum was higher than 3, this spectrum was considered as outside the spectral variability of HOL. It was found that 98.85% of DPB and 99.53% of EBRW selected spectra were included in the spectral variability of HOL. To conclude, the results suggest that milk MIR prediction equations developed on HOL spectra can be applied to DPB and EBRW without spectral extrapolation.

Key Words: mid-infrared spectrometry, spectral variability, prediction equation

2658 Investigating relationships between the host genome, rumen microbiome, and dairy cow feed efficiency using mediation analysis with structural equation modeling.

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The rumen microbiome is crucial for converting feed into milk, and the efficiency of this process affects the profitability and sustainability of the dairy industry. The rumen microbiome explains part of the variation in feed efficiency. We aimed to reveal relationships between the host genome, rumen microbiome, and dairy cow feed efficiency using structural equation models. The specific objectives were to infer the mediation effects of the rumen microbiome on feed efficiency, estimate the direct and total heritability of feed efficiency traits, and calculate the direct and total breeding values of feed efficiency traits. Data consisted of dry matter intake, milk energy, and residual feed intake records, SNP genotype data, and 16S rRNA rumen microbial abundances from 448 mid-lactation Holstein cows. We implemented structural equation models such that the host genome directly affects the phenotype and the rumen microbiome, whereas the microbiome affects the phenotype, partially mediating the effect of the host genome on the phenotype. We found that 7% to 30% of rumen microbes had structural coefficients different from zero. We grouped microbes into 3 groups that could have different uses in dairy farming. The first group of microbes, with low heritability ($h^2 < 0.10$) but significant causal effects on feed efficiency, are attractive for feed management. Conversely, the other 2 groups of microbes, with moderate heritability ($h^2 > 0.10$), significant causal effects, and genetic covariances and causal effects with the same or opposite sign to feed efficiency, are attractive for selective breeding, improving, or decreasing the trait heritability, respectively. In general, including different microbes in genomic models tends to decrease the trait heritability rather than increase it. The change in total heritability ranged from −15% to +5% depending on the microbial group and phenotype. Our findings provide useful information to target rumen microbes that can be manipulated through selection or management interventions to improve feed efficiency traits.

Key Words: rumen microbiome, feed efficiency, structural equation model

2659 Effect of preweaning plane of nutrition and age on blood DNA methylation of dairy calves.

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The effects of the environment on an individual after birth have shown to have long-term consequences by altering gene expression through DNA methylation. Among many environmental factors, the diet is known to modulate cellular signaling, tissues synthesis, and many other biological functions. Considering that the calf's diet could have a programming effect on its future productivity, we evaluated how the amount of milk replacer (MR) fed to dairy calves before weaning could affect their blood DNA methylation profiles. A total of 12 Holstein female calves were selected from a larger study in which 6 calves were fed 8.3 L/d of MR (ReCan), and 6 calves were fed MR ad libitum (AdLib). Pairs of calves from both treatments were matched so that date of births, birth weights, and success of passive immunity transfer were similar among the 2 groups, and that only the median MR intake was different. Feeding treatments were given from birth to weaning (76 d of age), and on average, the MR intake was 7.0 ± 0.20 L/d and 9.7 ± 0.20 L/d ($P < 0.001$) for ReCan and AdLib, respectively. Blood samples were collected at birth and at d 76, and whole-genome DNA methylation analyses were performed on all samples using enzymatic methyl sequencing. Reads were aligned to a reference genome using Bismark and methylation analysis were performed using the R library Methylkit. Methylation differences were identified throughout the genome using sliding regions of 500 pb with a step of 250 bp. Age-related contrast analysis (birth vs. weaning) revealed that DNA from weaned calves is significantly ($P < 0.001$) hypermethylated compared with DNA at birth. We defined differentially methylated regions (DMR) as regions overlapping promoters with a q-value <0.05 and an absolute methylation difference higher than 10%. Over the 549,010 analyzed regions, we identified 1,619 DMR associated with 1,056 unique genes from which 640 and 416 were respectively hyper- and hypo-methylated in AdLib compared with ReCan. The differences in promotor regions could influence gene regulation in white blood cells, especially regarding cytokines, and could affect subsequent response to immune challenges.

Key Words: DNA methylation, epigenetic, immune function

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2660 Effects of yeast inoculation on the fermentation profile and aerobic stability of rehydrated corn grain silage. M. P. Rodrigues, M. R. Pupo*, E. C. Diepersloot, and L. F. Ferraretto, *University of Wisconsin-Madison, Madison, WI.*

This experiment aimed to evaluate the effects of yeast inoculation on the fermentation profile and aerobic stability of rehydrated corn grain silage stored for 30 or 90 d. Corn grain was rehydrated targeting 70% DM and inoculated with increasing concentrations of yeast isolated from a high-moisture corn sample. Treatments were prepared as follows: yeast colonies were isolated, diluted in distilled water, and used to inoculate material targeting yeast counts of 5.3, 6.3, and 7.3 log cfu/g of rehydrated corn grain, or the same amount of distilled water as control. Silage was ensiled in quadruplicate for each storage length using nylon-polyethylene standard barrier vacuum pouches (1,000 g fresh of material). Data for each storage length were analyzed separately using the PROC GLIMMIX of SAS with fixed effect of yeast inoculation and evaluated for linear and quadratic effects of increasing yeast inoculation. After 30 d, silage pH had a quadratic association ($P = 0.03$) with inoculation of yeast counts, where it was greatest for 5.3 log cfu/g and lowest for the control. Both lactic ($P = 0.80$) and butyric ($P = 0.10$) acid concentrations were unaffected by treatments. Acetic acid concentration increased linearly ($P = 0.001$), and ethanol concentration had a quadratic relationship ($P = 0.01$) with increasing yeast counts. Concentrations of ammonia-CP ($P = 0.55$) and total acids ($P = 0.38$) were similar among treatments. No differences were observed ($P = 0.23$) for aerobic stability. After 90 d, lactic acid concentration increased linearly ($P = 0.001$) with increasing yeast counts. No differences were observed for acetic acid ($P = 0.94$) and ammonia-CP ($P = 0.58$) concentrations. Butyric acid concentration decreased linearly ($P = 0.04$), whereas ethanol concentration increased linearly ($P = 0.001$) with increasing yeast counts. No differences were observed ($P = 0.97$) for aerobic stability. Greater ethanol concentration for both storage lengths suggests an increase in the activity of supplemental yeast in rehydrated corn grain silage, but without negative effects on aerobic stability.

Key Words: fermentation, yeast, corn

2661 Effect of high-flavonoid corn hybrids on ruminal fermentation and methane emission in vitro. C. J. Eifert*, B. P. Panek, T. Lesko, N. Stepanchenko, C. V. Almeida, L. F. Martins, S. F. Cueva, D. E. Wasson, A. Richards, S. Issabekova, S. Chopra, and A. N. Hristov, *The Pennsylvania State University, University Park, PA.*

Flavonoids are secondary plant metabolites that have been shown to reduce CH_4 emission in vitro. The objective of the present study was to determine the effects of 2 high-flavonoid (HiFlav) corn hybrids on ruminal fermentation in vitro. Repeated incubations were carried out for 48 h at 39°C using an automated gas production system. A nonflavonoid expressing hybrid was used as a control (CON). All hybrids were tested as fresh (FRS) and ensiled (ENS; for 45 d at room temperature) and incubated at a 1% (wt/vol) inclusion rate. Total gas production was continuously measured, and headspace gas samples were taken at 48 h and analyzed for CH_4; liquid samples collected at 48 h were analyzed for volatile fatty acid (VFA) and ammonia concentrations. Data were analyzed using PROC MIXED of SAS with corn type (CON or HiFlav), processing method (FRS or ENS), and the interaction in the model. The FRS treatments produced 13% and 16% more ($P < 0.01$) total gas (TG) and CH_4 (as mL/g substrate dry matter), respectively, than ENS, but there was no difference in TG and CH_4 production between CON and HiFlav. The concentration of CH_4 in TG was not affected by HiFlav and tended to be slightly greater (3%; $P = 0.08$) for FRS than ENS. The total VFA concentration was not different ($P \geq 0.21$) between FRS and ENS and HiFlav and CON. The proportion of acetate in VFA was 2% greater ($P < 0.01$) and that of propionate 4% lower ($P < 0.01$) for FRS versus ENS. The acetate concentration was not different between HiFlav and CON, but that of propionate was 6% lower ($P < 0.01$) for HiFlav versus CON. The concentration of butyrate was greater (3%; $P < 0.01$) for FRS than ENS and for FLA versus CON (11%; $P < 0.01$). The acetate:propionate ratio was 6% greater ($P < 0.01$) for FRS than ENS and 7% greater ($P < 0.01$) for HiFlav versus CON. Overall, in this in vitro experiment, ensiling decreased TG production, had no effect on CH_4, and resulted in increased acetate:propionate ratio. HiFlav corn did not affect CH_4 production, but shifted VFA profiles. Further investigation is required to determine the effects of HiFlav corn on rumen fermentation, digestibility, and animal physiology in vivo.

Key Words: flavonoid, ruminal fermentation, methane

2662 Hybrid rye harvested at two maturities and its effects on yield, composition, and fermentation profile. L. Garcia*[1], C. M. Rundle[2], and F. C. Cardoso[1], [1]*University of Illinois at Urbana Champaign, Urbana, IL,* [2]*KWS Cereals USA, Champaign, IL.*

Our objective was to determine the effects of ensiling time (0, 30, 60, 90, and 150 d) on fiber composition of hybrid rye (KWS Progas, KWS Cereals USA, Champaign, IL) harvested at 2 maturities. Treatments were replicated once and assigned to one of two 10-acre plots of hybrid rye as follows: hybrid rye harvested at boot stage (BOO) and hybrid rye harvest at milky stage (MIL). Baled hybrid rye was transported by flat rack wagons (E-Z Trail Inc., Arthur, IL) from field to scale where each wagon weight was recorded. A total of 6 samples were removed directly from the bales using a forage sampler (Nasco Education, Fort Atkinson, WI) at their respective ensiling time then frozen for later analysis. Statistical analyses were conducted using the MIXED procedure of SAS. At harvest, the average DM content for BOO and MIL was 16.9 ± 0.01, and 29.0 ± 0.06%, respectively. Hybrid rye yield for BOO and MIL was 8.46 and 13.6 tons/acre, respectively. Rye silage in MIL had higher ($P = 0.002$) concentrations of ADF (39.0%) than rye silage in BOO (34.0%). Rye silage in MIL had higher ($P = 0.03$), concentrations of NDF (58.3%) than rye silage in BOO (54.8%). There was a treatment × time point interaction ($P = 0.03$) in which rye silage in BOO had a lower concentration of starch at d 0, 30, and 60 (0.50%, 0.75%, and 0.15%, respectively) than rye silage in MIL (2.90, 2.70, and 1.85%, respectively). Rye silage in MIL had higher ($P = 0.04$) concentrations of total acids (6.25%) than rye silage in BOO (4.19%). There was a treatment × time point interaction ($P = 0.03$) in which rye silage in MIL had a higher concentration of acetic acid on d 60, 90, and 150 (1.08%, 1.03%, and 1.33%, respectively) than rye silage in BOO (0.48%, 0.63%, and 1.00%, respectively). In conclusion, days ensiled negatively affected the nutritive profile and fermentation quality of hybrid rye harvested at milky stage but not for hybrid rye harvested at boot stage.

Key Words: hybrid rye, maturity, fermentation

2663 Effect of hybrid type and region on DM yield and nutritive value of whole-plant corn forage. E. C. Diepersloot*[1], N. Lobos[2], and L. F. Ferraretto[1], [1]*Department of Animal and Dairy Sciences,*

University of Wisconsin–Madison, Madison, WI, [2]Pioneer Nutritional Sciences, Corteva Agriscience, Johnston, IA.

The objective of this retrospective study was to evaluate the effect of corn hybrid type and region on the nutrient composition of whole-plant corn forage using a data set of corn forage samples (n = 6,323) grown multiple years (2017–2021). Corn forage samples differed in hybrid type, including conventional and brown midrib (BMR), and region, including the Upper Midwest (UM; Wisconsin and Minnesota), Northeast (NE; New York, Pennsylvania, and Vermont), Mideast (ME; Michigan and Ohio), and California (CA). Data were analyzed as a completely randomized design with fixed effects of hybrid type and region, and hybrid (year) was included as a random effect. An interaction between hybrid type and region was observed ($P = 0.04$ and $P = 0.001$, respectively) for starch and 240 h undigested NDF (uNDF) concentration. For starch concentration, BMR hybrids had on average 3 percentage units less starch than conventional hybrids. However, the starch concentration of BMR hybrids grown in CA did not differ from any other treatments and conventional hybrids grown in CA had a lower starch concentration than conventional hybrids grown in other regions. Conventional hybrids had a 1.3 percentage unit greater uNDF concentration than BMR hybrids, on average, although some differences among regions within hybrid type existed. Conventional hybrids had a 2.1 Mg/ha greater ($P = 0.001$) DM yield than BMR hybrids. Dry matter yield was also affected by region ($P = 0.001$), where CA and UM were 2.3 Mg/ha greater, on average, than other regions. The concentrations of NDF and NDF corrected for residual ash (NDFom) were also affected by region ($P = 0.001$ and $P = 0.001$). Both NDF and NDFom concentrations were greater in ME and UM than NE, although CA did not differ from any other regions. Overall, it appears that BMR hybrids had enhanced fiber quality due to the lower uNDF concentration, which came at the expense of reduced starch concentration and DM yield. Additionally, nutrient composition of corn forage varies by region, likely because of climate and other factors affecting growing conditions.

Key Words: BMR, corn silage, starch

2664 Effects of a lactic acid bacteria inoculant on characteristics and microbiology of almond hulls silage.

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Almond hulls (AH) are commonly used in dairy cow feeding, whether in fresh or dried form, yet their ensiling potential has been scarcely explored. Silage additives are frequently used to improve the ensiling process. This study evaluated the effects of a lactic acid bacteria (LAB) additive on the characteristics and microbiology of AH silage. Fresh AH was hand-cut into pieces approximately 1 × 1 cm and weighed into 10 vacuum bags (500 g of AH per bag); 5 bags were treated with LAB (*Lactobacillus buchneri* at 5×10^9 cfu/kg of fresh AH) and the other 5 bags received no additive (control). The LAB inoculant was homogenized in water (107 mL/bag) and the same water amount was added to control silages. The air inside the bags was extracted using a vacuum-packing machine, the bags were heat-sealed and stored in darkness at room temperature for 3 mo before being opened and their content analyzed. Ensiling microbiology was assessed by classical plate-culture techniques. Compared with control silage, the addition of LAB reduced pH (5.04 and 4.40 for control and LAB silage, respectively; $P < 0.001$) and increased lactic acid concentrations (10.5 and 16.8 g/kg DM silage; $P < 0.001$). There were no significant effects of LAB on silage DM (53.9% and 53.7% for control and LAB silages, respectively; $P = 0.579$). However, the addition of LAB increased DM losses from 1.86% to 3.65% ($P = 0.004$). No *Escherichia coli*, *Salmonella* spp., *Listeria* spp. *Clostridium perfringens*, and *Staphylococcus* coagulase-positive were detected in any silage. Adding LAB reduced the counts of both molds (2.80 and 1.54 log cfu/g fresh material for control and LAB silage, respectively; $P = 0.006$) and yeasts (4.66 and 1.51 log cfu/g; $P < 0.001$) in the silages, but had no effect on *Enterobacteria* spp. (1.20 and 0.95 log cfu/g; $P = 0.193$). The results indicate that using *Lactobacillus buchneri* as an bacterial inoculant can improve the quality of AH silage, but DM recovery can be reduced.

Key Words: almond hulls, silage, bacterial inoculant

2665 Can inoculants alter the starch-protein matrix in high-moisture corn?

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The objective of this study was to evaluate the effects of several inoculants on aerobic stability, N fractionation, and starch degradability of high-moisture corn (HMC). Treatments included (1) no additive (CON); the addition of (2) *Lactobacillus buchneri* NCIMB 40788 (LB); (3) LB and *L. hilgardii* CNCM I-4785 (LBLH); (4) LB, LH, and *Pediococcus pentosaceus* NCIMB 12455 (LBLHP); and (5) LB, LH, *P. pentosaceus*, and enzymes (xylanase and β-glucanase; LBLHPE). Flint type corn kernels (33.9% of moisture) were processed (diameter geometric mean = 0.98 mm) and ensiled in 5-L jars for 30, 60, 120, and 150 d, totaling 100 experimental units. Measurements included fermentation end products, aerobic stability, N fractionation, and in situ starch degradation at 7 h ($isSD_{7h}$). The trial was set up as a completely randomized design using a 5 × 4 factorial arrangement. Data were analyzed using the MIXED procedure of SAS, followed by a Tukey test at $P \leq 0.05$. Silages inoculated with LBLH had the greatest concentration of acetate and 1,2-propanediol, followed by the LBLHPE, LBLHP, LB, and CON treatments. There was an effect of time for aerobic stability, which increased from 30 to 120 d of conservation in all silages. Inoculated silages had longer aerobic stability (>240 h) than the control treatment (160 h) at 120 and 150 d of conservation. During the aerobic exposure, the peak of temperature was, on average, 39.6°C for control and 26.1°C for treated silages. Ammonia was greatest in LBLHP treatment, followed by LB, LBLH, LBLHPE, and CON silages. There were effects of inoculation and conservation time for B1, B2, and C fractions. The CON showed greatest concentration of C fraction (0.45% of CP) than the treated silages (0.25% of CP, on average). There was an effect of time for $isSD_{7h}$, which was greatest at 150 d (76.4% of starch), intermediate at 120 d (70.1% of starch), and lower at 30 and 60 d of storage (60% of starch, on average). Overall, inoculated silages changed HMC fermentation, enhanced the aerobic stability, and had a more intense degradation of starch-protein matrix. These findings warrant future investigations on the role of inoculants in increasing the starch degradability.

Key Words: inoculant, high-moisture corn, starch degradation

2666 Effect of homofermentative and heterofermentative bacterial inoculants on microbial community of alfalfa-bermudagrass silage mixtures.

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The objective of this study was to evaluate the effects of homofermentative (MC) and heterofermentative (FC) bacterial inoculants on bacterial diversity and community structure of alfalfa-bermudagrass silage mixtures harvested during the summer. Representative samples of 8 alfalfa-bermudagrass plots established in 2016 were harvested in 2 consecutive years (2020 and 2021). Samples were randomly harvested at the mid-bloom stage (34.2% DM for bermudagrass and 36.7% DM for alfalfa), chopped (19-mm), wilted, and ensiled in laboratory-scale mini-silos (2 kg each) in quadruplicate with one of the following treatments: (1) distilled water (control), (2) 150,000 cfu/g of *Lactococcus lactis* and *L. buchneri* (FC) and, (3) 150,000 cfu/g of *L. lactis* and *L. plantarum* (MC). At silo opening after 100 d, samples (100 g) were immediately stored at 80°C for bacterial community composition via 16s rRNA sequencing analysis after DNA extraction. Differentially abundant microbes at the genus level was determined using the linear discriminant analysis (LDA) effect size method (LEfSe). The rarefaction curves tended to plateau, and the average Good's coverage of all samples was greater than 99%, illustrating that the sequencing coverage was adequate. At silo opening, *Lactobacillus* genus dominated the bacterial community with relative abundance of 80.2%, 91.4%, and 93.1% in the control, FC, and MC treatment, respectively. The Chao1 index, a measure of β diversity, revealed a less diverse community in the FC and MC silages, compared with the control. Compared with the control, the LEfSe results showed that the relative abundance of *Lactobacillus* and *Weissella* were greater (LDA ≥ 4.0; $P \leq 0.05$) whereas that of *Enterococcus* was lower (LDA ≥ 4.0; $P \leq 0.05$) in FC. The relative abundance of *Lactobacillus*, *Acinetobacter*, and *Lactococcus* was greater (LDA ≥ 4.0; $P \leq 0.05$) in MC, compared with the control. Our results revealed that both FC and MC reduced the diversity of the bacterial community and increased the relative abundance of *Lactobacillus*, which is an indication of good fermentation quality.

Key Words: silage, inoculant, microbiome

2667 Effects of corn silage cutting height on in vitro and in situ digestibility parameters of neutral detergent fiber.

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The objective of this study was to evaluate the effect of corn silage (CS) cutting height (CH) on NDF degradability (NDFD) at multiple time points of fermentation through in vitro and in situ analysis. Additionally, the kd, fast, and slow pools of NDF, ruminal NDFD (rNDFD), and total-tract NDFD (ttNDFD) were estimated. Two CS from the same hybrid (B2782PWU), cultivated in the same area, were harvested at 2 different CH (40 or 65 cm). Samples were collected over the course of an 84-d experiment, with collections conducted every 14 d, each for 7 consecutive d to form a representative pool. Samples were dried, ground at 1 mm, and incubated in quadruplicate in 2 ruminally cannulated cows fed the same diet for 0, 2, 4, 8, 12, 24, 36, and 48 h. For in vitro analysis, samples were incubated in triplicate for 12, 24, 30, 48, 120, and 240 h. Data were analyzed by the PROC GLIMMIX in SAS 9.4. The in situ NDFD was 49.0% and 23.1% greater for CS65 than CS40 ($P < 0.05$) at 12 and 24 h, respectively. The 24 h kd tended to be greater ($P = 0.08$) for CS40 (3.86% vs. 3.54%/h). Estimated rNDFD and ttNDFD were 11.8% greater at 12 h ($P < 0.01$) and 7.0% at 24 h ($P = 0.01$) of incubation for CS65. The CS65 in vitro NDFD was 15.5% and 9.4% greater at 30 h ($P = 0.01$) and 120 h ($P = 0.03$), respectively, than CS40. The CS65 kd tended to be lower ($P = 0.08$) than CS40 (2.93 vs. 3.36%/h). The rNDFD and ttNDFD for CS65 were 8.0% higher at 30 h ($P = 0.01$). The fast pool did not differ, but the slow pool tended to be greater for CS65 ($P = 0.074$; 22.2% vs. 26.7%), whereas the undigested NDF (uNDF) was 18.1% greater for the CS40 ($P = 0.04$; 33.4% vs. 27.3% of NDF). The estimated rNDFD and ttNDFD using a 3-pool mechanistic model were 7.0% greater for CS65 ($P = 0.05$). Considering the weighted 2-pool mechanistic model, the kd was similar between silages, but the rNDFD and ttNDFD were 7.2% greater for CS65 ($P = 0.03$). Increasing the CH decreased uNDF and increased the estimated rNDFD and ttNDFD considering the 3- and 2-pool mechanistic models. Additionally, increasing CH increased in vitro NDFD at 30 and 120 h of incubation, whereas in situ NDFD was greater at 12 and 24 h of incubation.

Key Words: digestibility, fermentation, NDF degradability (NDFD)

Growth and Development 1

2668 Meta-analysis of the effect of milk feeding method on average daily gain, concentrate intake, and weight at weaning of dairy calves. B. D. Alcantara[1], J. P. Donadio*[1,2], K. T. De-Sousa[1], R. N. S. Torres[1], M. J. Hotzel[3], and M. Deniz[1], *[1]Grupo de Estudos em Bovinos Leiteiros, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil, [2]Programa de Pós-Graduação em Zootecnia, Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu, São Paulo, Brazil, [3]Laboratório de Etologia Aplicada e Bem-Estar Animal, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil.*

In this study, we evaluate the effect of milk supply methods on the performance variables of dairy calves using estimates from the peer-reviewed literature in a meta-analysis approach. Initially, 540 studies from 1980 to 2019 were retrieved from databases (Web of Science, PubMed, and Cab Direct) using search terms such as (1) population: calf, calves, heifers and dairy, milking, (2) comparison: environmental enrichment, nutritional enrichment, feeding enrichment, feeding method, artificial teat, nipple, bucket, and (3) outcome: performance. The resulting manuscripts underwent a 4-step Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) appraisal process and were excluded when they presented the wrong population (e.g., goats, cows, and rats), focused on other results (e.g., blood, glycemic index), or dealt with other subjects. We also selected studies that compared the offering of milk on open bucket and any other method of offering that stimulated the calf's natural suckling behavior (e.g., nipple bucket, nipple bottle, rubber teat) and that reported means and mean error. The weighted mean differences between milk feeding methods were analyzed for each variable using the Der-Simonian and Laird. This process resulted in a final sample of 13 articles, which were used for recording metadata. All studies (n = 13) evaluated average daily gain, 6 studies evaluated concentrate intake, and 5 studies evaluated weight at weaning. Calves that received milk from artificial teats tended ($P = 0.088$) to present (0.012 kg/day; CI = −0.01 to 0.02) higher average daily gain. There was no difference in concentrate intake (0.73 ± 0.61 kg/d; $P = 0.588$; CI = −0.03 to 0.05) and weight at weaning (63.52; ± 9.72 kg; $P = 0.218$; CI = −1.57 to 6,87) when compared open bucket to artificial teats. Despite offering milk from artificial teats stimulate the natural suckling behavior, there appears to not be a clear effect on the performance of dairy calves. However, the lack of positive effects of offering milk using artificial teats on evaluated variables should not be used as detrimental to its adoption because it could have a greater impact on calves' emotional states.

Key Words: calf nursing, performance, preweaning

2669 Effects of milk replacer fat levels on feeding intake and performance of male dairy calves. J. V. R. Lovatti*[1], J. M. V. Pereira[1], T. E. da Silva[1], A. J. Keunen[3], D. L. Renaud[2], and J. H. C. Costa[1], *[1]University of Vermont, Burlington, VT, [2]University of Guelph, Guelph, ON, Canada, [3]Maple View Agri, Palmerston, ON, Canada.*

The objective of this study was to determine the effects of fat levels in milk replacer (MR) on feeding intake and performance of dairy calves. Holstein male calves (n = 126) were housed individually and enrolled in a 91-d trial divided in 3 phases: preweaning (1–42 d), weaning (43–63d), and postweaning (64–91 d). Calves were randomly assigned to 3 treatments consisting of a MR with a palm and coconut fat blend with low fat level in relation to the crude protein level (17%; Low-fat [LF] %; n = 42), moderate fat (23%; Mod-fat [MF] %; n = 43), and high fat (29%; High-fat [HF] %; n = 41). The MR was fed twice daily (Mapleview Agri Ltd., ON, Canada; 26% CP; 130 g/L) following the step-up, step-down program: 520 g/d from d 0 to 6; 650 g/d from d 7 to 13; 910 g/d from d 14 to 20; 1,040 g/d from d 21 to 41; 910 g/d from d 42 to 48; 650 g/d from d 49 to 63. Calf starter (CS; 20% CP pellet + 4% straw) was fed ad libitum. Statistical analyses were performed using the PROC MIXED procedure of SAS. Overall, average daily gain (ADG) was greater for LF dairy calves during preweaning (LF = 0.90 ± 0.02, MF = 0.76 ± 0.02, HF = 0.74 ± 0.02 kg/d; $P < 0.01$) but did not differ among treatments across weaning and postweaning phases ($P = 0.17$). Final body weight (91 d) was greater for LF dairy calves across all phases (LF = 160.60 ± 1.80, MF = 147.15 ± 2.09, HF = 148.71 ± 2.67 kg; $P < 0.01$). Total DMI (MR + calf starter) was greater ($P < 0.001$) for LF calves due to greater grain intake across weaning and postweaning ($P < 0.001$). This led to greater metabolizable energy (ME) intake for LF calves during postweaning phase (LF = 13.88 ± 0.18, MF = 11.74 ± 0.22, HF = 11.95 ± 0.26 Mcal/kg; $P < 0.01$), and greater overall crude protein (CP) intake across weaning and post weaning ($P < 0.01$). Fat level in the MR did not affect overall feed efficiency ($P = 0.13$). Lower fat levels were associated with greater calf starter intake, consequently increasing ME and CP intake, positively affecting calf performance. Future research should investigate the effects of fat level in the feeding intake satiety and weight gain composition.

Key Words: nutrient intake, milk replacer composition, fat ratio

2670 Performance and beef grading of F1 Holstein cattle in northern Mexico fed in the preweaning stage with a high volume of milk at 14% solids. E. Carrillo-Moreno*[1], E. Carrillo[2], E. Perez-Rebolloso[3], M. Mellado[1], D. Carrillo-Moreno[1], and F. G. Veliz[1], *[1]Universidad Autónoma Agraria Antonio Narro, Torreón, Coahuila, México, [2]Instituto Tecnológico de Torreón, Torreón, Coahuila, México, [3]Centro de Bachillerato Tecnológico Agropecuario N°47, León Guzmán, Durango, México.*

The objective of this study was to evaluate the effect on performance parameters and carcass classification of F1 Holstein/beef cattle, with 2 different feeding programs in the pre-weaning stage (0–60 d of age), both groups with high milk volume (450 l/60d), but one group fed whole milk with milk replacer for 14% total solids (14% TS) or milk only (11% TS). For this study, 80 calves were completely randomly divided into 2 groups (40 calves/group): 14% TS or 11% TS. These 2 groups were subdivided by sex, leaving 4 groups (20 calves/group): males and females 14% TS group, or males and females 11% TS group. Data were analyzed by ANOVA with multiple comparisons (group × sex) and Tukey, using the Fit Model procedure of JMP 17 Pro (SAS Institute Inc., Cary, NC). At the end of the pre-weaning stage (60 d) the 14% TS groups were 6.61 kg ($P < 0.01$) heavier than the 11% TS groups (99.45 vs. 93.28 kg). In the same way, the average daily gain (ADG) of the 14% TS group was better ($P < 0.01$) than the 11% TS group (989 vs. 850 g/d), and there is a correlation between ADG at weaning and final weight ($R2 = 0.81$). The final weight was higher in the 14% TS group ($P < 0.05$) than the 11% TS group (535 vs. 523 kg). In the age at sale, there were no significant differences, but it was lower in the 14% TS group than in the 11% TS group (419 vs. 426 d). Carcass weight was greater in the 14% TS group ($P < 0.01$) than in the 11% TS group (310.68 vs. 299.88 kg). Beef grading of the carcass was better in the 14% TS group ($P < 0.05$) than in the

11% TS group, obtaining a higher percentage of animals classified as choice (19/40 = 47.5% choice vs. 14/38 = 36.84% choice). The ribeye area was also better for the 14% TS group ($P < 0.05$) than in the 11% TS group (12.73 vs. 11.91 cm). In conclusion, this study demonstrates the benefits of using a high-volume milk feeding program adjusted to 14% TS on the performance and carcass classification of F1 Holstein/beef cattle and describes the possible scope of a beef on dairy program to improve the profitability of the dairy industry.

Key Words: beef on dairy, growth, preweaning

2671 Association between water consumption and solid feed intake in dairy calves.

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The objective of this retrospective cohort study was to investigate the association between the amount of water and solid feed a calf consumes in the first 84 d following arrival at a commercial calf rearing facility. Holstein and crossbred calves between 5 and 14 d of age on arrival were offered milk, up to 8 L per day at 130 g of milk replacer per L, from arrival to 63 d. Solid feed, composed of 4% straw and a pellet (19.8% crude protein and 1.64 Mcal/kg) was offered ad libitum from arrival to 84 d and weighed back weekly to note refusals. Water was available to calves via a nipple or bowl, which was connected to a calf-specific water line with water meters attached. The meters were read on a weekly basis to determine water consumption. Mixed linear regression models, with a random effect for arrival group and a fixed effect for body weight at arrival, were built to explore associations between grain and water intake using Stata 18. Data were available from 346 calves from 6 groups. Over the 84-d period, calves consumed on average (± SD) 150.82 ± 32.54 kg of calf starter and 363.78 ± 103.82 L of water. For every 1 L of water consumed over the 84-d period, starter consumption increased by 0.24 kg (95% CI: 0.22–0.27; $P < 0.001$). Furthermore, every kg increase in body weight at arrival was associated with a 0.87 kg (95% CI: 0.27–1.48; $P = 0.005$) increase in starter intake over the 84 d under observation. When evaluating each wk after arrival independently, it was found that for every 1 L of water consumed, starter consumption increased during the 12 wk after arrival (Table 1). In conclusion, providing access to drinking water to calves could promote an increase in calf starter intake.

Key Words: calf, drinking water, body weight

Table 1 (Abstr. 2671). Predicted grain intake for every 1-L increase in weekly calf starter for 346 calves

Wk after arrival	Predicted grain intake (kg)	95% CI	*P*-value
1	0.02	−0.002 to 0.03	0.09
2	0.03	0.01 to 0.06	0.01
3	0.08	0.04 to 0.11	< 0.001
4	0.05	0.01 to 0.09	0.008
5	0.07	0.03 to 0.1	< 0.001
6	0.17	0.13 to 0.21	< 0.001
7	0.26	0.23 to 0.30	< 0.001
8	0.26	0.24 to 0.28	< 0.001
9	0.24	0.22 to 0.26	< 0.001
10	0.24	0.22 to 0.26	< 0.001
11	0.21	0.19 to 0.23	< 0.001
12	0.22	0.19 to 0.24	< 0.001

2672 The effect of pair housing on immune development in response to weaning and social mixing in dairy calves.

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Most dairy operations in the United States house calves individually before weaning and social mixing. As a result of individual housing, dairy calves have shown increased amounts of stress behaviors during the weaning and social mixing transition and often have an increase in disease occurrence. It is unclear how isolation housing may be affecting calves' immune development during this time period. This study assessed the impact of housing type before weaning on immune development during weaning and postweaning social mixing. A total of 30 Holstein heifer calves were enrolled in 1 of 2 treatment groups within 7 d after birth: pair housed (n = 20; 10 pairs) or individually housed (n = 10). Calves in both treatment groups were housed in outdoor hutches, (2 hutches side-by-side for each pair; one hutch for individual calves), and managed via the same standard operating procedures. All calves were weaned from d 43 to 56 and spent an additional 7-d adjustment period, after weaning, in the hutches. Calves were socially mixed within their treatment group on d 63 of life. Whole blood was collected 1 wk before the start of weaning, on d 43, 44, 46, 48, and 50, and on d 63, 64, 66, 68, and 70. White blood cells were harvested from the whole blood and used for the determination of gene expression through qPCR. A 2-sample *t*-test showed no difference between the average CTs of the treatment groups in the gene expression PCR assay: IL1β (25.7 vs. 25.7, $P = 0.59$), TNFα (28.7 vs. 28.7, $P = 0.75$), MPO (32.3 vs. 32.4, $P = 0.79$), SELL (24.3 vs. 24.4, $P = 0.28$), and TLR4 (29.7 vs. 29.9, $P = 0.35$). Findings from this research will be used to better understand the impact of housing on immune development during the weaning and postweaning social mixing transition phases in dairy calves.

Key Words: cytokine, welfare, housing management

2673 Investigating effect of Holstein heifer growth pattern on first-lactation performance based on cluster analysis.

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It is important to identify key factors that affect first lactation performance and determine the optimal growth pattern for heifers. Cluster analysis was employed to examine the growth modes of Holstein heifers and the factors influencing their first lactation performance. Data from 2481 Holstein cows from Chinese dairy farms, covering the period from birth to first lactation production. The data set comprises information on birth weight, weaning weight, 6-mo body weight, 12-mo body weight, age at first breeding (AFB), age at first calving (AFC), average daily gain at weaning (ADG1), average daily gain from weaning to 6 mo (ADG2), average daily gain from 6 mo to 12 mo (ADG3), total days with disease before calving, and frequency of disease before calving for the cluster analysis. The data were divided into training and test sets in a 9:1 ratio. The sum of squared errors was calculated using the R (version 4.1.3) "factoextra" package to determine the optimal number of clusters, which was then followed by k-means cluster analysis. After clustering, we used the "FeatureImpCluster" package to calculate the importance of variables in the clustering results. A non-parametric Kruskal-Wallis ANOVA with multiple comparisons was conducted. The study revealed that the clustering results were divided into 3 categories: Excellent Level Heifer, Good Level Heifer, and Medium Level Heifer. The key factors affecting first lactation production performance were the total days with disease before preweaning, weaning weight, frequency of disease before calving, and ADG1. Collectively, the heifer growth model that performs the best indicates that the average weaning weight reached 103.25 kg. The ADG1 increased rapidly to 1.11 kg/d, whereas the recommended range for average ADG3 is between 0.63 and 0.71 kg/d, with a maximum of 0.63 kg/d. The recommended age range for average AFB is between 13.37 and 13.51 mo old, whereas the recommended age range for average AFC is between 22.90 and 23.82 mo old.

Key Words: heifer, first-lactation performance, cluster analysis

2674 Evaluation of the NASEM (2021) energy requirement models by using dairy-beef crossbred calf data. T. A. Klipp*, A. J. Carpenter, and J. A. D. R. N. Appuhamy, *Iowa State University, Ames, IA.*

The objective of this study was to evaluate the NASEM (2021) energy requirement models against the data from young dairy-beef crossbred (DBC) calves. Data were obtained from a study where 117 newborn Angus × Holstein bull calves were assigned to a high (HS: 26% starch, 20% protein, 7% fiber) or a low starch (LS: 13% starch, 20% protein, 11% fiber) calf starter feed (CSF) until 9 weeks of age. Calves were fed with a milk replacer (MR; 20% fat and 22% protein) until weaning at 8 wk of age. The data included 1,027 records of weekly body weight (BW), average daily gain (ADG_observed), and corresponding MR solid and CSF intake (kg/d). The metabolizable energy (ME) content of MR was 4.81 Mcal/kg of DM, and that of CSF was estimated with a dairy-calf model accounting for the nutrient digestibility varying with age (Quigley et al., 2019). The estimated ME contents of HS and LS varied between 2.25 and 3.33, and 2.08 and 3.13 Mcal/kg of DM, respectively. The ME of maintenance (ME_m) was calculated as $ME_m = 105.8 \times BW^{0.75}$ by adjusting for the changes in ME requirement for thermoregulation as described in NASEM (2021). The ME_m was subtracted from ME intake (MEI; MEI = ME from MR + ME from CSF) to calculate the MEI available for growth (MEI_g). The MEI_g allowable daily gain (ADG_predicted) was calculated by rearranging the NASEM (2021) equation to predict the retained energy (RE; $RE = EBG^{1.1} \times EBW^{0.205}$, where EBG = average daily gain × 0.91 and EBW = BW × 0.93) with the 0.55 energy efficiency. The mean square prediction error (MSPE) and the square root of it (RMSPE) were calculated. The MSPE was decomposed to mean (MB), slope (SB), and random bias (RB). The RMSPE was 0.37 kg/d, which was 51% of the mean ADG_observed. The MB, SB, and RB represented 13.5%, 5.5%, and 81% of the MSPE. The CSF type did not affect the prediction error, which increased with age. Overall, NASEM (2021) models were associated with significant bias in describing ME requirements of Angus × Holstein bull calves. Reparameterizing the extant models to represent energy digestibility and ME efficiencies of DBC calves may be required.

Key Words: calf, dairy-beef crossbred, energy model

2675 Development of body weight estimation equations using LiDAR scanning and machine learning in growing dairy cattle. Y. Kato*[1], Y. Shiotsuki[2], H. Takei[2], K. Azuma[3], M. Okimoto[3], R. Harada[3], T. Obitsu[1], and T. Sugino[1], [1]*The Research Center for Animal Science, Graduate School of Integrated Science for Life, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan,* [2]*BIPROGY Inc., Koto, Tokyo, Japan,* [3]*The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Shibuya, Tokyo, Japan.*

This study aims to develop equations for estimating body weight (BW) using body measurements obtained through LiDAR scanning. A total of 691 Holstein female calves and heifers (aged 0–25 mo) were used. LiDAR scanning on an iPad Pro was employed to capture 3D images of each animal's left and upper body sides, allowing for length of body measurements including chest girth, withers height, hip height, body length, horizontal body length, chest depth, and hip width. Additionally, chest girth was measured using a tape measure, and BW was determined using a static weighing station. Animals were categorized into 3 stages: calf (<18% of mature BW [MatBW] of 700 kg, n = 234), heifer before conception (18–55% of MatBW, n = 330), and heifer from conception to first calving (>55% of MatBW, n = 127) based on target weight guidelines for growing dairy cattle in NASEM (2021). The BW prediction equations incorporating body measurements at each stage were analyzed using a machine learning (a boosting tree) in JMP® Pro 17. A 75% training set was used to develop prediction models using body measurement data, with a 10% validation set to assess performance, and the remaining 15% as the final testing subset. Estimation accuracy was assessed by calculating the root mean square error (RMSE) between measured and estimated BW. For calves, the equation with 7 body measurements yielded the highest correlation coefficient and the lowest RMSE ($R^2 = 0.963$, RMSE = 4.85 kg). Similarly, for heifers before conception, the equation with 5 parameters excluding chest depth and hip width achieved the highest correlation coefficient and the lowest RMSE ($R^2 = 0.984$, RMSE = 10.2 kg). Furthermore, for heifers from conception to first calving, the equation with 3 parameters excluding chest depth, hip width, withers height, and hip height showed the highest correlation coefficient and the lowest RMSE ($R^2 = 0.995$, RMSE = 5.05 kg). These findings suggest that whole skeletal growth occurs predominantly in the initial growth phase, with growth rates gradually diminishing in the order of width, height, and length in growing dairy cattle.

Key Words: estimation of body weight, machine learning, growing dairy cattle

2676 CalfSim Tool: A free and user-friendly tool for tailoring calf nutritional strategies and growth. T. Da Silva* and J. Costa, *University of Vermont, Burlington, VT.*

Developing optimized nutritional strategies for preweaning calves is fundamental for ensuring their health, welfare, and future performance. To our knowledge, the availability of tools tailored for designing nutritional plans based on farm-specific conditions is often limited. Therefore, we developed the CalfSim Tool, a free and user-friendly tool that aims to allow consultants and researchers to simulate calf performance under different nutritional plans. As basis for developing the tool, the equations were used for calf starter intake and nutritional requirements presented in the National Academies of Sciences, Engineering, and Medicine (NASEM, 2021). In determining the metabolizable energy (ME; Mcal) of the calf starter, in addition to its chemical composition, we used the cumulative intake of non-fibrous carbohydrates (NFC) as an indicator of gastrointestinal tract maturity. The maximum calf starter ME value was achieved with an accumulated NFC consumption of 15 kg (Quigley, 2019). We developed the CalfSim Tool using the package and framework for web application development called Shiny as a backbone, available in the R programming language (R Core Team version 4.2.0). In utilizing the CalfSim Tool, the user is required to input information regarding calf initial weight (kg), weaning age (d), average temperature (°C), nutritional composition of whole milk or milk replacer, and the number of scenarios to be simulated. Within

each scenario, the user has the flexibility to specify the daily quantity of milk or milk replacer provided to the calves. The user must enter the nutritional composition of the calf starter to estimate its ME value. After entering the information, the user must run the model and analyze the outcomes summarized in a dashboard, comparing each simulated scenario in terms of key performance indicators, such as final weight (kg), average daily gain (kg), and age at accumulated consumption of 15 kg NFC. Thus, the CalfSim Tool can help farmers and consultants to simulate and optimize nutritional plans for calves, guaranteeing performance based on farm-specific conditions.

Key Words: energy, modeling, optimization

Lactation Biology 2

2677 Repeated biopsies performed in calves, heifers, and first lactation cows. A. L. Vang*, W. S. Frizzarini, H. H. Webster, T. Cunha, J. R. R. Dorea, and L. L. Hernandez, *University of Wisconsin–Madison, Madison, WI.*

Mammary gland development research has often required culling of calves and heifers for tissue collection, as mammary biopsies have historically been performed exclusively in lactating dairy cows. The objective of this research was to investigate the utility of mammary biopsies in calves and heifers for research purposes, and their impact on subsequent lactation performance in the adult phase. Ultrasound-guided udder biopsies were performed on 8-week-old calves (n = 14) and 10-week-old calves (n = 113) with a novel technique using a disposable biopsy punch. Repeated biopsies were performed on 36 of the 113 animals biopsied at 10 wk of age at 6, 9, and 12 mo of age; 5, 7, 8 mo gestation; and 1, 45, 90, 150, and 270 DIM. This technique required incisions ranging between 0.5 cm and 1.5 cm depending on punch size and yielded 1 mm by 0.5 to 1 cm of tissue from calves using the 2-mm punch and 4 to 5 mm by 2 to 4 cm of tissue from heifers using the 6-mm punch. Tissue glue was used to close incisions in calves and sutures were used in heifers. Incisions healed and sutures were removed within 7 to 10 d post-biopsy. Of the 445 total biopsies performed to date, few complications have been encountered (e.g., infection, excessive bleeding). Linear mixed models with repeated measures were used to analyze milk production in the days surrounding the biopsy procedure with minimal effects detected ($P > 0.05$). The model included days in relation to biopsy (−2, −1, 0, 1, 2) as a fixed effect and animal as a random effect. The described technique is a safe, reliable method for mammary biopsies in cattle beginning at 8 weeks of age and can be used to obtain repeated tissue collections in heifers through the first lactation with low risk for infection and long-term tissue damage. The method has also been successfully replicated in multiparous cows in separate studies. This work will allow for longitudinal assessment of manipulations in early life on milk yield.

Key Words: mammary gland biopsy, calf, heifer

2678 Impact of residual milk, teat size, and cow age on hyperkeratosis in Holstein cows. T. B. P. Silva, B. B. Silva, A. L. Silva, and P. P. Rotta*, *Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*

This study aimed to assess the correlation between hyperkeratosis (HY) and residual milk, teat size, cow age, and milk yield (MY) in Holstein cows. A total of 37 Holstein cows, producing an average of 32.8 ± 7.61 kg/d of milk, and 153 ± 99 DIM, were included in the study. The cows were milked 3 times daily. Following the standard milking procedure, a manual milking session was conducted to eliminate any residual milk. The residual milk was collected and weighed. This process of collecting residual milk was carried out for each teat of all animals during every milking session throughout the day. The relationships between the total residual milk and total MY and the age of the animals were analyzed using linear regression, using the lm function of R. The presence or absence of HY was analyzed by ANOVA. The influence of the teat size and the age of the animals on the presence of HY was analyzed by binomial odds ratios, using the glm function of R. For all analyses, significance of 0.05 was considered. Anterior teats showed increased susceptibility to odds ratio for HY as teat size increased (odds ratio = 1.23 and 1.21 for the left and right anterior teats, respectively). There was no correlation between residual milk and HY score, and no correlation between residual milk and total MY was observed. When examining the total residual milk volume in relation to age, we noted a positive correlation: as the cows advanced in age, specifically in months, there was a corresponding increase in residual milk volume. There was an effect between the age of the animal and the presence of HY for the left rear teat and the front teats. Anterior quarters showed a greater susceptibility to HY, especially in cows with larger teats. Cow age was found to influence both residual milk and the presence of HY. No significant relationship was observed between residual milk and teat size or the presence of HY. The findings of this study suggest that older animals exhibit a higher incidence of incomplete milking. This observation underscores the importance of considering age as an additional criterion for replacement in the selection process.

Key Words: hyperkeratosis, milking, teat size

2679 Single cell multi-omics reveal the lactating mechanisms of hypothalamus-pituitary-mammary gland axis on dairy cows. C. Zhang* and H. Liu, *Zhejiang University, Hangzhou, China.*

The hypothalamus and pituitary gland play an important role in many physiological processes, such as growth, metabolism, and lactation in dairy cows. However, many of their underlying interactive and integrated mechanisms remained unclear until now. Here, samples were collected from the hypothalamus, pituitary gland, and mammary gland of dairy cows (n = 2). We applied single-nucleus RNA and spatial transcriptomics to generate an atlas of hypothalamus-pituitary-mammary gland axis during lactation of dairy cows. We obtained 30,654 high-quality single nuclei, among which 8,071 were from hypothalamus, 6,686 from pituitary, and 15,897 from mammary gland cells. Moreover, we identified the oligodendrocyte, astrocytes, and microglia, as well as the neuronal subtypes in the hypothalamus. Analyses of cell-type-specific transcriptomics showed the gene expression profile associated with oligodendrocyte differentiation, and more striking, glutamatergic and GABAergic neurons, actively functioning in energy metabolism and stress responses, which were presumed to exert a remarkable effect on lactating mammary gland. Cluster analysis further demonstrated the different hormone-producing cell types in the pituitary. For the mammary gland, we characterized the principal cellular subtypes and identified a distinct alveolar cell subset that exhibits elevated expression of HSP90. The analysis showed that ATF3 could be critically involved in the regulation of stress responses. This hypothesis was supported by in vitro experiments with bovine mammary epithelial cells, which elucidated that ATF3 influences cell fate, the synthesis of milk composition, and the expression of hormone receptors. In addition, by employing the integrative analysis of spatial transcriptomics, we generated an in situ map of cell types, which would provide a comprehensive understanding of functional tissue organization and cell-to-cell interaction. Together, our study offers a rich resource to elucidate the stress response and lactation regulation tailoring by the hypothalamus-pituitary-mammary gland axis in dairy cows.

Key Words: dairy cow, single-nucleus transcriptomics, spatial transcriptomics

2680 Milk vein blood flow measured via Doppler ultrasound to estimate mammary plasma flow at calving and day 7 of lactation in multiparous Holstein cows. A. J. Fischer-Tlustos*[1], D. J. Seymour[1,2], K. Klein[1], J. Petrou[1], C. McQuaig[1], E. Ribeiro[1], J. M. Leão[3], C. A.

Redifer[4], A. M. Meyer[4], J. P. Cant[1], and M. A. Steele[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Trouw Nutrition Research and Development, Boxmeer, the Netherlands,* [3]*Saskatoon Colostrum Co. Ltd., Saskatoon, SK, Canada,* [4]*Division of Animal Sciences, University of Missouri, Columbia, MO.*

The Fick principle is commonly used to estimate mammary plasma flow (MPF) as an indicator of mammary metabolic activity; however, this method requires total milk AA output to determine MPF. As such, estimation of MPF via the Fick principle is not possible during the dry period when mammary metabolic activity may be an important predictor of production success during the fresh period. The aim of this study was to determine the relationship between milk vein blood flow (MVBF) measured via Doppler ultrasound and MPF estimated via the Fick principle during the fresh period. Parity 2 (P2; n = 11) and 3 (P3; n = 10) Holstein cows were fed a standard lactating diet (NE_L = 1.67 Mcal/kg DM). At calving (d 0) and d 7 ± 1 relative to calving, MPF was estimated via the Fick principle using Phe and Tyr as internal markers, and MVBF was measured via Doppler ultrasound of both milk veins at 2 and 8 h after colostrum collection (d 0) and at the morning milking on d 7. Serum was analyzed for Phe and Tyr by UPLC, and milk was analyzed for N content by Dumas combustion. Mixed model linear regression was performed using the GLIMMIX procedure of SAS considering the fixed effects of MVBF and sampling day, and the random effect of cow. There was no relationship ($P > 0.70$) between MVBF and MPF for P2 cows, possibly due to the high individual variation in MVBF observed in P2 cows. A quadratic relationship ($P = 0.04$; $R^2 = 0.47$) was present for P3 cows with day-specific intercepts observed at d 0 (1699.5 ± 354.68; $P = 0.005$) and d 7 (1497.7 ± 339.86; $P = 0.007$). The coefficients for the linear and quadratic terms were −3.69 ($P = 0.036$) and 0.0032 ($P = 0.04$), respectively. Using the regression equation for MPFon d0 and total MVBF, average MPF for P3 cows was estimated to be 909.3 ± 135.84 L/h at wk 1 relative to calving. These data indicate that MVBF, a non-invasive technique, may be used in future studies as an indicator of mammary metabolic activity in response to management and nutritional strategies aimed at optimizing the prepartum process of colostrogenesis in P3 cows.

Key Words: mammary plasma flow, milk vein ultrasound, fresh period

2681 Heat stress affects mammary gene expression and milk production in Saanen goats.

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Stress induces significant metabolic changes, resulting in increased reactive oxygen species and decreased levels of antioxidants. This study aimed to assess the effects of heat stress on gene expression in mammary tissue and their interactions with apoptosis rates and milk yield in Saanen goats. A total of 30 goats were subjected to treatments: heat stress (HS: under a climatic chamber, n = 15) or control (CT: under a thermoneutral environment, n = 15) on last 60 d of gestation. Respiratory frequency (RF), rectal temperature (RT) and cortisol (CORT) were measured biweekly. Milk yield and quality were assessed daily, and mammary tissue biopsies were taken on d 30 and 150 of lactation. The CORT concentration was measured by EIA analysis, and gene expression related to oxidative stress, apoptosis and milk synthesis were analyzed on epithelial cells obtained from mammary tissue by quantitative PCR. Data were analyzed using the GLM or MIXED procedure, the treatment effect was considered fixed, and day of lactation, time of sampling and animals were considered random effects. Statistical significance was defined as $P = 0.05$. During gestation, HS significantly increased RT (39.2 vs. 38.8 ± 0.05°C), RF (89 vs. 65 ± 1.81 movements/min), and CORT (14.25 vs. 6.05 ± 2.2 ng/mL) compared with CT ($P = 0.05$). However, there was no significant effect of HS during gestation on subsequent milk yield. There were no changes in bacterial counts in milk nor increases in clinical or subclinical mastitis rates in HS goats, and HS significantly increased the SCC in milk ($P = 0.05$), suggesting that RT higher than 39.5°C increased the apoptosis rate in mammary tissue. On 150 d of lactation, HS significantly increased the expression of *BAX*, ER_1, *INSR*, *SOD*, and *CAT* genes in mammary tissue ($P = 0.05$). These results were associated with the oxidative process, and it is possible to argue that higher SCC in the milk of healthy goats is due to cell apoptosis and greater exfoliation of mammary epithelial cells. Further studies are necessary to understand the heat stress effects on the synthesis of oxidant and antioxidant molecules and apoptosis rates in mammary tissue.

Key Words: mammary tissue, apoptosis, oxidative process

Production, Management, and the Environment 5

2682 A One Welfare perspective of dairy farmer well-being: The complexity of caring for production animals. A. Le Heiget[1], H. Tambadou[1], B. Zwick[1], V. Fraser-Celin[2], B. Hagen[1,2], A. Jones[1,2], J. Kinley[1], E. Pajor[1,3], J. C. Plaizier[1], K. Ominski[1], C. Winder[1,2], and M. King*[1], *[1]University of Manitoba, Winnipeg, MB, Canada, [2]University of Guelph, Guelph, ON, Canada, [3]University of Calgary, Calgary, AB, Canada.*

Dairy farmers are faced with many challenges that come with caring for animals, and there is growing concern about their mental health and well-being. To adequately address their needs, a deeper understanding of the interconnections between farmer well-being, technology, and animal health and welfare is needed. The objective of this qualitative study was to explore dairy farmer well-being in the context of animal health and welfare. Farmers were purposively sampled to ensure that a variety of perspectives were included. An interview guide was created in collaboration with industry stakeholders, academics, and dairy farmers, and was used by the lead author (ALH) to conduct semi-structured interviews from a constructivist paradigm. A total of 30 farmers (60% men and 40% women) from Western Canada (57%) and Ontario (43%) completed a demographic survey and a semi-structured interview by phone, online, or in person (June to October 2023). Interview recordings were transcribed by Otter.ai and then reviewed by 2 authors (ALH and MK). Transcripts were open coded by 4 authors (ALH, BH, AJ, and VFC) to create a codebook to help guide the iterative coding process conducted by 2 authors (ALH and VFC). NVivo was used to analyze the data with an approach informed by reflexive and codebook thematic analysis. Farmers had freestall housing (73%) or tiestalls (27%), and conventional milking systems (63%) or automated systems (37%). Themes primarily surrounded caring for livestock and the complexity of providing care. Farmers mentioned that the pressure of providing the best possible care to their herd was stressful, but caring for animals also gave them feelings of fulfilment, illustrating a paradoxical relationship of caretaking. Factors attributed to their capacity to care for their herd included climate concerns related to feed, the demanding nature of dairy farming, entanglement of work and life, labor challenges, financial stress, and sociopolitical and sociocultural challenges. These findings highlight the entanglement of dairy farmer well-being, animal care, and the environment, and the need for resources with (agri)cultural competency to support farmers.

Key Words: mental health, well-being, qualitative

2683 Transition cow health and management on pasture-based dairy herds: Results of a farmers' survey. L. Horan[1], J. Patton[1], C. G. McAloon[2], A. García-Muñoz[3], A. Reagan[1], J. F. Mee[1], and A. Valldecabres*[1], *[1]Teagasc, Animal and Grassland Research and Innovation Centre, Fermoy, Co. Cork, Ireland, [2]School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland, [3]Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, Valencia, Spain.*

This study aimed to describe transition period perceptions, disease incidence, and management strategies reported by farmers to inform and guide future research on pasture-based dairy farms. An online survey was distributed via text message among 3,899 Teagasc dairy advisory clients. The survey yielded 597 responses (15% response rate), of which 525 responses were deemed valid and suitable for analysis. Fresh cow diseases (e.g., milk fever, retained placenta, metritis, displaced abomasum, ketosis) were ranked to be of highest importance by nearly half of respondents (49%). Freshly calved cows' health was identified as critical and a good transition to set the cow up for lactation (86%). Incidence of disease was reported to be highest in fresh cows (first 3 wk after calving; 58%), at the end of the calving season (48%) and in multiparous cows (52%). Most respondents reported to treat 1% to 6% of their herd for milk fever (66%) and retained placenta (66%). A noticeable proportion of respondents indicated that milk fever (16%) and subclinical hypocalcemia (9%) were a significant (regularly treating severe cases with some cows lost/culled) or routine (regularly treating cows to control issues) problem in their herds. Correct management during the dry period was regarded as essential for future health and performance by 90% of respondents. Body condition monitoring was the most commonly reported dry cow management strategy by all herds (73%). Regarding fresh cow management, keeping freshly calved cows indoors for a period after calving (68%) and high-risk cows' calcium supplementation (57%) were the most commonly reported strategies. Management strategies varied between farms, and according to farm characteristics (size and calving pattern). In conclusion, the results from this survey suggest that research in transition cow health and management is warranted, and that in order to address reported concerns and optimize most commonly implemented management practices in pasture-based dairy herds, it should focus on milk fever, retained placenta, dry cow body condition monitoring, and fresh cow postpartum housing and calcium supplementation.

Key Words: grazing, transition disease, oral calcium

2684 Measuring skeletal muscle NADH production to assess feed efficiency and milk production. J. M. Lance*[1], L. T. Casarotto[1], D. O. Martinez[1], M. A. T. de Bari[1], H. Olmo[1], D. F. Binder[1], N. Jeronymo[1], J. Magalhães[1], B. J. Renquist[2,3], and G. E. Dahl[1], *[1]University of Florida, Gainesville, FL, [2]University of Arizona, Tucson, AZ, [3]Effani Agriculture Inc., Tucson, AZ.*

Identifying animals that more efficiently turn feed into milk will limit costs and improve sustainability. Residual feed intake can be calculated to allow selection for feed efficiency, but it is labor intensive and requires specialized equipment. We hypothesize that skeletal muscle metabolic rate is a phenotypic measure that can be applied to predict feed efficiency. We used a biopsy needle (18G) to collect triceps brachii muscle of Holstein cows (BW ± SEM; 662 ± 25 kg; lactation 1–4; n = 36) across the lactation cycle (dry, early, mid, and late) and from growing heifers before they enter production (417 ± 8 kg; n = 12). All cows were fed a production-stage appropriate total mixed ration. Biopsy metabolic activity was measured using the oxidation-reduction indicator resazurin, at 0 and 4 h later, followed by assessment of total DNA (May) or protein (October). The ln(fluor/DNA or protein) was regressed on the ln(DNA or protein) to get expected values and to calculate residuals (RES). Negative RES were indicative of cows that had higher than expected NADH production (less efficient), and positive RES were indicative of lower NADH production (more efficient). The RES in May was positively associated with the same measure in October ($P = 0.0251$; r = 0.44; n = 26). When a cow had 2 measures, their mean was used for future analyses. Growing heifers had more negative RES than dry, mid, and late lactation cows ($P < 0.05$). Lactation stage did not affect RES ($P > 0.05$). In a mixed model ANOVA that included lactation, genetic milk, RES, and all interactions (removed stepwise with $P > 0.30$), 305-d milk (previous lactation) was positively related to RES (1,502 ± 716 kg/RES unit; $P < 0.05$). Surprisingly, genetic prediction of milk was not associated with milk production ($P = 0.56$). When cows were segregated into efficient (top 50%) and inefficient (bottom 50%) groups, efficient cows produced 1,590 ± 658 kg more milk per lactation ($P = 0.02$). Together, these data suggest that skeletal muscle metabolic rate can be used to identify animals with differing maintenance energy requirements that result in differences in milk production.

Key Words: dairy cattle, energy expenditure, biopsy

2685 Enhancing FTIR-based machine learning models built from individual cow milk samples: Exploring the influence of pre-processing and number of farms.

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Dairy producers are constantly seeking more information about individual cows to aid in decision-making. Fourier transform infrared spectroscopy (FTIR)-based machine learning models (FTML) predicting important information from individual cow spectra have been developed in the last decade. We wanted to study the impact of preprocessing of the spectra (first derivative and filtering of the spectra using all, 516 [F15], or 212 [F16] datapoints), the number of farms (5 to 100), and the number of samples per farm (10 to 100) on the performance of FTML. A total of 400,955 individual milk samples were collected and analyzed by Lactanet between March 2019 and June 2022 to quantify metrics such as fat, lactose, BHB, and SCC. Samples were obtained from 288,119 Holstein cows housed in 3,046 commercial farms located in Quebec, Canada. FTML were trained and evaluated 10 times using random set of samples from 1,781 and 1,265 farms in the training and test sets, respectively and normalized root mean square error (NR) and R^2 were used to evaluate prediction performance. The performance of 31,680 FTML were analyzed using a linear mixed model with the metrics, pre-processing, number of farms and number of samples as fixed effects, and the set of samples used as a random effect. Optimal NR and R^2 of FTML were different depending on metrics ($P < 0.001$) and ranged from (NR = 0.005, $R^2 = 0.998$) for fat to (NR = 0.065, $R^2 = 0.234$) for SCC (Table 1). Filtering spectra improved performance ($P < 0.001$) and decreased the number of samples required to achieve optimal models, whereas applying the first derivative had no effect ($P \geq 0.66$). This study reveals that optimal FTML could be built from fewer samples using filtered spectra and no derivative, with a minimum of 10 farms and a total number of samples ranging from 100 (fat) to 1,800 (SCC).

Key Words: FTIR, machine learning, individual cows

2686 Effect of different training methods on adaptation of lactating primiparous cows to an automated milking system.

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The objective of this study was to determine the effect of automated milking system (AMS) training treatments on AMS ease of entry, kicking behavior, milk letdown, voluntary milkings, and milk yield of cows. A total of 48 primiparous Holstein dairy cows that had not previously been milked on an AMS were enrolled at 84.6 ± 12.3 DIM (mean ± SD). Cows were randomly allocated to 1 of 4 AMS training treatments (n = 12/treatment): control (CON), AMS environment exposure only (ENV), environment and concentrate provision (PEL), and environment and AMS mechanics exposure (included noises, robotic arm cleaning the teats; MEC). Cows in the ENV, PEL, and MEC treatments were subjected to training 4 times/d for 4 d, and then all cows were milked in the AMS on the evening of the fourth training day. Milking activity was recorded for the next 14 d on the AMS. Data were analyzed using mixed-effect repeated measures linear regression models. For the first milking, as compared with CON, ENV had a better ease of entry score (0 = easy to 3 = difficult) to the AMS (1.17 vs. 1.83; SE = 0.21, $P = 0.03$), and PEL tended to have a better ease of entry score (1.25 vs. 1.83; SE = 0.21, $P = 0.06$). As compared with CON, MEC had a better letdown score (0 = good letdown to 3 = poor letdown) (0.33 vs. 1.25; SE = 0.26, $P = 0.02$), and ENV tended to have a better letdown score (0.58 vs. 1.25; SE = 0.26, $P = 0.08$). As compared with CON cows, MEC had a lesser kicking score (0 = no movement to 2 = kicking) for the first milking (0.0 vs. 1.25; SE = 0.18, $P < 0.01$). PEL cows had fewer milkings (2.1 vs. 2.5 milkings/d; SE = 0.07, $P < 0.01$), and voluntary milkings (0.8 vs. 1.5 milkings/d; SE = 0.06, $P < 0.01$) across the 14 d after AMS introduction compared with CON cows. Compared with CON, MEC cows had greater milk yield (34.2 vs. 32.4 kg/d; SE = 0.48, $P < 0.01$), and ENV cows tended to have greater milk yield (33.6 vs. 32.4 kg/d; SE = 0.48, $P = 0.09$) across the 14-d period. The results demonstrate that only providing concentrate during AMS training may not provide enough exposure to aspects of an AMS to improve adaptation to that system, whereas exposing cows to the mechanics of the AMS before first milking may reduce kicking and improve milk yield.

Key Words: training, robotic milking, behavior

2687 Factors influencing Ontario dairy veterinarians' management and care of down dairy cattle.

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The objective of this cross-sectional study was to assess what management practices veterinarians recommended for down dairy cows in

Table 1 (Abstr. 2685). Optimal configurations of spectra pre-processing (derivative, filtering), farm numbers, sample numbers per farm, and total samples to enhance FTIR-based machine learning models' predictive performance (NR, R^2) of fat, lactose, BHB, and SCC

Item	Derivative	Filtering	Farms (n)	Samples/farm (n)	Total samples (n)	Optimal NR	Optimal R^2
Fat	No	F15	10	10	100	0.005^a	0.998^a
Lactose	No	F16	20	20	400	0.017^b	0.980^b
BHB	No	F16	10	15	150	0.042^c	0.838^c
SCC	No	F16	90	20	1,800	0.065^d	0.234^d

[a–d]Superscript letters indicate statistically significant differences observed among the results.

Ontario, Canada. An online survey was conducted between February and May 2021, distributed by email through the Ontario Association of Bovine Practitioners (OABP). The survey encompassed 75 questions, 38 of which pertained to down cow management. Descriptive statistics were computed using Python (version 3.11, 2022). A total of 48 Ontario bovine veterinarians responded, representing a response rate of 26.8%. Gender distribution was even (50.0%), and the majority of respondents were between 30 and 39 years old (41.3%). Variation was observed regarding the recommendations for providing feed and water to down cows; however, this could be due to each farm requiring a tailored approach. The majority suggested housing down cattle in individual pens (45.8%), followed by pasture (33.3%), special pens housing 3 or fewer animals (29.2%), and special pens housing 4 or more animals (4.2%). Regarding spacing allowance for a down cow, many veterinarians suggested 120 to 250 sq ft (53.3%) per cow, followed by providing a minimum of 100 sq ft (30.0%), 300 or more square feet (10%), or having "space to lunge" (6.7%). Additionally, there was substantial diversity in suggestions for lifting and moving. Recommendations for moving down cows included using a sled (62.5%), stone boat (56.3%), front-end loader bucket (45.8%), wheeled cart (20.8%), hip-lifter (2.1%), and "it depends" (2.1%). For lifting down cows, recommendations included using multiband slings (56.3%), hip lifters (43.8%), floatation tanks (25.0%), single belly slings (14.6%), ropes (4.2%), daisy lifters (2.1%), and hip lifters with additional straps (2.1%). The varying recommendations for down cow management highlights the need for more research in this area to determine best management practices. Furthermore, as herd veterinarians can influence producers' decision-making, it is critical to target this group of professionals with knowledge transfer on existing best management practices for care of down dairy cows.

Key Words: non-ambulatory, management practices, veterinarian

2688 Biocide tolerance of *Salmonella* isolates recovered from ill cattle.

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Cleaning and disinfection (C&D) is a common control method to reduce *Salmonella* transmission in cattle herds. Biocide choice is a critical component of C&D protocols, yet little is known about *Salmonella* susceptibility to commercial products. Our objective was to determine the minimum bactericidal concentration (MBC), defined as ≥3 log reduction, of 6 biocides used in livestock production or veterinary medicine. We hypothesized that some label guidelines would not significantly reduce *Salmonella.* To assess this, a 48-well microtiter plate assay was developed to evaluate Clorox® Germicidal Bleach, chlorine dioxide, chlorhexidine gluconate, KennelSol, Rescue, and VirkonS®. A bacterial inoculum of 10^5 to 10^6 cells mL^{-1} was challenged with each biocide. The lowest biocide concentration tested was based on label use directions and increased to 12 to 14 times the initial concentration. A neutralizer was used to inactivate biocides and simulate a 10-min contact time between bacteria and biocide. A second assay was run concurrently, using sterile water as a sham for neutralizer. A total of 6 isolates each from *Salmonella* serovars Dublin, Newport, and Typhimurium recovered from ill cattle were tested in duplicate. Bacteria were enumerated to determine log reductions after biocide exposure. Concentrations listed on KennelSol and VirkonS® labels resulted in eradication of all isolates with or without neutralization. When neutralized, Rescue required a 2- to 4-fold concentration above label guidelines to achieve biocidal effects but suggested use concentrations eradicated isolates after 24 h in sterile water. For all isolates, Clorox® MBC were either 625 or 1,250 ppm (ppm) within the label guideline for hard, nonporous surfaces (2,400 ppm). However, the 200 ppm guidance for food contact surfaces failed to significantly reduce any isolates tested, which is a public health concern for cattle-derived *Salmonella* strains that are transmitted through the food supply. Assay results identified rapidly effective biocides and, as isolates were recovered from ill cattle, are pertinent for guiding biocide choice in dairy herds experiencing salmonellosis outbreaks.

Key Words: *Salmonella*, disinfection, biocide

2689 Implementation of the CNCPS requirements for lactation in the Italian Mediterranean buffalo.

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Intensification of dairy buffalo farming is linked to the risk of increased nitrogen excretion. Thus, prediction of nutrient requirements and nitrogen efficiency in buffaloes is essential. Requirements for milk production in the Cornell Net Carbohydrate and Protein System (CNCPS) are calculated from inputted milk yield, fat, and true protein (TP). When milk protein is quantified as crude protein (CP), a correction factor TP/CP (cf TP/CP) is applied. The study's objective was to quantify CP and non-protein nitrogen (NPN) and determine the parameters explaining the cf TP/CP. Milk samples from 40 buffaloes (parity: 3.56 ± 1.22; DIM: 135.91 ± 16.64) were collected and analyzed, via Fourier transform mid-infrared, for fat, CP, lactose, casein, urea, acetone, BHBA, and NPN. We used PROC CORR of SAS to determine correlations between DIM and milk components. A backward stepwise regression with significance at $P < 0.05$ was applied to determine the variables which best explained TP, NPN, and cf TP/CP. The variance inflation factor was considered to avoid collinearity. Metabolizable energy (ME) and protein (MP) requirements for lactation were estimated using the calculated cf TP/CP and assuming the following animal input: DIM = 70; yield = 14.3kg; fat = 7.20; and CP = 4.30. Casein, CP, and TP were negatively correlated with NPN (<-0.92; $P < 0.01$), and NPN with cf TP/CP (0.99; $P < 0.01$); acetone was negatively correlated with CP, casein, TP, and cf TP/CP (<-0.41: $P < 0.01$); BHBA was negatively correlated with DIM and cf TP/CP (<-0.34; $P < 0.05$). In addition, CP was positively correlated with casein, TP, and cf TP/CP (>0.94; $P < 0.01$), and acetone with BHBA (0.88; $P < 0.01$); fat was positively correlated with acetone and BHBA, and acetone with NPN (>0.42; $P < 0.01$). In addition, fat and casein better predicted TP, NPN, and cf TP/CP ($P < 0.01$). The cf TP/CP was 0.928 ± 0.016, which is lower than what is used in dairy cows. When estimating the requirements with the buffalo cf TP/CP, the MP requirements were 43.1 g/d less than without the correction factor, and 11.3 g/d less than when using the dairy cow correction factor. In conclusion, prediction accuracy affects protein utilization and, therefore, it may affect nitrogen excretion.

Key Words: milk true protein, dairy buffalo, energy and protein requirements

2690 Evaluation of presynch-based protocols with Ovsynch in postpartum Nili-Ravi Buffalo.

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Presynchronization is a potential reproductive managemental tool in the dairy industry. The present study investigated the effectiveness of presynch-based synchronization protocols in postpartum Nili-Ravi buffaloes. A total of 237 cyclic buffaloes were randomly assigned to one of the 3 protocols: (1) eCG-Ovsynch protocol (Equine Chorionic gonadotropin; 500 IU, IM 84 h before Ovsynch part; n = 72); (2) G7G-Ovsynch (n = 90) composed of PGF2α followed by GnRH 48 h later, and then 7 d after the GnRH shot, a standard Ovsynch protocol was introduced; and (3) Ovsynch protocol (n = 75). Artificial breeding was done after heat detection using Estrotect. Pregnancy per artificial insemination (P/AI) was diagnosed by ultrasonography on d 30 post-breeding. Blood sampling was done before presynch, Ist GnRH, and PGF2α of breeding protocol for the P4 profile. Pregnancy data (%) was analyzed using the Chi-squared test and plasma P4 profile (ng/mL) by ANOVA using SAS. The P/AI on d30 post breeding was 44% (33/75), 58.33% (42/72), and 53.33% (48/90) in the Ovsynch, G7G-Ovsynch, and eCG-Ovsynch groups, respectively ($P = 0.21$). On d 60 after AI, P/AI was recorded to be 40% (30/75), 54.16% (39/72), and 46.66% (42/90) in the Ovsynch, G7G-Ovsynch, and eCG-Ovsynch groups ($P = 0.23$), respectively. The P/AI on d 90 after AI remained unchanged in both presynch-based Ovsynch protocols as compared with the Ovsynch (36%; 27/75) protocol ($P = 0.08$). Pregnancy loss on d 60 was 9%, 7%, and 12.5% in corresponding groups ($P = 0.84$), whereas overall pregnancy loss was 18.2%, 7%, and 12.5% in the Ovsynch, G7G-Ovsynch, and eCG-Ovsynch groups ($P = 0.01$), respectively. Based on the P4 profile (from PGF2α to GnRH-II), the G7G-OvSynch protocol has resulted in higher circulatory P4 ($P < 0.05$) in comparison with the Ovsynch protocol in the same window. In conclusion, although statistically insignificant, G7G-Ovsynch has resulted in higher P/AI (>15%) and P4 profile compared with the Ovsynch protocol. The findings of the current study with a limited number of subjects also warrant further studies with more animals in the future to validate these results.

Key Words: presynch, Nili-Ravi buffalo, pregnancy rate

2691 Influence of management and storage factors on detection of HSP70 in raw bovine milk. M. R. H. Rakib*[1,2], V. Messina[1], J. I. Gargiulo[1,3], N. A. Lyons[4], I. N. Pathirana[5], and S. C. Garcia[1], [1]*School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Camden, NSW, Australia,* [2]*Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh,* [3]*NSW Department of Primary Industries, Menangle, NSW, Australia,* [4]*DairyNZ, Hamilton, New Zealand,* [5]*Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka.*

Heat shock proteins play a crucial role in maintaining cellular function during elevated temperatures. Recently, heat shock protein 70 (HSP70) has been detected in bovine milk through a modified ELISA method; thus, it could serve as a biomarker for heat stress in dairy cows. However, the optimal management and storage of milk samples is yet to be determined. The aim of this research was to optimize management and storage of milk samples to improve HSP70 detection via ELISA method. Milk samples were collected from 20 Holstein Friesian cows (third parity in early lactation) at The University of Sydney's dairy farm (New South Wales, Australia). Samples were centrifuged (1,000 × *g* for 10 min at 4°C) as skim milk (skimmed before storage [SBS] and after storage [SAS]) and subjected to different storage conditions: (1) room temperature (T_1, ~22°C), (2) refrigerated (T_2, 4°C), (3) frozen (T_3, −20°C), and (4) adding bronopol (preservative) and refrigerated at 4°C (T_4). Samples were analyzed every 2 d until d 15 ($D_1, D_3\ldots, D_{15}$) using an in-house competitive ELISA method (sensitivity range 7.82–4,000 ng/mL) to detect HSP70. A linear mixed model was used to compare differences between milk handling (SBS and SAS), treatments (storage conditions and time) and their interactions on HSP70 levels. Results revealed significant ($P < 0.05$) effects on HSP70 levels between milk handling and treatments from D_1 to D_{15}. Higher levels of HSP70 were observed for SBS and SAS for T_2, followed by T_3, T_4, and T_1 until $D_{3,}$ then slightly decreased to $D_{9.}$ An increase in HSP70 levels were observed from D_{11} to D_{15} in both SBS and SAS, possibly indicating the involvement of other proteins beyond HSP70, where further research is required. In conclusion, milk samples can be stored as whole milk at 4°C (with or without bronopol) or −20°C until D_3 to avoid degradation of proteins, and then processed to skim milk for detection of HSP70 via ELISA method. To our knowledge, this is the first study to demonstrate the influences of management and storage on detection of HSP70 in raw bovine milk, offering practical guidelines for farmers in preserving sample integrity for subsequent HSP70 detection.

Key Words: ELISA, heat shock protein 70 (HSP70), milk

2692 Negative impacts of postpartum NSAID treatment in dairy cattle in a commercial farm setting. L. Olthof*[1], P. Descole[2], and B. Bradford[1], [1]*Michigan State University, East Lansing, MI,* [2]*Progressive Dairy Solutions, Oakdale, CA.*

Improving health in transition dairy cows is a priority to for dairy farm profitability and sustainability. Some previous studies have shown positive production, reproduction, and health impacts of nonsteroidal anti-inflammatory drug (NSAID) treatment within 48 h of calving. The objective of this study was to assess the impact of postpartum acetylsalicylic acid (aspirin) treatment on productivity, health, and herd retention outcomes of cows on a commercial dairy farm. A total of 428 dairy cows entering lactation 2 or greater were enrolled in a randomized study, with even-numbered cows treated with aspirin (n = 218) and odd-numbered cows not treated (n = 209). Aspirin-treated cows received 4 boluses (31.1 g aspirin each) at ~36 h after calving and another 4 boluses at ~60 h after calving. Treatment assignments were relatively balanced over time, genetic merit for milk, fat, and protein yield was similar between treatment groups, and parity distribution was similar. Repeated measures models accounting for parity and treatment were used to evaluate effects on milk production at wk 4, 8, 12, and 16 (DairyComp 305) and fat and protein concentrations at first and second DHI test. Pre-treatment cases of twinning, dystocia, and retained placenta (1, 36, and 0 vs. 2, 32, and 3 vs. control vs. aspirin) were similar across treatment groups. Aspirin treatment tended to decrease milk yield by ~2% (54.9 vs. 56.0 ± 0.52 kg/d; $P = 0.08$) over the first 100 DIM, with no significant effects on concentrations of fat or protein. Aspirin significantly increased incidence of metritis (1.4% vs. 5.9%; Fisher's exact test $P = 0.02$). Other recorded diseases had similar incidence across treatments, and there was no apparent treatment effect on hazard of leaving the herd by 100 DIM. In conclusion, postpartum aspirin treatment increased metritis incidence and tended to reduce productivity in multiparous cows. We speculate that adverse effects of restraining cows and administering boluses led to a net negative effect of this treatment protocol.

Key Words: periparturient, aspirin, nonsteroidal antiinflammatory drug

2693 Impact of farm size on dairy cow efficiency in small-scale dairy systems in the highlands of Mexico. J. Velarde-Guillen*, C. G. Martínez-García, and C. M. Arriaga-Jordán, *Universidad Autónoma del Estado de México, Instituto de Ciencias Agropecuarias y Rurales, Toluca, Mexico.*

Efficiency in dairy cows has been studied widely around the world. On the other hand, small-scale dairy systems (SSDS), from 3 to 30 cows, are key to combating poverty and hunger. Nevertheless, feeding costs represent 70% to 80% of the production costs, which decrease the revenue of the SSDS. However, few studies have focused on the relationship between efficiency (milk yield and feeding costs) and herd size in SSDS. The aim of this work was to estimate the impact of the farm size in the feed efficiency and feeding costs in SSDS. Data from 294 SSDS from the highlands of Mexico, recollected in 2011 (Alfonso-Ávila et al., 2012; Fadul-Pacheco et al., 2013), 2019 (García-Villegas et al., 2020, 2021) and 2023 (project in process), were used. Data were analyzed using the IBM SPSS Statistcs software. Farms were classified by the number of dairy cows: very small (<6 dairy cows), small (7–14 dairy cows) and medium (>15 dairy cows). Feeding costs were estimated using the data of 2011, 2019, and 2023. The costs were updated to September 2023. Feed efficiency was estimated in terms of kg of energy protein corrected milk (EPCM)/kg of DMI. Energy and protein corrected milk was estimated using the equation of Niu et al. (2018): 12.95 × milk fat (kg/d) + 7.65 × milk protein (kg/d) + 0.327 × milk yield (kg/d); and DMI (and the ME) were obtained from the aforementioned studies. The lowest feeding costs occurred in medium-sized farms ($US3.04), whereas the highest costs were in very small farms ($US4.47). However, the quality of the feed (MJ/kg DM) was higher in medium-sized farms (9.85 MJ/kg DM), whereas in the very small farms, the quality decreased (9.45 MJ/kg DM). In the same line, feed efficiency was higher in the medium farms (1.29 kg EPCM/kg DMI), followed by small farms (0.87 kg EPCM/kg DMI) and very small farms (0.84 kg EPCM/kg DMI). In conclusion, feed efficiency in SSDS is related to the farm size, in which the medium-sized farms present higher feed efficiency, a better use of the feed (lower feed costs) and a higher quality of feed (in terms of energy, MJ/kg DM).

Key Words: dairy efficiency, feeding cost, methane

2694 Use of the VaDia vacuum recorder for detection of factors related to mastitis and somatic cell count in organic dairy herds.

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The objective of the study was to evaluate the use of a vacuum recording device to detect factors related to mastitis and somatic cell count in organic dairy herds. The study was conducted at the University of Minnesota West Central Research and Outreach Center (Morris, MN) organic dairy. A total of 155 Holstein and crossbred certified organic cows were monitored for 5 morning milkings from December 2023 to January 2024. Holstein cows (n = 35) were compared with Grazecross crossbred cows (n = 31) composed of the Normande, Jersey, and Viking Red breeds and ProCross crossbred cows (n = 89) composed of the Montbéliarde, Viking Red, and HO breeds. VaDia vacuum recorders (Biocontrol, Rakkestad, Norway) were attached to the milking cluster during milking. In this cross-sectional study, 259 individual cow milking observations were analyzed. The VaDia recorder collected individual cow vacuum events during the 5 milkings. VaDia Suite software analyzed milking data for average milk flow, let down time, peak flow, and overmilking time. Daily milk weights were recorded with an AfiMilk and AfiLab milking system (Kibbutz Afikim, Israel). Independent variables for statistical analysis were fixed effects of genetic group (HO vs. Grazecross crossbred vs. ProCross crossbred) and lactation number. Machine on time was not different ($P > 0.10$) for breed groups (4 min 49 s for Holstein, 3 min 58 s for Grazecross, 4 min 45 s for ProCross). Mean milk flow rate was 5.27 L/min for first lactation cows and 6.37 L/min for second and greater lactation cows ($P < 0.05$). Average vacuum across the groups was 10.2 Hg ($P > 0.10$). The correlation between milk production at milking time and machine on time was 0.34 ($P < 0.01$). The milk let down time averaged 24.5 to 27.3 s and was not different between groups ($P > 0.2$), and it ranged from 5 to 55 s across all of the milkings. Milking vacuum dynamics are useful to determine milking parlor efficiency in an organic dairy herd.

Key Words: organic, milking, mastitis

2695 Rectal temperature is positively correlated with body surface temperature using infrared thermography in Holstein dairy calves.

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The aim was to evaluate the rectal (RT), eye, flank, and perineal temperature, and correlations between a data logger temperature and image infrared thermography in Holstein dairy calves under heat stress in the first 28 d after birth. A total of 35 calves (15 male and 20 female) were individually housed in pens and randomly allocated into 2 treatments: (1) control (CTRL; body weight at birth = 35.6 ± 4.18 kg; temperature-humidity index [THI] = 66; n = 17) and (2) heat stressed (HS; body weight at birth = 36.0 ± 5.13 kg; THI = 82 during 9 h/d; n = 18). Both groups received 6 L of milk divided into 2 meals (0730 and 1430 h) and concentrate (crude protein = 192 g/kg of DM; crude energy = 4.05 Mcal/kg of DM) ad libitum. The RT were measured daily at 6 and 10 a.m. and at 2 and 4 p.m. determined with the data logger (model: DS1921H2kb; Thermodata Pty. Ltd., Australia). Additionally, eye, flank, and perineal temperature were measured using an infrared thermography camera (FLIR T420; FLIR Systems Inc., Wilsonville, OR). Data were analyzed using R software with ANOVA, at 95% confidence interval in a completely randomized design, where the treatment was the main plot, and week was the subplot. Pearson correlation between the RT measured with the data logger and eye, flank, and perineal temperature was performed. Statistical significance was declared at $P \leq 0.05$. RT increased by 0.4 degrees in HS calves (CTRL = 38.7 vs. HS = 39.1°C; $P < 0.01$). Eye, flank, and perineal temperature were greater at 10 a.m., 2 p.m., and 4 p.m. in HS calves compared with CTRL ($P < 0.05$), but not at 6 a.m. The RT measured with a data logger was positively correlated with eye ($r = 0.52$; $P < 0.01$), flank ($r = 0.42$; $P < 0.01$), and perineal temperature ($r = 0.50$; $P < 0.01$). Calves under heat stress had increased rectal temperature and surface temperature of the eye, flank, and perineal area. The substantial correlation between the logger and body surface temperature measured with image infrared thermography indicates that it can be used for non-invasive measures of heat stress in Holstein calves.

Key Words: correlation, infrared thermography, surface temperature

2696 Characterization of milk production, milking frequency, and rumination outcomes of early-lactation Jersey cows diagnosed with concurrent hyperketonemia and hypoglycemia in an automated milking system.

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The objective was to characterize the milk yield, milking and refusals frequency, concentrate intake, and rumination of early-lactation Jersey cows diagnosed with hyperketonemia (HK), hypoglycemia (HG), or concurrent HK and HG in an automated milking system (AMS). Glucose and BHB concentrations were measured in whole blood using a hand-held device (FreeStyle Optium Neo, Abbott Laboratories), from Jersey cows between 3 and 9 d postpartum. This high-producing Jersey herd has 3 compost barns with free-flow traffic access to 6 AMS robots (Astronaut A4, Lely Industries). A total of 432 cows were categorized into 4 groups: NORM (BHB <1.2 mmol/L and glucose >2.2 mmol/L; n = 158), HK only (BHB ≥1.2 mmol/L; n = 5), HG only (glucose ≤2.2 mmol/L; n = 200), and concurrent HK and HG (HKHG; n = 69). Statistical analysis was carried out in SAS using the MIXED and the GLIMMIX procedures, with metabolic group, parity, time, and their interactions as fixed effects. Milk yield until 100 DIM was greater ($P < 0.01$) for HKHG cows (35.21 ± 0.09 kg/d), followed by HG (34.46 ± 0.05 kg/d), NORM (32.29 ± 0.06 kg/d), and HK cows (30.64 ± 0.35 kg/d). Daily milking frequency was greater ($P < 0.01$) to HKHG animals (3.14 ± 0.03), intermediate to HG (3.12 ± 0.02), and lowest for NORM and HK (2.98 ± 0.02 and 2.93 ± 0.10). Daily milking refusals were greater ($P < 0.01$) in normoketotic cows compared with HK ones (3.62 ± 0.01 vs. 3.20 ± 0.03 refusals/d), whereas normoglycemic cows had greater ($P < 0.01$) refusal frequency than HG cows (3.81 ± 0.02 vs. 3.52 ± 0.01 kg/d). Concentrate intake was higher ($P < 0.01$) in HKHG and HG cows (7.18 ± 0.03 and 7.16 ± 0.02 kg/d), intermediate in NORM (6.77 ± 0.02 kg/d), and lowest in HK (6.40 ± 0.10 kg/d). Rumination rate was greatest for HG cows (461.0 ± 0.7 min/d), intermediate for HKHG and NORM (451.8 ± 1.2 and 451.2 ± 0.9 min/d), and lowest for HK (409.4 ± 4.7 min/d). Postpartum hypoglycemic Jersey cows (HG or HKHG) produced more milk, ruminated more, and showed better milking outcomes than NORM cows and HK-only animals.

Key Words: β-hydroxybutyrate, ketosis, transition period

2697 Resveratrol improves the antioxidant status of heat-stressed dairy cows. R. N. Mini Ravi*, M. S. Hasan, K. Frady, Z. Yu, J. Cantet, and A. Ríus, *The University of Tennessee, Knoxville, TN.*

Resveratrol is a plant-based polyphenolic compound with antioxidant and anti-inflammatory properties. A study was designed to ascertain the effects of resveratrol on the antioxidant status and milk yield of heat-stressed lactating cows. A total of 48 Holstein cows were housed in a sand-bedded freestall barn and fed a typical corn silage-based TMR to collect baseline data for 14 d. Based on days in milk, milk yield, and parity (mean ± SD, 126 ± 33 d, 49.5 ± 8.8 kg/d, 2.3 ± 1, respectively) cows were randomly allocated into 2 groups as control (CON, with no resveratrol, n = 24) and resveratrol (RES, TMR top-dressed resveratrol at 500 mg/cow per d, n = 24) for 21 d. The sprinklers and fans in the barn were disabled during the treatment period to expose cows to the prevailing summer conditions of temperature and humidity. Plasma samples were harvested to conduct total antioxidant capacity (TAC), malondialdehyde (MDA), and superoxide dismutase (SOD) antioxidant assays. Data were analyzed using the mixed procedure with repeated measures in SAS with a significance level of $P \leq 0.05$. Treatment, d, parity, and their interactions were deemed as fixed effects and cow included as random. An average THI of 75 ± 4 was maintained throughout the study. The TAC analysis showed a treatment by parity interaction, indicating that RES increased TAC in parity 2 animals (1.01 ± 0.03 vs. 1.13 ± 0.02 mM; $P = 0.025$). The SOD activity showed a treatment by parity interaction and RES increased SOD activity in parity ≥3 cows (0.72 ± 0.16 vs. 1.49 ± 0.16 U/mL; $P = 0.003$). The MDA activity was not altered ($P = 0.175$). Additionally, simple linear regression analyses showed that the magnitude of reduction in milk yield associated with elevated THI was smaller in RES compared with CON group irrespective of parity (regression coefficients −0.92 and −1.11; $P < 0.001$). In summary, resveratrol supplementation could be beneficial to maintain antioxidant status and milk yield in heat-stressed dairy cows.

Key Words: heat stress, oxidative stress, resveratrol

2698 Impact of heat stress abatement on humoral immunity in preweaned calves prior to and following bovine respiratory disease. C. G. Savegnago*[1], A. M. Roper[1], T. N. Marins[1], J. Gao[1], G. G. Cunha[1], N. L. P. Kant[1], C. R. Czaykowsky[2], B. Credille[2], Q. Huo[3], and S. Tao[1], *[1]Department of Animal and Dairy Science, University of Georgia, Athens, GA, [2]College of Veterinary Medicine, University of Georgia, Athens, GA, [3]Department of Chemistry and NanoScience Technology Center, University of Central Florida, Orlando, FL.*

Heat stress negatively affects immune function of dairy cattle and potentially increases the risk of death due to bovine respiratory disease (BRD). To examine the impact of heat abatement on humoral immune response to experimentally induced bacterial bronchopneumonia, preweaned Holstein bull calves (n = 8/treatment, d of age = 21) were randomly assigned to 2 treatments based on body weight and serum brix refractometry: (1) non-cooled (NC) and (2) cooled (CL). All calves were housed in the same general area, but CL calves were provided with shade and fans, whereas NC calves were not. Calves were vaccinated with INFORCE 3 at −14 d of the experiment (DOE), and with Bovi-Shield GOLD® 5 at 7 DOE. On 22 DOE, all calves were inoculated with 10^7 cfu *Mannheimia haemolytica* at the tracheal bifurcation. Serum was collected at −2, 1, 3, 7, 14, and 21 DOE and 0, 6, 12, 24, 48, 96 h following inoculation to measure brix using a digital refractometer and D2Dx immunity score. The D2Dx immunity test is a rapid blood test that evaluates humoral immune response by detecting reactions between nanoparticle pseudo-pathogen probes and major immune molecules, including immunoglobin G and M and complement proteins. A higher D2Dx score indicates greater circulating humoral immunity. Additional serum was collected at −14, 2, 7, 14, and 21 DOE to measure antibodies (Ab) for bovine viral diarrhea type 1a, 1b (BVD1b), and 2; bovine rhinotracheitis; parainfluenza-3 virus; and bovine respiratory syncytial virus. Data were analyzed by the mixed procedure of SAS. Before inoculation, treatment did not ($P = 0.23$) affect serum brix, but the D2Dx score tended ($P = 0.10$) to decrease in NC calves over time relative to CL. The CL calves tended to have greater ($P = 0.06$) BVD1b Ab than NC at 7 DOA, but treatment did not affect other Ab. Following inoculation CL calves had greater ($P = 0.02$) serum brix but lower ($P = 0.03$) D2Dx score than NC calves. In conclusion, heat abatement helped maintain greater humoral immunity, but elicited stronger humoral responses (e.g., greater reduction in D2Dx score) following inoculation.

Key Words: bovine respiratory disease, dairy calf, D2Dx™ immunity test

2699 The effects of feeding zeolite or acidogenic diet in the close-up period to multiparous Jersey cows on milk, health events, and culling. A. Ravelo[1], M. L. Stangaferro*[2], M. J. Thomas[2], L. Caixeta[1], and R. Couto Serrenho[3], *[1]Department of Veterinary Population Medicine, University of Minnesota, Saint Paul, MN, [2]Dairy Health & Management Services LLC., Lowville, NY, [3]Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to evaluate the effect of feeding zeolite (ZLT; n = 756) or an acidogenic (DCAD; n = 750) diet during the close-up period on milk yield (MY), health events, and culling. Multiparous Jersey cows were randomized to one of 2 close up pens (CUP) where they were fed the assigned diet (TRT; ZLT or DCAD), and the TRT offered was switched every 4 wk (6 periods). Cows were also randomized to receive Ca supplementation (CaS+ = 756, CaS−= 750) postpartum. Milk yield and components data (monthly test-day; TD) were analyzed using a repeated mixed model. Final milk models included TRT, TD (1–4), their interaction, CaS, and parity (2 vs. 3+) as covariates, and the random effect of cow and TRT within period to account for pen effect (df <12). Final mixed logistic regression models used to compare health events (displaced abomasum, milk fever, retained placenta, metritis, and mastitis) and culling by 60 DIM included TRT, parity, and random effect TRT within period. TRT had no effect on fat %, protein %, or LS SCC. An interaction of TRT by parity by TD was detected for milk, fat, and protein yield (kg), and 3.5% FCM; thus, data were stratified by parity. In second parity cows, an interaction of TRT and CaS was detected for MY. For parity 2 cows, within TRT, CaS did not affect MY. Of the CaS− parity 2 cows, the DCAD group (34.8 kg [33.6, 35.9]) yielded more milk than ZLT (33.2 kg [32.0, 34.4]; $P = 0.03$); no differences were detected within CaS+ cows. No effect was observed in parity 3+. The incidence of milk fever was 0.01% (n = 7) on the DCAD and 0.004% (n = 3) in the ZLT. There was no effect of TRT on the occurrence of ≥1 disease by 60 DIM (OR: 0.79; 0.52, 1.22; $P = 0.3$). The odds ratio when comparing the ZLT to the DCAD group for culling by 60 DIM was 1.23 ([0.86, 1.76]; $P = 0.2$). When considering only CaS− cows, MY in ZLT group was less than in DCAD. Overall, there were no biologically relevant differences on MY, health events, or culling. This supports our hypothesis that using ZLT is a viable option for dry cow close-up management.

Key Words: mineral binder, negative DCAD, performance

2700 Assessing sustainable source of encapsulation for dietary calcium chloride: Impacts on key serum and urine markers in dairy cows. B. Pauwelyn[1], P. Mannina[2], R. Spaepen[1], D. Dubey[1], E. E. Gültepe*[1], and F. Nuyens[1], *[1]Kemin Europa NV, Herentals, Belgium, [2]Kemin Cavriago, Cavriago, RE, Italy.*

The EU's 2018 Renewable Energy Directive increased attention on palm oil use in feed due to environmental concerns. The objective of this pilot trial was to validate the effects of supplementation of a new generation sustainable encapsulated dietary calcium chloride (enCaCl) on key serum markers and urine pH. Multiparous ($\bar{X}$:3.8) cows were randomly assigned to receive 180 g/head per d (144 g/d of $CaCl_2$) with standard enCaCl (CON; n = 19; NutriCAB, Kemin Animal Nutrition and Health, Belgium) or enCaCl with alternative palm oil-free coating agents (POF; n = 17; NutriCAB *ef*) as a top-dress starting from 3 wk before the expected calving date until calving. Cows were housed in 1 of 2 dry cow pens according to relevant groups, then transferred to lactation pens after calving, and were milked twice daily. Blood samples were collected at calving (d 0), d 1, d 2, d 3, d 4, and d 7 from the tail vein. Spot urine sampling was performed to evaluate net acid excretion. Robust fit outliers with Huber M-estimation (K = 4) were executed for data quality. Data were analyzed using the Fit Model procedure with REML (JMP 16.1, SAS). The model incorporated fixed effects for group, DIM, and 2-way interactions with random effect of cow as experimental unit. The results were presented as Lsmeans, and significance was established at $P < 0.05$. Serum levels of tCa (2.15 vs. 2.09 ± 0.04 mmol/L), P (1.38 vs. 1.44 ± 0.10 mmol/L), Mg (0.95 vs. 0.96 ± 0.02 mmol/L), and Ca:P ratio (1.61 vs. 1.54 ± 0.09) in cows fed with the POF had similar responses to those in cows fed with the CON ($P > 0.05$). Although all mentioned parameters were significantly changed over time (DIM; $P < 0.01$), no interaction was observed between treatment and DIM ($P > 0.05$). Serum NEFA (0.59 vs. 0.61 ± 0.07 mmol/L) and BHBA (0.65 vs. 0.63 ± 0.05 mmol/L) levels were similar in POF vs. CON cows. Urine pH levels did not change in POF versus CON cows (6.57 vs. 6.20 ± 0.25; $P > 0.05$). Evidence indicated that sustainable source of encapsulated $CaCl_2$ variant performs similarly to the current version of encapsulated $CaCl_2$ in terms of key Ca metabolism markers.

Key Words: encapsulation, calcium, sustainability

2701 Effects of three different preventive protocols for ketosis on milking and reproductive performance of dairy cows. A. Lisuzzo[4], A. Valenza[2], A. Biancucci[4], M. Gianesella[4], E. Fiore[4], and A. Bach*[3,4], *[1]Institució de Recerca i Estudis Avançats (ICREA), Barcelona, Spain, [2]Marlex Recerca i Educació, Barcelona, Spain, [3]Ceva Salute Animale, Milano, Italy, [4]University of Padova, Padova, Italy.*

The objective was to assess the effect of different approaches to prevent ketosis on milking and reproductive performance of dairy cows. A total of 540 Holstein-Friesian cows were randomly selected from a commercial farm at the beginning of the dry period and randomly allocated to 4 groups: no supplementation (CTR), a complete preventive protocol (CPP) consisting of an intramuscular (IM) injection of 125 mL of a solution containing amino acids, inositol, and cyanocobalamin (Bogras) at 15 and 12 d before calving, followed by 7 IM doses on alternate days until 12 DIM of 20 mL of a solution containing acetylmethionine, α-lipoic acid, and cyanocobalamin (Erbacolina Plus); a simplified preventive protocol (SPP) consisting of 125 mL of Bogras and 70 mL of Erbacolina Plus at −12 and 7 d relative to calving; and a protocol consisting of an oral dose of 35.2 g of monensin (Kexxtone) at 21 d before calving (MON). All cows received the same pre- and postpartum diets. Cows were blood sampled at −21, −7, 7, 14, and 28 d relative to calving to determine serum β-OH-butyrate (BHB) concentration. Individual milk yield until 307 DIM and all reproductive events were recorded daily. Time-series data were analyzed using a mixed-effects model for repeated measures, reproductive data were analyzed with logistic regression. The serum BHB concentration was lower ($P < 0.05$) in CPP and SPP than in CTR and MON cows between 14 and 55 DIM. Overall, CTR and CPP cows produced less ($P < 0.05$) milk (42.0 and 41.7 ± 0.49 kg/d, respectively) than cows on SPP (42.9 ± 0.49 kg/d) or MON (43.5 ± 0.49 kg/d), but MON cows produced more ($P < 0.05$) milk during the first 150 DIM, whereas SPP cows produced more ($P < 0.05$) milk after 210 DIM. Cows were first serviced at 82.5 ± 1.4 DIM. Pregnancy per artificial insemination (AI) at first AI was greatest ($P < 0.05$) in SPP cows (56%), followed by CTR and MON cows (49.9%), and CPP (41.3%). In conclusion, CPP and SPP were more effective than CTR and MON reducing postpartum serum BHB levels. Cows in MON produced more milk during the first phase of the lactation curve, but had worse reproductive performance at first AI.

Key Words: amino acid, fertility, metabolism

2702 Supplementing a probiotic to early-lactation Holstein cows challenged by mycotoxins. 1. Milk production. L. D. Roth*[1], M. Stangaferro[2], M. Thomas[2], C. E. Knoblock[1], and H. Goetz[2], *[1]Agrarian Solutions, Nappanee, IN, [2]Dairy Health and Management Services, Lowville, NY.*

The objective of this study was to evaluate the effects of a probiotic on early-lactation Holstein cows challenged by mycotoxins. The study was conducted from August 2022 to May 2023 on a commercial dairy farm in New York. Before calving, Holstein cows were blocked by lactation and calving date then randomized to pen and base ration without mycotoxin protection (133 primiparous and 330 multiparous cows; CON) or to a base ration plus DTX (9 g DTX concentrate/cow per d; 131 primiparous and 332 multiparous cows; DTX). At calving, cows entered CON or DTX pens. Cows were fed once daily, and refusals were measured according to the dairy's standard protocol. Cows were milked 3 times/d and daily milk weights recorded for the first 150 d of lactation. Milk components and SCC were evaluated by pen 2 times/wk. Individual cow was the experimental unit for daily milk yield, and pen (4 per research group) was the experimental unit for dry matter intake (DMI), milk components, and SCC. Data were analyzed using Stata. The TMR mycotoxin contamination did not differ for DON (1.57 ppm CON, 1.56 ppm DTX, $P = 0.60$) or zearalenone (150 ppb CON, 143 ppb DTX, $P = 0.08$). The daily DMI by week of trial was similar ($P = 0.52$) and averaged 27.8 kg for CON and 27.5 kg for DTX pens. Daily milk yield was similar ($P = 0.33$) for primiparous cows (37.3 kg for CON, 37.1 kg for DTX). Daily milk yield for multiparous cows was greater ($P = 0.001$) for DTX (53.5 kg) than CON (52.3 kg) cows. Milk components were similar between treatments for milk fat (4.15% for CON, 4.12% for DTX, $P = 0.75$) and milk crude protein (3.16% for CON, 3.17% for DTX, $P = 0.94$). The pen-level SCC (× 1,000/mL) was higher ($P = 0.02$) for DTX (161.3 for DTX) than CON (143.0). These results indicate that supplementing a probiotic increased daily milk production for early-lactation multiparous cows challenged by a mycotoxin-contaminated diet.

Key Words: probiotic, milk production, mycotoxin

2703 Supplementing a probiotic to early-lactation Holstein cows challenged by mycotoxins. 2. Reproduction.

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A probiotic was evaluated with early-lactation Holstein cows challenged by mycotoxins from August 2022 to May 2023. Holstein cows pre-calving were blocked by lactation number and calving date, then randomized to a control (base ration without mycotoxin protection; 133 primiparous and 330 multiparous cows; CON) or treatment (base ration plus 9 g DTX concentrate/cow per d; 131 primiparous and 332 multiparous cows; DTX). At calving, cows entered either CON (4) or DTX (4) pens to start the study, with primiparous and multiparous cows penned separately within each research group. All cows were synchronized with Presynch-Ovsynch to receive first service at 73 ± 3 d. Embryos were transferred (ET) to 90% of primiparous, 99% of second- and third-lactation cows, with the remainder of primiparous and multiparous cows receiving sexed Holstein semen (AI). Fourth- and greater-lactation cows received conventional beef semen. Pregnancy diagnosis was conducted at 28 to 34 d post-service and reconfirmation at 61 to 67 d post-service. Individual cow was the experimental unit and pen was statistical model factor. The TMR mycotoxin contamination did not differ for DON (1.57 ppm CON and 1.56 ppm DTX, $P = 0.60$) or ZEA (150 ppb CON, 143 ppb DTX, $P = 0.08$). The proportion of pregnant cows at 28–34 d post-service was greater ($P = 0.03$) for DTX (45.0%) than CON (37.7%), and greater ($P = 0.02$) for AI (48.6%) than ET (38.4%). Pregnancy losses did not differ ($P = 0.88$) between the CON (19.6%) and DTX 19.1%) cows. The proportion pregnant cows at 61–67 d post-service tended to greater ($P = 0.06$) for DTX (36.4%) than CON (30.3%) cows, and greater ($P = 0.01$) for AI (42.0%) and ET (29.8%) cows. Supplementing DTX improved the proportion pregnant cows at 28 to 34 d post-service when early-lactation cows were fed a mycotoxin-contaminated diet.

Key Words: probiotic, mycotoxin, proportion pregnant

2704 Feed nutritive analysis affects predicted methane emissions, a case study.

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Mitigating enteric CH_4 emissions is important, but directly measuring CH_4 emissions is difficult. Hence, dairy farm carbon footprint and emission studies have used CH_4 predictive equations. The objective of this study was to determine if predicted enteric CH_4 output differed when using farm-level intensively sampled feed analysis data compared with feed library or laboratory regional nutrition analysis data. To evaluate the impact, exemplary data from 2 dairies sampling feed for nutrition analysis 2 to 3 times weekly (TV) over a 12-plus-month period were applied to a high-forage (HF; 62% forage, 32.6% NDF) and low-forage (LF; 42% forage, 31.8% NDF) diet. The TV diet NDF concentrations were then compared with diet NDF concentration using feed library (BV) or regional laboratory (RA) NDF values for the time period. The average NDF in TV diets for HF was 29.8% and 31.8%, and LF 28.5% and 30.2% for farm A and farm B, respectively. Dietary NDF using RA was 32.0% and 33.7% in HF and 30.9%, and 32.4% in LF for farm A and farm B, respectively. Calculated dietary NDF for these data were then applied to the US and EU methane emissions predictive equations proposed by Niu et al., 2018, using DMI and NDF inputs. Data for HF and LF diets were then analyzed using the LM procedure in R 2023.12.0. The NDF data source, farm, and their interaction were included as fixed effects and were significant for all models tested ($P < 0.01$). The EU equation applied to LF estimated CH_4 yield (g/d) to be 480.0, 481.6, and 473.7 for BV, RA, and TV, respectively. Applied to HF, CH_4 yield (g/d) was estimated at 483.8, 485.5, and 478.5 for BV, RA, and TV. The US equation applied to LF estimated CH_4 yield (g/day) to be 445.5, 446.7, and 440.7 for BV, RA, and TV, respectively. Applied to HF, predicted CH_4 yield (g/d) was 448.3, 449.6, and 444.4 for BV, RA, and TV. The predicted methane production was lowest for TV in all models tested. This study exhibits that the difference in TV, BV, and RA for feed NDF significantly affects predicted methane production. The accuracy of dietary NDF content should be considered when establishing a CH_4 baseline on farms.

Key Words: methane emissions, dairy sustainability, neutral detergent fiber

2705 Monensin effects on the behavior of late-lactation Holstein cows.

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Multiple strategies have been tested in the attempt to reduce enteric methane yield from dairy cattle. Among them, supplementation with monensin is being tested to determine its association with reduction in enteric CH_4 yield and intensity in dairy cows. In the present study, automated monitoring devices (Nedap, Groenlo, the Netherlands) were used to assess behavioral activity of late lactation cows supplemented with Rumensin (monensin; TRT; n = 5) or non-supplemented (CON; n = 5). Cows received a leg tag to measure daily lying time, number of steps and standing bouts and a neck tag to measure eating and rumination time. Behavioral activities were recorded for 21 d before supplementation began and for 28 d during Rumensin supplementation. The supplement treatment consisted of 300 mg/day per cow of Rumensin in 300 gr/day per cow of dried distillers grains (DDG), whereas the control consisted of only DDG, both top-dressed on a common TMR. All cows were housed in a sand-bedded freestall barn equipped with a cooling system including shade, fans, and soakers. Statistical analyses were performed using the PROC MIXED procedure of SAS. The model included the fixed effect of treatment, time, and the interaction between treatment and time. The cow within treatment was used as a random effect. No differences in behavioral activities were observed between the groups (TRT and CON) during the 21-d period before Rumensin supplementation started. During the 28 d of Rumensin supplementation, daily eating time did not differ between TRT and CON cows ($P = 0.94$). Rumination (538 ± 31.1 vs. 432 ± 32.3 min/d; $P = 0.04$) and lying (838 ± 26.9 vs. 740 ± 27.2 min/d; $P = 0.03$) times were greater in TRT compared with CON cows. Non-supplemented cows (CON) tended to be more inactive (790 ± 37.1 vs. 686 ± 37.0 min/d; $P = 0.08$), had more steps per day (3,675 ± 164 vs. 2,875 ± 162 steps/d; $P < 0.01$) and spent more time standing (701 ± 26.7 vs. 599 ± 26.5 min/d; $P = 0.02$) compared with TRT cows. No differences between TRT and CON cows ($P = 0.40$) were observed in standing bouts per day. The present results suggest that Rumensin is associated with positive behavioral activities changes in late-lactation dairy cows.

Key Words: methane, monensin, behavioral activity

2706 Association of individual cow milk fatty acids and milk production.

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Milk fatty acid (FA) groups, de novo (DN), mixed, and preformed (PF), indicate the origin of milk FA synthesis, which can be the mammary gland, body tissue reserves, or diet. Understanding the associations of milk FA groups with milk performance at the individual cow level may provide insight into making management and dietary decisions. Milk samples (n = 13,447) were collected the morning milkings from 1,735 Holstein cows from a herd milking 3 times daily and averaging 90 lbs of milk/cow. Milk samples were analyzed for FA groups (g/100g fat), fat, true protein, and lactose. Time periods of the first test (FT; 30 ± 3 DIM), peak milk (PT; 77 ± 40 DIM), and mid-lactation (MT; 100 ± 3 DIM) were selected. Linear models were fit with the FA group, parity (1 vs. ≥2), their interaction, and DIM treated as fixed effects and the month of sampling as the random effects. Across all periods, PF was positively associated with test day milk yield and cumulative milk yield through 150 DIM. In contrast, DN was negatively associated with test day milk yield at FT and PT, and negatively associated with cumulative milk yield through 150 DIM across all periods ($P < 0.1$). The relationship between FA and component yields differed among the periods. Energy-corrected milk yield was negatively associated with DN at FT ($P = 0.04$), not associated with DN at PT ($P = 0.21$), and positively associated with DN at MT ($P < 0.01$). The fat yield was negatively associated with DN at FT but was positively associated with DN at PT and MT ($P \leq 0.01$). In contrast, fat yield was positively associated with PF at FT and negatively associated with PF at PT and MT ($P \leq 0.03$). Protein yield was positively associated with DN for multiparous cows and negatively associated with DN for primiparous cows at FT and PT ($P < 0.01$), and positively associated with DN for all parities at MT ($P < 0.01$). The association of milk FA with milk and component yields suggests that milk FA may be a useful management tool. However, the variable associations between FA groups and milk performance outcomes at different lactation stages and parities highlights the need for tailored management decisions based on milk component testing.

Key Words: de novo, preformed, milk analysis

2707 Combining nitrate with biochar to inhibit methanogenesis in vitro.

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Enteric CH_4 contributes to climate change. Biochar (BC) is redox-active and is proposed to inhibit CH_4 production. Feeding NO_3^- inhibits methanogenesis, but NO_2^- accumulation can limit its adoption. We studied the direct effect of BC (a product of hardwood gasification) and its potential to embed NO_3^- for more complete reduction to non-additively interact to suppress CH_4 production in vitro. We expected a greater interaction of BC and NO_3^- if we increased the forage/concentrate ratio due to more potential acetate and CH_4 formation from more degraded NDF (NDFD). We used a randomized complete block design with 200-mL flasks of buffered, anaerobic rumen fluid from Jersey cows in 3 random incubation blocks with 11 duplicated treatments that were assessed by orthogonal comparisons using a mixed model. Diets were low (LF) and high (HF) forage, or LF containing almond hulls (AH); the LF and HF diets were without or with BC (6% of DM) and without or with NO_3^- (4% of DM). The AH treatments were without or with BC only. Gas production was derived using Mylar balloons during the 24-h incubation. Main effects of BC and NO_3^- in the HF diet reduced ($P \leq 0.04$) CH_4 production by 84 and 70%, respectively. The NDFD was decreased by NO_3^- by 8.9% ($P = 0.04$). The CH_4/NDFD interacted ($P = 0.06$) in HF diets, with the BC/NO_3^- combination having marginally lower CH_4suppression than individual treatments. Although CH_4 was not affected ($P > 0.10$) by treatments in LF diets, NO_3^- decreased acetate but increased the propionate molar percentage (all $P < 0.05$). Adding AH increased acetate, butyrate, and propionate, but lowered the higher chain VFA molar percentage ($P \leq 0.06$). Adding AH did not affect CH_4 production. Interactions ($P \leq 0.02$) of BC and NO_3^- for NH_4-N concentration in both diets support our hypothesis of a BC matrix mediating NO_3^- reduction, but minimal analyzed NO_3^- and NO_2^- showed complete reduction by 24 h. We dosed $(^{15}NH_4)_2SO_4$ and $^{15}NaNO_3$ to estimate microbial N production, which did not differ among treatments. Our BC appeared to limit H_2 production or sink H_2 to compete with methanogenesis. Although BC and NO_3^- did not interact, further study is needed with lower doses and longer microbial adaptation.

Key Words: biochar, methane, rumen

2708 Metabolic stress related biomarkers assessed by milk infrared are associated with differences in fertility.

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One of the main factors influencing fertility in dairy cattle is the metabolic status of animals, especially in the postpartum stage, when ovarian activity begins and cows undergo insemination. Our study sought to infer associations between milk infrared-derived predictions of blood biomarkers for metabolic stress response and fertility traits within a multibreed population of 83,056 dairy cows. The hematochemical

parameters (28 metabolites) were predicted using Fourier-transform infrared spectroscopy (FTIR) applied to milk and equations previously developed ($0.48 \leq R^2 \geq 0.87$) on an independent calibration database of 1,351 cows reared in 5 herds. Calibration equations were then applied to a population database of 159,151 FTIR milk spectral data, which were merged with fertility data collected by the Breeders Federation of Alto Adige from the northeast of Bolzano province in Italy. The days open (DO) was modeled as the hazard of becoming pregnant after calving at time *t* and was analyzed using a Cox's proportional hazards model. Models included the effect of the number of the lactation, the year of calving, the herd, the breed, the effect of FTIR-based predictions of metabolites (pMET), tested one at a time, and discretized based on percentiles (7 levels), and breed interacting with pMET. Among all the metabolites involved in the stress response, increasing levels of predicted ceruloplasmin (pCP), total reactive oxygen metabolites, and advanced oxidation protein products resulted associated with a significant decrease in the hazard ratio (HR) of achieving pregnancy. This impairment was particularly evident for pCP content, a proinflammatory protein. Beyond the clinical threshold of 2.3 µmol/L (located in the fourth percentile), the HR decreased significantly, dropping from an average value across breeds of HR = 2 (for the first pCP percentile) to HR = 0.8 (for the seventh pCP percentile). The results on the potential effects of pMET on DO are consistent with expected physiological patterns, and they appear to be promising tools for assessing and improving fertility in dairy cattle.

Key Words: infrared spectroscopy, blood metabolite, fertility

Ruminant Nutrition 5: Calves and Heifers

2709 Forage sources in total mixed rations on rumen fermentation and development of the gastrointestinal tract of dairy calves. A. F. Toledo*, J. G. Dantas, G. H. B. Silva, G. C. R. C. Evangelista, F. V. L. Barbosa, I. C. R. Oliveira, C. R. Tomaluski, and C. M. M. Bittar, *Dept. Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil.*

The objective of this study was to determine the effects of feeding forage sources, with different quality, on rumen fermentation and development of the gastrointestinal tract of dairy calves. A total of 48 Holstein calves were blocked and randomly assigned to 1 of 4 dietary treatments according to sex and body weight at 28 d of life. Treatments consisted of a no-forage coarsely ground starter (CON); or total mixed rations containing 7.5% on DM basis of Tifton hay of either medium quality (MH) or low quality (LH); or 10% on DM basis of corn silage (CS). Calves received 6 L/d of whole milk, a commercial pelleted starter ad libitum, and no forage during the first 28 d of life. After that, the solid diet was changed to the respective treatments. Rumen fluid samples were taken at wk 6, 8, and 10 by an oro-esophageal tube. Calves were gradually weaned from 52 to 56 d of age and 20 calves (5 per treatment) were harvested on d 70. The CON diet increased total volatile fatty acid concentration (89.9 CON; 68.5 MH, 65.7 LH, 71.5 CS ± 3.26 mM; $P < 0.01$) and decreased rumen pH compared with forage-containing diets (5.7 CON, 6.0 MH, 6.1 LH, 5.9 CS ± 0.08; $P < 0.01$). Nevertheless, there was no difference among diets for butyrate proportion in the rumen (8.5 CON, 8.0 MH, 8.4 LH, 8.8 CS ± 0.37 m*M*/100 m*M*; $P > 0.05$). Including forage tended to promote greater gut fill (8.9 CON, 10.1 MH, 10.8 LH, 9.5 CS ± 0.81 kg: $P = 0.07$). However, true empty body weight was not affected by the forage provision, regardless of the source (77.2 CON, 77.0 MH, 78.4 LH, 75.2 CS ± 5.74 kg; $P > 0.05$). Including fiber sources in the total mixed ration, with high energy content, resulted in small fermentation profile variation, with the benefit of lower rumen pH, but without negative effects on the performance of young dairy calves.

Key Words: empty weight, gut fill, VFA

2710 Evaluation of bovine colostrum replacer supplementation to improve weaning transition in dairy calves. K. Y. Edwards*[1], S. M. Bell[2], S. J. LeBlanc[1], T. J. DeVries[3], M. A. Steele[3], J. H. C. Costa[4], and D. L. Renaud[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Ringia Farms Ltd., Lakeside, ON, Canada,* [3]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [4]*Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT.*

During weaning, dairy calves often experience hunger, gastrointestinal challenges, and reduced weight gain. Decreased gain is associated with lower future production, so methods to mitigate the negative effects of weaning are needed. The objective of this study was to evaluate the efficacy of supplementing bovine colostrum replacer (CR) during weaning to reduce intestinal permeability and improve gain. Female Holstein calves (n = 65) were housed individually until d 70. Calves were fed milk replacer (MR; 150 g/L) 3 times daily up to 12 L/d from d 1 to 56. Calves were weaned over 8 d from d 57–64, receiving a total of 7.8 L in 2 meals from d 57 to 60 and 1 meal of 3.8 L from d 61 to 64. At d 57, calves were blocked by birthweight and randomly assigned to 1 of 2 treatments, equal in metabolizable energy, that were fed once daily during weaning from d 57 to 64 as one of their meals: control (CON; n = 31): 3.8 L of MR (150 g/L) fed by nipple bottle, or CR supplementation (COL; n = 34): a mixture of 1 L of CR (125 g/L) and 3 L of MR (150 g/L) with 3.8 L of the mixture fed by nipple bottle. Body weight (BW) was recorded at d 0, 57, 60, 64, 70, 77, and 84. Starter intake and respiratory score were measured daily from d 50 to 70, and fecal consistency was examined daily from d 56 to 70. Serum BHB and lung consolidation were evaluated at d 57, 64, and 70. Intestinal permeability was assessed by recovery of Cr-EDTA, lactulose, and D-mannitol from plasma after oral administration at d 56 and 65. Repeated measure mixed effects regression models were built with random effect of calf. There was no difference in BW between CON (90.8 ± 0.7 kg) and COL (90.8 ± 0.6 kg) at the start of weaning ($P = 0.97$); however, COL were 2.79 kg (95% CI: 0.90–4.68; $P = 0.004$) and 2.76 kg (95% CI: 0.86–4.65; $P = 0.004$) heavier than CON at d 77 and 84, respectively. Further, COL tended to gain 100 g/d more than CON from d 57 to 84 (95% CI: −10.41–207.13; $P = 0.08$). There were no differences in any other variable measured. Calves supplemented with CR once daily during the 8-d weaning had improved gain 2 and 3 wk after weaning, suggesting that CR supplementation during weaning may support gain, but the mechanism is unclear.

Key Words: gain, wean, supplement

2711 Health and performance of beef × dairy calves fed milk replacers of different fat levels and timing of varying protein sources. J. Stypinski*[1], A. Plumski[2], D. Ziegler[3], W. Hansen[3], M. Scott[3], and I. Salfer[1], [1]*University of Minnesota, St. Paul, MN,* [2]*University of Minnesota Southern Research and Outreach Center, Waseca, MN,* [3]*Milk Specialties Global, Eden Prairie, MN.*

Despite the growing prominence of beef × dairy breeding programs, research on optimal feeding practices for these calves is limited. The objective of the current study was to compare performance of crossbred calves consuming milk replacers (MR) with different fat concentrations (18% [18F] vs. 24% [24F]), and timing of varying protein sources (d 1 to 21: milk, plasma, wheat [MPW] only; d 22–63: MPW vs. milk, wheat [MW]). A total of 58 Angus × Holstein calves were enrolled in a 63-d trial beginning at 2 to 5 d of age and randomly assigned to one of 4 MR treatments: (1) MPW-MPW-18F, (2) MPW-MW-18F, (3) MPW-MPW-24F, and (4) MPW-MW-24F. The MR was fed at 0.34 kg MR per d (12.5% wt/vol in water) fed 2 times/d from d 1 to 56 and 0.34 kg/d (12.5% wt/vol in water) fed 1 time/d from d 56 to 63. Calves had ad libitum access to water and calf starter (CS). Feed intakes were recorded daily. Body weight (BW) was recorded on d 1, 21, 35, 49, 56, and 63. Data were analyzed as a linear mixed model, testing fixed effects of fat concentration, timing of protein source, and their interaction, and the random effect of source herd, with initial BW weight used as a covariate for growth and feed intake. The feeding MPW throughout the entire preweaning period tended to increase ADG from d 1 to 49 of life compared with MW (1.29 vs. 1.16; SEM = 0.058; $P = 0.08$). Milk replacer intake from d 1 to d 63 was increased by increasing fat concentration (1.36 vs. 1.33; $P < 0.001$)and decreased by feeding MPW through d 63 (1.34 vs. 1.35; SEM = 0.0053; $P = 0.01$). When MPW was fed during the entire preweaning period, CS intake was increased (1.38 vs. 1.16; SEM = 0.16; $P = 0.05$) and total feed intake tended to be increased (2.72 vs. 2.52; SEM = 0.16; $P = 0.07$). Feed efficiency and fecal scores were not affected by treatment ($P > 0.10$). Results from this study suggest that feeding only milk and wheat protein from d 22 to 63 reduces feed intake and growth of beef × dairy calves compared with inclusion of plasma protein during the entire preweaning period, whereas increasing fat concentration increased MR intake but did not affect growth.

Key Words: beef × dairy, milk replacer, calf nutrition

2712 Shifts in rumen profiles of Holstein steers fed antimicrobial and starch diets.

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We aimed to evaluate the effects of 2 diets differing in starch and antimicrobial content on the rumen metabolome and bacterial community and production of Holstein calves. Holstein steers between 3 and 7 d old (n = 72) were randomly allocated on birth date in a randomized block to control (CON; 38% starch; 50 ppm monensin and 20 ppm flavophospholipol) or treatment (TRT; 47.5% starch) diets (n = 36 calves/treatment; n = 6/replicate). Calves were fed milk powder twice daily for 42 d and ad libitum wheat straw in addition to pre-starter (d 0–24), starter (d 25–94), and finisher diets (d 95–452). Calves were housed indoors in pens of 6 with outdoor access. Concentrate intake and clinical signs were recorded daily. Rumen fluid was collected by stomach tube <3 h after feeding at d 100, 200, and 438 (14 d before slaughter) from 24 calves (2 calves/replicate; the second heaviest and second lightest per pen) and analyzed for pH, ammonia, lactic acid, and VFA concentrations, as well as bacterial 16S ribosomal DNA sequences. Fermentation and production markers were analyzed by mixed models that included the fixed effects of treatment and time, covariate of pen, and the random effects of ID and pen. The relative abundance of ruminal bacterial genera was center log-transformed and subjected to redundancy analysis with respect to treatment and time. All calves had metabolic profiles over 14.5 mo consistent with subclinical ruminal acidosis. The CON group had a higher final BW ($P = 0.033$) than TRT (575 and 565 ± 11.3 kg, respectively), but similar mean ADG ($P = 0.735$). Butyrate was 3.13 m*M* higher for TRT at d 438 than CON ($P = 0.046$). Ammonia was 6.0 m*M* higher for CON at d 100 and 5.67 m*M* higher at d 200 than TRT ($P < 0.001$). Acetate was 8.4 m*M* ($P = 0.041$) and propionate 18.1 m*M* higher ($P < 0.001$) for CON than TRT. Total VFA were higher for CON (138.4 ± 5.6 m*M*) than TRT (111.6 ± 5.6 m*M*; $P < 0.001$). The CON and TRT groups differed at each time ($P < 0.05$), with the variation in microbiota increasing over time (8.6%, 10.9%, and 19.2%, respectively), which suggests the different diets lead to different microbial successions. Antimicrobials and a low-starch diet produced better weights than a higher starch diet and had more fermentation control.

Key Words: acidosis, starch, microbiota

2713 Effect of maternal nutrition on calf morphometry at birth.

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Dam nutrient deficits result from various metabolic factors arising from maternal nutrition, diminished nutritional transport capacity by the placenta. Our objectives were evaluating the effects of maternal nutrition on calf at birth. Our hypothesis posits that calves from dams with high average daily gain (ADG) during gestation will exhibit greater birth weight. The experiment took place at the Dairy Research Facility at the Federal University of Viçosa. Sixteen 3/4 Holstein × Gyr calves, produced through the embryo transfer system, were used. They originated from Holstein × Gyr heifers, subjected to 2 nutritional plans since the 70th day of gestation: ADG 0.37 kg (MOD) and ADG 0.72 kg (HIG). Parturition was closely monitored, and a scoring system ranging from 1 to 5 was employed, with 1 indicating a normal delivery and 5 signifying a cesarean intervention. Upon birth, calves were weighed, subjected to morphometry measurements, and administered 15% of their BW in colostrum with 25% Brix within the first 2 h. They were weighed and measured every 30 d until reaching 90 d of age. The calves received transitional milk for 2 d, followed by 6 L of raw milk twice daily from d 3 until reaching 90 d. Starter feed was provided ad libitum, and after 40 d, hay was also made available ad libitum. Data analysis was performed using the MIXED procedure of SAS. The HIG dams encountered more challenges during delivery ($P = 0.02$) and necessitated more interventions. This phenomenon may be associated with a tendency for larger calves at birth (38.5 vs. 32.5; $P = 0.10$) and increased body length at birth (60.9 vs. 56.4 cm; $P = 0.01$). However, this difference was not evident at 31, 61 and 91 d. The calves' morphometry at 1, 31, 61, and 91 d demonstrated similarity ($P > 0.10$) in rump and withers height. Calves from HIG heifers tended to exhibit a larger thoracic perimeter until weaning ($P = 0.07$). Although calves from heifers fed for HIG exhibited greater body length than those from MOD heifers, they showed comparable morphometry measures and BW at weaning. Therefore, a moderate ADG during gestation does not seem to adversely affect 3/4 Holstein × Gyr calf development until weaning.

Key Words: fetal programming, calf, heifer

2714 The impact of colostrum fat concentration on IgG kinetics and metabolism in newborn Holstein-Angus crossbred calves.

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The composition of fat in whole colostrum and colostrum replacers (CR) varies greatly; ranging from less than 2% to greater than 20%, and it is independent of the protein and lactose content. The objective of this study was to explore the impact of colostrum fat concentration on immunoglobulin G (IgG) kinetics and metabolism throughout the first week of life. Holstein-Angus crossbred calves (n = 32; 8/sex per treatment) were enrolled at birth, blocked by sex, and randomly assigned to one of 2 treatments for the colostrum meals at 0 h and 12 h relative to birth: low fat (LOW; 16% IgG, 11% fat, 49% protein, 32% lactose CR) or high fat (HIGH; 12% IgG, 26% fat, 41% protein, 25% lactose CR). The meals were delivered at 3 g of IgG per kg of birth weight (BW), regardless of treatment. After 12 h, all calves received milk replacer (7.5% of BW as volume) twice daily. Calves were catheterized at birth, and blood was sampled hourly from 0 to 6 h, every 2 h from 6 to 12 h, and at 15 h, 18 h, 21 h, 24 h, 36 h, 48 h, d 3, d 4, d 5, d 6, and d 7 of life to analyze serum IgG concentrations and calculate average efficiency of absorption (AEA). On d 6 of life, blood samples were taken every 30 min from 0 to 4 h after the morning milk meal, as well as at 5 h, 6 h, and 8 h after feeding to assess insulin and glucose kinetics. All data were analyzed using a generalized linear mixed model in SAS 9.4 with treatment as a fixed effect, sex as a random effect, and repeated measures where appropriate. For serum IgG concentrations there was an interaction between treatment and time, where the LOW calves had greater ($P < 0.01$) serum IgG concentrations beginning at 18 h after colostrum and lasting until d 7 of life. LOW calves also had increased ($P = 0.03$) AEA when compared with HIGH calves. Insulin and glucose concentrations were not affected ($P > 0.05$) by treatment, nor was the insulin to glucose ratio. These results suggest that low fat colostrum allows for increased IgG absorbance without affecting glucose metabolism in the first week of life.

Key Words: colostrum composition, metabolism, IgG kinetics

2715 The effect of milk oligosaccharides on calf fecal microbiome composition and functional potential.

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Colostrum and transition milk are rich in milk oligosaccharides (MO), which are known to be beneficial for health and development. Supplementation of MO in milk replacer (MR) could support performance and health of calves. Three types of MO 2′-fucosyllactose (2′FL), 3′-sialyllactose (3′SL), 6′-sialyllactose (6′SL) were supplemented to MR and provided to calves for 9 weeks to evaluate the impact on fecal microbiome. Two-week old male surplus dairy calves (45.5 kg ± 2.9 kg SD) were randomly assigned to 4 groups of 100 calves: control (standard MR), B (0.005% 2′FL), C (0.025% 3′SL + 0.015% 6′SL), and D (0.005% 2′FL, 0.025% 3′SL + 0.015% 6′SL). Calves were fed MR 3L twice daily at 150g/L with a 4 week step-down plan. Fecal samples were collected from 6 calves per group at d 29 (T1) and 57 (T2) after arrival. Total fecal DNA was extracted to perform metagenomics and assess fecal microbial diversity, abundance and metabolic activity potential. The treatment effect on microbiome data was evaluated per time point using 2 methods: (1) Kruskal-Wallis test and post-hoc Dunn, and (2) Maaslin2 using arcsine square-root transformation and adding compartment as fixed effect. There were no differences for α and β diversities between the groups but fecal microbial abundance varied between groups. Relative comparisons between MO groups and control revealed inhibition of pathogens and negative weight-associated bacteria. More specifically, at both time points, 2′FL (B) promoted carbohydrate-polymer degrading bacteria, whereas they were inhibited by 3′SL and 6′SL (in C and D). Instead, sialyllactose promoted SCFA-producing and health beneficial bacteria. At T1, health beneficial activities, such as biotin synthesis and isoprenoid biosynthesis, were promoted in B and C, whereas D enhanced lactate production. At T2, a promotion of amino acid biosynthesis in B and C and SCFA production in groups C and D were seen. The results indicate a modulating effect on fecal microbiota in both composition and functional potential which differs depending on the type of MO fed. By steering the microbial composition and metabolic activity to a more positive situation, MO can support health and development of calves.

Key Words: calf, oligosaccharide, microbiome

2717 Effects of pre-weaned calves' daily gain and feed intake on first-lactation performance: A meta-analysis.

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The aim of this study was to investigate the impact of pre-weaned calves' daily gain and feed intake on first-lactation milk yield and milk composition through meta-analysis. The analysis included peer-reviewed articles published from 1997 to 2021, and 13 studies met the inclusion criteria. Inclusion criteria were: The study examines the impact of pre-weaned calf nutrition on the performance of first-lactation cows. It reports on the feed intake and daily weight gain during the pre-weaned calf period, as well as the first-lactation 305-d milk yield, milk fat yield, and milk protein yield. Abstracts were excluded from all the analysis. The response variables were: first-lactation 305-d milk yield, milk fat and milk protein yield. The explanatory variables were the average daily gain (ADG), liquid feed dry matter intake (LDMI), and starter dry matter intake (SDMI) of pre-weaned calves. The data were analyzed using the R 4.1.3 "Meta" package. The analysis of quadratic regression showed a significant correlation ($P < 0.01$) between 305-d milk yield and average daily gain (ADG) in the first calf. The highest values for 305-d milk yield and milk yield were achieved when pre-weaned calves reached ADG of 0.65 kg/d and 0.64 kg/d, respectively. Multiple regression analyses indicated that the productive performance of a cow's first lactation is synergistically affected by ADG, LDMI, and SDMI. The multiple regression model showed that ADG accounted for 15.3% and 14.8% of the variance in 305-d milk and fat yield, respectively, in first the lactation. Additionally, LDMI explained 24.5%, 23.6%, and 22.4% of the variance in 305-d milk, fat, and protein yield in first lactation, whereas SDMI accounted for 60.2%, 61.6%, and 77.6% of the variance in 305-d milk, fat, and protein yield in first lactation. Collectively, providing sufficient liquid and starter feed to pre-weaned calves can result in an average daily gain of more than 0.64 kg/d. Combined with optimal management strategies for dairy calves and heifers, this can improve the production performance of first lactation cows.

Key Words: pre-weaned calves, average daily gain, first-lactation performance

Ruminant Nutrition 5: Carbohydrates and Lipids

2718 Effect of ensiled alpha amylase-enhanced corn grain at 2 concentrations in lactating dairy cow diets. W. R. Silva[1], A. J. C. Silva[1], M. A. Tiengo[1], R. B. Silva[2], R. A. N. Pereira[3], T. J. DeVries*[4], and M. N. Pereira[1], *[1]Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, Minas Gerais, Brazil, [2]Better Nature Research Center, Lavras, Minas Gerais, Brazil, [3]Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, Minas Gerais, Brazil, [4]Department of Animal Biosciences, University of Guelph, Guelph, Ontario, Canada.*

This experiment evaluated the effect of α-amylase-enhanced corn (AAC; Enogen, Syngenta) on lactating dairy cows fed diets with ensiled mature kernels at 2 concentrations. A total of 20 individually housed Holstein cows, arranged in 4 × 4 Latin squares (21-d periods), were exposed to each of 4 treatments in a 2 × 2 factorial combination of corn concentration (C) and type (T): High (28.7% starch, 24.6% corn, 2.5% citrus pulp) versus Low (21.5% starch, 14.1% corn, 13.8% citrus pulp) and AAC (48.8% vitreousness) vs. isogenic control (CTL. 51.1% vitreousness). Kernels were ground each week, hydrated (62.2 ± 1.3% DM for AAC and 63.1 ± 1.4% DM for CTL) and ensiled for 28 ± 3 d. Feces and urine were collected from d 19 to 21 of each period. Nutrients in feces were used to calculate the digestibility. The N retention was calculated: N intake − (N in milk + feces + urine). The statistical model included the effects of square, cow(square), period, C, T, and C × T interaction. Milk yield (34.1 kg/d) and DMI (22.8 kg/d) did not differ ($P \geq 0.32$). High reduced the daily secretion of ECM (33.2 and 34.4 ± 1.10 g/d) and increased milk protein concentration (3.12 and 3.09 ± 0.050%) relative to Low ($P \leq 0.04$). Cows fed Low-AAC had the highest ECM/DMI (1.54 vs. 1.48, $P \leq 0.05$). There was a tendency for AAC to increase the total-tract NDF digestibility (49.9% vs. 48.0%, $P = 0.09$), but starch digestibility did not differ (98.4%, $P \geq 0.15$). High reduced the ruminal acetate to propionate ratio (2.45 and 2.95 ± 0.122) and pH (6.59 and 6.74 ± 0.089) relative to Low ($P \leq 0.05$). Cows fed High-AAC had lower MUN than Low-AAC (17.4 vs. 18.8 mg/dL) and plasma urea-N was lower on High-AAC than on High-CTL and Low-AAC (17.2 vs. 19.0 and 18.7 mg/dL, $P \leq 0.05$, C × T). High reduced the excretion of N in urine ($P \leq 0.01$) relative to Low (211.2 vs. 232.7 g/d; 35.6% vs. 39.3% of intake). The N retention was higher on High-AAC than on Low-AAC and Low-CTL (15.6 vs. 8.8 and 9.6 g/d, $P \leq 0.10$, C × T). Overall, Low increased milk solids secretion, N loss in urine, and the ruminal acetate to propionate ratio. Cows fed AAC tended to have improved feed efficiency and higher total-tract NDF digestibility than cows fed CTL.

Key Words: α-amylase, starch, feed efficiency

2719 Substituting dried ground corn with rehydrated corn or high-moisture corn with vitreous endosperm for lactating dairy cows. B. M. Martins[1], C. S. Bittencourt[1], T. A. S. Silva[1], L. H. R. Silva[1], E. Detmann[1], A. L. Silva[1], M. I. Marcondes[2], and P. P. Rotta*[1], *[1]Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, [2]Washington State University, Pullman, WA.*

This study aimed to evaluate the effects of substituting dried ground corn (DGC) with rehydrated ground corn silage (RGC) or high moisture corn silage (HMC) on nutrient intake and digestibility, milk yield (MY) and composition, and blood parameters in dairy cows. A total of 9 Holstein dairy cows, including 6 rumen-cannulated and 3 non-cannulated cows, were used in a replicated 3 × 3 Latin squares design, blocked by MY and DIM. The cows were fed a TMR diet with 22% starch twice a day. The 3 treatments included: (1) a control diet with concentrate based on DGC; (2) a diet with replacement of DGC with RGC; and (3) a diet with replacement of DGC with HMC. All treatments used the same corn hybrid with 88% vitreousness and ensiled for 258 d. Total fecal production was collected over a period of 3 d to estimate total digestibility, and ruminal digesta flow was determined by sampling omasal digesta using the double marker system every 9 h for 3 d. Blood sampling was conducted 4 h after the morning feeding. Additionally, milk sampling was carried out over 3 d for each milking time. The collected data underwent ANOVA using the "lmer" function from the "lme4" package in R. Results revealed that DMI was greater ($P = 0.03$) for HMC diet compared with DGC. However, no differences were observed for DMI when comparing DGC and RGC or RGC and HMC. Total digestibility of DM, NDF, and starch did not show differences ($P < 0.10$) among treatments. However, total digestibility for CP was greater ($P < 0.01$) for HMC compared with DGC. Starch rumen digestibility was similar ($P = 0.35$) among treatments. A tendency ($P = 0.09$) for greater MY was observed for HMC, with no difference between DGC and RGC. Fat-corrected MY was greater ($P = 0.05$) for HMC compared with DGC, but no difference was observed between DGC and RGC. MY/DMI was similar ($P = 0.60$) among treatments. The concentration of NEFA was greater ($P < 0.01$) for both RGC and HMC compared with DGC. In conclusion, the findings suggest that HMC enhances dairy cow performance more effectively than RGC and DGC, but no significant improvements were observed when using RGC compared with DGC.

Key Words: corn, cow, vitreous

2720 Effects of abomasal sodium cholate infusion on milk production, nutrient digestibility, and metabolome in lactating cows. M. You*, F. A. Gutierrez-Oviedo, A. Javaid, N. D. Senevirathne, and J. W. McFadden, *Cornell University, Ithaca, NY.*

Dietary fatty acid (FA) supplementation is an approach to enhance the energy density of cow diets; however, saturated FA have a low digestibility. Bile salts, which are emulsifiers, may enhance saturated FA digestion. To investigate, 6 multiparous Holstein cows (94 ± 37 DIM) underwent a 3 × 3 incomplete Latin square design across 2 squares. The experiment featured 3 treatments administered as continuous abomasal infusates at 5 L per day over 14-d periods: water (control), 15 g/d sodium cholate (SC; low dose), and 60 g/d sodium cholate (SC; high dose). Cows were fed a corn silage-based diet formulated to contain 5.5% ether extract (~4% FA; dry matter basis). Plasma and fecal samples were collected at 9-h intervals during the last 3 d of each period and subsequently combined into a composite by period. The statistical model comprised the fixed effects of treatment, period, square, and the random effect of cow. Milk yield, DMI, milk fat, and milk FA profile were unchanged ($P > 0.05$). Milk protein yield (1.49, 1.49, and 1.43 kg/d for control, low dose, and high dose, respectively; $P = 0.06$) tended to be modified by treatment. Milk solids yield (6.28, 6.24, and 6.07 kg/d for control, low dose, and high dose, respectively; $P < 0.05$) was modified by treatment. Apparent total-tract digestibility of dry matter ($P < 0.05$) and crude protein ($P < 0.05$) were modified by treatment (e.g., 55%, 58%, and 50% CP digestibility for control, low dose, and high dose, respectively). Digestibility of total FA, or 16C or 18C FA, was not changed ($P > 0.05$). Metabolomic analysis via mass spectrometry demonstrated that the low dose reduced plasma levels of betaine, chenodeoxycholate, creatinine, lactate, methionine, and uridine compared with the control ($P < 0.05$). Both low and high doses led to increased fecal glycocholate levels compared with the control, with the high dose showing the most significant elevation ($P < 0.05$). The results of this study indicate that the treatment with SC did not enhance fat digestion in lactating cows, possibly because SC was excreted from the body in a glycine-conjugated form.

Key Words: bile acid, emulsifier, digestibility

2722 Effect of increasing dietary fat by feeding oilseeds on melting properties of milk fat in Holstein cows.

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Whole cottonseed (WCS) and roasted soybeans are commonly fed as economical sources of rumen-available unsaturated fatty acids (FA). Increasing dietary fat with oilseeds commonly decreases milk de novo FA (FA <16C) and increases preformed FA (FA >16C) but has limited effect on PUFA, as the unsaturated FA are extensively biohydrogenated in the rumen. Our objective was to determine the melting properties of butter oil of cows fed increasing levels of WCS and high oleic roasted soybeans. Butter oil was prepared by centrifugation, and melting points and solid fat content (SFC) was determined using differential scanning calorimetry (DSC). Data were analyzed using JMP Pro 16 with a model that included the random effect of cow and period and fixed effect of treatment. In the first experiment, 4 primiparous and 8 multiparous Holstein cows were arranged in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were WCS provided at 0%, 3.4%, 6.8%, and 9.9% of dietary DM with WCS substituted for cottonseed hulls and soybean meal. Increasing WCS had no effect on the peak melting temperature ($P > 0.05$) or SFC at room temperature (20°C; $P > 0.05$). In the second experiment, 8 primiparous and 8 multiparous Holsteins were arranged in a replicated 4 × 4 Latin square design with 21 d periods. Treatments were 0%, 5%, 10%, and 15% of roasted high oleic soybeans substituted for soybean hulls and heat treated and solvent extracted soybean meal. The HO soybeans, when fed at 15%, decreased peak melting temperature from 14.27 ± 0.18°C to 13.9 ± 0.18°C ($P < 0.05$) and decreased SFC at 20°C from 27.57 ± 1.6 to 21.58 ± 1.6 ($P < 0.05$). In conclusion, although increasing dietary fat with oilseeds decreases de novo synthesized FA and increases preformed FA it has a limited impact on melting properties of milk fat until very high feeding rates.

Key Words: buttergate, physical properties, unsaturated fatty acids

2723 The effect of palmitic acid fat supplements on the melting properties of milk fat of Holstein cows.

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The melting properties of milk fat are important for butter spreadability, and the effect of diets fed to dairy cows on these properties is not well characterized. The objective was to characterize the thermal properties of milk fat from cows fed fat supplements enriched in high palmitic acid (PA). The hypothesis was that feeding PA would increase butter oil melting temperature and the percentage of solid fat content (SFC) at room temperature. Butter oil was prepared from 2 experiments by high-speed centrifugation and characterized using differential scanning calorimetry (DSC) and nuclear magnetic resonance (NMR). Data were analyzed using JMP Pro 16 with a model that included the random effect of cow and period and fixed effect of treatment. In the first experiment, 6 primiparous and 6 multiparous Holstein cows were arranged into a 3 × 3 Latin square. Treatments were a no supplement control (CON), a high-palmitic acid fat supplement at 2% DM of the CON diet (PA), and a diet that induced milk fat depression (MFD) by feeding a high starch and unsaturated FA diet. The PA supplement increased butter oil SFC at 20°C and 10°C ($P < 0.05$) as determined by both NMR and DSC. Butter oil from MFD also had a higher SFC than CON but was lower than PA. The SFC of butter oil from PA was 41.2% and 23% at 10°C and 20°C, respectively, whereas SFC in MFD was 28% and 20%. The second experiment used 12 multiparous lactating Holstein cows in a 3 × 3 Latin square. Treatments were low-fat control (CON) and a fat supplement either high in PA or a blend of PA and stearic acid (SA). Fatty acids were supplemented at 2% of the DM. The PA supplement increased SFC at 20°C ($P < 0.05$) observed by both DSC and NMR. The onset of peak melting temperature was higher in PA compared with blend and control ($P < 0.05$), and the temperature at which butter was completely melted was also higher ($P < 0.05$) in PA-fed cows. In conclusion, feeding supplements high in PA increased melting points and SFC, which are expected to decrease the spreadability of butter at room temperature.

Key Words: saturated fat, buttergate, physical properties

2725 Investigating the effects of fat supplementation on fecal metabolites using nuclear magnetic resonance.

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Nuclear magnetic resonance (NMR) is commonly used for untargeted metabolomics of small molecules in aqueous samples, but there is limited data showing its use for fecal metabolite analysis in dairy cows. The objective was to determine the ability of NMR to detect differences in the fecal metabolome of cows fed fat supplements that differ in fatty acid profile. A total of 8 primiparous and 8 multiparous Holstein cows (>70 DIM) were blocked by parity in a 4 × 4 Latin square design with 21-d periods. The treatments were (1) low-fat control (CON), (2) high-fat control (HF), (3) high oleic supplement (HO), and (4) high palmitic supplement (HP). Fecal samples were collected at 2000 h on d 20 of each period. Samples were snap frozen in liquid N, stored at −80°C, thawed, diluted in D_2O with 1 m*M* of 3-(trimethylsilyl) propionate-2,3-d4 (TSP) as an internal shift reference, homogenized, snap frozen and rethawed, and centrifuged to remove particles. The supernatant was analyzed on a Bruker Avance NEO 600 MHz spectrometer with enhanced sensitivity for ^1H. A one-dimensional NMR spectrum was acquired for all samples using the nuclear Overhauser effect spectroscopy pulse sequence and 2-dimensional NMR experiments performed on select samples. Chenomx 9.0 was used for corrections and reference calibrations for each sample. Data were analyzed in JMP Pro 17 and the model included the random effects of cow and period and the fixed effects of treatment, parity, and their interaction. Preplanned contrasts tested the effect of the addition of fat (CON vs. HF), increasing oleic acid (HF vs. HO), and increasing palmitic acid (HF vs. HP). In total, 16 metabolites were quantified. The HF diet decreased fecal alanine and valerate concentrations compared with CON ($P < 0.05$). Increasing palmitic acid increased fecal concentrations of glucose, isoleucine, leucine, and valerate ($P < 0.04$). Other metabolites that were not changed by treatment included acetate, formate, isovalerate, and propionate. Overall, NMR was successful in quantification of 16 small metabolites in feces and may be useful for some experiments investigating hindgut fermentation or other aspects of gastrointestinal physiology.

Key Words: palmitic acid, oleic acid, NMR

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2728 Chemical characterization and comparison of two new high protein corn milling coproducts. C. M. Harris*[1], S. Tilton[2], A. L. Carroll[1], and P. J. Kononoff[1], [1]*University of Nebraska–Lincoln, Lincoln, NE,* [2]*The Andersons Inc., Maumee, OH.*

Accurate chemical characterization of feedstuffs is integral to understanding the nutritional content and value of dairy feeds. Recently, some dry corn milling plants have modified facilities to mechanically separate fiber-containing particles from a portion of plant and yeast based nitrogenous particles, resulting in a high-protein processed corn milling coproduct. The objective of this study was to chemically characterize 2 novel, high-protein corn milling coproducts resulting from 2 different processes and compare their chemical composition. Over a ~5-mo period, independent samples (n = 5) of each feed were obtained from 2 different production processes. These 2 feeds are described as ANDVantage 40Y and ANDVantage 50Y and differ because the production process of the latter includes a propriety step that aims to increase protein purity of the coproduct. Using a completely randomized design, samples were analyzed for chemical composition and compared. Based on a DM basis, ANDVantage 40Y and ANDVantage 50Y differed in CP (45.1 vs. 53.9 ± 0.38%), methionine (0.94 vs. 1.17 ± 0.033%), lysine (1.12 and 1.51 ± 0.058%), ADF (15.4 vs. 19.4 ± 1.17%), neutral detergent insoluble CP (37.6 vs. 38.3 ± 3.41% of CP), acid detergent insoluble CP (3.61 vs. 5.82 ± 0.955% CP), and sulfur (0.57 vs. 0.704 ± 0.0078%). In contrast, these feeds contained similar concentrations of NDF (average 38.0 ± 3.41%), phosphorus (average 0.439 ± 0.0437%), and total fatty acids (average 10.7 ± 0.358%). As expected, these feeds contained more CP than traditional DDGS, but we also observed that they contained similar concentrations of fiber and less phosphorus. Overall, this study highlights the need for routine characterization of the chemical composition of feeds originating from the dry corn milling process.

Key Words: coproduct, chemical composition, high protein

2729 Protein and carbohydrate subfractions in the newly developed blended fat stimulated feed product (BFSFP) in comparison with commercial protein and energy feeds. U. Ihsan, L. L. Prates, and P. Yu*, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.*

The objectives of this study were to compare protein and carbohydrate subfractions in the newly developed blended fat stimulated feed product (called BFSFP) with commercial protein and energy feed products for lactating dairy cows. The new blended fat stimulated feed products with 3-batch samples (BFSFP1, BFSFP2, BFSFP3) were developed. Barley grain samples (n = 3) and canola meal samples (n = 3) were obtained from Canadian Feed Research Center (CFRC). The detailed chemical compositions were determined according to AOAC standard and feed analysis methods. The protein and carbohydrate subfractions were evaluated using the updated Cornell Net Carbohydrate and Protein System. The treatment design was a one-way structure. The experimental design was a completely random design with feed treatments as a fixed effect. The data were analyzed using the Mixed procedure of SAS. The results showed that compared with commercially available energy-rich and protein-rich feeds, the BFSFP had a lower ($P < 0.05$) fast degradable protein fraction (PA2: 4.9 vs. 9.1, 3.4%DM), intermediately degradable protein fraction (PB1: 23.4 vs. 31.6, 8.7%DM), and undegradable protein fraction (PC: 0.14 vs. 0.30, 0.06%DM) than canola meal but higher ($P < 0.05$) than barley grain. The BFSFP had lowest ($P < 0.05$) slowly degradable protein fraction (PB2: 4.3 vs. 11.2, 6.2%DM). For carbohydrate subfractions, the BFSFP had lower ($P < 0.05$) immediately degradable carbohydrate fraction (CA4: 7.4 vs. 9.3, 2.7%DM) and undegradable carbohydrate fraction (CC: 7.3 vs. 19.1, 1.9%DM) than canola meal but higher ($P < 0.05$) than barley grain. The BFSFP had lowest ($P < 0.05$) intermediate degradable carbohydrate fraction (CB2: 4.3 vs. 11.2, 6.2%DM) but highest ($P < 0.05$) slowly degradable carbohydrate fraction (CB3: 30.0 vs. 8.5, 16.6%DM) than canola meal and barley. In conclusion, the newly developed BFSFP had unique protein and carbohydrate subfractions in comparison with the commercially available energy-rich and protein-rich feeds.

Key Words: blended fat stimulated feed product, protein and carbohydrate subfraction, rumen degradation

2730 Evaluation of olive cake inclusion on TMR and wheat silage fermentation quality and aerobic stability. C. Härter*[1], J. Falson[1], T. Pozada[1], J. Schafhauser[2], and C. Rabelo[1], [1]*Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil,* [2]*Embrapa Clima Temperado, Pelotas, Rio Grande do Sul, Brazil.*

The olive cake (OC) is a wet by-product from the olive production chain, which has potential for pollution. In this regard, ensiling total mixed ration (TMR), which is composed of dried ingredients, may allow for the utilization of wet agro-industrial by-products (without compromising silage fermentation) in livestock production. Therefore, the objective of this study was to evaluate the effect of the inclusion of different doses of crude OC on the fermentation process, chemical composition, and aerobic stability of TMR silages. Secondarily, wheat forage was assessed as a basis for constituting a TMR. In a completely randomized design, the treatments studied were exclusive wheat silage and TMR silages (wheat forage, ground corn, soybean meal, and minerals) composed of different doses of OC (0%, 5%, 10%, and 20%, as DM basis; the treatments were named as TMR0, TMR5, TMR10, and TMR20, respectively). Laboratory silos (PVC tubes with a capacity of 2.5 L) were used, and they remained closed for 97 d. After the silos were opened, the aerobic stability of the silages was evaluated for 10 d. We tested 4 orthogonal contrasts, as follows: (1) Wheat vs. TMR; (2) TMR0 vs. TMR with OC; (3) TMR5 vs. TMR10; and (4) TMR10 vs. TMR20. The OC doses determined before beginning the experiment were corrected to the DM content of each ingredient and, indeed, the OC doses used in the current study were 0%, 4%, 8%, and 17% (on DM basis). The DM losses were greater in the TMR silages containing OC (TMR0 = 1.39% DM and TMR with OC = 2.63% DM; $P = 0.05$). The inclusion of OC did not increase effluent production (general average = 9.38 kg/t fresh matter; $P = 0.66$). Aerobic stability, aerobic deterioration, heating rate and DM losses after the exposure of TMR silages to oxygen were unaffected by the inclusion of OC. Wheat silage had low DM loss (1.43% of DM), aerobic stability higher than 80 h and good chemical composition (CP = 11.1% of DM and NDF = 51.8% of DM). We concluded that a OC can be included in a TMR up to 17% based on DM, and the wheat forage can be used for silage production either exclusively or as based-forage for a TMR.

Key Words: by-product, fermentation, olive pomace

2731 The effect of red sorghum on the rumen protozoa and fermentation characteristics of dairy cows. R. Yi[1,2], Y. Yang[1,2], S. Vigors[2], J. Xu[1], D. Bu[1], and L. Ma*[1], [1]*State Key Laboratory of Animal*

Nutrition and Feeding, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China, [2]School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.

This trial aimed to evaluate the effect of red sorghum as a supplement on rumen protozoa and fermentation characteristics in vivo. Lactating Holstein dairy cows (n = 22) were allocated to either the control or red sorghum supplement groups (0 and 83.2 mg/g, n = 11 per group) using a randomized block design, according to body weight (695 ± 98 kg), days in milk (192 ± 33 d), parity (2 ± 1), and milk yield (25.6 ± 4.6 kg) with feeding 49 d. Rumen fluid was collected by oral stomach tubing method on d 0, 28, and 49 before the morning feeding. Rumen protozoal cells were morphologically identified and counted using microscopy. Ammonia nitrogen and microbial protein were tested by colorimetric determination using a spectrophotometer, and concentrations of volatile fatty acids were determined using gas chromatography. Estimated methane production was calculated according to VFA concentration. Data were analyzed using the generalized least squares procedure (GLS) of R 1.4.1717. Statistical significance was declared at $P < 0.05$, and trends at $0.05 \le P < 1$. Compared with the control group, there was a tendency for decreased *Ophryoscdex* and increased *Dasytricha* with the inclusion of RS ($P = 0.088$ and $P = 0.089$, respectively). Compared with D28, total protozoa and *Diplodinium* ($P < 0.05$, respectively) were decreased and a tendency of decreased *Entodinium* compared with d 49 ($P = 0.089$), whereas treatment main effects were not identified ($P > 0.05$). There were no changes in *Epidinium* and *Isotricha*. Compared with d 0, the ammonia-nitrogen, microbial protein, and estimated methane production were increased in cows on d 49 ($P < 0.05$). Compared with d 0 and d 28, TVFA, butyrate, and valerate were increased , and a tendency for an increased propionate, whereas acetate, isobutyrate, isovalerate, and A:P ratio were decreased on d 49. In conclusion, the inclusion of red sorghum had the potential ability to modify the profiles of rumen protozoa, whereas its effect on fermentation characteristics requires further validation.

Key Words: red sorghum, rumen protozoa, dairy cows

2732 Impact of *Saccharomyces cerevisiae* strain CNCM I-1077 on feed efficiency in dairy cows. K. R. Oliveira*[1], P. T. R. Salgado[1], J. V. C. Rodrigues[1], C. S. Bittencourt[1], I. D. R. Ponciano[1], A. L. Silva[1], E. Chevaux[2], L. J. Mari[3], and P. P. Rotta[1], *[1]Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, [2]Lallemand SAS, Blagnac, Occitanie, France, [3]Lallemand Brasil Ltda., Aparecida de Goiânia, Goiás, Brazil.*

The high inclusion of forage and non-forage fiber sources in dairy diets highlights the continued opportunity to enhance fiber digestibility to optimize the use of alternative fibrous feed sources. This study aimed to evaluate the impact of *Saccharomyces cerevisiae* strain CNCM I-1077 on milk production, intake, total apparent digestibility, and feed efficiency in Holstein cows. Eight lactating Holstein cows, producing an average of 30.1 ± 2.56 kg/d of milk and at 175 ± 26 DIM, were included in the study. Rations, formulated based on NASEM (2021) recommendations, were provided as a TMR twice daily (41.4% NDF, 22.0% starch, and 16% CP). The study employed a crossover design with 2 treatments: control and live yeast (LY; 2×10^{10} cfu/d). Live yeast group had their cannulas opened to directly inoculate the LY into the rumen, thoroughly mixed with the ruminal content. To minimize the potential effects of oxygen resulting from open cannulas, control group cows also had their cannulas opened, and the ruminal content was similarly mixed. Following a 21-d adaptation period, milk samples were collected from each cow during milking over 3 consecutive days (d 22–24) and analyzed for fat, protein, and lactose content. Intake was monitored over 5 consecutive days (d 22–26). Total fecal outputs were collected on d 25 and 26, dried, ground, and analyzed for composition. Data were analyzed using a mixed model, with treatment and period as fixed effects and cows set as random. Milk yield did not differ significantly between treatments. Similarly, no significant differences were observed in milk constituents. However, cows fed with LY exhibited lower DMI and greater DM digestibility, with no difference observed for NDF digestibility. Evaluation of feed efficiency, expressed as milk/DMI, milk/OMI, FCM/DMI, and FCM/OMI, revealed that the LY diet presented the highest values. The substantial enhancement of feed efficiency (1.40 vs. 1.24 for FCM/DMI) evidences a better feed valorization, achieved by a reduction in DMI. Live yeast improved DM digestibility and feed efficiency in lactating dairy cows.

Key Words: digestibility, intake, live yeast

2733 Effects of *Ligilactobacillus agilis* W70 on nitrogen utilization in lactating dairy cows. X. Duan[1,2], Y. Liu[1], L. Ma[1], and D. Bu*[1], *[1]State Key Laboratory of Animal Nutrition and Feeding, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, [2]School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

This experiment was conducted to evaluate the effects of dietary protein and *Ligilactobacillus agilis* W70 on lactation performance, rumen fermentation, blood parameters and nitrogen metabolism in lactating dairy cows. Sixteen Holstein cows were evaluated in a replicated 4 × 4 Latin square with 2 levels of CP (16.02 vs. 17.00%) and 2 levels of W70 (0 vs. 1×10^{12} cfu). The cows were randomly assigned 4 treatment groups: low-protein no additive (LN), low-protein W70 additive (LA), high-protein no additive (HN), high-protein W70 additive (HA). Cows were fed individually. Experimental periods lasted 28 d, including 7 d washout and 21 d treatment periods, with measures performed in the last 3 d of the treatment period. Data were averaged per period and per animal and analyzed using the Mixed procedure of SAS version 9.4. Statistical significance was declared at $P < 0.05$, and trends at $0.05 \le P < 1$. Increasing feed conversion efficiency and nitrogen utilization efficiency were induced by supplementation with W70 either in low-protein or high-protein diets of lactating cows, but with no effect on milk production. Supplementation of W70 significantly reduced dry matter intake, ruminal ammonia nitrogen, and nitrogen intake. Milk yield, milk urea nitrogen, milk fat yield, milk protein yield, fat corrected milk yield, energy corrected milk yield, milk yield/DMI, FCM/DMI, and ECM/DMI all increased in response to higher levels of dietary protein, whereas DMI and the content of milk fat, milk protein, and milk lactose were unaffected. The high-protein diet significantly increased the acetate/propionate ratio and ammonia nitrogen and reduced isovalerate. Cows fed a high-protein diet had a higher milk nitrogen, nitrogen intake, retained nitrogen, absorbed nitrogen, retained nitrogen proportion, absorbable nitrogen proportion, and lower fecal nitrogen than those receiving low protein. These results suggest that W70 additives are beneficial to feed conversion efficiency and nitrogen utilization efficiency, which we suggest is due to reduced ammonia nitrogen in rumen. A high dietary protein level is beneficial to milk yield.

Key Words: dairy cow, nitrogen metabolism, rumen fermentation

2734 Effects of oral administration of *Megasphaera elsdenii* on health and production on commercial dairy farms. T. M. Nelson*[1], A. L. Kerwin[1], L. N. Ferro[1], C. M. Ryan[1], D. M. Barbano[1], C. J.

Louder[2], G. P. Gengelbach[2], and T. R. Overton[1], [1]*Cornell University, Ithaca, NY,* [2]*Axiota Animal Health, Fort Collins, CO.*

Our goal was to determine the effects of post calving administration of a *Megasphaera elsdenii* (Lactipro FLX) bolus (MEB) on inflammation, subclinical ketosis, health, and milk yield of dairy cattle. Holstein cows (n = 840) on 2 commercial farms entering first and greater lactations were randomly assigned to receive a MEB or control bolus (CON) within 24 h of calving. The 2 farms had different milk production during the first 70 DIM (47 vs. 39 kg/d). Blood and milk samples were collected once per week for the first 2 wk, body condition scores (BCS) and locomotion scores (LS) were assessed in the first week, with a follow-up ~70 DIM. Rumination was monitored until 70 DIM, and herd records were obtained to analyze milk yield and health events. Milk composition was determined, and serum samples were analyzed for haptoglobin and β-hydroxybutyrate (BHBA). Performance and blood biomarkers were evaluated using mixed effects models with repeated measures when applicable (SAS v. 9.4). We found that MEB had no difference in energy corrected milk (43.2 vs. 42.9 ± 0.61 kg/d, $P = 0.69$), but there was a treatment by farm effect where MEB cows in the high production herd tended to have more de novo fatty acids (1.08 vs. 1.05 g/100g of milk, $P = 0.13$). There was a treatment by parity interaction on pen rumination deviation, such that MEB cows had lower rumination deviation ($P = 0.02$), and primiparous cows given MEB had higher rumination compared with CON cows in the first 70 DIM ($P = 0.03$). Cows in the herd with high production and administered MEB tended to have a lower incidence of hyperketonemia (BHBA >1.2 mmol/L) in the first 2 weeks of lactation ($P = 0.15$), as well as a treatment by farm effect where cows in the lower production herd administered MEB tended to lose less BCS in the first 70 DIM (−0.12 vs. −0.16 ± 0.02 BCS, $P = 0.10$). Overall, cows administered MEB tended to have a lower cull rate in the first 60 DIM (16/398 vs. 26/402, $P = 0.12$). This study provides evidence that cows administered MEB after calving had improved transition to lactation, resulting in fewer cows leaving the herd in early lactation.

Key Words: transition cow, herd health, rumination

2735 Effect of feed additives and organic trace minerals on performance of lactating cows.

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This study evaluated the effects of different feed additives on intake, digestibility, milk yield and composition, and feed efficiency in dairy cows. A total of 39 Holstein cows (201.3 ± 70.0 DIM; 651.6 ± 36.1 kg BW, and 33.4 ± 0.49 kg/d milk yield) were blocked (n = 13) according to parity, DIM, and milk yield, and were randomly assigned to one of the following treatments: (1) monensin (Rumensin 200, Elanco) at 15 mg/kg of DM (MON); (2) phytogenic feed additives (Digestarom Dairy, DSM-Firmenich) at 2 g/cow per d (PFA); and (3) PFA + autolyzed yeast (AY) at 15 g/cow per d + organic trace minerals as carbo-aminofosfoquelates (OTM; DSM-Firmenich) at 30 g/cow per d (PAC). Additives were provided mixed into the concentrate. Cows were allowed a 2-wk covariate period, and treatments were applied for the following 9 wk. Samples of TMR and refusals were analyzed for particle size distribution and chemical composition. Fecal samples were collected for 3 consecutive days during covariate period, wk 3, 6, and 9, at 9 h-intervals. Fecal excretion was estimated based on indigestible NDF. Cows were milked 2 × daily and samples were collected 3 times weekly for analysis. Data were analyzed using the MIXED procedure of SAS 9.4. Orthogonal contrasts evaluated treatment differences (MON vs. PFA and PAC; and PFA vs. PAC). Cows fed PFA and PAC showed lower intakes of DM ($P = 0.038$), OM ($P = 0.035$), CP ($P = 0.049$), and NDF ($P = 0.017$), increased yields of FCM ($P = 0.016$, + 1.5kg) and ECM ($P = 0.047$, + 1.25 kg), milk fat percentage ($P = 0.003$) as well as milk fat yield ($P = 0.002$), and feed efficiency ($P = 0.044$) when compared with MON. Digestibility did not differ between treatments ($P > 0.05$). Cows fed PFA and PAC tended to have lower SCC than MON ($P = 0.066$), and cows fed PAC had lower SCC than PFA ($P = 0.026$). Treatment PAC showed lower milk urea nitrogen in comparison to PFA. The MON treatment sorted against larger particles ($P = 0.006$) and in favor of smaller particles ($P = 0.037$) compared with the other treatments. Feeding phytogenic additives, alone or in combination with AY and OTM, improves milk fat, FCM, and feed efficiency without altering nutrient digestibility compared with MON supplementation in dairy cows.

Key Words: milk yield, efficiency, somatic cell count

2736 Effects of red clover isoflavone on lactational performance, milk quality, and nitrogen metabolism in dairy cows.

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Reducing nitrogen loss and improving nitrogen utilization efficiency reduce economic costs and the environmental impact of nitrogen emission. Red clover isoflavone has the advantage of biological safety. The study aimed to investigate the effect of red clover isoflavone on lactational performance and nitrogen metabolism in dairy cows. A total of 68 Holstein cows (days in milk: 165 ± 21 d; body weight: 710 ± 75 kg; parity: 2.3 ± 1.21; milk yield: 33.93 ± 3.81 kg/d) were chosen in a randomized complete block design. Cattle were divided into 5 blocks by milk yield and randomly assigned to 4 treatments (17 cows per treatment). The experimental period was 84 d, during which cows were fed a basal diet supplemented with red clover extract at 0, 2, 4, or 8 g/kg of total mixed rations (TMR) on a dry matter basis, which corresponds to red clover isoflavones of 0 g/kg, 0.46 g/kg (0.31 g/kg formononetin and 0.15 g/kg biochanin A), 0.93 g/kg (0.67 g/kg formononetin and 0.26 g/kg biochanin A), and 1.83 g/kg (1.32 g/kg formononetin and 0.52 g/kg biochanin A), respectively. Data analyses were conducted by the MIXED procedure in SAS. Red clover extract had no effect on dry matter intake ($P = 0.31$), but significantly increased the milk yield of cows by 4.65% to 9.64% ($P < 0.01$) and feed efficiency by 6.58% to 10.53% ($P < 0.01$). Additionally, red clover extract enhanced the yield of milk components (fat, protein, lactose, nonfat milk solids, and total milk solids; $P < 0.03$). With respect to nitrogen metabolism, red clover extract reduced nitrogen intake, plasma urea nitrogen, total amino acid, urinary nitrogen, and increased microbial crude protein by 782.61 to 956.41 g/d ($P < 0.02$), supporting increased nitrogen utilization from 26.32% to 30.31% ($P < 0.01$). Except for NDF and starch, the nutrient apparent digestibility increased with red clover extract ($P < 0.04$). Supplementation of red clover isoflavone had no effects on plasma energy metabolism ($P > 0.29$). Our findings revealed that red clover extract contributed to measurable changes in lactational performance, milk quality, and nitrogen metabolism in dairy cows.

Key Words: lactational performance, nitrogen metabolism, red clover isoflavone

2737 Impact of a microencapsulated blend of fermentation extracts, essential oils, and organic acids on the productivity of dairy cows.

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This field study aimed to evaluate a novel blend of fermentation extracts, essential oils, and organic acids [P(F+EO+OA), Jefo Nutrition, QC, Canada] on cows' productivity. The blend was rumen-protected and engineered for synchronized release and optimal synergy of its active ingredients throughout the small intestine. The trial was conducted on a commercial Holstein dairy farm with an automated milking system (AMS). A total of 210 lactating cows were randomly allocated, within stage of lactation and parity (157 ± 96 DIM, 2.9 ± 0.9 lactations), to receive 0 or 10 g/d of the blend via compact dispensers linked to the AMS. Cows received a partially mixed ration consisting of corn silage, haylage, high-moisture corn, and a supplement. The cows received pelleted feed geared to production level via the AMS. Milk yield, composition, offered pellets, and rumination time were recorded for 6 mo and were analyzed using the mixed procedure of SAS Studio. The model included the fixed effects of treatment, parity, month, and their interaction and accounted for repeated measurements. Reproductive data were analyzed using Chi-squared. Energy-corrected milk (ECM) was greater ($P < 0.05$) for multiparous cows receiving P(F+EO+OA) throughout the trial and ranged from 1.2 to 3.8 with an overall average of 2.0 kg/d (45.0 vs. 43.0, respectively). This response was associated with an increase in milking frequency and amount of offered pellets (3.1 vs. 2.8 ± 0.01 milkings/d and 4.4 vs. 3.9 ± 0.02 kg/d for treatment and control, respectively). However, the increase in offered pellets (0.5 kg/d) can explain a part of the increase in ECM (50% on energy equivalence), and the remaining part may be explained by the treatment. Rumination time was increased across parities by treatment. Further, more treatment cows were confirmed pregnant by 100 DIM compared with control (51% vs. 35%, respectively, $P < 0.05$; odds ratio = 1.94). However, 66% of cows across treatments were confirmed pregnant by 120 DIM. Our findings support that supplementing P(F+EO+OA) can likely promote productivity. Research to elucidate the mode of action is required.

Key Words: milk yield, reproduction, gut health

2738 Impact of feeding calcareous marine algae to Holstein dairy cows on lactational performance.

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Calcified red seaweed remains (*Lithothamnion calcareum*; calcareous marine algae, CMA) are a bioavailable source of macrominerals, specifically calcium and magnesium. This study evaluated feeding CMA against traditional macromineral sources in Holstein cows. A total of 31 multiparous cows and 9 primiparous heifers were housed and fed using Calan gates at the East Tennessee Research and Education Center Little River Dairy Unit from −28 to 120 d in milk (DIM). Data were collected from 30 to 120 DIM for dry matter intake (DMI), milk production and components, and production efficiency (MEff) from 21 treatment (TRT) and 19 control (CON) cows balanced for parity and projected calving date. The pre- and postpartum total mixed rations (TMR; 38.2% forage dry matter) were formulated to partially replace calcium carbonate ($CaCO_3$), magnesium oxide (MgO), and sodium bicarbonate ($NaHCO_3$) with CMA. The CON lactating TMR was formulated to provide 1.5% dry matter (DM) as $CaCO_3$, 0.3% DM as MgO, and 1.5% DM as $NaHCO_3$. The TRT lactating TMR was formulated with 0.4% DM CMA (0.3% $CaCO_3$, 0.1% MgO, and 0.7% $NaHCO_3$ DM replaced, respectively). The GLIMMIX procedure of SAS 9.4 was used to determine the impact of treatment, DIM, temperature humidity index (THI), and projected 305-d mature equivalent milk yield (PMEM) and their 2-way interactions with significance set at $P \leq 0.05$. Stepwise backward elimination was used to remove non-significant interactions. Under non-heat stress conditions, TRT cows ate more compared with CON (29 vs. 25.3 ± 0.7 kg/d; $P < 0.01$). Treatment MEff was decreased compared with CON under heat stress (1.6 vs. 1.8 ± 0.1; $P < 0.01$) and non-heat stress (1.6 vs. 2.0 ± 0.1; $P < 0.01$). However, TRT cows maintained MEff in heat stress compared with non-heat stress conditions (1.6 vs. 1.6 ± 0.1; $P = 0.06$), whereas CON cows reduced MEff during heat stress (1.7 vs. 1.8 ± 0.1; $P < 0.01$). Experimental diets did not significantly affect body weight, milk, fat, and protein yield, or reproduction. These results indicated CMA can partially replace $CaCO_3$, MgO, and $NaHCO_3$ in the ration with minimal impacts on performance.

Key Words: heat stress, milk yield, feed additive

Ruminant Nutrition 5: Gut Physiology, Fermentation, and Digestion

2739 Effects of different cobalt sources on ruminal fermentation of lactating dairy cow diets in a dual-flow continuous culture system. M. U. Siregar*[1], J. A. Arce-Cordero[1,2], G. K. Salas-Solis[1], A. C. Silva Vicente[1], J. R. Vinyard[1], E. Sarmikasoglou[1], M. L. Johnson[1], R. R. Lobo[1], S. W. Ma[1], C. Hammond[1], K. S. Alves[1], S. Castillo[1], L. M. Katz[1], M. Socha[3], A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*Universidad de Costa Rica, San Jose, San Jose, Costa Rica,* [3]*Zinpro Corporation, Eden Prairie, MN.*

The objective of this study was to evaluate the effects of different sources of cobalt in a lactating dairy cow diet on in vitro ruminal fermentation. All diets provided the same amount of supplemental Co (1 mg per kg of diet DM) included in a premix with a targeted inclusion level of 2.5% of diet DM. Treatments were cobalt carbonate ($CoCO_3$), cobalt amino acid (CoAA), cobalt pectin (CoPec), and the combination of cobalt pectin and cobalt amino acid (CoPec+CoAA). All diets had the same nutrient composition (30.5% NDF, 16.3% CP, and 30.9% starch) and fermentors were fed 106 g/d (DM basis), split into 2 feedings (DM basis). WE used 8 dual-flow continuous culture fermentors in a replicated 4 × 4 Latin square. Experimental periods were 10 d (7 d adaptation and 3 d sample collection). Composite samples of daily effluent were collected and analyzed for VFA, digestibility of DM, OM, NDF, CP, and starch; and flow and metabolism of N. Samples of ruminal content and pH were collected from each fermentor at 0, 1, 2, 4, 6, and 8 h after feeding to determine lactate, NH_3-N, and pH kinetics. All data were analyzed using the Mixed procedure of SAS. The combination of CoPec+CoAA had the greatest NDF degradability among the treatments. Regarding N metabolism, bacterial N and N efficiency were greater for CoAA compared with CoPec+CoAA, $CoCO_3$ and CoPec. There were no effects of treatment on the concentrations of any VFA. There were neither treatment effects nor interactions between treatment and time for pH, NH_3-N, and lactate concentrations. With regards to lactate pool, we did not observe treatment effects for L-lactate; however, we observed treatment effects for D-lactate and total lactate. There were no differences in vitamin B_{12} flow among treatments. In conclusion, different sources of Co affected ruminal fermentation, CoAA improved bacterial N and N efficiency (SEM = 0.07, $P < 0.05$ and SEM = 2.71, $P < 0.05$, respectively), which is promising. The CoPec+CoAA treatment had the greatest NDF degradability (SEM = 3.89, $P < 0.01$), which is an important parameter and could improve energy supply.

Key Words: cobalt carbonate, NDF degradability, nitrogen efficiency

2740 Effects of dietary *Aspergillus oryzae* prebiotics and isoacids on ruminal fermentation in a dual-flow continuous culture system. M. L. Johnson*[1], C. A. Hammond[1], G. K. Salas-Solis[1], M. U. Siregar[1], A. C. S. Vicente[1], E. Sarmikasoglou[1], V. Oliveira[1], A. P. Faciola[1], M. Socha[2], and C. Cassady[3], [1]*University of Florida, Gainesville, FL,* [2]*Zinpro Corporation LLC, Eden Prairie, MN,* [3]*Biozyme Incorporated LLC, St. Joseph, MO.*

The inclusion of isoacids in dairy cattle diets have the potential to improve ruminal fiber digestibility and protein utilization by stimulating ruminal microorganisms important for fiber degradation. *Aspergillus oryzae* prebiotics (AOP) are used within dairy diets to increase nutrient utilization by stimulating rumen fungal and bacterial populations, subsequently improving fiber digestion. Potential synergistic effects of the combination of isoacids and AOP are not well-defined. Therefore, the objective of this study was to determine the effects of including isoacids, AOP, and the combination of the 2 on ruminal fermentation using a dual-flow continuous culture system. The experiment was conducted in a 4 × 4 Latin square design with a 2 × 2 factorial arrangement, combining the 2 factors (1) isoacids and (2) AOP. Treatments were randomly assigned as (1) control (basal diet without the inclusion of isoacids nor AOP); (2) ISO (basal diet plus the inclusion of 0.24 g/d of isoacids); (3) AOP (basal diet plus the inclusion of 0.18g/d AOP); and (4) ISOAOP (basal diet plus the inclusion of both 0.24 g/d of isoacids and 0.018 g/d of AOP). The fermentors were provided diets containing 16% CP, 32% NDF, and 30% starch. Each experimental period was 20 d long where the first 17 d were used to adapt the fermentors to each treatment and the last 3 d were used to assess pH, ammonia, volatile fatty acids, nitrogen metabolism, and nutrient degradability. Experimental data were analyzed using the Mixed procedure of SAS. Preliminary data indicate that there was a significant effect of the ISO × AOP interaction within 24 h ammonia composite samples ($P = 0.03$) and there were no significant effects of treatments for pH and ammonia kinetics. Our results demonstrate that the inclusion of isoacids and AOP allowed for a similar pH and ammonia concentration among treatments. Further analyses will need to be completed to further understand the potential effects of isoacids and *Aspergillus oryzae* prebiotics on volatile fatty acid concentration, nitrogen metabolism, and microbial protein production.

Key Words: isovalerate, 2-methylbuyrate, fermentation

2741 The effects of dietary inclusion of a fat-protein matrix on intake and flow of long-chain fatty acids, amino acids, and nitrogen in a dual-flow continuous culture system. J. R. Vinyard[1], M. L. Johnson*[1], G. K. Salas Solis[1], A. C. Silva Vicente[1], M. U. Siregar[1], E. Sarmikasoglou[1], C. Hammond[1], S. W. Ma[1], R. R. Lobo[1], L. Girardin[2], R. Dreger[2], and A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*O&T Farms, Regina, SK, Canada.*

The inclusion of n-3 fatty acids (FA) in human diets has been demonstrated to be anticarcinogenic and prevent cardiovascular disease. This has increased interest in increasing these FA in dairy products by including them in the diets of cows. Thus, the objective of this study was to determine the effects of feeding increasing amounts of LinPRO-R, a rumen-protected matrix of fat and protein from flaxseed and peas (LIN; approximately 21% fat, 24% CP, and 5.4% n-3 FA; as % of DM), using a dual flow continuous culture system. The treatments were: 0% (0P), 2.5% (2.5P), 5% (5P), and 7.5% of DM (7.5P) inclusion of LIN in a TMR with LinPRO-R replacing portions of dried distiller's grains and Ca salts of long FA in the diet to maintain similar levels of CP and EE. Twice daily, 53 g of DM of each TMR (16% CP, 33% NDF, 28% starch, 4.2% EE; as % of DM) was fed to each fermentor. The treatments were arranged in a replicated 4 × 4 Latin square using 8 dual-flow continuous culture fermentors. Four 10-d experimental periods (7 d adaptation and 3 d sample collection) were done. Samples were collected from fermentor effluents at 3, 6, 9, and 24 h after morning feeding and composited to determine LCFA and AA concentrations and N balance. Data were analyzed using the Glimmix procedure of SAS with square, fermentor within square, period, and the fermentor by treatment interaction being random effects. The AA intake was similar across treatments; however, the intake of saturated FA (SFA) decreased and unsaturated FA and polyunsaturated FA intakes increased with increasing dietary LIN. There was no effect of treatment on AA or N flow, RDP and RUP fractions of the diet, or the efficiency of bacterial nitrogen and nitrogen use. Increasing dietary LIN decreased C16:0, 9c-C18:1, and SFA and increased C18:3n-3, C20:1n-9, C22:1n-9, and C24:0. Thus, the inclusion of LIN in the diet increased the outflow of polyunsaturated FA without altering the AA flow and N balance within the system, indicating that LIN inclusion might allow greater concentrations of n-3 FA to flow into the small intestine.

Key Words: flaxseed, fermentation, n-3

2742 Effects of monensin and cashew nut-shell extract on bacterial community composition in a dual-flow continuous culture system. E. Sarmikasoglou*[1,2], P. Sumadong[3], L. F. Roesch[2], S. Halima[2], C. Hikita[4], T. Watanabe[4], and A. P. Faciola[2], [1]*Michigan State University, East Lansing, MI,* [2]*University of Florida, Gainesville, FL,* [3]*Khon Kaen University, Khon Kaen, Thailand,* [4]*SDS Biotech K.K., Tokyo, Japan.*

The objective of this study was to evaluate the effects of including monensin and 2 doses of cashew nut-shell extract (CNSE) in a high producing dairy cow diet on ruminal bacterial communities. A dual-flow continuous culture system was used in a replicated 4 × 4 Latin Square design. A basal diet was formulated to meet the requirements of a cow producing 45 kg/d of milk (17% crude protein and 27% starch). There were 4 experimental treatments: the basal diet without any feed additive (CON), 2.5 μM monensin (MON), 100 ppm CNSE (CNSE100), and 200 ppm CNSE (CNSE200). Samples were collected from the fluid and solid phases at 3, 6, and 9 h after feeding; composites of all time points were made for each fermentor within their respective phases. Bacterial community composition was analyzed by sequencing the V4 region of the 16S rRNA gene using the Illumina MiSeq platform. Treatment responses for bacterial community structure were analyzed with the PERMANOVA test run with the R *Vegan* package. Treatment responses for correlations were analyzed with the CORR procedure of SAS. Orthogonal contrasts were used to test the effects of ADD (CON vs. MON, CNSE100, and CNSE200); MCN (MON vs. CNSE100 and CNSE200); and DOSE (CNSE100 vs. CNSE200). Significance was declared at $P \leq 0.05$. The abundance of *Bacteroidetes* in liquid phase increased ($P = 0.03$) in response to MON compared with CNSE treatments. In addition, the abundance of *Synergistetes* in solid phase increased ($P = 0.04$) in response to CNSE100 compared with CNSE200. The abundance of *Sharpea* ($P < 0.01$), *Mailhella* ($P = 0.05$), *Ruminococcus* ($P = 0.03$), *Eubacterium* ($P = 0.01$), and *Coprococcus* ($P < 0.01$) in liquid phase and the abundance of *Ruminococcus* ($P = 0.03$) and *Catonella* ($P = 0.02$) in solid phase decreased, whereas the abundance of *Syntrophococcus* ($P = 0.02$) increased in response to MON when compared with CNSE treatments. Moreover, *Sharpea* in liquid phase increased ($P = 0.01$) by CNSE100 compared with CNSE200, whereas the abundance of *Rarimicrobium* in the solid fraction decreased ($P = 0.04$) by CNSE200 compared with CNSE100. Overall, MON and CNSE affected a few ruminal bacteria genera.

Key Words: anacardic acid, cardol, ionophores

2743 Carnosic acid can modulate rumen fluid fermentation and microbial abundance: An *in vitro* approach. J. Halfen*, L. Buraschi, T. Fernandes, and J. S. Osorio, *School of Animal Science, Virginia Tech University, Blacksburg, VA.*

This study aimed to evaluate the effects of increased concentrations of carnosic acid (CA) on rumen fluid fermentation and microbial abundance in an in vitro model. About 500 mL of ruminal fluid was collected into a thermo container from a mid-lactation cannulated dairy cow immediately after the morning meal and transferred to the laboratory (~30 min). Rumen fluid was strained through 4 layers of cheesecloth and diluted (1:4, vol/vol) in Goering and Van Soest medium under CO_2 flux. The buffered ruminal solution (20 mL) was distributed into Hungate tubes containing ~200 mg of a commercial total mixed ration (TMR) as the control group (CON), or TMR plus CA at concentrations of 1.54 mg (CA1); 0.15 mg (CA2); 0.0154 mg (CA3); and 0.0015 mg (CA4). Carnosic acid (97% purity, TCI America) was diluted in ethanol, added to the Hungate tubes, and freezer-dried before adding the TMR. Treatment tubes were filled with CO_2, closed with a septum stopper, and then placed in a shaker incubator and agitated at 40 rpm for 0, 6, 12, and 24 h at 39°C. At the end of each incubation time, tubes were placed in ice to stop the fermentation. The pH, ammonia, VFA, and bacteria abundance were analyzed in all samples. Data were analyzed using the MIXED procedure of SAS. A greater ($P = 0.05$) propionic acid and a trend ($P = 0.07$) for lower butyric acid were observed in CA1 compared with the other groups. A positive linear increase in propionate was observed as CA was increased from the control to CA1 ($P < 0.01$), whereas a linear decrease was observed in butyrate ($P < 0.01$) and acetate ($P = 0.02$) as CA was increased from the control to CA1. A lower ($P < 0.05$) abundance of *Prevotella albensis* and *Prevotella ruminocola* was observed in CA1 compared with the others. A trend ($P = 0.06$) for a linear increase in *Megasphaera elsdenii* abundance was observed as CA increased from the control to CA1. These results indicate that the highest inclusion rate of carnosic acid (CA1) positively modulated ruminal fermentation and microbial abundance in vitro.

Key Words: carnosic acid, in vitro, ruminal bacteria

2744 Pure plant secondary metabolites for impacts on in vitro methane production. J. Stypinski*[1], D. J. Heuschele[2], and I. J. Salfer[1], [1]*University of Minnesota–Twin Cities, Saint Paul, MN,* [2]*Plant Science Research Unit, United States Department of Agriculture, Saint Paul, MN.*

Enteric fermentation from ruminants is a major source of anthropogenic methane emissions. Many feed additives, some of which additives are naturally occurring in plants, have shown promise in reducing these emissions. Plant secondary metabolites (PSM) are associated with antimicrobial activity, as they protect the plant from pathogens, which suggests potential antimethanogenic activity. Therefore, the objective of the current study was to screen 30 pure PSM produced by a variety of forages with confirmed antimicrobial activity for gas production (GP) and composition using in vitro techniques. We inoculated 1 g of a ground total mixed ration consisting of a 50:50 ratio of forage to concentrate with 100 mL of a 20:80 ratio of rumen fluid-buffer mixture. Pure PSM were included at a rate of 25 mg per g of feed. Each experimental run consisted of 2 rumen fluid-buffer blanks and 2 control jars without added PSM. Each compound was evaluated twice per run and included in 2 runs for a total of 4 observations per PSM. Rumen fluid was collected from 2 mid-lactation Holstein cows consuming the same basal ration. Jars were fitted with gas pressure analyzers to record cumulative GP for 48 h, when a 10 mL gas subsample was taken for compositional analysis using gas chromatography. Data were analyzed using a mixed model with fixed effects of treatment and random effects of experimental run. Treatments were compared against the control flask (CTL). Interestingly, adamantane increased ($P < 0.01$) GP by 117% compared with CTL. Thymol and monensin (included as an internal control) reduced ($P < 0.01$) GP by an average of 58.0%. Alpha-pinene increased ($P < 0.01$) methane concentration by 145%, whereas no PSM reduced methane concentration. Adamantane significantly increased ($P < 0.01$) methane production by 297%, whereas abscisic acid, quercetin, rutin, thymol, uridine, and vitexin reduced ($P < 0.01$) methane volume by an average of 60.7%. This suggests that feeding certain PSM may be an effective methane mitigation strategy.

Key Words: methane, secondary metabolites, rumen fermentation

2745 Metagenomic analysis of microbial communities, enzymes, and metabolic pathways related to methane metabolism in the rumen supplemented with red seaweed *Gracilaria chorda*. Y. Kim*[1], Y. Son[2], J. H. Song[2], and H. G. Lee[1], [1]*Department of Animal Science and Technology, Sanghuh College of Life Sciences, Konkuk University, Seoul, Republic of Korea,* [2]*HAESONG S&T Co., LTD., Gwangju, Republic of Korea.*

This study aimed to investigate the effects of red seaweed *Gracilaria chorda* on microbial compositions, genes, and metabolic pathways in the rumen through metagenomic analysis. Rumen fluids incubated with either substrate alone (control) or 5% of *G. chorda* on an organic matter basis (treatment) were used in a semi-closed in vitro system. Samples underwent 16S rRNA sequencing and subsequent bioinformatics analysis. Gas production remained unaffected, whereas CH_4 production ($P < 0.01$) and proportion ($P < 0.01$) decreased by 18.9% and 11.0%, respectively, compared with the control. No significant changes were observed in pH or total and individual volatile fatty acids, except for the decreased molar proportion of butyrate ($P < 0.01$). The treatment exhibited a significant decrease in the relative abundance of the genus *Methanobrevibacter* ($P = 0.04$) and a tendency for decreased CH_4 metabolism ($P = 0.06$). Notably, there were significant reductions or tendencies to decrease in the number of enzymes, including *cobU*/*cobT* ($P = 0.06$), *cobS*/*cobV* ($P = 0.06$), *cobP*/*cobU* ($P = 0.06$), *cbiB*/*cobD* ($P = 0.10$), *cobQ*/*cbiP* ($P = 0.05$), *cobH*/*cbiC* ($P = 0.02$), *cbiD* ($P = 0.01$), *cbiG* ($P = 0.02$), *cobM*/*cbiF* ($P = 0.07$), and *cobJ*/*cbiH* ($P = 0.02$), all involved in porphyrin, particularly cobalamin, metabolism ($P = 0.05$), with a strong positive correlation with CH_4 production (r = 0.92; $P < 0.01$). Furthermore, an increase in the relative abundance of propionate metabolism ($P = 0.02$) was observed in the treatment, along with an elevated relative abundance of genes *acyP* ($P = 0.03$) associated with the acetate synthesis pathway. In contrast, the relative abundances of the family *Lachnospiraceae* ($P = 0.02$), genus *Butyrivibrio* ($P = 0.02$), and genes *fadN* ($P = 0.02$) and *gcdH* ($P = 0.04$), associated with butyrate synthesis, were decreased in the treatment group. These findings suggest that anti-methanogenic secondary metabolites within *G. chorda* may impede cobalamin synthesis from cobalt, consequently reducing the activity of the cobalamin-dependent coenzyme M methyltransferase step of rumen methanogenesis.

Key Words: rumen methane, *Gracilaria chorda*, metagenomics

2746 Effect of different doses of an enzymatic complex with main activity of xylanase on gas production using an in vitro technique. C. Rivoir*[1], A. Daudet[1], A. Casal[1], M. A. Bruni[1], A. I. Trujillo[1], V. Nenov[2], and P. Chilibroste[1], [1]*Universidad de la Republica, Facultad de Agronomía, Paysandú, Uruguay,* [2]*Huvepharma NV, Antwerp, Belgium.*

The study aimed to determine the effect of different doses of an enzymatic complex with main activity of xylanase (HX, Hostazym® X, Huvepharma, Bulgaria) on in vitro gas production (GP) in a total mixed ration (TMR) compost barn dairy system. Four treatments (T) were compared according to the dose of the HX: 0 (T0), 5 (T5), 10 (T10) and 15 (T15) g/d consumed by fistulated dairy cows in mid lactation. A balanced 4 × 4 Latin square was used as experimental design. The inoculum came from fistulated cows fed a TMR ad-libitum and supplemented twice a day (after am and pm milking) with HX. The T were run with 10 replicate amber glass bottles in each run with the TMR as a substrate (0.5 g DM/bottle per run). A total of 84 mL of culture medium plus 20 mL of inoculum and the corresponding HX dose (liquid form) were sealed with rubber stopper and aluminum seals and incubated in a water bath at 39°C. The GP was recorded at 2, 4, 6, 8, 12, 16, 24, 26, 48, 72, and 96 h of incubation. The accumulated GP (mL/g OM) was fit using the model $Y = (B) \times (1 - EXP(-C \times (h - L)))$ proposed by Mertens and Loften (1980) employing SAS®. Terms in the model included square, period and cow. The inclusion of HX increased total GP (Table 1) where the higher dose (T15) produced 14.6% more gas than T0. A significant positive linear response was found on GP to increasing the amount of HX on the diet. The inclusion of HX increases GP thought with the same rate and lag time as the control T, indicating a greater degradation of the substrate, microbial and fermentative activity in the rumen, suggesting potential effects on efficiency in nutrient utilization.

Table 1 (Abstr. 2746). Kinetics parameters for accumulated gas production per gram of OM incubated[1]

Model parameter	T0	T5	T10	T15	T	Lin	Qua
B (mL)	258^{c}	287^{bc}	283^{bc}	296^{ab}	0.025	0.009	0.228
C (%/h)	0.039	0.037	0.037	0.037	0.715	0.388	0.636
L (h)	0.313	0.621	0.367	0.27	0.874	0.837	0.572

[a–c]Within a row, different superscript letters indicate significant differences between treatments.

[1]B = maximum possible gas production (mL); C = degradation rate; L = lag time. Contrast effect of the dose response Lin = lineal and Qua = quadratic response.

Key Words: xylanase, gas production, dose response

2747 Assessment of botanicals as methane and ammonia inhibitors in *in vitro* ruminal fermentation. F. Ferrero*[1], B. Tugnoli[2], R. Majer[2], F. Ghiaccio[1], A. Federiconi[1], A. Palmonari[1], A. Formigoni[1], A. Piva[1,2], and E. Grilli[1,3], [1]*DIMEVET, University of Bologna, Bologna, Italy,* [2]*Vetagro S.p.A., Reggio Emilia, Italy,* [3]*Vetagro Inc., Chicago, IL.*

The use of botanicals is a promising strategy to reduce methane and ammonia coming from ruminal fermentation. The aim of this study was to assess the effects of different botanical additives on ruminal total gas, methane, and ammonia production in in vitro Gas Endeavor® automated system. In a first study, 6 distinct botanicals, including garlic essential oil (GEO), thymol (THY), saponin mix (SAP), anethol (ANT) onion extract (OE), thyme essential oil (TEO) and cinnamaldehyde (CIN), were tested in triplicate at a concentration of 100 ppm. In a second study, GEO, THY, and ANT were tested in 8 replicates at 250 ppm. A control group (untreated) was run concurrently in both studies. Rumen fluid sampled from 3 nonlactating Holstein cows was used as inoculum. Fermentations were conducted over a 24-h period, measurements of total gas and methane production were taken at h 24, and ammonia analysis was performed using a Urea/BUN Color kit. Data were analyzed using a 2-way ANOVA. No significant differences were observed at 100 ppm. However, GEO, THY and ANT additives tended to reduce methane production ($P < 0.15$). At 250 ppm, all 3 botanicals reduced total gas (−66.63%, −63.88%, and −39.22%) and methane production (−50.25%, −32.03%, and −15.42%), respectively for ANT, THY, and GEO, compared with control ($P < 0.05$). The GEO group also showed a reduction in the methane to total gas ratio (−29.76%) and in ammonia production (−25.35%) compared with control ($P < 0.05$). Although the 100-ppm concentration was insufficient to observe effects of these botanicals on fermentation, the 250 ppm, permitted to detect significant reductions for THY, ANT, and GEO treatments. Particularly, GEO demonstrated a more targeted action toward methanogens and ammonia producers, and therefore, it is subjected to further investigation.

Key Words: methane, ammonia, botanical

2748 Effect of plant extracted hydrolysable tannin (PEHT) on accumulative gas production kinetics and methane emission of the prairie blend pelleted feed products in dairy cows. T. Hidayat*, M. E. Rodriguez Espinosa, and P. Yu, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.*

The objectives of this study were to study dosage effect of plant-extracted hydrolyzable tannin (PEHT) as a phytochemical feed additive on in vitro accumulative gas production kinetics and methane (CH_4) emission of blend pelleted feed products at 0, 2, 4, 12, and 24 h incubation. We added 4 levels of PEHT (0, 2, 4, 6%) to canola meal and pea in 2 different ratios (CMP1: 50:50; CMP2: 70:30) to make prairie blend pellet feed products. The accumulative gas production kinetics and methane emission were determined using in vitro systems. Ruminal fluid was obtained from rumen-fistulated dairy cows. The experimental design was a RCBD with the dosage level as a fixed effect and the in vitro run with fistulated animals as a random effect. The data were analyzed using the Mixed procedure of SAS. Polynomial contrasts were used to determine linear and quadratic relationships. The 2 blend products were also compared. The results showed that with increasing PEHT level, the asymptotic production (a) was linearly decreased ($P < 0.05$) from 110.5 (control) to 89.7 mL/g DM (6%). The average production at half of asymptotic production (AP) was also linearly decreased ($P < 0.05$) from 11.9 (control) to 8.3 mL (6%). However, the gas production fractional rate (c, %/h) was not affected by PEHT. In comparison between the 2 blend pellet products, CMP1 had higher asymptotic production (113.3 vs. 77.9 mL/g DM, $P = 0.003$), lower lag time (1.01 vs. 3.19 h, $P = 0.006$) and lower AP (10.5 vs. 8.5 mL, $P = 0.094$). At short incubation times (2 h, 4 h, 12 h), the CH_4 production was linearly decreased ($P < 0.05$) from 2.77 to 1.88 ($P = 0.073$), 4.39 to 3.00 ($P = 0.018$) and 8.31 to 6.95 mL/g ($P = 0.012$) with increasing PEHT dosage. However, after longer incubation (24h), the PEHT effect on CH_4 disappeared. In conclusion, adding PEHT to prairie blend pellet feed products significantly affected accumulative gas production kinetics and decreased methane emission at short incubation times.

Key Words: plant extracted hydrolysable tannin (PEHT), prairie blend pelleted feed product, accumulative gas production kinetics and methane emission

2749 Hydrolysed yeast to influence *in vitro* rumen fermentation. H. Schulze*[1] and T. Rinttilä[2], [1]*Livalta/AB Agri, Peterborough, United Kingdom,* [2]*Alimetrics Research Ltd., Espoo, Finland.*

This study evaluated the impact of hydrolyzed, dried yeast (HY; Livalta™Cell HY40, Peterborough, United Kingdom) on rumen in vitro fermentation parameters in low and high energy fermentation substrates. We applied a 2 × 5 experimental design, with 2 substrates consisting of a blend of corn silage and concentrate (75:25 for low energy; 50:50 for high energy) and 4 different dosages of HY (0 [negative control; NC], 25, 50, 100 mg/g) as well as a positive control (PC; 0.15 mg/g monensin sodium). The *Saccharomyces cerevisiae* yeast contains 40% yeast cell wall and >36% CP. Rumen fluid of 2 fistulated cows fed a high energy ration was mixed and used as inoculum. The in vitro experiment used 6 vessels (40 mL) per treatment, except for NC, which had 12 replicates. Total gas production kinetics were measured at 0, 3, 6, 9, and 12 h, whereas SCFA and pH were measured at 0, 6, and 12 h. Total eubacterial density and digestibility of insoluble dry matter (DM) were measured at 0 and 12 h. Cumulative methane production was assessed after 12 h of fermentation. Statistical analysis was performed using Student's *t*-tests, comparing each treatment with the NC within each fermentation substrate. The results showed that there were no significant effects on total eubacterial counts or DM digestibility. In both rations, the PC reduced gas production and SCFA production, particularly acetic acid, compared with the NC. Overall, the addition of HY significantly stimulated bacterial fermentation as evidenced by increased gas and SCFA production. HY had no impact on propionate, but decreased acetate and increased butyrate proportion compared with the NC. Although HY enhanced overall fermentation, it also reduced methane production, especially at higher doses similar to the PC. Additionally, the inclusion of HY appeared to enhance protein fermentation, represented by an increase in branched chain fatty acids. It can be concluded that HY can modify rumen microbial activity but further research on its prolonged impact on microbial composition and activity is required when directly applied to the cow.

Key Words: hydrolysed yeast, in vitro fermentation, microbial activity

2750 Effect of exogenous fibrolytic enzymes (Hostazym X) on *in vitro* rumen fermentation of dairy cows. A. A. Molho-Ortiz*[1], C. C. Márquez-Mota[1], A. Romero-Pérez[1], E. Vargas-Bello-Pérez[2,3], and L. Corona-Gochi[1], [1]*Universidad Nacional Autónoma de México, Facultad de Medicina Veterinaria y Zootecnia, Departamento de Nutrición Animal y Bioquímica, Ciudad Universitaria, CDMX, México,* [2]*Universidad Autónoma de Chihuahua, Facultad de Zootecnia y Ecología, Chihuahua, Chihuahua, México,* [3]*University of Reading, School of Agriculture, Policy and Development, Reading, Berkshire, England.*

The objective of this study was to evaluate the effects of Hostazym X (HX), a commercial preparation of fibrolytic enzymes in the ruminal fermentation of dairy cattle, using a semi-automated in vitro gas production technique. Hostazym X was evaluated in 4 different levels plus a negative control (0, 5, 10, 15, 20 EPU/g) in a completely random experimental design with orthogonal polynomials array. The experimental diet consisted of 51.02% commercial concentrate, 20% corn silage, 20% oat hay, 3.91% alfalfa pellets, and 5.07% soybean meal. Gas production was measured after 72 h of incubation. All samples were collected after 72 h. The following variables were evaluated: maximum volume of gas (maxV), fermentation rate (S), latency phase (L), hydrogen potential (pH), ammonia ($N\text{-}NH_3$), total gas production (TGP), corrected by dry matter (TGPDM) and degraded DM (TGPDMD), DM degradation (DMD), in vitro DM digestibility (IVDMD), in vitro NDF digestibility (IVNDFD), volatile fatty acid production (total VFA; acetic, propionic, isobutyric, butyric, isovaleric, valeric, acetate/propionate ratio), and methane estimation. Data analysis was performed in R Studio. In general, the application of HX did not affect the maxV produced, S, L, pH, $N\text{-}NH_3$, and variables related to gas production. However, it increased DM digestibility (5.47%, $P < 0.05$) and tended to increase the NDF digestibility (2.47%, $P = 0.06$). All levels of HX reduced the total production of VFA (10.16 m*M*, $P < 0.05$). Moreover, different levels of HX increased ($P < 0.05$) the percentage of acetate (1.22%), propionate (1.55%), and butyrate (0.76%), and decreased ($P < 0.05$) the percentage of isobutyrate (0.16%), valerate (1.99%), and the ratio of acetic to propionic acid (0.15 units). As for the methane production estimation, the use of different levels of HX reduced (10.6%, $P < 0.05$) methane compared with the control. The present study indicates that applying HX to dairy cow diets has the potential of improving ruminal degradation, modifying the proportion of VFA, making fermentation more efficient, reducing the acetate/propionate ratio, and reducing methane production from rumen fermentation.

Key Words: fiber, digestibility, methane

2751 Effects of isopropyl ester of hydroxy analog of methionine (HMBi) on *in vitro* rumen fermentation, enzymatic activity and nutrient digestibility.

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The study aimed to explore in vitro ruminal effects of isopropyl ester of hydroxy analog of methionine (HMBi) to enhance understanding of rumen degradable portion of it. In Experiment 1, HMBi (KMFD, KESSENT® MF Dry, Kemin Animal Nutrition and Health, Belgium) was added to a basal feed (10% as fed) and incubated for 24 h in Hohenheimer Futterwerttest (HFT) and Daisy incubator (3 runs × 6 replicates). Basal feed was incubated as a control (CON). In Experiment 2, an in vitro 24-h rumen incubation was performed followed by a radial enzyme diffusion (RED) test of KMFD to observe the enzymatic activities (EA) of amylase (Amy), cellulase (Cel), and xylanase (Xyl) over time (n = 60, expressed as area of hydrolysis). A mixed model was used with REML (JMP 16.1, SAS) with fixed effects of treatment and random effects of each run of incubators. In RED, time was assigned as a fixed effect and each Petri was considered a random effect. After 24 h of incubation in HFT, KMFD did not affect pH, nor the molar level of acetic, isobutyric, isovaleric, valeric, caproic, and lactic acids, as well as total gas and methane production (mL/g DM). Molar concentrations of ammonia-N (20.3 vs. 17.4 ± 1.2), butyric acid (8.2 vs. 7.2 ± 1.0), and Ace:Pro ratio (5.2 vs. 4.8 ± 0.1) decreased with KMFD supplementation compared with CON, whereas propionic acid increased ($P < 0.05$). In addition, KMFD improved in vitro (%) total true digestibility and nutrient digestibility compared with CON ($P < 0.05$). Percentages of IVTD, IVTDDM, IVTDNDF, and IVTDOM were 71.6 versus 74.2 ± 1.5%; 68.7 versus 71.6 ± 1.6%; 19.7 versus 27.1 ± 4.2%, and 59.6 versus 63.4 ± 2.3% for KMFD versus CON, respectively. Compared with time-zero, Xyl EA linearly decreased (1.9 vs. 1.4 ± 0.1), whereas Cel EA increased (3.1 vs. 4.1 ± 0.1) after 2 h ($P < 0.05$) over time. The initial Amy EA was maintained by 12 h ($P > 0.05$) and increased at 24 h (3.3 vs. 4.0 ± 0.2, $P < 0.05$). In conclusion, the observed reduction in the rumen-free ammonia-N and alterations in some rumen fermentation patterns and EA with KMFD supplementation suggest an effect of a degradable portion of its HMBi concentration on nutrient utilization under in vitro rumen conditions.

Key Words: hydroxy analog of methionine (HMBi), rumen, enzyme

Table 1 (Abstr. 2752). Effect of XPC and NutriTek on ruminal VFA production (m*M*) using alfalfa haylage (AH), sorghum Sudan haylage (SSH) and corn silage (CS) as substrates

Forage	Treatment	Acetate	Propionate	Butyrate	Total VFA
AH	Control	33.82^{b}	15.63^{c}	3.25^{c}	53.58^{b}
	XPC	36.31^{ab}	16.83^{b}***	3.43^{b}*	57.57^{a}*
	NutriTek	36.96^{a}*	18.03^{a}****	3.63^{a}****	59.64^{a}***
	SEM	0.82	0.18	0.04	1.01
SSH	Control	24.42^{b}	16.66^{c}	3.78^{a}	45.21^{c}
	XPC	27.65^{a}**	18.50^{b}****	4.03^{a}	50.56^{b}****
	NutriTek	29.22^{a}***	19.77^{a}****	4.05^{a}	53.54^{a}****
	SEM	0.70	0.22	0.29	0.75
CS	Control	27.11^{b}	23.83^{b}	3.58^{a}	54.78^{b}
	XPC	30.12^{ab}	25.19^{ab}	4.09^{a}	59.81^{a}*
	NutriTek	30.48^{a}*	26.64^{a}**	4.08^{a}	61.56^{a}**
	SEM	0.98	0.55	0.81	1.32

$^{a\text{-}c}$Means within a column within a common forage source without a common superscript letter differ ($P \leq 0.05$).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

2752 Postbiotics of *Saccharomyces cerevisiae* fermentation product increase volatile fatty acid production in an in vitro rumen model on forages from Latin America.

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The objective of this study was to evaluate the effect of the *Saccharomyces cerevisiae* fermentation products Diamond V Original XPC (XPC) and NutriTek® (NutriTek) on in vitro volatile fatty acid (VFA) production of various forage sample types (alfalfa haylage [AH], sorghum Sudan haylage [SSH], and corn silage [CS]) obtained from Latin America. To each bottle, treatments were included based on their respective feeding rates, XPC (14 g/hd per d) and NutriTek (19 g/hd per d) along with 0.3 g of forage. Each bottle was inoculated with 40 mL of buffered rumen fluid from cows on a diet consisting of 65% forage and 35% concentrate mix and was incubated for 24 h. We performed 2 experiments to obtain 10 replicates per treatment and forage. Each forage was analyzed separately using the GLM model of JMP. Means were compared using Tukey analysis, and significance was defined as $P \leq 0.05$, as compared with the control. Individual and total VFA concentration (m*M*) are reported in Table 1. Results demonstrate that use of XPC and NutriTek with Latin American forages increase ruminal VFA production over control with greater responses with NutriTek. In conclusion, the use of *Saccharomyces cerevisiae* fermentation products enhances VFA production across all Latin American forages tested.

Key Words: XPC, NutriTek, VFA

Ruminant Nutrition 5: Protein and Amino Acids

2753 Effects of feeding controlled-energy and high-energy diets with rumen-protected lysine and methionine prepartum on metabolic markers of Holstein cows. E. O'Meara*[1], D. del Olmo[2], J. Aguado[2], J. Drackley[1], and F. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*Kemin Industries, Inc., Des Moines, IA.*

This study aimed to determine the effects of rumen-protected lysine (RPL) and methionine (RPM) fed in the same AA/metabolizable energy (ME) ratio in prepartum diets with different net energy of lactation (NE_L) concentrations on the metabolic markers of Holstein cows. A total of 62 multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, and body condition score during the far-off dry period, were assigned to 1 of 3 dietary treatments. Prepartum (−21 d to expected calving), animals were fed a controlled-energy diet (1.45 NE_L, Mcal/kg of DM) with RPL and RPM (both from Kemin Industries Inc., Des Moines, IA; CEAA; 0.15% RPL and 0.09% RPM of DMI); an controlled-energy diet without RPL and RPM (control; CENAA); or a high-energy diet (1.71 NE_L, Mcal/kg of DM) with RPL and RPM (HEAA; RPL 0.22% and RPM 0.12% of DMI). Postpartum, cows received the same lactation TMR (1.73 NE_L, Mcal/kg of DM) without RPL and RPM (CENAA, n = 19) or with RPL and RPM (CEAA, n = 21; and HEAA, n = 21; 0.38% RPL and 0.15% RPM of DMI) until 70 d relative to calving (DRC). Blood samples were taken on −7 ± 4.7, 3 ± 1.1, 28 ± 1.6, and 70 ± 2.1 DRC. Statistical analyses were performed using the MIXED procedure of SAS. Two treatment contrasts CENAA vs. CEAA and CEAA vs. HEAA were compared. Cows in CEAA had greater ($P < 0.04$) serum concentrations of glutamate dehydrogenase (16.7 U/L), aspartate aminotransferase (57.1 U/L), and gamma-glutamyl transferase (20.5 U/L) than cows in CENAA (11.6 U/L, 48.8 U/L, 17.3 U/L, respectively) prepartum. Serum urea nitrogen was greater ($P = 0.02$) for cows in CEAA (10.5 g/dL) compared with cows in CENAA (9.58 g/dL) postpartum. Cows in HEAA had greater ($P < 0.05$) total protein (6.97 g/dL) and globulin (3.32 g/dL) concentrations than cows in CEAA (6.71 g/dL and 3.11 g/dL, respectively) postpartum. In conclusion, cows in CEAA seemed to have increased protein metabolism prepartum and cows in HEAA had increased inflammatory markers postpartum.

Key Words: methionine, lysine, liver

2754 Evaluation of the NASEM milk protein prediction model and amino acid efficiency of utilization in Brazilian dairy farms. J. H. Carneiro*[1,3], J. P. A. Rezende[3], R. Almeida[2], and M. A. C. Danes[3], [1]*Dairy Inside Consult, Castro, Paraná, Brazil,* [2]*Federal University of Paraná, Curitiba, Paraná, Brazil,* [3]*University of Lavras, Lavras, Minas Gerais, Brazil.*

The NASEM (2021) milk protein yield (MPY) prediction equation includes independent and additive effects of digestible energy intake and absorbed grams of essential amino acids (EAA). Our objective was to evaluate the NASEM MPY prediction and EAA use efficiency in pens of Holstein cows in commercial farms. Data collected in 12 herds in Southern Brazil were used. All cows were housed in freestall or compost barn and fed TMR. Each farm was visited at least once a month in 2022. For each of the 89 pens of lactating cows (8,345 cows, 50–325 per pen), data on milk production, DM intake, DIM, parity, milk composition, body weight, and diet composition were compiled. All data from each pen were entered in NASEM software to predict MPY and efficiency of utilization for each EAA. Pens were divided by observed MPY levels in 3 clusters (low, medium and high production). Within each cluster, NASEM MPY prediction was compared with the observed MPY using the root mean square error (RMSE) and the concordance correlation coefficient (CCC). Additionally, the MIXED procedure of SAS with fixed effect of cluster and random effect of pen was used to evaluate the effect of clusters on number of protein sources used in the diets and EAA efficiency. Model evaluation is presented in Table 1. The NASEM MPY equation performed well in the low and medium clusters, but not so well in the high group. The number of protein sources in the diet was greater ($P < 0.05$) in the high and medium clusters than in the low production cluster. For all 3 EAA, use efficiency was greater for the high-producing cluster and lower for the low-producing cluster ($P < 0.01$). Both the greater pull effect in the higher production groups and the better combination of AA from more protein sources could explain better AA efficiencies.

Key Words: amino acid efficiency, model validation, protein source

Table 1 (Abstr. 2754). Evaluation of NASEM MPY equation by production cluster

Item	Low	Medium	High
No. of pens	17	55	17
Observed MPY, g/d	970	1217	1574
Predicted MPY, g/d	933	1125	1340
RMSE, g/d	64.7	113.6	240.4
CCC	0.73	0.44	0.31
Predicted AA efficiency			
His	0.73^c	0.80^b	0.87^a
Met	0.69^c	0.74^b	0.81^a
Lys	0.64^c	0.72^b	0.80^a
No. of protein sources	2.9^b	4.0^a	4.4^a

2755 Effects of 2-hydroxy-4-(methylthio)butanoate supplementation on blood metabolites of transition dairy cows. J. C. S. Lourenço*[1], R. Locatelli-Dittrich[1], I. A. M. A. Teixeira[2], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*University of Idaho, Twin Falls, ID.*

The objectives of this study were to evaluate the effects of methionine analog (HMTBa) supplementation on blood metabolites of transition dairy cows in a commercial dairy herd located in Paraná State, Southern Brazil. A total of 150 Holstein animals (62 nulliparous, 47 primiparous, and 41 multiparous) were assigned into 4 treatments: no supplementation (No-HMTBa), 35 g/d of methionine analog supplementation only in the prepartum (Pre-HMTBa) or only in the postpartum (Post-HMTBa), and 35 g of HMTBa per cow/d both prepartum and postpartum (P&P-HMTBa), in a randomized block design. Treatments were provided individually and top-dressed once a day mixed with 65 g per cow/d of corn meal, during 25 ± 6.2 d prepartum and 30 ± 8.5 d postpartum. An equivalent amount of corn meal was provided to the control animals. Serum blood samples were obtained on −14, −7, +1, +5, +12, and +21 d relative to calving. Blood samples were collected at 7:00 a.m. in prepartum and at 12:00 a.m. in postpartum, whereas the diets were provided at 8:00 a.m. in prepartum, and at 5:00 a.m. in postpartum. Data were analyzed as a mixed model, considering the fixed effects of prepartum and postpartum HMTBa supplementation, parity, time, and their interactions, and the random effects of block and cow within treatment. There was an effect for BHB concentrations, where the Pre-HTMBa group showed lower ($P = 0.02$) values compared with the No-HMTBa group

Table 1 (Abstr. 2756). Effects of feeding heat-treated soybean meal at different inclusion rates on intake, milk production and components, CH_4 production and respiratory quotient of lactating Jersey cows

	Treatment[1,2]					
Item	HTSBM	BM + SBM	HTSBM + CM	CM+BM	SEM	*P*-value
DMI, kg/d	19.5	19.5	19.7	20.3	0.60	0.38
Milk yield, kg/d	31.0	31.4	31.2	32.0	0.90	0.60
ECM, kg/d	36.9	37.9	38.0	38.6	1.17	0.36
Fat, kg/d	1.46	1.51	1.54	1.54	0.05	0.36
Protein, kg/d	1.02	1.05	1.03	1.06	0.03	0.18
CH_4 production, L/d	451	441	392	424	23	0.25

[1] HTSBM = heat-treated soybean meal diet; BM + SBM = blood meal and soybean meal diet; HTSBM + CM = heat-treated soybean meal diet and canola meal diet; CM + BM = canola meal and blood meal diet.

[2] Least squares means; largest standard error of treatment mean is shown.

(0.66 vs. 0.69 ± 0.01 mmol/L, respectively). There was a trend ($P = 0.06$) toward an increase in blood urea nitrogen concentration in prepartum for Pre-HMTBa group compared with the No-HMTBa group (28.1 vs. 26.8 ± 0.25 mg/dL, respectively). There were no effects for GGT, AST, triglycerides, cholesterol, glucose, albumin, bilirubin, and total calcium. The results of this study suggest that HMTBa supplementation has a modest impact on blood metabolites in transition dairy cows and only during the prepartum period.

Key Words: amino acid, beta-hydroxybutyrate, methionine analog

2756 The effect of replacing common protein sources with heat-treated soybean meal on milk production in lactating Jersey cows. K. A. Harpestad*, K. K. Buse, A. L. Carroll, and P. J. Kononoff, *University of Nebraska–Lincoln, Lincoln, NE.*

The cow's requirements for protein and amino acids can be met by feeding different feedstuffs. The objective of this study was to evaluate the impact of feeding heat-treated soybean meal (HTSBM; Aminoplus, AgProcessing Inc., Omaha, NE) in partial or full replacement of 3 common high protein feedstuffs namely soybean meal (SBM), canola meal (CM), and blood meal (BM) on feed intake and milk production. The 4 isonitrogenous diet treatments contained the same proportions of forage, but different concentrate mixes were made (% DM basis): HTSBM (10.3% HTSBM, 3.78% SBM, no CM, and no BM); BM + SBM (no HTSBM, 9.60% SBM, no CM, and 2.22% BM) HTSBM + CM (5.13% HTSBM, 5.00% SBM, 5.13% CM, and no BM); and CM + BM (no HTSBM, 3.85% SBM, 8.05% CM, and 2.2% BM). These formulations were fed to 12 multiparous Jersey cows (104 ± 4 DIM) in a 4 × 4 Latin square with 4 periods of 28 d. Data were analyzed with the Glimmix procedure of SAS (9.4) with fixed effects of treatment, square, and period nested in square and random effects of cow nested within square. No difference was observed in DMI, milk yield, or yield of fat or protein. As expected, methane did not differ across treatments (427 ± 23 L/d; Table 1). These results indicate that formulations containing HTSBM can be used to partially replace SBM, completely replace BM and CM, and to maintain milk production and yield of fat and protein.

Key Words: heat-treated soybean meal, blood meal, canola meal

2757 A meta-analysis to identify factors affecting milk component responses to metabolizable methionine fortification with Smartamine M or MetaSmart evaluated based on NRC 2001. L. Bahloul[2] and N. L. Whitehouse*[1], [1]*University of New Hampshire, Durham, NH,* [2]*Adisseo, Commentry, France.*

The objective is to establish response equations on milk protein and fat contents (MPC and MFC) and yields (MPY and MFY) to rumen protected methionine (RPM) supplementation and to determine factors affecting the responses. A meta-analysis was applied on peer-reviewed data (82 treatments, 26 publications from 2003 to 2023) with a variance-covariance GLM model (Minitab V18). Lactating dairy cows consumed 22.7 ± 3.91 kg DM, 160.2 ± 15.8 g CP/kg DM, 1.59 ± 0.07 Mcal NE_L/kg DM, and 2.14 ± 0.40 mMet%MP. Robust within-study models were obtained to predict MPC ($R^2_{adj} = 0.89$, root mean square error [RMSE] = 0.05%), MPY ($R^2_{adj} = 0.94$, RMSE = 45.0g/d), MFC ($R^2_{adj} = 0.89$, RMSE = 0.14%) and MFY ($R^2_{adj} = 0.97$, RMSE = 59.0g/d) to RPM based on metabolizable methionine (mMet) expressed as the percentage of metabolizable (%m) protein (%mMet). The MPC and MPY models suggested an increase of 0.6% mMet resulted in an increase of 0.14% and 54g/d, respectively. The within-study slopes of MPC and MPY were positively affected by the metabolizable Lys (mLys)/mMet ratio, %mHis ($P < 0.01$), NE_L, EE, %mArg ($P < 0.05$). Within-study slopes of MPC were positively affected by bacterial MP ($P < 0.05$). For the same %mMet content, MFC increased with RUP, %mArg, %mIle, %mThr and decreased with %mLeu ($P < 0.05$) where MFY increased with DMI, EE, NE_L, Bacterial MP, MP, RDP, RUP, %mHis, and mLys/mMet ratio and decreased with %mLeu ($P < 0.05$). The MFC and MFY indicated an increase of 0.14% and 66g/d, respectively, by increasing 0.6% mMet. Within-study slopes of MFY were positively affected by EE, %mHis, %mVal, and mLys/mMet ratio ($P < 0.05$) and negatively affected by %mIle ($P < 0.05$). For the same %mMet, MFY increased in early lactation, DMI, EE, NE_L, bacterial MP, %mHis, and mLys/mMet ratio ($P < 0.05$), and decreased with %mLeu ($P < 0.05$). Lastly, MFC decreased with %mLeu ($P < 0.05$). In summary, it is possible to predict the responses of lactating dairy cows as function of %mMet and determine factors that could affect these responses. These results provide deeper insight into biological processes and may help improve dietary formulation.

Key Words: meta-analysis, methionine, amino acid

Small Ruminants 2

2758 Assessment of fatty acid profile of rumen content and biohydrogenation patterns of lambs fed diets with tannin-enriched diets. A. B. M. Lima[1], H. N. Parente[1], H. S. Cavalcante[1], P. G. B. Gomes[3], J. S. Oliveira[3], D. O. Maia[1], G. O. Melo[1], F. N. S. Santos[1], G. A. A. Castelo Branco[1], M. R. Santos[1], and M. O. M. Parente*[1,2], *[1]Federal University of Maranhão, Chapadinha, Maranhão, Brazil, [2]Federal University of Piauí, Teresina, Piauí, Brazil, [3]Federal University of Paraíba, Areia, Paraíba, Brazil.*

The tannin compounds (TC) have a modulating effect on ruminal fermentation because of the antibacterial effects, and they can exert an inhibitory effect on ruminal biohydrogenation (RBH), increasing the accumulation of RBH intermediate fatty acids (IFA) and unsaturated fatty acids (UFA) in the rumen and in final products. The faveira (*Parkia platycephala* Benth) is a legume tree that occurs naturally in the Amazon region of Brazil, and its pods (FP), rich in TC, are used in ruminants' diet. The objective of this study was to evaluate the effects of increasing levels of FP on fatty acids (FA) profile of rumen content and RBH estimate. A total of 28 lambs (initial body weight 20 ± 0.4 kg) were distributed in a completely randomized design in individual pens for 60 d. The experimental diets were: a control diet containing 700 and 300 g/kg of concentrate and Tifton hay 85, respectively (basal diet, 0FP); the basal diet with 100g/kg FP added (100FP); the basal diet with 200g/kg FP added (200FP); and the basal diet with 300 g/kg FP added (300FP). At the end of the experiment, the lambs were slaughtered, and samples of the ruminal digest content were collected from each lamb, frozen, freeze-dried, milled, and stored at −20°C until analysis by gas chromatography. The RBH estimates (g/100g) for 18:1 *c*-9, 18:2n-6, and 18:3n-3 were obtained using the diminishing abundance of these FA, proportional to the sum of C18-carbon FA, between diet and rumen digesta, assuming that no losses of C18 FA occur in the gastric compartments. When significant treatment effects were found ($P < 0.05$), polynomial contrasts were used to determine the linearity and quadratic effects of different levels of FP inclusion. The FP addition promoted a quadratic effect ($P = 0.011$) on 18:0, and linearly increased ($P < 0.01$) the 18:1 *t*9, 18:1 *t*11 and 18:2n-6 in rumen content. However, FP linearly decreased the RBH of C18:1 *c*9 and C18:2n-6, without change in the RBH of C18:3n-3 and the RBH completeness (86 ± 0.8 and 75 ± 3.6 g/100g respectively). The FP addition reduces RBH of 18:1 *c*9 and 18:2n-6, increasing IFA in rumen content.

Key Words: antibacterial effect, *Parkia platycephala* Benth, rumen bacteria

2759 Evaluation of slow-release urea encapsulated in calcium alginate microparticles in sheep diets. L. R. Bezerra*[1,2], E. C. Silva Filho[2,3], J. M. Pereira Filho[1,2], M. A. Fonseca[4], and J. P. F. Oliveira[5], *[1]Federal University of Campina Grande, Postgraduate Program in Animal Science and Health, Patos, Paraiba, Brazil, [2]CNPq-Brazil Researcher, Brasília, Distrito Federal, Brazil, [3]Federal University of Piaui, Teresina, Piaui, Brazil, [4]University of Nevada, Reno, NV, [5]Federal University of Sergipe, Nossa Senhora da Gloria, Sergipe, Brazil.*

In ruminant nutrition, regulating ammonia release in the rumen is crucial for improving the conversion of dietary nitrogen into microbial protein, thus enhancing productive efficiency. We obtained and characterized slow-release urea (SRU) using calcium alginate as the encapsulating agent and tested in sheep diets from the intake, digestibility, nitrogen (N) balance, and blood parameters. Four SRU were developed from the external ionic gelation technique: SRU was encapsulated into a calcium alginate matrix and oven-dried, enriched or not with sulfate, and SRU freeze-dried/lyophilized with or without sulfate. The addition of SRU oven-dried without sulfate presented a greater yield and encapsulation efficiency than the other formulations and it was tested. A total of 24 noncastrated male Santa Ines sheep (average BW of 22 ± 3.0 kg) were distributed in a completely randomized design with 4 treatments and 6 replications: a control diet with free urea (U) and 3 proportions of SRU (1%, 1.5%, and 2% of total DM). Sheep were previously adapted, and the SRU or U were mixed in a total ratio concentrate containing BRS-Capiaçu (*Pennisetum purpureum*) hay as roughage (63%) and a concentrated mixture (37%) containing ground corn, soybean meal, and mineral mixture. The data means were compared by the Tukey test, considering differences when $P \leq 0.05$. The addition of SRU$_{1\%}$ promoted a higher intake of DM, CP, and NFC compared with U and a higher intake of NDF compared with U and SRU$_{2\%}$ ($P \leq 0.05$). The TDN intake was higher for sheep fed SRU$_{1\%}$ than SRU$_{1.5\%}$ but did not differ from U and SRU$_{2\%}$. The SRU$_{2\%}$ addition promoted higher NDF digestibility than U ($P \leq 0.05$). Sheep receiving SRU$_{1\%}$ presented higher N-ingested, N-urinary, and N-retention than the U diet, without effect compared with other SRU levels. Sheep receiving U had higher blood urea nitrogen (BUN) compared with SRU ($P \leq 0.05$), without a difference between SRU levels. The SRU using a calcium alginate matrix encapsulating is recommended for sheep diets at 1.0% because it improves intake, digestibility, and N-balance, and reduces BUN and urea intoxication risk. *We thank CNPq (INCT), CAPES, and FAPESq-PB (Brazil).*

Key Words: ammonia, extrusion, rumen

2760 Assessing feeding behavior and physiologic parameters of sheep fed faveira pods as replacement of hay. G. K. M. Vilela[1], H. N. Parente[2], F. S. Campos[2], V. C. Sousa[2], D. M. Pereira[2], G. O. Melo[2], W. S. Almeida[2], G. T. A. Silveira[2], E. F. A. Sousa[2], and M. O. M. Parente*[1], *[1]Federal University of Piauí, Teresina,Piauí, Brazil, [2]Federal University of Maranhão, Chapadinha, Maranhão, Brazill.*

In arid regions, the search for feed strategies is constant. *Parkia platycephala* is a legume tree found in the Amazon region, and its fruits, faveira pods (FP), have matured and fallen; they contain approximately 10% CP (DM basis). This research aimed to test the replacement of FP by roughage on ingestive behavior and physiologic parameters. A total of 14 sheep, mixed breed (average initial weight of 21 ± 2.4 kg) were assigned to a completely randomized design with 2 treatments and 7 replications. The treatments were defined as the Control diet (CON) containing 30% Tifton 85 hay and 70% concentrate (16% CP) and non-forage (NF) diets containing 30% FP as replacement of hay and 30% whole corn as replacement of ground corn (16.4% CP), DM basis. Sheep were individually confined for 60 d, with 15 d for diet adaptation and 45 d for experimental period. Animals were monitored every 5 min for 24 h on the 30th day of the experiment, according to the following activities: eating, rumination, and idle. The animals' physiologic responses were evaluated at 06:00; 10:00; 14:00 and 18:00 h over 10 consecutive experimental days, in which the following parameters were measured: rectal temperature (RT), body temperature (BT), and respiratory rate (RR). The effects of experimental diets were evaluated using variance analysis. The physiologic parameters were analyzed as repeated measures using the PROC MIXED. Effects were declared significant at $P < 0.05$. The replacement of hay by FP did not change ($P = 0.198$) the time spent eating, but animals fed CON spent more time ($P < 0.001$) in rumination activity (82 and 34 min for CON and NF diets, respectively) and less time in idle (108 and 161 min for CON and NF). There was observed an hour effect ($P < 0.05$) for all physiologic variables, but there was no observed effect ($P > 0.05$) of diet and diet × hour interaction (RR: 41.9 and 44.3; BT: 36.3 and 36.5, and RT: 39.1 and 39.0 for CONT and NF, respectively). It is concluded that FP can replace hay in diets of confined sheep without detrimental effects on physiological variables and feeding behavior.

Key Words: non-forage diet, *Parkia platycephala*, rumination

2761 Assessment of milk yield and composition of West African Dwarf goat fed silage with *Zingiber officinale* as an additive. G. Ibhaze*, *Federal University of Technology, Akure, Ondo State, Nigeria.*

Ginger (*Zingiber officinale*) is a perennial plant that may act as a pronutrient because of the vast active ingredients it has which improve intake and digestion and consequent optimum utilization of feed by the animals. It has been reported to be a promising natural galactagogue, which could induce systemic vasodilation and increased blood supply to the mammary gland. Ginger belongs to the class of feed materials known as phytogenics. The objective of the study was to evaluate the milk yield, milk composition such as protein, fat, ash, solid-not-fat, lactose, calcium, potassium, and phosphorus in lactating does fed silage with *Zingiber officinale* as additive. A total of 12 animals were milked on both halves and samples collected into 500-mL vials and stored in a refrigerator at 4°C at the nutrition laboratory of the Federal University of Technology, Akure, until analyzed. The milk samples were analyzed for composition using the AOAC (2012) method. All data obtained were subjected to ANOVA, and means were compared by the methods of Duncan's multiple range test using SAS 2010 version 9.3. Milk yield results ranged from 178.74 to 306.64 g/day, with animals fed a diet cotaining 400 g/kg ginger recording the highest milk yield of 306.64 g/day. Nutrient concentration showed that animals on a diet having 200 g of ginger had the highest protein content of 37.54 g/kg. Fat content decreased (32.30–30.05 g/kg) as the ginger addition increased. No significant ($P > 0.05$) difference was observed in the minerals examined. In conclusion, milk yield of West African Dwarf goats could be improved by the addition of ginger up to 400 g/kg.

Key Words: milk, *Zingiber officinale*, goat

2762 Effect of grape pomace in the diet of ewes during the mid-lactation period on milk composition and coagulation properties and methane emissions. S. Carta*[1], R. Steri[2], A. Cesarani[1], D. Meo Zilio[2], F. Correddu[1], M.F. Lunesu[1], R. Rubattu[1], and A. Nudda[1], *[1]University of Sassari, Sassari, Italy, [2]Council for Agricultural Research and Economics, CREA Research Centre for Animal Production and Aquaculture, Monterotondo, Roma, Italy.*

Grape pomace (GP), a byproduct rich in bioactive compounds, could be included in an animal's diet to improve the animal's performance and the quality of the livestock products. The aims of this study were to evaluate the effect of the inclusion of GP in the diet of ewes during the mid-lactation on milk composition, milk coagulation properties (MCP), and methane emissions (ME) using a remote laser CH_4 gas detector. A total of 24 Sarda dairy sheep, homogeneous for BW (58.0 ± 0.75 kg) and MY (2.4 ± 0.007 kg), were divided in 3 groups fed a total mixed ration: the first group without any supplementation (CON), the second group supplemented with 100 g/d per head of GP (GP100), and the third group supplemented with 150 g/d per head of GP (GP150). The trial lasted 7 wk, with 1 wk of adaptation and 6 wk of sampling. Individual feed intake was recorded daily with an individual automatic feeding system (Biocontrol AS, Rakkestad, Norway). Individual milk samples were collected weekly. The ME were measured during the last week of the trial for 3 consecutive days during the morning and evening milking. Data were analyzed with a mixed model in which the diet, the time, and their interaction were used as fixed effects and the animals as random. We found that MY and fat and protein content were not influenced by the diet. The SCC tended to be lower for animals fed the highest doses of GP compared with the other 2 groups (2.32, 2.41, and 1.95 for CON, GP100, and GP150, respectively, $P = 0.09$). This parameter had an effect on milk pH, that is higher in CON and GP100 compared with GP150, and also on MCP. Milk coagulation time was lower in GP150 than the GP100. The GP inclusion did not affect the ME, expressed as g of CH_4 per kg of MY. The ME were found equal to 15.6, 13.4, and 15.7 g CH_4/kg of MY in CON, GP100, and GP150, respectively ($P = 0.86$). The results of this work suggested that 150 g of GP could be included in sheep diet without change on production traits, but with potential positive effect on mammary gland health. *This work was financially supported by NextGenerationEU, PNRR M4C2, and CN00000022 AGRITECH.*

Key Words: dairy sheep, grape pomace, CH_4 emission

2763 Retention time of digesta in the gastrointestinal tract of lambs fed whole-plant sesame silage. M. J. de Araújo*, J. P. Carvalho Neto, S. J. A. Vallecillo, A. F. Carvalho, A. R. de Sousa, D. L. S. Jácome, T. P. Dias-Silva, and A. L. C. Gurgel, *Federal University of Piauí, Bom Jesus, Piauí, Brazil.*

Digesta mean retention time (MRT) is an important factor on digestive efficiency of ruminants. This study examined the effect of the replacement of whole-plant corn silage (WPCS) by whole-plant sesame silage (WPSS) in diets on the MRT of both particles and solutes and fiber digestion in the gastrointestinal tract (GIT) and GIT segments of growing lambs using the slaughter technique. A total of 32 castrated crossbred lambs with an initial BW of 21.1 ± 1.89 kg were distributed in a randomized block design with 4 replacement levels: (control [no sesame], 333, 670, and 1,000 g/kg of DM) in 8 replications. The feed was offered as a TMR using a forage/concentrate ratio of 40:60. The MRT of particles was estimated by indigestible NDF (MRT_{iNDF}), and the MRT of solute was determined by Co-EDTA (MRT_{Co}). The data were analyzed in a randomized complete block design, and orthogonal polynomial contrasts were used to determine linear and quadratic effects of substitution levels when it was significant ($P \leq 0.05$). The results showed that DMI (% BW) was not affected by the diets ($P > 0.05$). The ratio of iNDF/NDF for both ingested diet and GIT digesta linearly increased as WPSS in the diet increased ($P = 0.001$). No influence was observed on MRT_{iNDF} in the reticulorumen (RR), omasum, small intestine, cecum, colon-rectum segments, and total GIT ($P > 0.05$). However, the MRT_{iNDF} in the abomasum quadratically changed as WPSS increased in the diet, with the smallest value (2.6 h) observed at the level of 670 g/kg DM ($P = 0.01$). The MRT_{Co} in the RR ($P = 0.03$) and total GIT ($P = 0.04$) linearly increased but linearly decreased in the abomasum ($P = 0.04$) with increasing WPSS in the diet were observed. Furthermore, cecum MRT_{Co} quadratically decreased ($P = 0.01$) as the WPSS in the diet increased and reached the smallest values (0.58 h) at the level of 333 g/kg DM. No influence was observed on MRT_{Co} in the omasum, small intestine, and colon–rectum segments ($P > 0.05$). The results suggest that WPSS had a greater influence on MRT_{Co}. On average, the forestomach was responsible for 96% of the total NDF digested (across all treatments).

Key Words: fiber digestion, iNDF, *Sesamum indicum*

2764 Encapsulation of vitamin D_3 and curcumin combination by sulfur-saturated bovine lactoferrin-alginate complex coacervates for enhancing the immune response: *In vitro* study. A. Mora-Gutierrez*[1], M. T. Núñez de González[1], S. Woldesenbet[1], R. Attaie[1], and Y. Jung[2], [1]*Cooperative Agricultural Research Center, Prairie View A&M University, Prairie View, TS,* [2]*Statistical Consulting Center, Department of Statistics, Texas A&M University, College Station, TX.*

The poor water solubility and bioactivity of lipophilic bioactive compounds can be improved using delivery systems. Solubility and bioactivity of vitamin D_3-curcumin combination could improve by the entrapment of such lipophilic bioactive compounds into the biopolymer system of sulfur-saturated bovine lactoferrin-alginate complex coacervates. A suitable viscosity and high emulsion stability could increase encapsulation efficiency. The study aimed to characterize and evaluate in vitro the encapsulated vitamin D_3-curcumin combination by sulfur-saturated bovine lactoferrin-alginate complex coacervates for enhancing the immune response. Encapsulation efficiency reached a maximum of 90.4% (vitamin D_3) and 91.8% (curcumin). The degradation of vitamin D_3 and curcumin was negligible based on the results from the HPLC technique. Monocyte cells, which turn into macrophages and boost the immune system, were isolated from the blood samples of Alpine goats. The macrophages were then incubated with mastitis-associated bacteria *Escherichia coli*, the naturally occurring curcumin, and the encapsulated vitamin D_3-curcumin combination. Immunomodulatory assay data were evaluated by ANOVA and the means were separated using the Tukey's test to determine significant differences ($P < 0.05$). The results indicated that the encapsulated vitamin D_3-curcumin combination significantly ($P < 0.05$) stimulates the intracellular killing of *E. coli* by macrophages compared with naturally occurring curcumin. Because vitamin D_3 and curcumin work differently with the immune system, we found that these 2 lipophilic bioactive compounds are more effective synergistically. Our data indicated a potential immunomodulatory role of the encapsulated vitamin D_3-curcumin combination from sulfur-saturated bovine lactoferrin-alginate complex coacervates during bacterial infection.

Key Words: vitamin D_3, curcumin, immunity

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