ADSA 2021
Annual Meeting

Abstracts

adsa.org/2021

Abstracts of the 2021 American Dairy Science Association® Annual Meeting

Journal of Dairy Science®

Volume 104, Supplement 1

JOURNAL OF DAIRY SCIENCE® SINCE 1917
1800 S. Oak St., Ste. 100, Champaign, IL 61820 Phone 217-356-5146 | Fax 217-378-4083 | http://www.journalofdairyscience.org

Paul Kononoff, Editor-in-Chief (22)
University of Nebraska–Lincoln; pkononoff2@unl.edu; 402-472-6442

Invited Reviews

Kerst Stelwagen, Editor (21)
SciLactis Ltd.

Dairy Foods

Bioactivity and Human Health
Dairy Product Microbiology and Safety
Food Systems and Environment

Michael Miller, Senior Editor (23)
University of Illinois

Olivia McAuliffe, Editor (21)
Teagasc

Milena Corredig, Editor (22)
Aarhus University

Chemistry and Materials Science
Processing and Engineering
Sensory Analysis

Federico Harte, Senior Editor (21)
Penn State University

Scott A. Rankin, Editor (23)
University of Wisconsin–Madison

Jay Amamcharla, Editor (22)
Kansas State University

Production

Animal Nutrition

Jeffrey L. Firkins, Senior Editor (23)
The Ohio State University

Zhongtang Yu, Editor (21)
The Ohio State University

Alex Bach, Editor (21)
IRTA

Barry Bradford, Editor (22)
Michigan State University

Andre Brito, Editor (22)
University of New Hampshire

Farm Systems and Environment

Albert De Vries, Senior Editor (21)
University of Florida

Robin White, Editor (21)
Virginia Tech

Genetics and Genomics

Andrés Legarra, Senior Editor (23)
INRA

Nicolo Macciotta, Editor (21)
University of Sassari

Christine Baes, Editor (23)
University of Guelph

Health, Behavior, and Well-being

Stephen LeBlanc, Senior Editor (21)
University of Guelph

Dan Weary, Editor (21)
University of British Columbia

Wolf Heuwieser, Editor (21)
Freie Universität Berlin

Jessica McArt, Editor (22)
Cornell University

John Middleton, Editor (23)
University of Missouri

Olga Wellnitz, Editor (23)
University of Bern

Physiology

Stephen Butler, Senior Editor (21)
Teagasc

Gerd Bobe, Editor (23)
Oregon State University

Laura Hernandez, Editor (21)
University of Wisconsin

JOURNAL MANAGEMENT COMMITTEE

T. Schoenfuss (22)
University of Minnesota

A. Lock (23)
Michigan State University

S. Clark (24)
Iowa State University

S Greenwood (25)
University of Vermont

Matthew C. Lucy
University of Missouri

Board Liaison
Paul J. Kononoff
University of Nebraska

Ex officio
S. M. Pollock
L. Adam

EDITORIAL BOARD

A. Abuelo (23) USA
S. Alcaine (21) USA
S. Anand (23) USA
F. Bargo (23) Argentina
J. Barlow (21) USA
D. S. Beggs (22) Australia
D. Berry (21) Ireland
D. Bickhart (22) USA
M. Bionaz (21) USA
J. Boerman (23) USA
A. Bouwman (23) the Netherlands
R. Brandsma (21) USA
J. Bromfield (23) USA
T. Byrne (21) New Zealand
M. Castillo (23) Spain
T. M. Casey (22) USA
R. Cerri (21) Canada
P. Chaivisuthangkura (22) Thailand
E. Cuttance (23) New Zealand
J. Dalton (23) USA
S. Davis (21) New Zealand
M. de Veth (22) USA
S. Drake (21) USA
J. Dubuc (22) Canada
M. Endres (23) USA
P. Erickson (21) USA
A. Faciola (21) USA
L. Giblin (23) Ireland
J. Giordano (21) USA
C. Girard (23) Canada
B. Gredler-Grandl (21) Switzerland
G. Habing (23) USA
T. Hackmann (22) USA
K. Harvatine (21) USA
B. Heins (23) USA
A. Hristov (22) USA
R. Jimenez-Flores (23) USA
Y. Kim (23) Korea
V. Krömker (23) Germany
C. Kuhn (21) Germany
R. Laven (21) New Zealand
C. Lee (23) USA
X. Li (21) China
A. Lock (21) USA
L. Ma (23) USA
S. Mann (21) USA
M. Marcondes (23) Brazil
S. McDougall (21) New Zealand
B. M. Mehta (21) India
S. Meier (21) New Zealand
E. Miller-Cushon (21) USA
R. Mor (21) India
R. Narasimmon (21) USA
C. Oberg (23) USA
G. Opsomer (22) Belgium
B. Pardon (23) Belgium
P. Pinedo (22) USA
N. Raak (23) Denmark
M. Rhoads (22) USA
D. Roy (23) France
D. Sepulveda (23) Mexico
L. Shalloo (21) Ireland
N. Silvia del Rio (23) USA
A. Sipka (21) USA
M. A. Steele (21) Canada
L. Tauer (23) USA
P. Toral (22) Spain
S. Tsuruta (21) USA
T. Vasiljevic (22) Australia
M. A. G. von Keyserlingk (21) Canada
R. Wadhwani (21) USA
J. Wang (22) China
L. Ward (21) USA
M. Wattiaux (23) USA
M. Wiedmann (23) USA
C. A. Wolf (22) USA
Q. Zebeli (21) Austria

FASS PUBLICATIONS STAFF (journals@assochq.org)

Susan M. Pollock, Managing Editor
Louise Adam
Mandy Eastin-Allen
Sharon Frick
Katy Henkel
Christine Horger
Irene Kaufman
Ron Keller
Lisa Krohn
Theresa Lawrence
Shauna Miller

ADSA BOARD

President
R. Erdman
University of Maryland

Vice President
P. Kindstedt
University of Vermont

Treasurer
T. McFadden
University of Missouri

Past President
R. Jiménez-Flores
The Ohio State University

Director
B. Briczinski (21)
National Milk Producers Federation

Director
M. A. G. von Keyserlingk (21)
University of British Columbia

Director
M. Miller (22)
University of Illinois

Director
M. VandeHaar (22)
Michigan State University

Director
R. Kapoor (23)
Dairy Management Inc.

Director
X. Zhao (23)
McGill University

Journal of Dairy Science (ISSN 1525-3198) is published online (http://www.journalofdairyscience.org) monthly on behalf of the American Dairy Science Association® by FASS Inc., Champaign, IL 61820, and Elsevier Inc., 360 Park Avenue South, New York, NY 10010-1710. Business and Editorial Office: 1600 John F. Kennedy Blvd., Ste. 1800, Philadelphia, PA 19103-2899. Customer Services Office: 3251 Riverport Lane, Maryland Heights, MO 63043.

ADSA 2021 Program Committees

Overall Program Committee
Mike VandeHaar (chair)
Dave Everett
Trevor DeVries
Rani Govindasamy-Lucey
Kevin Harvatine
Michael Miller

Animal Behavior and Well-Being
Amber Adams-Progar (chair)
Alex Bach
Barbara Jones

Animal Health
Eduardo de Souza Ribeiro (chair)
Sabine Mann
Johan Osorio

Breeding and Genetics
Francisco Penagaricano (chair)
Daniela Lourenco
Brad Hein
Paul VanRaden
Natascha Vukasinovic

Dairy Foods
Dave Everett (chair)
Rani Govindasamy-Lucey
Sam Alcaine
Sanjeev Anand
Rohit Kapoor
Haotian Zheng

Extension Education
Noa Roman-Muniz (chair)
Gustavo Schuenemann
Dale Moore

Forages and Pastures
Matt Akins (chair)
Luiz Ferraretto
Kathy Soder
Ken Griswold

Growth and Development
Peter Erickson (chair)
Sonia Vázquez Flores
Kimberley Morrill

Lactation Biology
FenQi Zhao (chair)
Sha Tao
Adam Geiger
Rupert Bruckmaier

Milk Protein and Enzymes
Dave Everett (chair)
Lloyd Metzger
Rodrigo Roesch
Rafael Jimenez-Flores
Donald McMahon
Phoebe Qi
Yves Pouliot
Christina Levendoski
Hadi Eshpari

Physiology and Endocrinology
Massimo Bionaz (chair)
Angel Abuelo
Eshan Khafipour

Production, Management, and the Environment
Jennifer Heguy (chair)
Abigail Carpenter
Chaouki Benchaar

Reproduction
Stephen LeBlanc (chair)
Luciana Caixeta
Anna Denicol

Ruminant Nutrition
Joe McFadden (chair)
Ranga Appuhamy
James Tully
Agustin Ríus
Dengpan Bu

Small Ruminant
Noemi Castro (chair)
Benjamin Wenner
Jerry Roberson

Teaching/Undergraduate and Graduate Education
Abigail Carpenter (chair)
Michel Wattiaux
Juan Marcos Fernandez

ADSA Southern Section Symposium
Agustin Ríus

ADSA Graduate Student Symposium
Heather McCain-Keefer (chair)
Holly Miller

Graduate Student Competition: ADSA Dairy Foods Oral
Beth Briczinski (chair)
Hari Meletharayil
Neha Singh

Graduate Student Competition: ADSA Dairy Foods Poster
Khilendra Bhanduriya (chair)
Hadi Eshpari

Graduate Student Competition: ADSA Production Oral (MS/PhD)
Jamie Jarrett (chair)
Corwin Nelson
Laura Hernandez
Virginia Brandao
Mike Socha

Graduate Student Competition: ADSA Production Poster (MS/PhD)
Kamal Mjoun (chair)
Jeff Weyers
Pablo Pinedo
Ben Enger
Kayla Rink
Lorenzo Hernandez Castellano

ADSA Southern Section Oral Competition
Agustin Ríus

ADSA SAD Undergraduate Oral and Poster Competitions
Elizabeth Eckelkamp (chair)
Molly Kelley

NANP Nutrition Models Workshop
Tim Hackmann

Citing ADSA 2021 Meeting Abstracts (XXX = page number from this abstract collection):

Author, A., B. Author, and C. Author. 2021. Presentation title. J. Dairy Sci. 104(Suppl. 1): XXX. (Abstr.)

ABSTRACTS

American Dairy Science Association®

Sunday, July 11, to Wednesday, July 14, 2021

Contents

STUDENT COMPETITIONS

ORAL PRESENTATIONS

POSTER PRESENTATIONS

SYMPOSIA

FALL WEBINAR SERIES

Late-Breaking Abstract Session

LB100 Timing of gut closure: Temporal and regional propensity for IgG uptake in the neonatal bovine gut. K. Schalich*, C. Mondoza, M. Das, L. Francoeur, S. Gurung, M. Vogellus, C. Detrick, and V. Selvaraj, *Cornell University, Ithaca, NY.*

Neonatal calves are born agammaglobulinemic, so the enteric absorption of sufficient colostrum immunoglobulin G (IgG) is essential for immediate protection against pathogens (transfer of passive immunity, TPI). Still, the regional (duodenum, jejunum, ileum) and temporal capacity for IgG absorption in the calf gut remains inadequately defined. In this study, we investigated neonatal small intestine samples (every 100 cm through the duodenum, jejunum, and ileum) in 0, 12, 24, 48 h and 7-d old Holstein calves (n = 3–4/time point) for gene expression of the IgG receptor FCRN (genes *FCGRT/B2M*). Unexpectedly, we found a significant temporally increasing expression of *FCGRT* across all gut regions ($P < 0.001$), indicating that *FCGRT* has sustained yet differing contributions to enteric immune support and is not restricted to or a marker for TPI. We then pivoted to biomarker discovery to identify the transcytosis mechanism specific for sorting IgG cargo (endosomes) to interpret active IgG absorption. By analyzing an existing neonatal gut transcriptome, we identified a novel apical endosome glycoprotein, *MAMDC4*, involved in directed transcytosis of endocytosed cargo. Expression of *MAMDC4* was consistent with a short-lived role in IgG absorption across all intestinal regions ($P < 0.01$). Investigation into genes with similar developmental expression patterns and confirmed intracellular trafficking functions identified key transcytosis machinery (*DAB2, MYO6, RAB14, DNAJC13, KIF3B*), allowing insights into the bovine enterocyte mechanism in TPI for the first time. Temporal expression indicates IgG absorption can occur throughout the small intestine with different regional declines, yet persist in the gut with levels not significantly different between 12h and 48h ($P > 0.13$, all regions), suggesting the capacity for active IgG absorption out to 48h of neonatal life. Fundamental knowledge gained on the timing, regional capacity, and process of IgG absorption through expression of novel intestinal biomarkers for gut closure can be used to design precision IgG feeding programs for improved TPI in neonatal calves.

Key Words: immunoglobulin G, gut closure, calf

LB101 Application of actinidin in milk protein hydrolysis. S. Kaur*[1], T. Huppertz[1,2], and T. Vasiljevic[1], [1]*Institute for Sustainable Industries & Liveable Cities and College of Health and Biomedicine, Victoria University, Melbourne, VIC, Australia,* [2]*FrieslandCampina, Amersfoort, the Netherlands,* [3]*Food Quality & Design Group, Wageningen University & Research, Wageningen, the Netherlands.*

Actinidin, a plant protease, has been frequently used in the food industry, although its application has not been assessed fully in dairy systems. The research aim was to establish operational parameters (kinetics, temperature, pH, enzyme to substrate ratio, time) relevant to its application in hydrolysis of whey protein isolates (WPI), whey protein concentrates (WPC) and milk protein concentrates (MPC). The degree of hydrolysis (DH) was determined by trinitrobenzenesulfonic acid (TNBS) at an enzyme to substrate ratio of 1:100 and 1:500 and at 15, 35, 40, 55 and 60°C for all 3 substrates. The pH was not controlled. The DH increased with rise in temperature and the maximum degree of hydrolysis was achieved at 60°C for all 3 dairy systems. A lower E:S ratio (1:100) resulted in a greater degree of hydrolysis of whey proteins. Altering the ratio did not result in substantial change of %DH of MPC. Cleavage of proteins was clearly time dependent ($P < 0.05$) while pH, although not controlled, did not change significantly ($P > 0.05$) during this time. PAGE analysis revealed that actinidin mainly acted on α-lactalbumin and α_s-casein in WPI and MPC, respectively. Kinetic parameters from Lineweaver-Burk plot were determined at 60°C using WPI and MPC as a substrate showing that WPI hydrolysis was characterized with lower Km, higher K_{cat} and higher V_{max} as compared with MPC.

Key Words: plant proteases, hydrolysis, milk proteins

LB102 Induction of leaky gut by repeated intramuscular injections of indomethacin to Holstein bull calves. L. R. Cangiano*[1], B. K. McNeil[1], L. N. Buss[1], I. R. Ipharraguerre[2], C. Villot[3], T. J. DeVries[1], and M. A. Steele[1], [1]*Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Human Nutrition and Food Science, University of Kiel, Kiel, Germany,* [3]*Lallemand SAS, Blagnac, France.*

The objective of this study was to evaluate the efficacy of repeated intramuscular indomethacin injections to induce leaky gut in calves. Indomethacin is a cyclooxygenase inhibitor that depletes protective prostaglandins from the gut mucosa, inducing damage to the intestinal epithelium. Fifteen Holstein bull calves of 25 ± 2 d of age distributed across 2 experimental periods and stratified by BW were randomly assigned to 1 of 3 treatments: (1) Control (CTL), saline injection; or either (2) Low (INDO-L), or (3) High intramuscular indomethacin injections (INDO-H) dosed at 1.2 and 2.4 mg/kg BW, respectively. The challenge consisted of sequential indomethacin or saline intramuscular injections every 12 h for 48 h, after a 21-d adaptation period. During the challenge, intake and BW were measured daily and rectal temperature, fecal scores, and blood were sampled every 12 h. Gut permeability was measured the day before the challenge and during the last day of challenge by comparing plasma concentrations of orally dosed lactulose, D-mannitol, and Cr-EDTA markers. Data were analyzed using a generalized linear mixed-effect model with repeated measures, including the fixed effects of treatment, time, and their interactions, and the random effect of period. Overall, no negative clinical signs were detected during the challenge. Starter and milk intake, and ADG were similar between treatments ($P > 0.19$), and no effect on rectal temperature, or fecal scores was detected ($P > 0.28$). Pre-challenge, the concentrations of blood markers were similar for all animals ($P > 0.32$); however, on the last day of the challenge, INDO calves had a higher area under the curve for the concentrations of lactulose and Cr-EDTA ($P < 0.02$) compared with CTL. Furthermore, the difference between pre and post-challenge concentrations of lactulose and Cr-EDTA was greater in INDO calves ($P < 0.05$). Additionally, INDO-H calves had higher plasma cytokine concentrations of MIP-1β and IL-6 ($P < 0.03$) compared with CTL. In conclusion, this is the first evidence that repeated indomethacin injections may induce symptoms of leaky gut in suckling calves.

Key Words: gut barrier function, inflammation, intestinal permeability

LB103 Altering lactation ruminal microbiota in dairy cows using early-life rumen content dosing. M. S. Cox*[1,2], P. J. Weimer[3], A. J. Steinberger[1,2], J. H. Skarlupka[1,2], and G. Suen[1], [1]*Department of Bacteriology, University of Wisconsin-Madison, Madison, WI,* [2]*Microbiology Doctoral Training Program, University*

of Wisconsin-Madison, Madison, WI, [3]*US Dairy Forage Research Center, USDA Agricultural Research Service, Madison, WI.*

Improving milk production efficiency (MPE) by modulating the rumen microbial community is an attractive area of active research. Rumen bacterial community composition (BCC) has been implicated in MPE, but the adult rumen microbiota is highly stable and largely resistant to perturbation. We sought to influence the establishment of the ruminal BCC by early intervention in pre-weaning dairy calves. Two cannulated lactating Holstein cows historically disparate in MPE (high and low) were selected as donors. Three cohorts of heifer calves were dosed by oral gavage with rumen inocula sourced from the high-efficiency donor (HE, $n = 9$), the low-efficiency donor (LE, $n = 9$), or an autoclaved 50:50 mixture from the 2 donors that served as a microbe-free control (C, $n = 6$). Dosings were performed at birth and biweekly through 8 weeks of age. Cows were raised according to standard practices on the farm through the first lactation. Roughly 8 weeks before parturition, cows were ruminally cannulated. Samples of solid and liquid-phase rumen contents were collected in early, middle, and late lactation for assessment of BCC by 16S rRNA amplicon sequencing. Donor BCC differed throughout the inoculation period in bacterial community structure ($P < 0.001$). During lactation, rumen solid BCC in treatment cows differed by dosing cohort (adonis, $P = 0.004$), and bacterial community structure in HE cows was different from both LE cows ($P = 0.009$) and C cows ($P = 0.009$). Rumen liquid BCC also tended to differ by inoculation group ($P = 0.059$). These results demonstrate that early intervention in rumen microbial community development can have permanent impacts on ruminal BCC through adulthood. Future work will correlate these bacterial findings to production and feed intake and demonstrate that microbial communities can be leveraged to manipulate MPE and improve outcomes for producers.

Key Words: rumen microbiota, milk production efficiency, inoculation

LB104 Clinical and inflammatory response to eucalcemia during a lipopolysaccharide challenge in early postpartum dairy cows.

T. L. Chandler*, T. A. Westhoff, A. S. Sipka, and S. Mann, *Cornell University, Ithaca, NY.*

Hypocalcemia during endotoxemia and sepsis is conserved across species, and Ca therapy may exacerbate the inflammatory response. Postpartum cows experience systemic inflammation and hypocalcemia, but it is unknown how Ca administration affects inflammation. Our objective was to describe the clinical and inflammatory response to an intravenous (IV) lipopolysaccharide (LPS) challenge in postpartum cows with or without IV Ca to maintain eucalcemia. Cows ($n = 16$, 8 ± 1 DIM) were enrolled in a matched-pair randomized controlled design to receive IV Ca (IVCa) or 0.9% NaCl (CTRL) during an IV LPS challenge (25, 40, or 45 ng/kg BW over 1 h). Ionized Ca (iCa) was monitored cow-side and IV Ca infusion adjusted in a eucalcemic clamp for 12 h following challenge. Body temperature, heart rate (HR), respiratory rate (RR), and rumination were monitored and blood sampled for analysis of haptoglobin and serum amyloid A (SAA). Repeated measures ANOVA with baseline covariates were analyzed in PROC MIXED (SAS v. 9.4) with fixed effects of treatment (trt), time, trt × time, and random effect of pair(dose). Body temperature increased in all IVCa but not all CTRL animals following challenge (trt × time $P = 0.02$), and max temperature was 40.0 vs. 40.6 ± 0.2°C for CTRL and IVCa, respectively ($P = 0.08$). Blood iCa was maintained within $\pm 2.5\%$ of baseline in IVCa (trt × time $P = 0.01$) and 15 ± 5 g of Ca was infused to maintain eucalcemia whereas blood iCa decreased to 1.00 ± 0.04 mM in CTRL during challenge. Both HR and RR changed over time ($P \leq 0.001$) but did not differ by trt ($P > 0.47$). Rumen contractions decreased ($P = 0.02$) in both groups but the pattern of rumination depended on trt (trt × time $P = 0.08$). Rumination time was greater ($P = 0.07$) in IVCa during the first 12 h, but similar ($P = 0.97$) in both groups in the 24 h following challenge. Haptoglobin and SAA increased ($P \leq 0.0001$) similarly ($P \geq 0.31$) in both groups. We noted variation in the individual animal response to LPS that appeared larger compared with other models in mid- to late-lactating cows. These differences may be particular to the postpartum period and may be partially explained by endotoxin tolerance.

Key Words: calcium, inflammation, transition period

LB105 Glutathione reduced triacylglycerol content and altered lipid metabolism in primary liver cells enriched with hepatocytes.

K. Gallagher*, Z. Zhou, and C. Collings, *Department of Animal Science, Michigan State University, East Lansing, MI.*

Fatty liver is a metabolic disorder characterized by hepatic triacylglycerols (TAG) accumulation in dairy cows. Research into other species suggests glutathione can potentially prevent hepatic TAG accumulation, but cannot be concluded in ruminants. Our objectives were to quantify intracellular TAG content and expression of genes related to lipid metabolism in bovine primary liver cells enriched with hepatocytes (PLEH) exposed to glutathione. The PLEH were isolated from 3 nonpregnant mid-lactation multiparous Holstein cows previously. A customized medium (CM) was created to mimic the profile of circulating AAs, glucose, insulin, choline, and albumin levels observed for dairy cows on d 4 postpartum. Treatments were CM and GSH (CM + 50µM glutathione). After 72 h, neutral lipids were stained with a fluorescent dye to quantify intracellular TAG content using flow cytometer. Expression of genes controlling lipid metabolism and mitochondria copy number were also quantified in PLEH after 72 h. Treatment effects were determined using PROC MIXED in SAS. As expected, GSH reduced intracellular TAG compared with CM ($P < 0.01$). Compared with CM, GSH supplementation led to greater ($P < 0.05$) expression of genes regulating fatty acid oxidation (*ACADL, ACADM, ACOX1, ECHS1, UCP2*), suggesting enhanced fatty acid oxidation in response to GSH. Although no significant difference ($P = 0.32$) in mitochondria copy number was observed between CM and GSH, expression of a key mitochondrial transcription factor (*TFAM*) and genes encoding components of mitochondrial respiratory chain complexes (*SDHA, COX6A1, ATP5F1B)* were greater ($P < 0.05$) in GSH treated PLEH compared with CM. Additionally, GSH treatment resulted in lower expression of *GSR* ($P = 0.01$) and *NFKB1* ($P < 0.01$) compared with CM, suggesting the potential benefit of GSH supplementation in antioxidant metabolism and inflammatory response. Overall, results indicate that GSH altered lipid metabolism and reduced intracellular TAG level in PLEH. In vivo studies are required to examine the effects of glutathione on liver TAG and metabolism in the early lactation.

Key Words: glutathione, lipid metabolism, hepatocyte

LB106 Bar-coded cattle facilitate video tracking on farm.

N. Sadrzadeh*, B. H. Eagan, B. Foris, M. A. G. von Keyserlingk, and D. M. Weary, *University of British Columbia, Vancouver, BC, Canada.*

Increased use of technology on dairy farms can provide data that help inform management decisions. Data on cow location and behavior may, for example, serve as an early indicator of problems with cow health. The use of radio frequency identification (RFID) technology is common on farms, but camera-aided identification has the potential to be more

universally applicable and cost effective. In this study, we tested the applicability of a video-based barcode detection algorithm to identify cows. A barcode represents data in a visual, machine-readable form. We attached unique 6.4 × 6.4 cm barcodes printed on waterproof vinyl paper and glued to the collars of 15 cows housed in a group of 36. We obtained video recordings of cows' drinking behavior using a fisheye IP camera with a resolution of 2 MP placed above a water trough. A human video observer recorded the time and duration of individual cow visits to the water trough for 2 h after milking; these data were compared with that from an algorithm that automatically identified bar-coded cows visiting the drinker. All cows visiting the drinker were successfully detected by the algorithm, however, some cows not visiting but standing in close proximity were also detected, resulting in a low number of false positive detections. Adjusting algorithm parameters may help further improve detection accuracy. We conclude that simple barcodes attached to the collars of cows can be used to reliably track individual cow location and behavior using video.

Key Words: precision livestock, computer vision, animal welfare

ADSA Graduate Student Dairy Foods Oral Competition

SC100 Application of bulk nanobubbles generated by acoustic cavitation to improve the processability of milk protein concentrates. K. S. Babu* and J. Amamcharla, *Kansas State University, Manhattan, KS.*

Bulk nanobubbles (BNBs) are nano-scaled bubbles (50–700 nm) with unique properties and offer potential applications in the dairy and food industries. In the present study, a continuous acoustic cavitation technique at 20 kHz capable of delivering up to 1.5 kW of power with a flow cell design was employed to generate BNBs. The first objective of this study was to characterize the BNBs in deionized (DI) water in terms of concentration and mean diameter using a nanoparticle tracking system. The concentration and mean diameter of BNBs were 8.66×10^8 particles/mL and 231.5 ± 5.3 nm, respectively. BNB concentration significantly increased ($P < 0.05$) with the increase in amplitude. The second objective of this study was to evaluate the presence of BNB on the processability of milk protein concentrate (MPC) dispersions. Two lots of MPC powders were obtained from a commercial manufacturer. Randomized complete block design with total solids (TS; 15 and 19%) and amplitude (0, 50, 75, and 90%) as independent factors were studied. MPC powders were reconstituted to desired TS and exposed to acoustic cavitation as per the experimental design using a flow cell at 10 mL/min. The treated MPC dispersions were analyzed for rheology, functional properties, and microstructure. The apparent viscosity of the untreated MPC was 67.15 and 203.84 mPa·s at 100 s^{-1} for 15 and 19% TS, respectively. The viscosity decreased to 5.95 and 16.81 mPa·s at 100 s^{-1} for 15 and 19% TS, respectively, after the acoustic cavitation. The viscosity significantly decreased ($P < 0.05$) at all the amplitudes studied. The mean particle size of the untreated MPC was 207.07 and 309.88 nm for 15 and 19% TS, respectively. The mean particle size decreased to 160.95 and 167.03 nm for 15 and 19% TS, respectively, after the acoustic cavitation. Notably, at 19% TS, the particle size significantly decreased ($P < 0.05$) at all the amplitudes studied. In conclusion, the BNB treatment helps to reduce the viscosity and therefore this study suggests the potential of using BNB treatment for a more energy-efficient drying process.

Key Words: milk protein concentrate, nanobubbles, acoustic cavitation

SC101 Rheological characteristics of fibrillated model milk protein concentrates during acid gelation. G. Rathod* and J. Amamcharla, *Kansas State University, Manhattan, KS.*

Whey proteins in milk are globular proteins which can be converted into fibrils to enhance functional properties such gelation, emulsification, and foaming. A model fibrillated milk protein concentrate (F-MPC) was developed by mixing micellar casein concentrate (MCC) with fibrillated milk whey proteins. Similarly, control model MPC was obtained by mixing MCC with milk whey proteins. The resulted control model MPC and F-MPC contained 5% protein and casein-to-whey proteins ratio similar to milk. The objective of the current study was to understand the rheological characteristics of control MPC and F-MPC during acid gelation using small-amplitude oscillation. Initially, F-MPC and Control model MPC dispersions were heated to 90°C for 10 min, cooled to 30°C, acidified using glucono-δ-lactone (GDL; 2%wt/wt), and poured in cup and bob geometry of the rheometer maintained at 30°C. Gel formation was continuously monitored with small-amplitude oscillation (constant frequency: 1 Hz and strain: 0.5%) along with continuous pH measurement until the pH reached to 4.6. All the experiments were conducted in duplicate. Results showed that the maximum storage modulus (G') of acid gel of F-MPC was 546.9 ± 15.5 Pa, which was significantly ($P < 0.05$) higher than acid gel made from control model MPC (336.9 ± 11.3 Pa), indicating a firmer gel of F-MPC. Confocal microscopic images also showed significantly higher casein-whey protein interactions in F-MPC compared with control MPC. Complex viscosity was also significantly ($P < 0.05$) higher for F-MPC (90.0 ± 2.5 Pa·S) than control (55.8 ± 1.9 Pa·S). Buffering capacity was observed to be similar for Control and F-MPC until the pH reached to 5.1 and thereafter F-MPC showed a higher buffering capacity. So, it can be concluded that acid gel produced from F- MPC was stronger and more elastic than control model MPC. Fibrillated model MPC can be used to improve gelation in acid gel-type products.

Key Words: fibrillated model milk protein concentrate, acid gelation, rheology

SC102 Application of beta-serum in ice cream manufacturing. K. Rathnakumar*[1], S. Anand[1], and S. I. Martinez Monteagudo[2], [1]*South Dakota State University, Brookings, SD,* [2]*New Mexico State University, Las Cruces, NM.*

Beta-serum (BS) is the aqueous product (serum) produced after the phase inversion during the manufacture of anhydrous milk fat. Although its gross composition resembles that of nonfat dry milk (NFDM), BS contains about 6–8% of phospholipids (PLs) on dry basis. Such concentration of PLs may improve the emulsification during ice cream. The objective of this work was to evaluate the effect of BS on selected quality parameters of ice cream. A secondary objective was to monitor the presence of PLs within the serum phase at different processing steps of ice cream manufacture, including mixing, pasteurization, freezing, and melting. An ice cream formulated with NFDM was used as a control treatment. Ice cream mix formulated with and without BS was centrifuged (4,000 rpm for 30 min) to separate the serum phase (upper phase) and solid phase (lower phase), which were analyzed for total lipids and total phospholipids. Additionally, the presence of PLs in both phases was monitored through confocal laser scanning microscopy. Overall, the majority of PLs were found at the bottom phase during mixing, pasteurization, and melting. The particle size and zeta potential measurements were observed to be 474.6 ± 13.21 and 564.8 ± 12.7 nm, −37.9 ± 5.90 and −43.1 ± 4.90 mV for the control ice cream and B-serum ice cream, respectively. The PLs reported after meltdown using a sieve (0.833mm) for IC control and B-serum were 58.03 ± 4.10 and 63.47 ± 3.02%, respectively, while before meltdown it was reported 4.04 ± 1.49 and 11.27 ± 0.56%, respectively. The flow cure indicated a shear-thinning behavior for both samples. Therefore, results document the presence of PLs in the manufacturing of ice cream and significant quality attributes in ice cream with the addition of beta-serum.

Key Words: beta-serum, phospholipids, confocal laser scanning microscopy

SC103 Physicochemical characteristics and microstructural studies of a novel probiotic whipped cream product. K. Gaba*[1,2] and S. Anand[1,2], [1]*Midwest Dairy Foods Research Center, Minneapolis, MN,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

With the increasing trend of keto diets, the marketability of fat-enriched dairy products is expected to rise at a CAGR of 5.5% in the next 5 years but there exists an unhealthy image related to saturated fatty acids. Nonetheless, the incorporation of healthy ingredients in dairy fat products can help attenuate the negative mindset of consumers. Previously, we developed a spray-dried whey protein-probiotic powder of *Bifidobacterium animalis*, and *Lactobacillus acidophilus* that retained the probiotic counts of 8.50 log cfu/mL for 28 d. In this study, we prepared probiotic whipped cream by using the cream with 42.53% fat, pasteurized at 85°C for 5 min, homogenized at 3,500 rpm for 1 min, and refrigerated at 4°C for 24 h to enhance the partial coalescence. The probiotic powder was inoculated at 0.1%, and 1% levels, and whipped in a kitchen mixer at 4°C in 3 batches of 100 g and stored at 4°C for microbiological, physicochemical, and statistical analysis with one-way ANOVA. The probiotic whipped cream showed higher viability of probiotics with an average overrun, foam stability, storage modulus(G′), loss modulus(G″) of 148.64%, 19.83%, 13,132.67 Pa G′, 2,659.834 Pa G″, respectively in 0.1% treatment and 151.4%, 11.14%, 14,657.82 Pa G′ and 3,148 Pa G″ in 1% treatment indicating strong viscous protein network as compared with the control with 161.32% overrun, 15.17% foam stability, 9,038.35 Pa G′, 2,187.15 Pa G″. Morphological analysis was also done using confocal laser scanning microscopy dual stain technique, Nile Red for the fat phase and Fluorescein isothiocyanate for the proteins at 40× and 60× objective under white light followed by fluorescent and it was observed that the incorporated whey protein hydrolysates surrounded the air bubbles effectively indicating the effective emulsification process to avoid the re-coalescence of fat droplets and thus providing extra stability to the product. Hence, the whey protein-probiotic formulation not only provided functional and bioactive benefits but also improved the stability and whipping properties of the product without intervening with the fat matrix of whipped cream.

Key Words: probiotic, physiochemical, microscopy

SC104 Modifications in flowability and water-holding capacity of MPC85 treated with pulsed electric field processing.

S. Raghunath*, T. C. Schoenfuss, and K. P. Mallikarjunan, *University of Minnesota-Twin Cities, Saint Paul, MN.*

Milk protein concentrate (MPC) is a milk-derived high-quality protein consisting of both casein and whey proteins in their native state. The scope of this study is to improve the functional characteristics of MPC using pulsed electric field (PEF) and to understand the structural changes in the protein due to the treatment. Commercial MPC 85 was reconstituted to 10% total solids and subjected to the various treatment temperatures (25, 45, 65°C), electric field strength (EFS; 4, 12, 20 kV/cm) and frequency (30, 165, 300 Hz) with PEF. Face centered central composite response surface design was used to determine the experimental conditions for the pilot-scale study along with controls. The samples were spray-dried and analyzed for water holding capacity (WHC) with enzymatic gelation at 15% protein and for flowability by determining the avalanche energy of the protein powders using a Revolution powder analyzer. The lower the avalanche energy, the better flowability of the protein powders. The avalanche energy for flowability followed a quadratic model and the optimum condition was determined to be 27°C, EFS at 12.84 kV/cm and frequency at 33.80 Hz which gave a minimum of 23.79 kJ/kg for avalanche energy. This resulted in a percentage decrease of 59% from commercial sample control (58.2 kJ/kg) and 22% from respray dried control. The PEF treatment at 25°C with 20 kV/cm and 300 Hz increased WHC to 88.37% which is an increase of 24.41% in comparison to commercial control (71.03%). The combination of lower temperature and higher EFS and frequency was required to improve the WHC of MPC85. The protein powder flowability was optimized and WHC was found to be improved following the treatment, which may be possibly due to the change in the protein structure with partial unfolding. Experimental results proved that PEF can be used as a treatment for enhancing the water holding capacity and flowability and aid in the development of novel milk proteins products with improved functionality.

Key Words: processing, milk protein, flowability

SC105 Colloidal dispersion of casein micelles with pea protein via homogenization.

A. Krentz*, I. García-Cano, J. Ortega-Anaya, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

The dairy industry struggles to maintain the attention of consumers in midst of declining milk sales. Current trends create an opportunity to incorporate plant protein with milk protein, for potential applications to produce a high-protein, multi-sourced, functional ingredient. Plant proteins can be challenging to use in food because of their low solubility and undesirable off-flavors. However, casein micelles provide a particular system optimal for protein blending along with high sensory acceptability. The objective of this study was to create a stable colloidal dispersion with caseins and pea protein with 2 goals: 1) to improve the solubility of pea proteins, and 2) to preserve the functional properties of milk protein. Three blends were created with varying ratios of casein-to-pea protein (90:10, 80:20, 50:50). The mixtures were subjected to 3 cycles of homogenization using a bench-top GEA 2-stage homogenizer at 4,000 psi maintaining 4–10°C and then pasteurized at 63°C for 30 min. The resulting blends were homogeneous liquids, with no precipitate formation. Protein profiles obtained via RP-HPLC analysis and SDS-PAGE showed that some of the various pea proteins had incorporated with the casein micelles. The pea proteins that interacted with the caseins were isolated and sequenced. The amino acid sequencing indicated that vicilin, an insoluble pea protein, was identified as the main protein incorporated within the caseins. These results support our hypothesis that cold-temperature homogenization can successfully be used to create a colloidal dispersion in which insoluble pea protein may be incorporated with casein micelles in an aqueous solution. Additionally, upon coagulation of the liquid blends, protein gels were formed with textures similar to commercial queso fresco, and hard tofu. Further sensory analysis will need to be conducted. This research highlights a promising application for other plant proteins to be utilized within the dairy industry to help drive future product innovation while also meeting current processing conditions and consumer demands.

Key Words: casein, pea protein, homogenization

SC106 The impact of varying casein and pectin concentration on the stability of high protein, ambient stored cultured milk beverages.

D. Wilbanks*[1], S. Yazdi[2], and J. Lucey[1,3], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Arla Foods, Viby J, Denmark,* [3]*Center for Dairy Research, Madison, WI.*

Shelf-stable cultured milk beverages that have high protein levels have proven difficult to successfully manufacture. With increasing protein level, it is difficult to prevent rapid phase separation and gel formation in cultured beverages, even with the inclusion of stabilizers such as high methoxy (HM) pectin. To limit protein aggregation in cultured milk beverages, we investigated micellar casein as an alternative to milk due to the absence of whey proteins, which can contribute to increased gel strength in cultured products. In this study, micellar casein dispersed in ultrafiltered milk permeate was fermented to pH 4.1, blended with HM pectin, homogenized, pasteurized, and bottled for storage at ambient temperature for 6 mo. Utilizing response surface methodology with a central composite rotatable design, the protein and pectin content were varied between 5 and 9% and 0.0–1.0%, respectively. The elastic modulus, loss tangent, and yield stress of these beverages were measured during storage to observe the extent of bond restructuring, while particle size and visual phase separation were also measured to determine stability. Response variables were measured initially after pasteurizing the beverages and after 1 and 6 mo of storage at ambient temperature. All samples quickly formed gels after homogenizing, regardless of the pectin level. The stiffness (elastic modulus) of all samples increased throughout storage and was determined mainly by the protein content. In samples aged for 6 mo, high levels of pectin reduced gel stiffness. At 6 mo of storage, yield stress values were significantly lower for beverages with <7.5% protein when they were stabilized with >0.85% pectin. Prediction models for visual phase separation in beverages stored for 1 or 6 mo were significantly impacted by the protein content, with increasing instability at lower protein levels. This study showed that high levels of pectin (>0.85%) can be effective in reducing bond formation in aged samples, although all samples did form a gel.

Key Words: casein, pectin, rheology

SC107 Effect of temperature and protein concentration on the viscosity and distribution of protein types within liquid micellar concentrate.

J. Pranata*[1], M. Dunn[2], D. M. Barbano[1], and M. Drake[2], [1]*Cornell University, Ithaca, NY,* [2]*North Carolina State University, Raleigh, NC.*

Micellar casein concentrate (MCC) is a dairy protein ingredient produced by the microfiltration (MF) of skim milk to remove up to 95% milk derived whey proteins. MCC offers a bland flavor free from volatile sulfur compounds, but it exhibits a temperature dependent thickening behavior. The objectives of this study were: 1) to measure the viscosity of liquid MCC at different protein concentrations and temperature and 2) to determine the cause of temperature dependent viscosity changes in MCC by characterizing the soluble protein composition of the aqueous phase around the casein (CN) micelles in liquid MCC. Skim milk (ca. 350 kg) was pasteurized (72°C for 16 s) and filtered through a ceramic MF system to make MCC. Apparent viscosity (AV) from MCC (6, 8, 10, 12% protein) at 4, 20, and 37°C were measured using a rotational viscometer (Brookfield). AV increased ($P < 0.05$) with decreasing temperature and the increase in AV was nonlinear as the protein concentration increased. We hypothesized that the large increase in AV was caused by an increase in soluble protein concentration in the aqueous phase around the micelles. To investigate this, the nitrogen distribution (Kjeldahl) and soluble protein composition (SDS-PAGE) of the ultracentrifugation (UC) supernatants of skim milk, 6.93% MCC, and 11.51% MCC were determined after UC (100,605 × g for 2 h at 4, 20, and 37°C). The percent total nitrogen (TN), CN as a percentage of TN, and concentrations of β-CN and α_s-CN in the MCC supernatants increased with both decreasing temperature and increasing protein concentration. The concentrations of β-CN and α_s-CN in the aqueous phase around the micelles were influenced ($P < 0.05$) by temperature and a temperature by protein concentration interaction effect. These results demonstrate that the migration of casein monomers out of the micelles at low temperature induces a sharp viscosity increase in liquid MCC and when protein concentration was increased, the increase in viscosity was due both to an increase in phase volume of the micelles in the MCC and migration of CN out the micelles.

Key Words: micellar casein concentrate, viscosity

SC108 Understanding the dynamics of molecular dissociation during membrane filtration of milk concentrates.

O. Coskun*[1], L. Wiking[1], S. R. Yazdi[3], V. Rauh[3], and M. Corredig[1,2], [1]*Aarhus University, Department of Food Science, Aarhus N, Denmark,* [2]*iFood Center, Aarhus University, Department of Food Science, Aarhus N, Denmark,* [3]*Arla Innovation Center, Aarhus N, Denmark.*

The processing history during membrane concentration of milk causes differences in the functional behavior of milk proteins. This work investigates molecular dynamics and colloidal behavior of casein micelles (CM) during membrane filtration, under different processing conditions. Concentrates prepared with ultrafiltration (UF) or microfiltration (MF) in combination with diafiltration (DF) were compared at the same CM volume fraction, but with different amounts of serum proteins. DF was conducted while maintaining the volume fraction of the CM to 2× (corresponding to Φ 0.2), to minimize osmotic stress effects. After extensive DF, the retentates were then further concentrated up to 8×. The filtration system used is a patented Vibro technology (SaniMembranes, Denmark), operating at low TMP, with minimum fouling. The protein content of the concentrates reached between 80 and 93% based on a dry basis. 87% of whey protein was lost in MF with extensive DF. Particle size, viscosity, calcium and phosphate composition of the samples were analyzed. Size exclusion chromatography was used to observe differences in the soluble aggregates prepared by centrifugation at 100,000 × g for 1h at 20°C. The protein composition quantified by RP-HPLC. A concentration up to 6–8× was reached with no changes in the apparent diameter ($P > 0.05$). The higher amount of protein present in the serum fraction resulted in a higher viscosity for UF samples compared with MF. The amount of unsedimented protein did not change with the extent of DF, in spite of the dramatic decrease in soluble calcium in both MF and UF processes; however, the ratio of α_{s1}-, β- and κ-casein to total casein dissociated from the CM increased with DF. Further concentration from 2× to 4× after DF increased the extent of micellar dissociation: while the α_{s1}- and κ- increased, the β-casein/total casein ratio decreased in the serum phase. These results demonstrate the importance of controlling processing conditions to be able to target the technological functionality of the concentrates.

Key Words: casein, serum phase, membrane filtration

ADSA Graduate Student Dairy Foods Poster Competition

SC109 Determining the types of *Bacillus* endospores in whey protein concentrate and nonfat dried milk powders.

S. Jha*[1,2] and S. Anand[1,2], [1]*Midwest Dairy Foods Research Center, Minneapolis, MN,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Several spore-forming bacterial species are commonly encountered in dairy powders. These are known to produce thermally resistant endospores. Determining the types of endospores formed by these bacteria could thus be useful in developing control strategies to improve their microbial quality and further application in product development. In the current study, samples of whey protein concentrate (WPC) and nonfat dried milk powder (NFDM) were analyzed for the presence of different types of *Bacillus* endospores, based on their thermal activation treatments. Twenty-five samples of each of WPC and NFDM were procured from commercial dairy plants. The 11 g of each sample was reconstituted in 99mL of phosphate buffer saline. A 20 mL portion of the respective reconstituted sample was heated at 80°C/12min for regular spores (SP), 100°C/30min for high-heat resistant spores (HHRS), and 106°C/30min for specially thermoresistant spores (STS). After the heat treatment, the samples were cooled to room temperature, and the desired serial dilutions were plated on the tryptic soy agar. The plates were incubated for 24 h at 37°C for mesophiles, and 55°C for thermophiles. The experiments were performed in duplicates and the means were compared using ANOVA. Several spore-forming species were identified based on colony morphology followed by MALDI-TOF. In WPC the samples containing the mesophilic spores were found to be 64% SP, 20% HHRS, and 16% STS. The samples containing thermophilic spores were 73% SP, 14% HHRS, and 13% STS. Similarly, in NFDM the samples containing the mesophilic spores were 95% SP and 5% HHRS. The samples containing thermophilic spores were 60% SP and 40% HHRS. This distribution reveals the higher incidence of SP in the dairy powders that could play a vital role as the powders are intended for reconstitution before use. MALDI-TOF analysis revealed *Bacillus licheniformis* and *Bacillus cereus* as the predominant species in WPC and NFDM, respectively. Some other prominent *Bacillus* species identified were *B. pumilus, B. subtilis,* and *Geobacillus stearothermophilus*.

Key Words: endospore, mesophile, thermophile

SC110 Influence of sampling intervals on the standard plate counts of milk samples.

R. Kalita*[1,2], S. Anand[1,2], and G. Djira[3], [1]*Midwest Dairy Foods Research Center, Minneapolis, MN,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [3]*Department of Mathematics and Statistics, South Dakota State University, Brookings, SD.*

High accuracy in sampling is critical in determining the microbiological quality and safety of milk and dairy products. The issue arises with the lack of a standard approach to lower the variability of sampling results of low count milk (LCM) and high-count milk (HCM). It may occur in extended and multi-processing conditions that are triggered by the heterogeneous and aggregating nature of bacteria. This study focuses on the influence of sampling intervals on the microbial count variability of LCM and HCM samples. For a pilot-scale study, 380 gallons of raw whole milk (<4°C) were spiked with *Bacillus licheniformis* vegetative cells (4 log cfu/mL) and pasteurized at 72°C/16 s at a 0.5 gpm flow rate for 12 h. Samples (100mL each) were drawn aseptically at intervals 0, 4, 8, and 12 h from each of the raw (HCM) and pasteurized (LCM) sides of the unit operation using commercially available a port-septum-based sampling device with needle insertion. Two continuous composite samples (2-L each) were also collected during the 12 h run in sterilized sampling bags at 2.5mL/min rate and held at < 4°C. Samples were analyzed at each interval for standard plate counts using tryptic soy agar and incubated at 32°C for 48 h. The study was done in duplicates and ANOVA was used to compare the microbial counts. Mean counts for the individual samples at 0, 4, 8, 12 h., and the composite samples at 12 h., were found to be 4.18 ± 0.10, 4.29 ± 0.12, 4.24 ± 0.12, 4.41 ± 0.12, and 4.38 ± 0.13 log cfu/mL for the HCM, and 1.52 ± 0.13, 1.57 ± 0.13, 1.66 ± 0.12, 1.69 ± 0.12, and 1.78 ± 0.10 log cfu/mL for the LCM, respectively. Results showed an increasing trend in the counts for LCM with no significant difference between the intervals ($P > 0.1$). The microbial counts of samples from various intervals compared with composite samples were also not significantly different ($P > 0.1$) for both LCM and HCM. Under the conditions of analysis, a single sample of 100 mL volumes at any interval during processing is as effective as composite sampling for estimating the microbial quality of milk under prolonged dairy processing conditions of 12 h.

Key Words: sampling, variability, intervals

SC111 A genotypic evaluation of environmental *Listeria* isolates from a dairy plant.

S. Minj*[1,2], N. Singh[1,2], S. Anand[1,2], J. G. Hernandez[3], and B. Kraus[4], [1]*Midwest Dairy Foods Research Center, Minneapolis, MN,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [3]*Young Brothers Seed Technology Lab, South Dakota State University, Brookings, SD,* [4]*Wells Enterprises Inc., Le Mars, IA.*

Listeria species are ubiquitous in nature. Despite improvements in hygiene protocols, some strains of *Listeria* can persist for long periods in dairy plant environment and pose a risk of product cross-contamination. A genotypic evaluation of environmental *Listeria* isolates can thus provide an insight into their significance from a product cross-contamination and safety perspective. In this study, whole-genome sequencing was conducted to determine the genotypic variations and associated phenotypic expressions of 6 *Listeria* isolates (*Listeria innocua* 634–2; *Listeria innocua* 634–34-S-5; *Listeria innocua* 634–34-S-6; *Listeria welshimeri* 634–3; *Listeria welshimeri* 634–253-S-5, and *Listeria monocytogenes* 315-S-1) obtained from the processing environment of a commercial dairy plant. Genomic DNA was extracted using the Wizard Genomic DNA extraction kit and run through whole-genome sequencing on the Illumina MiSeq platform using V2 chemistry with 2 × 250 paired-end chemistry. The genome was fed into the RAST database to develop the annotations. The results revealed at least 13 types of phenotypic responses related to the genotypic variants of the *Listeria* isolates. Overall, the genotypic variants differed with the species and within the strains. Some of the common variants and features identified in the isolates were virulence (DNase, phage terminase, tRNA-Arg-ACG), cell signaling (NAG-IIA, NAG-IIB), phage immunity (CRISPR proteins), osmotic stress (CadA), oxidative stress (YRKL), and antibiotic resistance (BaiE, Lde). While *L. monocytogenes* was positive for all these phenotypic characteristics, *L. innocua* and *L. welshimeri* isolates lacked variants for motility (ActA), biofilm formation (AgD), and acid tolerance (AdiA), making them less resilient to environmental stress. Such information about the genes and their role in expressing phenotypic

attributes can help create more robust interventions for the control of *Listeria* in a dairy plant environment.

Key Words: genotype, phenotypic, sequencing

SC112 A comparison of various methods to measure the insoluble calcium content in cheese.

A. V. Swaminathan*[1] and J. A. Lucey[1,2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

The functionality of cheese like low-moisture part-skim (LMPS) Mozzarella is greatly impacted by solubilization of insoluble calcium phosphate during cheese manufacture. Measuring the insoluble calcium content (INSOLCC) in cheese at various points during manufacture would help to predict its functionality. The current method we use to measure INSOLCC in the final cheese involves acid-base titration, but it takes time to perform these titrations (several h), which could be a concern due to rapid pH changes impacting INSOLCC during cheesemaking process. We evaluated a simple water-soluble calcium (WSC) extraction method as an alternative method to quantify INSOLCC. The WSC method involved adding 40 g of water (55°C) to 4 g of curd, homogenizing with a hand-held mixer for 2 min, and centrifuging at 10,000 × g for 10 min. Supernatants were stored before measuring total calcium in soluble phase by ICP. We compared INSOLCC obtained by both WSC and acid-base titrations for commercial string cheeses made by both direct acid (DA) and starter cultures (SC) (n = 3). DA cheese had significantly lower INSOLCC than SC cheese. At 2 d of storage, DA and SC cheeses had an INSOLCC level of approximately 12 and 21 mg Ca/g protein, respectively, as measured by acid-base titration. Slightly higher INSOLCC levels (2–3 mg) were observed with WSC method possibly due to incomplete extraction of all soluble calcium from the curd matrix. We are currently comparing INSOLCC results of these cheeses measured by another method, i.e., cheese aqueous phase ("juice") using high hydraulic pressure. The cheese juice method does not involve any dilution of cheese so can be considered a good reference method. The cheese juice method results would clarify if our WSC method requires a correction factor. We are also using WSC method to evaluate changes in INSOLCC during manufacture of LMPS Mozzarella. In initial trials, we made experimental LMPS cheese under following cheesemaking conditions: pH of renneting = 6.6, pH of draining = 5.8, and final pH (1 d) = 5.2. The INSOLCC levels were 34, 26, and 20 mg Ca/g protein in the initial milk, curd at draining, and final cheese, respectively.

Key Words: cheese, insoluble calcium

SC113 Gel structure formation as functions of solids concentration and thermal denaturation in heated yogurt mix.

E. J. Donald, J. S. Myers*, and K. A. Schmidt, *Kansas State University, Manhattan, KS.*

Gel formation and quality in yogurt may be manipulated by altering milk solids non fat (MSNF) concentration or varying mix heating temperatures. The strength of yogurt gels is partially due to the interactions of β-Lactoglobulin (β-Lg), α-Lactalbumin (α-La) and κ-casein which aggregate forming complexes with casein micelles typically during mix heating. Dependent upon the temperatures, different associations and bonds form. While β-Lg starts to denature and interact at ~70°C, α-La denatures and interacts at 80°C. When larger aggregates are formed, a firmer texture results. This study focused on yogurt produced from 9 and 12% MSNF heated at 70, 75, or 85°C for 30 min as a means to maximize gel firmness. Mixes were heated to the respective temperature and held for 30 min, cooled, inoculated and incubated at 42°C to pH 4.6 then stored at 4°C. Yogurts were analyzed for textural and rheological properties and syneresis; while mixes were assessed for denaturation, measured by protein fractionation and the fluorescence of advanced Maillard products and soluble tryptophan (FAST) index. The firmest yogurt was made from a 12% MSNF mix heated at 85°C for 30 min (12–85), 310.8 g, while the least firm yogurts were made from 12 to 70, 9–70 and 9–75 mixes, ~144.2 g. Storage and loss moduli showed the 12–85 yogurt to be the most viscous, 283.4 Pa and 93.1 Pa, while 9–70 yogurt was the least viscous, 31.1 Pa and 12.2 Pa, respectively. Unaffected by MSNF, the degree of denaturation was ~53.5% at 85°C, 33.5% at 75°C, and 9.9% at 70°C. However, the FAST index was greatest in 12–85, 54.5, and least in 9–70, 7.7. Syneresis was greatest in 9–85, 7.38%, and least in the 12% MSNF mixes, ~0.46%. Increased MSNF correlates with increased protein content, resulting in additional denaturation and aggregation upon heating, translating to a denser protein gel network. Understanding the relationship between gel formation, MSNF and heating allows for conditions to be optimized to yield yogurts with maximum firmness. Further research to determine how MSNF concentration affects denaturation and aggregation may potentially lead to the production of firmer yogurts.

SC114 Milk phospholipids influence adhesion of bifidobacteria and lactic acid bacteria to human intestinal cells and their inflammatory response.

E. Kosmerl*, J. Ortega-Anaya, I. García-Cano, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Milk phospholipids (MPLs) are highly regarded for their health-promoting properties spanning from neurocognitive functions to modulation of the gut microbiota and more. However, little is understood about their mechanisms and interactions with beneficial bacteria, such as bifidobacteria and lactic acid bacteria (LAB). We hypothesize one mechanism is that MPLs improve the adherence of bifidobacteria and LAB to human intestinal epithelial cells and shift intestinal cell cytokine production toward an anti-inflammatory state. To test this hypothesis, 4 strains (*B. lactis* BB-12, *B. infantis* ATCC 15697, *L. delbrueckii*, *L. casei*) were selected, diluted to an optical density of 0.1 (600 nm), and incubated in MRS + 0.05% L-cysteine media supplemented with or without 0.5% MPLs at 37°C. These bacteria were then collected during the late exponential growth phase for imaging by TEM, measurement of ζ-potential and adhesion properties, and inflammatory response of HT29-MTX human intestinal cells. The ζ-potential of ATCC 15697 decreased significantly from −17.3 ± 4.6 mV to −24.3 ± 7.0 mV in response to MPLs, and increased adhesion to HT29-MTX cells. Similarly, MPLs shifted the ζ-potential of *L. delbrueckii* from −12.5 ± 2.9 to −18.0 ± 4.4 mV. Preliminary results also suggest that MPLs increase the adhesion of *L. casei* to HT29-MTX cells from 8.0% to 13.9%. The bacteria preincubated with MPLs were applied to HT29-MTX cells for assessment of cytokine (MCP-1, TNFα, IFNγ and IL-10) expression changes by qPCR. After 3 h incubation, *L. delbrueckii* had the most observable shift toward an anti-inflammatory state. In the presence of MPLs, *L. delbrueckii* increased anti-inflammatory IL-10 expression by 2.2-fold and decreased the expression of proinflammatory cytokines MCP-1, TNFα, and IFNγ by 1.37-, 4-, and 50-fold, respectively. Similar trends were observed for both BB-12 and *L. casei*. Taken together, these results shed light on one mechanism of MPLs in the promotion of human health and suggest MPLs may be able to improve probiotic efficacy.

Key Words: probiotics, gut microbiota, HT29-MTX

SC115 Reducing the sugar in school lunch chocolate milk.

R. P. Nakamura*[1], H. R. M. Keefer[1], D. M. Barbano[2], and M. A. Drake[1],

[1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

The federal school lunch program mandates caloric and nutrient content of school lunches. This program also requires schools to provide a 240 mL serving of flavored or unflavored skim or 1% fat milk. Chocolate milk remains the most popular milk among school children but added sugar and total calories of chocolate milk are a concern. The current school lunch chocolate milk (SLCM) formulation contains 19.5 g of sugar per serving, which includes 7.5 g of added sucrose. The objective of this study was to evaluate sucrose replacement in SLCM by non-nutritive sweeteners (allulose, mixed sugar syrup (glucose, fructose, allulose), monk fruit, stevia, or sucralose). Power function curves were first generated for each sweetener to determine isosweet taste equivalency to 5.8% (wt/wt) sucrose in water (the sweet taste intensity of SLCM) using magnitude estimation scaling (MES) with a trained panel. Identified isosweet taste concentrations for each sweetener in water were confirmed by paired comparison tests with consumers (n = 40). Subsequently, SLCM with no added sugar (skim milk, cocoa powder, stabilizer) was high temperature short time processed (78C, 26 s) followed by addition of each sweetener, including sucrose at the current SCLM formulation of 7.5 g per 240 mL serving. The SLCM sweetened with sucrose served as the target control. Isosweet taste concentrations for each sweetener in SLCM were confirmed by MES scaling and paired comparison testing as previously described. Chocolate milks with each sweetener at isosweet taste intensity were subjected to descriptive sensory analysis and temporal dominance of sensations profiling (TDS) to characterize other sensory attributes. At isosweet concentrations, SLCM with allulose followed by SLCM with mixed sugar syrup or sucralose had sensory profiles most similar to SLCM with sucrose by both trained panel and TDS. Chocolate milks with monkfruit or stevia had low bitter taste intensities, later sweet taste onset and lower viscosity than SLCM with sucrose. This study establishes a platform for sucrose replacement in SLCM.

Key Words: chocolate milk, school lunch, sugar reduction

SC116 A physicochemical investigation of flux decline in ultrafiltration of acid whey from tvarog production. J. Tarapata* and J. Zulewska, *University of Warmia and Mazury, Olsztyn, Poland.*

Due to increasing production of tvarog (acid coagulated cheese), popular in Eastern Europe, there is a need for optimized acid whey processing. The main challenge in protein concentration by means of ultrafiltration (UF) is membrane fouling. Thus, this study aimed to investigate flux decline in UF of rather rarely processed acid whey obtained during tvarog cheese and lactose-free tvarog cheese manufacture. Acid whey (normal (AW-N) and lactose-free (AW-LF)) was ultrafiltered using 20 nm ceramic membrane, at 50±1°C, cross-flow velocity of 4.1 m/s, and transmembrane pressure of 0.3 MPa, as recommended by membrane manufacturer. The average time of whey processing was 180 min. The reference process was UF of sweet whey (SW) conducted under the same conditions. The physicochemical properties of feed, permeate, retentate and 2 foulants streams obtained after UF: foulants located on the membrane surface and inside the pores were investigated. The average (n=3) flux decline was 26±4, 33±1 and 37±1% for SW, AW-LF and AW-N, respectively. The permeation fluxes (77±2 and 92±2 kg/m^2h) for AW-LF and AW-N were considerably higher ($P < 0.05$) than calculated critical value (~64 kg/m^2h) indicating protein deposition on membrane surface. SDS-PAGE electrophoresis showed that all major whey proteins were present in stream containing surface foulants. β-lactoglobulin and α-lactalbumin did appear to be the major cause of pore blocking. Dynamic Light Scattering analysis of particle size distribution showed multimodal size distribution in permeate streams shifted toward smaller particle sizes in contrast to unimodal (SW, AW-LF) and bimodal (AW-N) particle size distribution in feed. Higher Ca content in AW-LF and AW-N than in SW ($1174^{a}\pm24$ mg/L and $1180^{a}\pm74$ mg/L vs $366^{b}\pm50$ mg/L) contributed to significantly higher Ca content in stream containing internal foulants ($6.9^{a}\pm0.2$ mg/L and $3.7^{b}\pm0.4$ mg/L vs $2.2^{c}\pm0.8$ mg/L). The knowledge generated in this study advances the understanding of fouling in UF of polydisperse systems like tvarog acid whey in which diffusive transport is complex and not yet clearly understood.

Key Words: acid whey ultrafiltration, fouling, critical flux

SC117 Consumer understanding of fluid milk and cheese composition and processing. A. N. Schiano* and M. A. Drake, *North Carolina State University, Raleigh, NC.*

Ultrapasteurized and filtered (ultrafiltered or microfiltered) milk products are becoming common in the United States, but there is concern that consumers may form negative uninformed perceptions of these milks or cheeses made with them. The objective of this study was to explore consumer understanding of milk processing and constituents. We investigated 4 questions: (1) Does the average dairy product consumer (DPC) understand the basic nutrients in fluid milk and cheese? (2) Do they understand the basic processes for fluid milk and cheese? (3) Do different processes impact perception or purchase intent? (4) Does explaining a process change consumer beliefs about and purchase intent for dairy products? Qualitative interviews (n=54) and an online survey (n=1,210) were conducted with DPCs. Data were evaluated by nonparametric and parametric statistical analyses. The average DPC could recall key words related to dairy processing and composition, but was largely unfamiliar with these subjects. Highly educated and older consumers were more likely than other consumers to have a better understanding of dairy composition and nutrition ($P < 0.05$). Processing-related descriptors (e.g., ultrapasteurized or ultrafiltered) in ingredient statements were generally overlooked on labels; 34% of DPCs read the labels on dairy products. The majority (>80%) of DPCs were unfamiliar with filtered milk, but uninformed perceptions were generally positive. Consumers unfamiliar with processing methods were likely to assume that additional processing methods or terms increased the price of a dairy product ($P < 0.05$). Purchase intent for fluid milk and cultured dairy products was not impacted when non-conventional processing terms such as ultrafiltered or microfiltered were included in the ingredients statement ($P > 0.05$). This impact was consistent for fluid milk and Cheddar cheese but not for cottage cheese, suggesting possible product-specific effects. Providing a definition of filtration increased consumer understanding, positive beliefs, and purchase intent for fluid filtered milk and cheese made with it. Educating consumers through on-package labeling or marketing messaging should be investigated for ultrapasteurized or filtered milk products.

Key Words: consumer, ultrafiltration, ultrapasteurization

SC118 The impact of encapsulation on vitamin premix stability and flavor in ready-to-mix protein beverages. H. R. M. Keefer* and M. A. Drake, *North Carolina State University, Raleigh, NC.*

Vitamin fortification of ready-to-mix (RTM) protein beverages is desirable to many consumers. However, vitamins are unstable and degradation can lead to off-flavors in fortified foods. Encapsulation is used to improve stability and shelf life, but studies have not investigated shelf life of vitamin premixes or potential flavor impacts of these premixes in foods. The objective of this study was to determine encapsulated

(EN) and unencapsulated (UNEN) vitamin premix stability at 2 temperatures (21°C and 35°C) and to determine flavor effects on fortified ready-to-mix (RTM) protein beverages. Two vitamin premixes (EN and UNEN, vitamins B1, B2, B3, B5, B7, B9, D, E, and A) were obtained in duplicate lots. The premixes (subsampled into moisture barrier pouches) were stored at 21 and 35°C and sampled monthly through 6 mo. At each time point, fat- and water-soluble vitamin concentrations were documented by UPLC with a photodiode array detector. Additionally, at each time point, premixes were evaluated in vanilla whey protein RTM beverages (15 g protein/240 mL serving) by descriptive sensory analysis. Vitamin degradation and beverage sensory properties were compared using 2-way ANOVA (EN/UNEN × vitamin premix storage temperature) with Fisher's least significant difference (LSD) for means separation. All vitamins evaluated decreased in concentration with storage time and decreased faster at 35°C compared with 21°C with the highest degradation noted in vitamins A and B1 ($P < 0.05$). Vitamin loss in encapsulated premixes was slower than that in unencapsulated premixes at both storage temperatures ($P < 0.05$). A distinct perfumey/carrot flavor was documented by mo 1 in RTM beverages with EN and UNEN stored at 35°C, by mo 2 in RTM beverages with UNEN at 21°C, and by mo 3 in RTM beverages with either premix at both storage temperatures. The perfumey/carroty flavor was attributed to vitamin A degradation. This study demonstrates vitamin premix stability and its role in sensory properties of RTM protein beverages.

Key Words: vitamin premix, stability, protein beverages

SC119 Consumer perception of natural hot pepper cheeses.

C. M. Racette* and M. A. Drake, *North Carolina State University, Raleigh, NC.*

Hot pepper cheese (HPC) is a growing category of flavored natural cheese. With increasing consumer interest in this category, it is important for manufacturers to understand consumer perceptions of HPC. The objective of this study was to evaluate consumer perception of HPC using a combination of quantitative survey methods and consumer evaluation of actual HPCs. An online survey (n = 510) was conducted to understand drivers of purchase for the hot pepper cheese category. Hot pepper cheese consumers answered Maximum Difference (MXD) exercises and an Adaptive Choice Based Conjoint (ACBC) activity focused on hot pepper cheese attributes. Subsequently, natural HPC (n = 6) were manufactured in duplicate with different hot pepper blends with a range of heat/burn intensities and distinct colors. Trained panel profiling and a consumer acceptance test (n = 194 consumers) were applied to the cheeses. Univariate and multivariate statistics were used to evaluate the data. Three clusters of consumers were identified from the survey. Cluster 1 (n = 175) placed value on visual characteristics of HPC, while cluster 2 (n = 152) preferred milder HPCs. Cluster 3 (n = 183) showed the most interest in novel HPCs, such as those made with habanero peppers or white Cheddar cheese. Conceptually, the overall ideal HPC was a Monterey Jack with medium-sized, multicolored pieces of jalapeno peppers and a moderate heat/burn intensity. Heat/burn intensity and type of cheese were the most important attributes in the MXD exercise followed by price and appearance. Trained panelists confirmed that the 6 HPC used for consumer testing had a distinct range (low to high) of heat/burn intensity. Consumer overall liking increased as heat/burn intensity increased to a certain point, indicating consumers have an optimal point for heat/burn in HPCs. Consumers also preferred HPCs with multicolored pepper pieces over those with a singular pepper color, which was consistent with ACBC results. This study demonstrates that most HPC consumers prefer HPCs with higher heat/burn intensity and are also motivated by visual characteristics of HPCs.

Key Words: hot pepper cheese, consumer, drivers of liking

ADSA Graduate Student Production MS Oral Competition

SC120 Effects of weaning strategies on intake, growth, and health in Holstein dairy calves. A. Wolfe*[1,2], P. Rezamand[2], B. Agustinho[2], D. Konetchy[2], and A. Laarman[1], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*University of Idaho, Moscow, ID.*

The weaning transition is a stressful time for young dairy calves, yet little is known about stress indicators at different ages. An objective of this study was to examine the effects of calf weaning age (6 vs. 8 wk) and pace (abrupt vs. gradual) on health and production parameters in dairy calves. Holstein calves (n = 40), blocked by sex and birth weight, were assigned to one of 4 treatments (n = 10 per treatment): Early-Abrupt (EA), Early-Gradual (EG), Late-Abrupt (LA), and Late-Gradual (LG). Milk replacer (24% CP, 17% fat; restricted up to 1,200 g/d) was fed twice daily; water, calf starter (18% CP), and alfalfa hay were fed ad libitum. Daily intakes of milk replacer, calf starter, and forage were recorded. Body weight, health measures, and fecal scores were obtained before, and after weaning. Calves were orally bolused with a rumen pH logger for the last 3 d of the weaning transition and rumen pH was measured continuously. Data were analyzed with age and pace as fixed effects. Age at weaning, but not pace, tended to increase respiration (39.9 vs. 43.8 bpm, $P = 0.07$). Heartrate was higher in abrupt than in gradual groups ($P < 0.01$). Fecal score was greater in EG than in EA (0.76 vs. 0.22, $P = 0.02$). Body core temperature showed no significant difference among treatments in age or pace. During the weaning transition, average daily gain increased from EA to LA (0.35 vs. 1.05 kg/d, $P = 0.01$). Grain intake change during weaning increased by age ($P < 0.01$) regardless of weaning pace. Forage intake change during weaning tended to increase by pace ($P = 0.07$) and is most evident by the increase from EA to EG (0.04 vs. 0.18% BW, $P = 0.02$). Minimum rumen pH tended to decrease by age ($P = 0.08$) regardless of weaning pace. Mean rumen pH increased from EG to LG (7.07 vs. 8.02, $P = 0.03$) and from LA to LG (6.9 vs. 8.02, $P = 0.01$). The duration of subacute ruminal acidosis tended to increase from EA to LA (0 vs. 271.44 min/d, $P = 0.05$). Overall, intake, growth, and health parameters suggest calves are more resilient to weaning stress by 8 weeks. Forage intake and rumen pH data suggest gradual weaning confers physiological benefits to the rumen.

Key Words: weaning, health, calves

SC121 Effect of fibrolytic enzyme on lactational performance, feeding behavior, rumen fermentation and nutrient digestibility in dairy cows fed whole-plant faba bean silage-based diet. J. Yang*[1], D. Christensen[1], H. Lardner[1], V. Guevara-Oquendo[1], B. Refat[1], O. AlZahal[2], and P. Yu[1], [1]*University of Saskatchewan, Saskatoon, SK, Canada,* [2]*AB Vista, Marlborough, United Kingdom.*

The objectives of this study were to evaluate the effects of pre-treating whole-plant fava bean silage-based diet with exogenous fibrolytic enzyme derived from *Trichoderma reesei* (FETR; mixture of xylanase and cellulase; AB Vista, Wiltshire, UK) on lactational performance, digestibility, rumen fermentation characteristics, and feeding behavior in lactational dairy cows. The animal trial was conducted using 8 Holstein dairy cows (BW = 710 ± 44 kg and DIM = 121 ± 17) with 4 enzyme treatments (0, 0.5, 0.75, and 1 mL of FETR /kg dry matter of silage) in a double Latin square design. The cows were given a total mixed ration (TMR) composed of 49% (DM basis) forage and 51% concentrates. The enzyme levels used in this trial were selected from previous in situ and in vitro studies which showed positive responses on whole-plant fava bean silage. The enzyme treatments were sprayed directly on the silage 1 h before feeding. The response of NDF digestibility and digestible NDF to the increasing level of FE was linear ($P < 0.05$), where the enzyme group (0.5 mL of enzyme/kg of silage DM) exhibited the highest NDF digestibility (48.5%). The enzyme application significantly affected on percentage of milk fat and milk fat yield which was linearly ($P < 0.05$) differed among treatments, being highest (4.35%, 1.82 kg/d) for low dosage groups. The milk yield in Control averaged 41.2 kg/d with 4.35% fat. Both energy (ECM, $P = 0.018$) and fat-corrected milk yield (FCM, $P = 0.058$) were linearly affected or tended to be affected by fibrolytic enzyme dose level. Adding enzyme to the diet had linearly ($P < 0.05$) affected FCM production efficiency (kg of FCM/kg of DMI). The ECM production efficiency (kg of ECM/kg of DMI) was cubically ($P < 0.05$) affected by the enzyme application. Consequently, this study demonstrated positive effects of pre-treating whole-plant fava bean silage with lower dose level (0.5 mL of FETR /kg dry matter of silage) of fibrolytic enzyme to dairy cows which could benefit the development of a new and alternative feeding strategy in Western Canada.

Key Words: faba bean silage, fibrolytic enzyme, dairy cow

SC122 Physiological effects of the SLICK1 allele of the prolactin receptor (*PRLR*) gene in Holstein calves during heat stress. A. T. Carmickle*[1], C. C. Larson[2], F. Sosa[3], L. M. Jensen[3], M. Haimon[3], P. J. Hansen[3], and A. C. Denicol[1], [1]*Department of Animal Science, University of California-Davis, Davis, CA,* [2]*Okeechobee County Cooperative Extension Service, University of Florida IFAS Extension, Okeechobee, FL,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The SLICK1 allele of the prolactin receptor (*PRLR*) gene, i.e., slick mutation, has been shown to help mitigate the detrimental effects of heat stress (HS) in lactating Holstein cows. The goal of this study was to evaluate whether inheritance of the SLICK1 allele would alter physiological responses of growing Holstein female calves to HS. Holstein cows in 3 dairy farms located in south Florida were inseminated with semen from 2 heterozygous slick Holstein sires to produce slick and non-slick calves. The experiment was performed in July 2020 when calves born in group 1 (12 slick and 18 non-slick calves) were 7–8 mo. and group 2 (n = 8 slick and 8 non-slick calves) were 10–55 d of age. Rectal temperature (RT), respiration rate (RR; breadths/min), surface temperature (ST; infrared thermometer), and sweating rate (SR; transepidermal water loss instrument) were measured between 1200 and 1400 H. Dry bulb temperature, relative humidity and dew point were recorded every 15 min for the duration of data collection and used to calculate the temperature-humidity index (THI). ST and SR measurements were obtained from a shaved area posterior to the shoulder blade and an unshaved area immediately next to it. Data were analyzed using the GLIMMIX procedure of SAS version 9.4. The model included the effects of genotype, farm, sire, and the interactions between genotype and farm, sire and farm, and sire and genotype. THI was included as a covariate. Significant effects were considered as those with $P < 0.05$. The THI varied between 80.5 and 84 during the collection period. Both young and older slick calves had lower RT compared with non-slick calves (group 1: 40.2 ± 0.1 vs 40.6 ± 0.1°C; $P < 0.01$; group 2: 39.0 ± 0.2 vs 39.7 ± 0.3; $P < 0.05$). In group 1, slick daughters of sire 1, but not sire 2, had lower RR (sire 1: 84 ± 8 vs 108 ± 7; sire 2: 86 ± 8 vs 75 ± 7; $P < 0.05$). Group 2 slick calves had lower ST in the shaved skin (33.9 ± 0.5 versus 36.1 ± 0.8°C; $P < 0.05$). Results confirm that inheritance of the SLICK1 allele can improve ability of growing Holstein calves to regulate body temperature during HS. Funding provided by the Holstein Association USA Research Grant Program and L.E. "Red" Larson Endowment.

Key Words: Slick, Holstein, thermotolerance

SC123 Effects of partially replacing corn with molasses, untreated whey, and treated whey on in vitro ruminal fermentation in a dual-flow continuous culture system. A. D. Ravelo*[1], B. C. Agustinho[2], J. A. Arce-Cordero[1], H. F. Monterio[1], S. L. Bennett[3], E. Sarmikasoglou[1], J. R. Vinyard[1], E. R. Q. Vieira[4], R. R. Lobo[1], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Moscow, ID,* [3]*Department of Animal Science, Penn State University, University Park, PA,* [4]*Department of Animal Sciences, Federal University of Tocantins, Brazil.*

The objective of this study was to evaluate the effects of partially replacing dietary corn with by-products in lactating dairy cow diets. Using 8 fermenters in a 4 × 4 replicated Latin-square with 4 periods of 10 d each, we tested 4 treatments: 1) control with corn – CON, 2) molasses - MOL, 3) untreated whey (WHEY), and 4) whey treated with a caustic agent (TWHEY). Diets were formulated by replacing 4% of corn starch for sugars from the by-products, represented as water-soluble carbohydrates (WSC), in the rations. The fermenters were fed twice daily. After 7 d of adaptation, in the last 3 d of each period samples were collected for VFA, lactate, and ammonia analysis, while pH was monitored at 0, 1, 2, 4, 6, 8, and 10 h post morning feeding. Pooled samples from effluent containers were also collected for analyses of VFA and lactate, nutrient digestibility, and N metabolism. Statistical analysis was performed with MIXED procedure of SAS using multiple comparisons and significance was declared when $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. There tended to be an increase in pH in MOL and TWHEY compared with WHEY ($P = 0.06$). Total VFA concentration increased in WHEY compared with MOL ($P = 0.02$). Acetate to propionate ratio increased in TWHEY compared with CON ($P = 0.03$); however, only tendencies where observed in the acetate and propionate molar proportions ($P = 0.07$; $P = 0.09$). Isobutyrate molar proportion was increased in CON ($P = 0.03$). Caproate molar proportion was increased in TWHEY ($P = 0.03$). Lactate concentration was greatest in MOL ($P < 0.01$). There were no differences in N utilization among treatments. Additionally, there was increased digestibility of WSC in MOL, WHEY, and TWHEY compared with CON ($P < 0.01$). There were no differences in other nutrients digestibility. Based on these results, it can be concluded that dietary inclusion of molasses (6.58%), whey (4.37%), or treated whey (4.61%) as replacements of corn has minimum effects on ruminal fermentation and nutrient utilization in dairy diets.

Key Words: digestibility, fermentable carbohydrates, starch

SC124 Effects of reducing dietary cation-anion difference on total-tract digestibility and urine and fecal outputs of lactating dairy cows. H. M. Zynda*[1], J. E. Copelin[1], W. P. Weiss[1], F. Sun[2], and C. Lee[1], [1]*The Ohio State University, Wooster, OH,* [2]*Origination O2D, Woodbury, MN.*

Ammonia emissions from manure can be mitigated by reducing the pH of manure slurry through direct addition of strong acids. However, this increases manure management costs and health hazards for farm operators. Reducing the dietary cation-anion difference (DCAD; Na + K − Cl − S) of a lactating cow diet may be an option to reduce manure pH through reducing urine pH. However, it was regarded that increasing DCAD above the normal level (about 200 mEq/kg DM) has potential to increase DM and NDF digestibility and production, meaning that reducing DCAD affects those negatively. We determined the effects of reduced DCAD on nutrient digestibility and manure characteristics. Twenty-seven mid-lactation cows were blocked by parity and DIM in a randomized complete block design and assigned to 1 of 3 isoenergetic and isonitrogenous diets: CON, with DCAD of 192 mEq/kg DM; MID, CON but with reduced DCAD (101 mEq/kg DM); and LOW, a diet with DCAD 1.2 mEq/kg DM. An anionic product (MegAnion) replaced urea and some soybean meal in MID and LOW to lower DCAD. The experiment lasted 7 wk (1-wk covariate followed by 6-wk data collection). Spot fecal sampling was conducted to determine nutrient digestibility with indigestible NDF as a marker. Data were analyzed using the MIXED procedure, block was a random effect and diets, repeated wk, and interactions were fixed effects. Nutrient intake and total-tract digestibility of DM, OM, CP, and NDF did not differ among treatments (66, 67, 69, and 39%, respectively). Fecal output was not different among treatments, but urine output (20.4, 17.0, and 23.1 kg/d; quadratic $P = 0.04$) was greater for LOW, which numerically decreased the weight ratio of feces to urine from 3.2 to 2.6. Urine pH was decreased quadratically (8.26, 8.05, 6.38 for CON, MID, and LOW, respectively; $P < 0.01$). In conclusion, reducing DCAD did not negatively affect nutrient digestibility, contrary to previous literature. Additionally, decreasing the ratio of feces to urine with decreased urine pH indicates feeding reduced DCAD has potential to reduce NH_3 emissions from manure.

Key Words: dietary cation-anion difference, urine, feces

SC125 Bio-based plastics demonstrate degradation during in vitro fermentation. H. Galyon*, S. Vibostok, G. Ferreira, A. Whittington, and R. Cockrum, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Replacing agricultural plastics with bio-based polyhydroxyalkanoates (PHA) could mitigate deleterious effects of plastic ingestion in domestic ruminants. The objective of this study was to determine PHA and copolymer ruminal degradability. Commercial PHA, polybutylene succinate adipate (PBSA), and PBSA/PHA pellets, PBSA/PHA filament, and forage controls were incubated in Daisy^{II} Incubator jars for 0, 3, 6, 12, 24, 48, 96, and 240 h in F57 and R510 bags. Digestion kinetic parameters were estimated using PROC NLIN in SAS according to the predicted digestibility equation. Thermogravimetric and differential scanning calorimetry analyses were conducted on samples incubated for 0, 24, 96, and 240 h in F57 bags. Mass loss was analyzed using PROC MIXED in SAS with fixed effects of bag type, treatment, and time and random residual error. Onset degradation temperature (T_o) and melting peak temperature (T_m) were analyzed with fixed effects of treatment and time and random residual error. The filament had 0.90 ± 0.10% ($P < 0.01$) more mass loss than the average of the remaining biopolymers at 240 h. Remaining biopolymers did not differ from one another. Mass loss was significant by 96 h but did not change thereafter ($P < 0.01$). Degradation kinetics demonstrated that biopolymer treatments were still in the exponential phase of degradation at 240 h, though projected degradation did not exceed 2% after 500 h. T_o decreased only from 0 to 24 h for PBSA (151.05 ± 15.75°C, $P < 0.01$), biopolymer blend (119.50 ± 15.75°C, $P < 0.01$), and the filament (114.00 ± 15.75°C, $P < 0.01$). PHA T_o decreased only for 0, 24, and 96 h consecutively (269.55, 213.15, 144.60°C, respectively; $P < 0.01$). T_m differed across incubation time within the blend and filament in a non-specific manner ($P < 0.01$), but did not differ for PHA and PBSA. Based on these preliminary findings, we suggest the first stage of degradation is occurring within 24 h and a filament copolymer blend degrades more quickly. However, though polymer chains are breaking down, mass loss was unsubstantial by 240 h. Further ruminal degradation studies of biopolymers are warranted to elucidate maximum degradation.

Key Words: bio-based plastics, degradation kinetics

SC126 Modeling B_9 and B_{12} synthesis and use within the rumen in dairy cows.

V. Brisson*[1], C. Girard[2], J. Metcalf[3], D. Castagnino[3], J. Dijkstra[4], and J. Ellis[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [3]*Trouw Nutrition Canada, Guelph, ON, Canada,* [4]*Wageningen University and Research, Wageningen, the Netherlands.*

The long-accepted idea that B-vitamin (BVit) needs of ruminants are entirely supported by the rumen microbiota's ability to synthesize them has been challenged by positive responses to rumen-protected BVit supplementation. While BVit deficiencies are rare in dairy cows, signs of subclinical deficiency could have an important impact on their overall health and milk production efficiency. The objective of this study was to create empirical models through meta-analyses, to describe the apparent ruminal synthesis (ARS), representing the summation of BVit synthesis and degradation by the rumen microbiota. Data utilized were from 340 individual lactating cows from 16 published studies, from which saved diets, containing no B-vitamin supplementation, and duodenal samples were analyzed for B-vitamin content, creating the database used in the present study. A Spearman correlation matrix was first constructed between all potential driving variables to assess for collinearity and guide model creation. A suite of potential models (with study treated as a random effect) were then developed in GLIMMIX. Finally, statistically significant models were chosen based on concordance correlation coefficient (CCC). The best performing models were: Folate ARS (mg/d) = −65.4 (±12.08) + 1.6 (±0.29) × DMI (kg/d) + 0.14 (±0.034) × Microbial N flow (g/kg of DM) + 0.10 (±0.020) × Digestible starch (g/kg of DM); CCC: 0.789 and Cobalamin ARS (mg/d) = −5.0 (±3.25) + 0.47 (±0.056) × DMI (kg/d) + 0.045 (±0.0191) × Total VFA (mM); CCC: 0.914. These models indicate that while the digestible starch content increases folate ARS, it is not a main contributing factor in the ARS of cobalamin. The latter can be explained by either a decreased utilization or increased synthesis of cobalamin, as demonstrated by its positive relationship with VFA concentration. These findings suggest that folates and cobalamin may be synthesized and used by different rumen microbial populations indicating that BVit supply and requirements need to be evaluated individually in lactating dairy cows, as they are a very heterogeneous group of molecules with different properties.

Key Words: meta-analysis, folate, cobalamin

SC127 Association of pretreatment somatic cell counts with bacteriological cure.

J. Williamson* and V. Ryman, *University of Georgia, Athens, GA.*

Milk somatic cell count (SCC) increases during mastitis. Previous work demonstrated associations between pre-treatment SCC and a cure or failure of the antibiotic regimen. Preliminary data conducted at UGA demonstrated that a lower SCC at the time of intramammary antibiotic therapy was associated with bacteriological cure of mastitis. Thus, we hypothesized that when antibiotic therapy is administered according to specific SCC guidelines, cure rates are increased. Aseptic milk samples were collected from quarters of lactating cows experiencing clinical or subclinical mastitis (n = 36). Clinical mastitis was diagnosed by a trained milking technician and subclinical mastitis was diagnosed as a SCC >200,000 cells/mL and bacterial growth present at time of treatment. Following detection and collection of the d 0 (D0) milk samples, intramammary antibiotic therapy, SpectramastLC, was administered once/day for 5 d. The SCC was enumerated and the milk sample cultured for bacteria. Milk samples were streaked onto trypiticase soy agar plates with 5% sheep blood and incubated at 37°Celcius for 48 h. Samples were collected 14 d (D14) and 28 d (D28) post-treatment. A bacteriological cure was confirmed when both the D14 and D28 samples were free of bacteria. Average SCC at each time point was statitistically evaluated utilizing a one-way ANOVA procedure, with $P < 0.05$ deemed statistically signficant. In quarters that cured, the average D0 SCC was 552,800 cells/mL. In contrast, the D0 SCC of quarters that failed to cure was significantly higher at 1,436,900 cells/mL ($P = 0.02$). The SCC decreased in cured quarters on D14 (248,000 cells/mL) and D28 (191,700 cells/mL). While there was a numerical decrease in SCC at D14 in quarters that failed to cure (1,104,400 cells/mL), ultimately the SCC rose again at D28 (1,723,500 cells/mL). Overall, the cure rate was 59%. As SCC increased, the % of quarters that cured decreased. For example, quarters treated with SCC ≤250,000 cells/mL, demonstrated a cure rate of 73%. Whereas quarters treated with SCC ≤550,000 cells/mL, demonstrated a cure rate of 59%. In conclusion, current data collected suggests that quarters averaging 1.4 million cells/mL before therapy will fail to cure, whereas quarters with SCC closer to 550,000 cells/mL will successfully cure. Ultimately, we hope to evaluate the impact of modifying a mastitis control program on economic and health parameters of a dairy operation.

Key Words: mastitis, bacteriological cure, intramammary therapy

SC128 Effects of heat stress abatement strategy during the preweaning on lying behavior and behavioral responses and pain sensitivity following disbudding of Holstein calves.

A. B. Montevecchio*[1], W. Frota[1], V. R. Merenda[1], J. G. Martin III[2], and R. C. Chebel[1], [1]*Department of Large Animal Clinical Sciences and Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Dairy Design Engineers, Gainesville, FL.*

Our objectives were to determine the effects of heat stress abatement strategies on behavior and disbudding related behavior and mechanical nociceptive threshold (MNT). At birth, male calves were assigned to: HS (n = 20) - hutches outside, covered with plywood (50% hutch area), SH (n = 21) - hutch in a barn with no cooling, and SHF (n = 19) - hutch in a barn with ceiling fans. Calves were fitted with data loggers on the hind leg and lying behavior was recorded from 1 to 70 d of age. At 32 ± 8 d of age, calves were disbudded using hot iron. On the day of disbudding, MNT was determined using an algometer in 4 points surrounding each horn. Cornual nerves were blocked with lidocaine and the disbudding was carried out ~30 min later. Calves were evaluated for MNT at h 1, 2, 3, 24, and 72 and d 7 and 14 after disbudding and for behavioral responses (ear and tail flick, headshake and rubbing, foot stamping, and restlessness) at h 1, 2, and 3 after disbudding. Data were analyzed by ANOVA and logistic regression. Calves in the SHF treatment (18.5 ± 0.2 h/d) had greater lying time than calves in the HS (18.0 ± 0.2 h/d; $P = 0.09$) and SH (17.8 ± 0.2 h/d; $P < 0.01$). Calves in the SHF treatment had ($P < 0.01$) the fewest lying bouts per day; thus, the SHF treatment had ($P \leq 0.01$) the longest lying bout duration. Within 3 h of disbudding, the number of disbudding related behaviors was not ($P = 0.60$) different among treatments, but the interaction between treatment and hour affected ($P = 0.04$) MNT. At 1 h after disbudding, calves in the

HS (6.9 ± 0.2 kgf) treatment had ($P < 0.01$) greater MNT than calves in the SH (6.2 ± 0.2 kgf) treatment. Treatment did not ($P = 0.62$) affect wound healing 7 d after disbudding, but a greater percentage of calves in the HS treatment had ($P = 0.03$) abnormal wound healing 14 d after disbudding (HS = 36.4 ± 8.3, SH = 15.2 ± 5.9, SHF = 10.7 ± 5.3%). The data demonstrate improvement in welfare measured by greater lying time and lying bout duration and improves wound healing following disbudding when heat stress abatement is utilized.

Key Words: heat stress; disbudding; behavior

ADSA Graduate Student Production MS Poster Competition

SC129 Evaluating skim milk as a substrate for the biomanufacturing of value-added ingredients and products. L. Wise*, *Cornell University, Ithaca, NY.*

Recent decreases in skim milk consumption have challenged the dairy industry, with resultant increases in waste and reduction in its use in foods despite its nutritional quality. Repurposing skim milk is therefore crucial to avoid loss and provide alternative uses. For example, valuable products can be derived from fermenting lactose in skim milk. In fact, several yeast species can ferment lactose into ethanol. Alternatively, when lactose is hydrolyzed into glucose and galactose, *Brettanomyces* yeast can selectively ferment glucose into ethanol, leaving behind galactose, a useful ingredient in snacks, sweeteners, pharmaceuticals and galactooligosaccharides. The goal of this study is to screen parameters for optimal ethanol and galactose yields in skim milk fermentations using *Brettanomyces claussenii and Kluyveromyces marxianus*, 2 lactose-hydrolyzing yeasts, as well as *Saccharomyces cerevisiae and Brettanomyces bruxellensis*. A minimum of 5×10^6 cfu/mL of each species was pitched into 2 bottles of skim milk, one treated with lactase, the other without. Triplicate fermentations were run and pH measured continuously. Cell counts and densities were determined on alternate days, and fermentations run until all densities were level for 3 straight days. HPLC samples were drawn at the beginning and end of each replicate to isolate the contents of lactose, glucose, galactose, ethanol and organic acids. Residual galactose levels in *B. claussenii* were erratic and lower than anticipated. The highest ethanol productions were recorded in *K. marxianus* and *B.* claussenii, particularly without lactase, where average final ethanol concentrations approached 2.5%. The variance yielded a P-value < 0.0001, meaning ethanol production differed across species. A Tukey's test confirmed that ethanol production in *B. claussenii* and *K. marxianus* was different from that of the 2 other species. Further research will aim to improve fermentation stability and the sensory properties of the fermented milk, and optimize ethanol and galactose yields using response surface methodology.

Key Words: lactose, ethanol, galactose

SC130 Impact of increasing dietary cottonseed on rumen fermentation, nutrient digestibility, and microbial community composition in continuous culture fermenters. C. Castro*, N. Baghme, and F. Batistel, *Utah State University, Logan, UT.*

In this study, we determined the impact of increasing dietary cottonseed (CS) on rumen fermentation, nutrient digestibility, and microbial community composition. The study was conducted as a replicated 4 × 4 Latin square using continuous culture fermenters (n = 8). Treatments were a control diet without CS, and the control diet plus 5, 10, or 15% of CS. The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate fed twice daily. The prokaryotic community was determined by sequencing the V4 region of the 16S rRNA gene, and protozoa were counted. Data were analyzed using a mixed model including the fixed effect of treatment and the random effects of period and fermentor. Linear, quadratic and cubic contrasts were tested. No treatment effect was observed for NDF and starch digestibility as well acetate concentration ($P \geq 0.23$). Butyrate and ammoniacal N concentrations increased (Quadratic, $P \leq 0.05$), while propionate concentration tended to decrease (Quadratic, $P = 0.08$) as CS was added. Increasing CS in the diet, decreased protozoa count and relative abundance of archaea (Linear, $P = 0.01$). Analysis of the phyla abundance showed that 6 phyla were affected by treatments. The most abundant phyla across treatments were the *Firmicutes* and *Bacteroidetes*. *Firmicutes* abundance increased whereas *Bacteroidetes* decreased with the addition of CS (Linear, $P \leq 0.05$). *Lachnospiraceae* and *Prevotellaceae* were the most abundant bacteria families in all the samples. No treatment effect was observed for *Lachnospiraceae*, while *Prevotellaceae* abundance tended to decrease as CS was added to the diet (Linear, $P = 0.07$). The 2 most abundant genera across samples were *Prevotella* and *Pseudobutyribrio,* and the addition of CS decreased the abundance of both (Linear, $P \leq 0.05$). Our preliminary results indicate that increasing levels of CS up to 15% DM diet do not negatively impact fiber and starch digestion in the rumen. Furthermore, the inclusion of CS affected the microbial community composition at different levels.

Key Words: bacteria, fiber, protozoa

SC131 Earlier administration of an internal teat sealant in primigravid dairy heifers to prevent intramammary infections at calving. L. R. Larsen*[1], P. H. Baker[1], K. M. Enger[1], L. E. Moraes[2], and B. D. Enger[1], [1]*The Ohio State University, Wooster, OH,* [2]*The Ohio State University, Columbus, OH.*

Intramammary infections (IMI) are common in primigravid dairy heifers and negatively impact future milk production. Bismuth subnitrate-based internal teat sealants (ITS) have been used to prevent prepartum IMI in dairy heifers by creating a physical barrier within the teat, preventing pathogens from entering the gland, though determination of when to administer ITS in heifers has yet to be investigated. The objectives of this study were to determine if infusion of ITS during first gestation reduces the risk of IMI at calving and if administering ITS at different stages of gestation (75 vs 35 d prepartum) differentially affects IMI prevalence at calving. A total of 270 heifers were utilized at a single farm. One-quarter of each heifer was randomly assigned to receive ITS 75 d prepartum (TS75), another quarter of each heifer received ITS 35 d prepartum (TS35), while the remaining 2 quarters served as control quarters (CON). After calving, aseptic colostrum samples were collected from all quarters and were cultured to determine quarter infection status. Data were analyzed using PROC GLIMMIX to yield odds ratios (OR); ITS treatment and quarter location (fore or rear) were included as fixed effects while heifer was a random effect. For major mastitis pathogens, CON quarters were 3.5 times (95% CI: 1.5–8.0) and 3.4 times (95% CI: 1.5–7.8) more likely to be infected at calving than TS75 and TS35 quarters, respectively. For minor mastitis pathogens, CON quarters were 5.2 (95% CI: 3.0–9.2) and 4.9 (95% CI: 2.8–8.7) times more likely to be infected than TS75 and TS35 quarters, respectively. Odds of IMI at calving was not affected by quarter location ($P = 0.07$) and were similar between TS75 and TS35 quarters for both major (OR = 1.0) and minor (OR = 1.1) mastitis pathogens. Results indicate that ITS administration at either 75 and 35 d prepartum significantly reduced IMI prevalence at calving in primigravid dairy heifers and that there was no disadvantage to earlier ITS application. Farm factors may influence timing of heifer IMI and earlier administration of ITS provides an extended period of protection for the developing gland.

Key Words: mastitis, heifer mastitis, IMI

SC132 Effects of treating soybean meal on ruminal fermentation, microbial growth, nutrient digestion, and nitrogen partitioning in a dual-flow continuous culture system. A. Bahman*[1], H. F. Monteiro[1], A. D. Ravelo[1], J. Arce-Cordero[1], T. S. Winowiski[2], and A.

P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*Borregaard USA Inc., Rothschild, WI.*

The objective of this study was to evaluate the effect of 3 different protein supplements on ruminal fermentation, nutrient digestibility, microbial efficiency, and ruminal nitrogen partitioning. The treatments were: a control soybean meal (SBM), the control soybean meal treated with 0.75% amino resin (ARSBM), and the control soybean meal heat-treated (HTSBM). Experimental design was set up as a replicated 3 × 3 Latin square with 6 fermenters in a dual-flow continuous culture system. Treatments were randomly assigned within Latin square for each period. Each fermentor was fed 106 g/d of diet DM equally distributed in 2 feeding times per day at 0800 and 1700. Diets were formulated to contain 16% CP, 30% NDF, and 30% starch across treatments. The experiment consisted of 3 experimental periods, each lasting for 10 d, for a total of 30 d of fermentation. The first 7 d of each period was considered adaptation, and the last 3 d were used for sampling and data collection. On d 8 and 9, samples were collected for pH, volatile fatty acid (VFA), lactate, and NH_3-N kinetics. Days 8, 9, and 10, samples were collected for VFA and NH_3-N pools, digestibility measurements, and bacterial analysis. Data were analyzed using the GLIMMIX procedure of SAS and significance was declared when $P \leq 0.05$. No effects were observed for pH or lactate. Ammonia concentrations were significantly different among treatments. Compared with the control SBM (16.91 mg/dL), both the ARSBM (8.24 mg/dL) and the HTSBM (7.99 mg/dL) had lower NH_3-N concentrations, indicating a lower ruminal protein degradation and greater ruminal undegraded protein supply. Our preliminary NH3-N results indicate that the treatment of soybean meal could be effective in increasing ruminal undegraded protein levels in the diet.

Key Words: rumen-undegradable protein, rumen-degradable protein, ruminal ammonia

ADSA Graduate Student Production PhD Oral Competition

SC133 Diurnal variation of milk fatty acids in early-lactation Holstein cows with and without hyperketonemia. C. Seely*[1], K. Bach[2], D. Barbano[3], and J. McArt[1], [1]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [2]*Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [3]*Department of Food Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY.*

Estimates of milk constituents by Fourier-transform mid-infrared (FTIR) analysis offer a promising tool in identifying dairy cows experiencing excessive energy deficit (EED). Our objectives were to describe the diurnal variation of FTIR estimates of milk fatty acids (FA) and the effect of hyperketonemia on this variation. Blood samples were collected bihourly for 5 d from multiparous Holstein cows (n = 28) enrolled between 3 and 9 DIM. Cows were fed ad libitum once daily at 0900 h, and milk samples were collected thrice daily at 0600 h (M1), 1400 h (M2), and 2200 h (M3) for d 2, 3, and 4 of the study period. Cows were classified as hyperketonemic (HYK; n = 13) if their average daily blood BHB was ≥1.2 mmol/L for ≥3 d or HYK negative (non-HYK; n = 15) if their average daily blood BHB was ≥1.2 mmol/L for ≤2 d. Milk samples were analyzed via FTIR for de novo, mixed, and preformed FA yield and relative percentage (g/100 g FA; rel %), and yield of C18:0 and *cis*-9 C18:1. Generalized linear mixed models were used to analyze concentrations of FA over time and differences between HYK groups. De novo FA rel % were greater in non-HYK cows than HYK cows ($P = 0.01$; 18.7 ± 1.4% and 15.8 ± 1.5%, respectively) and differed by milking time ($P < 0.001$), with peak concentrations occurring at M1 and nadirs at M2. The HYK cows had a greater rel % of preformed FA than non-HYK cows ($P = 0.03$; 51.1 ± 3.0% and 46.2 ± 2.8%, respectively) and differed by milking time ($P < 0.001$), reaching a nadir at M1 and peaking at M2. The yield of preformed FA was greater in HYK cows compared with non-HYK cows ($P = 0.05$; 336.0 ± 25.8 g and 298.0 ± 24.1 g, respectively) and differed by milking time ($P = 0.001$), with the greatest yields occurring at M2 and lowest yields at M1. Yields of C18:0 and *cis*-9 C18:1 varied by milking time ($P < 0.001$); both with nadirs at M1 and peaks at M2. The yield of C18:0 was greater in HYK cows than non-HYK cows ($P = 0.01$; 84.6 ± 5.6 g and 72.1 ± 5.5 g, respectively). Our findings support the use of FTIR estimates of milk FA to identify cows experiencing EED, however time relative to feeding should be considered when analyzing milk FA.

Key Words: hyperketonemia, FTIR, fatty acids

SC134 Barriers to implementing transition cow protocols in the United States. A. Sen* and E. Eckelkamp, *University of Tennessee Institute of Agriculture, Knoxville, TN.*

Transition cow protocols (TCP) can help producers to ensure successful fresh-cow transitions. Our objective was to assess barriers to TCP implementation in US dairy farms. A survey was distributed across the US electronically, by mail, and through ads in popular dairy magazines in October 2020. Data were collected on producers' farm demographics, existing TCP, fresh-cow management, and perceived transition cow disease (TCD) prevalence and TDC impacts on-farm profitability. Frequencies were calculated using the FREQ procedure of SAS 9.4. We received 71 responses across the US, with 68% of responses from Tennessee. Respondents were farm owners (63%; n = 45), managers (21%; n = 15), or other (16%; n = 11). Most (55%; n = 39) herds had 100 to 500 cows, only 13 farms had <100 and 19 farms had >500 cows. Rolling herd mean production was <7,727 (20%; n = 14), 7,727 to 12,272 (65%; n = 46), or >12,272 (15%; n = 11) kg/lactation. Bulk tank somatic cell count was <250,000 (53%; n = 37), 250,000 to 399,000 (46%; n = 32), or 400,000 to 749,000 (1%; n = 1) cells/mL. Only 24% (n = 16) of the respondents had a written TCP posted in the farm. Most producers never checked for rectal temperature (56%; n = 38), ketosis (57%; n = 38), displaced abomasum (49%; n = 33), and body condition score (38%; n = 25) and never provided propylene glycol (52%; n = 33) or transition cow paste (57%; n = 38) to fresh cows in the first 21 d after calving. Only 48% (n = 34) checked for subclinical diseases. Most respondents could clearly define the transition period (71%; n = 50) and knew how to implement TCP (70%; n = 49) and its importance (86%; n = 60). However, respondents agreed that having space for providing separate housing (46%; n = 32), maintaining and analyzing fresh-cow records (49%; n = 36), and disease testing costs (38%; n = 26) were problems. Most respondents (85%; n = 55) perceived a negative impact of TCD on their farm profitability. However, their perceived disease incidences were ≤20%. Reduced incidences may be the result of not checking for TCD. Future research showing economic benefits of TCP might encourage its implementation among dairy producers.

Key Words: transition cow protocol, implementation, barrier

SC135 In vitro effects of 5-hydroxytryptophan dose and time exposure on primary bovine mammary epithelial cell gene expression. S. Field*[1], V. Ouellet[2], C. Sheftel[1], L. Hernandez[1], and J. Laporta[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Animal Sciences, Université Laval, Québec City, QC, Canada.*

Peripheral serotonin (5-HT) acts in a biphasic fashion to regulate mammary calcium metabolism during lactation and mammary involution. The objective was to examine the in vitro effects of increasing 5-Hydroxytryptophan (5-HTP, serotonin precursor) concentrations and exposure times on the gene expression of markers involved in involution, tight junctions, extracellular matrix (ECM) remodeling, and cell proliferation in primary bovine mammary epithelial cells (pBMEC). pBMECs were plated on dishes coated with rat-tail collagen in growth medium (DMEM:F12, 10% fetal bovine serum, 10 µg/mL insulin, 25 ng/mL epidermal growth factor, 10 ng/mL hydrocortisone, 10 mM sodium acetate and 1:100 antibiotic/antimycotic) at 37°C with 5% CO_2 for 7 d until confluency. Four doses of 5-HTP (0, 50, 200, 500 µM; dose) were then added and harvested after 8, 12, and 24 h, which was repeated 3 times. pBMEC RNA was extracted to quantify gene expression using the Fluidgm Delta-Gene Assay. Data were analyzed by PROC MIXED in SAS with fixed effect of 5-HTP dose independently at 8, 12 and 24 h. At 8 h, all 5-HTP doses downregulated expression of *BCL2* and *MAPK10* ($P < 0.07$). At 12 h, genes related to apoptosis and autophagy were impacted; *TGFB1* was downregulated and *PTGES* was upregulated by all doses ($P < 0.10$), whereas *IGFBP3* and *ATG5* were upregulated by the 50 µM and downregulated by the 200 µM doses ($P < 0.10$). Cell survival genes (e.g., *ATK2, IGF1R*; $P < 0.03$) were upregulated by all doses, and *OCLN*, a tight junction gene, was downregulated by all 5-HTP doses ($P = 0.05$). At 24 h, genes related to apoptosis/autophagy were different; *AIFM1* was downregulated and *ATG3* upregulated by all doses ($P < 0.05$), while *BAX* was downregulated by the 200 µM dose ($P = 0.04$). *MMP14*, an ECM remodeling gene, was downregulated by the 200 and 500 µM 5-HTP doses ($P = 0.005$). *TJP1*, a tight junction gene, was downregulated by the 200 µM and upregulated by the 500 µM doses ($P = 0.10$). Increasing local 5-HT actions within the mammary gland might hasten molecular events that promote an involution-like state, which might aid in accelerating mammary gland involution and cell turnover during the dry period in dairy cattle.

Key Words: 5-HT, involution

SC136 Isoprostanes increase endothelial cell barrier integrity independent of altered inflammatory gene expression.

A. Putman* and L. Sordillo, *Michigan State University College of Veterinary Medicine, East Lansing, MI.*

Dysregulated inflammation and oxidative stress contribute to the pathophysiology of several economically important diseases of dairy cattle. A hallmark of these processes is the peroxidation of polyunsaturated fatty acids in cellular membranes, leading to the generation of lipid mediators that regulate many aspects of the inflammatory response. Endothelial cells are crucial for appropriate inflammatory responses, partly because they are responsible for maintaining an effective vascular barrier and forming specialized lipid mediators known as isoprostanes (IsoP). Previous work has indicated that certain IsoP can modulate inflammatory outcomes, such as gene expression. As the biological action of IsoP is poorly described, the objective of the present study was to investigate the ability of IsoP to impact barrier integrity and inflammatory gene expression during acute bovine inflammation. Bovine aortic endothelial cells (n = 4) were treated with 15 ng/mL lipopolysaccharide (LPS), 10 nM omega-6- (15-F_{2t}-IsoP) or omega-3-derived (15-F_{3t}-IsoP) IsoP, or LPS cocultured with IsoP. Barrier integrity was assessed with electric cell-substrate impedance sensing (ECIS) for 24 h. Relative mRNA expression of thromboxane receptor, nuclear factor kappa B, and inducible nitric oxide synthase was assessed with reverse transcriptase qPCR after 1, 4, 8, and 12 h treatment incubations. Statistics were run with the PROC MIXED procedure in SAS 9.4 ($P < 0.05$). A trend toward increased barrier resistance was noted in cells treated for 8–24 h with LPS and 15-F_{2t}-IsoP relative to LPS alone. However, mRNA expression of genes associated with inflammation was not different between treatment groups during coinciding time points, suggesting that the increase in barrier integrity is likely not due to alterations in inflammatory gene transcription. This study benefits the dairy industry by describing how IsoP may influence inflammation in bovine cells. Future studies should be directed toward further comparing effects of omega-6- and omega-3-derived IsoP, which may implicate dietary modifications capable of mitigating inflammation in dairy cattle.

Key Words: isoprostane, oxidative stress

SC137 Clinical criteria used for defining puerperal metritis in dairy cows: A scoping review.

A. Garzon*[1], F. Samah[1], G. Habing[2], and R. Pereira[1], [1]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA,* [2]*Department of Veterinary Preventive Medicine, Ohio State University, Columbus, OH.*

Puerperal metritis (PM) is a common infectious disease in dairy cattle. Currently there is little agreement between clinical signs for defining PM in literature and on farms. This information gap has resulted in disparities related to recommendations for the treatment of affected cows. Our objective was to systematically review the literature for clinical signs used as diagnostic criteria for PM, including local (vaginal discharge) and systemic signs of infection (fever, drop in milk). The Preferred Reporting Items for Systematic Review and Meta-Analysis extension for Scoping Reviews (PRISMA-ScR) protocols was used to screen commonly used databases. One reviewer screened title/abstract (n = 2,096) followed by full-text screening of selected articles (n = 396) by 2 reviewers (n = 174). Descriptive statistics and multiple correspondence analysis were computed to evaluate the association among variables for definition and diagnosis of PM and year of publication of included articles. The most frequently cited reference article (37.5%) for the definition of PM was published in 2006, followed by articles published between 1998 and 2009 (13%). In 40.2% of articles, no reference was provided for the PM definition. For the definition of PM, the vaginal discharge (VD) was described in terms of color, odor, and viscosity. Among colors, the terms used were red-brown (61.4%), red (5.1%), brown (8.6%), chocolate (4%), white (1.7%), yellow (0.5%), pink (5.7%), or gray (0.5%); VD color was not reported in 24.1% articles. The VD odor was described as fetid (75.8%), putrid (5.1%), foul (10.3%), or other (5.7%) (e.g., abnormal, malodorous); but not mentioned in 7.4% of articles. The VD viscosity was described as watery (74.1%), purulent (27%), mucopurulent (8.6%), thin (4%), serous (2.8%) or abnormal (2.3%), and was not mentioned in 11.5% of articles. Fever was included in 59.7% articles as a criterion for PM diagnosis. The most used rectal temperature threshold was ≥39.5°C (56.8%), followed by ≥39.2°C (2.8%). Approaches used for VD evaluation included rectal palpation (37.3%), gloved hand (18.4%), Metricheck (9.8%) or speculum (5.7%); for 28.7% of articles diagnostic tools used were not mentioned. This review describes a disparity in robust and clear criteria used to diagnose PM in literature. Although select consensus articles are available, it is common not being used as reference, and further high-quality research is needed to identify a standard case definition for PM

Key Words: metritis, cattle

SC138 Adipogenesis and lipogenesis are modulated by cannabinoids in dairy cows.

M. N. Myers*[1], C. J. Rendon[1], M. Zachut[2], J. Tam[3], and G. A. Contreras[1], [1]*Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI,* [2]*Department of Ruminant Science, Institute of Animal Sciences, Volcani Center, Rishon LeZion, Israel,* [3]*Obesity and Metabolism Laboratory, Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.*

Intense adipose tissue (AT) lipolysis and suppressed adipogenesis and lipogenesis are attributes of the periparturient period of dairy cows. The extent of these processes recedes with the progression of lactation; however, if dysregulation occurs, disease risk is exacerbated. Cannabinoids (CBs) activate CB receptor 1 (CB1) in monogastric AT and enhance the adipogenic and lipogenic capacity of adipocytes. The effect of CBs on dairy cow AT is currently unknown. An in vitro cell culture model was used to determine the effects of the synthetic CB arachidonyl-2’-chloroethylamide (ACEA) with or without the CB1 inverse agonist rimonabant (RIM) in dairy cow adipocytes. Subcutaneous AT was collected from multiparous, nonlactating, non-gestating Holstein cows (n = 8). Stromal vascular fraction was obtained via collagenase digestion. Pre-adipocytes were isolated and induced to differentiate upon confluency in standard differentiation medium for 14 d. Adipocytes were exposed on d 2 of differentiation to ACEA at 0 (CON), 1 (ACEA1), 5 (ACEA5), or 10 (ACEA10) µM with or without RIM pre-treatment at 0.1 µM for 48h. Cell viability, adipogenic capacity, and lipogenesis were evaluated using commercially available plate-based assays and the IncuCyte S3 system. The adipogenic efficiency was calculated as the number of cells with 1+ lipid droplet over the total number of cells per well. Cell viability was not affected by ACEA or RIM treatments. Statistical analyses were performed using a mixed effect linear model in

JMP. Adipogenesis was enhanced ($P < 0.05$) by ACEA10 (54.5 ± 4.3%), but not ACEA5 (50.05 ± 4.3%) or ACEA1 (50.89 ± 4.3%) compared with CON (44.23 ± 4.3%). The presence of RIM (46.25 ± 4.1%) reduced adipogenic capacity across all ACEA-treated cells compared with those unexposed to the CB1 inhibitor (53.59 ± 4.1%, $P < 0.05$). Increased lipid accumulation (i.e., lipogenic capacity, reported in RFU/ng DNA) was observed in ACEA10 (3,115.43 ± 278) compared with CON (1,849.45 ± 278), and lipid content was reduced in ACEA10+RIM (2,681.19 ± 278) but not in CON+RIM (1,840.40 ± 278). Collectively, these findings suggest that adipogenesis and lipogenesis may be enhanced through activation of CB1 by CBs in dairy cow AT.

Key Words: cannabinoids, adipogenesis, lipogenesis

SC139 Exploring Lys metabolism in lactating cows.

L. R. Rebelo*, W. P. Weiss, and C. Lee, *Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.*

Lys utilization, excretion, and contribution to other amino acids (AA; transamination after oxidation) were explored using a stable isotope technique. Four ruminally cannulated cows in a 4 × 4 Latin square (17-d periods) received Lys at 0, 25, or 50 g/d into the abomasum via continuous infusion for the last 5 d (L0, L25, or L50, respectively) or rumen-protected Lys at 105 g/d (42 g/d Lys) for 17 d in each period. The body labile Lys pool was labeled with ^{15}N-Lys via continuous abomasal infusion (last 5 d) during which total collection of milk, urine, and feces and blood sampling were conducted. Steady-state enrichment of ^{15}N in samples occurred on d 4. Digestible Lys (dLys) supply was calculated by ^{15}N absorbed (infused – fecal excretion) divided by ^{15}N enrichment of milk Lys (representing ^{15}N enrichment of body labile Lys). ^{15}N partitioned into milk and urine was used to calculate the transfer of dLys to milk and urine. ^{15}N enrichment of blood and milk AA was determined for Lys contribution to other AA assuming zero net Lys uptake of peripheral tissues. Data were analyzed using PROC MIXED of SAS (cow as random and treatment as fixed effects). All data from L0 were removed from analysis due to issues of abomasal infusion. Fecal ^{15}N excretion was 12% of ^{15}N-Lys infused across treatments, indicating free Lys in the small intestine is not 100% digestible. Increasing Lys supply from L25 to L50 increased dLys ($P = 0.05$) from 132 to 152 g/d and increased dLys that was utilized for milk (108.1 to 117.5 g/d, $P = 0.02$) and numerically increased that was excreted in urine (23.6 to 34.2 g/d; $P = 0.21$). Proportion of dLys transferred to milk (80%) and urine (20%) were not affected by dLys supply. ^{15}N enrichment occurred in a few AA in plasma (Glu and Ala), but the enrichment occurred for almost all milk AA (relatively high in Glx, Asx, and branched-chain AA). The enrichment in milk AA was significantly or numerically greater for L50 vs. L25. In conclusion, results confirmed large flexibility of the mammary glands to supply AA through transamination. Increasing dLys increased Lys uptake by the mammary gland but also increased Lys oxidation contributing to other AA for milk protein.

Key Words: Lys metabolism, mammary glands, stable isotope

SC140 The effects of the COVID-19 pandemic, gender, and institution on student performance in an undergraduate animal science course.

J. R. Vinyard*[1], F. Peñagaricano[2], and A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Wisconsin–Madison, Madison, WI.*

In March 2020 in the United States, university students were forced to abruptly transition from traditional in-person courses to having their courses online. This transition, along with the increases in stress and anxiety due to the pandemic, could have potentially affected overall student performance in lecture-based courses. Therefore, the objective of this study was to compare the overall student scores (n = 768) in a lecture-based undergraduate animal nutrition course taught by the same professor. The course used for this study was taught at 2 institutions (University of Florida; UF and University of Nevada, Reno; UNR) over 7 years (2014–2017 at UNR and 2018–2020 at UF). Student performance was evaluated using both quizzes and exams from 2014 through the spring semester 2020 and only exams were used for summer and fall semesters of 2020. Students were classified as high performing students if they scored ≥95% and low performing students if they scored ≤70%. The variables that were evaluated were the effects of institution, gender (male or female), and class format (online or in person). The course was taught in person at UNR and in person and online at UF. The spring semester of 2020 was taught in person until March, but was switched to online when UF closed, and was considered an online semester for this analysis. As the course was only taught online at UF, the variable format was assessed using only UF records. Data were analyzed using logistic regressions, using the GLM procedure of SAS. The probability that students were high performing was not affected by gender or institution, nor was either gender more likely to perform highly than the other in a given institution. Interestingly, format online had a positive, desirable effect on the probability that students were high performing. The probability that students were low performing was not affected by gender or format, nor was either gender more likely to be low performing in a given format. However, if a student performed poorly in the class, they were more likely to have taken the course at UNR. Therefore, the format online, due to COVID-19 pandemic, had positively impacted the probability that students will perform high in an undergraduate animal nutrition course.

Key Words: animal nutrition, online learning, student performance

SC141 Effect of intravenous lipid infusion on blood metabolite concentrations and immune functions of dairy cows.

N. Vanacker*[1,2], R. Blouin[1], and P. Lacasse[2,1], [1]*Université de Sherbrooke, Sherbrooke, QC, Canada,* [2]*Agriculture and Agrifood Canada, Lennoxville, QC, Canada.*

During the transition period, dairy cows often experience negative energy balance which induces metabolic and immunological disturbances. Our previous work has shown a relationship between the decrease of immune functions and the increase in blood nonesterified fatty acid (NEFA) concentration. Nevertheless, it is difficult to determine the exact contribution of NEFA on the immune system as other metabolic and hormonal perturbations occur simultaneously during the transition period. In the present study, we have determined the impact of NEFA on immune functions using an experimental model designed to assess their effects independently of energy balance and hormonal changes. Six dry and non-pregnant cows were infused with either sterile water or a lipid emulsion (Intralipid 20%, Frenesius Kabi, Toronto) at a rate of 1 mL/kg/h for 6 h using a Latin square design. Data were analyzed with the MIXED procedure of SAS and differences were considered significant at $P < 0.05$. Blood concentration of NEFA, β-hydroxybutyrate (BHBA) and glucose were measured every hour throughout the infusion period as well as 1h and 24h after the end of infusion. Proliferation of lymphocytes, secretion of INF-γ, phagocytosis of neutrophils and oxidative burst were evaluated before, during and after infusion. For NEFA, BHBA and glucose, treatment × time interaction ($P < 0.01$) occurred. NEFA and BHBA levels were greater in the serum of cows infused with Intralipid ($P < 0.01$) for 1 h and thereafter when compared

with the control condition. Glucose levels also increased in response to Intralipid infusion from 2 h until 1 h after the end of treatment ($P < 0.001$). Maximum blood concentrations of NEFA, BHBA and glucose in control and Intralipid infused cows were, respectively, 0.06 ± 0.10 mM vs 1.39 ± 0.10 mM, 0.70 ± 0.05 mM vs 1.06 ± 0.05 mM and 4.56 ± 0.27 mM vs 6.90 ± 0.27 mM at 24 h post-Intralipid treatment, all blood metabolite concentrations returned to control levels as measured before infusion. Lymphoproliferation was decreased ($P < 0.01$) starting at 3 h after the start of the Intralipid infusion. Secretion of INF-γ was also reduced ($P < 0.05$) by Intralipid infusion. The Intralipid infusion did not affect neutrophil's phagocytosis and the oxidative burst. These results confirm that NEFA inhibits lymphocyte functions independently of energy balance. They also suggest that high blood lipid concentration causes insulin resistance.

Key Words: energy balance, insulin resistance, immunosuppression

ADSA Graduate Student Production PhD Poster Competition

SC142 First-lactation milking performance of dairy heifers fed pre- or probiotic diets in the prewean period.

P. M. Lucey*[1], I. Lean[2], E. Block[3], and H. A. Rossow[1], [1]*Veterinary Medicine Teaching and Research Center, UC Davis, Tulare, CA,* [2]*Scibus, Camden, NSW, Australia,* [3]*Arm & Hammer Animal and Food Production, Princeton, NJ.*

The focus of the pre-wean period is growth, rumen development and reducing risk of disease. Success during this period can impact a heifer's survival and performance in the milking herd. The objective of this analysis is to compare the first-lactation 305-d milk, fat, and protein production, age of calving, and survival to the milking herd, between comparable groups of heifers who were randomly assigned to a control (CON), a *Bacillus subtilis* probiotic (PRO), mannan-oligosaccharide as a prebiotic (PRE), or both agents in combination (SYN). Each supplement was mixed into the milk feed twice daily and fed the entire duration of the pre-wean period. A total of 1,801 Holstein heifer calves at a commercial dairy were randomized into each treatment group at birth. Bodyweight was measured at beginning and end of the pre-wean period and ADG was compared between treatment groups. Fixed linear regression models were created to analyze first-lactation 305-d milk, fat and protein yield in kg. Logistic regression was performed on the odds of survival to the milking herd. Ordinal logistic regression was performed on the age in months at first calving. In 305 d, cows in CON produced 10,911 (± 114) kg milk, 456 (± 5) kg fat, 349 (± 3) kg protein, cows in PRE produced 11,106 (± 120) kg milk, 469 (± 5) kg fat, 357 (± 4) kg protein, cows in PRO produced 11,168 (± 118) kg milk, 468 (±5) kg fat, 359 (± 4) kg protein, cows in SYN produced 11,175 (± 116) kg milk, 473 (± 5) kg fat, 358 (± 4) kg protein. Cows in SYN yielded 15 kg more milk fat than cows in CON ($P = 0.03$), no other differences in milk production were seen. The proportion of calves that entered the milking herd was 78% (± 2) for CON, 75% (± 2) for PRE, 77% (± 2) for PRO and 79% (± 2) for SYN. No difference in odds of survival or age at calving was seen between treatments. Though many environmental factors impact the adult performance of dairy animals, examining these data on a single site, with homogenously managed dairy cattle, and a pre-wean dietary difference may add information the potential impact of pre-wean diet on future milk production.

Key Words: probiotic, prebiotic, milkfat

SC143 Impacts of in utero heat stress on the gastrointestinal morphology of dairy calves.

B. D. Davidson*[1], S. L. Field[1], B. Dado-Senn[1], M. A. Steele[2], G. E. Dahl[3], and J. Laporta[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Late-gestation heat stress in dairy cows negatively impacts offspring absorption of immunoglobulin G (IgG) from colostrum. Herein, the objective was to quantify the gastrointestinal tract (GIT) structure of in utero heat-stressed or cooled heifer calves at birth. For the last ~56 d of gestation (THI ≤68), pregnant dams were either heat-stressed (HT, shade; n = 41) or cooled (CL, shade, fans, soakers; n = 41) and gave birth to calves which were in utero heat-stressed (IUHT) and in utero cooled (IUCL), respectively. At birth, calves were weighed, fed 3.78 L of high-quality colostrum within 2 h, and blood was collected at 24 h (n = 15/treatment). Apparent efficiency of absorption (AEA) of IgG was calculated by measuring IgG in fed colostrum and 24 h serum. Within 4.6 ± 2.3 h of birth, a subset of calves was euthanized (n = 8/treatment, no colostrum) to harvest the GIT. Full tract weights (including stomach) were obtained, and a cross-section of jejunum was fixed for 18 h in 10% neutral buffered formalin, dehydrated, and embedded in paraffin. Jejunal morphology was assessed from sectioned tissues stained with Hematoxylin and Eosin. Villi length, width, and crypt depth were measured with Keyence BZ-X800 Analyzer software (n = 20 villi/calf). Data were analyzed using PROC MIXED in SAS, with in utero treatment as a fixed effect. Colostrum IgG concentration fed to calves was not different (136.1 vs. 122.5 ± 13.4 g/L; IUHT vs. IUCL, respectively, $P = 0.32$), but AEA of IgG was reduced in IUHT relative to IUCL heifers (23.5 vs. 33.3 ± 3.1%. $P = 0.004$). Weight for the GIT at birth was not different between treatments (1.4 vs. 1.4 ± 0.07 kg, $P = 0.98$). Villi length (802.4 vs. 796.6 ± 95.5 µm, $P = 0.95$), villi width (102.9 vs. 112.9 ± 8.1 µm, $P = 0.24$), and crypt depth (249.5 vs. 235.0 ± 9.6 µm, $P = 0.16$) from the jejunum were not different between IUHT and IUCL calves. In utero heat stress does not seem to alter the morphology of the jejunum. These results suggest there might be structural differences in other portions of the intestine (i.e., ileum or duodenum) or negative effects at the cellular and molecular level which impact IgG absorption of in utero heat-stressed calves.

Key Words: immunoglobulin, jejunum, villi

SC144 Effects of Mg sources and buffer inclusion on high-producing dairy cows` performance.

R. R. Lobo*[1], J. A. Arce-Cordero[1], M. N. Marinho[1], S. So[2], A. Ravelo[1], B. C. Agustinho[3], J. Vinyard[1], M. L. Johnson[1], E. Sarmikasoglou[1], H. Monteiro[1], and A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*Khon Kaen University, Khon Kaen, Thailand,* [3]*University of Idaho, Moscow, ID.*

The objective of this study was to evaluate sources of Mg and buffer inclusion, on high-producing dairy cows' performance. Sixty Holstein cows (initial milk production of 40.5 kg and 100 DIM) were arranged into blocks according to parity, DIM, and milk production, a total of 15 blocks with 4 animals in each were formed. Animals in each block were assigned into one of 4 treatments: 1) MgO; 2) MgO + Na sesquicarbonate (Na sesq); 3) proprietary mineral formulation (PMF); 4) PMF + Na sesq. During 60 d animals were fed a corn silage-based diet with similar chemical composition (16.3% CP, 26.8% NDF, 31.8% starch, 0.67% Ca, and 0.27% Mg, on a DM basis) in individual Calan-gates and treatments were top-dressed. Animals were milked twice a day and milk samples were collected 2 d a week for 7 weeks on both morning and night milking. The experiment was carried out as a randomized block design with a 2 × 2 factorial arrangement, data were analyzed using the MIXED procedure of SAS, orthogonal contrasts were used to evaluate the effects of Mg source, buffer, and interactions. Partial data are presented in Table 1. Sources of Mg and buffer did not affect milk yield. Interactions were observed on DMI, feed efficiency, and energy-corrected milk; however, neither Mg source nor buffer effects were observed in any of these parameters. In summary, the replacement of MgO by PMF had no major effects on DMI, feed efficiency, and milk yield and composition.

Table 1. Effects of source of Mg and buffer (+) on performance of high-producing dairy cows

Parameter	MgO	MgO+	PMF	PMF+	SEM	P-value[1]
DMI, kg d^{-1}	22.0	23.1	23.0	21.2	0.53	0.03 (I)
FE, kg milk kg^{-1} DMI	1.8	1.6	1.7	1.7	0.05	0.02 (I)
Milk yield, kg day^{-1}	39.8	38.2	38.5	36.9	1.13	NS
ECM, kg^{-1} kg DMI	1.6	1.5	1.5	1.6	0.04	0.04 (I)
MUN, mg dL^{-1}	12.0	12.4	11.5	11.4	0.38	0.06 (S)
Fat, kg d^{-1}	1.1	1.2	1.1	1.1	0.05	NS
Protein, kg d^{-1}	1.2	1.2	1.2	1.1	0.03	0.09 (S)
Fat, %	3.0	3.2	2.9	3.2	0.13	0.07 (B)
Protein, %	3.1	3.2	3.1	3.2	0.04	0.06 (B)

[1] P-values of the contrasts of main effects of Mg source (S), buffer (B), and interaction (I). NS = nonsignificant.

Key Words: mineral source, MgO, Na sesquicarbonate

SC145 Factors impacting pregnancy at first service in lactating cows.

F. M. Masia*[1,2], M. B. Piccardi[1,2], M. G. Molina[1,3], M. G. Balzarini[1,2], and R. L. De la Sota[2,4], [1]*Facultad de Ciencias Agropecuarias–UNC, Córdoba, Córdoba, Argentina,* [2]*CONICET, Córdoba, Córdoba, Argentina,* [3]*Instituto de Ciencias Básicas y Aplicadas–UNVM, Villa María, Córdoba, Argentina,* [4]*Facultad de Ciencias Veterinarias–UNLP, La Plata, Buenos Aires, Argentina.*

During the periparturient period, dairy cows experience an increase in the prevalence of metabolic and production-related diseases. These health problems not only cause reductions in milk production and increases in production costs but also reduce the reproductive performance of cows. This study aimed to estimate the relative contribution of factors affecting the likelihood of pregnancy at the first service. We analyzed 66,935 lactations from 20 Argentinian dairies as function of 2 factors: parity season (warm: vs cool) and occurrence of early postpartum diseases (retained placenta 35%, hypocalcemia 3%, displacement of the abomasum 2%, metabolic diseases 36%, and mastitis 24%). Lactations were categorized according to the occurrence of diseases as follows: diseases appearing within 21 DIM, between 22 and 42 DIM, and without any disease. A logistic regression model was adjusted in JMP (v14.2) including parity, season, occurrence of postpartum diseases and their interaction independently for primiparous and multiparous cows. Parity and season affected the pregnancy odd ratio (OR) ($P < 0.0001$) while their interaction was not significant. Primiparous and multiparous cows without postpartum disease were 1.19 (OR, 95% CI: 1.13–1.26) and 1.23 (OR, 95% CI: 1.17–1.29) times more likely to conceive at the first service than those with a disease event during the first 21 d in lactation, respectively. The odds of pregnancy at first service were not affected by the occurrence of disease between 22 and 42 DIM. Primiparous and multiparous cows that calved during the cool season were 1.68 (OR, 95% CI: 1.59–1.77) and 1.61 (OR, 95% CI: 1.53–1.69) times more likely to become pregnant at first service than those who started in the warm season, respectively. We concluded that in addition to parity season, the occurrence of health events during the first 21 d postpartum impacts the reproductive efficiency at the first service of dairy herds.

Key Words: conception rate, health issue, logistic regression

SC146 White wastewater recovery from dairy industries: Evolution of bacterial ecosystem during treatment by reverse osmosis.

S. Alalam*, J. Chamberland, A. Bérubé, Y. Pouliot, S. Labrie, and A. Doyen, *STELA Dairy Research Center, Institute of Nutrition and Functional Foods (INAF), Department of Food Sciences, Laval University, Quebec, QC, Canada.*

White wastewater (WW) is a dairy effluent, generated during cleaning of equipment, after the first hydraulic flush. Pressure-driven membrane technologies, mainly reverse osmosis (RO), are interesting to recover its valuable dairy components and generate process water. In a sustainability context, different valorization processes were proposed. However treated effluents are generally of lower quality than that required for different water applications, while any study was focused on the microbiological quality of milk by-products. Consequently, the purpose of this study was to characterize the microbial ecosystem and the chemical composition, of WW generated during the cleaning procedure of pasteurizers from 2 Canadian dairy plants, and their subsequent RO retentate and permeate generated at 50°C in recirculation mode. Bacterial ecosystem was performed through a metabarcoding approach coupled to quantitative PCR targeting the 16S ribosomal RNA (rRNA) gene. Results demonstrated that bacterial communities identified in concentrated WW were different depending on their sampling site. The WW of the dairy plant #1 was dominated by psychrotrophic bacteria (*Pseudomonas* abundance of 51%), but there was no increase of the total number of 16S rRNA genes in the retentate throughout RO. Thermophilic bacteria (*Streptococcus* abundance of 71%), were the most abundant genus in the initial samples of WW of dairy plant #2. During their recirculation in the RO system, the total number of 16S gene copies in the RO retentate increased significantly ($P < 0.05$) from 6.9 ± 0.5 log 10/mL to 9.6 ± 0.6 log 10/mL after 20 h. Both RO concentrates showed similar chemical compositions to that of skim milk and could potentially be reused in cheese milk. However, RO should be done at 10°C to avoid the development of thermophilic bacteria. Consequently, considering that RO generates permeate requiring minimal treatments to be recycled in the dairy plant, RO represents an interesting strategy to reduce the environmental impact of dairy effluents.

Key Words: white wastewater, reverse osmosis, metabarcoding

SC147 Synchrotron-based study to determine the inherent molecular structure changes induced by steam pressure times in faba bean seeds.

M. Rodríguez*, D. Christensen, R. Newkirk, Y. Ai, V. Guevara, and P. Yu, *University of Saskatchewan, Saskatoon, SK, Canada.*

Synchrotron technology is currently a valuable instrument to examine detailed intrinsic molecular features in a variety of materials including feed ingredients. Heat processing methods commonly used in the feed industry alter the physicochemical structure of feeds, modifying their degradation behavior when fed to livestock animals. Traditional research tools are unable to detect processing induced molecular structure changes associated with nutrient supply. Hence, this study aimed to determine the extent of protein molecular structure modifications related to steam pressure processing times in fava bean seeds. Analyzed samples belong to CDC Snowbird variety heated at 121°C for 0, 30, 60, 90, and 120 min. Seeds were pre-processed into cross-sections (6 μm thickness) and then fixed into BaF2 windows (3 windows/sample) for molecular analyses. This study was performed using the infrared beamline at the Advanced Light Source-ALS (Berkeley, CA) where spectra were collected in the mid-infrared region from 4,000 to 750 cm^{-1}. Molecular data were analyzed using OMNIC 7.3 software and SAS software 9.4

(SAS Institute, Inc., Cary, NC) was used for statistical analysis with significance declared at $P < 0.05$. Results showed variations in the spectra protein related areas and heights with a lower amide I area ($P < 0.01$) found at 0 min (47.53 AU, infrared absorbance units) compared with 30 min (54.16 AU) and 120 min (57.01 AU). Amide II area was higher ($P = 0.01$) at 30 min (24.36 AU) compared with 60 and 90 min (22.06 and 22.16 AU, respectively). The α-helix to β-sheet ratio was higher ($P < 0.01$) at 0 min (1.09 AU) compared with all heating times (avg. 1.01 AU). Protein molecular structure changes associated with heat processing can be directly identified using synchrotron technology. The increased knowledge in the area of feed molecular structure will benefit the application of precise dairy feeding strategies as we get a better understanding of the close relationship between individual inherent structure characteristics of each feed nutrient and the overall degradation behaviors of feedstuffs when fed to cattle.

Key Words: synchrotron, dairy cattle, faba beans

SC148 Production and temporal plasma metabolite effects of soybean meal versus canola meal fed to dairy cows during the transition period and early lactation.

J. Kuehnl*[1] and K. Kalscheur[2], [1]*University of Wisconsin–Madison, Department of Animal and Dairy Science, Madison, WI,* [2]*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Replacement of soybean meal (SBM) with canola meal (CM) in diets fed to early-lactation dairy cows increases milk production. Our objective was to determine production and temporal plasma metabolite effects of dairy cows fed isonitrogenous diets formulated with SBM or CM during the transition period and early lactation. Multiparous Holstein cows ($n = 79$), blocked by calving date, enrolled in a 2 × 2 factorial arrangement of treatments in a randomized complete block design. Cows consumed diets containing SBM or CM as the main protein source from wk −3 to +16 relative to calving. Half of the cows consumed each diet pre- (PRE) and postpartum (POST), with half of each PRE group switching protein source at calving. Milk production, components, DMI, and BW were determined weekly. Plasma was collected twice weekly from the coccygeal artery in wk −3 to +8, composited by cow for each wk, and evaluated using ELISA and enzymatic colorimetry. Data were analyzed using the MIXED procedure of SAS. Fixed effects were wk, prepartum diet, postpartum diet, and all 2- and 3-way interactions. Block was included as a random effect. Cows fed CM increased DMI both PRE and POST compared with cows fed SBM (15.3 vs. 14.5 ± 0.3 kg/d; 26.2 vs. 25.0 ± 0.4 kg/d; $P < 0.05$). Milk production tended to increase for cows fed POST CM compared with POST SBM (52.8 vs. 50.9 ± 0.7 kg/d; $P = 0.09$). An interaction between PRE and wk was detected for ECM ($P < 0.05$) and FCM ($P = 0.05$). Cows fed POST CM decreased in MUN compared with POST SBM (12.9 vs. 13.7 ± 0.2 mg/dL; $P < 0.05$). Diet did not affect BW, BW change, feed efficiency, components, plasma insulin, IGF-1, or glucose ($P > 0.1$). Plasma triiodothyronine decreased both PRE and POST for cows fed CM compared with cows fed SBM (1.38 vs. 1.54 ± 0.03 ng/mL; 1.14 vs. 1.35 ± 0.03 ng/mL; $P < 0.01$). Plasma growth hormone decreased for cows fed the PRE CM compared with PRE SBM (5,050 vs. 6,230 ± 382 pg/mL; $P < 0.05$). Altogether, our data demonstrates that the early-lactation dairy cow fed a diet formulated with CM increases production and DMI and alters physiological status.

Key Words: canola meal, transition period, early lactation

ADSA Graduate Student Southern Section Oral Competition

SC149 Effect of homofermentative and heterofermentative bacterial inoculants on quality, fermentation, and digestibility of alfalfa-bermudagrass mixture ensiled in the summer. M. D. Idowu*[1], V. Bryant[1], T. H. Terrill[1], Z. Estrada-Reyes[1], J. J. Tucker[2], L. L. Baxter[3], I. M. Ogunade[4], and A. A. Pech-Cervantes[1], [1]*Fort Valley State University, Fort Valley, GA,* [2]*Department of Animal and Dairy Science, University of Georgia, Tifton, GA,* [3]*Department of Crop and Soil Sciences, University of Georgia, Tifton, GA,* [4]*West Virginia University, Morgantown, WV.*

Interseeding alfalfa (*Medicago sativa*) into warm-season perennial grasses like bermudagrass (*Cynodon dactylon*) has been proposed to increase the sustainability of forage production in the southeastern United States through improved forage quality, yield, and animal performance. The objective of this study was to evaluate the effects of homofermentative (MC) and heterofermentative (FC) bacterial inoculants on the quality, fermentation, and in vitro digestibility of an alfalfa-bermudagrass mixture ensiled during summer. Representative samples of 8 alfalfa-bermudagrass plots established in 2016 were randomly harvested at the mid-bloom stage (34.2% DM bermudagrass and 36.7% DM alfalfa) and chopped to 19-mm length at the University of Georgia, Tifton campus. Samples were then transported, wilted, and ensiled using food-grade pouches at Fort Valley State University, Fort Valley, GA, 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). Treatments were ensiled following a 3 × 5 factorial arrangement for 0, 4, 18, 32, and 100 d in quadruplicate. Samples were analyzed for pH, chemical composition, and organic acids. Samples from 100 d were analyzed for yeast, molds, and aerobic stability. Data were analyzed using the NLME package of R, including the effects of treatment, day, and interactions. Botanical composition analysis revealed a greater proportion of alfalfa (95.5%) than bermudagrass (9.5%). Inoculation with FC increased pH, NDF and, ADF at d 18, 32 and, 100 compared with MC and control ($P < 0.05$), but decreased CP in silage ($P < 0.05$). Conversely, inoculation with MC decreased ADF content in silage compared with FC and control ($P < 0.01$), but increased yeast ($P < 0.01$), IVDMD and IVNDFD ($P = 0.06$) after 100 d of ensiling. Compared with MC and control, inoculation with FC increased ($P < 0.01$) acetate and propionate concentrations at d 18 and 100, but decreased lactate concentrations ($P < 0.01$). However, bacterial inoculation did not affect ($P > 0.90$) both DM recovery and aerobic stability of silages. Ensiling alfalfa-bermudagrass mixtures with homofermentative inoculants increased the quality and digestibility of forages harvested in the summer.

Key Words: alfalfa, homofermentative, heterofermentative

SC150 Daylength exposure in dry cows associated with colostrum production. K. Alward*, A. Nin-Velez, A. Connor, J. Duncan, and R. Cockrum, *Virginia Tech, Blacksburg, VA.*

Pre-weaned calf deaths account for more than $100 million in losses annually in the US Diarrhea is the primary cause of death, with half being attributed to failure of passive transfer of immunity; primarily caused by inadequate quality or insufficient colostrum consumption. Based on previous research showing altered photoperiod during the dry period increased milk production, we hypothesized that decreased photoperiod during this period would subsequently improve the quality and volume of colostrum produced. To test this hypothesis, colostrum data were collected from Jersey cows (n = 43) from 2 farms and compared with photoperiod length during the dry period. Daily light exposure was determined via local weather station sunrise/sunset data and was averaged for the far-off and close-up stages of the dry period. Within 12 h. of calving, colostrum volume was measured and quality tested with a Brix refractometer. Colostrum was analyzed for composition (i.e., fat, protein, solids nonfat, lactose, somatic cell count, acetate, urea and β-hydroxy butyrate) at a commercial DHIA laboratory. Data were analyzed using R Version 4.0.3. Regression coefficients of far-off and close-up photoperiod exposure were determined against colostrum measures. Covariates included were farm, dry period length, year and season of collection. Day length averaged 771.8 + 14.9 min. for far-off and 853.2 + 14.4 min. for the close-up period. Dry period length ($\beta = 0.16$, $P < 0.01$) and day length ($\beta = 0.04$, $P < 0.05$) during the close-up dry period were significant predictors of colostrum volume, both increasing colostrum volume. For Brix score, dry period length ($\beta = 0.08$, $P < 0.05$), and day length during the far-off ($\beta = 0.06$, $P < 0.01$) were significant predictors and increased Brix score while close-up ($\beta = 0.07$, $P < 0.01$) dry period photoperiod exposure decreased Brix score. Photoperiod exposure was not a significant predictor of any colostrum composition markers ($P > 0.05$). Based on these results, we suggest that limiting daily light exposure during the close-up dry period could improve colostrum quality without compromising colostrum components, however colostrum volume could be reduced.

Key Words: photoperiod, colostrum, dry cows

SC151 Effects of a living mulch system on dairy cattle production in the southeastern of United States. Z. Yu*, J. M. Cantet, R. Nave, and A. G. Rius, *The University of Tennessee Institute of Agriculture, Knoxville, TN.*

The use of living mulch systems of clover spp. for corn production have the potential to provide benefits to the row crop and the availability of forage mass to sustain grazing of livestock. Our objective was to evaluate dairy cattle productivity grazing clover-based living mulch systems. The 4 living mulch systems used in the study were: 1) grain corn planted into white clover (G-WC), 2) silage corn planted into white clover (S-WC), 3) grain corn planted into crimson clover (G-CC), and 4) silage corn planted into crimson clover (S-CC). Following corn harvest, 12 dairy heifers in spring (465.3 ± 57 Kg) and 36 dairy heifers in fall (522.5 ± 77 Kg) were allocated to grazing mulch forage and determine the effect of living mulch system on cattle body weight, average daily gain (ADG), and energy metabolism for 4 weeks. Body weight records and blood samples were collected biweekly throughout the study. Plasma was harvested and used to determine nonesterified fatty acids (NEFA). Data were analyzed using PROC MIXED in SAS with cows as a random effect and time as a repeated measure. The statistical model included mulch system, time, and interaction. Differences among means were tested using Tukey' test. Significant effects were declared at $P < 0.05$ and tendencies at $0.05 \leq P \leq 0.10$. Spring results showed that body weight and ADG were not affected by mulch system ($P > 0.10$). Plasma NEFA concentrations tended to be lower in G-CC (0.130 mMol/L) than in S-CC and G-WC (0.231 and 0.216 mMol/L, [SEM = 0.0657], $P = 0.075$). Fall results showed that the ADG of S-WC heifers tended to be greater than that of G-CC counterparts (0.810 and 0.270 Kg/day, respectively, $P =$

0.053, SEM = 0.3180), although body weight did not change ($P > 0.10$). Heifers allocated to G-CC and S-CC presented greater concentration of NEFA than those grazing WC (0.446 and 0.454 mMol/L, vs 0.330 and 0.291 mMol/L, $P = 0.002$, SEM = 0.151). These results suggest that living mulch systems in the southeast provide forage mass to sustain productivity of dairy heifers in fall and spring.

Key Words: living mulch system, white clover, crimson clover

ADSA-SAD Dairy Foods Oral Competition

SC152 The effects of light oxidation on milk flavor and quality. Y. M. Longenecker*, K. E. Kaylegian, and D. R. Olver, *The Pennsylvania State University, University Park, PA.*

When consumers reach for a jug of milk in the dairy aisle of a grocery store, they expect the milk to be high quality and fresh with no off-flavors or odors present. The milk should smell and taste clean with a slight cooked note resulting from pasteurization. When off-flavors or off-odors can be detected in milk by the consumer, this can negatively affect sales. There are 3 categories of off-flavors associated with milk that are important to note: absorbed, bacterial, and chemical. The chemical category encompasses the most common off-flavor found in fluid milk: oxidized, caused by light-induced oxidation. To the consumer, the oxidized flavor will have a similar taste to cardboard or burnt feathers and in more extreme cases, may exhibit a strong odor. Light-induced oxidization occurs when the milk is exposed to even the slightest levels of UV light. This light stems from many sources, including sunlight, fluorescent lighting, and light-emitting diode (LED) lighting, among others. The UV light initiates reactions that affect proteins and fats in the milk and cause a buildup of compounds that lead to the development of the cardboard and burnt feather off-flavors. It can also decrease nutrients in milk such as riboflavin and vitamin A. In recent years, there has been a shift back to the original glass and clear plastic jugs for transparency for the consumer. However, these containers allow light to pass through to the product inside, thus increasing the light-induced oxidized flavors in milk. With LED lighting becoming more prominent in dairy retail cases, recent studies have compared the effects of fluorescent and LED lighting on light-induced oxidation. These results have encouraged processors to continue to find innovative ways to package and store milk to prevent oxidized flavor and nutrient loss. The dairy industry is continuing to develop solutions to this problem by creating new types of light-blocking containers that still allow for consumer transparency. Ultimately, it is best for milk to be exposed to light as little as possible to ensure that light-induced oxidation is not affecting the flavor and quality.

Key Words: light oxidation, milk quality

SC153 COVID aid: Differing effects on the milk market. K. Hillis* and J. Bohlen, *University of Georgia, Athens, GA.*

The dairy industry took a particularly hard hit in the face of the global COVID-19 pandemic, with national “stay at home” orders and supply chain disruptions leading to dumped milk and struggling producers. At the height of the pandemic, Dairy Farmers of America (DFA) estimated that their producers were dumping as much as 3.7 million gallons of milk every day. In response, the federal government released the $19 billion relief bill known as the CFAP, or Coronavirus Food Assistance Program. This bill provided money for food purchases as well as direct payments to farmers. The USDA’s Farmers to Families Food Box program also bought surplus dairy products to provide to disadvantaged families across the US. While these new governmental policies provided much needed cash to farmers, some market analysts worry about the effects of a market thriving on temporary relief versus natural market patterns. Coupled with this is the potential issue of unequal distribution of relief relative to the various milk markets of the US Such that hard cheeses werer favored in food redistribution programs and cultured products were left floundering. Class I milk, with its high perishability, is also not easily stored long-term and less likely to be used by some government programs. This preferential support for class III milk over classes I and II also has a farther-reaching implication- a negative producer price differential (PPD) on milk checks. If class III prices are substantially higher than other classes, a negative PPD can result. The 2018 Farm Bill included a reformulation of class I prices based on historical averages between class III and class I milk, with the intention of improving risk management for class I producers and processors. Unfortunately, this formula has multiplied the effect of volatile prices between the 2 classes due to COVID Aid. Class III milk market continues to do much better due in large part to government intervention in the class III market. In fact, the recovering market for class III milk in June 2020 resulted in a record PPD of -$7.05. These factors leave many market analysts wondering whether COVID Aid will help or hurt the dairy market as a whole. Moving forward, it may be wise for producers to be cautious when it comes to anticipating or viewing their farm’s profitability relative to support and relief programs. Many of these may provide a temporary masking to market trends and favor certain product markets, which can further exacerbate market volatility.

Key Words: COVID-19, Coronavirus Food Assistance Program (CFAP), milk market

SC154 Upcycling whey through fermentation for an ethanol product. C. Putman* and D. Winston, *Virginia Tech, Blacksburg, VA.*

Whey has been a challenge to dispose of in an environmental and economical way for many years. It previously has been spread on farm land, used in animal rations, and incorporated into whey protein powder. These have all been temporary solutions that end up regressing due to the expense of unsustainable practices. With the ratio of whey to cheese being 9:1, the dairy industry is left with a large quantity of whey to properly dispose of in a feasible manner. Specifically, for small creameries that produce cheese, discarding of the whey can be bothersome. Disposal of whey for these cheese creameries is difficult because of the fee that comes with bringing it to a municipal water center. Through partnerships and contracts with small distilleries, both dairy and ethanol industries are able to be more sustainable. Fermentation of whey can be used to produce clear spirits such as vodka and gin. In traditional liquor procedures, a “mashing” process is needed to extract and convert grain starch into fermentable sugars. By using whey, the mashing process can be eliminated because lactose is able to be broken down by a yeast, *Kluyveromyces marxianus*, to fermentable sugar monomers. Use of whey in ethanol production allows for less water and carbon dioxide emissions since the mashing process utilizes a large quantity of those. When using whey in the fermentation process of ethanol, distilleries use 0.44 kg less water and produce 8.45 kg less carbon dioxide per 750 mL bottle than traditional procedure. By producing ethanol products with whey as a core ingredient, the dairy industry is able to reduce the environmental implications that come from whey disposal as well as reduce fees and expenses. The alcohol industry benefits by limiting their resources used in the process and gaining a marketing edge on other alcoholic beverage companies that use the traditional process.

Key Words: fermentation, whey, ethanol

SC155 Effects of dairy probiotics on human health. K. Edwards*, G. Mazon, and J. H. C. Costa, *Dairy Science Program, University of Kentucky, Lexington, KY.*

Probiotics are nonpathogenic microorganisms that aim to improve the health of the host (Williams et al., 2010). Probiotics are available in many forms such as capsules, powders, and various yogurts and dairy drinks. Probiotics in fermented milk have been used for centuries. In fact, the use of fermented dairy products has been mentioned in biblical scriptures and has been prescribed as medication by early scientists such as Hippocrates (Lourens-Hattingh et al., 2001). More recently, scientists started investigating the effects of dairy probiotic products as functional foods. The most common probiotics in fermented dairy products are *Bifidobacterium* and *Lactobacillus*. Such microorganisms are non-pathogenic, resistant to the gastrointestinal environment, and have shown benefits to human health (Shah et al., 2011). One clinical trial showed that the consumption of yogurt containing *Lactobacillus acidophilus* was related to a 3-fold decrease in vaginal infections when given to patients with chronical candidal vaginitis (Hilton et al., 1992). Furthermore, the consumption of yogurt containing *Bifidobacterium* improved bowel movements in constipated women when compared with yogurt without the probiotic (De Paula et al., 2008). The daily consumption of kefir containing *Lactobacillus* by overweight adults was associated with decreases in intestinal inflammation and improvement in overall mood when compared with daily consumption of plain milk (Praznikar et al., 2020). In addition to the positive effects on the health of adults, dairy probiotics have also been shown to benefit children. For instance, a study with more than 80,000 infants in Japan showed the consumption of yogurt at least thrice per week was associated with a significant decrease in the incidence of gastroenteritis (Nakamura et al., 2019). In summary, dairy probiotics have shown positive effects on human health ranging from decreases in infections and inflammations to improvements in the mood of humans. Most of the research on dairy probiotics focuses on yogurt and kefir. Thus, future research should investigate the benefits of added probiotics to other dairy foods on human health.

Key Words: yogurt, kefir, functional foods

SC156 Cheese intake improves cognitive function.

T. Van Hout* and E. Eckelkamp, *University of Tennessee Institute of Agriculture, Knoxville, TN.*

Dairy products in the diet can have a positive effect on patients experiencing heart disease, obesity, cancer risks, and other chronic diseases. Cheese intake has been shown to improve cognitive function and delay the onset of cognitive decline. Alzheimer's disease in particular could be impacted by cheese consumption. Alzheimer's disease is defined as a specific neural disease accounting for 60 to 80% of dementia cases. Dementia has been defined as the general condition presenting a decline in memory, reasoning skills, and other general cognition skills. In a recent study, researchers in the United Kingdom explored the impact of different diets on fluid intelligence from 2006 to 2016. Fluid intelligence was defined as abstract reason and problem-solving ability. The study considered the following factors: family history, presence of genetic risk factors for Alzheimer's (APOE4 allele), diet, and alcohol consumption. All respondents ($n = 1,929$) were asked to complete a fluid intelligence test of 13 questions within 2 min across 3 time periods (2006 to 2010, 2011 to 2013, and 2015 to 2016, respectively). Respondents would take one test per year cohort, 3 tests total. Respondents were grouped based on family history of Alzheimer's disease or dementia and presence of the APOE4 allele. Respondents who consumed cheese daily had the greatest fluid intelligence score compared with respondents who never, rarely, or weekly consumed cheese. Daily cheese consumption showed a positive impact on fluid intelligence score for individuals without a family history of Alzheimer's. A positive impact was also present for individuals with and without the APOE4 allele. Other studies have shown fermented dairy products had a positive impact on preventing cognitive decline and Alzheimer's disease in mice models. Additional studies have also shown cheese consumption improved cognitive performance in healthy, elderly, and overweight individuals. The consumption of cheese should be further explored to identify and fully understand the impacts on cognitive function in humans.

Key Words: Alzheimer's disease, dairy products, fluid intelligence

ADSA-SAD Dairy Production Oral Competition

SC157 Concentration of anti-Müllerian hormone in dairy heifers is positively associated with long-term reproductive merit and productive herd life. A. Rauton* and J. Bohlen, *University of Georgia, Athens, GA.*

The original and traditional role of anti-Müllerian hormone (AMH) is related to sexual differentiation in early gestation. In the presence of AMH, male embryos experience regression of the Müllerian ducts while female embryos, lacking AMH, rely on the same ducts to give rise to important reproductive organs such as the oviducts, uterus, cervix, and vagina. In recent years significant attention has been paid to the possibility that AMH may be used as an indicator of the ovarian reserve and thus a biomarker for female fertility. Numerous studies to date have established the positive correlation between AMH and antral follicle county (AFC). However, the primary use of AMH has been as an assessment tool for donor animal selection for embryo flushing with higher AMH values in animals generally linked to higher embryo recovery rate. More recent information indicates that AMH may be used as a much more robust tool for establishment of reproductive merit and ultimately productive life. A recent study conducted at Michigan State University sought to analyze the established correlation between AMH and AFC as well as their association with ovarian function and fertility as an indication of an animal's longevity in the dairy herd. Using 281 Holstein heifers, researchers took a single AMH measurement at 11 to 15 mo of age. Subsequent data to include lactation performance, calving intervals, and cull rates were recorded on animals allowed to complete, at a minimum, 2 lactations. Results indicated that the lowest AMH heifers corresponded to not only a reduction in reproductive metrics (pregnancy rate and cull reason) but also herd productive life. This study, along with others having similar findings, suggest that AMH has promising potential to offer a more user friendly and reliable diagnostic tool for evaluating an animal's long-term reproductive performance and productive life before her first lactation.

Key Words: anti-Müllerian hormone, fertility, productive life

SC158 Feeding *Asparagopsis taxiformis* to inhibit dairy cattle methane production. K. A. Forbes* and D. R. Olver, *Pennsylvania State University, University Park, PA.*

Methane emissions from livestock are a growing concern in today's society. According to EPA estimates, agricultural activities accounted for 9.6% of total US greenhouse gas emissions in 2019, and enteric fermentation was responsible for 27% of total methane emissions resulting from anthropogenic activities. Diet alterations are one pathway to reduce livestock methane emissions. Researchers are investigating the antimethanogenic effects of various feed ingredients. Seaweed, particularly *Asparagopsis taxiformis*, contains bromoforms that inhibit the production of methane. Early trials at James Cook University and UC Davis with beef cattle along with studies on dairy cattle at Penn State show promising results. Methane levels in the short term were reduced by including low levels of *A. taxiformis* in diets, while milk production remained largely unaffected. Feeding *A. taxiformis* is not without its challenges. The bromoforms in seaweed are sensitive to both light and heat and may lose efficacy during storage. The composition of seaweed can vary greatly depending on time of harvest, water temperatures, and other variables. Palatability is also a major issue with feeding seaweed. Cows fed higher levels of *A. taxiformis* exhibited a decrease in DMI. Another concern is the adaptability of the rumen microbes to this diet. In the short term a marked decrease in methane emissions has been observed, but there is a lack of data long-term to determine if this decrease is sustained. Additionally, it has not been demonstrated that maintaining a sustainable supply of the seaweed is feasible. *A. taxiformis* is not found in the wild globally, and to grow an amount necessary to feed even a fraction of the United States' dairy cattle population, large-scale seaweed farming will be necessary. Finally, there are regulatory concerns related to feeding seaweed to livestock. Despite these challenges, future seaweed research may lead to other promising methods to reduce greenhouse gas emissions from enteric fermentation.

Key Words: *Asparagopsis taxiformis*, methane

SC159 Environmental enrichment to promote health and well-being for preweaned calves. S. Thomas*, K. Daniels, and D. Winston, *Virginia Tech, Blacksburg, VA.*

Contemporary calf housing systems in the United States typically meet all basic needs for survival, but frequently lack environmental enrichments for calves such as interactive objects for grooming and social partners. Enrichment items, such as social companionship and brushes, can promote natural behaviors, decrease negative and boost positive affect, improve overall biological function, and provide good calf welfare. Compared with individual housing, provision of one or more social companions can impact feeding, play and grooming behaviors. Provision of brush access to preweaned dairy calves is gaining popularity but impacts on calf well-being based on housing system (individual, pair, or group housed) and type of brush (stationary or mechanical) remain largely unknown. Available literature suggests that when comparing brush types (stationary or mechanical), calves seem to prefer mechanical brushes because they offer grooming in hard to reach areas. Also, calves tend to use brushes most often in the minutes surrounding meal time although variability in brush use suggests individual preferences. In conclusion, social companionship and access to brushes increases environmental complexity for preweaned calves and may have favorable impacts on certain calf behaviors without having negative effects on growth.

Key Words: calves, environmental enrichment, well-being

SC160 Milk production and price trends reflected in the Federal Milk Marketing Orders. L. Foley* and E. Eckelkamp, *University of Tennessee Institute of Agriculture, Knoxville, TN.*

The dairy industry pricing system has evolved over the past century with multiple authoritative governing bodies, the most prominent being the Federal Milk Marketing Orders (FMMO) that have been influential since the 1930s. The original goal of the FMMO was to provide consumers adequate access to essential, affordable dairy products through orderly milk movement from producers to processors. This was accomplished through grouping areas within a region and providing a price structure based on different milk classes (I, II, III, and IV). The perceived role and consequences of the FMMO differ across regions, and between producers and processors. The dairy industry has changed to a market with an abundance of raw product, greater variety of selection, and greater product availability. Previous research was used to create a timeline of the FMMO, dairy production changes, and the FMMO's intended and unintended consequences. Several studies have analyzed this complex system to determine the efficiency and profitability of marketing dairy products. Key consideration points were: milk classification, regional orders, misconceptions of the FMMO's role, key issues identified within the FMMO, and the current role of the FMMO. Common misconceptions included full milk price governance, class I regulation, and a guaranteed market for producers. Through the FMMO, an unintended consequence was the stability in the FMMO minimum price compared with the volatility and increased dairy production costs. With the price control exerted by the FMMO, adjustments that have been made have not kept up with changing farm and processing technologies or consumer offerings in the retail market. Limited updates have resulted in unbalanced prices paid by consumers, artificially reducing the price ceiling for both producers and processors. With the competitiveness of the food industry, the FMMO has become a major influential aspect of dairy marketing that should reflect the concerns of producers, processors, and consumers. The volatility of production and retail markets led to constant evolution, and the accompanying regulatory measures could be misconstrued by individual perceptions of their function.

Key Words: milk pricing, consumer trends, regulations

SC161 Acidosis in dairy calves: Why we should worry and how can we manage it?

C. Langford*, G. Mazon, and J. H. C. Costa, *Dairy Science Program, University of Kentucky, Lexington, KY.*

Ruminal acidosis is a metabolic disorder caused by the decrease in ruminal pH that can affect the animal's metabolism, feeding behavior, and consequently production (Krause and Oetzel, 2005). Studies show that not only adult cows but calves are also susceptible to ruminal acidosis, especially during weaning (Gelsinger et al., 2020). Li et al. (2019) reported a significant impact of ruminal acidosis on the development of the ruminal epithelium in dairy calves. Additionally, the authors reported that calves with induced acidosis gained less weight when compared with a control group. Also, Gelsinger et al. (2019) reported that calves with grain-induced acidosis had lower dry matter intake and body weight when compared with a control group. In dairy cows, one of the many prevention methods for acidosis is the use of probiotics. Probiotics are live bacteria that aid in bringing balance to the gut microbiome (Hume, 2011). In a recent study with mature cows, Mazon et al. (2020) reported that cows drenched with a probiotic containing *Megasphaera elsdenii* experienced shorter and less intense acidosis periods when compared with control cows. However, the effects of *M. elsdenii* in dairy calves are contradictory. Muya et al. (2015) reported that calves supplemented with *M. elsdenii* at 14 d of age had greater dry matter intake, greater weaning weight, and tended to have greater average daily gain when compared with control calves. Also, the same author reported that calves supplemented with *M. elsdenii* had greater reticulorumen weight and papillae density than control calves. However, Yohe et al. (2018) did not observe any effects of the *M. elsdenii* probiotic on body weight, dry matter intake, or ruminal fermentation in calves. Yet, research shows that most beneficial results of probiotics in calves were observed when animals were supplemented during a bout of illness (Cangiano et al., 2020). There is still limited research on the possible connection between acidosis and feeding behavior. As a result, there is a need for more research to understand the effects of acidosis and if intervention with probiotics is associated with feeding behaviors in calves and cows.

Key Words: *Megasphaera elsdenii,* probiotics, weaning

ADSA-SAD Original Research Oral Competition

SC162 Once-a-day milking to limit milk production and effect on udder firmness. G. M. DeMers* and C. D. Dechow, *The Pennsylvania State University, University Park, PA.*

Milking cows once a day (1×) is a management practice utilized in some low-input grazing systems but rarely practiced on non-grazing farms with high milk production. However, it may be a viable solution in high-producing herds to help limit milk production in times of oversupply such as during the COVID-19 pandemic. Our objectives were to determine how milk production decreases with 1× milking in late lactation Holsteins and to determine effects on udder firmness. Cows in the trial were assigned to 1× (n = 15) or twice a day (2×) milking (n = 14) 1 month before dry-off. Milk yield was recorded from December 18, 2020, through February 24, 2021, resulting in 285 1× and 307 2× daily observations. Udder firmness was measured on 8 dates with a hand-held fruit penetrometer and resulted in 110 observations. The penetrometer was depressed into the rear quarters 4 inches from the top of each quarter without breaking skin to a depth of 2 cm. Milk yield was evaluated with the MIXED procedure of SAS with effects of milking frequency, day of treatment (DOT) from 0 to 28, and a covariate of yield before trial enrollment as fixed effects. Date was treated as a random effect with observations repeated over DOT with cow as the subject. Least squares means (LSM) were derived for milking frequency with differences declared significant at $P < 0.05$. Udder firmness was evaluated with a model that included fixed effects for milking frequency, daily milk yield, and their interaction. This model contained the same random and repeated structure as that used for daily milk yield. The LSM of daily yield for 1× milking was 16.5 kg and for 2× milking was 22.6 kg ($P < 0.05$), corresponding to a 27% decrease in daily yield. The effect of milking frequency on udder firmness was significant with LSM of 3.5 kg/cm^2 and 2.1 kg/cm^2 of pressure for 1× and 2×, respectively. Pressure increased with yield, especially for 1× milking, with regression coefficients of 0.08 and 0.19 kg/cm^2 per kg of milk for 1× and 2×, respectively. These findings imply that milking 1× in the last month of lactation could help to reduce milk supply in high-producing herds and that udder firmness will increase compared with cows milked 2×.

Key Words: 1× milking, udder firmness

SC163 Evaluating the impact of bacterial load and IgG concentration in colostrum on passive transfer in dairy calves. S. Johnson* and J. Bohlen, *University of Georgia, Athens, GA.*

Calves are born with little to no immunoglobulins due to a unique fetal-dam relationship in utero and are extremely susceptible to disease within the first few days of life. The main source of immunoglobulins to a newborn calf comes via colostrum. However, several variables impact colostrum quality, which ultimately impact the ability of a calf to utilize its immunoglobulins and nutrient rich components to the fullest. The objective of this study was to investigate 1) colostrum handling methods and colostrum quality on passive immunity in calves and 2) to describe the bacterial population of colostrum. For objective 1, calves (n = 22) received either fresh (n = 5) or previously frozen (n = 17) colostrum. Colostrum was evaluated using Brix refractometry with a Digital-Dairy DD3 before feeding. Success or failure of passive transfer in calves (n = 22) was determined via blood collection at 48–60 h of age. Using the DD3, blood serum was evaluated for IgG (n = 22 calves) and total proteins (TP) (n = 16 calves) with effective passive transfer confirmed at 8.3% and 5.2 g/dL, respectively. For objective 2, colostrum samples (n = 39) were evaluated for bacterial profile using on-farm methods with Petrifilm and with laboratory microbiology. Bacterial counts were determined with Petrifilm using a 1:125 direct inoculation with 1 mL of the diluted colostrum and colonies counted at 48 h after incubation at 35°C. Further an aliquot of each sample was sent for more specific bacteriology by lab analysis. Variables were analyzed using correlation coefficients, *t*-tests and linear models. Average Brix readings for fresh versus previously frozen colostrum were 26.3% and 22.1%, respectively and were not different ($P > 0.05$). Colostrum Brix reading was positively correlated with calf IgG ($r = 0.45$) and calf IgG was positively correlated with TP in calf serum ($r = 0.97$). Bacterial load as determined by Petrifilm colony counts nor bacterial type as determined by laboratory analysis influenced the Brix reading of colostrum. In total there were 29 species of bacteria isolated from colostrum samples sent for bacterial analysis. In highest abundance were bacilli, found in 61.5% of samples, *Pseudomonas* found in 33.3% of samples and coagulase (-) found in 20.5% of samples. Further work is necessary to evaluate the method of handling on bacterial species and the impact of these bacteria on immunoglobulin absorption and gut microbiome establishment.

Key Words: bacteria, colostrum, passive transfer

SC164 Enhancing postweaning prepubertal mammary growth through application of estrogen. I. Leonard*, E. Harrison, M. Ellett, C. Parsons, A. Lengi, B. Corl, and K. Daniels, *Virginia Tech, Blacksburg, VA.*

Enhanced prepubertal mammary growth has potential to increase first-lactation milk yield in dairy heifers. An earlier experiment showed a pronounced mammogenic effect of estrogen when applied to newly weaned heifers for 2 wk. Estrogen effects beyond 2 wk are not well characterized. The objective was to determine the effects of exogenous postweaning estrogen administration on mammary growth in Holstein heifers. Twelve Holstein heifers (74 ± 9 d of age, 79 ± 13 kg BW) were blocked by weight into 2 groups. Six heifers received an estradiol implant (EST; Compudose, 25.7 mg estradiol; Elanco) and 6 heifers received a blank implant (CON). Heifers were housed and fed in a single group and received a 16% CP, 3% fat (as-fed) heifer grower feed. Heifers were weighed weekly and slaughtered at 150 kg BW. At slaughter, udders were removed, weighed, and subsampled. Data were analyzed using treatment (CON, EST) as the fixed term; heifer was the experimental unit. Neither age at slaughter (127 ± 5 d and 125 ± 5 d, respectively), nor BW at slaughter (150 ± 4 kg and 154 ± 4 kg) were affected by treatment. Total udder mass (671 ± 89 g and 799 ± 89 g,), mass of right half parenchyma (27.2 ± 3.7 g and 27.8 ± 3.7 g), and mass of right half mammary fat pad (220 ± 29 g and 261 ± 29 g) did not differ by treatment. Average teat length was greater in EST compared with CON (4.0 ± 0.1 cm and 2.8 ± 0.1 cm, respectively). Estrogen receptor-α intensity in parenchyma, assessed by Western blot analysis, was greater in CON compared with EST (6.54 ± 0.97 a.u. and 1.51 ± 0.97 a.u., respectively). Overall, and in contrast to previous research, exogenous estrogen applied to Holstein heifers for 56 d from 79 ± 13 kg to 152 ± 4 kg BW did not increase mammary growth. Perhaps the duration of estrogen exposure, age, weight, or body composition at application, or some combination of the previous ultimately drive mammogenic efficacy of estrogen implants in prepubertal heifers.

Key Words: heifer, mammary growth, estrogen

SC165 How personality affects dairy heifer response to music genres: An early investigation.

R. Hall*, M. C. Cantor, M. Griffin, A. Martin, R. Drake, J. Doak, G. Mazon, and J. H. C. Costa, *Dairy Science Program, University of Kentucky, Lexington, KY.*

The objective of this study was to evaluate if personality traits of heifers were associated with the response to 2 music genres. Holstein heifers (n = 6) at 355 ± 10 d of age (mean ± Sd) were first evaluated for personality traits using a 5-min novel environment test with a randomized testing order. The test pen consisted of a 48-m^2 pen with floor markings and a Bluetooth speaker placed opposite of the pen entrance. A bucket containing grain was placed by the entrance of the pen before the music exposure to potentially remove negative associations the animals had with the test environment. Once heifers consumed grain, they were exposed for 3 min to either an upbeat or a slow song in a randomized order. Approach to the speaker was recorded for each animal as they entered a 1-m radius from the speaker. The test ended if the approach occurred before 3 min. After the first song, heifers were given a 2 h rest period before being retested. From the novel environment test, heifers were classified as exploratory (EX) or fearful (FE) using video software to analyze their reactions. Differences between personality traits were assessed using paired *t*-tests. Three heifers were considered FE: high escape behavior, high vigilance behavior at pen entrance, less distance traveled across quadrants, as well as many vocalizations. Three heifers were EX: high quadrants crossed, approached camera, touched pen sides, sniffed floor, low to no vocalizations. For the music test, observers recorded latency to approach the grain bucket, time attentive toward speaker once music was playing, escape behavior, vocalizations, and latency to approach the Bluetooth speaker. Fearful heifers crossed quadrants less often when compared with exploratory heifers (FE = 4.5 ± 3.6, EX = 12.0 ± 2.3 times). Latency to approach grain was lower for EX heifers (FE = 3.0 ± 0.0, EX = 1.1 ± 0.0 min). There was no effect seen of music genre on latency to approach the music, or duration of time spent attentive. Regardless of the music genre, FE heifers tended to spend more time lookingf at the music source (FE = 29.0 ± 8.0 and EX = 14.0 ± 1.0), were less likely to approach the speaker, and maintained a further distance from the speaker.

Key Words: behavior, management, welfare

SC166 Location differences in Tennessee dairy operations.

A. McCalmon*, S. Schexnayder, A. Sen, D. Bilderback, and E. Eckelkamp, *University of Tennessee Institute of Agriculture, Knoxville, TN.*

Tennessee (TN) is geographically divided into East, Middle, and West regions that have distinct features and farmer populations. The objective of this study was to observe differences across the regions in farmer ages, years owned, herd size, labor force, milk marketing strategy, herd production, and bulk tank somatic cell count (BTSCC). Data for this project was a subset of a larger study. A survey was electronically distributed and mailed to all permitted TN dairy operations in August 2019. Ninety surveys were returned and 88 provided the data needed in the analyses representing 48% of TN dairy operations at the time of the survey. The total responses provided for East (n = 43), Middle (n = 36), and West (n = 9) TN were used in all analyses. The MEANS procedure of SAS 9.4 was used to describe farmer age, years owned, herd size, labor, herd production, and BTSCC by location. The FREQ procedure of SAS was used to describe the current and preferred milk marketing strategy by location. The MIXED procedure of SAS was used to determine the impact of location on farmer age, years owned, herd size, family labor, non-family labor (NFL), herd production, and BTSCC with significance of $P < 0.10$. The CORR procedure of SAS was used to determine relationships between significant variables ($P < 0.10$). Across locations, 47% of producers preferred a different milk market than they currently had (52% vs. 24% current vs. preferred milk cooperatives, respectively; 41% vs. 53% current vs. preferred independent plant contracts; and 7% vs. 22% current vs. preferred on-farm processing or a combination of other marketing strategies). East TN had the largest mean herd size followed by Middle and West (340 ± 53, 180 ± 58, and 99 ± 116 cows, respectively; $P = 0.05$). East TN also had the most NFL compared with Middle and West (5 ± 1, 3 ± 1, and 1 ± 2 workers, respectively; $P = 0.07$) However, no other variables were significantly different across locations. Non-family labor was positively correlated with herd size ($P < 0.001$), indicating larger herds had more non-family labor. Although production and BTSCC did not differ across TN, significantly larger herds were present in Middle and East TN. More research is needed to determine barriers to expansion in West TN and access to NFL.

Key Words: labor, survey, marketing strategy

SC167 Is greater milk production associated with cows who have a greater probability of ruminating while lying down?

C. J. McWilliams*, A. J. Scwhanke, and T. J. DeVries, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to determine if there is an association of position while ruminating (standing vs lying) with production in dairy cows. Data from 30 lactating Holstein dairy cows was assembled from 2 studies in which cows were milked by automated system (AMS) and fed a PMR in feed bins that recorded intake behavior. Rumination and lying behavior were monitored used automated neck- and leg-based sensors, respectively. Each cow was monitored over 2 separate, 2-wk treatment periods. To estimate position while ruminating, for each 2-h period of the day for each cow, a conditional probability (ranging from 0 to 1) was calculated to determine the probability that during that period any rumination time and lying time were occurring at the same time. These probabilities (RwL), and all behavioral data, were summarized per cow per 2-h interval throughout the day, and then averaged per day and then per 2-wk period, along with milk yield and component data. Cows averaged (mean ± SD) 1.9 ± 1.1 lactations, 85.5 ± 55.2 DIM, and 668.5 ± 96.0 kg BW at the start of their respective studies. Data included rumination time (557.7 ± 41.1 min/d), lying time (703.9 ± 65.3 min/d), idle standing time (520.1 ± 83.2 min/d), PMR feeding time (204.7 ± 48.5 min/d), PMR DMI (21.8 ± 4.6 kg/d), AMS pellet (4.6 ± 1.6 kg/d), total DMI (26.4 ± 4.5 kg/d), and DMI%BW (3.96 ± 0.43%). Production data included milk yield (42.4 ± 7.2 kg/d), 4%FCM (37.6 ± 7.5 kg/d), milk fat content (3.75 ± 0.51%) and yield (1.56 ± 0.39 kg/d), and milk protein content (3.21 ± 0.32%) and yield (1.35 ± 0.31 kg/d). Behavioral predictors and production outcomes associated with RwL probability (mean = 0.19 ± 0.02; range = 0.14–0.23) were tested in mixed-effect linear regression models. RwL probability was associated ($P \leq 0.05$), with greater rumination time (+0.0002/min), lying time (+0.0002/min), and lesser idle standing time (−0.0001/min). RwL probability was predictive of total DMI (kg/d = $-7.0 + 32.4 \times \text{RwL} + 0.04 \times \text{BW}$; $P = 0.075$), milk fat content (% = $0.64 + 5.77 \times \text{RwL} + 0.50 \times \text{DMI\%BW}$; $P = 0.06$) and milk protein content (% = $2.05 + 6.01 \times \text{RwL}$; $P = 0.004$), but not associated with any measures of milk yield. The results indicate that cows who have greater probability of ruminating while lying down spend more time ruminating, lying, spend less time standing inactive, and consume more total DM and produce milk with greater milk fat and protein content.

Key Words: behavior, rumination, components

SC168 Effect of feeding rumen-protected methionine and lysine with or without an animal-derived source of rumen-undegradable protein on performance of mid-lactation cows.

M. Machiela*[1], A. N. Negreiro[1], J. de Souza[2], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Perdue AgriBusiness, Salisbury, MD.*

We determined the effect of supplementing rumen-protected methionine (Met) and lysine (Lys) with or without an animal-derived source of rumen undegradable protein (RUP) on the yields of milk and milk components of mid-lactation dairy cows. Thirty-six Holstein cows (138 ± 31 DIM) were used in a 3 × 2 incomplete Latin square arrangement of treatments with 2 consecutive 35-d periods with the last 5 d of each period used for sample and data collection. Treatments were: 1) control diet containing 16.5% CP (10% RDP + 6.5% RUP) without supplemental AA (CON); 2) 16.5% CP (10% RDP + 6.5% RUP) diet with rumen-protected Met and Lys (ML); and 3) 16.5% CP (9.7% RDP + 6.8% RUP) diet supplemented with rumen-protected Met and Lys and an animal-derived source of RUP (MLO). All diets were formulated to contain (% diet DM) 30.0% NDF, 28.5% starch, and 5.0% fat. Diets 2 and 3 were supplemented to provide 24 g and 14 g of metabolizable Lys and Met, respectively. The statistical model included the random effect of cow and the fixed effects of treatment, period, square, and their interactions. Preplanned contrasts were: 1) overall effect of AA supplementation (PROT) compared with CON; and 2) effect of AA supplementation (ML vs. MLO). Results are presented in the following sequence: CON, ML, and MLO. Compared with CON, PROT increased the yields of 3.5% FCM (42.3, 44.6, 44.7 kg/d; $P = 0.03$), ECM (42.8, 44.3, 44.2 kg/d; $P = 0.02$), milk fat (1.57, 1.61, 1.63 kg/d; $P = 0.04$), milk protein (1.26, 1.33, 1.31 kg/d; $P = 0.01$), and milk protein concentration (3.05, 3.12, 3.14%; $P < 0.01$). PROT tended to increase the yields of milk (41.3, 42.8, 42.2 kg/d; $P = 0.05$) and milk lactose (1.98, 2.06, 2.04; $P = 0.05$). We did not observe differences between ML and MLO for yields of milk, 3.5% FCM, ECM, milk fat, or milk protein (all $P > 0.35$). However, compared with MLO, ML decreased milk fat (3.77 vs. 3.95%; $P < 0.01$) and milk lactose concentrations (4.81 vs. 4.83%; $P = 0.04$). In summary, PROT treatments increased the yields of milk fat and protein, although no differences were observed between ML and MLO.

Key Words: amino acids, dairy cows, milk components

SC169 Associations between activity patterns during the dry period and calving events in dairy cattle. M. Shabloski*[1], A. A. Barragan[1], E. Hovingh[1], and L. da Costa[2], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.*

The aim of this study was to evaluate associations between activity patterns (i.e., lying time, number of lying bouts, and lying bout duration) during the 14 d prepartum and calving events in dairy cattle. Heifers and cows from 4 large dairy farms (700–2,800 milking cows) located in Pennsylvania were enrolled in this trial. Based on cow conception date information, obtained from on-farm records, cows that were between 20 and 30 d from expected calving date were fitted with a HOBO accelerometer on the rear right leg. Only cows that had complete activity data for 14 d before the actual calving date were included in the study (heifers, n = 66; cows, n = 70). A calving record sheet was developed for farm personnel to collect calving-related events (i.e., calving difficulty [no assistance, mild assistance, severe assistance]; calf presentation [forward, backward, breech, large calf], calf limb position [extended or deviated], twin births, stillbirth, and calf sex). The data were analyzed using the MIXED procedure of SAS. Heifers spent less time lying down compared with cows during the 14 d before calving (heifer = 766.01 ± 46.25 min/d; cow = 789.36 ± 46.72 min/d). Heifers and cows that had a female calf spent more time lying down (female calf heifer/cow = 793.66 ± 46.78 min/d; male calf heifer/cow = 761.71 ± 46.21 min/d), had more lying bouts (female calf cow = 11.81 ± 0.72 bouts/d; male calf cow = 10.44 ± 0.71 bouts/d), and had shorter lying bouts (female calf cow = 74.15 min, 95% CI:57.90–94.93; male calf cow = 82.49 min, 95% CI:64.25–105.88) compared with heifers and cows that had a male calf. Heifers and cows that experienced a severe assisted calving spent more time lying down during the 14 d before calving compared with cows that experience either normal or mild assisted calvings (no assistance calving cows = 786.68 ± 44.84 min/d; mild assistance calving cows = 722.75 ± 48.85 min/d; severe assistance calving cows = 823.63 ± 53.32 min/d). The study results suggest that monitoring cow behavior before calving may have the potential to predict important calving events, ultimately helping farmers to plan farm management practices accordingly.

Key Words: dry period, activity patterns, calving events

SC170 Assessment of the effects of intrauterine dextrose infusion after calving on uterine health, metabolic stress, systemic inflammation, and daily milk yield in healthy postpartum dairy cows. H. Mattice*[1], E. J. Arrieta[2], A. A. Barragan[1], S. Bas[3], E. Hovingh[1], and M. Martinez[1], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica, Heredia, Costa Rica,* [3]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.*

The objective of this study was to assess the preventive effects of intrauterine infusion of a 50% dextrose solution after calving on clinical metritis incidence, metabolic stress (i.e., body condition score [BCS], β-hydroxybutyrate [BHB] concentration), systemic inflammation (i.e., haptoglobin [HP] concentration) and daily milk yield in postpartum dairy cows. Cows from a dairy farm located in southwest Pennsylvania were screened for eligibility at 4 ± 1 DIM (study d 0). Cows that presented a normal vaginal discharge, did not show any clinical signs of disease (e.g., lameness, mastitis), and had a normal rectal temperature (i.e., <39.7°C) were blocked by parity and randomly assigned to one of 3 groups: 1) SAL (n = 46): one aseptic intrauterine infusion of saline solution (1 L/cow); 2) DEX (n = 44): one aseptic intrauterine infusion of a 50% dextrose solution (1 L/cow), and 3) CON (n = 45): no treatment. Cows were re-screened 7 d after enrollment (study d 7) to assess presence of fetid red-brownish vaginal discharge (i.e., clinical metritis). BCS was assessed and blood samples were collected at enrollment and at study d 7, 14 and 21. Daily milk yield for the first 60 DIM was collected from the on-farm computer records. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized complete block design. There was no difference on daily milk yield between treatement gorups. A greater proportion of cows in the SAL and DEX groups tended to have clinical metritis compared with CON cows (SAL = 39.76 ± 9.26%; DEX = 38.57 ± 9.18%; CON = 16.33 ± 6.29%). Furthermore, SAL cows had higher HP (SAL = 132.45 ± 28.48 µg/mL; DEX = 86.52 ± 29.34 µg/mL; CON = 49.74 ± 21.45 µg/mL) and BHB concentrations (SAL = 1.32 mmol/L, 95% CI: 1.16–1.5; DEX = 1.24 mmol/L, 95% CI:1.07–1.42; CON = 1.02 mmol/L, 95% CI:0.88–1.16) at study d 7 compared with DEX and CON cows. These results suggest that postpartum intrauterine preventive treatments in healthy cows may interfere with normal uterine involution processes, impairing subsequent uterine health.

Key Words: calving, dextrose, clinical metritis

SC171 Valorization of buttermilk powder to produce a milk fat globule membrane (MFGM)-enriched ingredient. A. Carroll*, J. Ortega-Anaya, and R. Jiménez-Flores, *Food Science Department, The Ohio State University, Columbus, OH.*

The objective was to use buttermilk powder (BMP) to prepare an ingredient enriched in milk fat globule membrane (MFGM), reduced in casein and whey proteins, and suitable for beverage industry applications. A method for isolating and concentrating MFGM from rehydrated buttermilk was developed. Ammonium hydroxide (NH_4OH), rennin, and acetic acid treatments with or without trisodium citrate (TSC) were tested. NH_4OH and TSC were expected to solubilize casein micelles; rennin and acid treatments were expected to precipitate out caseins. The best method was chosen based on qualitative observations of the degree to which sodium dodecyl sulfate-PAGE (SDS-PAGE) and thin layer chromatography (TLC) showed the presence of MFGM proteins or phospholipids in treated samples. Rennin precipitation (30°C, 1% $CaCl_2$, pH 5.47, 5% rennin), followed by cheesecloth straining, addition of TSC (2.5%) to the liquid fraction, ultrafiltration (50 kDa; 4× concentration, 5× diafiltration), and centrifugation of the retentate was determined to be the best method to obtain a MFGM enriched semi-solid pellet ("the ingredient"). SDS-PAGE results showed this treatment had the most consistent enrichment in MFGM proteins, with a large reduction in casein proteins. Bands with molecular weights of 169, 80, 58, 38, 21, and 20 kDa were detected, corresponding to MFGM proteins xanthine oxidase/dehydrase, butyrophilin, and lactadherin/periodic acid Schiff base 6 and 7 doublet, and to the milk proteins casein, β-lactoglobulin, and α-lactalbumin. Difficulties separating out whey proteins could be due to protein-protein aggregates induced by heat treatment of BMP. TLC results confirmed the presence of MFGM phospholipids (sphingomyelin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine). Using the ingredient, several prototype MFGM enriched, probiotic fruit-flavored beverages were made. Valorization of

BMP can reduce dairy industrial waste and expand MFGM nutritional benefits to the general population beyond infant formula.

Key Words: milk fat globule membrane, buttermilk powder, dairy waste reduction

SC172 Effects of prolactin on de novo fatty acid synthesis in mammary epithelial cells. A. Beach*, A. C. Hruby, G. Kawecki, and M. D. Hanigan, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

Prolactin has been demonstrated to positively influence mammary cell activity. Thus, prolactin is added to media to stimulate milk component production in bovine mammary epithelial cells (BMEC), but the amount used greatly varies. For instance, lactogenic hormones such as prolactin were used in media in concentrations as high as 10 mg/L. Further, cell culture grade prolactin is not always available. Thus, the objective of this study was to determine prolactin concentrations required to induce milk fatty acid production in BMEC. The study was a complete randomized design consisting of 6 treatments with 3 replicates per treatment. Treatments were varying prolactin concentrations of: 1) 0 mg/L, 2) 0.25 mg/L, 3) 0.5 mg/L, 4) 1 mg/L, 5) 2.5 mg/L, and 6) 5 mg/L. The base medium was DMEM/F12 supplemented with 10% FBS. Primary BMEC were obtained from the State Key Laboratory of Animal Nutrition in Beijing, China. Cells were seeded in the presence of 10% FBS, and medium was changed daily. Upon reaching > 90% confluency, the FBS was removed from the medium and insulin (5 mg/L), transferrin (5 mg/L), hydrocortisone (1 mg/L), EGF (0.01 mg/L), sodium acetate (5 mM), and prolactin (as specified for each treatment) was applied for 48 h followed by fatty acid starvation media for 24 h composed of a custom DMEM/F12 without fatty acids. ^{13}C-labeled acetate was added to the medium for 4 h to assess rates of de novo fatty acid synthesis. Methyl esters of the fatty acids harvested from each plate were created and analyzed for isotopic enrichment using an ion trap mass spectrometer linked to a gas chromatograph. Enrichment was corrected for background and expressed as an isotope ratio (IR). Enrichment data were checked for normality; C14:0 and C18:0 IR were adjusted by taking the inverse. Data were analyzed in R studio using a linear model (lmer package). The isotope ratios indicate there were no differences among treatments in fractional incorporation of labeled acetate into C12:0, C14:0, or C18:0 ($P > 0.05$). Thus, prolactin may be excluded or used in low concentrations for as many as 2d when examining milk fatty acid synthesis.

Table 1. Fatty acid isotope ratios (IR) in response to varying prolactin concentrations

Fatty	Prolactin (mg/L)							
acid	0	0.25	0.5	1	2.5	5	SEM	*P*-value
C12:0	0.30	0.30	0.25	0.32	0.31	0.21	0.04	0.37
C14:0[1]	2.96	3.31	3.16	3.31	3.19	3.14	0.15	0.61
C18:0[1]	17.0	12.6	13.5	15.1	14.3	15.1	2.22	0.79

[1] Data expressed as inverse of IR.

Key Words: cell culture, isotope

SC173 Evaluation of the use of intervaginal temperature monitors to assess postprandial body temperature changes. G. Combs*[1], L. Cavani[1], F. Baier[1], M. Martin[1], S. Erb[1], M. VandeHaar[2], J. Koltes[3], K. Weigel[1], F. Peñagaricano[1], and H. White[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Michigan State University, East Lansing, MI,* [3]*Iowa State University, Ames, IA.*

Fermentation is a main energy source in dairy cows and measuring postprandial temperature changes may offer insights into how differences in fermentative efficiency impact overall feed efficiency. The objective of this analysis was to explore postprandial temperature change (ΔT) using intravaginal temperature sensors to identify patterns that may be indicative of individual animal variation in efficiency. Eighteen mid-lactation Holstein cows (8 multiparous, 10 primparous; 180 ± 17 DIM) were housed in a freestall pen with Insentec feeders for a 45d feed efficiency study. Intravaginal Thermocron temperature logging iButtons mounted on a non-medicated CIDR Cattle Insert recorded temperature, recording in 5-min intervals for 14d. Meal bouts were defined as the largest (MAX; greatest DMI/d) and meals that were at least 5% (FIVE) or 10% (TEN) of daily DMI from Insentec data. Temperatures were averaged over 15-min intervals for 120 min post-feeding, deviated (ΔT) from 15-min pre-feeding, and averaged over 14 d (ΔT_{avg}). Residual feed intake (RFI) was calculated by regressing DMI on milk energy output(parity), median DIM(parity), metabolic BW(parity), and change in BW(parity). Negative RFI cows (more feed efficient; n = 10) were compared with positive RFI cows (less feed efficient; n = 8). The ΔT_{avg} for each period post feeding for low and high RFI was compared. In general, ΔT_{avg} 45 min postprandial was MAX: −0.161 ± 0.052°C; TEN: −0.167 ± 0.086°C; FIVE: −0.161 ± 0.049°C. Most cows had ΔT <0°C by 120 min (MAX: n = 8; TEN: n = 12, FIVE: n = 11). Visual assessment of ΔT graphs suggest an average nadir between 45 and 60 min. Comparing ΔT at a potential nadir may be insightful of high efficiency (MAX: −0.177 ± 0.071°C; TEN: −0.177 ± 0.122°C; FIVE: −0.168 ± 0.036°C) vs. low efficiency cows (MAX: −0.142 ± 0.045°C; TEN: −0.137 ± 0.101°C; FIVE: −0.171 ± 0.040°C). A linear regression comparing RFI to ΔT 45 to 60 min postprandial was not significant (e.g., FIVE: $P = 0.253$, adj. R^2: 0.023). Intravaginal ΔT may reflect postprandial changes in fermentation and should be further explored to identify differences potentially attributed to individual cow variance in efficiency.

Key Words: feed efficiency, body temperature

SC174 Probiotic survivability and health-promoting benefits of the bioaccessible compounds in fermented camel milk after in vitro digestion by INFOGEST2.0: Comparative study with fermented bovine milk. A. Al-Hammadi* and M. Ayyash, *United Arab Emirates University, Al-Ain, United Arab Emirates.*

This project aimed to 1) investigate the probiotic survivability in fermented camel milk under in vitro gastrointestinal digestion, 2) examine the health-promoting benefits of the bioaccessible compounds in the fermented camel milk after in vitro digestion, 3) investigate the differences in the health benefits before and after the in vitro digestion, and 4) identify the bioaccessible compounds in the fermented camel milk. Camel and bovine milk were fermented individually using starter bacteria (SC, 2 strains of *Lactobacillus bulgaricus*), a potential probiotic (Pro, *Lactococcus lactis*) or a combination (SC+Pro) thereof, followed by storage for 21 d. Each experimental fermented product was performed in triplicates and samples at d 1 and d 28 were analyzed in duplicates (n = 6). The significant effect of the milk type (camel vs bovine milk) and culture type (SC, SC+Pro, and Pro) was examined by 2-way ANOVA using XLSTAT software. Means comparisons were carried out using fisher's tests ($P < 0.05$). The in vitro antioxidant activities, antidiabetic properties, ACE-inhibition, and anticancer activities were assessed before (water-soluble extract) and after (bioaccessible) the in vitro digestion using INFOGEST model. The viable numbers of lactobacilli and lactococci populations in the fermented milk and control were enumerated during the digestion steps (before in vitro digestion, and after the oral, gastric, and intestinal digestion steps. Lactobacilli were enumerated on MRS agar and plates were incubated anaerobically at 37°C for 48 h using anaerobic incubator with CO_2 5.0%. Lactococci were enumerated on M17 agar and plates were incubated aerobically at 37°C for 24 h. The bioaccessible fraction of fermented camel milk exhibited higher biological functionality compared with fermented bovine milk. ACE-inhibition ranged from 64 to 86% and 68–85% in the bioaccessible fractions from fermented camel and bovine milk, respectively. Antiproliferative activity against HT-29 colorectal cancer cell line of the bioaccessible fractions from fermented camel and bovine milk ranged from 2.5 to 11.7% and 1.5–4.0%. Untargeted metabolomics demonstrated noticeable discrimination between the camel milk samples fermented with different cultures, indicating that changing the culture type affected the metabolites qualitatively and quantitatively.

Key Words: camel milk, fermented product, bioaccessible

SC175 Low-fat Akawi cheese made of bovine-camel blended milk: Rheological properties, texture profile, and microstructure.

S. Al-Madhani* and M. Ayyash, *United Arab Emirates University, Al-Ain, United Arab Emirates.*

Due to the poor rennet-induced gel formation of camel milk. Cheesemaking from camel milk alone is a challenge. Therefore, mixing camel milk with bovine milk would be more visible and practical to dairy manufacturers. Thus, this study aimed to investigate the chemical composition, rheological properties, texture profile, and microstructure of low-fat akawi cheese (LAFC) made of blended camel milk with bovine milk. LAFC was made of bovine milk only (BM, control), bovine milk blended with 15% (CM15%), or 30% (CM30%) camel milk. LAFCs were stored at 4C for 28 d. The experimental cheeses were sampled on d 1 and d 28. Chemical composition (moisture, protein, fat, carbohydrates, ash), rheological properties (viscoelastic properties and thermal behavior), texture profile (hardness, cohesiveness, gumminess), and microstructure were assessed on d 1 and d 28. Each experimental cheese was made in duplicate, and samples were analyzed in triplicate (n = 6). One-way ANOVA was performed to assess the significant effect of the camel milk on the cheese parameters ($P < 0.05$) at each storage time. A means comparison was performed using Tukey's test. XLSTAT software was employed to run the analysis. The magnitude of storage modulus G′ was significantly higher than the loss modulus for cheeses made of camel milk 15% and camel milk 30% during all storage periods. The elasticity is higher than the viscosity, with the BM having the highest elasticity, followed by CM-15% and, lastly, the CM-30%. Cheeses on d 0 exhibited more hardness, springiness, and chewiness results than after storage on d 28. the pH of cheeses with camel milk blend declined after 28 d of storage. The microstructures of the blended Akawi cheeses were more closed compared with bovine milk only. In conclusion, camel milk affected the rheological, textural, and microstructural properties of low-fat akawi cheese.

Key Words: camel milk, Akawi cheese, rheological properties

SC176 Functional properties of the bioaccessible compounds of low-fat Akawi cheese made of bovine-camel blended milk subjected to in vitro digestion by INFOGEST2.0.

M. Alameri* and M. Ayyash, *United Arab Emirates University, Al-Ain, United Arab Emirates.*

Camel milk was known for its nutritional and health benefits for a long time. The potential bioactive properties of casein protein in camel milk have been explored widely. Camel milk forms a very weak gel induced by rennet. Therefore, this study aimed to 1) make a low-fat Akawi cheese (LFAC) produced from blended camel and bovine milk, and 2) assess the health-promoting benefits of the bioaccessible compounds of the LFAC after in vitro digestion using the INFOGEST2.0 system. LFACs were made from bovine milk (BM) only, blended with camel milk by 15% (CM15%), and 30% (CM30%) followed by storage for 28 d at 4°C. All LFACs were in vitro digested and a dialysis membrane (10000 MWCO) was employed to assess the bioaccessible compounds. The antioxidant activities, α-glucosidase, and α-amylase inhibitions, and ACE-inhibition were employed to assess the health-promoting benefits. The proteolytic hydrolysis was performed using the OPA test and degree of hydrolysis. One-way ANOVA was carried out to examine the significant effect of camel milk addition at each storage time. Tukey's test was performed for means comparison ($P < 0.05$). XLSTAT software was used for statistical analysis. Results showed that pH decreased significantly during storage for all treatments. The antioxidants activity increased in the bioaccessible samples for all the treatments compared with pH4.6-water-soluble extract (WSE; before digestion). Blended Akawi cheese (CM30%) also displayed higher potential antidiabetic activities compared with bovine cheese only. The antihypertensive property via angiotensin-converting enzyme (ACE) inhibition was found to be high for mixed Akawi cheese (CM15% and CM30%) on d 1 and d 28. The degree of hydrolysis (DH%) was higher in Akawi cheese CM30% compared with bovine only. Overall, the blended Akawi cheese exhibited superior antioxidant, antidiabetic, and antihypertensive properties compared with Akawi cheese made from bovine milk.

Key Words: Akawi cheese, camel milk, antioxidants

Animal Behavior and Well-Being I

100 Effects of shade and sprinklers on physiological responses of heat stress on grazing dairy cows. C. A. Becker*, A. R. Woolums, B. B. Karisch, M. X. S. Oliveira, and A. E. Stone, *Mississippi State University, Mississippi State, MS.*

This study was conducted at the Mississippi State University Bearden Dairy Research Center from July 1, 2019 to July 30, 2019. The objective of the study was to monitor effects of heat stress on grazing dairy cows by comparing daily milk yield, reticulorumen temperature (RT; SmaXtec, Graz, Austria), respiration rate (RR), milk fat percent, milk protein percent, somatic cell score (SCS), hygiene score, and body condition score (BCS). Three treatments were studied: shade (portable shade structure), sprinkler (portable polyvinyl chloride pipe sprinkler system), and control (no heat abatement). Each treatment was replicated 3 times (n = 3) with 3 s trimester, lactating cows (2 Holstein and one Jersey) in each pen (27 cows total enrolled in study). Pens were balanced by parity, days in milk, and days in gestation. Ambient temperature and relative humidity were averaged and split into 3 time periods: morning (0000 h to 1159 h), afternoon (1200 h to 1759 h), and evening (1800 h to 2359 h). The MIXED procedure of SAS was used to evaluate the effects of treatment, time of day, and treatment × time of day interaction on milk yield, reticulorumen temperature, and respiration rate. For milk yield, milk fat percent, milk protein percent, somatic cell score, hygiene score, and body condition score treatment, week, and treatment × week effects were evaluated. Pen nested within treatment was used as a random variable to account for pen being the experimental unit. Comparison of effects were conducted using LSMEANS. No significant difference occurred for milk yield between treatments (11.51 ± 0.54 kg/d, 11.35 ± 0.54 kg/d, and 12.82 ± 0.54 kg/d for control, shade, and sprinkler, respectively). However, a significant week effect occurred for milk yield. Sprinkler cows had a significantly lower RR (66.0 ± 6.70 breaths/min) compared with control cows (93.34 ± 6.54 breaths/min). No significant difference in RR occurred between the shade and control groups or the sprinkler and shade groups. A treatment × time of day interaction occurred for RR, RT, milk fat percent, milk protein percent, SCS, and BCS. Hygiene scores were lower for the sprinkler group (1.47 ± 0.16) compared with the shade (2.39 ± 0.16) and control group (2.44 ± 0.16). Providing sprinklers for grazing dairy cows may decrease some of the negative physiological effects associated with heat stress to a greater extent when compared with shade or no heat abatement.

Key Words: heat stress, grazing, sprinkler

101 *Salix* extract and flunixin meglumine dosage to minimize inflammation after disbudding in dairy calves. K. Sharpe*[1], B. Heins[1], M. Endres[2], and H. Phillips[2], [1]*West Central Research and Outreach Center, Morris, MN,* [2]*University of Minnesota, St. Paul, MN.*

The objective of the study was to determine the dosage of *Salix* extract (Mountain Rose Herbs, Eugene OR) from white willow bark that would minimize inflammation in dairy calves during disbudding. Calves were Holstein and crossbred bull calves (n = 25; 4 to 9 wk of age) that were housed in an automatic calf feeder group fed 8 L/d. A randomized crossover design with repeated measures was used for the study. During each of the 2 treatment periods, calves were randomly assigned to 1 of 5 treatment groups: 1) No treatment (CON; n = 5), 2) Flunixin meglumine (FM; 2.2 mg/kg BW; n = 5), 3) low dose of *Salix* extract (LD; 225 mg/kg BW; n = 5), 4) medium dose of *Salix* extract (MD; 450 mg/kg BW; n = 5) or 5) high dose of *Salix* extract (HD; 900 mg/kg BW; n = 5). Flunixin meglumine was administered via venipuncture, and *Salix* extract was administered orally via a rumen bolus. A 7-d washout period was used to minimize carry-over effects between periods; treatment sequences were orthogonal. Blood samples via venipuncture were collected 5 min before and 1, 2, and 4 h after treatment. The samples were sent to Iowa State University for analysis. Concentration of flunixin meglumine and *Salix* extract in the blood were determined using HPLC. Inflammation was determined by measuring prostaglandin E2 (PGE2) metabolites in the blood. Dependent variables for analysis were PGE2 and *Salix* extract concentrations. Independent variables were breed, period, treatment, time, the interaction of period and treatment, and the interaction of time and treatment. Calf body weight was a covariate, and calf within period was a random effect within the model. There were no differences among treatments for *Salix* extract concentration in blood serum across period and time. The PGE2 concentration was 5,229 pg/mL for CON; 3,393 pg/mL for FM; 5,167 pg/mL for LD; 3,329 pg/mL for MD; and 3,842 pg/mL for HD. Differences were not significant among treatments or period. In summary, *Salix* extract did not provide reduction in inflammation compared with control for hot iron disbudding in dairy calves.

Key Words: disbudding, flunixin meglumine, *Salix*

Animal Health I

102 The economic impact of suboptimal mobility in spring-calving, pasture-based dairy herds. A. H. O'Connor*[1,2], E. A. M. Bokkers[2], I. J. M. de Boer[2], H. Hogeveen[3], R. Sayers[1], N. Byrne[1], E. Ruelle[1], and L. Shalloo[1], [1]*Teagasc, Animal and Grassland Research and Innovation Center, Moorepark, Fermoy, Co. Cork, Ireland,* [2]*Animal Production Systems group, Department of Animal Science, Wageningen University & Research, the Netherlands,* [3]*Business Economics group, Department of Social Sciences, Wageningen University & Research, the Netherlands,* [4]*Mathematical and Statistical Methods - Biometris, Department of Plant Sciences, Wageningen University & Research, the Netherlands.*

Suboptimal mobility refers to any abnormality to a cow's gait which causes a deviation from the optimal walking pattern of a cow. So far, the economic consequences of suboptimal mobility has been investigated mainly for non-pasture-based systems. The aim of the current study was to determine the economic consequences, specifically on farm net profit, associated with varying prevalence of suboptimal mobility within spring calving, pasture-based dairy herds. A new submodel was developed (Mobility submodel) predicting mobility scores (on a 4-point, 0–3 scale) was developed and integrated within the existing Pastured Based Herd Dynamic Model (PBHDM). The PBHDM simulates the operation of a spring calving, pasture-based dairy farm with a daily time step. The Mobility submodel predicts claw disorders initially and then the mobility score of each individual cow in the herd. The impact of a cow having suboptimal mobility was also simulated, whereby cows with a mobility score >0 have an increased risk to be culled and cows with a mobility score >1, also have a reduced milk yield. The impact on farm net profit was simulated based on production and reproductive effects of individual animals as well as the associated treatment costs. Several scenarios were simulated based on a varying cow-level value for a genetic predisposition for suboptimal mobility and a varying value representing the herd-level management factors (good, average, poor and very poor) . This 'good' herd scenario (with a 3% prevalence of suboptimal mobility) achieved about €4,000 more farm net profit compared with the 'poor' herd scenario (with a 47% prevalence of suboptimal mobility) per year, for a 100-cow herd. The decrease (5% compared with the 'good' herd) in farm net profit is due to reduced milk yield, increased culling, and treatment costs for mobility issues.

Key Words: lameness, grass-based, claw disorders

103 Assessment of the association between week of diagnosis of hyperketonemia and variations in milk characteristics, herd removal, and reproductive performance in dairy cows. Z. Rodriguez*[1], G. Cramer[1], E. Shepley[1], P. P. C. Ferro[1], N. L. Moraes[1], M. I. Endres[2], and L. S. Caixeta[1], [1]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN,* [2]*Department of Animal Science, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN.*

Hyperketonemia (HYK) is commonly diagnosed between 3 and 14 d postpartum in dairy cattle. In this study, we aimed to evaluate whether the week postpartum when HYK is diagnosed is associated with variations in milk yield and composition, herd removal, and reproduction in multiparous cows. Blood samples from multiparous Holstein cows (n = 383) in a single dairy herd in Minnesota were collected at 7 ± 2 and 14 ± 2 d postpartum for the diagnose of HYK (BHB ≥1.2 mmol/L). Milk yield and characteristics (fat, protein, somatic cell count) were obtained for the first 10 mo. of the lactation. Reproductive performance and herd-removal records were obtained from farm management software. Milk yield and characteristics were analyzed using a generalized estimated equation with an exchangeable correlation structure among monthly measurements within individuals. To evaluate pregnancy rate by 150 d and herd removal by 60 d we performed Cox proportional hazard models. Potential confounder variables included BCS, parity, and calving ease. The prevalence of HYK was 22.7% at 7 d and 17.0% at 14 d. Hyperketonemia positive cows (HYK+) in wk 1 (W1) postpartum produced on average 1,067 kg (95% CI: 305, 1,830) less milk during the first 10 mo. than hyperketonemia negative (HYK-) cows. Also, HYK+ in W1 had a higher fat % in milk than HYK- cows (mean = 0.27%, 95% CI: 0.07, 0.46). HYK+ cows in wk 2 (W2) postpartum showed no difference in milk yield (mean = 0.05 kg, 95% CI: −2.48, 2.58) nor milk fat % (mean = 0.14%, 95% CI: −0.07, 0.36) when compared with HYK- cows. The risk of being removed from the herd by 60 d was 3.9 times (95% CI: 1.66, 9.30) higher for HYK+ than HYK- cows in W1, while no difference was observed in W2. The risk of becoming pregnant by 150 d was lower for HYK+ cows in W1 than W2 (HR W1 = 0.70, 95%CI: 0.48, 1.01; HR W2 = 1.09, 95%CI, 0.74, 1.62). Cows diagnosed with HYK during W1 had lower milk yield and milk fat %, higher risk of being removed by 60 DIM and lower risk of becoming pregnant than HYK- cows. These parameters were not affected when HYK+ was diagnosed in WK2.

Key Words: hyperketonemia, milk, performance

104 Relationship between serum metabolites and milk fatty acid with periparturient diseases in Pacific Northwest dairy farms. C. Y. Tsai*[1], H. H. Hung[1], T. Weber[1], Q. Huo[2], and P. Rezamand[1], [1]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*Department of Chemistry and NanoScience Technology Center, University of Central Florida, Orlando, FL.*

During the periparturient period, dairy cows mobilize stored nutrients to support fetal development and milk production. The objective of this study was to determine the relationship between the serum metabolites in dairy cows and calves, and milk fatty acid composition with the health status of the periparturient cows. Blood samples from periparturient cows were obtained (farm A, n = 645; farm B, n = 559, respectively) on d-21, d-7, d+1, d+7 and d+14 relative to calving. Blood samples of calves were obtained within the first 4 d of life (farm A, n = 429; farm B, n = 428, respectively). Sera were analyzed for α-tocopherol, β-carotene, retinol, haptoglobin (Hpt), β-hydroxybutyrate (BHB), and glucose (calves only). In addition, 115 healthy and mastitic and lameness cows were randomly selected for milk fatty acid composition (gas chromatography) and serum type1/type 2 immunity balance analysis (D2Dx immunity test). The type 1/type 2 immune balance was evaluated by measuring the relative quantity ratio of immunoglobulin G_1 and G_2 subclasses (IgG_1/IgG_2). Health records were categorized based on the occurrence of postpartum diseases. Data were analyzed using linear mixed models in SAS with significance declared at $P \leq 0.05$. Results showed that cows with pneumonia and lameness had lower serum α-tocopherol and retinol compared with that of healthy cows at postpartum (farm B). Serum Hpt was lower when cows had pneumonia at d +1 (farm A). Greater Hpt was observed with retained placenta (RP) at d +1 and pneumonia (farm B). Serum BHB was greater at d +7 and +14 for cows with lameness and RP (farm B). The D2Dx immunity test score was greater at d +14 for diseased cows corresponding to a decreased relative quantity ratio of IgG_1/IgG_2. Serum glucose in calves was greater when the calves were born from the RP dams. No significant difference in milk fatty acid composition between diseased and healthy cows was observed. In summary, disease affects the lipid-soluble vitamins status and serum metabolites of periparturient cows, and consequently calves may experience health issues.

Key Words: serum metabolites, health status, dairy cow

105 Heat stress in transition dairy cows is associated with impaired production, health, and reproduction.

P. R. Menta*[2], V. S. Machado[2], J. M. Piñeiro[3], W. W. Thatcher[4], J. E. P. Santos[4], and A. Vieira-Neto[1], [1]*Kansas State University, Manhattan, KS,* [2]*Texas Tech University, Lubbock, TX,* [3]*Texas A&M University, College Station, TX,* [4]*University of Florida, Gainesville, FL.*

Objectives were to determine the association between heat stress (HS) exposure during the transition period with production, health, and reproduction in dairy cows during the first 90 d postpartum. Holstein cows (n = 5,722; from 2 California dairies and 3 years) were categorized into groups based on temperature-humidity index (THI) exposure during pre (Pre; −28 to −1 d relative to calving) and early postpartum (Post; 1 to 28 d relative to calving) periods as: TN-TN (mean THI Pre = 57.9 and Post = 63.7), TN-HS (mean THI Pre = 68.2 and Post = 72.0), HS-TN (mean THI Pre = 71.4 and Post = 65.6), and HS-HS (mean THI Pre = 72.2 and Post = 72.7). Data from nulliparous and parous cows were analyzed separately using mixed models. In nulliparous cows, exposure to HS during the Pre and/or Post was associated ($P < 0.05$) with a 1.7-kg/d reduction in milk yield and 15%-point increase in incidence of metritis compared with TN-TN. Postpartum HS was associated ($P < 0.05$) with increases of 4.4%-point in incidence of retained placenta and 2.0%-point in incidence of mastitis, but a reduction in 5.3%-point in pregnancy at first AI and an increase in 4.5%-point in pregnancy loss compared with Post TN. In parous cows, exposure to HS during the Pre and/or Post was associated ($P < 0.05$) with a 2.4-kg/d reduction in milk yield compared with TN-TN. Postpartum HS was associated ($P < 0.05$) with an increase in 6.3%-point in incidence of metritis and tended to be associated ($P = 0.08$) with an increase in 1.9%-point in mastitis incidence compared with Post TN. There was an interaction ($P = 0.01$) between HS exposure Pre and Post and pregnancy per AI, because Post HS was associated with reduced pregnancy in 10.6-% point within Pre TN cows, whereas no difference was found within HS Pre cows. The data suggest that Post HS is associated with performance losses to a greater extent than Pre HS and that nulliparous and parous cows are prompt to losses associated with exposure to HS during the transition period. This data set concurs with results from manipulative experiments that evaluated the loss of milk yield in cows under HS but adds insights to the associations between HS and health and reproduction.

Key Words: dairy cow, heat stress, transition period

106 Whole transcriptome-based competing endogenous (ce) RNA network analysis revealed ochratoxin A-induced intestinal tight junction damage through WNT/Ca^{2+}signaling pathway.

X. Yang*[1,2], N. Zheng[1,2], Y. N. Gao[1,2], and J. Q. Wang[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

The harm of ochratoxin A (OTA) to humans and livestock remains a global concern in public health because it is a widespread environment pollutant that has negative effects on both humans and animals in a continuously exposed environment. Recently, the compromised intestinal barrier caused by OTA has aroused widespread concern. The aim of this study was to reveal the underlying mechanism of OTA-induced tight junction (TJ) proteins damage, the important component of intestinal barrier through in vivo whole transcriptome analysis combined with in vitro functional verification. There were 619 differential expression (DE) mRNAs, 71 DE miRNAs, 493 DE lncRNAs and 144 DE circRNA. Bioinformation analysis revealed that regulated TJ protein related mRNA expression (*FZD4*, *WNT5a*, *Axin2*, *Cav1*, *ACTG1* and *CCND1*) were perturbed, which activated the WNT/Ca^{2+}signaling pathway possibly regulated by some lncRNAs (*Zeb1*, *Stk36*, *Phkb*, *Prss23*, *Inpp5e* and *Gm28588*) and miRNAs (miR-1258-x, mmu-miR-1258–3p, mmu-miR-122–5p, miR-205-z, mmu-miR-1981–5p, mmu-miR-1943–5p and mmu-miR-146b-5p). Competing endogenous RNA (ceRNA) network revealed that lncRNA *Zeb1* regulated *FZD4* binding with *WNT5a* to release the Ca^{2+} by targeting miR-1258-x to reduce the expression of TJ proteins, thus damaging the function of intestinal barrier. As the RT-qPCR results depicted, 16 selected randomly DERNAs were consistent with results by RNA-seq. In vitro Caco-2 cells experiment verified that the increase of Ca^{2+} level was involved in OTA-induced decreased the expression of TJ proteins. Taken together, these results will help us to find the target of OTA compromise the intestinal barrier, which can provide the basis for preventing the hazard and risk of OTA.

Key Words: ochratoxin A, whole-transcriptome, tight junction

107 Feeding *Saccharomyces cerevisiae* fermentation products lessens the severity of a viral-bacterial co-infection in preweaned calves.

P. O. McDonald*[1], C. Schill[1], T. W. Maina[1], B. Samuel[1], I. Yoon[2], and J. L. McGill[1], [1]*Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, Ames, IA,* [2]*Diamond V, Cedar Rapids, IA.*

Bovine respiratory disease is the leading cause of mortality in preweaned dairy calves and weaned dairy heifers. In prior research, *Saccharomyces cerevisiae* fermentation products (SCFP; SmartCare and NutriTek; Diamond V) reduced clinical signs and lung pathology following experimental bovine respiratory syncytial virus (BRSV) infection in preweaned dairy calves. The objective of this study was to determine the effect of SCFP supplementation on the outcome of a viral-bacterial co-infection. Twenty-eight, 1–2 d old Holstein-Angus cross calves with adequate passive transfer were enrolled in the study. Calves were assigned to 2 treatment groups (14/group): 1) control, base milk replacer and calf starter; or 2) SCFP treated, milk replacer with 1 g/d SmartCare and calf starter top-dressed with 5 g/d NutriTek. One calf in each group was euthanized during the feeding period, so 13 calves/group were included in the challenge period. Calves were infected with ~10^4 (Median Tissue Culture Infectious Dose, $TCID_{50}$) BRSV on d 21, followed 6 d later by intratracheal inoculation with ~10^7 colony-forming units of *Pasteurella multocida* (PM, strain P1062). One control calf reached clinical endpoint and was humanely euthanized on d 8 post viral infection. The remaining calves were euthanized on d 10 post infection. Calves receiving SCFP had a tendency toward reduced thoracic ultrasonography scores on d 7 and 10 post infection ($P = 0.02$ and $P = 0.09$) and lower lung pathology scores at necropsy ($P = 0.06$). No differences ($P = 0.40$) between treatments were observed in lung viral loads; however, BRSV was detected in the lungs of fewer SCFP fed animals compared with controls (5/12 controls vs. 3/13 SCFP). No differences in bacterial lung loads were detected between treatments ($P = 0.34$). Results from this study suggest that supplementing with SCFP may improve the outcome of a respiratory viral-bacterial co-infection in preweaned calves.

Key Words: *Saccharomyces cerevisiae* fermentation products, bovine respiratory disease, preweaned calves

Breeding and Genetics I

108 Response to ad libitum milk allowance by crossbred dairy and dairy-beef calves in an automated feeding system. S. C. Arens*, B. J. Heins, and M. M. Schutz, *University of Minnesota, St. Paul, MN.*

The objective of this study was to determine growth and milk consumption traits of Pure Holstein, ProCROSS, Grazecross, and Limousin crossbred dairy and beef calves fed alternative milk allowances in an automated group feeding system. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN. Sixteen Holstein, 24 ProCROSS, 6 Grazecross, and 22 Limousin crossbred heifers, and 23 Limousin crossbred bulls were randomly assigned to one of 2 treatments from September 2019 to June 2020. Calves were introduced to the Holm & Laue HL 100 calf feeder (Holm & Laue GmbH & Co KG, Westerronfeld, Germany) at 5 d. Treatments were 8 L/d (8L) or ad libitum (AL) milk allowance. Calves were weaned from the automated feeder at 56 d. Data were analyzed using PROC MIXED of SAS. Independent variables for analyses were fixed effects of birthweight, season of birth, breed group, treatment group and breed group by treatment interaction. Limousin crossbred bull calves had the greatest (1.1 kg/d, $P < 0.01$) average daily gain (ADG) and Holstein heifer calves had the least (0.96 kg/d) ADG. Calves fed AL had a greater ($P < 0.01$) ADG than calves fed 8L (1.13 kg/d versus 0.88 kg/d, respectively). Limousin crossbred bull calves (101.6 kg) had the heaviest ($P < 0.01$) weaning weight and Holstein heifer calves (98.1 kg) had the lightest weaning weight. Weaning weight was also heavier ($P < 0.01$) for AL calves compared with the 8L calves (107.4 kg versus 91.4 kg, respectively). Grazecross calves (1,427 mL/min) had the fastest drinking speed ($P < 0.01$) and Holstein heifer calves (1,193 mL/min) had the slowest drinking speed. The calves fed 8L had faster drinking speed (1,476 mL/min, $P < 0.01$) than calves fed AL (1,106 mL/min). Limousin crossbred heifer calves had the least milk cost ($151.66, $P < 0.01$) and ProCROSS heifer calves had the greatest milk cost ($181.32). As expected, AL calves had larger milk cost ($193.66, $P < 0.01$) than the 8L calves ($138.82). The results from this study show advantages to feeding calves 8L or AL with an automated feeding system depending on breed.

Key Words: automated feeder, crossbreeding, milk allowance

109 Identification of genomic regions associated with dry matter intake, energy-corrected milk, and metabolic body weight in 2 stages of lactation using whole-genome sequence data. K. Houlahan*[1], T. C. S. Chud[1], F. Miglior[1,2], P. Stothard[3], G. A. Oliveira Jr.[1], F. S. Schenkel[1], and C. F. Baes[1,4], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Lactanet, Guelph, ON, Canada,* [3]*Faculty of Agricultural, Life and Environmental Science, University of Alberta, Edmonton, AB, Canada,* [4]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, BE, Switzerland.*

Feed efficiency is an important aspect of dairy cattle productivity. Animals that consume less feed while producing the same amount of milk have the potential to reduce costs without reducing production. The physiology of lactation must be taken into account when selecting for improved feed efficiency. Peak lactation occurs 6 to 8 weeks after calving and coincides with a period of negative energy balance; in early lactation, cows use more energy than they can consume. This requires body store mobilization to meet their energy demands. It is therefore important to consider a feed efficiency related trait as 2 traits, the first before the peak of lactation (calving to 8 weeks of lactation) and the second after the peak of lactation (9 to 43 weeks of lactation). A genome-wide association study was performed using imputed whole-genome sequence to better understand the genomic architecture of traits that underlie common measures of feed efficiency. Deregressed estimated breeding values of 1,163 Holstein cows for dry matter intake, energy-corrected milk, and metabolic body weight at 2 time periods of the lactation were used as pseudo-phenotypes. Tag SNP were selected based on linkage disequilibrium to deal with collinearity. Significant tag SNP ($P < 0.01$E-06) on BTA 7, 10, 11, 14, 15, 19, 22, 23, and 25 were found for dry matter intake, energy-corrected milk, and metabolic body weight pre-peak lactation and post-peak lactation. Functional analyses of the candidate genes and QTL were also subsequently performed. Candidate genes found in more than 2 traits were *ARSB*, *OTX1*, *MSI2*, *MYO1D*, and *LDLRAD3*. The results show promise for attaining a better understanding of the genomic markers associated with traits that underlie common measures of feed efficiency in dairy cattle.

Key Words: feed efficiency, GWAS, whole-genome sequence

Dairy Foods: Cheese I

110 Lactoferrin could alleviate liver injury caused by Maillard reaction products with furan ring through regulating necroptosis pathway.

F. Linlin*[1,2], L. Huiying[1,2], Y. Qianqian[1,2], W. Jiaqi[1,2], and Z. Nan[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

The Maillard reaction (MR) is common during heat processing of food, especially dairy processing, diverse Maillard reaction products (MRPs) are generated during early, middle and late stage of MR, including N(ε)-2-furoylmethyl-l-lysine (furosine), pyrraline and 5-hydroxymethylfurfural (5-HMF). Moreover, there are accumulating evidences showing that the consumption of MRPs might lead to multiple diseases, such as cataract, diabetes, degenerative diseases, atherosclerosis, chronic renal failure, etc. We constructed HL-7702 cell (a normal hepatic cell line) model and ICR mouse model, and investigated the toxic effects of furosine, pyralline and 5-HMF on cell viability in vitro, as well as on liver function and liver pathological state in vivo. Additionally, the effects of lactoferrin in alleviating liver injury, as well as regulating necroptosis pathway, were evaluated. First, the contents of MRPs in milk powder samples were detected by UHPLC, and results showed that the content of furosine, 5-HMF and pyralline was 1 401.40 ± 76.70 mg/kg, 1.20 ± 0.15 mg/kg and 0.86 ± 0.097 mg/kg, respectively. The 3 MRPs were further proved to significantly inhibit HL-7702 cells viability in vitro, and 100 mg/L LF (selected by CCK8) could alleviate the damage. To further explore the related mechanisms of LF protective effects, we constructed acute toxicity model using ICR mice. The hematoxylin-eosin (HE) staining of liver tissue in ICR mice showed that the 3 MRPs (0.10 g/kg b.w.) caused occasional cytomorphosis and slight edema in some areas in the liver tissue, and lactoferrin (0.25 g/kg b.w.) showed protective effects in these damages. Meanwhile, the levels of necroptosis pathway related RIPK1, RIPK3, MLKL and p-MLKL, as well as inflammatory factors TNF-α and IL-1β, both in hepatic cells and liver tissue were detected by Western blotting. Results showed that RIPK1, RIPK3, p-MLKL, TNF-α and IL-1β were significantly upregulated by MRPs compared with the control ($P < 0.05$), meanwhile significantly downregulated by lactoferrin ($P < 0.05$). All these results indicated that lactoferrin could protect liver injury caused by MRPs with furan ring structure, which mainly through activated RIPK1/RIPK3/p-MLKL necroptosis pathway and downstream inflammatory reaction.

Key Words: lactoferrin, furosine, 5-hydroxymethylfurfural (5-HMF)

111 The combination of lactoferrin and linolenic acid inhibits colorectal tumor growth through activating AMPK/JNK-related apoptosis pathway.

F. Linlin*[1,2], L. Huiying[1,2], Y. Qianqian[1,2], W. Jiaqi[1,2], and Z. Nan[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Colorectal cancer is a common cause of death with few available therapeutic strategies, and the preventive complexes in adjunctive therapy are urgently needed. Increasing evidences have shown that natural ingredients, including lactoferrin, oleic acid, docosahexaenoic acid (DHA) and linolenic acid, possess anti-inflammatory and antitumor activities. However, investigations and comparisons of their combinations in colorectal tumor model have not been reported, and the mechanism is still unrevealed. In the study, we examined the viability, migration, invasion and apoptosis of HT29 cells to choose the proper doses of these components and to select the effective combination in vitro. BALB/c nude mice bearing colorectal tumor were used to explore the role of selected combination in inhibiting tumor development in vivo. Additionally, metabonomic detection was performed to screen out the specific changed metabolites and related pathway. The results demonstrated that lactoferrin at 6.35×10^{-5} M, oleic acid at 1.77×10^{-4} M, DHA at 1.80×10^{-4} M, and linolenic acid at 1.52×10^{-4} M significantly inhibited the viabilities of HT29 cells ($P < 0.05$). The combination of lactoferrin (6.35×10^{-5} M) + linolenic acid (1.52×10^{-4} M) exhibited the strongest activity in inhibiting the migration and invasion of HT29 cells in vivo and suppressing tumor development in vitro ($P < 0.05$). Furthermore, the lactoferrin + linolenic acid combination activated p-AMPK and p-JNK, thereby inducing apoptosis of HT29 cells ($P < 0.05$). The present study was the first to show that lactoferrin + linolenic acid combination inhibited HT29 tumor formation by activating AMPK/JNK related pathway.

Key Words: lactoferrin, oleic acid, DHA

112 Anti-inflammatory actions of acetate, propionate, and butyrate on fetal mouse jejunum cultures ex vivo and immature small intestinal cells in vitro.

S. N. Huang*[1,2], Y. N. Gao[1,2], Z. W. Wang[1,2], X. Yang[1,2], J. Q. Wang[1,2], and N. Zheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Necrotizing enterocolitis (NEC) is an intestinal disease that frequently occurs in premature infants. Presently, there is no effectual therapy for NEC. Therefore, the key to reduce the incidence rate of NEC is to take effective intervention measures as early as possible. Short-chain fatty acids (SCFAs) (acetate, propionate and butyrate) are produced by intestinal microbial fermentation. SCFAs could also be obtained directly from bovine milk, which is rich in SCFAs (about 10% of milk fat). SCFAs has been reported to play anti-inflammatory actions in mature intestinal cells. However, little studies focus on their roles in immature small intestine. Here, we evaluated the anti-inflammatory actions of SCFAs ex vivo with ICR fetal mouse jejunum cultures, and explored the potential anti-inflammatory regulators through RNA-seq, and then verified them in vitro with human fetal small intestinal epithelial FHs 74 Int cells. In this study, we found that acetate, propionate, and butyrate decreased IL-1β-induced production of CXCL2 ex vivo and IL-8 and IL-6 in vitro significantly ($P < 0.05$). Furthermore, the inhibitors of NF-κB p65, JNK1/2, and ERK1/2 pathways, which selected from RNA-seq and depressed by SCFAs, also significantly decreased IL-8 and IL-6 productions induced by IL-1β ($P < 0.05$). Consequently, our results implied that acetate, propionate, and butyrate ameliorated the fetal small intestine inflammatory response induced by IL-1β through inhibiting ERK1/2 pathway, NF-κB p65, JNK1/2, and ERK1/2 pathways, or NF-κB p65 and ERK1/2 pathways, respectively. Therefore, these findings showed that SCFAs were beneficial for treatment of NEC.

Key Words: SCFA, inflammation of immature small intestine, inflammatory cytokines

113 Aflatoxin B_1 and aflatoxin M_1 induce compromised intestinal integrity through clathrin-mediated endocytosis.

Y. N. Gao*[1,2], X. Y. Bao[1,2], J. Q. Wang[1,2], and N. Zheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Aflatoxin M_1 (AFM_1) could be present in the milk of dairy cows and human nursing mothers and in mammals that consume a diet containing aflatoxin B_1 (AFB_1). High AFM_1 occurrence and contamination levels in milk samples were reported. With the growing diversity and complexity of diet, humans could be exposed to AFB_1 and AFM_1 through daily consumption of contaminated agricultural products, milk, and dairy products. The intestine represents the first barrier against external contaminants; however, evidence about the combined effect of AFB_1 and AFM_1 on intestinal integrity is lacking. To fill this knowledge gap, we determined the changes of intestinal barrier integrity of mice and differentiated Caco-2 cells treated with individual AFB_1, AFM_1 and the combination of both mycotoxins (AFB_1+AFM_1). In vivo, the results showed that the serum biochemical parameters related to intestinal barrier function, ratio of villus height/crypt depth and distribution pattern of tight junctions were significantly affected in mice exposed to 0.3 mg/kg b.w. AFB_1 and 3.0 mg/kg b.w. AFM_1. In vitro results on differentiated Caco-2 cells showed that both toxins provoked a decrease in cell viability determining by CCK-8 assay. AFB_1 caused a significant ($P < 0.05$) decrease of 10% in cell viability at 1 μg/mL, whereas AFM_1 treatment led to a similar decrease in cell viability at 4 μg/mL. Permeability measurement on FITC-dextran through determining the intensity of fluorescence showed that AFB_1 at 0.5–1 μg/mL and AFM_1 at 8 μg/mL were found to exert negative effects ($P < 0.05$) on paracellular resistance. Furthermore, 4 μg/mL AFM_1 aggravated AFB_1-induced compromised intestinal barrier, as demonstrated by the significant ($P < 0.05$) downregulation of tight junction proteins and their redistribution, particularly internalization. Adding the inhibitor chlorpromazine illustrated that clathrin-mediated endocytosis partially contributed to the compromised intestinal integrity. Synergistic and additive effects were the predominant interactions, suggesting that a combined consumption of commodities contaminated by several mycotoxins could lead to a higher risk of adverse effects than consumption of commodities contaminated by only one mycotoxin.

Key Words: aflatoxin B_1, aflatoxin M_1, intestinal epithelial barrier

114 Effect of different thermal processes of bovine milk on the gut microbiota composition of rat.

H. G. Yang*[1,2], H. Y. Li[1], Q. Q. Yao[1], N. Zheng[1], and J. Q. Wang[1], [1]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Sericultural & Agri-Food Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, P. R. China.*

While thermal processing can eliminate potential pathogens in raw milk, it can also cause complex physicochemical changes such as Maillard reactions (MR) including isomerization, denaturation, etc. During these reactions, natural components are modified in structure or concentration, while new products are generated. In this study, we conducted a rat feeding model to investigate whether milk, treated with different thermal processing conditions, can modulate the gut microbiota composition. Three types of solid diet were produced, which was mainly made from pasteurized (P; 75°C/15 s), ultra-heat-treated (UHT; 135°C/4 s) and in-canned (C; 120°C/15 min) bovine milk, respectively. Thirty male Wistar rats (4-week old) were randomly divided into 3 groups (n = 10 per group, one rat per cage), and the rats had free access to diet and water. In the following experimental period, fresh feces samples were collected once each week (4 weeks, totally), and DNA was extracted immediately. The V3-V4 region of the 16S rRNA gene was amplified, and the data from high-throughput sequencing was mapped to a reference database. Sequence similarity more than 97% was regarded as one OTU (operational taxonomic units). Based on this, the abundance of each level (kingdom, phylum, class, order, family, genus) in every sample was calculated, respectively. ANOVA and Tukey's pairwise comparisons were conducted to test statistical difference between groups in each level, and significance was defined as $P < 0.05$. At phylum level, it was shown that the P group had a significantly higher abundance ratio of *Firmicutes*/*Bacteroides* compared with the other 2 groups. Furthermore, lefse analysis (LDA Effect Size) revealed that the predominant bacteria species from the P group were *Ruminococcus*, *Adlercreutzia*, and *Dehalobacterium*, while *Desulfovibrio* was relatively enriched in UHT milk feeding, and content of *Proteobacteria* markedly increased in the C group. Considering rising *Proteobacteria* was reported to be related to many diseases or intestinal disorders in previous studies, it might be a signal implying that over-heated milk had potential adverse effect on human health. To sum up, this study demonstrated that different thermal processed milk could alter the intestinal microbiota diversity, suggesting more attention to the temperature/time of dairy production is needed.

Key Words: bovine milk, thermal processing, gut microbiota

115 Alkaline phosphatase inhibits *Cronobacter sakazakii* LPS-induced liver injury by regulating miR146a expression.

H. M. Wu*[1,2], H. Y. Li[1,2], N. Zheng[1,2], and J. Q. Wang[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Cronobacter sakazakii is a foodborne pathogen, which can cause a variety of diseases and even life-threatening infections in infants, with a mortality rate of 40% to 80%. Lipopolysaccharide (LPS) is a potential virulence factor of *Cronobacter sakazakii*. After milk powder sterilization, LPS can remain in dairy products and pose a threat to infant health. Alkaline phosphatase (ALP) is a natural enzyme in all raw milk. ALP enzyme is used as a safety indicator of pasteurized milk because its thermal stability is greater than that of pathogens that may exist in milk. ALP can reduce the toxicity of lipopolysaccharide in enteritis and nephritis models, and can effectively treat or prevent sepsis, early pregnancy defects, enteritis, alcoholic liver, acute kidney injury and other diseases. However, the mechanism of oral ALP protecting liver tissue from LPS stimulation is still unclear. In this study, the acute liver injury model induced by *Cronobacter sakazakii* LPS (200μg/kg) was constructed in vivo, to verify the protective mechanism of ALP (200 U/kg) on mice liver. Next, ELISA, quantitative reverse transcription PCR (RT-qPCR) and Western blotting were used to detect the levels of inflammatory factors at protein level and RNA level, to confirm the inflammation in liver tissue caused by LPS. This study found that oral ALP does not cause liver damage and can effectively prevent the immune response triggered by LPS. Oral ALP can significantly reduce the concentration of LPS in blood ($P < 0.05$). In LPS stimulated mice, the expression of mir146a in the liver of ALP mice was increased, while the expression of TNF-α/IL-1β/NF-κB was significantly decreased ($P < 0.05$). At the same time, we found that ALP decreased the expression of TNF-α/IL-1β/NF-κB, which was related to the increase of mir146a. In conclusion, ALP can reduce the degree of liver inflammation by upregulating mir146a levels in liver tissues and hepatocytes and inhibiting the expression of TNF-α/IL-1β/NF-κB.

Key Words: lipopolysaccharide, alkaline phosphatase, acute liver injury

116 The effect of therapeutic administration of β-lactam antibiotics to mastitic cows on the bacterial community and antibiotic resistance patterns in milk.

L. Dong*[1,2], L. Meng[1,2], H. M. Liu[1,2], H. M. Wu[1,2], N. Zheng[1,2], and J. Q. Wang[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Dairy cows with mastitis are frequently treated with antibiotics. The potential effect of antibiotics on the milk microbiome is still not clear. Therefore, the objective of this research was to investigate the effect of 2 commonly used cephalosporins on the milk microbiota of dairy cows and the antibiotic resistance genes in the milk. The milk samples were collected from 7 dairy cows at the period before medication (d 0), medication (d 1, 2, 3), withdrawal period (d 4, 6, 8) and the period after withdrawal (d 9, 11, 13, 15). 16S rRNA sequencing was applied to explore the microbiota changes, and antibiotic resistance patterns were investigated by qPCR. The microbiota richness and diversity in each sample were calculated using the Chao 1 (richness), Shannon and Simpson (diversity) index. The cephalosporins treatment significantly lowered the Simpson diversity value at the period of withdrawal ($P < 0.05$). Members of the Enterobacter genera were the most affected bacteria associated with mastitis ($P < 0.05$). Meanwhile, antibiotic resistance genes in the milk were also influenced by antibiotic treatment. The cephalosporins treatment raised the proportion of *bla*TEM in milk samples at the period of withdrawal ($P < 0.05$). Therefore, the treatment of cephalosporins led to significant change in the milk microbiota and increase of β-lactam resistance gene in the milk at the time of withdrawal period.

Key Words: dairy cow mastitis, cephalosporins, milk microbiome

117 A study of environmental *Listeria* isolates from a dairy plant reveals a relationship of their genotypic variability with phenotypic expression and biofilm formation on clay brick floors.

N. Singh*[1,2], S. Anand[1,2], J. L. G. Gonzalez Hernandez[3], and B. Kraus[4], [1]*Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [3]*Young Brothers Seed Technology Lab, South Dakota State University, Brookings, SD,* [4]*Wells Enterprises Inc., Le Mars, IA.*

Listeria forms resilient biofilms in dairy plants. Three environmental *Listeria* isolates: *L. monocytogenes (Lm), L. innocua (Li)*, and *L. welshimeri (Lw)*, were compared for their cell surface properties, and biofilm formation on clay brick floors. For estimating cell attachment and biofilm formation, floor chips ($1\text{x}1\text{cm}^2$) were immersed in sterile distilled water, containing 6 log cfu/mL of individual isolate, and held for 1h and 48 h at 22.4°C in a shaker incubator. The attached cells were retrieved using 3M sponge sticks and plated on brain heart infusion agar. Three trials were conducted, with samples drawn in triplicates, and the means were compared by ANOVA. For genetic analysis, the genomes were assembled using CLC Genomics Workbench. The water contact angle of clay brick chips, measured by Sessile drop device, was less than 90° (43.3 ± 0.78), indicating it to be hydrophilic and thus supporting *Listeria* attachment. The *Lm* and *Lw* resulted in a greater attachment than *Li*, also supported by their respective hydrophobicity values (20.13%, 21.15%, and 13.88%), as determined by hexadecane method. The zeta potential values, determined by Zeta sizer, were comparable (−15.8 mV, −16.2 mV, and −15.5 mv, respectively). The adhesion potential of isolates, as log counts, showed variability (*Lm* 2.91 ± 0.16, *Li* 2.73 ± 0.04 and *Lw* 2.89 ± 0.40) that exhibited itself in the biofilm formation: *Lm* ($2.50 \pm 0.14/\text{cm}^2$), *Li* ($2.72 \pm 0.26\ /\text{cm}^2$), *and Lw* ($3.32 \pm 0.18/\text{cm}^2$). The presence of genes related to capsular glycan, cell wall/ capsular LTP, biotin biosynthesis, and carbohydrate metabolism, associated with amino sugars such as chitin, were correlated with biofilm formation of the 3 isolates. Whereas in the case of *Lw*, the presence of N- acetylglucosamine biosynthesis gene (NAG) was also detected. It is anticipated that biotin, chitin, and LTP genes might help the organism to colonize and NAG, when expressed, might interfere with biofilm formation resulting in sporadic presence of *Lw* as compared with *Lm* and *Li*. The study provides evidence that the initial attachment of *Listeria* species to floor surface is critical for biofilm formation.

Key Words: *Listeria*, environmental, biofilm

118 The use of protective lactic acid bacteria adjunct cultures to decrease the incidence of gas defects in aged Cheddar cheese.

R. Crompton*, T. S. Oberg, and D. J. McMahon, *Utah State University, Logan, UT.*

Late gas formation in Cheddar cheese, expressed as splits and cracks, continues to be a problem in the dairy industry, causing a negative economic impact on dairy manufacturers due to consumer dislike and downgrading of products. The phenomenon of CO_2 production in cheese is caused by heterofermentative non-starter lactic acid bacteria (HFNSLAB) which metabolize the residual hexose sugars in aging cheese. The aim of this research was to use galactose positive, lactose negative lactic acid bacteria as adjunct cultures to reduce gas production in aged cheese challenged with known HFNSLAB. Four cultures were selected for use, 2 commercially available and 2 from the Utah State University (USU) culture collection. Separate Cheddar cheese vats were produced at USU and inoculated with either *Lacticaseibacillus rhamnosus, Pediococcus acidolactici*, *Lacticaseibacillus paracasei* and *Latilactobacillus curvatus* at a level of 10^5 cfu/mL into the milk the same time as the starter culture. A control vat was made with no added adjunct. The cheese was pressed, refrigerated overnight then ground using a 3mm grinding head. Two 10-pound batches of the ground cheese were then inoculated with one of the following challenge cultures: *Limosilactobacillus fermentum, Levilactobacillus brevis, Lentilactobacillus parabuchneri,* and *Paucilactobacillus wasatchensis*, at a level of 10^4 cfu/g. Two 10-pound batches were not inoculated as a control. The inoculated ground cheese was pressed, cut into 7 one-pound blocks and vacuum sealed. Samples were prepared in quadruplicate. Gas production in the cheese were determined every week for 16 weeks by measuring the height of the gas in the vacuum bags with a ruler. Results showed that samples inoculated with *Lev. brevis* had profuse gas production, but there was a reduction in gas levels in samples treated with *P. acidolactili*, *Lat. curvatus* compared with the other samples. Samples treated with *Lcb. rhamnosis* showed a reduction in gas compared with control when challenged with *Len. parabuchneri* and *Plb. wasatchensis*. These results show the potential of adjunct cultures to reduce late gas formation in Cheddar cheese.

Key Words: late gas defects, lactic acid bacteria, adjunct culture

119 New insights into the creaming reaction in a model processed cheese system.

A. H. Vollmer*[1], N. N. Youssef[1], I. Kieferle[2], and U. Kulozik[2], [1]*Utah State University, Department of Nutrition, Dietetics, and Food Sciences, Logan, UT,* [2]*Technical University of Munich, Chair of Food and Bioprocess Engineering, TUM School of Life Sciences Weihenstephan, Freising, Germany.*

A change in the apparent viscosity of processed cheese during the cooking step has been reported repeatedly in the scientific literature and is commonly referred to as 'creaming' or 'creaming reaction'. Creaming is technically important as it affects the texture and functional properties of the final product. Some creaming is desirable, but over-creaming is not. Handling of an over-creamed product is not only more difficult during manufacturing, but the final product will also show texture defects. While the creaming phenomenon is principally known, its underlying mechanism is still not clear. A basic model has been proposed which describes the reaction as primarily protein-based through the restructuring of casein. Since the viscosity profile can change drastically with process and compositional parameters, it becomes apparent that the current model is not painting the complete picture. High-resolution transmission electron microscopy (TEM) was employed to gain a better understanding of how the protein network changes and possibly interacts with the fat phase during prolonged cooking times (up to 410 min at 90°C with constant shear at 7.93 rpm) in a model processed cheese system consisting of micellar casein concentrate powder, sunflower oil, water, and a mixture of melting salts. Ingredients were pre-mixed, transferred to the pre-heated geometry of a rheometer, continuously stirred under heat, and samples for TEM were taken after a cool-down period at pre-determined times which corresponded to characteristic points in the viscosity profile. Each time point was sampled twice for a total of 12 independent runs. Results reveal an extraordinary complexity of novel ultrastructural changes of the protein network which also clearly interacts with the fat phase, a continuous emulsification of the fat phase and a progressive separation of protein components within the matrix. Taken together, these results shed new light on the complex structure formation processes that take place during the creaming reaction. The knowledge gained here is an essential prerequisite for targeted structural design of this versatile dairy product.

Key Words: creaming reaction, transmission electron microscopy, rheology

120 Pasture and non-pasture-based feeding systems influence aroma-active compounds in raw bovine milk.

H. J. Clarke[1,2], M. G. O'Sullivan[2], J. P. Kerry[2], and K. N. Kilcawley*[1], [1]*Teagasc Food Research Centre, Moorepark, Fermoy, Cork, Ireland,* [2]*University College Cork, Cork, Ireland.*

Aroma-active compounds in raw bovine milk produced from cows fed perennial ryegrass (*Lolium perenne* L.; GRS) or cows fed total mixed ration (mixture of grass silage, maize silage and concentrates; TMR) were identified by sorptive extraction (Hi-Sorb) coupled with gas-chromatography-olfactometry. Five trained panelists evaluated each sample in duplicate including aroma extract dilution analysis. Over 108 volatile organic compounds (VOC) were identified in raw GRS and TMR milk, 15 of which were found to be significantly ($P = 0.001$) different using the Independent Samples t-Test, thus, highlighting that feeding system influences the VOC profile of raw bovine milk. Moreover, using olfactometry, 34 VOC were found to be aroma active in raw GRS milk and 36 in raw TMR milk, therefore ~30% of the VOC in raw milk influence sensory perception. The odor profile of raw GRS milk was dominated by methanethiol, furfural, benzaldehyde, 1-octen-3-ol, phenylethyl alcohol and maltol which correlated with 'cheesy', 'nutty', 'sweet', and 'green' aromas. Raw TMR milk had a much different aroma profile dominated by furfural, 2,5-dimethylpyrazine/2,3-dimethylpyrazine, 2-pentylfuran, benzaldehyde, 1-octen-3-ol, p-cresol/2-pyrrolidinone and 3/4-ethylphenol which correlated with 'roasted', 'smokey', 'animal', and 'pungent' odors. While the intensities varied, numerous compounds contributed to the aroma profile of both raw GRS and TMR milk. Raw TMR milk had a higher overall odor intensity which could be due to the diverse source components in the TMR feed as well as direct inhalation of volatiles due to the reduced air circulation from being housed indoors. This is the first study to identify individual aroma-active VOC in raw bovine milk that differ due to feeding system and that are impacting on sensory perception. This further highlights the significance of pasture-based dairy systems in quality milk production.

Key Words: pasture, milk, aroma

121 Emulsions stabilized by whey protein aggregates: Impact of particle structure on the oil-water interfacial characteristics and emulsion stability.

A. Christenson* and H. Zheng, *North Carolina State University, Raleigh, NC.*

Emulsions stabilized by whey protein aggregates (WPAs) are of interest in food research for their improved stability and functionality. Amphiphilic aggregates or Janus particles (JPs) can be formed by aggregation of whey proteins into various structures. Three WPAs, including fractal (FA) and spherical aggregates (SA) and microgels (WPM) have been manufactured by heating WPI dispersions (5 and 10%, wt/wt) at 80, 85 and 90°C, respectively. However, the o/w emulsification mechanism of JPs is not fully understood. In this study we investigated the impact of structural nature of WPI-JPs on emulsion droplet surface excess concentration (Γ, mg/m^2), which measured 26.7, 1.4 and 0.7 mg/m^2, respectively, suggesting mono- and multilayer protein films. Data were analyzed using one-way ANOVA with Tukey's HSD test. Aggregate morphology was confirmed by TEM. Surface hydrophobicity significantly ($P < 0.05$) increased when converting native WPI to JPs; WPI < WPM < FA < SA, and Z-average diameters of JPs for WPM, SA and FA were 1,020, 4560 and 174 nm, respectively. SA had the most negative zeta potential (−77mV). Oil-in-water emulsions (0.8% protein wt/vol, 20% fat vol/vol) were stabilized by each JP and WPI (control) and droplet size measured (via laser diffraction) at d 0 and 10 to determine stability against coalescence, measuring 13.3, 7.3, 10.8 and 13.6%, respectively. Emulsion stability was not significantly different ($P > 0.05$) between JPs and WPI.

Key Words: emulsions, aggregates, rheology

Forages and Pastures I

122 Yeast population and dry matter losses of sugarcane silage inoculated with *Lactobacillus buchneri*. G. B. Neto*[1], A. W. P. Freitas[1], R. B. Botelho[3], C. A. Rosa[4], and J. P. Sampaio[5], [1]*Animal Science Institute of Department of Agriculture and Food Supply, Ribeirão Preto, São Paulo, Brazil,* [2]*Animal Science Institute of Department of Agriculture and Food Supply, Ribeirão Preto, São Paulo, Brazil,* [3]*AnimaDepartment of Agriculture and Food Supply, Ribeirão Preto, São Paulo, Brazil,* [4]*Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, São Paulo,* [5]*Universidade Nova de Lisboa, Lisboa, Portugal.*

Sugarcane has limitations to the ensiling process due to fermentation of sugars and organic acids by yeasts, producing alcohol and gas. The present study was performed to investigate the yeast population and dry matter losses of sugarcane silage inoculated with *Lactobacillus buchneri* (2.5 × 10^{-10} cfu g^{-1}). The sugarcane (*Saccharum officinarum* L.) cultivar IAC-SP93–3046 (28.3° Brix, 36%DM) was chopped and sprayed with inoculant solution and ensiled in plastic buckets with a volume of 20 L, provided with Bunsen valves. Silos were weighed after filling and at the end of ensiling period to assess gravimetric DM losses (DML), gas losses (GL) and effluent losses (EL). The silos were opened after 114 d and the DML were calculated, according to the equation: DML = (FMs – FMo) × 100 / FMs, in which: DML = dry matter losses (% of DM); FMs = weight of forage mass at sealing; FMo = weight of forage mass at opening. The EL were calculated, according to the equation: EL = EW × 1000 / FF, in which: EL = effluent losses (kg/t of fresh forage), EW = effluent weight and FF = fresh forage. Yeast counts were done in silage water extract (25 g silage and 225 mL deionized water, blended for 4 min) sequentially diluted and plated in Petrifilm YM 3M, 48h at 30°C. The data were submitted to one-way analysis (GLM procedure in SAS) and means were compared by Tukey test ($P < 0.05$). The high density of the silos led to subestimation of effluent losses as it was not removed from the silos during ensiling. The additive was capable of reducing yeast counts and dry matter losses during fermentation of sugarcane silage (Table 1).

Table 1. Losses of sugarcane silage inoculated with *Lactobacillus buchneri*

Item	Control	*L. buchneri*	SEM	*P*-value
Density, kg m^3	881	885	16.391	0.815
pH (0 h)	3.13	2.90	0.081	0.024
pH (2 4h)	3.31	3.03	0.072	0.008
DM (%) initial	36.47	36.81	1.616	0.022
DM (%) final	28.14	32.90	0.400	0.358
DM losses, (%DM)	32.26	19.68	5.260	0.042
Gas losses, (%DM)	16.21	10.10	2.533	0.041
EL, kg t^{-1}	64.06	64.42	5.280	0.936
Brix	15.53	20.70	2.714	0.080
Yeast (0 h), log cfu g^{-1}	4.79	2.95	0.986	<0.001
Yeast (24 h), log cfu g^{-1}	6.00	4.47	0.212	0.391

Key Words: *Lactobacillus buchneri*, silage losses, yeast

Lactation Biology I

123 The impact of incomplete milking on mammary gland transcriptome changes under 3× milking frequency.

W. Li*[1], J. Kuehnl[2], T. Walker[2], and L. Hernandez[2], [1]*US Dairy Forage Research Center, Madison, WI,* [2]*Department of Dairy Science, University of Wisconsin–Madison, Madison, WI.*

Increased milking frequency (3× vs. 2× per day) and incomplete milking (IM) have differential effects on milk yield and mammary gland physiology. In our previous work, we have demonstrated that cows milked 3× tended to have increased milk production rate (MPR) and significantly increased milk fat percent compared with the cows milked 2× daily. Additionally, IM has significantly negative impacts on milk production (70% vs. 100% milking). However, the underlying molecular mechanisms remain uncharacterized. Our main objective is to study the impacts of IM on the mammary gland transcriptome profile in cows milked 3× daily. Four cows were included in this study. Within each cow, a contralateral half-udder was randomly assigned to be either IM (30% milk remaining in the gland) or milked completely (CM). Total RNA sequencing was used to identify differentially expressed genes (DEGs) ((fold-change > = 2, normalized read count ≥ 1 and $P < 0.1$) between the IM and CM mammary glands. Alignment of reads to the *Bos taurus* genome (UMD 3.1), DEG analysis between the 2 treatments, and pathway analysis were done using STAR (v.2.5.2b), Cufflinks (Cuffdiff, v.2.2.1), and DAVID (v.6.8), respectively. A total of 456 of the DEGs were upregulated (URG), while 104 DEGs were downregulated (DRG) in CM. URGs were predominantly involved with glycoprotein (181, $P \ll 0.0001$) and disulfide bond synthesis (155 genes, $P \ll 0.0001$). DRG were involved with regulation of cell differentiation (22, $P \ll 0.001$), extracellular organelle (23, $P \ll 0.001$) and response to cyclic AMP (5, $P \ll 0.001$). Notably, glycoproteins were reported to play a key role in the biomodulatory properties of milk and ultimately determine the nutritional quality of milk. The disulfide isomerase family of endoplasmic reticulum proteins had a reported function in immunity and inflammation. Our results provided a transcriptome level understanding of the impact of incomplete milking on mammary gland. Such knowledge may facilitate future targeted-gene based strategies for improving mammary gland health and milk production rate in dairy cattle.

Key Words: incomplete milking, mammary gland transcriptome

124 Factors influencing milking performance of teatcup liners.

J. F. Penry*[1], J. Upton[3], P. D. Thompson[2], G. A. Mein[4], and D. J. Reinemann[2], [1]*Dairy Australia, Melbourne, Victoria, Australia,* [2]*The University of Wisconsin–Madison, Madison, WI,* [3]*Animal and Innovation Research Centre, Teagasc, Fermoy, Co Cork, Ireland,* [4]*Scientific adviser, Werribee, Victoria, Australia.*

Experiments assessing the relationship of factors influencing liner performance conducted at the University of Wisconsin–Madison Dairy Cattle Centre, using a single, highly instrumented quarter milking device and 2 methods of estimating liner overpressure, are summarized. Liner performance can be described as milking gentleness, speed and completeness of milk-out. In addition to milking vacuum and pulsation settings, liner performance is influenced by liner geometry, material and teatcup shell mounting tension. The liner applies a compressive force (liner compression or LC) to the teat-end during the closed (d) phase and part of the closing (c) phase and opening (a) phase of pulsation. Effective pulsation circulates blood within the teat and limits congestive effects which reduce the teat-canal cross-sectional area (CA) during the open (b) phase of pulsation. Liner compression cannot be directly measured easily but can be estimated through a biologically relevant measurement described as overpressure. Eight liners assessed at UW-Madison showed a range in overpressure measurements from 0 to 18 kPa. Based on results from a central composite experimental design using 9 unique combinations of vacuum and pulsation applied to 8 cows (32 teats), the predictors of milk flow rate (MFR, kg/min) and teat-end congestion during the peak milk flow rate period were: liner compression (kPa overpressure), teat-end vacuum (kPa), mouthpiece chamber vacuum (kPa) and the open (b) phase of pulsation (ms). Increasing teat-end vacuum and open (b) phase time increased MFR but reduced CA. Increasing mouthpiece chamber vacuum reduced both MFR and CA. Liners with a higher LC had an increased range of potential MFR across combinations of teat-end vacuum and open (b) phase time. A 2 × 2 factorial experiment assessing teat-end and mouthpiece chamber vacuum demonstrated that milking conditions designed to induce teat-barrel congestion also increased teat-end congestion. Combined, these experiments illustrate the importance of balancing milking speed and milking gentleness and highlight the importance of characterizing LC in liner performance assessment.

Key Words: liner performance, liner compression, milk flow rate

125 Correlation of plasma purine derivatives with production, rumen fermentation and blood biochemical parameters of lactating dairy cows.

M. Li[1,3], S. G. Zhao*[1,2], N. Zheng[1,2], and J. Q. Wang[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [3]*College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, P. R. China.*

Purine derivatives are often used to calculate rumen microbial protein yield. Although studies on the relationship between purine derivatives and microbial protein yield have made great progress, the factors affecting the purine derivatives are still not clear, especially the individual physiological factors of dairy cows influencing on purine derivatives has not been reported. Because blood was easier to collect, we hypothesized plasma purine derivatives were influenced by individual physiological factors in dairy cows. Studying on plasma purine derivatives can provide a basis for optimizing the prediction model of microbial protein yield based on purine derivatives. The purpose of this experiment was to reveal the influencing factors of plasma purine derivatives. This study analyzed the Spearman correlation of plasma purine derivatives with production, rumen fermentation and blood biochemical of lactating dairy cows by SPSS 26.0. There were 2-hundred and 84 healthy dairy cows in second lactation between 24 and 77 d in lactation, milk yield was recorded and milk samples were collected 3 times a day in the morning, noon and night, rumen fluid and blood were collected within 2 h after feeding. Thereby we tested milk components, rumen fermentation parameters, blood biochemical parameters, plasma Δ^{15}N and purine derivatives. The results showed that days in lactation, milk protein yield and milk yield were correlated with purine derivatives ($P < 0.05$). Ruminal isovalerate and valerate were negatively correlated with purine derivatives ($P < 0.05$), butyrate was positively correlated with purine derivatives ($P < 0.05$). Meanwhile, the plasma Δ^{15}N and creatinine were negatively correlated with purine derivatives ($P < 0.05$), free fatty acids and glucose

were positively correlated with purine derivatives ($P < 0.05$). In conclusion, the days in lactation, milk protein yield, milk yield, isovalerate, valerate, butyrate, plasma Δ^{15}N, creatinine, free fatty acids and glucose effect plasma purine derivatives.

Key Words: plasma purine derivatives, rumen fermentation, blood biochemical

126 Rapamycin alleviated lipopolysaccharides-induced inflammatory response in bovine mammary epithelial cells via autophagy and NF-κB/MAPK pathway. L. B. Xu*, Y. F. Ren, W. Lan, P. F. Hou, J. X. Liu, and H. Y. Liu, *College of Animal Sciences, Zhejiang University, Hangzhou, P.R. China.*

Mastitis is a prevalent disease of dairy cows, which results in significant economic losses for dairy producers. Previous work showed that rapamycin had anti-inflammatory effect. However, the association between rapamycin and inflammatory response in bovine mammary epithelial (MAC-T) cells and the underlying mechanism need to be further investigated. Establishment of inflammatory model of MAC-T cells was performed first by using different concentration of lipopolysaccharides (LPS). After that, the MAC-T cells were randomly cultured in standard medium (CON), standard medium with 100 μg/mL LPS (LPS), standard medium with 10 mmol/L rapamycin (RAP), or standard medium with 100 μg/mL LPS plus 10 mmol/L rapamycin (LPS+RAP) for 24 h. Cells and culture supernatant were collected at the end of treatment. Incubation with 100 μg/mL LPS for 24 h significantly increased the IL-8, IL-1β and TNF-α concentrations in MAC-T cells ($P < 0.01$), while 10 mmol/L rapamycin administration abolished the elevated inflammatory cytokines production induced by LPS treatment ($P < 0.05$). Compared with the LPS group, the MAC-T cells in LPS+RAP group exhibited lower phosphorylation and translocation of p65 protein in nuclear factor-kB (NF-κB) signaling pathway ($P < 0.01$). The LPS+RAP stimulation significantly reduced the c-Jun NH2-terminal kinase (JNK) and p38 protein phosphorylation in mitogen-activated protein kinase (MAPK) signaling pathway than those with LPS treatment ($P < 0.01$). In addition, rapamycin supplementation significantly elevated the autophagy of MAC-T cells with or without the presence of LPS ($P < 0.01$). These results indicated that rapamycin may alleviate LPS-induced inflammatory response via autophagy and NF-κB/MAPK pathway in MAC-T cells, which give a reference for the therapeutic potential of rapamycin in mastitis.

Key Words: rapamycin, inflammation, NF-κB/MAPK

127 Mitochondrial adaptations in liver and skeletal muscle of lactating dairy cattle. V. R. Favorit*[1], A. N. Kavazis[2], W. R. Hood[2], P. Villamediana[1], and A. L. Skibiel[1], [1]*University of Idaho, Moscow, ID,* [2]*Auburn University, Auburn, AL.*

Lactation alters nutrient and energy demands, requiring metabolic adaptations in mammary and extramammary (e.g., liver and skeletal muscle) tissues. Mitochondria are essential to the bioenergetic capacity of tissues, yet mitochondrial adaptations for lactation are understudied. Mitochondria produce ATP through oxidative phosphorylation, which results in formation of reactive oxygen species (ROS) that must be neutralized by antioxidants. We assessed temporal variation in mitochondrial function and oxidative stress across lactation. Liver and skeletal muscle (biceps femoris) biopsies were collected from multiparous Holsteins (n = 11) at early (8 ± 2 d in milk [DIM]), peak (75 ± 4 DIM), and late lactation (199 ± 6 DIM). Milk yield (MY) was recorded the day before biopsy. Mitochondria were isolated from liver and skeletal muscle and coupling for complex I (NADH-linked) and II (FADH$_2$-linked) of the electron transport chain was measured as the respiratory control ratio (RCR, maximal/basal respiration). Isolated mitochondrial ROS emission and tissue superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity, and oxidative damage (i.e., protein carbonyls) were also quantified. Changes in mitochondrial function and oxidative stress across lactation and in relation to MY were assessed using general linear mixed models with lactation stage and MY as fixed effects and cow ID as a random effect. Liver complex II RCR increased with MY ($P = 0.02$), indicating greater coupling of fatty acid oxidation to ATP production. Liver mitochondrial ROS emission was highest at peak lactation ($P < 0.001$) but no change in skeletal muscle ROS was detected ($P = 0.19$). Muscle SOD activity increased ($P = 0.03$) and GPx activity decreased ($P < 0.01$) across lactation. Liver antioxidant activity was similar across lactation ($P > 0.1$). Protein carbonyl content of muscle was highest at early and late lactation ($P < 0.001$) but was not associated with lactation stage for liver ($P = 0.65$). These results suggest that greater MY is associated with enhanced liver complex II mitochondrial efficiency but also greater ROS emission when energy demand is highest at peak lactation.

Key Words: oxidative phosphorylation, metabolism

128 Effects of di-2-ethylhexyl phthalate (DEHP) exposure on lactation performance in mice. P. Hou*, Y. Zhan, C. Wang, and H. Liu, *Zhejiang University, Hangzhou, China.*

Di-2-ethylhexyl phthalate (DEHP), as a type of environmental endocrine disruptors (EEDs), can interfere with the synthesis, release, transport and metabolic process of normal endocrine substances after entering the body. However, the effects of DEHP on lactation is still unclear. In the present study, 32 pregnant female Institute of Cancer Research mice were intragastrically administered with 500 mg/kg body weight of DEHP or corn oil (CON) from pregnancy d 8 until lactational d 12. Integrated transcriptomic and metabolomics analysis of mouse mammary gland was performed in combination with serum hormone and histopathological evaluation to investigate the potential mechanism. The results showed that DEHP exposure significantly decreased milk yield ($P < 0.05$) and food intake ($P < 0.05$). In serum, the concentration of PRL ($P < 0.05$), GH ($P < 0.05$), IGF-1 ($P < 0.05$) and Insulin ($P < 0.05$) were significantly decreased and the concentration of leptin ($P < 0.05$) were significantly increased after DEHP exposure. Moreover, DEHP exposure induced changes in mammary morphology with smaller shaped and isolated alveoli and widened interstitial spaces. Metabolomic analysis showed that carbohydrate metabolism, lipid metabolism and amino acid metabolism were the major metabolic pathways involved in DEHP-induced poor lactation performance. Meanwhile, transcriptomic analysis showed that 91 differential expressed genes were found in significantly enriched metabolic pathways, which were mainly involved in catalytic activity. Notably, a fully connected network of metabolites and genes in these pathways was built, which can increase the credibility of the selected metabolites. In summary, DEHP exposure induced poor lactation performance by altering hormone levels, damaging mammary gland structure and disordering metabolism of precursors of milk synthesis.

Key Words: di-2-ethylhexyl phthalate (DEHP), lactation, omics

129 Identification and network analyses of long noncoding RNA (lncRNA) regulating cattle lactation. Y. Xing[1], H. Lyman[2], B. Davis[1], I. Korf[2], D. Lemay[2,3], A. Cullum[4], J. Dobson[4], K. Oden[4], A. Molenaar[5], K. Singh[4], C. Couldrey[8], R. Weikard[6], C. Kühn[6,7], and M. Rijnkels*[1], [1]*Department of Integrative Veterinary Biosciences, Texas A&M university, College Station, TX,* [2]*Genome Center, University of*

California-Davis, Davis, CA, [3]USDA ARS Western Human Nutrition Center, Davis, CA, [4]AgResearch Ltd, Ruakura Research Station, Hamilton, New Zealand, [5]AgResearch Ltd., Grassland Research Center, Palmerston North, New Zealand, [6]Institute of Genome Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, [7]Faculty of Agricultural and Environmental Sciences, University Rostock, Rostock, Germany, [8]LIC, Hamilton, New Zealand.

LncRNAs are non-protein-coding RNA molecules larger than 200 nt that regulate gene expression at transcriptional or post-transcriptional levels. In this study LncRNA were predicted based on mammary gland RNA-seq data from 16 Holstein-Frisian animals at peak lactation and virgin using FEELnc. Differential gene expression between lactation and virgin was determined. LncRNA overlap with lactation QTL and conservation in mammals was determined. Weighted gene coexpression network analyses (WGCNA) were used to identify correlations between co-expressed gene modules and lactation or virgin state, or differentially expressed lncRNAs. Furthermore, gene sets based on these analyses were used as candidate genes to conduct Regulatory Impact Factor Analysis (RIF) and PCIT analysis. Pathway and GO enrichment analyses were conducted for gene modules, and lncRNA correlation partners from lactation and virgin networks. 2,210 lncRNA transcripts (1,500 loci) were predicted. 88 loci (102 transcripts) were new lncRNA genes. A total of 373 differentially expressed lncRNA genes (636 transcripts) were identified. 55 lncRNA and 117 positional partner RNA genes overlapped with lactation QTLs. 39.4% and 53.2% of lncRNA transcripts were conserved in mouse and human respectively. Of these, 45.3%, 53.9% had lncRNA as biotype respectively. 58.9%, and 68.7% of the partner RNA transcripts were conserved in mouse and human respectively, more than 67% of these are protein coding. Based on the RIF-PCIT constructed networks, 69 lncRNA transcripts are key regulators in lactation and 116 in the virgin. Of these, 10 and 16 were transcripts from predicted new lncRNA genes respectively. A proportion of the key regulators' network partners were in the same WGCNA gene module. Overall, 100 out of 185 key regulators were conserved in at least one other mammal, several have known mammary gland function. Genes regulated by key-regulatory lncRNA are involved in Milk protein, PI3K-Akt, Calcium, and prolactin signaling pathways among others. The lncRNAs identified are potentially important regulators of lactation in livestock, human and mouse.

Key Words: long noncoding RNA, lactation, dairy cattle

130 Segmenting mammary gland tissue of preweaned dairy calves using spatial pyramid pooling networks.

D. A. B. Oliveira*[1], T. Bresolin[1], S. G. Coelho[2], M. Magalhaes[3], C. Lage[2], L. G. R. Pereira[1,3], L. Hernandez[1], and J. R. R. Dorea[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [3]*Brazilian Agricultural Research Corporation – Embrapa, Juiz de Fora, MG, Brazil.*

Mammary gland development of dairy cows can be detrimentally affected by environmental effects, including suboptimal nutritional strategies and heat stress early in life. These negative impacts can drastically reduce lactation performance and feed efficiency of adult animals. A novel, non-invasive, and simple technology to evaluate mammary gland characteristics is ultrasonography. Recently, machine learning algorithms have been used in medical image analyses for soft tissue segmentation, creating a powerful support tool for clinical diagnostic, feature extraction, and pattern recognition. In this context, the objective of this study was to investigate the use of spatial pyramid pooling deep networks for segmenting parenchymal tissue in mammary gland ultrasound images from pre-weaned dairy calves. A total of 405 ultrasound mammary gland images (front and rear quarters) were obtained from 29 crossbred F1 Holstein × Gyr, at wk 1, 2 and 3 of age, using a B-mode ultrasound equipped with microconvex transducer at a frequency of 6 MHz (DP 2200, Mindray, Shenzhen, China). Parenchyma tissue was manually labeled using VGG Image Annotator. We trained the deep neural networks in a leave-one-animal-out schema, where for each iteration ($n = 29$) all images of a given animal was removed for final validation. Due to the small training set (405 images), we proposed a novel data augmentation procedure that uses a polar transformation to enable shifting of the ultrasound image in a similar way a moving probe would. The average F1-score in wk 1 was 54% on the testing set; in wk 2, 70%; wk 3, 75%. Our results indicated that for images where parenchyma was very small (wk 1) the algorithm did not perform well, but as the mammary gland developed, the identification and segmentation of the parenchymal tissue drastically improved. A larger data set and high-resolution images could potentially improve deep learning performance to segment small tissues. Our results show that using deep learning networks to segment parenchymal tissue in mammary gland ultrasound images can be a promising tool for tissue identification and feature extraction.

Key Words: neural network, parenchymal tissue, polar transformation

Physiology and Endocrinology I

131 Fuel oxidation and heat production differences between high and low feed efficient dairy cows.

K. M. Kennedy*, H. M. Hammon, and B. Kuhla, *Institute of Nutritional Physiology, Dummerstorf, Germany.*

Our objective was to determine if lactating dairy cows grouped by high or low feed efficiency differed in fuel oxidation and heat production across 2 nutritional levels. From a herd of 15 dairy cows (parity = 2), 10 cows were retrospectively grouped into either a high (H) or low (L) feed efficiency group (n = 5/group) based on weekly energy-corrected milk (kg) divided by dry matter intake (kg; ECM/DMI) from wk 4 through 30 of lactation. Cows were fed a common ration throughout lactation. In wk 5.70 ± 0.82, cows spent 2 d in respiration chambers (RC) in which CO_2, O_2, and CH_4 gases were measured every 6 min for 23 h. Measured CO_2 was corrected for CH_4 to calculate metabolic CO_2 and the latter used in subsequent energy calculations except heat production (HP). Fatty acid oxidation (FOX), carbohydrate oxidation (COX), metabolic respiratory quotient (RQ), and HP were calculated from gas measurements. Cows were fed ad-libitum (AD-LIB) on d 1 and had feed withdrawn (RESTRICT) on d 2. Data were analyzed with mixed models using SAS software. As planned, H (body weight (BW): 628 ± 58.1 kg; BCS: 2.45 ± 0.42) was more efficient than L (BW: 703 ± 73.5 kg; BCS: 2.95 ± 0.52) in feed efficiency (1.80 vs. 1.64; $P = 0.02$). Neither DMI, BW, milk yield nor COX differed between groups during the RC period ($P > 0.10$). However, HP (133 vs. 115 MJ/23 h; $P = 0.05$) and HP per metabolic BW (mBW) was greater for L than H (967 vs. 1,032 kJ/(kg of $BW^{0.75} \times 23$ h); $P = 0.04$). Additionally, FOX tended to differ between groups across days (interaction, $P \leq 0.06$), and L was greater than H during AD-LIB when unadjusted (1,607 vs. 1,011 g/23 h; $P = 0.01$) and adjusted for mBW [13.2 vs. 9.66 g/kg of $BW^{0.75} \times 23$ h); $P = 0.02$] but not during RESTRICT ($P > 0.10$). The RQ average tended to differ between groups across days (interaction, $P = 0.06$) with L tending to be lower than H during AD-LIB (0.85 vs. 0.89; $P = 0.07$) but not during RESTRICT ($P = 0.47$). Greater FOX likely contributed to the greater HP loss in L compared with H. Considering no differences in DMI, BW, or milk yield during the RC period, these results suggest that lower feed efficiency may result from greater HP loss.

Key Words: oxidation, efficiency, dairy cow

132 Physiological state influences circulating and mammary calcium and serotonin concentrations.

M. Connelly*, T. Walker, J. Kuehnl, S. Henschel, and L. Hernandez, *University of Wisconsin–Madison, Madison, WI.*

At the onset of lactation, the dairy cow experiences a dynamic change in calcium physiology as large amounts of calcium are lost through milk. Thus, normal calcium homeostatic mechanisms are challenged and mammary-derived responses, such as parathyroid hormone-related protein (PTHrP) and serotonin, aid in responding to this change in calcium metabolism. The objective of this study was to evaluate physiological adaptations in response to induced subclinical hypocalcemia in lactating and dry, non-pregnant dairy cows. Using a randomized complete block design, 12 dry, non-pregnant multiparous Holstein cows and 12 early-lactation (5–20 DIM) multiparous Holstein cows received either (1) a continuous 24-h intravenous solution of 0.9% saline or (2) 5% ethylene glycol tetraacetic acid (EGTA) in 0.9% saline (n = 6 lactating, n = 6 dry, non-pregnant/treatment) with the aim of maintaining blood ionized calcium (iCa) less than 1.0 mM. Mammary gland biopsies were taken immediately after termination of infusion and 48 h later. Mammary tissues were analyzed using qPCR to evaluate gene expression and normalized to the geometric mean of ribosmal protein S9, cyclophilin A, and keratin 8. Groups were compared using a mixed model ANOVA. Circulating serotonin concentrations and mammary serotonin content were elevated in early-lactation cows relative to dry, non-pregnant cows ($P = 0.002$ and $P = 0.03$, respectively), but were not different between chelated and non-chelated groups ($P > 0.05$). Interestingly, mRNA of tryptophan hydroxylase 1 was decreased in the mammary gland of lactating cows when compared with dry, non-pregnant cows ($P = 0.03$). Lactating EGTA-infused cows had increased PTHrP expression compared with dry, non-pregnant cows ($P < 0.01$), with mRNA expression of PTHrP robustly upregulated in lactating EGTA-infused cows compared with lactating saline-infused cows ($P < 0.01$). These data support calcium and serotonin metabolism differing among early-lactation and dry, non-pregnant cows. Further, these data suggest mammary-derived endocrine signals, such as PTHrP and serotonin, may be coordinators in the peripartal cow's adaptation to lactation.

Key Words: calcium, parathyroid hormone-related protein (PTHrP), serotonin

133 Effects of recombinant bST on mammary gland growth of Holstein × Gyr heifers.

A. L. L. Sguizzato*[1], S. E. F. Guimarães[1], G. M. Santos[2], F. A. Castaño[1], and M. I. Marcondes[3,1], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*Cenva Post Graduation, Viçosa, MG, Brazil,* [3]*Washington State University, Pullman, WA.*

New technologies to improve mammary gland (MG) growth in high-performance dairy heifers are warranted, especially if they could improve their future milk production. We aimed to evaluate the effects of rbST on MG growth of prepubertal Holstein × Gyr (HG) heifers. Thirty-four HG heifers with average initial body weight (iBW) of 218 ± 49 kg and 14 ± 4 mo of age received rbST (500 mg) or sodium chloride (0.9%) shots every 14 d for 84 d. They were fed a diet to gain 1 kg/d (NRC, 2001) with a metabolizable protein/metabolizable energy ratio of 42 g/Mcal. We performed ultrasound measurements in each MG quarter on d 1, 28, 56, and 84. Then, we evaluated the pixel value using ImageJ software, collecting 3 random squares of 4 mm^2 for parenchyma (PAR) and 16 mm^2 for fat pad (FP) areas. PAR would present pixel values close to 0 (black = hypoechoic), and FP would present pixel values close to 255,000 (white = hyperechoic; Albino et al., 2017). We also performed MG biopsy in 18 randomly selected heifers on d 86. A RT-PCR analysis was carried out to assess the effects of rbST on expression of *IGF1*, *IGF1R*, *IGFBP3*, *FASN,* and *ESR1*. All variables were analyzed using the GLIMMIX procedure (SAS 9.4) in a randomized block design, [using iBW (±56 kg) as blocking criteria, and repeated measures, if needed] adopting 5% as critical level of type I error. All interactions were non-significant ($P > 0.05$). There was a reduced pixel value for PAR and FP of rbST heifers, and no changes in gene expression, except for *IGFBP3* (Table 1). Although a rise in PAR pixel was noticed along the days, the PAR/FP ratio increased around 7% from d 1 to 84, suggesting PAR growth toward FP tissue. In conclusion, the reduced pixel value for PAR and FP observed in rbST heifers, would indicate a greater amount of proliferative tissue (i.e., epithelial cells) with lower FP accumulation in the gland.

Key Words: crossbred, prepuberty, somatotropin

Table 1 (Abstract 133). Ultrasound and gene expression results in the mammary gland of Holstein × Gyr heifers

			Day					P-value[1,2]	
Item	no rbST	rbST	1	28	56	84	SEM	Treatment	Day
Ultrasound									
Parenchyma	73.84	64.95	59.79^b	72.08^a	72.30^a	73.40^a	3.32	0.003	<0.001
Fat pad	119.96	113.86	103.04^c	124.31^a	122.71^a	117.59^b	2.02	0.031	<0.001
Genes									
IGF1	0.016	0.010					0.004	0.201	
IGF1R	0.019	0.028					0.006	0.337	
IGFBP3	0.278	0.151					0.035	0.023	
FASN	0.005	0.006					0.002	0.705	
ESR1	0.059	0.048					0.015	0.605	

[1] Means within a row with different superscripts differ ($P = 0.05$).

[2] All interactions were nonsignificant ($P > 0.05$).

134 Quantifying whole-body calcium flux following immune activation. J. Opgenorth*[1], E. J. Mayorga[1], M. A. Abeyta[1], S. Rodriguez-Jimenez[1], B. M. Goetz[1], A. D. Freestone[1], C. H. Stahl[2], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*University of Maryland, College Park, MD.*

To further understand immune activation-induced hypocalcemia, we evaluated whole-body Ca flux following an intramuscular lipopolysaccharide (LPS) challenge. Pigs (n = 12; 44 ± 2.6 kg) were randomly assigned 1 of 2 treatments: 1) saline control (CON; 2 mL sterile saline; n = 6) or 2) LPS (40 µg LPS/kg BW in 2 mL sterile saline; *Escherichia coli* O55:B5; n = 6). Pigs were housed in individual metabolism stalls and restricted access to feed and water for 2 h before and during the challenge. Immediately following treatment administration, urine and feces were continuously collected. Pigs were euthanized 6 h post-injection and the Ca content of the entire heart, kidneys, liver, and spleen and luminal contents of the stomach, small intestine and large bowel were determined; likewise, samples of skeletal muscle, pancreas, adipose tissue, bone marrow, urine, ascites, serum, and bile were obtained to determine Ca concentration. Data were analyzed with PROC MIXED. LPS increased rectal temperature (39.2 and 40.7°C, in CON and LPS, respectively; $P < 0.01$) and decreased circulating glucose (39%; $P < 0.01$). Serum ionized Ca decreased in LPS relative to CON 6 h after LPS administration (1.09 vs 1.28 mmol/L; $P < 0.01$). The estimated total circulating Ca was decreased ($P = 0.04$) in LPS vs CON (95 vs 135 mg; respectively). LPS increased fecal Ca (130%; $P = 0.05$), at least partially explained by increased fecal output (246 vs 104 g for LPS vs CON, respectively). Ca increased and tended to increase in skeletal muscle and kidneys (55 and 35%; $P \leq 0.09$), and numerically increased in the liver (28%; $P = 0.12$) and bone marrow (96%; $P = 0.12$) in LPS injected pigs. Considering its contribution to total empty carcass weight, Ca primarily was sequestered in skeletal muscle (806 mg increase) following LPS administration. LPS decreased Ca in adipose (55%; $P = 0.02$) and urine (75%; $P = 0.05$). Ca content did not differ in the heart, pancreas, spleen, ascites, or digesta within different segments of the gastrointestinal tract. In summary, immune activation causes severe hypocalcemia, which is primarily explained by Ca sequestration in skeletal muscle.

Key Words: immune activation, hypocalcemia

135 Effects of recombinant bST on carcass characteristics and blood metabolites of Holstein × Gyr heifers. A. L. L. Sguizzato*[1], M. S. Duarte[1], P. V. F. Correa[1], E. A. C. Lopes[1], and M. I. Marcondes[2,1], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*Washington State University, Pullman, WA.*

Recombinant bovine somatotropin (rbST) is frequently used to improve lean gain in animals fed high-performance diets. We aimed to evaluate the effects of rbST on performance, rib eye area (REA), back fat thickness (BFT), and blood metabolites of prepubertal Holstein × Gyr (HG) heifers fed a high-performance diet. Thirty-four HG heifers with average initial body weight (iBW) of 218 ± 49 kg and 14 ± 4 mo of age received rbST (500 mg) or sodium chloride (0.9%) subcutaneous shots every 14 d for 84 d. They were fed a diet to gain 1 kg/d (NRC, 2001) with a metabolizable protein/metabolizable energy ratio of 42 g/Mcal. Heifers were fasted weighted for 3 consecutive days, each 28 d to assess average daily gain (ADG). We performed carcass ultrasound (US) analyses on d 1, 28, 56 and 84, to measure the *gluteus medius* and the *biceps femoris* muscle intercessions, and the *longissimus dorsi*. Images were recorded and later analyzed for REA and BFT. Moreover, blood samples were collected on the same days to evaluate IGF-1 and insulin, and on d 84 to evaluate T_3 and T_4. All variables were analyzed using the GLIMMIX procedure of SAS 9.4 in a randomized block design, [using iBW (±56 kg) as blocking criteria, and repeated measures, when necessary] adopting 5% as critical level of type I error. There was no difference for ADG of heifers ($P = 0.730$), where they gained around 1.14 kg/d. The REA was 20% greater for animals receiving rbST ($P < 0.001$), but there was no effect of rbST on BFT ($P = 0.108$). Both characteristics increased along the days assessed ($P < 0.001$). We observed a treatment × day interaction on IGF-1, where heifers receiving rbST had greater IGF-1 concentrations, mainly on d 28 ($P = 0.005$). Insulin was greater on d 84 ($P = 0.025$), but no rbST effect was observed ($P > 0.05$). A tendency of increased T_3 ($P = 0.064$) and an increase in T_4 concentration were found in rbST heifers ($P = 0.002$). In summary, the use of rbST improves lean gain of high-performance HG heifers during the prepubertal phase by increasing IGF-1, $T_{3,}$ and T_4 concentrations. These differences among treatments can be responsible for promoting protein synthesis and deposition instead of adipose tissue.

Key Words: performance, ultrasound, hormones

136 Changes in biomarkers of metabolic stress during late gestation of dairy cows associated with colostrum volume and IgG content across seasons. R. M. Rossi*, F. Cullens, P. Bacigalupo, L. M. Sordillo, and A. Abuelo, *Michigan State University, East Lansing, MI.*

The objective of this study was to compare the metabolic status of dairy cows during the last 6 wk of gestation based on colostrum volume and IgG content across seasons during a year. For this, healthy Holstein cows were randomly selected from 3 commercial herds in Michigan. In each farm, 4 cohorts of 21 cows (one per season), stratified by parity, were enrolled. Cows were blood sampled weekly during the last 6 wk of gestation, and biomarkers related to nutrient utilization, oxidant status, and inflammation were quantified in serum. Cows were milked within 6h of calving and the volume of colostrum produced was recorded and an aliquot collected. Concentration of IgG was measured by radial immunodiffusion. Cows were grouped into high colostrum producer (HCP) or low (LCP) and high IgG (HG) or low (LG). For volume category, we arbitrarily defined 6 L of colostrum (4 L for first and 2 L for second feeding of calves) as the cut-off point, whereas for IgG we used the industry standard of $\geq$ 50g/L. Data were analyzed statistically through mixed models with repeated measures including the fixed effects of groups (HCP vs LCP or HG vs LG), time, and their interaction; and the random effects of cow, season, lactation number, and farm ($P < 0.05$). Compared with LCP, HCP cows had higher concentrations of albumin, antioxidant potential, BHB, magnesium, and lower cholesterol and oxidant status index. For IgG group, HG cows had higher concentrations of albumin, glucose, and total protein. Collectively, these data show that producing high volumes of colostrum or IgG is associated with changes in nutrient utilization pre-calving. Nevertheless, the differences observed did not result in changes in inflammatory status in either HCP or HG, suggesting that physiological homeostasis was not disrupted during late gestation in association with the colostrum variables studied. Moreover, our results suggest that greater availability of antioxidants might support the production of higher volumes of colostrum.

Key Words: oxidant status, colostrogenesis, immunoglobulins

Production, Management, and the Environment I

137 Milk production of grazing dairy cows fed a supplementary grain ration formulated to complement nutrients from pasture during autumn. M. Douglas*[1,2], M. Auldist[1,3], M. Wright[1], L. Marett[1,3], V. Russo[1,3], M. Hannah[1], S. Garcia[2], and W. Wales[1,3], [1]*Agriculture Victoria Research, Ellinbank, Victoria, Australia,* [2]*The University of Sydney, Camden, New South Wales, Australia,* [3]*Centre for Agricultural Innovation, School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Victoria, Australia.*

Formulating grain rations for grazing dairy cows can be improved by taking into account the expected nutrient supply from pasture, which is known to vary throughout the year. The objective of this experiment was to use the nutritive characteristics of perennial ryegrass to formulate grain rations for lactating dairy cows during autumn in Gippsland, Victoria, Australia to optimize milk production. Ninety-six spring-calving, Holstein-Friesian cows grazed pasture at an allowance of 36 kgDM/cow per day (measured to ground level) and supplemented with 6.0 kgDM/cow per day of oaten hay fed in the paddock and one of 4 grain rations fed in the parlor during milking: control (CON), consisting of wheat and barley grain (8.0 kgDM/cow per day); standard grain mix (SGM), consisting of wheat, barley and corn grain, and canola meal (8.0 kgDM/cow per day); designer grain mix 1 (DGM1) consisting of the same ingredients as SGM but formulated to complement the expected nutrients from pasture (8.0 kgDM/cow per day); and designer grain mix 2 (DGM2) consisting of wheat, barley and corn grain, urea and a fat supplement (7.5 kgDM/cow per day). Dry matter intake, milk production and BW were measured over a 14-d experimental period following a 14-d adaptation period. Data were averaged over time for each cow fed each ration and analyzed by ANOVA. There were no differences in pasture or hay DMI between cows fed any of the 4 rations, however cows fed DGM2 consumed less grain and had the lowest total DMI ($P < 0.05$). The CP intake from the grain ration was highest for cows consuming SGM ($P < 0.001$), however overall intake of CP was lowest for cows consuming CON but was not different for cows consuming the other grain rations ($P = 0.001$). Milk yield was greatest for cows fed SGM and DGM1, as were milk fat and protein concentrations ($P < 0.001$). We conclude that formulating a grain ration based on the nutritive characteristics of the pasture can result in a ration that complements the nutrient intake from pasture without a decrease in milk production.

Key Words: nutrient intake, pasture

138 Metabolomics analysis underlay mechanisms in the renal impairment of mice caused by combination of aflatoxin M_1 and ochratoxin A. Z. Wang*[1,2], Y. Gao[1,2], X. Huang[1,2], J. Wang[1,2], and N. Zhen[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Aflatoxin M_1 (AFM_1) and ochratoxin A (OTA) are pernicious mycotoxins widely existing in the environment. Co-occurrence of AFM_1 and OTA may cause additive or synergistic effects, posing greater risks to public health. However, nephrotoxicity and underlying mechanism induced by AFM_1 plus OTA still remain to be explored. In this study, CD-1 mice were treated with 3.5 mg/kg b.w. AFM_1, OTA, and AFM_1+OTA for 35 d, and LC-MS-based metabolomics method was applied to investigate metabolomic profiles of mice kidney. Subsequent experiments on human renal proximal tubular (HK-2) cells were performed to dig out the causal connections between distinguished differential metabolites and nephrotoxicity. The results showed that, compared with control, AFM_1 and OTA alone and in combination significantly reduced final body weight and kidney index, remarkably elevated blood urea nitrogen (BUN), serum creatinine (SCr), and uric acid (UA) values, and induced abnormal histological phenotypes (represented by H&E staining and Masson trichrome staining), indicating the occurrence of renal injury. Besides, by means of multivariate analysis of metabolomics, AFM_1+OTA caused more extensive disturbances in metabolic pathways in comparison with single toxin treatment. Among those differentially expressed metabolites affected by AFM_1+OTA, lysophosphatidylcholines (LysoPCs) were identified as the main type with significant upregulation. Western blotting of HK-2 cells further demonstrated that AFM_1+OTA significantly increased the expression of apoptotic factors (caspase 3, PARP, and SAPK/JNK), which can be relieved by baicalein (BE), a verified inhibitor of LysoPCs. It could be speculated that LysoPCs were the pivotal metabolites in response to the combined toxins engendering renal injury, which might be served as a sensitive and specific biomarker of nephrotoxicity. The results of this study underlay latent mechanisms in the renal impairment induced by AFM_1 and OTA, and importantly, pointed out the necessity of more attentions and relevant limitation standards of co-existed mycotoxins in the milk and food safety controlling.

Key Words: aflatoxin M_1, ochratoxin A, nephrotoxicity

139 Net partial cashflow of dairy cows with different voluntary waiting periods until first insemination. E. E. A. Burgers*[1,2], A. Kok[1], R. M. A. Goselink[2], H. Hogeveen[3], B. Kemp[1], and A. T. M. van Knegsel[1], [1]*Adaptation Physiology group, Wageningen University & Research, Wageningen, the Netherlands,* [2]*Wageningen Livestock Research, Wageningen University & Research, Wageningen, the Netherlands,* [3]*Business Economics group, Wageningen University & Research, Wageningen, the Netherlands.*

Based on modeling studies, a 1-yr calving interval is generally considered optimal from an economic point of view. Recently some dairy farmers are deliberately extending the voluntary waiting period for insemination (VWP) to extend the calving interval. Reasons to extend the VWP are to reduce the frequency of transitions such as dry-off and calving to improve health, to reduce labor related to these transitions, and to reduce the number of surplus calves. This study aimed to evaluate yearly net partial cashflow in a randomized controlled trial for individual cows with a VWP of 50, 125, or 200 d. The net partial cashflow included revenues and costs for milk yield, calves born, number of inseminations, concentrate supply, roughage supply, disease treatments, discarded milk due to disease treatments, culling, and labor (for calving cows, inseminations, and disease treatments). Holstein-Friesian dairy cows (n = 153) within one herd were blocked for parity, calving season, and 305-d milk yield, randomly assigned within the blocks to one of 3 VWP (50 d, 125 d, or 200 d; VWP50, VWP125, or VWP200), and monitored from wk 6 postpartum until 6 wk after the next calving. Revenues and costs were calculated per individual cow, and expressed per cow per year. Primiparous and multiparous cows were evaluated separately. Revenues from milk and costs for roughage and concentrate contributed most to the yearly net partial cashflow. For primiparous cows, the VWP did not affect yearly revenues from milk. For multiparous cows, yearly milk revenues were greater in VWP50 compared with VWP200 (€3,487 vs €3,089). For primiparous cows, the VWP did not affect yearly concentrate costs, while for multiparous cows, yearly concentrate costs were greater in VWP50 compared with VWP200 (€517 vs €413). Primiparous cows in VWP200 had lower yearly insemination costs compared with VWP50 (€26 vs €62), and multiparous cows in VWP200 tended to have lower yearly insemination costs compared with VWP50 (€37 vs €57). The VWP did not affect the net partial cashflow per cow per year for both primiparous and multiparous cows.

Key Words: economic result, extended calving interval, extended lactation length

140 Genome, ruminal metabolome, and milk associations in lactating Holsteins. H. M. Golder*[1,2], I. J. Lean[1,2], S. J. LeBlanc[3], T. Duffield[3], H. A. Rossow[4], R. Bogdanich[5], L. Hernandez[6], E. Block[7], and J. Thomson[8], [1]*Scibus, Camden, NSW, Australia,* [2]*Dairy Science Group, Faculty of Veterinary Science, The University of Sydney, Camden, NSW, Australia,* [3]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [4]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare, CA,* [5]*Cross Street Veterinary Clinic, Tulare, CA,* [6]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [7]*Arm & Hammer Animal and Food Production, Princeton, NJ,* [8]*Montana State University, Department of Animal and Range Sciences, Bozeman, MT.*

Holstein cows (n = 293; DIM = 11 to 110) from first to seventh parity from 36 farms in Canada, USA, and Australia were enrolled to examine associations among genome, ruminal metabolome, and milk composition and yield. Diets ranged from pasture supplemented with concentrates to TMR (NFC = 17 to 47 and NDF = 27 and 58% of DM). Rumen samples were collected < 3 h after feeding and analyzed for pH, and ammonia, D- and L-lactate, and VFA concentrations. Eigenvectors were produced using cluster and discriminant analysis from a combination of all rumen measures and were used to calculate the probability of the risk of ruminal acidosis based on proximity to the centroid of 3 clusters. DNA of sufficient quality was successfully extracted from whole blood (218 cows) or hair (65 cows) collected simultaneously with the rumen samples and sequenced using the Geneseek Genomic Profiler Bovine 150K Illumina SNPchip. Genome-wide association used an additive model and linear regression with principal components analysis (PCA) population stratification and a Bonferroni correction for multiple comparisons. Population structure was visualized using PCA plots. One farm from Australia had differentiated population structure when the first 2 PCA eigenvectors were plotted and data were coded by farm ID, hence these eigenvectors were included as covariates in the analysis to correct for underlying population structure or any underlying genetic variation such as regional or country variation. There were single genomic markers associated with milk fat yield, milk protein percent, ruminal acetate, butyrate, and isovalerate concentrations, and probability of acidosis. More than one genomic marker was associated with ruminal isobutyrate and caproate concentrations. No genomic markers were associated with milk yield, fat percent, protein yield, total solids, energy-corrected milk, SCC, ruminal pH, ammonia, propionate, valerate, total VFA, and D-, L- or total lactate concentrations. Genome-wide associations with the ruminal metabolome and milk yield and composition were present across a wide geographical and management range of farms, suggesting markers for ruminal acidosis susceptibility exist.

Key Words: acidosis, genome-wide association

141 Seasonal effects on multiparous lactating dairy cow behavior. I. M. Toledo*, L. T. Casarotto, and G. E. Dahl, *University of Florida, Gainesville, FL.*

Controlled studies have shown that heat stress abatement positively influences health, productivity, behavior and reproductive performance of dairy cows during all stages of the lactation cycle. Based on previous findings, the present study focused on a better understanding of how seasonal changes affect the behavior of multiparous lactating dairy cows kept in typical freestall housing with the objective to aid in the management of lactating cows exposed to variable environmental conditions. Automated monitoring devices (Nedap, Netherlands) were used to document behavioral activity of mature Holstein dairy cows during the "hot season" (HS; n = 19; July, August and September) and the "cool season" (CS; n = 15; December, January and February). 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. All cows were housed in sand-bedded freestall barns equipped with cooling systems (soakers and fans). Behavior was recorded for the first 9 weeks of lactation after calving. Statistical analysis was conducted using the mixed model of SAS. Average THI was 78 in the HS and 54 in the CS. Compared with CS, during HS cows spent less time eating (134 ± 13.1 vs 199 ± 14.2 min/d; $P < 0.01$), lying (717 ± 21.1 vs 814 ± 23.9 min/d; $P < 0.01$), and tended to spend less time ruminating (558 ± 25.8 vs 629 ± 28.2 min/d; $P = 0.07$). In addition, exposure to ambient heat resulted in increases in standing bouts (15 ± 0.7 vs 12 ± 0.7 stands/d; $P < 0.01$), and overall standing time (720 ± 21.3 vs 626 ± 24.0 min/d; $P < 0.01$) in HS compared with CS. No differences in number of steps (3,172 ± 138.7 vs 3,288 ± 156.7 steps/d; $P < 0.01$), was observed between HS and CS. In summary, exposure to high THI during lactation seems to negatively affect the behavior and consequent daily time budget of lactating Holstein cows, even under normal housing conditions with active cooling. A better understanding on how different seasons affect the daily time budget of lactating dairy cows may contribute to the development of more effective management strategies to decrease the negative effects of heat exposure.

Key Words: freestall housing, lying time, eating time

142 Feeding behavior of heifers monitored through computer vision systems. T. Bresolin*, F. Baier, J. Van Os, and J. R. R. Dorea, *University of Wisconsin–Madison, Madison, WI.*

Feeding behavior can be used as an important indicator of health issues, estrus events, feed intake, and welfare. However, in large dairy operations, the daily monitoring of feeding behavior is laborious, and the large number of animals becomes a limiting factor. Furthermore, validated methods of determining individual feeding behavior in group-housed settings are lacking. The objective of this study was to develop a computer vision system to individually monitor feeding behavior of group-housed dairy heifers. Eight Holstein heifers (96 ± 6 d old) were housed in a group and a total of 25,214 images were acquired using one RGB camera. Images were collected every second for a total period of 7 h of video streaming (13:30 to 20:30 h). A total of 2,209 images were randomly selected and each animal in the image was labeled with its respective identification. The label was annotated only on animals that were eating in the feed bunk (head through the feed rail). From the labeled images, 1,392 random images were used to train a deep learning algorithm for object detection YOLOv3 ("You Only Look Once") and the 154 images were used for tuning. A total of 663 images were used as a testing set to validate the trained algorithm. Analyses were implemented in Python using the computational resources provided by UW-Madison Center for High Throughput Computing (CHTC). The average precision for identifying individual animals in the testing set was 96.0%, and for each individual heifer was: 99.2%, 99.6%, 99.2%, 99.6%, 99.6%, 99.2%, 99.4%, and 99.6% for heifers 1–8, respectively. After identifying the animals at the feed bunk, we computed total time spent at the feed bunk, average time per visit, number of visits, and average interval between visits for each heifer using 8,883 sequential images from 4-time points, and the correlation between observed and predicted values were 0.99, 0.85, 0.33, and 0.49, respectively. Our results indicate that computer vision systems can be used to individually monitor feeding behavior of group-housed Holstein heifers, generating precise predictions of total time spent at the feed bunk and the average time per visit.

Key Words: deep learning, feeding behavior, machine learning

143 Predicting body weight of lactating and pregnant dry dairy cows using an RGB-D sensor camera.

L. M. Campos*[1], G. Morota[2], and M. D. Hanigan[1], [1]*Dairy Science Department, Virginia Tech, Blacksburg, VA,* [2]*Animal Science Department, Virginia Tech, Blacksburg, VA.*

Daily body weight (BW) measurements of lactating dairy cows can be used for deriving health diagnosis, reproductive efficiency, feeding strategies, and animal performance. Currently, animals are weighed using walk-over or stationary electronic scales. The former is not commonly used on production dairies due to cost and maintenance, and the latter is very labor inefficient. Thus, utilization of BW data for on-farm decision-making is not common. The objective of this study was to develop prediction equations relating body measurements derived from a red-green-blue (RGB) sensor camera equipped with depth-sensing devices (Intel RealSense D435) to measure BW. Ten lactating, Holstein cows (286 DIM) with an average initial BW (SD) of 780 (137) kg and 2 pregnant, nonlactating, Jersey cows with an initial BW of 520 (12) kg at 12- and 21 d ante-parturition were chosen to capture the variability in BW associated with physiological state, parity, and changes over time during the lactation cycle. Animals were video recorded (top view) every day for 30d while the animal was restrained on the walk-over scale for 10 s. The camera was set to capture depth images at 640X480 resolution and 30 frames per second. Body weights were recorded automatically 2x/d as the lactating animals transited the scale when exiting the parlor (2 a.m. and 2 p.m.), after milking. Nonlactating animals were manually weighed 1x/week until they calved. One lactating animal was removed from the study after the first week of measurements due to behavior inconsistency. Each video frame was processed by the MATLAB Image Processing Toolbox. Four morphological image descriptors, length, width, height, and volume, were extracted from depth images. The Pearson correlation coefficients between scale-based BW records (average of the AM and PM weights) and morphological image descriptors were 0.83 (length), 0.88 (width), 0.85 (height), and 0.91 (volume). A goodness of fit obtained by multiple linear regression when regressing observed BW on the morphological image descriptors was 0.92 in adjusted R2. Volume was most correlated with BW records. We conclude that the depth images derived from the RGB-D sensor camera could potentially provide an effective way to predict the BW of cows. Additional work is required to develop descriptors capable of predicting BW change over time when the frame size is not changing.

Key Words: image descriptors, multiple linear regression, prediction.

144 The effect of calcareous marine algae on feed intake, milk production, mineral status, energy metabolites, and inflammatory markers in transition dairy cows.

E. W. Neville*[1], A. G. Fahey[2], and F. J. Mulligan[1], [1]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland,* [2]*School of Agriculture and Food Sciences, University College Dublin, Dublin, Ireland.*

Feeding highly fermentable diets in early lactation is necessary to minimize the extent of negative energy balance but can lead to periods of low rumen pH and subsequently induce an inflammatory response. The objective of this experiment was to investigate the effects of supplementing calcareous marine algae (CMA) to cows during the transition period on feed intake, milk production, energy balance, serum mineral concentrations and inflammatory markers compared with a control in both the pre- and postpartum period. Twenty-two multiparous and 10 primiparous cows were assigned to 2 dietary treatments 25 d before expected parturition until 42 d postpartum. Cows were blocked by parity and randomly assigned to their treatments based on pre-experimental BCS (multiparous: 2.88 ± 0.33, primiparous: 3.3 ± 0.26), previous 305-d milk yield (7,009 ± 1,404) kg and fat and protein yield (556 ± 97) kg for multiparous cows and PTA for milk yield (255 ± 127) kg and fat and protein yield (22 ± 7) kg for primiparous cows. Cows were fed a negative DCAD (−50 mEq/kg) TMR based on maize silage, grass silage, and straw during the pre-partum and a 50:50 forage: concentrate TMR based on grass silage, maize silage and concentrate during the postpartum period. The 2 dietary treatments consisted of a control (CON) and calcareous marine algae (CMA) included at 0.42% and 0.47% DM for the pre- and postpartum periods, respectively. Data were analyzed using the MIXED procedure (SAS, version 9.4). The model included fixed effects of treatment, week, parity, and treatment by week interaction. The CMA treatment had higher dry matter intake in both the prepartum (1.08 kg, $P < 0.05$) and postpartum (0.94 kg, $P < 0.05$) compared with the control diet. The CMA treatment had reduced NEFA concentrations in plasma during the prepartum (−0.11 mmol/L, $P < 0.10$) and the postpartum (−0.03 mmol/L, $P < 0.05$) compared with the control treatment. Fat concentration (0.43%, $P < 0.05$), 4% fat-corrected milk (1.56 kg, $P < 0.05$) and energy-corrected milk (1.32 kg, $P < 0.10$) were higher on the CMA treatment compared with the control. Results from this experiment demonstrate the benefits, on feed intake and some production parameters, of supplementing CMA to dairy cows during the transition period.

Key Words: dairy cow, feed intake

145 Effect of evaporative cooling on systemic and mammary inflammatory responses of lactating dairy cows during summer.

Y.-C. Chen*[1], R. M. Orellana Rivas[1], T. N. Marins[1], V. Lacerda[1], Z. Wang[2], M. Garrick[1], H. Liu[2], J. K. Bernard[1], and S. Tao[1], [1]*Department of Animal and Dairy Science, University of Georgia, Tifton, GA,* [2]*College of Animal Sciences, Zhejiang University, Hangzhou, China.*

To examine the impact of evaporative cooling on systemic and mammary inflammation of lactating dairy cows during summer, multiparous Holstein cows (n = 30, parity = 2.4, DIM = 156 d) were randomly assigned to either cooled (CL) with fans and misters or not (NC). Temperature-humidity index averaged 78.4 during the 36-d experiment. Milk yield (MY) and DMI were recorded daily. Blood and milk samples were collected from a subset of cows (n = 18) on d −3, 1, 3, 7, 14, and 28 of the experiment to measure cortisol, interleukin (IL)10, tumor necrosis factor (TNF)α, haptoglobin (HP), and lipopolysaccharide (LPS) binding protein (LPSBP). Mammary biopsies were collected from another subset of cows (n = 12) on d −7, 2, 10, and 36 to analyze gene expression of TNFα, IL10 and HP. Fourteen cows received a bolus of LPS in left rear quarter on d 30. Blood and milk samples from the LPS infused quarter were collected at −4, 0, 3, 6, 12, 24, 48, 96 and 144 h relative to infusion to analyze inflammatory products. Data were analyzed using PROC MIXED of SAS. Significance and tendency were declared at $P \leq 0.05$

and $0.05 < P \leq 0.15$. Deprivation of cooling decreased ($P \leq 0.03$) MY and DMI. Plasma cortisol concentration of NC cows was higher on d 1 but lower on d 28 than CL (environment [E] × time [T]: $P < 0.01$). Deprivation of cooling tended to reduce serum IL10 concentrations on d 14 (E × T: $P = 0.02$) but did not affect circulating TNFα, HP, or LPSBP. Compared with CL, NC cows tended ($P = 0.08$) to have higher milk IL10 concentrations but did not affect TNFα or HP. No differences were observed in mammary gene expression of TNFα, IL10 and HP. MY reduced after LPS infusion but not affected by treatments. NC cows had similar circulating IL10 and TNFα but tended ($P = 0.06$) to have higher HP concentrations than CL. Milk IL10 and TNFα concentrations were higher ($P \leq 0.01$) 3 h after LPS infusion (E × T: $P \leq 0.15$) for NC cows compared with CL. In conclusion, deprivation of cooling had minimal effects on lactating cows' basal inflammatory status, but upregulated systemic and mammary inflammatory responses after mammary inflammation induced by LPS.

Key Words: lactation, inflammation, heat stress

146 The effect of time away from pasture on milk production and grazing behavior.

M. Douglas*[1], M. Wright[1], P. Alvarez-Hess[1], V. Russo[1,2], M. Hannah[1], W. Wales[1,2], and M. Auldist[1,2], [1]*Agriculture Victoria Research, Ellinbank, Victoria, Australia,* [2]*Centre for Agricultural Innovation, School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Melbourne, Victoria, Australia.*

The average herd size of Australian dairy farms has increased by 30% in the last 10 years. A challenge of larger herds in pasture-based systems is to provide optimal nutrition for cows that may have compromised time budgets due to long wait times at the parlor for milking and the need to walk long distances to and from the paddock each day. As there is a consistency in milking order, it is repeatedly the same cows that spend the longest time away from pasture. The objective of this experiment was to measure the effect of time away from pasture on DMI, milk production and grazing behavior of dairy cows in late lactation. For 15 d, 40 spring-calving, Holstein-Friesian cows were split into 5 groups of 8 cows and returned to a common paddock in 45-min intervals following milking. Pasture allowance was 25 kgDM/cow per day (measured to ground level) and cows were fed cereal grain (4.0 kgDM/cow per day) in the parlor during milking. Data were averaged over time for each group and analyzed by ANOVA, with the corresponding covariate data included in the model. Milk yield was greatest for cows that returned to the paddock immediately after milking and 45-min later, intermediate for cows that returned to the paddock 90- and 135-min later, and lowest for cows that returned to the paddock 180-min after the first cows ($P < 0.001$). Cows that returned to the paddock first produced 6.1 kg milk more than cows that returned to the paddock last ($P < 0.001$). This was due partly to the amount of pasture available as 38% of the pasture DM mass had been removed by the time the last cows returned to the paddock, with a trend for available ME in pasture to be lowest when the last cows entered ($P = 0.093$). RumiWatch devices (Itin+Hoch, Switzerland) showed that cows that returned to the paddock last spent more time eating than those that returned first ($P < 0.028$). We conclude that time away from pasture affects milk yield primarily by reducing the amount and quantity of pasture on offer to later cows, rather than by compromising their time budgets. Future research will look at strategies to re-allocate pasture allowance and the amount of grain offered to ensure a greater consistency of feed to all cows.

Key Words: time budget, milk yield

Reproduction I

147 Changes in uterine metabolome associated with metritis in dairy cows. C. C. Fgueiredo*, L. O. Balzano-Nogueira, D. Z. Bisinotto, A. C. Conesa, K. N. Galvão, and R. S. Bisinotto, *University of Florida, Gainesville, FL.*

The objective was to characterize changes in uterine metabolome associated with metritis in lactating Holstein cows. This prospective cohort study was conducted from February to November 2018 in 3 dairy herds located in Florida. Vaginal discharge was evaluated between 2 and 15 DIM using the Metricheck device and metritis was characterized by the presence of watery, fetid, reddish-brownish vaginal discharge (study d 0). Cows with metritis (MET; n = 24) were paired with counterparts without metritis of similar DIM and parity (NoMET; n = 24). Uterine lumen was flushed with 15 mL of saline solution using a plastic infusion pipette attached to a plastic syringe and cows with metritis received parenteral antimicrobial therapy. A second uterine flush was performed on d 6. A total of 8 cows with metritis underwent clinical cure at d 6. Uterine metabolome was evaluated using untargeted gas chromatography time-of-flight mass spectrometry (GC-TOF-MS). Normalized data were analyzed in R using Canonical Analysis of Populations (CAP), considering 4 groups as follows: MET_d0, NoMET_d0, MET_d6, and NoMET_d6. A MANOVA analysis was performed and a total of 657 out of 884 metabolites identified by GC-TOF-MS were considered statistically significant ($P < 0.05$). The first 2 canonical variables explained 67.2 and 25.2% of the total variation among groups respectively and exhibit mainly a separation given by cows with or without metritis on d 0. From these 657 metabolites, a total of 128 showed a quality of representation larger than 0.99 and are tightly associated with the metabolism of spermidine and putrescine, long-chain fatty acids (i.e., docosahexaenoic acid and arachidonic acid), histidine, β-alanine, pentose phosphate, propanoate, glutathione, arginine and proline, tryptophan, and aminoacyl-tRNA biosynthesis. This demonstrates that metritis is associated with changes in uterine metabolome before antimicrobial therapy is applied. Finally, CAP showed no significant differences between MET and NoMET on d 6; thus, supporting that uterine metabolome in cows treated for metritis converged to a pattern similar to that observed in non-affected counterparts.

Key Words: uterine health, disease

148 Use of recombinant bST to improve reproductive characteristics and oocyte quality. A. L. L. Sguizzato*[1], J. D. Guimarães[1], E. F. Santos[1], V. A. P. Alfradique[1], and M. I. Marcondes[2,1], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*Washington State University, Pullman, WA.*

Reducing the age at puberty is a wise alternative to reduce the nonproductive phase of a cows' life in the dairy herd. Thus, we aimed to evaluate the effects of recombinant bovine somatotropin (rbST) on the average number and size of follicles (ANF; ASF), the average size of ovaries (ASO), and oocyte quality of Holstein × Gyr (HG) heifers. Thirty-four HG heifers with average initial body weight (iBW) of 218 ± 49 kg and 14 ± 4 mo of age received either rbST (500 mg) or sodium chloride (0.9%) shots every 14 d for 84 d. They were fed a diet to gain 1 kg/d (NRC, 2001) with a metabolizable protein/metabolizable energy ratio of 42 g/Mcal. We performed rectal ultrasound measurements in each heifer every 7 d for 84 d. We collected data for ovaries and dominant follicle sizes, and number of follicles in each ovary. On d 84, heifers went through a 6 d-synchronization protocol for ovum pick-up (OPU). Then, oocytes were classified based on cytoplasm characteristics and the number of cumulus cell layers (Leibfried and First, 1979). All variables were analyzed using the GLIMMIX procedure of SAS 9.4 in a randomized block design, [using iBW (±56 kg) as blocking criteria, and repeated measures, when necessary] adopting 10% as critical level of type I error. The use of rbST did not affect ANF or ASF ($P > 0.353$); however, ANF and ASF changed over time ($P < 0.015$), following the follicular wave phase. There was a treatment × day interaction for ASO ($P = 0.050$), with the highest ASO on d 50 for rbST heifers. Phenotypical data presented a greater percentage of class 2 oocytes ($P = 0.062$; Table 1), but no differences for the other oocyte classes or number of follicles harvested ($P > 0.10$). In summary, the use of rbST on HG heifers does not improve reproductive traits and oocyte quality, although there was an increase in ASO.

Table 1. Phenotypical data from ovum pick-up procedure in Holstein × Gyr heifers

	Treatment			*P*-value
Phenotypical data	no rbST	rbST	SEM	Treatment
Follicles harvested	19.91	20.55	0.144	0.908
% Follicles recovered	40.89	38.33	0.090	0.772
% Class 1	11.24	21.58	0.085	0.322
% Class 2	32.54	13.57	0.085	0.062
% Class 3	31.19	39.91	0.086	0.484
% Class 4	25.03	25.03	0.075	0.999

Key Words: follicular development, ovary size, phenotypical classification oocytes

Ruminant Nutrition: General I

149 Effect of whole cottonseed fed at 15% of the diet on milk production and methane emissions.

K. Harvatine[1], Y. Adeniji*[1], R. Bomberger[1], and R. Goodall[2], [1]*Penn State University, University Park, PA,* [2]*Cotton Inc., Cary, NC.*

Cottonseed provides both fatty acids and fiber to a diet and may support increased milk fat yield through increasing absorbed fat while maintaining lower risk for diet-induced milk fat depression. Additionally, feeding unsaturated fat has been reported to decrease methane emissions in some experiments. The hypothesis was that feeding cottonseed at 15% of the diet would increase milk fat concentration and yield and decrease methane production. Sixteen multiparous cows were arranged in a crossover design with 21 d periods. Treatments were 15% whole cottonseed and a mixture of cottonseed hulls and soybean meal balanced to provide equal NDF and CP. Data were analyzed in JMP Pro 15 using a mixed model that included the random effect of cow and period and the fixed effect of treatment and a pretrial covariant. Whole cottonseed had no effect on DMI ($P = 0.16$) and milk yield ($P = 0.42$). Whole cottonseed increased milk fat concentration 0.18 units ($P = 0.02$) and yield 110 g/d ($P < 0.01$). Whole cottonseed decreased the concentrations of < 16 C and 16 C FA in milk fat and increased FA > 16 C and *trans*-10 C18:1 and *trans*-11 C18:1 (all $P < 0.001$). There was no effect on milk protein concentration and yield. Whole cottonseed had no effect on methane production per day (mean = 376 g/d; $P = 0.71$) or per unit of DMI (mean 13.8 g/kg DMI; $P = 0.16$) as measured using the GreenFeed system (C-Lock Inc., Rapid City, SD). In conclusion, addition of whole cottonseed increased milk fat yield through increased supply of absorbed fatty acids and did not impact methane production under these dietary conditions.

Key Words: milk fat depression, biohydrogenation, lipogenesis

150 Evaluation of a phytogenic feed additive on feed intake, milk production, and health in lactating Holstein cows.

A. Jantzi[1], C. Siberski[1], M. S. Mayes[1], S. Ramirez*[2], G. R. Murugesan[2,3], A. Tacconi[3], and J. Koltes[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*BIOMIN America Inc., Overland Park, KS,* [3]*BIOMIN Holding GmbH, Getzersdorf, Austria.*

Milk production requires energy and nutrients that may be repartitioned to immune function when health events (HE) occur such as mastitis and lameness. Phytogenic feed additives have been shown to have anti-inflammatory properties and may be an effective strategy to reduce health events and improve milk production. Therefore, this study objective was to assess the impact of a phytogenic feed additive (PFA) on health events, feed intake, and milk production in lactating Holstein cows (n = 108). Cows were allotted based on parity (1–6) and DIM (47–219) to 1 of 2 dietary treatment groups: 1) Control (CON), basal diet and 2) PFA, basal diet plus 2 g PFA/h/d that was top-dressed daily over 2 trial replicated periods (rep) for a total of 54 cows per treatment group. Individual cow feed intake was collected using the Calan Broadbent system, milk production measured daily, and components measured weekly. Health events (n = 21) were recorded in cooperation with ISU veterinary staff and included mastitis and lameness. The GLIMMIX procedure of SAS was used to analyze the effect of treatment, HE, and their interaction on response variables with parity, rep, and metabolic body weight as covariates and DIM within cow as repeated measure. An interaction ($P < 0.05$) was observed for DMI (kg/d) where CON cows reduced DMI when HE was present; whereas, PFA cows maintained DMI regardless of HE. No interaction was observed for ECM (kg/d) however, main effect of treatment and HE were both significant ($P < 0.05$). Regardless of HE, PFA had greater ($P < 0.05$) ECM produced compared with CON. Cows fed PFA had reduced ($P < 0.05$) health events compared with CON fed cows. Results of this study indicate that the use of phytogenics may be an effective strategy to improve milk production by potentially reducing health events.

Key Words: milk yield, health events, phytogenics

151 Effects of forage-to-concentrate ratio in diet of late-lactation dairy cows on performance and feed efficiency.

Y. A. Ben Meir, L. Lifshitz, S. Jacoby, J. Miron, and U. Moallem*, *Department of Ruminants Science, Volcani Center, Rishon LeZion, Israel.*

In our previous study we demonstrated the potential of improving feed efficiency in low deficient dairy cows by increasing the forage-to-concentrate ratio. The objectives of the present study were to examine the effects of different forage-to-concentrate ratio during late lactation on performance and efficiency. Forty-two late-lactation cows were divided into 2 groups by milk yield, DIM, parity, and BW, and fed TMRs contained: low forage (LF) – 1.78 Mcal NE_L/kg DM, 34% forage, and 17.5 forage NDF; or high forage (HF) – a diet contained 1.75 Mcal NE_L/kg DM, 41.5% forage, and 21.5 forage NDF. The differences in forage-to-concentrate ratio between diets was obtained by changing the proportions of ground corn and oat hay. The study continued 12 wks, and milk, feed intake, and body weights were determined daily, and milk solids every 10 d. Rumen samples were taken once 2 and 5 h post feeding, and fecal grab samples were collected at 3-h intervals over 2 consecutive days. were taken 8 times to evaluate the rumen environment and digestibility of diets. Data were analyzed with the PROC MIXED, and rumen and digestibility data with the GLM procedures of SAS. Milk yields were 5.2% (1.9 kg/d) and ECM 3.9% greater in the LF compared with the HF cows ($P < 0.05$), with no differences in 4% FCM. Fat percentage ($P < 0.03$), but not yields, was higher in the HF cows, whereas protein percentage and yields were higher in the LF cows ($P < 0.04$). No differences were observed in DMI or energy intake between cows; milk/DMI was greater in the LF than in the HF cows ($P < 0.002$), with no differences in 4% FCM/DMI or ECM/DMI. Rumination and resting times were greater in the HF cows, with no differences in body gain. Rumen ammonia was lower, and propionate and butyrate were higher in the LF than HF cows. Acetate/propionate was higher in the HF ($P < 0.001$) and total VFA concentration tended to be higher in the LF cows ($P < 0.06$). The apparent total-tract digestibilities of DM and all other nutrients were higher in the LF than HF cows ($P < 0.0001$), as well as the apparent digestible intake of DM ($P < 0.01$) and nutrients ($P < 0.006$). Feeding cows at late lactation with a diet containing high forage and low energy, reduced milk yields, diet digestibility, and feed efficiency for milk production; however, efficiencies for 4% FCM and ECM production were not different between groups.

Key Words: high forage diet, feed efficiency

152 Effects of encapsulated calcium gluconate on blood metabolites and health biomarkers in mid-lactation Holstein cows.

S. T. Quanz*[1], L. K. Mamedova[1,2], K. E. Griswold[3], S. K. Kvidera[3], R. S. Fry[3], and B. J. Bradford[1,2], [1]*Kansas State University, Manhattan, KS,* [2]*Michigan State University, East Lansing, MI,* [3]*Micronutrients, Indianapolis, IN.*

The aim of this study was to assess the effects of supplementing encapsulated calcium gluconate (CaG) on biomarkers of intestinal and metabolic health in mid-lactation cows. Thirty multiparous Holstein cows (94 – 197 d in milk) were blocked by parity, pre-trial milk yield, body weight and condition score, then randomly assigned to treatments within block. Control cows were fed a basal diet top-dressed with palm fat at 0.04% of projected DMI, whereas treatment cows were fed the basal diet top-dressed with CaG embedded in a palm fat matrix (Selko Cremalto; Trouw Nutrition USA) at 0.07% of projected DMI. Cows underwent a 2-week basal diet covariate period followed by a 12-week treatment period. Blood was collected once every other week (1700 h) and processed to collect plasma. Mycotoxicosis contributed to cows being removed for metabolic problems (3 Control, 1 CaG); other cows were removed for lameness (2 Control) and mastitis (1 CaG). Data from removed cows (n = 7) were retained in the analysis until disease diagnosis. Statistical models included fixed effects of covariate period data, treatment, week, and treatment × week, and the random effect of block. In addition, the fixed effect of covariate × treatment was included in models but removed if not significant. No effects of CaG on plasma concentrations of citrulline, β-hydroxybutyrate, haptoglobin, D-lactate, serum amyloid A, glucose, or insulin were observed ($P > 0.10$). Cows fed CaG tended to have a treatment × week interaction for lipopolysaccharide-binding protein (LBP; $P = 0.09$), an acute phase protein that is produced in response to inflammation, with a 30% decrease for CaG vs. control cows in wk 9, during a phase when diet-associated inflammation was resolving. Plasma free fatty acid concentration was increased 10% by CaG ($P < 0.05$). In summary, CaG caused a small increase in circulating free fatty acids and prevented a spike in plasma LBP in wk 9, during the period when metabolic problems occurred.

Key Words: prebiotic, inflammation, feed additive

153 The rumen and fecal bacteriome of Holstein cows differing in nitrogen efficiency.

V. M. De La Guardia-Hidrogo* and H. A. Paz, *Mississippi State University, Starkville, MS.*

The objective of this study was to evaluate the relationship between N efficiency (NEff) phenotype and the bacteriome communities in the rumen and feces of Holstein cows. A nutrient balance trial was conducted to determine the N partitioning of 23 mid-lactation Holstein cows (133 ± 37 DIM, 633.5 ± 49.8 kg of BW) under the same management and dietary conditions. Nitrogen efficiency was defined as N milk/N intake and cows with a NEff above and below 0.5 SD from the mean were identified as high (n = 5) and low (n = 6) NEff, respectively. Rumen samples were collected via esophageal tubing and fecal samples via fecal grab. The bacteriome composition of the samples was determined through 16S rRNA gene sequencing of the V4 region using the Illumina MiSeq platform. Bioinformatics analyses were conducted using QIIME2 (v2020.8) and statistical analyses were performed using R (v3.5.1). Nitrogen efficiency between high and low groups differed ($P < 0.01$) and averaged 30.3 ± 2.53% and 22.8 ± 1.55%, respectively. The proportion of N excreted in manure (urine + feces) per kg of milk was lower ($P < 0.01$) for high NEff cows (11.0 ± 0.59 g/kg) than in low NEff cows (14.3 ± 0.55 g/kg). The bacteriome community structure differed by sample site (PERMANOVA <0.01) but was similar within the rumen ($P = 0.86$) and feces ($P = 0.61$) of both NEff groups. Alpha diversity analysis supported a higher bacterial richness in the rumen of high NEff cows compared with low NEff cows ($P = 0.04$). At the ASV-level, differentially abundant features ($P \leq 0.05$) presenting strong correlations ($r \geq 0.70$) with NEff in the rumen corresponded to the families *Prevotellaceae, Spirochaetaceae*, *Selenomonadaceae*, *Rikenellaceae*, *Erysipelatoclostridiaceae*, *Oscillospiraceae* and*Acholeplasmataceae*, whereas in the feces corresponded to the families *Anaerovoracaceae*, *Bifidobacteriaceae*, *Christensenellaceae*, *Eubacteriaceae*, *Clostridia UCG*-014, and *UCG*-010. These findings suggest that the bacteriome composition across the gastrointestinal tract in dairy cows is a factor influencing NEff variation.

Key Words: dairy cow, bacteriome, nitrogen efficiency.

154 Effect of glycerol administration on dairy cow metabolism and performance: A meta-analysis.

B. Yanibada*[1], M.-P. Létourneau-Montminy[1], B. Médina[2], and D. E. Rico[3], [1]*Université Laval, département des sciences animales, Québec city, QC, Canada,* [2]*Probiotech International Inc., Saint-Hyacinthe, QC, Canada,* [3]*Centre de recherche en sciences animales de Deschambault (CRSAD), Deschambault, QC, Canada.*

As a main by-product of several industries, glycerol has generated great interest in dairy cow nutrition as a rapidly available energy source able to alter metabolism and reduce ketosis. Although several studies have been carried out in recent decades, outcomes remain variable. Thus, the objective of this study was to quantify the responses of dairy cows to glycerol supplementation through a meta-analysis. Fifty-four studies were selected including 72 trials. Data showed important variation within variables such as those related to cow (physiological status and breed), glycerol administration (purity, rate, length of use), and the basal diet composition. Subdatabases were created to accurately study cow responses to glycerol supplementation: low and high doses of glycerol (<800 g/d and >800 g/d respectively; n = 31 and 23 studies); physiological state (transition and lactating cows respectively; n = 22 and 32 studies) and combination of both (e.g., transition cows fed low doses of glycerol; n = 15 studies). Multiple regression models were fitted with the MIXED procedure of Minitab including the random effect of experiment. Volatile fatty acid composition, blood parameters, milk yield and composition were defined as Y variables, while glycerol amount (g/d), dry matter intake (kg/d) and the forage:concentrate ratio were defined as predictors. The best models were chosen based on the fitting quality (R^2 and Akaike Information Criterion (AIC)) and physiological knowledge. Analysis of the main database with all physiological stages (n = 54 studies) showed, for instance, an increase of milk yield ($P = 0.048$; $R^2 = 97.7\%$) and plasma glucose level ($P = 0.01$; $R^2 = 94.3\%$) with adding glycerol while plasma B-hydroxybutyrate (BHB) and plasma fatty acids concentrations were not modified. Moreover, in the transition cow subdatabase, glycerol increased plasma glucose linearly ($P = 0.02$; $R^2 = 94.7\%$) and decreased plasma BHB linearly ($P = 0.02$; $R^2 = 91.2\%$). These results indicate that glycerol administration improves metabolic status, especially during transition period.

Key Words: cow, glycerol, meta-analysis

155 Effects of fibrolytic and amylolytic compound enzyme preparation on rumen fermentation, serum parameters, and production performance in peak-lactation dairy cows.

Z. Liu[1], L. Wang[1,2], X. Chen[2], J. Yao[1], and Y. Cao*[1,2], [1]*Northwest A&F University, Yangling, Shaanxi, China,* [2]*Harvard Medical School, Boston, MA.*

The dietary supplement of exogenous enzymes is an environment-friendly and safe way to compensate the limitation of endogenous enzymes, improving nutrient utilization and production performance of livestock. The aim of this study was to evaluate a compound enzyme preparation consisting of fibrolytic (cellulase, xylanase, and β-glucanase) and amylolytic (amylase) enzymes on nutrient intake, rumen fermentation, serum parameters, milk yield and composition, and feed conversion rate (FCR) in peak-lactation dairy cows. 448 primiparous peak-lactation Holstein cows with similar body conditions were randomly allocated to control (CON, n = 224) and experimental (EXP, n = 224) groups as a completely randomized single-factor design. The control group was fed basic TMR diet without exogenous enzymes. The experimental group was dietary supplemented with compound enzyme preparation at 70 g/cow/d (cellulase 3,500 U/g, xylanase 2000 U/g, β-glucanase 17,500 U/g, and amylase 37,000 U/g). The experiment lasted 6 weeks, with 3 weeks adaptation period and 3 weeks experimental period. The supplementation of compound enzyme improved the dry matter intake ($P < 0.01$) but had no effect on the intake of other nutrients ($P > 0.05$). Moreover, the compound enzyme preparation increased the mole ratio of acetic acid and propionic acid ($P < 0.05$), the percentage of isobutyric acid ($P < 0.05$), decreased the percentage of propionic acid ($P < 0.05$) and valeric acid ($P < 0.05$), tended to improve the percentage of butyric acid ($P < 0.10$). However, no effect was found on pH, the concentration of total volatile acid (VFA) and other VFA ($P > 0.05$). Besides, exogenous enzymes improved the alkaline phosphatase activity ($P < 0.05$), albumin concentration ($P < 0.05$), ratio of albumin and globulin ($P < 0.05$), but had no effect on other serum parameters ($P > 0.05$). In addition, compound enzyme increased the yield of actual milk ($P < 0.05$), 4% fat-corrected milk ($P < 0.01$), milk fat ($P < 0.01$), milk protein ($P < 0.01$), tended to improve the yield of lactose ($P < 0.10$) and FCR ($P < 0.10$). However, no effect was observed on other parameters like milk fat and milk protein concentration ($P > 0.05$). In conclusion, the supplementation of fibrolytic and amylolytic compound enzyme preparation could affect rumen fermentation, increase milk yield, and improve milk quality in peak-lactation dairy cows.

Key Words: dairy cow, enzyme, lactation performance

156 Effect of a specific blend of flavonoids on lactation performance of multiparous Holstein cows.

S. Schuling*, D. Schimek, and M. Engeseth, *NutriQuest, Mason City, IA.*

Sixty multiparous Holstein cows were used in a randomized complete block design to determine the effect of a specific blend of flavonoids (FLV) on milk and ECM yield from 1 to 100 DIM. Cows were blocked before calving by parity, expected calving date, and previous 305ME milk yield. Cows within block were randomly assigned to one of 3 dietary treatments: 1) control diet (CON), 2) CON + 10 g/d FLV from 1 to 21 DIM (FLV21), and 3) CON + 10 g/d FLV from 1 to 100 DIM (FLV100). Cows were housed in a freestall barn equipped with 8 voluntary milking systems (VMS; DeLaval, Tumba, Sweden). Control diet contained 50% forage and 50% concentrate and was formulated for 26.6 kg of DMI. Pellet intake at the VMS was set to a maximum of 2.72 kg/visit and 8.17 kg/d, and FLV was included in the pellet for cows on FLV21 and FLV100. The partial mixed ration was fed once daily and number of milkings per cow per day was (mean ± SD) 3.00 ± 0.97. Milk was sampled every 3 weeks for analysis of fat, protein, MUN, and SCC. Blood samples were collected on 1, 10, 21 and 100 DIM. Body weights were measured day −21, 0, 21 and 100 DIM, and BCS was recorded by camera on the VMS at each milking. Data were analyzed using the GLIMMIX procedure of SAS. There was no effect of treatment on milk yield (mean ± SE; 57.17 ± 0.34 kg/d), BCS (3.11 ± 0.01), milk protein content (2.77 ± 0.01%) or yield (1.62 ± 0.01 kg/d), milk fat content (3.44 ± 0.05%), or somatic cell linear score (2.77 ± 0.04). Feeding FLV from 1 to 100 DIM tended to increase ECM compared with CON ($P = 0.08$; 59.9 vs. 52.7 ± 1.81 kg/d, respectively). Compared with CON, both FLV21 and FLV100 increased number of milkings/d (2.55, 3.31 and 3.22 ± 0.17), pellet intake (5.54, 7.66, and 6.58 ± 0.54 kg/d), final BW (665, 702 and 759 ± 11.02 kg), and plasma sirtuin concentrations (0.91, 1.06 and 1.49 ± 0.17 ng/mL, respectively). Feeding FLV100 tended to increase ECM yield by 7.2 kg/d compared with control. Increased circulating sirtuin concentrations, as observed presently, can protect cells against oxidative stress and regulate cell survival, providing a possible explanation for reported production outcomes.

Key Words: flavonoids, milk production, milk components

Ruminant Nutrition: Calves and Heifers I

157 Effect of weaning pace and age on the blood cells and haptoglobin concentration of Holstein dairy calves. A. Wolfe[1], B. C. Agustinho*[2], C. Y. Tsai[2], D. E. Konetchy[2], A. H. Laarman[1], and P. Rezamand[2], [1]*Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, AB, Canada,* [2]*Department of Animal, Veterinary & Food Sciences, University of Idaho, Moscow, ID.*

Weaning is a critical process to dairy calves, which can cause stress and stimulate body response. An objective of this study was to determine the effect of the weaning calves at 2 ages (49 vs. 63 d) and 2 weaning paces (abrupt over 3 d vs. gradual over 14 d) on the blood parameters. Forty Holstein dairy calves (20 male and 20 female), blocked by sex and body weight at birth, were randomly assigned in a 2 × 2 factorial arrangement of treatments (weaning age; weaning pace). The treatments included early-abrupt (EA), early-gradual (EG), late-abrupt (LA), and late-gradual (LG). The animals were housed in hutches (1 × 1.5 m) with free access to feed (alfalfa hay, starter grain) and water. Blood samples were obtained one-day post-weaning, and the blood counts and haptoglobin concentration were determined in a hematology analyzer. Data were analyzed using mixed model of SAS with significance declared at $P \leq 0.05$ and the tendency at $P \leq 0.10$. Results showed that animals weaned at the late-stage presented a greater haptoglobin concentration (12.3 vs. 11.5; $P < 0.01$) and hematocrit percentage (38.8 vs. 36.6; $P < 0.01$) in the blood when compared with early-weaned calves. In addition, hematocrit percentage ($P < 0.01$) was greater in animals weaned abruptly as compared with that for gradually weaned calves. There was a weaning stage × weaning pace interaction for neutrophil percentage ($P = 0.01$), lymphocyte percentage ($P = 0.05$), and absolute platelet numbers ($P = 0.02$) in the blood. Calves in LA had a higher neutrophil percentage and absolute platelet numbers compared with that for animals in the EA. On the contrary, lymphocyte was lower in LA compared with that for EA. Calves in LA showed a greater absolute platelet numbers compared with that for calves in LG (322 vs. 244). Overall, the weaning method, age at weaning, as well as the interaction of the 2 factors affected the blood cells and haptoglobin concentration of calves.

Key Words: stress, weaning, white blood cells

158 Effect of weaning pace and age on the gene expression of inflammatory markers in hepatic, rumen, and intestinal tissues of Holstein dairy calves. B. C. Agustinho*[1], A. Wolfe[2], C. Y. Tsai[1], D. E. Konetchy[1], A. H. Laarman[2], and P. Rezamand[1], [1]*Department of Animal, Veterinary & Food Sciences, University of Idaho, Moscow, ID,* [2]*Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, AB, Canada.*

Weaning of the dairy calves can induce stress, which may elicit inflammatory response; however, the literature is limited. Therefore, an objective of this study was to determine the effect of the weaning at 2 ages (49 vs. 63 d) and 2 weaning paces (abrupt vs. gradual) on local inflammation status. Forty Holstein calves were blocked by sex and body weight at birth and randomly assigned in a 2 × 2 factorial arrangement (weaning age; weaning pace). The treatments included early-abrupt (EA), early-gradual (EG), late-abrupt (LA), and late-gradual (LG). Liver, rumen, and large intestine tissues were obtained at termination (one-day post-weaning) from 20 male calves for gene expression analysis using RT-qPCR. The target genes included interleukin 6 (IL-6), interleukin 8 (IL-8), tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ), and nuclear factor κ-B (NF-κB). Cycle threshold (Ct) of target genes corrected by Ct of house-keeping genes (GAPDH and RPS-9) were used (ΔCt) for statistical analysis using the mixed model of SAS with significance declared at $P \leq 0.05$ and the tendency at $P \leq 0.10$. Results showed that calves weaned late-stage presented a reduced expression of IL-8 in the liver ($P = 0.04$) and in the large intestine ($P = 0.10$) when compared with that of the early-stage. In addition, the hepatic expression of IL-8 tended to reduce ($P = 0.09$) in calves weaned abruptly as compared with gradual pace. There was a weaning age × weaning pace interaction for the expression of IL-8 ($P = 0.05$), NF-κB in the rumen ($P = 0.10$); and TNF-α ($P = 0.02$), INF-γ ($P = 0.10$), and IL-6 in the large intestine ($P = 0.10$). Animals in LG weaning had a greater IL-8 expression in the rumen, and TNF-α and INF-γ in the large intestine than that of EG weaning. On the other hand, animals weaned in the EA group tended to have an increased NF-κB expression in the rumen compared with that for EG, and an increased IL-6 in the large intestine compared with that for LA. In summary, weaning pace, age at weaning, and the interaction of the 2 factors altered gene expression of inflammatory markers in the liver, large intestine, and rumen.

Key Words: stress, weaning

Ruminant Nutrition: Carbohydrates and Lipids I

159 Effect of increasing oleic acid concentration in a saturated fatty acid prill on milk yield in Holstein cows. R. Pierce, R. Bomberger, and K. Harvatine*, *Penn State University, University Park, PA.*

Fat supplements are commonly included in rations to increase energy intake and milk and milk component yield of dairy cows. Recent research indicates that the fatty acid (FA) profile of fat supplements may change fatty acid digestibility and metabolism with a resulting impact on milk production. The hypothesis of this experiment was that increased oleic acid levels in a saturated fatty acid supplement reacted with magnesium would increase milk yield without induction of milk fat depression. Eight primiparous and 8 multiparous cows (43.8 and 56.5kg milk and 74 and 84 DIM, respectively, at the start of experiment) were arranged in a 4x4 Latin square design with 21 d periods. One multiparous cow was removed from the study due to mastitis. Treatments were a control diet with no added fat and diets which included FA supplements at 1.1% of dry matter intake. The supplements contained either 5%, 10%, and 15% oleic acid (OA) at a percent of FA. The fat supplement contained a 1.13 ratio of C16:0 to C18:0 that was maintained as OA was increased. The FA were reacted with magnesium during prilling resulting in partial formation of magnesium salts. Data were analyzed using the random effect of cow and period and the fixed effect of treatment and preplanned contrasts tested the effects of the control diet vs fat addition and the linear effect of increasing OA level. Overall, there were no significant interactions between diet and parity. Milk yield, fat yield, protein yield, and fat percent were not affected by treatment. Fat percent was increased 0.18% ($P < 0.05$) with fat supplementation in primiparous cows but was not increased in multiparous cows ($P > 0.10$). Protein percent was decreased 0.06% ($P < 0.05$) in primiparous cows fed the diets with added fat and did not differ between OA levels. Dietary fat supplements tended to decrease milk protein in multiparous cows. The study suggests that replacing a constant ratio of C16:0 to C18:0 with OA does not negatively impact milk yield and milk fat yield.

Key Words: milk fat, fat supplement, emulsifier

160 Effect of dietary oilseed supplementation on milk production, milk composition, and milk fatty acids of dairy cows: A meta-analysis. G. Plata-Perez*[1], R. Vieyra-Alberto[1], O. Del Razo-Rodriguez[1], J. C. Angeles-Hernandez[1], E. Morales-Almaraz[2], F. Lopez-Gonzalez[2], and E. Vargas-Bello-Perez[3], [1]*Autonomous University of the State of Hidalgo, Tulancingo de Bravo, Hidalgo, México,* [2]*Autonomous Mexico State University, Toluca de Lerdo, Estado de México, México,* [3]*University of Copenhagen, Grønnegårdsvej, Frederiksberg, Denmark.*

Oilseed supplementation is a strategy to improve milk production and milk composition in dairy cows, however, response to this approach are inconsistent. Thus, the aim of this study was to evaluate the effect of oilseeds supplementation on milk production and milk composition in dairy cows via meta-analysis and meta-regression. A comprehensive and structured search was performed using the following electronic databases: Google Scholar, Primo-UAEH and Web of Science. To avoid review-bias, 3 independent analytic reviews were carried out by 3 field experts. The final database comprised 37 peer-reviewed papers that fulfilled the inclusion criteria. The response variables considered were: milk yield (MY), Atherogenic Index of Plasma (AIP), fat, protein, lactose, conjugated linoleic acid (CLA), unsaturated fatty acids (UFA), saturated fatty acids (SFA) and vaccenic acid (VA) content. The explanatory variables were breed, seed type (cottonseed, linseed, canola, soybean, sunflower), processing (extruded, ground, roasted, whole), intake level, experimental design, washout period and forage-to-concentrate ratio. The 'meta' package in R software was used to perform the meta-analysis. The random model was expanded to a mixed model to detect sources of heterogeneity using the 'Metafor' package. The inclusion of oilseed of dairy cow ration had a positive effect on CLA (>0.26 g/100g Fatty acids (FA); $P < 0.0001$), VA (>1.02 g/100g FA; $P < 0.0001$), UFA (>8.32 g/100g FA; $P < 0.0001$), also was observed a significant reduction of AIP (<1.01; $P < 0.0001$). In milk, a decrease of fat (<0.11%; $P < 0.001$), protein (<0.04%; $P < 0.0007$) and SFA (<7.51 g/100g FA; $P < 0.0001$) content was detected to oilseed supplemented animals. Desirable FA milk components (CLA, VA and SFA) were affected by breed, type, processing, and intake of oilseed. Animals fed sunflower showed the highest milk CLA content. Oilseed supplementation in dairy cow rations have a positive effect on desirable milk components for human consumption. However, animal response to oilseed supplementation depends on explanatory variables as type, level and processing of oilseeds and animal breed. Authors are grateful to Mexican Ministry of Education for funding this research project under grant UAEH-PTC-823

Key Words: lipids, biohydrogenation, fatty acids

161 Effect of folic acid supplementation on lactation performance of Holstein dairy cows: A meta-analysis. L. Wang*, Z. J. Li, X. J. Lei, and J. H. Yao, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

The purpose of this meta-analysis was to evaluate the effect of folic acid (FA) supplementation on milk production and composition in Holstein cow, which still remains controversial over published studies. China National Knowledge Infrastructure, PubMed and Science Direct databases were used for searching possible eligible studies. The screening criteria were as follows, participants were Holstein cow, interventions were FA supplementation, comparisons contained blank control with no other vitamins or amino acids added, outcomes included dry matter intake (DMI), milk yield, and milk fat, milk protein and milk lactose content. When multiple groups with different FA concentrations were designed in a study, the results of each outcome in all subgroups were combined into one group. Data were analyzed using the Stata 15.1 software, and sensitivity test, meta-regression and subgroup analysis were conducted when heterogeneity existed in outcomes. A total of 14 studies, involving 384 cows, were included in this meta-analysis. The results indicated that, with the supplementation of FA, milk yield was distinctly increased by 1.41 kg/d ($P = 0.001$), milk fat and milk protein were also significantly raised up by 0.73 g/kg ($P < 0.001$) and 0.84 g/kg ($P = 0.004$) respectively, whereas DMI and milk lactose had no obvious change ($P > 0.05$). Heterogeneity was only discovered in milk yield and milk protein, but sensitivity test showed the results were relatively stable. Year, initial weight and supplementary method were the 3 covariates that could strongly explain the heterogeneity in milk yield after meta-regression and subgroup analysis, and so as year did in milk protein. In conclusion, FA supplementation increased milk yield and milk fat and milk protein content in Holstein cow, but had no significant effect on DMI or milk lactose content. Cows in different years or different initial weights had noticeable differences in milk yield with different supplementary methods of FA.

Key Words: folic acid, Holstein cow, lactation performance

162 Effect of granule size on dissociation of calcium salts of palm fatty acid distillate.

W.-L. Ong[1], R. Kirkland[2], J. Newbold[3], and K.-H. Ng*[1], [1]*WIL@NUS Corporate Laboratory, Singapore,* [2]*Volac Wilmar Feed Ingredients Limited, Hertfordshire, UK,* [3]*Scotland's Rural College, Aberdeen, UK.*

Calcium salts of palm fatty acid distillate (CaS) are among the most common rumen-protected fat supplements used on dairy farms. Rumen-active oil sources have negative effects on rumen microbes, fiber digestion and milk fat production, and it is important to ensure supplements used are stable under rumen conditions but subsequently dissociate in the acidic conditions of the abomasum. In this study we investigated the effect of granule size of CaS (<0.5 mm, 1–2 mm, 2–3 mm, 3–4 mm) on dissociation pattern in different pH conditions across a range of time points from 0 to 24 h. Samples of CaS were sieved to different granule sizes. To understand the relative stability of the CaS of different sizes, we carried out in vitro dissociation study using a series of buffers with different pH values coupled with ICP-AES analysis to detect the released calcium (Ca). The CaS samples were pre-washed with water and dried at 60°C overnight. One gram of the prewashed and dried samples were either digested with 20 mL of 3M HCl (70°C, 2 h) for complete recovery of Ca or incubated with 40 mL each of buffers of different pH values at different time points at 39°C with shaking (200 rpm). The released Ca was separated from undigested solids by filtering and centrifugation. The Ca content in the digested solids and buffers was measured by ICP-AES. Release of Ca (%) per gram of CaS was determined as: % of Ca released = (amount of Ca released in the buffer/total amount of Ca in the CaS) × 100%. We found that at 16-h incubation, CaS of < 0.5 mm granule size would have 24.5% and 41.6% of Ca dissociated at pH 6.5 and pH 5.6. In contrary, for the large granule size of 3–4 mm, the Ca dissociation was 8.6% and 24.4%, respectively. Our results suggest that small granules break down more extensively in acidic conditions than larger granules and Ca release (dissociation) increases with incubation time at a specific pH.

Table 1. Effect of buffer pH on CaS over 24 h with particle size of <0.5 mm and 3-4 mm (% of Ca released)

Particle size and pH	1 h	2 h	3 h	4 h	8 h	12 h	16 h
<0.5 mm							
pH 3.7	52.6	70.4	71.8	68.9	95.0	98.5	99.8
pH 5.6	9.2	11.9	11.4	12.9	26.8	35.1	41.6
pH 6.0	10.7	13.7	14.5	16.0	20.7	23.6	25.9
pH 6.5	10.5	14.0	14.4	15.6	20.4	22.6	24.5
3–4 mm							
pH3.7	19.8	36.2	43.6	50.9	98.6	100.0	100.0
pH5.6	3.5	4.6	5.7	5.4	11.6	18.0	24.4
pH6	1.4	2.2	2.7	3.2	6.3	8.6	11.4
pH6.5	1.0	1.7	2.0	2.4	5.0	6.6	8.6

Key Words: calcium salts, dissociation, particle size

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion I

163 Effect of grazing and ration composition on methane production in mid to late lactation. T. Van De Gucht*[1], M. Thys[1], R. Delagarde[2], S. Benaissa[1], B. Ampe[1], N. Peiren[1], and L. Vandaele[1], [1]*Animal Sciences Unit, Flanders Research Institute for Agriculture, Fisheries and Food, Melle, Belgium,* [2]*PEGASE, INRAE, Institut Agro, Saint-Gilles, France.*

The objective of this study was to compare the enteric methane emission and milk production parameters in cows in mid to late lactation allowed either a 6-h restricted grazing period as a supplement to one of 2 partial mixed rations (PMR), or receiving a basal PMR in confinement without grazing (PMR-C). The 2 partial mixed rations were one basal (PMR-G) and one with increased inclusion of maize silage (PMR(CS)-G) (42.8 vs 55.4% maize silage). A balanced crossover design with 3 treatments and 3 successive 4-week treatment periods using 24 Holstein-Friesian cows was used. The cows were habituated to the basal PMR and consecutively to 6-h grazing during respectively 3 and 2 weeks preceding the experiment, as they were previously used to confinement. Fresh grass on pasture, water and PMR in the barn were provided ad libitum. Strip grazing was used to provide a daily fresh strip of palatable grass to motivate cows to graze. Cow data including milk yield, concentrate intake and PMR intake were recorded automatically. Fresh grass intake was estimated using the n-alkanes technique by providing C32 through oral boluses for 10 consecutive days. Emissions were measured using 2 GreenFeeds (one in the barn and one on pasture) throughout the day. Analysis was done using a linear mixed model with treatment, period and days in milk as fixed effects and cow as random effect. Results showed that dry matter intake was higher in confined cows (PMR-C) than grazing cows fed the same PMR (PMR-G), but not different from grazing cows fed a PMR with higher inclusion of maize silage (PMR(CS)-G). A significant reduction of absolute methane production (−12%) ($P <$ 0.001) was found in the grazing treatments compared with non-grazing cows. Methane yield (−7% and −10% g/kg dry matter intake (DMI)) and intensity (−8% and −12% g/kg fat and protein corrected milk (FPCM)) were significantly lower ($P < 0.001$ and $P = 0.003$ resp.) in the grazing treatments. FPCM was lower in PMR-G compared with confined cows (PMR-C), but not different from PMR(CS)-G. NDF content of the different rations was 341, 378 and 365 g/kg DMI for PMR-C, PMR-G and PMR(CS)-G respectively, and net energy of lactation (NEL) was 6.4, 6.8 and 6.4 MJ/kg DMI respectively. We concluded that grazing combined with an increased maize silage inclusion in the barn PMR (i.e., PMR(CS)-G) resulted in the best trade-off between milk production and methane reduction in cows in mid and late lactation.

Key Words: grazing, methane, emission

Ruminant Nutrition: Protein/Amino Acids I

164 Predictions of N use efficiency from natural ^{15}N abundance in periparturient dairy cows are impaired by the protein mobilization. M. Correa-Luna*[1], M. Larsen[2], C. Chantelauze[1], L. Bahloul[3], and G. Cantalapiedra-Hijar[1], [1]*Université Clermont Auvergne, INRAE, France,* [2]*Aarhus University, Denmark,* [3]*Adisseo France S.A.S., France.*

The natural ^{15}N enrichment of animal proteins over the diets (Δ^{15}N) is negatively correlated to N use efficiency (NUE) in ruminants and enables to capture animal-to-animal variation. This may represent a tool for genetic selection and precision feeding. However, the potential of Δ^{15}N as biomarker of NUE in periparturient dairy cows may be hampered by the strong protein mobilization supplying amino acids rich in ^{15}N compared with dietary substrates. Our objective was to examine the relationship between Δ^{15}N and NUE in the peripartum period. Plasma samples from 8 multiparous Holstein cows along with TMR feeds were collected in pre-calving (d-14) and in post-calving (d4, 15, 29 and 50) and analyzed for ^{15}N abundance by EA-IRMS. Values of Δ^{15}N from each cow and time (n = 39) was calculated as the ^{15}N difference between plasma and diets. In each instance NUE was calculated as the ratio of milk N from the N intake. Data were analyzed in R with Pearson correlations coefficients. A similar pattern was observed for the 8 cows immediately after calving, with a sharp increase in Δ^{15}N from d-14 toward d4 postpartum followed by an uninterrupted decrease from d4 to d50 postpartum ending closer to the pre-calving Δ^{15}N level. The peak in Δ^{15}N at d4 matched with the body weight loss (r = 0.77; $P = 0.04$) and with the expected high protein mobilization in this period. In addition, Δ^{15}N values at d4 were positively rather than negatively correlated with NUE (r = 0.88; $P = 0.01$). As the lactation progressed the relationship between Δ^{15}N and NUE became non-significant with slopes approaching zero. Given that protein mobilization does not generally occur beyond the very first weeks of lactation, we would have expected a negative correlation between NUE and Δ^{15}N in d50 but this was not observed. This study confirms the effect of lactation stage on the relationship between NUE and ?^{15}N. This suggests that, at least, in the first 50 d of lactation the biomarker has limitations to predict NUE. Moreover, this study confirmed that Δ^{15}N perceived the protein mobilization occurring during the early lactation.

Key Words: ^{15}N enrichment, N use efficiency, peripartum

Small Ruminant I

165 Reducing dietary rumen-degradable starch level can maintain colonic function and health of growing goats.

C. Jin*, J. Zhang, X. Lei, Z. Liang, and J. Yao, *Northwest A&F University, Yangling, Shaanxi, China.*

Starch-enriched diets have been proven to increase the risk of rumen subacute acidosis. In previous studies, rumen subacute acidosis also led to inflammation of hindgut. We hypothesized the regulation of starch digestion sites would influence the microbiota in hindgut. Microbiota-derived products might regulate the inflammatory signaling pathways in colonic mucosa and disrupt the colon hemostasis. Therefore, the present study was conducted to explore the critical factors for understanding of rumen-degradable starch (RDS) in maintaining hindgut homeostasis in ruminants. 40 healthy, weaned male goats (BW 13.6 ± 0.23 kg, 3-mo-old) were randomly enrolled to receive either a low rumen-degradable starch diet (LRDS, whole corn) or high rumen-degradable starch group (HRDS, crushed corn). Diets were formulated to be isoenergetic, isonitrogenous, and isostarch. Eight goats were slaughtered in each group at 6 mo of age to determine the intestinal function, bacterial flora stability and metabolites. The independent sample *t*-test in SPSS 20.0 was was used to evaluate the differences of fecal scores, pathology scores, bile acids, immunohistochemistry results and mRNA expressional levels of genes in colonic mucosa. The Mann-Whitney U test in SPSS 20.0 was used to evaluate the differences of the abundances of bacterial communities. The Spearman correlation coefficients and significance tests between bile acids and microbiota were calculated using bivariate correlation in SPSS 20.0. LRDS increased ($P < 0.05$) fecal scores and decreased ($P < 0.05$) colonic pathology scores. LRDS decreased the mRNA expression of DUOX2 ($P < 0.05$), IL-6 ($P < 0.05$), IL-22 ($P < 0.05$) and IFN-γ ($P < 0.05$) in colon mucosa. In comparison to the HRDS group, the relative abundances of *Fibrobacter*, *Lachnobacterium*, *Acidaminobacter* and *Beduinibacterium* significantly increased ($P < 0.05$), while the relative abundances of *Prevotella*, *Porphyromonadaceae_unclassified*, *Succinivibrio*, *Gloeobacter*, *Oligosphaera*, *Erysipelotrichaceae_unclassified* and *Flavonifractor* significantly decreased ($P < 0.05$) in the LRDS group. LRDS also decreased ($P < 0.05$) the content of primary bile acid and secondary bile acid in colonic lumen. Correlation analysis indicated that excessive bile acids promoted colon inflammation positively, while disrupting the homeostasis of colonic microbiota. In summary, reducing dietary RDS would maintain the homeostasis of colon by moderate bile acids.

Key Words: growing goats, microbiome, mucosa immunity

166 Selective treatment: Improving the safety and milk quality on dairy goat farms.

L. da Costa*[1] and M. Rovai[2], [1]*The Ohio State University, Columbus, OH,* [2]*South Dakota State University, Brookings, SD.*

This study aimed to evaluate the selective antibiotic therapy at dry-off to reduce the prevalence of IMI in dairy goats. Two dairy goat farms (farm 1 from Ohio with 178 does and farm 2 from South Dakota with 80 does) were enrolled in this study. Both farms use automatic milking and apply similar procedures for udder preparation (pre-dipping, wipe dry with a single-service paper towel, and post-dipping). Following cleaning, the teats were disinfected with 70% ethanol and dried with disposable paper towels. Udder-half milk samples were taken for bacterial culturing and SCC after first squirts were discarded, and 3 to 4 mL samples were collected into sterile tubes. Phase 0 was the baseline time before the trial started, and farmers used dry-off blanket therapy. The prevalence of bacterial isolation from the milk was determined in the subsequent lactation at dry-off (phase 1), where the bacteriological status of all quarters at dry-off was determined to assess the effectiveness of the practice later. Phase 1 prevalence of IMI was 23% (55/241) for farm 1 and 28% (28/101) for farm 2. The decision to use selective antimicrobial treatment at dry-off was based on culture – only culture-positive does receive treatment. Non-*Staphylococcus aureus* were the most common isolates for both farms. In the subsequent dry-off period (phase 2), based on bacteriological culture, the prevalence reduced to 16% (40/256) for farm 1 and 17% (13/75) for farm 2. The cost of treatment, calculated by farmers, was estimated to be on phase 0 equal to US$1,070, phase 1 equal to US$260, and phase 2 equal to US$166 without considering labor costs related to the antimicrobial usage. Milk quality also improved as the absolute difference between average SCC values from phase 1 to the average SCC values in phase 2 decrease in $893\text{x}10^{3}$ cells/mL for farm 1 and around $1{,}582\text{x}10^{3}$ cells/mL for farm 2. This preliminary report indicates that bacteriological culture could be a good strategy to reduce the cost and use of antibiotics while enhancing mammary health in dairy goats' farms.

Key Words: selective treatment, dairy goat, intramammary infection

Animal Behavior and Well-Being II

167 Opinions of Ontario producers on the use of pain control for disbudding and dehorning dairy calves.

J. Saraceni*[1], D. Renaud[1], E. Nelson[2], J. Van Os[3], C. Miltenburg[4], and C. Winder[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Sociology and Anthropology, University of Guelph, Guelph, ON, Canada,* [3]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [4]*Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada.*

Pain control for disbudding is a mandatory requirement for Canadian producers. The objective of the study was to explore the perspectives of Ontario dairy producers on the use of pain control when disbudding calves. Phone interviews were conducted with 29 Ontario dairy producers from September 2020 to January 2021. Interview questions concerned current practices, and motivations and barriers related to pain control use. Thematic analysis of interview transcripts was done using NVivo software. Full analysis has been completed on 14 interviews. All of these participants identified cautery disbudding as their primary method and used an analgesic and/or a local anesthetic. Meloxicam was used by 93% of participants, lidocaine was used by 86% of participants and 57% of participants used a sedative. The most common age for disbudding was 4 to 8 weeks old (43%) and 1 to 4 weeks old (36%). Overall, producers reported a positive view of pain control for disbudding, stating that it was necessary and provided a better disbudding experience for both the calf and the producer. Specifically, calf comfort and post-operative performance were identified as major motivations for pain control use. Additionally, participants cited pain control as making the procedure easier, and noted that regulatory bodies and outside sources (e.g., other farmers, the public, research) were influential on use of pain control. Cost was commonly cited as a barrier to pain control use, along with increased time associated with the procedure and issues with sedation (e.g., poor calf recovery). Perceived barriers that could prevent other producers from using pain control centered around the producer as an individual (e.g., lack of education, unwillingness to change practices, lack of motivation). Participants cited veterinarians as a key resource for removing barriers against pain control use, along with educational resources, regulations, incentives, and producer organizations. These findings identify potential avenues to increase adoption of pain mitigation for disbudding through better understanding of producers' motivations and barriers.

Key Words: interview, welfare, anesthetic

168 Impact of plane of nutrition and nonsteroidal anti-inflammatory drug treatment on wound healing following cautery disbudding in preweaned dairy calves.

C. N. Reedman*[1], T. F. Duffield[1], T. J. DeVries[2], K. D. Lissemore[1], C. B. Tucker[3], S. J. J. Adcock[4], and C. B. Winder[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Center for Animal Welfare, Department of Animal Science, University of California, Davis, Davis, CA,* [4]*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI.*

The objective of this 2 × 2 factorial study was to determine the impact of plane of nutrition, with 1 or 2 doses of a nonsteroidal anti-inflammatory drug (NSAID), on wound healing in calves disbudded by removal of the horn bud with a hot iron. Eighty heifers were randomly assigned to a high (15 L/d) or low (6 L/d) milk feeding. Calves were disbudded at 18–25 d of age and received a lidocaine cornual nerve block and a subcutaneous injection of meloxicam 15 min before disbudding (n = 80) and 3 d later (n = 40). Stage of wound healing, diameter, and depth of wounds were evaluated 2×/wk for 8 wk. Time to re-epithelization and 0 mm depth were analyzed using Cox regression models. Mixed-effects linear models with random effects for wound within calf within disbudding date were used for wound diameter and depth. Time to re-epithelization ranged from 28 to 59 d and time to depth of 0 ranged from 10 to 59 d after disbudding. At any time, high milk calves were 75% (HR:1.75, 95%CI:1.01–3.03, $P = 0.05$) and 98% (HR:1.98, 95%CI:1.16–3.40, $P = 0.01$) more likely to have had re-epithelization occur and a wound depth of 0mm, respectively, compared with low calves. Calves that received only 1 NSAID dose were 81% (HR:1.81, 95%CI:1.02–3.21, $P = 0.04$) and 63% (HR:1.63, 95%CI:0.93–2.87, $P = 0.09$) more likely to have re-epithelization occur and a wound depth of 0mm, respectively, compared with 2 dose calves. Interactions were detected between milk and NSAID treatment and time point after disbudding, for wound diameter and depth models. On the day of disbudding, the average diameter and depth of the wounds was 18.8 mm and 5.2 mm, respectively. For every 1 wk increase after disbudding, the mean diameter and depth of a wound for a high milk calf was 0.50 mm (95%CI: −0.60 to −0.39, $P < 0.001$) and 0.16 mm (95%CI: −0.22 to −0.094, $P < 0.001$) smaller, compared with a low calf. For every 1-wk increase after disbudding, the mean diameter and depth of a wound on a calf in the 2 dose NSAID group was 0.42 mm (95%CI:0.32 to 0.52, $P < 0.001$) and 0.14 mm (95%CI: 0.076 to 0.21, $P < 0.001$) larger, compared with 1 dose calves. Our results suggest that limit feeding calves slows wound healing, as does, to a lesser extent, an extra NSAID 3 d after disbudding. The latter may occur through suppression of inflammation.

Key Words: dehorning, inflammation, welfare

169 Dairy farmer, veterinarian, and hoof trimmer perceptions of barriers in lameness management.

E. Wynands*[1], S. Roche[2], G. Cramer[1], and B. Ventura[3], [1]*University of Minnesota, Department of Veterinary Population Medicine, St Paul, MN,* [2]*ACER Consulting, Guelph, ON, Canada,* [3]*University of Minnesota, Department of Animal Science, St Paul, MN.*

Lameness is a leading animal welfare concern in the dairy industry as it is painful and impacts cow longevity in the herd. Multiple stakeholders are involved in lameness management on a dairy farm, including farmers, hoof trimmers, and veterinarians. This qualitative study sought to explore perceptions of lameness and barriers to improved lameness management in these groups. Fourteen homogeneous focus groups were held in Minnesota, Wisconsin, and New York, USA from April 2017 to March 2020; 5 with farmers (n = 31), 4 with hoof trimmers (n = 32), and 5 with veterinarians (n = 25). The 1-h facilitated discussions were audio-recorded, transcribed verbatim, and common themes identified through thematic analysis. Lameness was perceived by participants as a complex disease and one in which the connections between pathogenesis, facilities, and management were not always well understood or easy to change. The complexity of the problem encompassed the lack of agreement on a definition of lameness, normalization to its signs, and the interconnectedness of lameness with other health and management issues. These ultimately appeared to contribute to resignation by participants that lameness was inevitable. Despite shared concerns about lameness among these groups, respondents reported a lack of

communication, especially between hoof trimmers and veterinarians. Participants also voiced a desire to work together more productively with hoof trimmers and veterinarians valuing the ability to deliver a consistent message to farmers. These findings suggest a need for increased efforts to facilitate collaboration between farmers, hoof trimmers, and veterinarians to improve lameness management on dairy farms.

Key Words: lameness, stakeholders, communication

170 Perspectives of Western Canadian dairy farmers on providing outdoor access for dairy cows. A. M. C. Smid*[1], P. H. J. Inberg[1], S. de Jong[1], S. Sinclair[1], M. A. G. von Keyserlingk[2], D. M. Weary[2], and H. W. Barkema[1], [1]*University of Calgary, Calgary, AB, Canada,* [2]*University of British Columbia, Vancouver, BC, Canada.*

Dairy cows are highly motivated to access pasture, especially at night. When pasture is not available, dairy cows show a partial preference for alternative types of outdoor access, spending half the night outside in summer on an outdoor sand or wood chip pack. In 2015, however, a survey with a 9% response rate reported that only 29% and 57% of Canadian dairy farms provided lactating and dry cows pasture access, respectively. To better understand reasons why dairy farmers choose to provide or not provide outdoor access, we studied the perspectives of dairy farmers located in the 4 Western Canadian provinces: British Columbia, Alberta, Saskatchewan and Manitoba. Data were collected via: 1) 11 focus group discussions with a total of 50 Western Canadian dairy farmers, and 2) semi-structured individual interviews with 6 dairy farmers of Hutterite colonies. Transcripts were analyzed using template analysis. Reasons to not provide outdoor access fell into 5 main themes: 1) adverse climate conditions, 2) negative implications of outdoor access for cow welfare including concerns about udder health, 3) concerns regarding decreases in profitability, 4) farm infrastructure not set up for outdoor access, and 5) higher ability to manage animals kept indoors. Reasons to provide outdoor access fell into the 5 main themes: 1) local climate conditions conducive for outdoor access, 2) beneficial effects of outdoor access on cow welfare including lower lameness prevalence, 3) increased profitability due to a premium milk price provided to farmers that allow outdoor access to their cows, 4) farm infrastructure that is set up for outdoor access, and 5) easier management of animals outdoors. We conclude that the decision to provide outdoor access depends on how farmers weigh these factors given the specific requirements of their farm. The results of this study may help guide debate on outdoor access for dairy cattle and can help to inform the public and policy makers on the complex issues affecting farmers' decisions to provide outdoor access to their cows.

Key Words: barrier, motivator, dairy farmer view

171 United States dairy producers' perceptions of male dairy calves. K. Creutzinger*[1,2], J. Pempek[2], S. Locke[2], D. Renaud[1], K. Proudfoot[3], K. George[2], D. Wilson[1], and G. Habing[2], [1]*University of Guelph, Guelph, ON, Canada,* [2]*The Ohio State University, Columbus, OH,* [3]*University of Prince Edward Island, Charlottetown, PEI, Canada.*

There is some evidence that calf-care practices differ between male and female calves on dairy farms of birth, however, it is unclear what influences dairy producer care decisions. The aim of this study was to characterize influencers of early-life care of male calves on dairy farms and dairy producers' perceptions of male calves. Fourteen dairy producers in Ohio and Indiana were individually interviewed using a semi-structured format to explore neonatal calf care, producer decision-making regarding care, and perceptions of male calves. Interviews were audio-recorded, transcribed, and thematically analyzed (NVivo) to identify common themes expressed by producers. Some producers acknowledged that they provided different care for male and female calves for some, but not all, routine practices. All producers reported dipping male calves' navels after birth. However, some mentioned providing poorer quality or less colostrum to males, and the majority rarely administered preventive health supplements. The cost of care for male calves in relation to calf sale price was reported as a major barrier. Producers also acknowledged the marketing route as a barrier to male calf care. Calves sold through auctions reportedly received low levels of care, including lower quality or volumes of colostrum compared with females. By comparison, calves sold to someone in the producer's local community or reared on the farm of birth reportedly received better care, such as high-quality colostrum, milk replacer rather than waste milk, and vaccinations. Reported reasons for calf care included slaughter withholding periods for antimicrobials and vaccines, accountability for calf health, the purchaser paying for additional supplementation, or a combination of these factors. Overall, these findings highlight some of the barriers to neonatal male dairy calf care at the dairy farm of birth. We suggest future extension and research efforts address these barriers to improve the care of male dairy calves.

Key Words: decision-making, surplus calves, veal

172 The effect of long-distance transportation on lying behavior of surplus dairy calves. K. Creutzinger*[1], H. Goetz[1], K. Proudfoot[2], and D. Renaud[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*University of Prince Edward Island, Charlottetown, PEI, Canada.*

The objective of this study was to evaluate the effect of transportation duration on calf lying behavior during and after transport. Male and female surplus dairy calves (n = 50) were enrolled on the day of birth on 4 dairy farms in Ontario, Canada. On the day of transport, calves from 2 to 22 d of age were randomly assigned to 1 of 3 treatments: 6, 12, or 16 h of continuous transportation in a gooseneck trailer (9.1 × 2.3 m) deep bedded with chopped straw. All calves were transported to a single calf-raiser facility. Daily lying time and bouts (transition from lying to standing) were recorded using data loggers from −2 to +5 d (0:00 a.m. – 23:59 p.m.) relative to transport. Mixed linear regression models were constructed to determine the effect of treatment on lying time and bouts each day and between days (including day as a repeated measure). Regardless of treatment, calves spent the least amount of time lying ($P < 0.001$) and had the most lying bouts ($P < 0.001$) on the day of transport compared with all other days. Lying time was greater the day after transport compared with 2 (1,149 vs. 1,092 ± 19 min; $P = 0.003$) and 3 d after arrival (1,089 ± 19 min; $P = 0.002$). On the day of transport, calves transported for 6 h spent more time lying than calves transported for 12 h (1,036 ± 26 vs. 921 ± 28 min; $P < 0.001$) or 16 h (905 ± 26 min; $P < 0.001$); there was no difference between 12 and 16 h ($P = 0.65$). The day after arrival, calves transported 6 h spent less time lying than calves transported for 12 h (1,107 ± 25 vs. 1,159 ± 26 min; $P = 0.05$) or 16 h (1,189 ± 25 min; $P = 0.002$); there was no difference between 12 and 16 h ($P = 0.25$). No treatment effects were found on any other days ($P > 0.05$). On the day of transport, calves transported for 6 h had fewer lying bouts than calves transported for 12 h (20 vs. 25 ± 2 bouts, $P = 0.02$) or 16 h (30 ± 2 bouts, $P < 0.001$), and calves had fewer bouts when transported for 12 compared with 16 h ($P = 0.02$). Preliminary results from this study show that resting ability is compromised by transportation.

Key Words: bull calf, welfare, veal

Animal Health II

173 Maternal choline supplementation to improve calf growth, health, and survival.

M. B. Poindexter*[1], G. Negro[1], M. J. Granados[1], P. G. L. Lima[1], D. C. Ramos[1], D. M. Burgos[1], F. X. Amaro[1], K. Estes[2], M. G. Zenobi[2], C. Zimmerman[2], J. E. P. Santos[1], and C. D. Nelson[1], [1]*University of Florida, Gainesville, FL,* [2]*Balchem Corp., New Hampton, NY.*

Objective was to determine if maternal choline supplementation in the last 3 wk of gestation affects incidence of disease and growth of Holstein heifer calves. Holstein cows (n = 1,003; 633 primiparous, 370 multiparous) were assigned randomly to 1 of 21 pens at 252 d of gestation and treatments were assigned to pens with 11 pens receiving control (505 cows) and 10 pens receiving rumen-protected choline (RPC, 498 cows) as a top dress at 12.9 g of choline ion per cow per day. Female calves were randomly assigned at birth to receive colostrum from dams fed control or RPC. The experiment was a split-plot design with the main plot being dam treatment containing 21 experimental units. The subplot was colostrum treatment containing 1,003 experimental units resulting in a 2x2 factorial arrangement of treatments for calves: NN (No RPC in utero + no RPC colostrum), CN (RPC in utero + no RPC colostrum), NC (No RPC in utero + RPC colostrum), or CC (RPC in utero + RPC colostrum). Calves were housed individually and weighed at 0, 30, 60, and 82 d of age. Pulmonary lesions were assessed by ultrasonography (0 = no consolidation to 5 = at least 3 consolidated lobes) and morbidity was evaluated daily. Data were analyzed by ANOVA, logistic regression, or the Cox's proportional hazard for a split-plot design using mixed models with fixed effects of in utero treatment, colostrum treatment, interaction between in utero and colostrum treatments, and random effects of pen nested within in utero treatment and sire of the calf. Maternal RPC did not support health, growth, or survival of calves by 82 d.

Key Words: calf, choline, health

174 Effect of feeding *Echinacea purpurea* to dairy calves on markers of immunity and inflammation.

B. K. McNeil*[1], D. L. Renaud[2], M. A. Steele[1], A. J. Keunen[3], 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,* [3]*Mapleview Agri Ltd., Palmerston, ON, Canada.*

Echinacea purpurea is a perennial herb that has demonstrated immunostimulatory and anti-inflammatory effects. The objective of this study was to investigate how supplementing calves with *Echinacea purpurea* affects blood immune and inflammatory markers. Male Holstein calves (n = 117), sourced from local farms or auction, arrived at a rearing facility between 5 and 14 d of age and were kept in 1 of 3 rooms (39 calves/room), beginning d 1 of the experiment. Within each room, calves were randomly assigned to an individual stall and 1 of 3 treatments: 1) control (C; n = 39), 2) 3 g of *Echinacea*/d split over 2 milk feedings from experiment d 14–28 (E14; n = 39), and 3) 3 g of *Echinacea*/d split over 2 milk feedings from experiment d 1–56 (E56; n = 39). *Echinacea* treatments were mixed into the milk replacer (MR). Calves received MR 2×/d for 56 d (total = 36 kg of MR) and had ab libitum water and starter access. Blood was collected from each calf on d 1, 14, 28, and 57, and assessed for various white blood cells (WBC) and haptoglobin. Serum total protein (STP) was determined on d 1. Data were analyzed in repeated-measures mixed-effect linear regression models. No treatment × day interactions were detected. STP did not differ among treatments (5.54 ± 0.10 g/dL; $P = 0.99$). Segmented neutrophils were higher ($P = 0.04$) in C calves ($3.91 \pm 0.25 \times 10^9$/L) than in E56 calves ($3.30 \pm 0.24 \times 10^9$/L), although neither differed ($P > 0.14$) from E14 calves ($3.48 \pm 0.25 \times 10^9$/L). Lymphocytes were higher ($P = 0.04$) in E56 calves ($4.40 \pm 0.13 \times 10^9$/L) and tended ($P = 0.07$) to be higher in E14 calves ($4.37 \pm 0.13 \times 10^9$/L) when compared with C calves ($4.02 \pm 0.13 \times 10^9$/L). The segmented neutrophil:lymphocyte ratio was smaller ($P \leq 0.03$) for both E14 calves (0.86 ± 0.07) and E56 calves (0.83 ± 0.07) when compared with C calves (1.04 ± 0.07). The total WBC count for C, E14, and E56 calves were 8.90 ± 0.30, 8.71 ± 0.30, and $8.52 \pm 0.29 \times 10^9$/L, respectively, and did not differ ($P = 0.68$). Haptoglobin was lower ($P = 0.05$) for E56 calves (0.21 ± 0.019 g/L) and tended ($P = 0.07$) to be lower for E14 calves (0.21 ± 0.020 g/L) when compared with C calves (0.26 ± 0.020 g/L). Overall, *Echinacea purpurea* supplementation was associated with markers of increased immunity and reduced inflammation, particularly when fed across the whole milk feeding period.

Key Words: feed additive, health, medicinal herb

Table 1 (Abstract 173). Effect of in utero and colostrum choline supplementation on calf performance and health

Variable	Incidence, % (no./no.)	NN	CN	NC	CC	*P*-value In utero	 Colos	 In utero × Colos
ADG at 82 d, g/d		639.5	631.5	638.1	646.8	0.97	0.31	0.22
Lung score 60 d (1-5)		1.74	1.70	1.74	1.78	0.99	0.67	0.67
Clinical disease								
Diarrhea	87.3 (876/1003)	89.5	85.4	88.4	89.3	0.46	0.58	0.44
Pneumonia	18.6 (187/1003)	17.4	20.0	17.8	20.1	0.89	0.79	0.92
Antimicrobial use	89.4 (897/1003)	90.8	86.6	89.0	90.9	0.88	0.68	0.23
Morbidity	90.7 (910/1003)	91.5	88.4	91.0	91.6	0.64	0.60	0.52
Multiple diseases	16.4 (164/1003)	15.3	17.0	15.1	17.9	0.90	0.86	0.96
Mortality	2.9 (29/1003)	2.3	2.2	5.3	1.8	0.17	0.45	0.19

175 Effects of yeast-derived products feeding on performance in dairy calves: A meta-analysis.

C. Zhang*, *Northwest A&F University, Xianyang, Shaanxi, China.*

The yeast-derived products (YDP) mainly include live yeast fermentation products (LYF) or yeast extracts (YE). However, the effects of YDP on calf performance is still controversial. Given that the nursing stage plays an important role on calf growth, a meta-analysis was conducted to determine the effects of YDP on average daily gain (ADG) and solid feed intake (SFI) in preweaning and postweaning calves. We retrieved articles by searching keywords including yeast etc and calf. Thirty articles from 1992 to 2021 were included in our study. After collecting the experimental treatment, number of animals, weaning stage, SFI, ADG, we used the StataSE 15 to conduct meta-analysis for Preweaning-ADG, Postweaning-ADG, Preweaning-SFI and Postweaning-SFI. Select the standardized mean difference (SMD) as the effect magnitude, calculate the 95% confidence interval (95% CI) and use the inverse variance to pool effect sizes. The chi-squared (Q) test and the I^2 statistic were determined to measure heterogeneity. Egger's linear regression asymmetry was used to examine the presence of publication bias. According to the kinds of YDP (LYF or YE), subgroup analysis was performed for each meta-analysis group. During preweaning, LYF feeding significantly increased the ADG (42 g/d) and SFI (0.46 kg/d), while YE had no significantly influence on the ADG and SFI of calves. During postweaning, YE feeding significantly increased the ADG (56 g/d) and SFI (0.27 kg/d), while LYF had no influence on the ADG and SFI of calves. Subgroup YE existed high heterogeneity and publication bias. In summary, we suggested that for calves, LYF would better be fed before weaning and YE would better be fed after weaning.

Table 1. Summary of YDP feeding of calves from meta-analysis

		No. of	SMD (95% CI)		Heterogeneity test	
Subgroup	Item	trials	Effect size	P	I^2/%	P
Preweaning						
LYF	SFI	17	0.46 (0.29, 0.64)	<0.05	21.7	0.20
	ADG	17	0.42 (0.25, 0.59)	<0.05	14.6	0.28
YE	SFI	10	0.04 (−0.02, 0.28)	0.73	82.6	<0.10
	ADG	11	0.30 (0.16, 0.44)	0.68	86.6	<0.10
Postweaning						
LYF	SFI	17	−0.04 (−0.18, 0.10)	0.57	0.0	0.65
	ADG	21	0.09 (−0.04, 0.22)	0.21	43.2	<0.10
YE	SFI	13	0.27 (0.04, 0.50)	<0.05	68.5	<0.10
	ADG	10	0.56 (0.24, 0.88)	<0.05	75.0	<0.10

Key Words: meta-analysis, calf, yeast-derived products

176 Fecal microbiota transplantation alters the intestinal microbiota and blood cytokines in preweaned dairy calves.

F. Rosa*[1,2], T. C. Michelotti[1], B. St-Pierre[3], E. Trevisi[4], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR,* [3]*Department of Animal Science, South Dakota State University, Brookings, SD,* [4]*Deparment of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

This study aimed to evaluate the changes in gut microbiota and blood cytokines during fecal microbiota transplantation in preweaned dairy calves. Healthy newborn Holstein calves (n = 8/TRT) were randomly assigned to either a baseline nutritional program (CON) or 1 × /d inoculations with 25 g of fecal donor material (FMT) mixed in the antibiotic-free milk replacer from 8 to 12 d of age. Body weight (BW) was recorded weekly. Blood samples were collected weekly for cytokines measurement by ELISA assay. Fecal samples were collected at 7, 14, and 35 d of age and immediately frozen in liquid nitrogen. Data were analyzed using the MIXED procedure of SAS, where treatment, time, and their interaction were the fixed effects in the model, and calf the random effect. The respective fecal bacterial compositions of donor material used for the FMT and of the calf samples were determined by MiSeq (2X300) sequencing of amplicons generated from the V1-V3 region of the 16S rRNA gene. Taxonomic composition was determined using the Ribosomal Database Project (RDB) classifier. Microbial composition differences in the feces of CON and FMT calves were assessed by Kruskal-Wallis test in R. A trend ($P = 0.06$) for greater BW (61.2 vs 57.7 kg) in FMT calves than CON was observed at 35 d of age. This was followed by lower ($P \leq 0.05$) IL-1β and IL-6 in FMT calves than CON during weaning at 42 and 49 d of age. *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* were the most highly represented phyla in the fecal material of the donor, accounting for 95.8% of the sequences. At 7 and 14 d of age, *Odoribacteraceae* abundance was higher in CON calves compared with FMT group, whereas at 14 d of age, the family *Succinivibrionaceae* from the *Proteobacteria* phylum was significantly enriched in FMT calves relative to CON. At 35 d of age, the relative abundance of *Enterobacteriaceae* was significantly lower in the feces of FMT calves relative to the CON. A persistent imbalance of the gut's microbial community has been associated with an abnormal increase in the abundance of *Enterobacteriaceae*, which may trigger calf scours. Thus, the lower abundance of *Enterobacteriaceae* in the FMT calves compared with the CON, along with a 3.5-kg increment in BW and no incidence of diarrhea at 35 d of age or before weaning, suggest that FMT-induced alterations in the gut microbial composition of preweaned calves may improve overall host performance.

Key Words: gut microbiota, feces, neonatal calves

177 Health outcomes for calves housed in a simplified group system.

B. M. Karle*[1], E. M. Abdelfattah[2], D. M. Brady Yount[1], G. S. Machado[1], M. D. Lowe[1], F. C. Ferreira[2,3], S. S. Aly[2,3], and T. W. Lehenbauer[2,3], [1]*Cooperative Extension, Agriculture and Natural Resources, University of California, Orland, CA,* [2]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Tulare, CA,* [3]*Department of Population Health & Reproduction, School of Veterinary Medicine, University of California, Davis, CA.*

Most California dairy calves are raised in individual hutches which facilitate their monitoring and handling. Previous studies show that group housing is beneficial to calf welfare and is associated with improved performance, yet concerns of increased disease transmission hinder its adoption. Our study aimed to compare the health outcomes for calves raised in individual hutches to those housed in groups. A total of 42 Holstein heifer calves on a Northern California dairy were systematically assigned by birth order to be either individually housed (IH, n = 21) or group-housed (GH, n = 21) in July–October, 2020. GH calves were kept in groups of 3 from a minimum of 7 d old until 70 d of age. Groups were constructed by assembling 3 polyethylene hutches with a 1.5 m × 3.6 m outside exercise area of wire panel fencing. Individual hutches consisted of one polyethylene hutch with a 1.5 m × 1.2 m outside exercise area. Calves were weighed on a platform scale and measured for height at birth and weaning. Diarrhea status was based on fecal scores of 1, 2 and 3 (normal, loose, watery diarrhea, respectively) and a score of 2 or greater was considered diarrhea. Respiratory disease was scored using the California BRD scoring system, with a score of ≥5 indicating BRD. Both scores were recorded daily for all calves throughout the preweaning period. A linear mixed model using Stata's mixed command was specified using a forward manual model building process with $P < 0.05$ for significance. Differences in weight at enrollment were adjusted for using a mixed model with group as a random effect and showed no significant difference in average daily gain between the housing types (GH = 0.665 kg/d, IH = 0.619 kg/d, $P = 0.32$). GH calves gained 0.225 cm/d in withers height compared with 0.216 cm/d for IH calves ($P = 0.58$). The cumulative incidence of respiratory disease in GH calves was 76.2% compared with 71.4% in IH calves, ($P = 0.73$). All calves had diarrhea at some point before weaning, hence comparison of diarrhea incidence between groups was not possible. These findings show evidence that simplified group housing provides benefits of GH without detrimental short-term impacts on calf growth and health.

Key Words: group housing, calves

178 Associations between feeding behaviors collected from an automated milk feeder and disease in group-housed dairy calves in Ontario: A cross-sectional study.

M. Conboy*[1], C. B. Winder[1], C. Medrano-Galarza[2], S. J. LeBlanc[1], D. B. Haley[1], J. H. C. Costa[4], M. A. Steele[3], and D. L. Renaud[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Programa de Especialización en Bienestar Animal y Etología, Facultad de Medicina Veterinaria, Fundación Universitaria Agraria de Colombia, Bogotá, Colombia,* [3]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [4]*Department of Animal and Food Sciences, University of Kentucky, Lexington, KY.*

The aim of this cross-sectional study was to assess if single time point of feeding behavior data could be used to aid detection of neonatal calf diarrhea (NCD), bovine respiratory disease (BRD), and general disease (GD) (having NCD, BRD and/or umbilical infection), in pre-weaned group-housed calves fed via an automated milk feeder (AMF). Eight dairy farms recruited from an online calf management survey were enrolled in the study. Farms were visited once each season, where calves were health scored and AMF data, corresponding to the same day of health scoring, were collected. Mixed linear regression models were used to identify associations between feeding behavior and NCD, BRD, or GD. Generalized linear mixed models were used to assess if the percentage of milk the calf consumed from their daily milk allotment was associated with the presence of BRD, NCD or GD. There were 523 total observations with 115, 130 and 210 events of BRD, NCD and GD, respectively. Calves with BRD consumed 63% less of their daily milk allotment, had 2 fewer unrewarded visits to the AMF, and drank 152 mL/min slower, compared with calves without BRD. Calves with NCD consumed 57% less of their daily milk allotment, consumed 758 mL less per day, and drank 92 mL/min slower, compared with calves without NCD. Calves with GD drank 50% less of their daily milk allowance, consumed 496 mL less per day, drank 80 mL/min slower, and had 2 fewer unrewarded visits to the AMF, compared with calves without disease. Sensitivity and specificity for disease detection were low when evaluating the feeding behaviors individually as determined by ROC curves. Parallel testing (where change in any parameter indicated disease) increased sensitivity to 0.82, 0.78 and 0.84, and a specificity of 0.26, 0.23 and 0.21, for BRD, NCD and GD, respectively. This study suggests that AMF data may be a useful screening tool to detecting diseased calves but should not be used as the sole method.

Key Words: health, automation, group housing

179 Are daily behavioral patterns in calves associated with response after antibiotic treatment for bovine respiratory disease?

M. C. Cantor*[1], D. L. Renaud[2], and J. H. C. Costa[1], [1]*Dairy Science Program, University of Kentucky, Lexington, KY,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

This study investigated the association of daily patterns of feeding and activity behaviors of dairy calves who failed to recover from bovine respiratory disease after treatment with antibiotics. Calves (n = 36, 18 response, 18 relapse) identified with BRD and fitted with an accelerometer (IceRobotics, Scotland) were enrolled. Calves were offered 10 L/d milk replacer and calf starter from automated feeders. Calf symptoms for BRD (Wisconsin Scoring, WI, USA) were recorded daily and lung ultrasounds were recorded twice weekly. A clinically sick calf was defined as a BRD score ≥5, and lung consolidation ≥3 cm^2 on ultrasound that received antibiotic treatment (d 0; enrofloxacin (1 mL/15 kg). Recovery status was evaluated from d 10 to d 13 after first antibiotics; a BRD score <5 was recovery (RESPONSE) and failure-to-recover (RELAPSE) was BRD score ≥5 and lung consolidation ≥3 cm^2 (re-treated on d 14). The MIXED procedure (SAS 9.4, Cary, NC, USA) evaluated the fixed effect of recovery (response or relapse) on relative changes in feeding behavior: [milk intake, drinking speed, visits, calf starter consumption] and relative changes in activity behaviors [lying time, total step count, lying bouts, and an acceleration activity index] for treatment d 0 (baseline) to d 10. Pairing of calves by age treated, birthdate, and sex was performed. Response calves took an average 6 ± 2 d to resolve symptoms. There was a positive association of recovery status with relative changes in lying time, suggesting relapsed calves had higher lying times than response calves ($P = 0.001$). For feeding behavior, a tendency was found for an interaction with recovery status and treatment day with relapse calves having lower relative changes in calf starter intake than response calves ($P = 0.07$). For activity behavior, a significant interaction with recovery status and treatment day was found, relapse calves had lower relative changes in the step count and in the activity index than response calves ($P \leq 0.04$). These results suggest there is a complex relationship between behavioral patterns and recovery status in calves that can be used as metrics to identify successful treatments.

Key Words: automated feeder, accelerometer, precision technology

180 Antimicrobial resistance in samples submitted from calves to a diagnostic laboratory in Ontario, Canada, between 2007 and 2020.

T. Uyama*[1], D. F. Kelton[1], J. T. McClure[2], S. J. LeBlanc[1], C. B. Winder[1], and D. L. Renaud[1], [1]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada.*

Sick farm animals are often treated with antimicrobials, which could increase the level of antimicrobial resistance (AMR) in the pathogens, as well as commensal organisms. Young calves are at a greater risk than older animals of excreting resistant bacteria, but few studies have investigated the prevalence of resistant organisms in diseased calves. The objective of this study was to describe the prevalence of AMR and investigate its temporal trend in key organisms isolated from biological samples obtained from diseased calves that were 0 to 2 mo of age. Diagnostic data were obtained from the Animal Health Laboratory of the University of Guelph between 2007 and 2020. Antimicrobial susceptibility results in bacterial isolates recovered from calves were used for analysis. The proportions of resistant isolates (R) and susceptible/intermediate isolates (S) were compared using a multivariable logistic regression model to investigate the association between the year the samples were obtained and the prevalence of AMR. A total of 64,675 bovine isolates were available and 3,526 isolates included AMR results. Isolates without information on the age of the animal, and those used for herd monitoring or research purposes were excluded. In total, 1,334 calf isolates with AMR results were evaluated. Samples were mostly collected from the gastrointestinal tract/feces (n = 571) and lung (n = 378). The most common isolates were *Escherichia coli* (n = 451), *Salmonella* spp. (n = 383), and *Pasteurella multocida* (n = 132). The proportion of *E. coli* isolates resistant to spectinomycin (2007:10%; 2020:54%) and the proportion of *Salmonella* isolates resistant to ampicillin (2007:25%; 2019:70%), sulfonamide (2007:38%; 2020:80%), and tetracycline (2007:38%; 2018:81%) increased significantly over the 14 years examined. The temporal trend of AMR in *P. multocida* isolates was not analyzed due to the small sample size. Among the ceftiofur-resistant *Salmonella* isolates, 73% were *Salmonella* Dublin. Although AMR data from a diagnostic lab are not generalizable to the entire calf population, these findings offer some insights into AMR in diseased calves and support the need for AMR surveillance in calves.

Key Words: antibiotic-resistant, gastrointestinal, pre-weaned

181 Colostrum management practices associated with failed transfer of passive immunity in dairy calves: A scoping review.

T. Uyama*[1], D. F. Kelton[1], C. B. Winder[1], J. Dunn[1], H. M. Goetz[1], J. T. McClure[2], S. J. LeBlanc[1], and D. L. Renaud[1], [1]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada.*

Failed transfer of passive immunity (FTPI) continues to occur commonly in the dairy industry and has significant effects on calf health. Various factors affect FTPI including colostrum management practices, but information on risk factors for FTPI remains piecemeal. The objective of this scoping review was to describe the breadth and key attributes of the literature that addresses colostrum management practices related to FTPI in dairy calves. Literature was searched through 8 databases with relevant search terms. After de-duplication, 3,729 records had titles and abstracts screened, with 1,217 references screened at full text, independently by 2 reviewers. Clinical trials and analytic observational studies were eligible for data extraction, resulting in 328 references. Among the 160 references evaluated (preliminary results), studies were primarily conducted in North America (38%) and Europe (16%). Outcomes measured to evaluate FTPI in calves included blood IgG concentration (90% of experiments), blood total protein concentration (41%), blood gamma-glutamyl transferase (3%) and Brix percent (2%). The proportion of calves with FTPI were further measured in 23% of the experiments. Research consisted of controlled trials (77%), and cohort (20%), and cross-sectional studies (3%). Among the controlled trials, common risk factors investigated were the source of colostrum (29%), pre-parturient nutrition of the dam (19%), and quantity of colostrum at the first feeding (15%). Among cohort studies, common risk factors investigated were the quality of colostrum (52%), age/parity of the dam (27%), difficulty of birth (18%), and the timing of the first feeding of colostrum after birth (18%). Among cross-sectional studies, the most common risk factors investigated were age/parity of the dam (60%) and route of feeding colostrum (60%). Although an array of different risk factors were investigated in the literature, future synthesis work, including meta-analysis, could quantify the effects of commonly assessed variables.

Key Words: passive transfer, newborn calves, immunoglobulin G

182 Total serum protein and its influence on morbidity, mortality, and performance of preweaned dairy calves from different genetic compositions.

M. B. Moreira[1], F. C. Ferreira*[2], and S. G. Coelho[1], [1]*Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [2]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare, CA.*

Our objective was to describe risk factors for failure of transfer of passive immunity (FTPI) and to investigate how FPTI is associated with the risk of morbidity, mortality, and growth of preweaned dairy calves from 2 genetic compositions in tropical conditions. We performed a retrospective cohort study and used pre- and post-birth calf-level data from 6,011 crossbred pre-weaned calves from a single herd, born between 2012 and 2018. We obtained information on calf pedigree, season of birth, occurrence of retained placenta (RP), total serum protein (TSP), morbidity [neonatal calf diarrhea (NCD), bovine respiratory disease (BRD), and tick-borne disease (TBD)], mortality, and weight gain. Genetic composition was predominantly dairy Gyr (PG, 0–50% Holstein breed), and predominantly Holstein (PH, > 50% Holstein breed). Calves received colostrum from their dams if Brix >22%, otherwise from a colostrum bank. Multivariate mixed logistic regression was performed (SAS 9.4). The optimal TSP cut-off points for predicting morbidity and mortality were 7.6 g/L and 6.9 g/L, respectively. The median value of TSP was 7.2 g/L. At cut-off points of 5.2 g/L, 7.6 g/L, and 6.9 g/L the prevalence of FTPI was 2%, 55%, and 31%. Significant results are presented ($P < 0.05$). PH calves had 1.3 greater odds of having FTPI, and 1.5 greater odds of disease. Calves born from multiparous cows and from calving followed by RP had greater odds of FTPI. Overall, the prevalence of disease was 53% (41% of the calves had NCD, 18% had BRD, 10% had TBD). Season, dam's parity order, sire PTA for milk, and weight at birth were associated with odds of disease. FTPI was not a good predictor for disease. The overall mortality rate was 6%. Calves PH had 3.0 greater odds of mortality than PG. Mortality risk varied according to an interaction between FTPI and the season of birth, but calves from sires with a positive PTA for milk had greater odds of dying. The average daily gain was 0.636 kg, and calves with FTPI (cut-off point of 5.2 g/L) gained less weight than calves without FTPI. Thresholds for FTPI may vary according to specific risk factors and calf raising conditions.

Key Words: transfer of passive immunity, crossbred, tropical

183 Characterizing and describing the literature on the impacts of umbilical care on health outcomes and risk factors examined for umbilical health in intensively raised bovines: A scoping review.

M. Van Camp*[1], C. Winder[1], D. Gomez[2], T. Duffield[1], and D. Renaud[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Clinical Studies, University of Guelph, Guelph, ON, Canada.*

The objectives of this scoping review were to describe and characterize the existing literature regarding umbilical health and identify current gaps in knowledge. Six databases were searched for relevant primary research. Eligible studies had to examine umbilical health in any intensively raised bovine population. There were 6,816 articles that fit within the search terms and had the title and abstract screened by 2 independent reviewers to ensure relevance. 617 articles were then screened at full text, with 155 articles meeting the review criteria. Of these, 22 were classified as descriptive, 24 clinical trials, and 109 analytical observational studies. The umbilical outcomes evaluated in descriptive studies were; omphalitis (n = 13) of which 10 did not provide a definition, parasitic infections of the navel (n = 7) and umbilical hernias (n = 3). Only one clinical trial (n = 1) examined treatment of a navel health outcome, the

remaining evaluated management factors that could be used to prevent a navel health outcome (n = 23). These outcomes included; umbilical infections (n = 17) of which 5 did not have definitions, navel myasis (n = 3), navel measurements (n = 5), umbilical hernias (n = 1) and umbilical edemas (n = 1). Analytical observational studies were further broken down into studies examining risk factors for umbilical health (n = 61), and studies examining umbilical health as a risk factor (n = 59). Studies examining risk factors for umbilical health assessed outcomes such as umbilical disease (n = 38) of which 16 provided no definition, conformation scores of the navel (n = 17), navel myasis (n = 2) and navel measurements (n = 6). Studies examining umbilical health as a risk factor assessed umbilical disease (n = 43) of which 13 provided no definition, navel dipping (n = 12), conformation scores of the navel (n = 2) navel measurements (n = 3), and umbilical cord drying times (n = 2). This review highlights the areas in need of further umbilical health research and emphasizes the importance of future studies to clearly define umbilical health outcomes of interest.

Key Words: navel, morbidity, omphalitis

184 Calf care practices and opportunities for improvement.

D. J. Wilson*[1], G. Habing[2], K. L. Proudfoot[3], S. M. Roche[4,1], and D. L. Renaud[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*The Ohio State University, Columbus, OH,* [3]*Atlantic Veterinary College, Charlottetown, PEI, Canada,* [4]*ACER Consulting, Guelph, ON, Canada.*

The objectives of this study were to describe neonatal calf care practices in Ontario, Canada and to evaluate ways to motivate improvements. A 45-min, online survey was made available through the Qualtrics platform to all Ontario dairy farmers (n = 3,367, identified through the provincial dairy association) from November 2020 to March 2021. Descriptive statistics of the preliminary data were generated using STATA 16. The survey had a 5% response rate (n = 172), with 78% of respondents being dairy farm owners. The median (IQR) herd size was 86 (57–130) and mean (SD) 305 d milk production was 10,675 (1,607) kg/cow. Veterinarians visited the farm most commonly every 2 weeks (50%) or monthly (30%). Calves received a mean (SD) of 3.6 (0.8) L of colostrum for their first feeding, which was frequently delivered using a bottle (68%) or esophageal tube feeder (23%). Colostrum was sourced mostly from the calf's dam (85%), and 96% of farmers reported targeting colostrum delivery at 1 to 6 h after birth. In the first week of life, calves received a mean (range) of 6.3 (2 to ad-libitum) L/d of milk, as milk replacer (38%), from the bulk tank (37%), or as waste milk (13%). When considering adopting a new calf care practice, improved health was often cited as "very important" (66%), with practices that mimic what would occur in nature less commonly cited (8%). Furthermore, 37% of farmers considered it "very important" for a new practice to be economical. When determining which calf care practices to use, the herd veterinarian's opinion was often cited as "very important" (41%), compared with the public or other dairy farmers (both at 7%). Of the farms using non-family labor for calf work (50%), 49% considered training these workers to be "somewhat" to "very" challenging. Our results suggest that on Ontario dairy farms, areas for improvement in calf care include the amount of milk offered to calves, and a need for assistance in training on-farm employees. Given dairy farmers' interest in calf health and their high valuation of herd veterinarians, veterinarians have the potential to be highly influential in motivating the implementation of best practices for neonatal calf care.

Key Words: calves, survey, motivation

185 Perspectives on male dairy calf care and opportunities to motivate change.

D. J. Wilson*[1], G. Habing[2], K. L. Proudfoot[3], S. M. Roche[4,1], and D. L. Renaud[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*The Ohio State University, Columbus, OH,* [3]*Atlantic Veterinary College, Charlottetown, PEI, Canada,* [4]*ACER Consulting, Guelph, ON, Canada.*

Our objectives were to compare male and female calf care practices, determine how dairy farmers perceive male calves, and investigate ways to motivate improvements. A 45-min, online survey was made available through the Qualtrics platform to all Ontario dairy farmers (n = 3,367, identified through the provincial dairy association) from November 2020 to March 2021. Statistics of the preliminary data were generated using STATA 16. The survey had a 5% response rate (n = 172) and was primarily filled out by farm owners (78%). Colostrum and milk feeding practices were similar between male and female calves, though male calves received less milk (6.4 vs 7.7 L) in the second week of life compared with females ($P = 0.001$). Male calves were mostly sold before 14 d (82%), and 11% were kept and raised for veal or beef. Farmers reported 46% of their calves were taken directly to a calf-raiser while 34% sold through an assembly station or auction. Most farmers (93%) agreed with the statement, "as a farmer, it is my job to provide optimal care to male dairy calves" and a range of agreement (48% agree, 30% neutral, 22% disagree) was reported for the statement, "male calves are a valuable part of the dairy industry." A low but notable proportion of farmers (15%) agreed that euthanizing male calves is a reasonable alternative when their selling price is very low. Farmers largely disagreed (73%) with the statement, "I cannot provide the level of care I wish to provide to male calves due to the financial cost." However, farmers frequently agreed that a price premium for more vigorous calves would be motivating (40% agreed), compared with a consistent baseline price (32% agreed) or a price discount for calves in poor condition (26% agreed). Approximately half (47%) of farms had received feedback on calf performance from their calf buyers, and 41% of those farmers had changed a management practice based on this. These results suggest most dairy farmers value male calves, but calves still experience challenges with milk feeding and marketing practices. Feedback from calf buyers along with price premiums for good quality male calves may motivate dairy farmers to improve their care.

Key Words: calves, survey, motivation

186 Effect of long-distance transportation on health of surplus dairy calves.

H. Goetz*[1], D. Renaud[1], C. Winder[1], J. Costa[2], D. Kelton[1], and K. Creutzinger[1], [1]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal and Food Sciences, University of Kentucky, Lexington, KY.*

Following arrival at raising facilities, calves experience high rates of disease; however, it is unclear if duration of transportation affects health. The objective of this study was to determine if transport duration affects calf health in the 2 wk after arrival at a veal facility. All surplus dairy calves born on 4 dairy farms in Ontario were eligible for enrollment (n = 50). Farms were visited daily before transport to enroll calves and perform health exams evaluating fecal and respiratory scores. On the day of transport, calves from 2 to 22 d of age were randomly assigned to 6, 12, or 16 h of transportation in a gooseneck trailer. Body weight was recorded upon arrival at the facility and daily health exams were performed for 14 d thereafter. Generalized linear regression models were built to assess the impact of transport duration on the number of days with abnormal fecal and respiratory scores. Calf age and weight at transport were not statistically different between groups. The proportion of days with an abnormal fecal score tended to be greater for calves transported for 16 compared with 6 h (Relative Proportion Ratio (RPR) = 1.63, 95% CI = 0.96 – 2.76, $P = 0.07$) but not 12 compared with 6 h (RPR = 1.46, 95% CI = 0.73 – 2.94, $P = 0.29$). As age increased, the proportion of days with an abnormal fecal score decreased (RPR = 0.87, 95% CI = 0.80 – 0.94, $P = 0.001$). The proportion of days with an abnormal respiratory score did not vary between 12 (RPR = 0.23, 95% CI = −0.72 – 1.19, $P = 0.63$) or 16 h of transportation (RPR = 0.64, 95% CI = −0.22 – 1.50, $P = 0.15$) compared with 6 h. Compared with calves less than 41 kg at arrival, calves weighing 41 to 47 kg were less likely to have abnormal respiratory scores (RPR = −1.11, 95% CI = −2.15 - −0.07, $P = 0.04$), but there was no difference for calves 47 to 52 kg (RPR = −0.06, 95% CI = −0.88 – 0.75, $P = 0.88$) or greater than 52 kg (RPR = −0.04, 95% CI = −1.04 – 0.96, $P = 0.94$). These preliminary results provide evidence that health after arrival at raising facilities are affected by calf age and weight and may be negatively affected by longer transportation.

Key Words: bull calf, morbidity, diarrhea

187 Predicting high somatic cell count cows at dry-off using behaviors measured with an ear-tag 3-axis accelerometer.

G. Vidal*[1], J. M. V. Pereira[2,5], C. F. de Assis Lage[2], D. R. Bruno[3], C. B. Tucker[4], N. Silva-del-Rio[2], B. Martínez-López[1], and F. C. Ferreira[2], [1]*Center for Animal Disease Modeling and Surveillance (CADMS), Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California-Davis, Davis, CA,* [2]*Veterinary Medicine Teaching and Research Center (VMTRC), Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Tulare, CA,* [3]*Cooperative Extension Fresno County, University of California, Fresno, CA,* [4]*Center for Animal Welfare, Department of Animal Science, University of California-Davis, Davis, CA,* [5]*Universidade Federal de Viçosa, Viçosa, Brazil.*

SCC is a good indicator of intramammary health status and can be used to select cows for selective dry-cow therapy. Our objective was to assess the classification performance of a machine learning algorithm on behavioral patterns with SCC class at dry-off (low ≤200,000 cells/mL < high). We enrolled 212 cows at dry-off (Jun-Nov 2020) from a 3,000-cow dairy in California. For the selected animals, hourly sensor (min/h) data measured by an ear-tag accelerometer for the 14 d before dry-off regarding rumination, eating, not active, active, and high activity behaviors were obtained from CowManager (Netherlands). At dry-off, individual, composite milk sample was taken for SCC determination at a DHIA laboratory. Hourly sensor data for each behavior were aggregated using the mean, sum, and variance at 2-time windows (12h and 6h), and 3 different time lags (2–7 d, 8–14 d, and 2–14 d before dry-off). For each behavior, random forest (RF) was used with aggregated sensor data. High versus low SCC class probabilities were ranked from high to low, and performance was assessed at different classification thresholds (20%, 30%, and 40%). After using 5-fold cross-validation, performance was evaluated in terms of sensitivity (Se), positive predictive value (PPV), and F_1 score for a combination of time window, time lag, and statistic used to aggregate each behavior data. The prevalence of high SCC at dry-off was 34%. There were no differences in RF performance across behaviors. For both time windows, mean F_1 score was the highest at the 30% threshold (mean 93% ± 0.6). Furthermore, PPV was 100%, and Se was 87% ± 1.0. However, at the 40% threshold, Se was 100%, and PPV was 86% ± 0.7. No significant differences were found between the 6 and 12 h time windows, but the later should be preferred due to model parsimony. Finally, at the 40% threshold, the highest PPV was yielded when the sum was used to aggregate the hourly sensor data for the 2–7 d time lag. Our results suggest that behavioral data collected via sensor can be used to classify cow with high SCC at dry-off, and the threshold should be chosen based on high SCC prevalence at dry-off at the farm level to maximize Se and PPV.

Key Words: machine learning, behavior, SCC

188 The association of prepartum urine pH and periparturient activity and rumination time on postpartum subclinical hypocalcemia dynamics in Holstein cows.

C. Seely* and J. McArt, *Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY.*

Dynamics of subclinical hypocalcemia (SCH) in early-lactation dairy cows have been associated with negative health outcomes and decreased production. Our objective was to investigate the association of SCH dynamics with prepartum urine pH, periparturient activity and rumination time, and serum total Ca (tCa). A prospective cohort of 89 multiparous Holstein cows from 2 herds in NY were classified into 1 of 4 SCH groups based on mean serum tCa at 1 and 4 DIM: normocalcemic (NC; [tCa] >1.89 mmol/L at 1 DIM and >2.25 mmol/L at 4 DIM, n = 30); transient SCH (tSCH; [tCa] ≤1.89 mmol/L at 1 DIM and >2.25 mmol/L at 4 DIM, n = 12); delayed SCH (dSCH; [tCa] >1.89 mmol/L at 1 DIM and ≤2.25 mmol/L at 4 DIM, n = 23); and persistent SCH (pSCH; [tCa] ≤1.89 mmol at 1 DIM and ≤2.25 mmol/L at 4 DIM, n = 24). Explanatory repeated-measures ANOVA models were used to analyze differences between SCH groups and changes over time for prepartum urine pH, activity and rumination time in the 10 d pre- and post-calving, and serum tCa for the first 10 DIM. Serum tCa was different by SCH group ($P < 0.001$) and was greatest in the NC cows. Prepartum urine pH was lower in the dSCH cows compared with the NC, tSCH, and pSCH cows ($P = 0.5$; 6.15 ± 0.36 and 6.44 ± 0.31, 6.52 ± 0.48, and 6.47 ± 0.32, respectively). Prepartum rumination time was lowest in the pSCH cows compared with the NC, tSCH, and dSCH cows ($P = 0.05$; 481.9 ± 19.8 min/d and 513.6 ± 17.8 min/d, 511.0 ± 27.5 min/d, and 520.3 ± 22.2 min/d, respectively). An interaction of DIM and SCH group was apparent for postpartum rumination time ($P < 0.001$) and was lower in the pSCH cows compared with the NC, tSCH, and dSCH ($P = 0.2$; 467.1 ± 24.1 min/d and 499.8 ± 21.6 min/d, 496.4 ± 33.5 min/d, and 485.8 ± 28.3 min/d, respectively). Postpartum activity time was greatest in the NC compared with the tSCH, dSCH, and pSCH cows ($P = 0.04$; 467.4 ± 22.3 min/d and 429.9 ± 34.6 min/d, 428.6 ± 27.9 min/d, and 421.4 ± 25.0 min/d, respectively). Our results suggest that periparturient activity and rumination time are associated with SCH dynamics.

Key Words: hypocalcemia, rumination, activity

189 Does rumination behavior in the first week postpartum impact the association between β-hydroxybutyrate and early-lactation milk yield?

C. Florentino*[1], Z. Rodriguez[1], E. Shepley[1], F. P. Mosca[1], G. Cramer[1], C. Rial[2], A. Laplacette[2], K. Borchers[3], D. Asper[3], M. Borchers[3], J. Giordano[2], and L. Caixeta[1], [1]*University of Minnesota, Saint Paul, MN,* [2]*Cornell University, Ithaca, NY,* [3]*Zoetis Inc., Kalamazoo, MI.*

Increased blood β-hydroxybutyrate (BHB), also known as hyperketonemia (HYK), is a common metabolic disorder in dairy cows associated with impaired health and decreased performance. Nonetheless, HYK in early lactation is not detrimental to all cows. Our objective was to evaluate whether rumination behavior influenced the association between HYK during the first week postpartum and milk yield during the first

90 DIM. In this observational trial, blood BHB was measured in dairy cows (n = 382) from a single farm in Minnesota once between 1 and 7 DIM. Rumination time (RT), used to quantify rumination behavior, was recorded daily using an automated monitoring system (Smartbow, Zoetis Inc.). Cows with blood BHB ≥ 1.2 mmol/L were deemed HYK positive (HYK+), otherwise HYK negative (HYK-). RT was categorized based on the distribution mean: cows with RT ≥ 22.5 min/h were considered high-RT, otherwise low-RT. The association between HYK and milk yield in the first 90 DIM across RT groups was analyzed using a multivariable mixed linear regression model, and included HYK group, RT group, the interaction between HYK and RT group, and parity. Cow was included as random effect. The prevalence of HYK was 18.6%. There was not enough statistical evidence (*P* = 0.38) to support the notion that the association between HYK and milk yield in early lactation differ according to rumination behavior in the first week postpartum. However, within high-RT, cows with HYK+ produced 3.4 more kg than HYK-; and within low-RT, cows with HYK+ produced 1.8 more kg than HYK- (Table 1). Although we did not have statistical power to observe an interaction between HYK and RT in our interim results, our results indicate that this research question merits further investigation.

Table 1. Difference in average milk yield (M Y) in the first 3 milk test-days across rumination time (RT) and hyperketonemia (HYK) groups in the first week postpartum.

RT group	HYK group	Average MY (kg/d)	Difference in MY (kg/d) [1]	*P*-value
High RT	HYK+	42.7	3.4 (0.13, 6.7)	0.03
	HYK−	39.3	Referent	—
Low RT	HYK+	39.2	1.8 (−1.5, 5.0)	0.45
	HYK−	37.4	Referent	—

[1] Mean difference (95% CI) in milk yield by HYK group within RT groups.

Key Words: hyperketonemia, rumination behavior, milk yield

190 Integration of statistical inferences and machine learning algorithms for prediction of metritis cure in dairy cows.

E. B. Oliveira*[1,2], F. C. Ferreira[1,2], K. N. Galvao[3], V. Y. Jason[4], I. Tagkopoulos[4], N. Silva-Del-Rio[1,2], R. V. Pereira[1], V. S. Machado[5], and F. S. Lima[1], [1]*Department of Population Health and Reproduction, University of California-Davis, Davis, CA,* [2]*Veterinary Medicine Teaching and Research Center, Tulare, CA,* [3]*Department of Large Animal Clinical Sciences and D. H. Barron Reproductive and Perinatal Biology Research Program, Gainesville, FL,* [4]*Department of Computer Science, Genome Center, and AI Institute for Next Generation Food Systems (AIFS), University of California-Davis, Davis, CA,* [5]*Department of Veterinary Sciences, Texas Tech University, Lubbock, TX.*

This study's objectives were to identify cow-level and environmental factors associated with metritis cure and to predict metritis cure using traditional statistics and machine learning algorithms. The data used for analysis were from a previous study comparing the efficacy of ceftiofur and self-cure for metritis. Cows were randomly allocated to receive 6.6 mg/kg of ceftiofur crystalline-free acid (Excede, Zoetis) at diagnosis and 3 d later (CEF, n = 275); or to remain untreated (CON, n = 275). Days in milk at metritis diagnosis (DIM D0), treatment, season of the metritis, month of metritis diagnosis, number of lactation, parity, calving score, dystocia, retained fetal membranes, body condition score at d 5 DIM, vulvovaginal laceration score, rectal temperature at the metritis diagnosis, fever at diagnosis, milk production from the day before to metritis diagnosis (MD), and milk increase slope from d 1 up to 5, 7 and 9 DIM (MI) were offered to univariate logistic regression, and then variables with *P* < 0.10 were included in the multivariable logistic regression model. Variables used for the univariate logistic regression were offered to a recursive feature elimination (RFE) to find an optimal subset of features for a machine learning algorithms analysis. Cows without vulvovaginal laceration and those developing metritis after first week postpartum had, respectively, 1.91 and 2.09 higher odds (*P* < 0.01) of curing of metritis. Each degree Celsius above 39.4°C leads to a lower odd (*P* = 0.02) to be cured. Also, greater MD and MI were associated (*P* < 0.01) with greater odds of metritis cure. Machine learning analysis showed that in addition to variables associated with cure by traditional statistical models, calving-related disorders, season, and month of metritis event help to predict metritis cure with a substantial degree of accuracy (≥70%). The current study suggests that cows developing metritis after 7 DIM, without vulva laceration and decrease in milk production from the day before to metritis diagnosis, and with a rectal temperature ≤39.40°C could be left untreated and cure.

Key Words: metritis cure, dairy cow, machine learning

191 Pattern of rumination time and physical activity captured by an ear-attached sensor before, during, and after the clinical diagnosis of metabolic-digestive disorders in lactating dairy cows.

C. Rial*[1], A. L. Laplacette[1], M. M. Perez[1], C. C. Florentino[2], F. Pena-Mosca[2], L. Caixeta[2], and J. O. Giordano[1], [1]*Deparment of Animal Science, Cornell University, Ithaca, NY,* [2]*College of Veterinary Medicine, University of Minnesota, St. Paul, MN.*

The objective of this prospective observational study was to characterize the pattern of rumination time (RT), and physical activity (PA) as measured by an automated health monitoring system based on an ear-attached sensor (SmartBow, Zoetis) immediately before, during, and after clinical diagnosis (CD) of metabolic-digestive disorders (MET-DIG) including displaced abomasum (DA), clinical ketosis (KET), and indigestion (IND). Rumination time and physical activity represented by active time (AT) data were collected from 481 lactating Holstein cows. Clinical health events were monitored daily until 21 DIM. Data were daily summed from −7 to 7 d relative to CD for cows diagnosed with at least one of the disorders of interest (MET-DIG; n = 74), DA (n = 18), KET (n = 28), IND (n = 18), and cows without clinical signs of disease (NCD; n = 417). Data for RT and AT were analyzed using ANOVA with repeated measurements. Fixed effects were group, time, and their interaction whereas parity and calving season were offered as covariates. Cow was included as a random effect and was the subject of repeated measurement analysis. Compared with NCD, cows in the MET-DIG group had reduced (*P* < 0.05) RT from −5 to 3 d after CD and reduced AT from −7 to 7 d after CD. On the day of CD, RT was 334 ± 14 min/d and AT was 582 ± 19 min/d for the MET-DIG group compared with RT of 534 ± 14 min/d and AT of 837 ± 8 min/d for the NCD group. For the comparison of cows in MET-DIG and NCD, parity and season of calving affected (*P* < 0.05) RT and AT. Compared with NCD, cows in the DA group had reduced (*P* < 0.05) RT and AT from −7 to 7 d after CD. Compared with NCD, cows in the KET group had reduced (*P* < 0.05) RT from −7 to 6 d and AT from −7 to 7 d after CD. Compared with NCD, cows in the IND group had reduced (*P* < 0.05) RT from −5 to −1 d after CD whereas AT differed (*P* = 0.05) for the 14-d period. We conclude that RT and PA monitoring data captured by an ear-attached sensor system might be used to identify cows with metabolic-digestive disorders including displaced abomasum, clinical ketosis, and indigestion.

Key Words: health, dairy cattle, rumination time and active time

192 Reticulo-ruminal temperature in the peripartum period was associated with the occurrence of health disorders after calving in dairy cows.

M. M. Perez*, C. Rial, and J. O. Giordano, *Cornell University, Ithaca, NY.*

Our objective was to compare reticulo-ruminal temperature (RRT) around calving for dairy cows with or without health disorders (HD) in the first 3 weeks after calving. Data for RRT was collected from Holstein cows (n = 731) using a bolus sensor (smaXtec Animal Care GmbH, Austria), and split for analysis in 3 periods; i.e., before (PreC), during (Cal), and after (PostC) calving. For PreC and PostC, RRT was averaged every 4 h from −60 to −5 h and from 5 to 60 h after calving, whereas for Cal RRT was averaged in 30 min periods from −4.5 to 4.5 h relative to calving. The occurrence of metritis, mastitis, ketosis, indigestion, displaced abomasum, and pneumonia was recorded daily. Cows were grouped based on the number of HD recorded: NoHD (n = 507), at least one HD in the first (HD1; n = 143), second (HD2; n = 50), or third (HD3; n = 31) week after calving. Data were analyzed using ANOVA with repeated measures. Fixed effects were HD group, time, parity (1, 2, 3+), and interactions. Calving season, number of calves born, calf sex, stillbirths, and calving ease were offered as confounders. Cow within group was a random effect. None of the confounders had an effect ($P > 0.10$) on RRT for PreC and Cal. Cows in HD1 had lesser ($P < 0.05$) RRT than NoHD for PreC (39.58 ± 0.02; 39.64 ± 0.01), tended ($P = 0.09$) to have lesser RRT for Cal (39.29 ± 0.02; 39.36 ± 0.01), and had greater ($P < 0.01$) RRT (39.43 ± 0.07; 39.32 ± 0.07) for PostC. Cows in HD2 and HD3 had similar RRT ($P > 0.1$) than cows in NoHD for all periods. For PostC, cows that calved during the warm season (39.52 ± 0.04) had greater ($P < 0.05$) RRT than cows that calved during the cold (39.47 ± 0.03) season and cows that had twins (39.57 ± 0.06) had greater ($P < 0.05$) RRT than cows with singletons (39.42 ± 0.02). We conclude that cows with at least one HD within the first week after calving had differences in RRT within 60 h before and after calving and thus, RRT might be used to identify cows with health disorders around calving. Also, season and twin births might affect the pattern of RRT after calving. Supported by USDA-NIFA 2017–67015–26772.

Key Words: automation, health, temperature

Breeding and Genetics II

193 Assessment of novel estrus-related traits derived from activity measurements in Holsteins. D. F. Cardoso*[1], C. A. Rosenberg[1,2], F. Malchiodi[2], and C. F. Baes[1,3], [1]*Center for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*The Semex Alliance, Guelph, ON, Canada,* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Automated activity monitors (AAM) have gained credibility as a technology for the accurate and timely identification of primary signs of estrus. Beyond being a promising management tool, AAM can also generate relevant estrus-related scores, such as measures of frequency, intensity, and duration, which may serve as indicator traits for reproductive efficiency. Here, we developed and estimated (co)variances of 5 AAM-based estrus traits in Holstein cows. Also, we assessed the genetic correlations with classical reproductive traits, such as non-return rate (NRR56, 0 for failure and 1 for success), number of services (NS), and days open (DO, interval from calving to conception). The studied population comprised 6,044 cows from 3 US herds with activity data monitored between Feb. 2018 and Nov. 2020. Scores of activity change (AC) were recorded every 2h, and values greater than 25 were treated as estrus. The novel traits consisted of days interval from calving to first estrus (ICFE) and from first to second estrus (IFSE), mean interval between estrus (MIE), estrus duration (ED), and estrus strength (ES). (Co)variances components were estimated using mixed linear animal models in the software Wombat, considering the fixed effect of parity (1 to 4), contemporary group (herd-year-season) at estrus (ED, ES), first estrus (MIE, IFSE) or previous calving (IFSE), and age at estrus as a covariable (linear and quadratic). Heritability estimates for the novel traits ranged from 0.04 ± 0.01 (ICFE) to 0.09 ± 0.02 (MIE). High positive correlations (0.81 ± 0.04) were observed between ED and ES, and both of them were negatively correlated with MIE and IFSE (ranging from −0.41 ± 0.15 to −0.78 ± 0.09). The interval from calving to first estrus was not significantly correlated with any of the other novel traits, however, it had favorable correlations with classical traits, including NRR56 (−0.52 ± 0.41) and DO (0.48 ± 0.28). Other favorable correlations between the novel and classic traits included a negative correlation between ES and DO (−0.45 ± 0.19). Despite the low heritabilities of the studied novel traits, our preliminary results suggest their suitability as indicators of reproductive performance.

Key Words: automated activity monitors, estrus, variance component

194 Novel fertility trait: Genetic parameters of reproductive tract size and position scoring. A. Martin*[1], G. de Oliveira[1], A. Madureira[2], R. Cerri[2], C. Baes[1,3], and F. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Applied Animal Biology, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada,* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Over the past decades, reproductive problems in Holstein cattle have brought the dairy industry to increase the effort to select for more fertile cows by investigating novel traits closer to animal biology. The size and position score (SPS) was recently developed in physiological studies as an indicator of pregnancy rate and number of services to conception. Cows are scored as 1, 2 or 3 based on the size of the reproductive tract and its position in the pelvis determined by transrectal palpation. A score of 1 (i.e., a small and compact uterus) is associated with higher fertility compared with other scores throughout all parities. However, the genetic background of this score trait has not yet been investigated. The objective of this study was to estimate genetic parameters of SPS to assess its potential as a novel fertility trait. Phenotypes were collected at the University of British Columbia research herd from 2017 to 2020 and consisted of 3,247 score records collected within and across lactations on 490 Holstein cows. The following univariate model was used to estimate variance components: $\mathbf{y} = \mathbf{Xb} + \mathbf{Z_a a} + \mathbf{Z_{pew} pe_w} + \mathbf{Z_{pea} pe_a} + \mathbf{e}$, where $\mathbf{y}$ is a vector of SPS phenotypes; $\mathbf{b}$ is a vector of fixed effects including year-season, weeks in milk, parity number, and linear and quadratic regression on body condition score; $\mathbf{a}$ is a vector of random animal additive genetic effects; $\mathbf{pe_w}$ and $\mathbf{pe_a}$ are vectors of random within- and across-lactation permanent environmental effects, respectively; and e is a vector of random residual effects; $\mathbf{X}$, $\mathbf{Z_a}$, $\mathbf{Z_{pew}}$, and $\mathbf{Z_{pea}}$ are corresponding incidence matrices. Variance components were estimated using restricted maximum likelihood method (AIREMLF90). The heritability estimate (±SE) was 10.6% (±2.6) and the repeatability within- and across-lactation estimates were 19.9% (±3.6) and 18.9% (±2.1), respectively. Although preliminary, these results are promising for SPS as a fertility trait, since its heritability estimate was higher than that of number of services to conception and pregnancy rate in Holstein cattle. Results will be validated with a larger data set in future studies.

Key Words: dairy cattle, variance component estimation, reproductive tract score

195 Genes and pathways associated with pregnancy loss in dairy cattle. A. Sigdel*[1], R. S. Bisinotto[2], and F. Peñagaricano[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*University of Florida, Gainesville, FL.*

Pregnancy loss directly impairs reproductive performance in dairy cattle. Here, we investigated genetic factors associated with pregnancy loss following detection of a viable embryo around 42 d of gestation until term. The objectives of this study were to perform whole-genome scans and subsequent gene-set analyses for identifying candidate genes, functional gene sets and gene signaling pathways implicated in pregnancy loss in US Holstein cows. Data consisted of about 58k pregnancy/abortion records distributed over nulliparous, primiparous, and multiparous cows. Threshold models were used to assess the binary response of pregnancy loss. Whole-genome scans identified at least 7 genomic regions on BTA2, BTA10, BTA14, BTA16, BTA21, BTA24 and BTA29 associated with pregnancy loss in heifers and lactating cows. These regions harbor several candidate genes that are directly implicated in pregnancy maintenance, and fetal growth, such as *CHST14*, *IGF1R*, *IGF2*, *PSEN2*, *SLC2A5*, and *WNT4*. Moreover, the enrichment analysis revealed at least 7 relevant processes, namely calcium signaling, cell-cell attachment, cellular proliferation, fetal development, immunity, membrane permeability, and steroid metabolism as significantly enriched with genes associated with pregnancy loss. Additionally, our pathway analysis revealed several significant gene signaling pathways that regulate placental development and fetal growth, including Wnt, Hedgehog, Notch, MAPK, Hippo, mTOR and TGFβ pathways. Overall, our findings contribute to a better understanding of the genetic and biological basis of pregnancy loss in dairy cattle and points out novel strategies for improving pregnancy success via marker-assisted breeding.

Key Words: gene sets, threshold model, whole-genome scans

196 Genetic and genomic evaluation of late-term abortion recorded through Dairy Herd Improvement test plans.

M. Neupane*, J. L. Hutchison, J. B. Cole, C. P. Van Tassell, and P. M. VanRaden, *Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA, Beltsville, MD.*

Late-term abortions cause significant economic loss and are of great concern for dairy herds. Late-term abortions ≥152 d and <251 d of gestation that terminate a lactation or initiate a new lactation have long been recorded in Dairy Herd Improvement (DHI). For 22.7 million DHI lactations, the average recorded incidence of late-term abortions across all years was 1.2%. However, the 1.3% incidence of abortions reported in 2012 has declined to < 1.0% incidence since 2015. Small adjustments were applied among the 82 million daughter pregnancy rate (DPR), 29 million cow conception rate (CCR), and 9 million heifer conception rate (HCR) records to more accurately account for late-term abortions. Fertility credits for CCR and HCR were changed to treat the last breeding as a failure instead of success if the next calving is coded as a late-term abortion. Similarly, when computing DPR, days open is now set to the maximum value of 250 instead of the reported days open if the next reported calving is an abortion. The test of these changes showed very small changes in SD and high correlations (0.997) of adjusted predicted transmitting abilities (PTA) with official PTA from about 20,000 HO bulls born since 2000 with >50% reliability. For late-term fetal survival as a trait, estimated heritability was only 0.001 and PTA had a SD of only 0.1% for recent sires with high reliability (>75%). Young animal genomic PTA have near 50% reliability but range only from −0.5 to +.4 because of the low incidence and heritability. Genetic trend was slightly favorable and late-term fetal survival PTA were correlated favorably by 0.08 with net merit, 0.38 with productive life, 0.32 with livability, −0.23 with daughter stillbirth, −0.25 with daughter calving ease, 0.18 with CCR, 0.12 with DPR, and −0.15 with gestation length. Thus, PTA for late-term abortions should not be needed as a separate fertility trait and instead these minor edit changes should suffice. PTA for earlier abortions would add little value because national evaluations for current fertility traits already accounted for those economic losses.

Key Words: fertility traits, genetic and genomic evaluation, late-term abortions

197 Evaluation of bull fertility in Italian Brown Swiss dairy cattle using cow field data.

H. A. Pacheco*[1], M. Battagin[2], A. Rossoni[2], A. Cecchinato[3], and F. Peñagaricano[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Italian Brown Breeders Association, Bussolengo, Verona, Italy,* [3]*University of Padova, Legnaro, Padua, Italy.*

Dairy bull fertility is traditionally evaluated using semen production and quality traits; however, these attributes explain only part of the differences observed in fertility among bulls. Alternatively, bull fertility can be directly evaluated using cow field data. The main objective of this study was to investigate bull fertility in the Italian Brown Swiss dairy cattle population using confirmed pregnancy records. The data set included a total of 420,512 breeding records from 1,260 bulls and 136,403 lactating cows between first and fifth lactation from 2000 to 2019. We first evaluated cow pregnancy success, including factors related to the bull under evaluation, such as bull age, bull inbreeding and AI organization, and also factors associated with the cow that receives the dose of semen, including herd-year-season, cow age, parity, and milk yield. We then estimated sire conception rate, considered as a phenotypic assessment of male fertility, using only factors related to the bull. Model predictive ability was evaluated using 10-fold cross-validation with 10 replicates. Interestingly, our analyses revealed that there is a substantial variation in conception rate among Brown Swiss bulls, with more than 20% conception rate difference between high-fertility and low-fertility bulls. We also showed that the phenotypic prediction of bull fertility is feasible, our cross-validation analyses achieved predictive correlations around 0.31 for sire conception rate. Improving reproduction performance is one of the major challenges of the dairy industry worldwide, and for this, it is essential to have accurate predictions of service sire fertility. This study represents the foundation for the development of novel tools that will allow dairy producers, breeders, and AI companies make accurate management and selection decisions on Brown Swiss male fertility.

Key Words: pregnancy records, service sire fertility, sire conception rate

198 Relationship of β-casein A2 genetics, production, and fertility of organic Holstein dairy cows.

B. J. Heins*[1], C. D. Dechow[2], and L. C. Hardie[2], [1]*University of Minnesota, Morris, MN,* [2]*Pennsylvania State University, State College, PA.*

The objective of the study was to determine milk production, fertility, and survival for β-casein A2 genotypes of organic Holstein cows. Holstein cows (n = 1, 982) from 13 dairy herds across the Midwest and Northeast were genomic tested with Clarifide Plus for A2 status. Two-hundred fourteen cows were A1A1 (11%), 848 cows were A1A2 (43%) and 920 cows were A2A2 (46%). In total, 2,249 lactation records were used with 1,025 from first parity and 1,224 from second parity and greater. Daily milk, fat, and protein production and SCS from milk recording were calculated with random regression. A lower limit of 50 d for days open (DO) was applied, and cows with more than 250 d for DO had DO set to 250 d. Independent variables for statistical analysis with PROC MIXED included the fixed effects of herd, parity, milk β-casein genotype (A1A1, A1A2, A2A2), and the interaction of milk β casein genotype and parity. Cow and birth date were random effects in the statistical model. Test-day milk production was not different ($P > 0.50$) for A1A1 (26.4 kg/d), A1A2 (27.5 kg/d), and A2A2 (27.5 kg/d) cows. Days open was not different ($P > 0.40$) for A1A1 (124 d), A1A2 (136 d), and A2A2 (144 d) cows. Furthermore, the number of times bred was not different ($P > 0.44$) for A1A1 (1.77), A1A2 (1.62), and A2A2 (2.13) cows. Survival to second lactation was 83% for A1A1 cows, 97% for A1A2 cows, and 95% for A2A2 cows. Results indicate no difference in production and fertility with regard to A1 or A2 genotype in organic dairy herds. Survival may be biased against the A1 genotype which is indicated by lower survival rates in first lactation.

Key Words: genetic selection, organic, A2 milk

199 Inheritance of a mutation causing neuropathy with splayed forelimbs in Jersey cattle.

A. Al-Khudhair*[1], D.J. Null[1], J. Cole[1], C. W. Wolfe[2], and P. M. VanRaden[1], [1]*USDA, Agricultural Research Service, Animal Genomics and Improvement Laboratory, Beltsville, MD,* [2]*American Jersey Cattle Association, Reynoldsburg, OH.*

A new undesirable genetic factor, known as "neuropathy with splayed forelimbs" (JNS), has been identified recently in the Jersey breed. Calves affected with JNS are unable to stand on splayed forelimbs that exhibit significant extensor rigidity and/or excessive lateral abduction at birth. Affected calves are generally alert at birth but exhibit neurologic symptoms including spasticity of head and neck and convulsive behavior. Other symptoms reported include dislocated shoulders, congenital craniofacial anomalies, and degenerative myelopathy. Inheritance of the undesirable genetic factor was determined from a study of 16 affected calves reported by Jersey breeders across the country. Their pedigrees all traced on both paternal and maternal sides to a common ancestor born in 1995. Genotypes revealed that JNS is attributable to a specific haplotype on *Bos taurus* autosome (BTA) 6, and about 6% of the genotyped Jersey population are now carriers of the haplotype. The region of shared homozygosity was further examined by sequencing, revealing missense variant rs1116058914 at base 60,158,901 of the ARS-UCD1.2 reference map as the most concordant with the genetic condition and most likely cause. The single base substitution (G/A) is in the coding region of the last exon of the ubiquitin C-terminal hydrolase L1 (*UCHL1*) gene that is conserved across species. Mutations in humans and gene knockouts in mice cause similar recessive symptoms and muscular degeneration. Since December 2020, carrier status is tracked with a haplotype and reported for all 303,087 genotyped Jersey animals. With random mating, about 300 affected calves per year would result from the 370,000 US Jersey cows in DHI. Selection and mating programs can reduce the number affected using either the haplotype status or a direct gene test in the future. Breeders should report calf abnormalities to their breed association to help discover new defects such as JNS.

Key Words: genetic defect, lethal recessive, carrier

200 Single-step genomic predictions for yield traits in US Holsteins with unknown parent groups and phenotype-pedigree truncation.

D. Lourenco*[1], A. Cesarani[1], Y. Masuda[1], S. Tsuruta[1], E. Nicolazzi[2], P. M. VanRaden[3], and I. Misztal[1], [1]*University of Georgia, Athens, GA,* [2]*Council on Dairy Cattle Breeding, Bowie, MD,* [3]*AGIL-USDA, Beltsville, MD.*

In this study we assessed the reliability and inflation of GEBV from ssGBLUP with unknown parent groups (UPG) only for the pedigree relationship matrix (**A**) and for both **A** and the pedigree relationship matrix among genotyped animals ($\mathbf{A}_{22}$). The first scenario was termed UPG1 and the second was UPG2. Six large phenotype-pedigree truncated Holstein data sets were used. The complete data included 80M records for milk, fat, and protein yield from 31M cows born from 1980 to 2017. Truncation scenarios included pruning of phenotypes for cows born before 1990 and 2000 combined with truncation of pedigree information after 2 or 3 ancestral generations. A total of 861,525 genotyped bulls with progeny and cows with phenotypes were used in the analyses. Reliability and inflation/deflation of GEBV were obtained for 2,710 bulls based on deregressed proofs (DRP), and on 381,779 cows born after 2014 based on adjusted phenotypes (predictivity). Reliabilities ranged from 0.54 to 0.69 for UPG1 and from 0.69 to 0.73 for UPG2. The regression coefficient of bull DRP on GEBV ranged from 0.77 to 0.94 for UPG1 and was 1.00 for UPG2. Cow predictivity ranged from 0.48 to 0.51 for UPG1, and 0.51 to 0.54 for UPG2. The regression coefficient of cow adjusted phenotypes on GEBV was 1.02 for UPG2 with the most extreme truncation. Overall, reliability and predictivity from ssGBLUP with UPG2 were not affected by phenotype-pedigree truncation. Computations with the complete data set took 58h with UPG1 and 23 h with UPG2 because the number of rounds to converge was twice as large in UPG1. Similar computations with truncation before 2000 took 36 h and 15 h. Old phenotypes (before 2000) did not impact the reliability of predictions for young selection candidates, especially in UPG2. Here we used a selected set of 861k genotyped animals, but tests with 3.4M genotypes confirmed the computational feasibility of ssGBLUP without loss in reliability. In ssGBLUP evaluations with missing pedigree, unknown parent groups assigned to both **A** and $\mathbf{A}_{22}$ provided accurate and unbiased evaluations regardless of phenotype-pedigree truncation scenario.

Key Words: large-scale genomic evaluation, unknown parent groups

201 Are indirect genomic predictions a good option as the number of genotypes continues to rise?

S. Tsuruta*[1], D. A. L. Lourenco[1], Y. Masuda[1], I. Misztal[1], and T. J. Lawlor[2], [1]*University of Georgia, Athens, GA,* [2]*Holstein Association USA Inc., Brattleboro, VT.*

As the number of genotyped animals continues to grow every year, the computational cost increases. One way to reduce the cost is to remove the older genotyped animals that have been culled and had no progeny nor phenotype. Another option could be indirect genomic predictions (IGP) for genotyped animals that have no progeny nor phenotypes. Assuming that these genotyped animals have no significant impact on the other genotyped animals, it is more practical to predict their genomic performance indirectly. The objective of this study is to conduct IGP for various genotyped animal groups for 18 linear type traits in US Holsteins using 2.3M genotyped animals and to investigate if the IGP are accurate and unbiased. Phenotypic records for 18 linear type traits used in December 2018 genetic evaluation in US Holsteins were provided by Holstein Association USA, and genotypes in December 2018 were provided by the Council on Dairy Cattle Breeding. The full data set consisted of 10.9M records up to 2018 calving, 13.6M animals in the pedigree, and 2.3M genotyped animals with 79K SNP. Genomic prediction was conducted with single-step genomic BLUP, applying the 18 multi-trait animal model to calculate direct genomic predictions (GEBV) for all genotyped animals, and then IGP were calculated for genotyped animals with no progeny nor phenotype by year from 2014 to 2018. To reduce computing costs, IGP were calculated by GEBV from randomly selected genotyped animals from 15K to 60K. R^2 in GEBV = $b_0 + b_1$*IGP ranged from 0.96 to 0.98 for males and from 0.95 to 0.96 for females for 18 traits. The high correlation (0.95) between b_0 and annual genetic gains for 18 traits indicates the bias in IGP due to directional selection, which can be adjustable. For practical genomic evaluation, 25K to 35K randomly selected genotyped animals from GEBV can be used to obtain accurate and unbiased IGP. The result in this study can be a practical solution when conducting a large-scale genomic evaluation and can make more frequent evaluation with lower cost when genotyped animals have no phenotypes nor progeny. Further coordination will be needed to determine how genotyped animals should be selected for IGP.

Key Words: ssGBLUP, indirect genomic predictions, US Holsteins

202 Automatic scaling in single-step genomic BLUP.

M. Bermann*, D. Lourenco, and I. Misztal, *The University of Georgia, Athens, GA.*

Single-step genomic BLUP (ssGBLUP) requires compatibility between genomic and pedigree relationships for unbiased and accurate predictions. Scaling the genomic relationship matrix (**G**) to have the same averages as the pedigree relationship matrix (i.e., scaling by averages) is one way to ensure compatibility. This requires computing both relationship matrices, calculating averages, and changing **G**, whereas only the inverses of those matrices are needed in the mixed model equations. Therefore, the compatibility process can add extra computing burden. In the single-step Bayesian Regression (SSBR), the scaling is done by including an average of the breeding values of the genotyped animals (μ_g) as a fixed effect in the model. In this study, such scaling called “automatic” was implemented in ssGBLUP via QP transformation of the inverse of the relationship matrix used in ssGBLUP. Comparisons involved a simulated data set, and the genomic relationship matrix was computed using different allele frequencies either from the current population (i.e., realized allele frequencies), equal among all the loci, or from the base population. For all the scenarios, we computed bias, accuracy, and dispersion. With no scaling, the bias expressed in terms of genetic standard deviations was 0.86, 0.64, and 0.58 with realized, equal, and base population allele frequencies, respectively. With scaling by averages, which is currently used in ssGBLUP, bias was 0.07, 0.08, and 0.03, respectively. With automatic scaling, bias was 0.18 regardless of allele frequencies. Accuracies were similar among scaling methods, but about 0.10 lower in the scenario without scaling. The GEBV were more inflated without any scaling, whereas the automatic scaling performed similarly to the scaling by averages. When μ_g was treated as random, with the variance equal to the mean difference between pedigree and genomic relationship matrices, the bias was the same as with the scaling by averages. The automatic scaling is biased, especially when μ_g is treated as a fixed effect and populations underwent strong selection.

Key Words: compatibility between genomic matrices, genomic selection, scaling

203 Partitioning SNP heritability in related individuals.

J. Jiang*[1], P. M. VanRaden[2], L. Ma[3], and J. R. O'Connell[4], [1]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [2]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD,* [3]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD,* [4]*Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.*

Partitioning SNP heritability by many functional annotations has been a successful tool for understanding the genetic architecture of complex traits in human genetic studies. Similar analyses are being extended to animal research, as (imputed) whole-genome sequence data of many individuals and various functional annotations have become available in livestock animals. Though many approaches have been developed for heritability partition (e.g., linkage disequilibrium score regression [LDSC] and Haseman-Elston regression [HE-reg]), they are mostly based on approximations tailored to human populations and few can produce statistically efficient estimates for animal genomic studies where individuals are often related. To tackle this issue, we present a stochastic MINQUE (Minimum Norm Quadratic Unbiased Estimation) approach for partitioning SNP heritability, which we refer to as MPH. We provide a theoretical analysis comparing LDSC and HE-reg with REML and MPH and demonstrate what LDSC and HE-reg (and similar methods) take advantage of in their approximations: sparse relationships between individuals and relatively weak linkage disequilibrium (LD). We also show that our method is mathematically equivalent to the Monte Carlo REML approach implemented in BOLT. MPH has 3 key features. First, it is comparable to genomic REML in terms of accuracy, while being at least one order of magnitude faster than GCTA and BOLT and using only ~1/4 of memory as much as GCTA, when applied to sequence data and many variance components (or functional annotation categories). Second, it can do weighted analyses if residual variances are unequal (such as DYD). Third, it works for many overlapping functional annotations. Using simulations based on a human pedigree and a dairy cattle pedigree, we illustrate the benefits of our method for partitioning SNP heritability in pedigree-based studies. We also demonstrate that it is feasible to efficiently partition SNP heritability for animal genomes with strong, long-span LD. MPH is freely available at https://jiang18.github.io/mph.

Key Words: SNP heritability, partition, functional annotation

204 Scalable mixed model approach for mapping omnigenic core genes.

J. Jiang*[1], P. M. VanRaden[2], L. Ma[3], and J. R. O'Connell[4], [1]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [2]*Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD,* [3]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD,* [4]*Department of Medicine, University of Maryland School of Medicine, Baltimore, MD.*

To make use of exploding genomic data, we present a scalable mixed model approach for genome-wide association studies (GWAS) that can handle millions of genotyped animals, which we refer to as SSGP. Using simulations, we show that our method is as accurate as EMMAX and is a few times faster than BOLT. SSGP can address the genomic inflation issue in large-scale GWAS in domestic animals. Substantial genomic inflation will arise in GWAS in the presence of polygenic inheritance, even when population structure and relatedness have been accounted for. This can be demonstrated by the non-centrality parameter (NCP) for a SNP that is in linkage disequilibrium (LD) with causal variants, $NCP_i \approx N\Sigma_j r_{ij}^2 q_j^2$, where NCP_i is the NCP for SNP i, r_{ij} is the correlation coefficient between SNP i and causal variant j, q^2 is the proportion of phenotypic variance explained by a causal variant, and N is the sample size. If polygenic effects are not well accounted for, the NCP for many tested SNPs may be big in large-scale GWAS, especially those in domestic animals that generally have small effective population sizes and strong, long-span LD on the genome. As a result, we may see significant loci everywhere on the genome, even though any causal variant alone has an undetectable effect. We illustrate this phenomenon by leave-one-chromosome-out (LOCO) GWAS with big cow data and simulations, given the fact that LOCO GWAS does not account for effects of causal variants on the same chromosome as a tested SNP. We also illustrate that only a few loci of significance can be found when whole-genome polygenic effects have been accounted for by SSGP. This finding is in line with the omnigenic core versus peripheral gene model that was recently proposed: the few SSGP significant loci correspond to core genes and those LOCO significant loci everywhere on the genome result from peripheral genes that each have a tiny effect. In summary, our method is useful for finding omnigenic core genes that matter in functional studies and targeted genome editing. SSGP is freely available at https://github.com/jiang18/ssgp.

Key Words: mixed model association, biobank-scale, omnigenic

205 Accounting for X chromosome and allele frequencies in genomic inbreeding estimation.

J. P. Nani* and P. M. VanRaden, *ARS-USDA, Beltsville, MD.*

Breeders for many decades used pedigrees to limit increases in inbreeding (pedF), but genomic measures of relationship and inbreeding can provide more precise control. Previous calculations ignored influence of the X chromosome (BTX) when estimating relationships. For mating programs, excluding BTX can cause an increase in inbreeding by mating 2 individuals with the same BTX. Numbers of SNP markers on BTX have increased recently in US national evaluations. The X-specific region has 3.0% of the 79,060 SNPs used and those are coded as 100% homozygous in males, causing homozygosity of females to appear 3% less than males. Allele frequency also has an impact on computing genomic inbreeding (genF). Correlations were high between genF computed using 0.5 ($genF_1$) or base population ($genF_2$) allele frequencies for most but not all breeds. Also, average genF was higher for males than females. The $genF_1$ was further adjusted for BTX ($genF_X$) to obtain better correlations across breeds and sex and to make $genF_X$ more similar to pedF. Haplotype-based inbreeding (hapF) was also estimated for comparison. Future inbreeding was estimated as half an animal's relationship to a recent reference population using pedigree (pEFI), 0.5 ($genFI_1$) or base frequencies ($genFI_2$) or adjusted for BTX markers ($genFI_X$). Definitions were compared for 3,280,753 genotyped animals of 5 breeds using a pedigree file of 86,924,013 animals. Smaller breeds were more sensitive to the use of different allele frequencies. Correlations with pedF were generally higher using genF_1 or genF_X compared with genF_2 (average correlations across breeds of 0.67, 0.67 and 0.54 respectively). Correlations with EFI were similar using genFI_1, genFI_2 or genFI_X (average correlations across breeds of 0.83, 0.84 and 0.83, respectively). Correlations of hapF with pedF were not higher than of genF_1 or genF_x with pedF (0.64). Use of genF_X and genFI_X did not affect the correlations within sexes but did improve the mean differences occurring between sexes. The adjustments allow simpler and more accurate comparisons of genomic and pedigree relationships.

Key Words: inbreeding, genomics, relationships

206 Identifying family clusters within the US Holstein population to manage genetic diversity.

Y. Steyn*[1], T. Lawlor[2], Y. Masuda[1], D. A. L. Lourenco[1], S. Tsuruta[1], and I. Misztal[1], [1]*University of Georgia, Athens, GA,* [2]*US Holstein Association, Brattleboro, VT.*

Reproductive technology has allowed a few bulls or families to have major genetic contributions on a population. Differences in frequency of alleles that are identical by descent between predominant families may be an indication of multiple paths, i.e., genetic redundancy, leading to a similar polygenic response. The objective of this study was to identify family clusters and their key founders within the Holstein population. Sires with the greatest number of progeny born after 1985 were selected. Final data included 1,145 genotyped sires from 7 countries with birth year ranging from 1962 to 2009. Number of progeny per sire varied from 312 to 49,146. A principal component analysis using the genomic relationship matrix identified founders for 7 potential families. Clustering analysis using k-means was used to separate animals into 5 clusters (C1 to C5) containing 171 (C1), 252 (C2), 200 (C3), 244 (C4), and 278 (C5) animals, respectively. Five clusters allowed for the major progenitors of the Holstein breed to be identified with high genetic relationships within cluster. The 2 most predominant families were C1 and C2. Two families were grouped together in C3, several families primarily used in Canada were in C4, and multiple families used in the US were in C5. While C3 and C4 contained almost no bulls born before 1985, older bulls occurred more in C1 and C5. Indirect genomic predictions (IGP) were obtained for 5 type traits. The family clusters were ranked based on these traits. Rankings were similar for all traits. Differences in trait means were largely due to groups peaking in popularity at different times. Increasing the number of clusters to 10 allowed the predominant sons within a family to be identified. Results from this study suggest that k-means clustering can be used to identify the most influential family groups. Further work will apply this clustering method to the current group of genomic tested selection candidates with the aim of having low within-cluster and high between-cluster variance. This can lead to greater across-family selection and a reduction in loss of genetic diversity while still improving the breed performance.

Key Words: genetic redundancy, optimal selection

Dairy Foods: Cheese II

207 Chemical and fatty acid composition of Manchego type and Panela cheeses manufactured from either hair sheep milk or cow milk. A. J. Chay-Canul[1], A. A. Ochoa-Flores[1], J. A. Hernández-Becerra[2], J. R. Velázquez-Martínez[1], J. M. Piña-Gutiérrez[3], L. E. Hernández-Castellano[4], P. Toro-Mujica[5], and E. Vargas-Bello-Pérez*[6], [1]*División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Tabasco, México,* [2]*División de Tecnología de Alimentos, Universidad Tecnológica de Tabasco, Villahermosa, Tabasco, México,* [3]*Rancho "El Rodeo." Carretera Villahermosa-Jalapa, Tabasco, México,* [4]*Department of Animal Science, AU-Foulum, Aarhus University, Tjele, Denmark,* [5]*Instituto de Ciencias Agroalimentarias, Animales y Ambientales (ICA3), Universidad de O'Higgins, San Fernando, Chile,* [6]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark.*

In tropical regions of Mexico, hair sheep are typically bred for meat production and milk is not relevant for farmers but could be used for cheese manufacturing. In addition, hair sheep adapt better to tropical conditions than cows. Therefore, this study aimed to compare the chemical composition and fatty acid (FA) profile of Manchego type cheese and Panela cheese made from hair sheep milk and compared with both types of cheese manufactured with cow milk as a reference. In addition, this study aimed to determine differences in sensory characteristics between Manchego type cheeses manufactured with either hair sheep milk or cow milk. Twenty-five hair sheep and 14 cow Manchego type cheeses were manufactured. In addition, 30 hair sheep and 15 cow Panela cheeses were manufactured. The chemical composition as well as the FA profile were determined in all cheeses. In addition, a sensory analysis was performed in Manchego type cheeses manufactured from either hair sheep milk or cow milk. The sensory panel comprised 92 untrained judges. Differences among treatments were determined using orthogonal contrasts between animal species, between cheese type, and between Manchego type cheeses. Moisture content was lower in Manchego type cheeses (37.5 ± 1.26 and 37.5 ± 1.26 g/100g in cheeses manufactured from hair sheep milk and cow milk, respectively) than in Panela cheeses (54.0 ± 1.26 and 56.1 ± 1.26 g/100g in cheeses manufactured from hair sheep milk and cow milk, respectively). Manchego type cheese manufactured from hair sheep milk contained more C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C18:2 *cis*-9,*cis*-12, total SFA, total short-chain FA, total medium-chain FA, total polyunsaturated FA (PUFA), and de novo FA than Manchego type cheeses from cow. Total content of short-chain FA was higher in hair sheep cheeses (24.4 ± 1.30 and 19.6 ± 1.30 g/100g in Manchego type and Panela cheeses, respectively) than in cow cheeses (8.89 ± 1.30 and 8.26 ± 1.30 g/100g in Manchego type and Panela cheeses, respectively). Manchego type cheeses from hair sheep milk obtained higher scores for odor (7.05), texture (6.82), flavor (7.16) and overall acceptance (7.16) than those made from cow milk (6.37, 6.12, 6.17 and 6.83, respectively). In conclusion, both Manchego type cheese and Panela cheese manufactured with hair sheep milk had a similar chemical composition and contained higher levels of short-chain FA, total PUFA and de novo FA than those manufactured with cow milk.

Key Words: sheep, cheese, milk

208 Impact of high pressure and low storage temperature on extending the shelf life of 2 types of string cheese. M. A. Becher*[1], S. Govindasamy-Lucey[2], J. J. Jaeggi[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.*

String cheese is a popular snack cheese known for its stringiness and firmness. Quality of stringiness is highest directly after cheesemaking and decreases over time due to proteolysis and calcium/casein changes. The aim of our study was to extend the stringiness/firmness of string cheese and reduce softening by slowing proteolysis during storage by using high-pressure processing (HPP) and reduced storage temperatures. There are 2 main methods of manufacturing string cheese: cultured and direct acidified (DA). Three samples of each type were obtained from 2 commercial plants. The first day after cheesemaking, HPP was applied, and cheeses were held at 4°C or 0°C (low temperature storage, LTS) for 6 mo. Composition, textural and sensory analyses were performed after 2 wk, 2, 4 and 6 mo. Cheese functionality was assessed using texture profile analysis (TPA). Sensory Spectrum and quantitative descriptive analysis were conducted with 10 trained panelists to evaluate texture, feathering and fiber quantity attributes. Multiple comparison ($\alpha = 0.05$, Duncan test) and split-plot design were used for statistical analyses. The DA cheese had higher moisture and pH but lower protein and calcium content. Insoluble calcium content was much lower in the DA cheese and decreased during storage in both cheese types. Higher levels of pH 4.6 soluble nitrogen were observed in DA cheeses; use of LTS and HPP reduced proteolysis for both cheese types. Both the initial TPA hardness and sensory hand firmness scores were lower in DA cheese compared with the cultured cheese; neither HPP nor storage temperature greatly impacted the hardness/firmness values. The DA cheeses had very low hardness/firmness values by 6 mo. The quantity of feathering (fine strings) and fibers (larger strings) were reduced after HPP, and both attributes decreased during storage for all treatments/cheese types. The DA cheese was less suitable than cultured string cheese for extension of shelf life. Future research will focus on changes in the cheese manufacturing process to increase the stringiness shelf life and reduce the softening of string cheese during storage.

Key Words: string cheese, high pressure

209 Textural characteristics of different varieties of commercial cow and goat milk cheeses stored under different temperature and time treatments. R. Paswan* and Y. W. Park, *Fort Valley State University, Fort Valley, GA.*

Texture plays an important role in food quality and consumer acceptability of cheeses, while the textural analysis of cheese is complicated and often confusing. Texture profile analysis utilizes the universal testing machine which mimics chewing through the use of large destructive shears, and the hardness, springiness and cohesive nature of the food are calculated. The purpose of this study was to compare textural properties of 5 different varieties of commercial cheeses as cow milk Gouda (CMG), goat milk Gouda (GMG), cow milk Cheddar (CMC), cow milk Monterey Jack (CMMJ) and sheep milk Manchego (SMM) cheeses, stored under 2 temperatures (4°C and −18°C) and 5 storage periods (0, 1, 2, 4 and 6 mon). The experimental cheeses were subdivided into 2 equal portions and subjected to texture analysis at each storage period. Textural characteristics of all cheese samples were determined using a texture analyzer TA-XT2 (Texture Technologies Corp.). Cheese samples were loaded on the analyzer in a suitable size of 2 × 2 × 2 cm with the speed of the probe set at 2 mm/sec. All the experimental cheeses were also analyzed for basic composition. The SMM had lowest moisture (%) followed by GMG cheese. The mean hardness (N) of the corresponding 5 cheeses at 4°C for 0 and 6 mon storage were: 5,581, 14,455; 5,580, 19,411; 5,548, 22,166; 5,574, 35,752; 4,849, 12,652, respectively, indicating hardness of all cheeses significantly ($P < 0.01$) increased during 6 mon refrigerated storage. All other textural indices were also elevated as storage period (SP) advanced with some variations. Effects of main factors, such as cheese type (CT) and SP on all textural parameters were significant ($P < 0.01$), while storage temperature (ST) significantly affected only springiness, gumminess and chewiness. The interactive term CT × SP significantly affected all textural parameters except cohesion and springiness while CT × St had a significant effect on hardness, gumminess and chewiness. It was concluded that textural characteristics of all 5 varieties of the commercial semi-hard cow, goat and sheep milk cheeses were significantly affected by CT, ST and SP.

Key Words: three-species milk cheese, textural properties, storage

210 Impact of flavor adjunct culture type and ripening temperature on the properties of direct-salted Gouda cheese.

Y. Gong*[1], S. Govindasamy-Lucey[2], J. Jaeggi[2], M. Johnson[2], and J. Lucey[1,2], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

Direct-salted block Gouda cheese allows manufacturers to produce Gouda using Cheddar equipment for end-use pizza applications. Though citric acid fermentation gives a characteristic buttery flavor to Gouda, it increases the danger of splits in block Gouda. To mitigate the splits/gas issue, the use of a non-citric acid fermenting buttery flavor producing adjunct (BFA) was investigated as an alternative to the traditional citric acid fermenters (CAF). Three groups of direct-salted, milled-curd block Gouda cheese with different flavor cultures (CAF, BFA, combination of both (COMBO)) were made (n = 4) using whey dilution (composition: 39.1 ± 0.4% moisture, 32.9 ± 0.4% fat, 24.0 ± 0.5% protein, 1.0 ± 0.1% salt). Cheeses were vacuum sealed and ripened at 2 temperatures (4 or 8°C). Composition, textural and sensory analyses were performed after 1d, 2wk, 1, 3, and 6-mo of ripening. Cheese functionality was assessed by texture profile analysis (TPA), dynamic low-amplitude oscillatory rheology, and sensory evaluation (Sensory Spectrum and quantitative descriptive analysis; 10 trained panelists) to evaluate flavor, texture, and pizza performance. A split-plot design (a = 0.05) was used for statistical analyses. Cheese pH increased during storage from 4.99 ± 0.01 (1 d) to 5.34 ± 0.05 (6 mo). Cheese made with CAF had significantly lower citric acid content, as expected. Lower ripening temperature (RT) and use of a BFA reduced the CO2 production (measured by Mocon's gas analyzer) during ripening. At 3 mo, splits were observed in the CAF and COMBO cheeses, but not in the BFA cheeses. All cheeses had similar TPA hardness. Rheological parameter crossover temperature (COT) was impacted by culture type and RT; COT was lower in the cheeses made with BFA and stored at 8°C, due to less insoluble Ca content. Primary proteolysis (pH 4.6 soluble N formed) was higher in cheeses stored at 8°C. Sensory evaluation of the melted cheese on pizza showed that all cheeses had a similar level of buttery flavor. Gouda stored at higher RT (8°C) was softer and less chewy. The use of BFA is a promising alternative to the CAF, mitigating gas production while maintaining the characteristic buttery flavor expected in Gouda.

Key Words: block Gouda, flavor culture

211 Gas production by *Paucilactobacillus wasatchensis* WDCO4 is accelerated in Cheddar cheese containing sodium gluconate.

D. J. McMahon*[1], K. Sorenson[2], M. Domek[2], M. Culumber[2], P. Sharma[1], and C. J. Oberg[2,1], [1]*Western Dairy Center, Utah State University, Logan, UT,* [2]*Department of Microbiology, Weber State University, Ogden, UT.*

It has recently been shown that nonstarter lactobacilli such as *Paucilactobacillus wasatchensis* can use 6-carbon gluconate as an energy source. Our objective was determine if adding sodium gluconate to curd is another risk factor for unwanted gas production and slit formation in cheese. Three vats of Cheddar cheese were made with no *Pa. wasatchensis* WDC04 (WDC04) added (Control) and 3 vats in which WDC04 was added at 10^4 cfu/mL to the milk. After milling the curd from the vats with WDC04 added was divided into 6 12-kg portion to which was added: (A) salt, or salt plus (B) 0.5% galactose (GAL) + 0.5% ribose (RIB), (C) 1% sodium gluconate (GLCN), (D) 1% GLCN + 0.5% RIB, (E) 2% GLCN, (F) 2% GLCN + 0.5% RIB. Cheeses were cut into 900-g pieces, vacuum packaged and stored at 12°C. Each month the bags were examined for gas production and looseness of the bag measured as distance from edge of the cheese block to where the 2 sides of the bag were held tightly together by the vacuum. Cheese was tested for lactose, GAL and GLCN and microbial numbers. Adding GLCN increased cheese moisture content by 0.5 to 1.5%. Lactococcal numbers in cheese decreased from an initial 10^8 cfu/g to 10^5 cfu/g after 16 wk in all cheeses. In the Control cheese (i.e., no WDC04 added), *Pa. wasatchensis* remained undetected (i.e., $<10^4$ cfu/g) while in cheeses A, C and E, *Pa. wasatchensis* counts increased to 10^7 cfu/g, and to 10^8 cfu/g when RIB was also added (cheeses B, D and F). By adding WDC04 to the milk, more gas was produced in the cheeses than in the Control, (*t*-test, $P = 0.02$). There was no significant difference ($P > 0.05$, LSD = 28 mm) in gas production for cheeses A, B, C or D with looseness of the bags measured at 24, 40, 34 and 46 mm, respectively. However, when RIB was added as well as GLCN to speed up growth of WDC04 during storage, (comparing C versus D, and E versus F) gas production increased (*t*-test, $P = 0.03$). There was no significant difference based upon adding 1% or 2% GLCN. GLCN concentration in cheeses C, D, E and F decreased from initial levels of 0.38, 0.42, 0.84 and 0.93%, respectively to 0.37, 0.10, 0.71 and 0.67% after 16 wk. Only cheese B contained GAL and it decreased from an initial 0.29% to 0.02% during 16 wk. In conclusion we found that adding sodium gluconate to curd increased gas production in cheese with more gas being formed when *Pa. wasatchensis* WDC04 grew to higher numbers.

Key Words: gluconate, galactose, nonstarter lactic acid bacteria

212 Comparison of 3 generations of fermentation-produced chymosin for the production and ripening of Cheddar cheese.

B. Li*[1], D. S. Waldron[1], M. A. Drake[2], J. Lyne[3], A. L. Kelly[1], and P. L. H. McSweeney[1], [1]*School of Food and Nutritional Sciences, University College Cork, Cork, Ireland,* [2]*Department of Food Science, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC,* [3]*Chr. Hansen A/S, Hoersholm, Denmark.*

The aim of this study was to evaluate the suitability of a new generation of fermentation-produced camel chymosin as a coagulant for Cheddar cheese manufacture. Milled-curd Cheddar-type cheese was manufactured using fermentation-produced bovine chymosin (BC), commercially available fermentation-produced camel chymosin (CC) or a commercially available modified fermentation-produced camel chymosin (mCC), added at the same milk coagulating activity (60 IMCU L^{-1}), and ripened for 180 d. No significant differences ($P > 0.05$) were found in moisture (BC: 37.77%, CC: 37.63%, mCC: 37.27%), fat (BC: 32.56%, CC: 32.13%, mCC: 31.90%), and protein (BC:26.10%, CC: 25.96%, mCC: 25.99%) contents or pH (BC: 5.22, CC: 5.22, mCC: 5.22) of cheese samples. The salt content of cheese mCC was significantly higher ($P < 0.05$) than that of cheese BC (BC: 1.23%, CC: 1.26%, mCC:

1.37%). From the levels of pH 4.6-soluble N as a percentage of total N (BC: 18.68%, CC: 13.64%, mCC: 9.86%) and urea-PAGE, proteolysis in cheese made with mCC was reduced compared with cheeses made with BC (18.68%) or CC (13.64%). Texture profile analysis, Schreiber meltability test, dynamic small-amplitude oscillatory rheology, and descriptive sensory analysis (6 trained panelists) were used to analyze the functionality of the cheese. Significantly higher ($P < 0.05$) instrumental hardness (>3,000 g) and sensory hardness (>0.6 out of a 15-point product-specific scale) were found in cheeses made using CC and mCC compared with cheese BC after 180 d of ripening. Descriptive sensory analysis results showed that cheese made with CC and mCC had significantly less ($P < 0.05$) sulfur and barny flavor; the brothy flavor and bitter taste of cheese mCC were also lowest. The meltablity of cheese made using mCC was found higher than that of cheese BC and CC at low temperature (<80°C), while cheese BC showed higher meltability than cheeses CC and mCC at high temperature (232°C). In conclusion, the modified camel chymosin appears to be suitable for the manufacture of Cheddar cheese with reduced proteolysis, higher hardness and meltability, and modified flavor.

Key Words: camel chymosin, proteolysis, sensory

Dairy Foods: Cheese and Microbiology

213 3D printing applications for cheese: Process evaluation and public perception. M. M. Ross*, S. V. Crowley, M. B. McCarthy, A. P. Morrison, A. M. Collins, J. Oliveira, S. Crotty, and A. L. Kelly, *University College Cork, Cork, Ireland.*

3D printing is an additive manufacturing (AM) technology that is used to make complex structures and geometries using a diverse range of materials, including food, with the potential to give users the freedom to design personalized meals and snacks. To achieve this, a greater understanding of the fundamental printability of food materials is required, along with consumer insights pertinent to the successful adoption of the technology. This multidisciplinary study involved a team of consumer scientists and food scientists, focused on the twin specific objectives of: (1) characterizing the 'printability' of processed cheese formulations, using rheological methods, and (2) determining the dominant factors influencing Irish consumers' willingness to try 3D-printed foods, using a combination of qualitative and quantitative methods. The pH and casein content of processed cheese formulations significantly ($P > 0.05$) affected its printability; 3D-printed processed cheese with higher pH was found to be significantly softer and led to less accurate prints (i.e., deviation from original model dimensions) in comparison to those with lower pH. Processed cheese formulations consisting of primarily young Cheddar mixed with mature Cheddar as the cheese source were determined as optimal for printing in terms of precise printing and suitable texture (i.e., extrudable, yet holds its structure). Printing temperature had a large effect on the microstructure and texture of printed processed cheese, where processed cheese printed at higher temperatures (65°C) showed larger fat droplets and were harder in texture than cheese printed at lower temperatures (45°C). Consumer studies indicated that there was a degree of skepticism and confusion among some participants about 3D food printing, although participants were also aware of the benefits afforded by 3D printing, including meal customization and tailored nutrition. Prevailing themes emanating from the consumer science study included novel food technology neophobia, trust in science and consumer innovativeness, among others. In conclusion, the formulation and technology of processed cheese will influence its suitability as a printing material, while consumer education around the technology will be key to enabling successful future adoption.

Key Words: 3D printing, processed cheese, rheological methods

214 Use of wear behavior and material properties for predicting shreddability of process cheese. J. D. Young, D. J. McMahon, and P. Sharma*, *Utah State University, Logan, UT.*

Mass loss during shredding of cheeses blocks is a matter of concern for cheese manufactures as it incurs significant financial losses to the commercial operations. In this study we investigated the applicability of wear behavior and other material properties in predicting shreddability of process cheese (PC). We prepared 18 batches of PC samples with spectrum of shreddability by manipulating amount of intact casein (as reflected by weighted average age of natural Cheddar cheese; 1–102 d) and TSC content (2–3%). Wear behavior for PC was determined by measuring penetration depth and mass loss at 5°C using a pin-on-disk tribological attachment to MCR 302 rheometer at sliding velocity of 50 mm/s, and 1 N normal force. Rheological characterization was performed at 5°C using both small oscillatory shear and large amplitude oscillatory shear tests. Texture Profile Analysis performed at 25% compression, 1 mm/s cross head speed, and 5°C. Shreddability tests were performed at 5°C on the Texture Analyzer with a grating rig attachment and 15 mm/s cross head speed. A shreddability index (SI) was developed from shred characteristics obtained after passing them through a mechanical sieve. Mechanical sieve data in terms of length of the shreds, quantity of fines and adhesion to the surface was used for developing SI. We found that with increasing weighted age of natural cheese, PC samples became softer, more viscous, indicated by a decrease ($P < 0.05$) in comples modulus (G*) values (at 0.01% strain), gumminess and hardness; leading to increase in penetration depth and mass loss. Age of natural cheese had a positive impact ($P < 0.05$) on the work to grate (extent of difficulty in grating) and negative impact on crumbliness. A good correlation was found between SI and tribological, rheological and material properties. Various multiple regression models were used on selective variables for testing their ability to predict SI. A model having mass loss, penetration depth, rate of wear, work to grate, G*, and gumminess, as predicting variables, was found the best fit ($R^2 = 0.82$). However, the model with wear data only, was also able to predict the shredding behavior with acceptable confidence ($R^2 = 0.74$). These results suggest potential use of wear data for predicting shreddability of cheese and for minimizing material losses at commercial scale operations.

Key Words: wear data, shreddability, process cheese

215 Effect of emulsifying salt, and age of natural cheese on microstructure, wear behavior and grating properties of process cheese. J. D. Young, A. H. Vollmer, D. J. McMahon, and P. Sharma*, *Utah State University, Logan, UT.*

Wear behavior is closely related to processability for food materials such as process cheese (PC). During the process of slicing or shredding a block of cheese, significant amount of material loss can occur due to sticking of PC to the moving parts of machinery or crumbling during high-speed operations. Wear techniques measure mass removal from the surface of an object over time because of relative motion. In this study we investigated effects of the PC formulation on wear, structural, and textural properties. PC samples in 18 batches were prepared using RSM full factorial design varying trisodium citrate (TSC) levels (2–3%) and weighted average ages of lab-scale natural Cheddar cheese (1–102 d) indicating different extent of proteolysis with 2 sets of quadruplicate batches. Each batch of PC was standardized to 42% moisture, 2.0% sodium chloride, and pH 5.5. Microstructure of the PC samples was characterized using confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM). Wear measurements in terms of penetration depth (mm) and mass loss (g) were performed at 5°C with a pin-in-disk attachment to MCR 302 Rheometer, using 1N tribological force and 50mm/s sliding speed. Texture profile analysis and grating properties of PC were measured using Texture Analyzer at 5°C. Grating rig test was performed to determine work to grate and crumbliness. CLSM and TEM micrographs of PC indicated that size of fat globules decreased with increasing both age of natural cheese and TSC concentration. Penetration depth and mass loss increased linearly ($P < 0.05$) as natural cheese age increased, indicating strong influence of extent of proteolysis. With larger proportion of aged cheese in the PC formulation, hardness (N) and gumminess (N) both decreased ($P < 0.05$), suggesting softer PC matrix due to lack of enough intact casein levels. With age of natural cheese, work needed to grate a block of PC increased, and crumbliness decreased, indicating loss of shredding property. Effect of TSC on these properties was not significant, as solid fat at 5°C, largely dominated the material characteristics. The findings of this study provide useful information to cheese manufacturers for optimizing formulation and processing condition to attain desirable shredding property with minimal material loss.

Key Words: microstructure, wear behavior, process cheese

216 The synergism of lactic acid bacteria and its impact on their bioactivities in a consortium.

S. Badiger*, I. García-Cano, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Lactic acid bacteria (LAB) have been used in food fermentations and as probiotics. A consortium of LAB can lead to wider range of benefits and flavor profile, if added in the right combination. This is because they can react differently when present in different combinations. To better understand the synergism of LAB and its impact on bioactivities, 3 lactic strains (*L. plantarum*, *L. reuteri* and *P. acidilactici*) were chosen. They were grown in MRS media (in triplicates) in 7 combinations: individually, 2 strains combined, and all 3 combined, while maintaining the same optical density at inoculation. Growth curves for every species in every combination were obtained using RT-qPCR and differentiating DNA probes. Simultaneously, bioactivities such as proteolytic, antibacterial, lipolytic and β-galactosidase were measured at different growth stages for these combinations. The time points in the growth curves were compared using ANOVA, and it was seen that the growth of *L. plantarum* was higher in the presence of *L. reuteri* but lower in the presence of *P. acidilactici* ($\alpha = 0.05$). Interestingly, the *L. plantarum-P. acidilactici* had highest proteolytic activity and this could be due to antagonism occurring between the 2 bacteria. It was also seen that the combinations having *L. reuteri* had higher antibacterial and β-galactosidase activities. These results imply that bioactivities observed in LAB consortia are driven by the type of bacteria present and not the number of species or number of cells present. Additionally, there is a strong correlation between synergism and the bioactivities observed. An understanding of these correlations can help in better selection of bacteria in a consortium based on the application.

Key Words: consortia, lactic acid bacteria, bioactivities

217 Comparative peptidomic and metatranscriptomic analyses reveal improved γ-aminobutyric acid production machinery in *Levilactobacillus brevis* NPS-QW 145 cocultured with *Streptococcus thermophilus* ASCC1275 during milk fermentation.

T. T. Xiao*, *The University of Hong Kong, Hong Kong, China.*

The high γ-aminobutyric acid (GABA)-producing bacterium *Levilactobacillus brevis* strain NPS-QW 145, along with *Streptococcus thermophilus* (one of the 2 starter bacteria used to make yogurt for its proteolytic activity), enhances GABA production in milk. However, a mechanistic understanding of how *Lb. brevis* cooperates with *S. thermophilus* to stimulate GABA production has been lacking. Comparative peptidomic and metatranscriptomic analyses were carried out to unravel the casein and lactose utilization patterns during milk fermentation with the coculture. We found that particular peptides hydrolyzed by *S. thermophilus* ASCC1275 were transported and biodegraded with peptidase in *Lb. brevis* 145 to meet the growth needs of the latter. In addition, amino acid synthesis and metabolism in *Lb. brevis* 145 were activated to further support its growth. Glucose hydrolyzed from lactose present in milk by *S. thermophilus* 1275 was metabolized by *Lb. brevis* 145 for ATP production. In the stationary phase, under acidic conditions due to the accumulation of lactic acid produced by *S. thermophilus* 1275, the expression of genes involved in pyridoxal phosphate (coenzyme of glutamic acid decarboxylase) metabolism and glutamic acid decarboxylase (Gad) in *Lb. brevis* 145 was induced for GABA production. Based on the widely reported contribution of lactic acid bacteria (LAB) and GABA to human health, the elucidation of interactions between the 2 groups of bacterial communities in the production of GABA-rich milk is important for promoting the development of functional dairy food and may provide new insight into the development of industrial GABA production.

Key Words: *Levilactobacillus brevis*, *Streptococcus thermophilus*, GABA

218 Milk fat globule membrane phospholipids modify adhesion of *Lactobacillus* to mucus-producing Caco-2/Goblet models by altering their cell envelope.

J. Ortega-Anaya* and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

The importance of milk components such as the MFGM and probiotics such as *Lactobacillus* are highly regarded for their health-promoting properties. *Lactobacillus* produce metabolites with direct effect in the intestinal epithelial cells, and MFGM improves gastrointestinal status by various mechanisms. Interaction between beneficial bacteria and milk fat is a naturally occurring phenomenon in any dairy product; however, its mechanism has not been fully characterized. In this work, we studied the effect of milk phospholipids (MPL) in the adhesion of 4 strains of *Lactobacillus* (*L. acidophilus, L. casei, L. delbrueckii* and *L. plantarum*) to mucus-producing Caco-2/goblet cell cocultures. We found that the treatment modified the bacterial surface electronegativity, measured by their ζ-potential values, which correlated with improved adherence to the intestinal model. This is particularly evident when the cells acquire more electronegativity (Table 1). Moreover, we utilized an original means of characterizing the adhesion utilizing quartz crystal microbalance (QCM-D). All strains studied, experienced alterations of adhesion parameters, either physicochemical or kinetic (Table 1). Furthermore, by imaging bacterial cells by TEM, we identified that *L. acidophilus* and *L. casei* metabolize MPL, which improved their adhesion to inorganic surfaces such as gold; whereas *L. delbrueckii* and *L. plantarum* accumulated MPL irreversibly bound to the bacterial surface, instead of metabolizing it resulting in a decreased adhesion to gold. In conclusion, we found that MPL treatment modifies the cell surface of *Lactobacillus* enhancing significantly the adhesion of *L. casei* and *L. delbrueckii* to intestinal models and will in turn, potentially increase their residence time in the human intestine with the concomitant benefits to the consumer.

Table 1. Adhesion parameters tested on *Lactobacillus* upon treatment with MPL

		Intestinal model	QCM-D	
Strain	Δ ζ-potential (mV)	Δ Adhesion to Caco-2-goblet (×10^3 cfu/mL)	Mass (ng/cm^2)	Kd (×10^3 ng/cm^2)
L. acidophilus	−4.0*	0.0013	654.3*	266.2*
L. casei	−2.2*	1.162*	817.7*	190.3*
L. delbrueckii	−6.4*	1.238*	525.8*	461.4*
L. plantarum	−2.1	−0.786*	562.0	277.2*

*Denotes significant difference.

Key Words: MFGM, *Lactobacillus*, Caco-2/goblet

Physiology and Endocrinology II

219 Opportunities and limitations of milk mid-infrared spectra–based estimation of acetone and β-hydroxybutyrate for the prediction of hyperketonemia in dairy cows. M. O. Caldeira[1], D. Dan[1], A.-L. Neuheuser[1], R. Stürmlin[1], C. Weber[1], D. L. Glauser[2], M. Stierli[2], U. Schuler[3], J. Moll[3], S. Wegmann[3], R. M. Bruckmaier[1], and J. J. Gross*[1], [1]*Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Suisselab AG, Zollikofen, Switzerland,* [3]*Qualitas AG, Zug, Switzerland.*

Subclinical (SCK) and clinical (CK) ketosis are metabolic disorders responsible for big losses in dairy production. Although the suitability of Fourier-transform mid-infrared spectrometry (FTIR) to predict hyperketonemia in cows was repeatedly studied, little is known about its suitability in predicting hyperketonemia in individual samples, e.g., in small dairy herds. The objective of the present research was to determine the applicability of FTIR spectrometry in the individual screening for SCK and CK. In experiment 1, blood and milk samples were taken biweekly after calving from Holstein (n = 80), Brown Swiss (n = 72) and Swiss Fleckvieh (n = 58) cows. In experiment 2, cows diagnosed with CK (n = 474) and 420 samples with blood β-hydroxybutyrate [BHB] < 1.0 mmol/L were used to investigate if CK would have been detectable by FTIR-predicted BHB and acetone from a preceding milk control. In experiment 3, correlations between data from an on-farm automatic milk analyzer and FTIR-predicted BHB and acetone from the monthly milk controls were evaluated. Pearson correlation coefficients between the target variablese were calculated by the CORR procedure of SAS. Receiver operating characteristic (ROC) curves were created to calculate sensitivity and specificity for milk BHB detection via FTIR analysis. Hyperketonemia was diagnosed in the majority of blood samples during the first 8 weeks of lactation. Thresholds for SCK and CK were considered at blood BHB concentrations >1.0 mmol/L and >2.0 mmol/L, resp. In exp. 1, correlations between blood BHB and FTIR-predicted BHB and acetone were low (r = 0.37 and 0.12, respectively, $P < 0.0001$), as well as the percentage of true positive values (11.9% and 16.6%, respectively). No association of FTIR-predicted ketone bodies with the interval of milk sampling relative to CK diagnosis was found in exp. 2. In exp. 3, data obtained from the automatic milk analyzer were moderately correlated with the same day FTIR-predicted BHB analysis (r = 0.61). In conclusion, the low correlations with blood BHB and the small number of true positive samples discourage the use of milk mid-infrared spectrometry analyses as the only method to predict hyperketonemia at the individual cow level.

Key Words: mid-infrared spectrometry, acetone, ketosis

220 Effects of body condition on the insulin resistance, lipid metabolism, and oxidative stress of lactating dairy cows. J. J. Wu*, J. X. Liu, and D. M. Wang, *Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China.*

An appropriate body condition score (BCS) plays an important role in maintenance of health of lactating dairy cows. Here, we aimed to investigate insulin sensitivity and oxidative status in late lactating dairy cows with different BCS. Forty-two multiparous Holstein cows were selected with similar parity (2.45 ± 0.92 SD), days in milk (200 ± 21.98 SD), and milk yield (33.6 ± 4.94 SD), and were fed the same diet for a month during which the BCS was recorded 3 times. The cows were then divided into 3 groups based on BCS: low BCS (BCS ≤2.75; n = 12), medium BCS (3.0 ≤ BCS ≤3.5; n = 15) or high BCS (BCS ≥3.75; n = 15). On d 30, blood samples were collected from coccygeal vein to determine biochemical and hematological parameters. All data analysis was performed with the PROC MIXED of SAS software, with BCS as fixed variable and each cow within the BCS group as a random variable. The concentration of insulin was higher and nonesterified fatty acid tended to be higher, but the revised quantitative insulin sensitivity check index (RQUICKI) was lower in high BCS cows than in low and medium BCS cows ($P < 0.01$, $P = 0.06$, and $P < 0.01$, respectively), suggesting that insulin resistance exacerbates lipolysis in high BCS cows. Serum concentrations of very low-density lipoprotein, apolipoprotein A-I, and apolipoprotein B were lower in high BCS cows than in low or medium BCS cows ($P = 0.03$, $P = 0.02$, and $P = 0.01$, respectively). Although low BCS cows had higher reactive oxygen species levels ($P < 0.01$) than did high BCS cows, no differences were found in the malondialdehyde concentration, superoxide dismutase, glutathione peroxidase, and paraoxonase activities among cows with different BCS. The percentage of granulocytes tended to be higher ($P = 0.07$) in medium BCS cows than in high BCS cows, with no differences found in leukocyte counts, red blood cell profiles and platelet profiles in the 3 groups. These results indicate that compared with medium and low BCS cows, high BCS cows at late lactation stage have greater lipolysis and lower antioxidant potential due to greater insulin resistance.

Key Words: body condition score, insulin resistance, oxidative stress

221 Muscle proteomes of periparturient dairy cows exposed to circadian disruption indicate increased mitochondrial oxidation and cellular stress. C. J. McCabe*[1], U. K. Aryal[2], J. P. Boerman[1], and T. M. Casey[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Bindley Proteomics Core, Purdue University at Discovery Park, West Lafayette, IN.*

In the transition from late gestation to early lactation, dairy cows experience increased nutrient requirements that feed intake alone cannot support. Muscle tissue serves as a key nutrient reservoir dairy cows utilize to meet both energy and amino acid requirements. Circadian clocks are integrated with the metabolic system and act as homeostatic regulators so that organisms can anticipate regular environmental changes. Our previous studies found disrupting circadian rhythms by disrupting light-dark cycles in late-gestation dairy cows increased insulin resistance and decreased milk production. Our objective was to understand how circadian disruptions during late gestation affected muscle metabolism by analyzing the proteome. At 5 wks before expected calving (BEC), multiparous Holstein cows were assigned to control (CON; n = 8) or 6 h forward phase shift (PS, n = 8) of the light-dark cycle every 3 d. At calving, all animals were exposed to CON light-dark cycles to 60 d postpartum (PP). Muscle biopsies were collected from *longissimus dorsi* muscle at 21 d BEC and at 21 d PP. Proteins were isolated and liquid chromatography tandem mass spectrometry was used to measure the proteome. Data were mapped to 891 proteins in the Uniprot *Bos taurus* database. Abundance of high confidence proteins (n = 3/treatment/time point) were compared between treatments at 21 d BEC and 21 d PP, and within treatments between time points using *t*-test analysis run in Metaboanalyst. At $P < 0.1$, 116 proteins were different between PS and CON at 21 d BEC, and 121 proteins at 21 d PP. Between pregnancy and lactation 134 proteins were differentially abundant in CON, and

145 in PS. Proteins that increased in abundance between pregnancy and lactation regulated β oxidation and glycolysis. At both time points, PS cows exhibited an oxidative stress proteome signature indicated by a greater abundance of proteins associated with mitochondrial output, antioxidants, and heat shock proteins. Proteome profiles indicate that increased energetic demands of lactation are supported by increased fatty acid oxidation and glycolysis in muscle tissue. Chronic disruptions of circadian rhythms increased mitochondrial oxidation and cellular stress and emphasize the need to minimize dairy cow circadian disruptions.

Key Words: proteomics, muscle biopsy, mitochondria

222 Anandamide enhances barrier integrity of bovine vascular endothelial cells during endotoxin challenge via cannabinoid receptor-1 activation.

C. C. F. Walker* and L. M. Sordillo, *Michigan State University, East Lansing, MI.*

Dysfunctional inflammation and the targeted breakdown of the vascular endothelium are part of the pathology of acute coliform mastitis. Despite medical interventions being available, there is need for improvement of the inflammatory function of dairy cows. Inflammatory involvement of the endocannabinoid (EC) system has not only been shown in humans and rodents, but recently also in bovine. The EC arachidonoylethanolamide (AEA) is a natural ligand of the endocannabinoid receptor 1 (CB1) and improves network formation and proliferation in human and rodent endothelial cell models, the exact mechanism of action, however, is unknown. We have recorded increased plasma AEA during acute coliform mastitis in vivo and elevated CB1 and 2 expression in cultured bovine aortic endothelial cells (BAEC) challenged with endotoxin (LPS), further supporting the bovine EC system involvement in inflammation. During prior experiments using the electric cell-substrate impedance sensing (ECIS) technology, AEA treatment increased barrier resistance of LPS challenged BAEC, indicative of increased barrier integrity. Rimonabant (RIMO), a CB1 inverse agonist used in previous bovine EC studies, was used to test the involvement of CB1 in increased barrier resistance observed in LPS challenged BAEC treated with AEA. Confluent primary BAEC cells in ECIS arrays were treated with 25 ng/mL of LPS for 6–8 h before addition of RIMO. Statistical analysis was done using SAS9.4 with a 2-way repeated-measures ANOVA and Dunnett's multiple comparison. Rimonabant doses of 0.1, 0.5, and 1 μM decreased barrier resistance compared with the LPS control within the first 2 h of treatment, with a loss of effect at 4 h. Timing and effect of RIMO coincide with effects of AEA on barrier resistance, suggesting CB1 involvement. However, AEA and RIMO were shown to activate non-CB receptors involved in network formation and proliferation, such as transient receptor potential vanilloid-1, and further in vitro modeling is necessary to fully elucidate the mechanism of increased BAEC barrier resistance in response to AEA.

Key Words: coliform mastitis, endocannabinoids, inflammation

223 Energy balance, calcium homeostasis, and bone metabolism: Preliminary data.

M. O. Matthaei[1], J. Rehage[1], G. Szura[1], I. Leiter[1], K. Hansen[2], S. Daenicke[3], D. von Soosten[3], S. Kersten[3], U. Meyer[3], and M. R. Wilkens*[2,4], [1]*Clinic for Cattle, University of Veterinary Medicine Hannover, Foundation, Hannover, Lower Saxony, Germany,* [2]*Institute of Physiology and Cell Biology, University of Veterinary Medicine Hannover, Foundation, Hannover, Lower Saxony, Germany,* [3]*Institute of Animal Nutrition, Federal Research Institute for Animal Health, Braunschweig, Lower Saxony, Germany,* [4]*3Department of Animal Sciences, Animal Nutrition Physiology, University of Goettingen, Goettingen, Lower Saxony, Germany.*

At calving, the increase in Ca demand induces bone mobilization. As recent studies suggest that the bone formation marker osteocalcin (OC), especially in its undercarboxylated form (ucOC), has endocrine functions, this preliminary study aims to explore potential interactions between Ca homeostasis, OC and intermediary metabolism in transition cows. We analyzed serum samples from d −7, d −3, d +1, d +3 and d +7 relative to calving from 15 multiparous cows for total Ca, intact OC (iOC), ucOC, insulin, glucose, nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB). Insulin sensitivity was estimated by calculating the revised quantitative insulin sensitivity index (RQUICKI). Pearson correlation coefficients (r) were calculated separately for cows entering the second lactation and older animals. In cows >second lactation, serum total Ca on d −3 correlated with serum concentrations of glucose (r: 0.859, $P < 0.01$) and BHB (r: −0.794, $P < 0.05$) on d +3. In younger animals (second lactation), there were correlations between Ca on d +3 with NEFA (r: −0.880, $P < 0.01$) and RQUICKI (r: 0.814, $P < 0.05$) on d +7. Independent of age and iOC, we found large variations for serum ucOC already before parturition (a.p.). Therefore, we allocated the cows retrospectively to 3 groups with mean serum ucOC of 0.74 ng/mL, 1.51 ng/mL and 4.28 ng/mL on d −7 (low ucOC, medium ucOC and high ucOC). High ucOC and low ucOC cows showed more or less stable plasma concentrations of ucOC throughout the entire observation period, while the concentrations in medium ucOC animals decreased to 0.64 ng/mL after calving (p.p.). A mixed-effects model (time and group) revealed a significant effect of group for RQUICKI ($P < 0.01$). In the low ucOC group, RQUICKI was lowest a.p. and increased p.p., while medium ucOC cows showed an opposite pattern and high ucOC animals were characterized by the highest RQUICKI a.p. and p.p. From our preliminary data it can be concluded that low serum Ca precedes disturbances of energy metabolism. The role of ucOC and its potential endocrine functions seems to be more complex, but prepartum ucOC might turn out to have a diagnostic potential in respect to metabolic disturbances in early lactation.

Key Words: bone turnover, mineral homeostasis, intermediary metabolism

224 Effects of dietary octanoic acid supplementation on performance and rectal temperature in heat-stressed lactating cows.

L. Han* and C. Dechow, *Department of Dairy and Animal Science, The Pennsylvania State University, State College, PA.*

The acylated form of ghrelin (AG) reportedly inhibits heat production by modulating feed intake and thermogenesis. Octanoic acid (OA) is the primary substrate of the ghrelin acylation process. The objective of this project was to investigate the effect of dietary OA supplementation on AG concentration and performance under moderate heat stress. Eight multiparous lactating Holstein cows were assigned to the base diet only (control) or base diet plus a top dress of 550 g/cow/day OA with a 7-d adaptation and 7-d data collection period. Cows were fed once daily at 0800 h at approximately 110% of expected DMI. Rectal temperature was recorded at 0730 h (RT1) and 1600 h (RT2) daily. Milk yields were recorded daily, milk and blood samples were collected on d 6 and 7 of each data collection period for determination of milk components and AG; a log-transformation was applied for analyses of AG (LAG). The average daily minimum, average, and maximum THI during the experiment were 68, 74, and 78, respectively, which was above the heat stress threshold for lactating cows (72 daily average THI). A series of repeated-measures analyses of LAG, RT1, RT2, DMI, milk yield, milk fat, and protein concentration were used to determine effects of OA, one of daily minimum, average, or maximum THI, and their interaction. The repeated effect was test day with the subject of cow and significance

was declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Compared with control, OA decreased DMI (18.7 vs. 21.2 kg, $P < 0.05$), decreased RT2 (39.21 vs. 39.47°C, $P < 0.01$), and tended to decrease RT1 (38.85 vs. 38.90°C, $P < 0.1$). There was no effect on milk yield (33.7 for OA vs. 33.5 kg), milk fat (4.12 vs. 4.25%), milk protein (2.90 vs. 2.89%), or LAG (3.94 vs. 4.01 $\log_e$ pg/mL). Higher minimum THI was associated with higher RT1 (slope = 0.01, $P < 0.05$) whereas higher maximum THI was associated with higher RT2 (slope = 0.04, $P < 0.05$). There was an OA by maximum THI interaction on RT2 with the slope larger for control than OA (0.040 vs. 0.091, $P < 0.05$). Dietary OA helped maintain body temperature during heat stress, but not by facilitating activation of ghrelin.

Key Words: heat stress, octanoic acid, ghrelin

Production, Management, and the Environment II

225 Precision milking management strategies to improve automatic milking systems performance. L. Fadul-Pacheco*[1], E. Rolli[2], D. Reinemann[3], and V. E. Cabrera[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Ederer Dairy Supply, Plain, WI,* [3]*Biological Systems Engineering, University of Wisconsin–Madison, Madison, WI.*

Automatic milking systems (AMS) give the opportunity to manage milking permission (MP, i.e., controls time interval for a cow to be allowed for milking again according to the estimated milk accumulated in the udder) more precisely individual or group. We hypothesized that cow traffic and thus overall AMS milk harvesting per unit of time could be improved by optimizing MP settings, because cows are milked with fuller udders. The aim of this study was to assess the impact of MP changes in a robotic farm with 2 AMS DeLaval serving one pen with free-flow traffic pattern, with an average of 69 cows/AMS, 1.7 milkings/cow per d, and 1,935 kg of milk/AMS per d. Data from milk harvesting and idle time were extracted from the DelPro software (DeLaval) 2 wk before and 2 wk after MP were implemented. MP for all cows were changed twice: 1) from 9.5 to 10.5 kg and then 2) from 10.5 to 11.4 kg of milk (each, a cautious about a 10% increase based on farmer's decision to avoid radical changes). Data were grouped by AMS and day. A generalized linear model was conducted to predict milk yield (kg milk/AMS per d) as a function of: idle time per cow (min/AMS per cow/d), number of cows (NC) per AMS per d, MP change, total milkings/AMS per d. The adjusted R^2 and the RMSE of the model were 76% and 112, respectively. The MP treatment had no effect on milk yield per cow ($P = 0.09$ and 0.25, respectively) and idle time tended to decrease by −3.6 per AMS/cow per d ($P = 0.07$). The model predicts that the NC could be increased by 4.2 per AMS ($P < 0.01$). The total milkings/AMS could also be increased (+7.9 milkings/AMS/d; $P < 0.001$). With these changes the farm could produce an additional 98 kg/AMS/day, which would translate to an extra revenue of $12,600/AMS per yr for a milk price of $0.35/kg. Hence, optimizing MP thresholds, a precision milking strategy, could increase the performance of the AMS by improving cow traffic and allowing more cows/AMS per d and producing more milk/AMS/day. This work was supported by the Food and Agriculture Cyberinformatics and Tools grant no. 2019–68017–29935/project accession no. 1019780 from the USDA-NIFA.

Key Words: milking permission, automatic milking systems

226 Using social network analysis to identify cows' affinities in automatic milking systems. L. Fadul-Pacheco*[1], M. Liou[2], D. Reinemann[3], and V. E. Cabrera[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Statistics, University of Wisconsin–Madison, Madison, WI,* [3]*Biological Systems Engineering, University of Wisconsin–Madison, Madison, WI.*

Social network analysis (SNA) explores complex relational patterns in communities. Dairy cows are social animals and understanding their mutual affinities could help improve management and welfare. The study objective was to perform an exploratory analysis using SNA to identify potential affinities between cows. Data from a guided-flow dairy with an average of 50 cows in an automatic milking system (AMS) was used. Cow traffic data from a 1-mo period was extracted from the DelPro software (DeLaval). To consider cow affinity, we followed 3 different approaches, all of which were captured as scores and depended on when cows passed through the sorting gate (SG): 1) 15 min window: All cows that went through the SG in this window were considered to have a relationship; 2) Continuous: Assigned inversely to the proximity in minutes that the cows passed through the SG; and 3) Lag sequence: An exponentially decreasing score assigned according to the sequence of 2 cows passing through the SG, for up to 4 passes apart. All scores were normalized by the total time each pair of cows spent together in the pen because it was different for some cows. Although results varied substantially depending on the approach used, we identify 3 cows with the highest scores, no matter the approach used. Cows with higher scores were the most active cows (number of gate passes [average] was significantly higher for these cows than for all cows, 666 vs. 322 times/mo, $P = 0.02$). Two of these cows were in their second and one on her third lactation. We also identified 3 other cows that had much lower scores than all other cows, who were the older cows in the pen (>8 yr; ≥ fifth lactation). Although these are preliminary results, this study may help understand cows' interactions and use such information for better management. We are exploring the same analysis in a longer period of time, the effect of resocialization on cow performance, and if breaking affinities can alter the level of stress of cows. This work was supported by the Food and Agriculture Cyberinformatics and Tools grant no. 2019–68017–29935/project accession no. 1019780 from the USDA-NIFA.

Key Words: social network analysis, automatic milking system, cow behavior

227 Association between body weight change in early lactation and milk production variables in automatic milking system farms. M. Peiter*, L. Caixeta, and M. Endres, *University of Minnesota, St. Paul, MN.*

The objectives of this study were to investigate (1) the association between fat-to-protein ratio (FPR) and change in body weight (CBW) in early lactation; and (2) the association between early-lactation CBW and 90-d milk yield (90MY). Retrospective daily cow data were collected from the Lely T4C software on 33 farms using automatic milking system (AMS). Cows were categorized by parity into P1, P2, or P3+. Data for FPR, successful milkings, and concentrate intake in the AMS were averaged for each 3-d period from 1 to 30 DIM. The CBW (kg/d) was calculated over 7, 15, and 30 DIM, as the regression slope coefficient related to DIM = 1, and used as outcome in 3 mixed linear regression models, with FPR, milkings, concentrate intake, parity, DIM category, and interactions with parity and DIM as initial explanatory variables. Backward elimination was used until all factors had a $P < 0.05$. Cow nested within farm (n = 5,569) was the random effect in the models. The 90MY was the outcome variable in 3 other models, with each CBW variable, parity, and their interaction as explanatory variables. Cow nested within farm (n = 4,437) was the random effect. FPR had a trend ($P = 0.07$) for a positive association with 7-d CBW for P1, and it was negatively associated with 7-d CBW for P2 ($P = 0.02$) and P3+ ($P < 0.0001$). Milkings had a positive association with 7-d CBW for P1 ($P < 0.0001$), a negative association for P2 ($P < 0.0001$), and no association for P3+ ($P = 0.62$). Concentrate intake had a negative association with 7-d CBW for P1 ($P < 0.0001$), a positive association for P2 ($P < 0.0001$), and no association for P3+ ($P = 0.25$). FPR was positively associated with 15-d CBW for P2 ($P = 0.04$) and had a negative association for P3+ ($P = 0.002$). FPR had a positive association with 30-d CBW for P2 only ($P = 0.05$); number of milkings was negatively associated with

30-d CBW ($P = 0.03$). The 7-d CBW was not associated with 90MY ($P = 0.53$); however, 15-d and 30-d CBW had a negative association with 90MY ($P < 0.0001$). In summary, CBW was associated with both FPR and 90MY. Further research is warranted to investigate CBW as a predictor of transition cow health disorders in AMS herds.

Key Words: body weight, automatic milking systems, fat-to-protein ratio

229 Farmers' perceptions on implementing automatic milking systems in large US dairies. C. F. A. Lage*[1], D. R. Bruno[2], M. I. Endres[3], and F. C. Ferreira[1], [1]*Department of Population Health and Reproduction, University of California, Tulare, CA,* [2]*University of California Cooperative Extension, Fresno, CA,* [3]*University of Minnesota, St Paul, MN.*

The adoption of automatic milking systems is trending among large dairies in the US. Our objective was to describe the perceptions of US producers on the implementation of AMS in dairies with ≥7 AMS units installed. Farmers (n = 16), representing an estimated 16% of the dairies within these criteria in the United States, from 9 (CA, IN, MN, NH, NY, PA, TX, WA, WI) states selected by convenience responded a survey online or by phone. These preliminary results show that the MIN, median, and MAX of AMS units/farm and cows on AMS/farm were 7, 12, and 36 and, 400, 720, and 2,200, respectively. Flow systems reported were free-flow (69%), guided-flow (19%), and both (12%). New barns were built by 75% of the respondents. The main reasons for adopting AMS were to improve cow`s welfare and reduce labor cost (83%), improve herd performance (75%), and to reduce number of employees (75%). Most of the respondents (88%) believe AMS reduced the labor on their dairy, and half of respondents reported a ≥20% reduction in the number of full-time employees after the transition. A large proportion (67%) of respondents kept their conventional parlor after installing AMS, but 50% plan on having all their cows on AMS in the future. The main reasons to keep the conventional parlor were because the farm was still transitioning to AMS (70%), and to milk hospital cows (70%) and fresh cows (60%). Among the respondents, 57% would do something different during the AMS installation process, including better training of employees before transitioning (21%) and modifications on barn design (36%). Overall, respondents strongly agreed that AMS improved the quality life of their cows (50%), and improved cow's milk production and reproductive performance (36%). However, respondents were neutral on the statement that AMS has improved herd profitability (50%). Most respondents would recommend AMS to other farmers, whereas 27% responded that the recommendation would depend on farm aspects. Overall, producers reported that AMS have met their expectations about improving animal production and welfare; however, the economic aspect of the investment is not yet clear and would benefit from further investigation.

Key Words: automatic milking systems (AMS), large dairies, perception

230 Association of housing and management practices with bulk tank fatty acid profile in farms with automated milking systems. M. M. D. Castro*[1,2], R. D. Matson[2], D. E. Santschi[3], M. I. Marcondes[4], and T. J. DeVries[2], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada,* [4]*Washington State University, Pullman, WA.*

Milk fatty acid (FA) profile can be divided into: 1) de novo (C4-C14), which are synthesized in the mammary gland; 2) preformed (≥C18), which are absorbed from blood and originate from mobilized adipose tissues or dietary fat, and 3) mixed (C16), which has both origins. Our objective was to associate housing and management factors with bulk tank FA profile in farms with automated milking systems (AMS). We visited 124 commercial Canadian AMS dairy farms located in Quebec (n = 56) and Ontario (n = 68) from April to September 2019 and collected housing and management information. Bulk tank milk samples were collected for every farm milk shipment from 04/2019 to 04/2020 in Quebec and from 08/2019 to 05/2020 in Ontario. Milk samples were analyzed for FA using Fourier-transform mid-infrared spectroscopy. Univariable models were used to screen independent variables in mixed-effect linear regression models and variables with $P < 0.25$ were offered to multivariable models, with herd as experimental unit and region as random effect. Across herds, milk yield averaged 35.9 ± 0.4 kg/d, with 3.97 ± 0.01% fat and 3.09 ± 0.01% protein, while FA profile averaged 26.2 ± 0.1, 33.1 ± 0.1, and 40.7 ± 0.2 g/100g of FA for de novo, mixed and preformed, respectively. FA yield averaged 0.34 ± 0.01, 0.44 ± 0.01, and 0.54 ± 0.01 kg/d for de novo, mixed and preformed, respectively. Greater frequency of partial mixed ration delivery (>2x/d vs 1 and 2 x/d) was positively associated with de novo FA ($P = 0.08$) and mixed FA ($P = 0.03$), and negatively associated with preformed FA ($P = 0.08$), when expressed as g/100g of FA. Yield (kg/d) of milk (+2.1), fat (+0.08), de novo FA (+0.018), mixed FA (+0.022), and preformed FA (+0.039) were all greater ($P \leq 0.04$) on-farm using an automated feed pusher compared with non-automated. As compared with a mattress stall base, herds using deep bedding had greater ($P \leq 0.03$) yield (kg/d) of milk (+2.6), fat (+0.075), de novo FA (+0.016), mixed FA (+0.021), and preformed FA (+0.027). The demonstrated associations with milk FA profile indicate that bulk tank FA profile can be used as a tool to monitor and adjust management and housing in AMS farms.

Key Words: fatty acid, de novo, robotic milking

231 Identifying on-farm factors associated with the level of free fatty acids in bulk tank milk. H. M. Woodhouse*, D. F. Kelton, T. J. DeVries, and S. J. LeBlanc, *University of Guelph, Guelph, ON, Canada.*

Elevated free fatty acids (FFA) in bulk tank milk are a recent concern in the dairy industry. Concentrations of FFA >1.2 mmol/100g of milk fat are associated with undesirable milk characteristics, such as off-flavor, rancidity, reduced frothing ability, and inhibited milk fermentation and cheese coagulation. Previous research indicates that physical and chemical agitation result in milk fat globule membrane breakage to release triglycerides that dissociate into FFA. It is hypothesized that milking system type, pipeline diameter, and fat supplementation in the diet affect FFA. An observational case-control pilot study was conducted to identify on-farm factors associated with elevated FFA in bulk tank milk. 50 dairy farms in Ontario, Canada were visited once to complete a survey, measure pipelines, and gather feeding information. Bulk tank FFA data were obtained from the Dairy Farmers of Ontario and used as the outcome variable for analysis. Univariable and multivariable linear regression analyses were used to identify explanatory variables associated ($P < 0.10$) with bulk tank FFA. One farm was excluded from analysis due to a biologically implausible high FFA level. Of the 49 analyzed farms, 15 were tie-stall, 27 were freestalls with parlors, and 8 were freestall with robotic milking. The mean bulk tank FFA level was 1.13 mmol/100g of fat (SD = 0.30, range = 0.69–1.92 mmol/100g of fat). Univariable linear regression analysis indicated that a decreased bulk tank capacity ($P = 0.10$), smaller pipeline diameter ($P = 0.09$), absence of a plate cooler (P

= 0.01), and pasture access ($P = 0.06$) were associated with higher FFA concentrations. 63% of farms fed a fat supplement (range = 100–360 g/head/d), but this was not associated with FFA level in the univariable analysis ($P = 0.27$). Milking system was also not associated with FFA level ($P = 0.26$). In the final multivariable regression model, pipeline diameter <2 inches and the absence of a plate cooler were associated with a greater FFA concentration, but these variables explained only a small proportion of the FFA variability (adjusted $R^2 = 22\%$). The results suggest that more factors affecting FFA need to be investigated.

Key Words: milk fat, non-foaming, lipolysis

232 Prediction of mastitis from routine milk mid-infrared spectral data in dairy cows.

L. Rienesl*[1], A. Koeck[2], N. Khayatzadeh[1], L. Dale[3], A. Werner[3], C. Grelet[4], N. Gengler[5], F. Auer[6], C. Egger-Danner[2], J. Leblois[7], and J. Sölkner[1], [1]*University of Natural Resources and Life Sciences (BOKU), Vienna, Austria,* [2]*ZuchtData EDV-Dienstleistungen GmbH, Vienna, Austria,* [3]*Landesverband Baden-Württemberg für Leistungs- und Qualitätsprüfungen in der Tierzucht e.V. (LKV), Stuttgart, Germany,* [4]*Centre Wallon de Recherches Agronomiques (CRA-W), Gembloux, Belgium,* [5]*Université de Liège (ULg), Gembloux, Belgium,* [6]*LKV Austria Gemeinnützige GmbH, Vienna, Austria,* [7]*European Milk Recording (EMR), Ciney, Belgium.*

Mid-infrared (MIR) spectroscopy is the method of choice to determine standard milk components. Since milk composition is related to health and metabolic status of a cow, MIR spectra could be potentially used for disease detection. The aim of this study was to develop a discriminant model to predict mastitis events from routine MIR spectral data. The data were from the Austrian milk recording system and its health monitoring. It included test day data (recorded every 30 to 40 d) and mastitis diagnoses (acute/chronic) of Fleckvieh, Brown Swiss and Holstein Friesian cows. Diagnoses were linked with adjacent test-day records. Records within 21 d before/after diagnosis were defined as mastitis cases, records outside this period were considered to be healthy. As MIR predictor variables, first derivatives of selected wavelengths, corrected for days in milk, were used. The data set contained roughly 1.2 Mio. records of 96,200 cows and was randomly split into calibration (70%) and validation (30%). Three discriminant models with different sets of predictor variables were developed and evaluated: MIR, SCS and MIR+SCS. Breed and parity were used as fixed effects in all models. Predictions were done with partial least squares by using the function trainControl of R package 'caret', indicators of model fit were sensitivity, specificity, balanced accuracy (mean of sensitivity and specificity) and AUC (area under receiver operating characteristic curve). Results were extracted for different time windows (days of test-day date before or after mastitis diagnosis). Best prediction accuracies were obtained for the model MIR+SCS, where AUC was 0.75 in the overall time window (−21 to +21 d) and 0.83 in time window 0 to +7 d. Corresponding values for MIR were 0.68 and 0.75, for SCS they were 0.72 and 0.82. In all models, AUC values were higher for acute mastitis when test day was close to diagnosis, while for test days further from diagnosis AUC values were higher for chronic mastitis. Results indicate that mastitis may be predicted reasonably accurately with highest accuracies when combining MIR spectra and SCS. Further studies on genetic correlations of clinical mastitis, SCS and MIR predicted mastitis are following.

Key Words: mid-infrared (MIR) spectroscopy, somatic cell score, dairy cow

233 Effect of antimicrobial treatment on the dynamics of ceftiofur resistance in *Enterobacteriaceae* from adult California dairy cows.

D. Sheedy*[1], E. Okello[1,2], D. Williams[1], K. Precht[1], E. Cella[1], T. Lehenbauer[1,2], and S. Aly[1,2], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare, CA,* [2]*Department of Population Health & Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA.*

Dairy farm use of antimicrobial drugs (AMD) is a known risk factor for the selection and propagation of antimicrobial resistance (AMR); however, the dynamics of this relationship are poorly understood on commercial dairies. Our objective was to investigate the rate of change in counts of enteric ceftiofur-resistant *Enterobacteriaceae* in early-lactation dairy cows, mainly in response to AMD treatment. A prospective cohort study was performed on 2 commercial dairies in California's San Joaquin Valley over 2 seasons, enrolling a total of 96 cows. Individual cow fecal samples were collected from pre-parturition to 60 d in milk, 3 times weekly. Lactose fermenting *Enterobacteriaceae* were enumerated by spiral plating fecal samples on MacConkey agar impregnated with 0, 1, 8, 16 and 30 µg/mL ceftiofur. Analysis was performed using mixed effect negative binomial regression models for AMR bacteria counts over time and mixed effect interval regression models for mean inhibitory concentration (MIC) over time. Ceftiofur hydrochloride was administered to 15 enrolled cows and penicillin administered to 2 cows. There was no growth of *Enterobacteriaceae* at 8 µg/mL ceftiofur, the Clinical and Laboratory Standards Institute clinical breakpoint for ceftiofur resistance, in 88% of samples collected from non-treated cows. Samples from treated cows had peak counts of resistant *Enterobacteriaceae* during AMD treatment and returned to pre-treatment levels 3–4 d post treatment. The MIC peaked during treatment and returned to baseline levels by 7–8 d. Our study showed that detectable levels of ceftiofur resistance were very low in early-lactation dairy cows. The concentration of ceftiofur-resistant fecal commensal *Enterobacteriaceae* rapidly increased and decreased following systemic ceftiofur treatment. Our results suggest that the effect of systemic ceftiofur therapy on the resistance level of enteric *Enterobacteriaceae* in early-lactation dairy cows was limited in extent and duration.

Key Words: antimicrobial resistance, medically important antimicrobial drugs, bacteria

234 Addition of nano-sized zinc oxide exerts no adverse effect on lactation performance and mammary health of lactating dairy cows.

J. Cai*, C. Miao, J. X. Liu, and D. M. Wang, *Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China.*

Nanoscale enhances the tissue permeability of substances and thus makes it possible to improve the feed bioavailability. However, limited information is available on the effects of nano-feed additives in dairy cows. This study evaluated the effects of adding nano-sized zinc oxide (nZnO) to the diet on lactation performance, health status and mammary tight junction in dairy cows. Thirty lactating cows (days in milk: 158 ± 43.2; BW: 694 ± 60.5 kg) were selected and assigned to 3 groups: basal diet (control, 69.6 mg zinc/kg DM), basal diet supplemented with zinc methionine (Zn-Met, 109.6 mg zinc/kg DM), basal diet supplemented with nZnO (109.6 mg zinc/kg DM). The experiment lasted for 10 weeks, with the first 2 weeks as adaptation. Lactation performance, mammary tight junction and health indicators were measured every other week. All data were analyzed using SAS software version 2000 and using the covariance type AR (1) for repeated measures. No difference was observed in feed intake, milk yield and milk composition

among 3 groups. Milk zinc in the nZnO and Zn-Met groups (3.93 and 3.89 mg/L) was higher than that in the control (3.79 mg/L) ($P < 0.01$), with no difference between nZnO and Zn-Met groups. Plasma zinc was the highest in nZnO (1.29 mg/L), followed by the Zn-Met (1.25 mg/L), and the lowest in the control (1.21 mg/L) ($P < 0.01$). Milk zinc accumulation calculated by area under 4-h curve in nZnO and Zn-Met groups was higher than that in control (16.1 and 15.7 vs. 15.0, $P < 0.05$). Compared with the control, the milk/blood ratio of zinc was higher in nZnO ($P < 0.05$), but not in Zn-Met group, indicating that the nZnO was more efficient than Zn-Met in mammary delivery. No significant differences were found in oxidative stress variables and mammary tight junction indicators among 3 groups. In conclusion, nZnO improved zinc bioavailability without impairing lactation performance, health status, and mammary permeability in dairy cows, thus it can be used as a potential zinc feed additive for lactating cows.

Key Words: nano-sized zinc oxide, lactation performance, oxidative stress

235 Association of de novo fatty acids in bulk tank milk with herd performance in commercial dairy farms.

D. Warner*, C. Boulanger, J. Prince, R. Roy, S. Adam, R. Lacroix, and D. E. Santschi, *Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

De novo milk fatty acids (DN) are synthetized in the mammary gland from volatile fatty acid precursors and are associated with ruminal function. Rapid analysis of DN from routinely collected bulk tank samples through Fourier-transform infrared (FTIR) analysis could therefore be a valuable tool to monitor herd performance. The objective was to evaluate relationships between DN in bulk tank milk through FTIR and herd performance across 96 commercial Holstein herds in Quebec, Canada. Herds in tie stalls (n = 59) and free stalls (n = 37) were selected based on DN concentration and milk fat yield at test day during the 3 mo before the respective farm visit (July–December 2020). Herds were categorized in 4 clusters as high DN–low fat (HDN-LFY; n = 30; 1.00 ± 0.045 g/100 g of fatty acids and 1.34 ± 0.099 kg fat/cow, mean ± SD), low DN–low fat (LDN-LFY; n = 25; 0.90 ± 0.055 and 1.35 ± 0.157), high DN–high fat (HDN-HFY; n = 24; 1.01 ± 0.041 and 1.45 ± 0.231), and low DN–high fat (LDN-HFY; n = 17; 0.91 ± 0.029 and 1.48 ± 0.311). Data on feed rations and milk yields at nearest test date were assembled, and margin over feed costs (MOFC) were calculated. Linear mixed-effect model implementation in R was used to assess differences in herd performance for clusters and housing with farm location and visit date (month) as random effects. Milk yield per cow was on average 2.5 kg/d lower ($P \leq 0.001$) for high vs. low DN. Feed costs per hectoliter of milk did not differ among clusters. Yet, MOFC per unit of milk was on average greater ($P < 0.05$) for high DN by CAN$2.9/hL of milk and by CAN$0.3/kg of milk fat. MOFC per lactating cow did not differ among clusters but was CAN$1.2 greater ($P < 0.05$) for freestall herds. These results suggest that high DN herds might have adopted more favorable herd management practices resulting in lower overall production costs. Associations with farm management practices collected during the farm visits are addressed by ongoing research. Continuous monitoring of DN in bulk tank milk through FTIR analysis can be a valuable dairy management tool for producers and advisors.

Key Words: fatty acid, mid-infrared spectroscopy, decision support

236 Effects of limits in milking capacity, housing capacity, or fat quota on economic optimization of dry period lengths.

P. Pattamanont and A. De Vries*, *University of Florida, Gainesville, FL.*

The economically optimal dry period length (DPL) of dairy cows remains a topic of interest. Increasing daily milk production and improved management of the transition period require frequent evaluation of the optimal DPL. The economically optimal DPL also depends on the most limiting farm resource such as milking capacity, housing capacity or fat quota. Therefore, the objective of this study was to determine economically optimal DPL under farm constraints on milking capacity, housing capacity, and fat quota given variations in 12 input factor levels. We developed a deterministic whole herd simulation model, including a nonlinear optimizer of the DPL in the first 3 parities. The model included estimates of milk, fat, and protein yield deviations in the subsequent parity and hazard ratios of culling risk and pregnancy rates as functions of the DPL in the current parity. In addition to a one-factor-at-a-time analysis, we used a Definite Screening Design and a space-filling design with Latin hypercube sampling to determine important linear and curvature effects of input factors and their interactions. Results indicated that the economically optimal DPL were typically between 35 and 50 d under a large variation in input factor levels. The opportunity costs of the same DPL in all parities were small compared with optimal policies where the DPL were allowed to vary between parities. The DPL under the parlor constraint were generally approximately 6 d longer compared with the optimal DPL under the housing constraint and 4 d longer compared with the optimal DPL under the quota constraint. Opportunity costs compared with 50 d dry were often small, but in some cases large. A formal global sensitivity analysis revealed important interactions of input factors that were not discovered with one-factor-at-a-time analyses. In conclusion, economically optimal DPL were often shorter than typically are recommended. Limits on milking capacity increased the optimal DPL. A global sensitivity analysis revealed important interactions of input factors

Key Words: dry period, economics, profit

237 Milk flow from 31 to 60 seconds can identify delayed milk ejection.

P. Bacigalupo-Sanguesa*[1,2], P. L. Ruegg[1], B. Norby[1], R. Thomson[1], and R. Erskine[1], [1]*Michigan State University, East Lansing, MI,* [2]*Michigan State University Extension, East Lansing, MI.*

The objective of this study was to identify an interval of milk flow, from a parlor recording system, that would best identify individual cows with delayed milk ejection. Using digital recorders, we measured vacuum in the short milk tube and mouthpiece chamber of 2 liners per cluster during a single milking for 98 cows. Simultaneously, we collected milk flow dynamics from the milking system software (DeLaval DelPro Software). Milking vacuum data were used to calculate time to milk let-down after unit attachment. Milking ejection was categorized as delayed (>30 s) or normal (≤30 s). Individual let-down time and milk ejection category were matched to milk flow during 4 time intervals: <15 s, 15–30 s, 31–60 s, and 61–120 s. For statistical analysis, we used milk let-down time and milk ejection category as independent variables and milk flow as dependent variable. Univariate regression and ANOVA of the 4 time intervals on milk let-down time and milk ejection category, respectively, identified milk flow between 31 and 60 s as the best time interval to predict delayed milk ejection (Table 1). Delayed milk ejection can decrease milk production and impair teat health. The use of on-farm technology to identify and monitor delayed milk ejection incidence could help producers and consultants to address the underlying causes and improve milking efficiency.

Table 1. Regressions of milk flow time intervals and milk let-down time and ANOVA of milk flow time intervals and milk ejection category

Milk flow time interval (s)	R^2		*P*-value
0-14	0.218		0.15
15-30	0.0007		0.80
31-60	0.1015		0.001
61-120	0.0635		0.01
	Delayed milk ejection LSM	Normal LSM	
0-14	0.74	0.57	0.31
15-30	3.75	4.36	0.31
31-60	6.62	6.31	<0.0001
61-120	5.77	7.13	0.04

Key Words: delayed milk ejection, milk flow

Reproduction II

238 Replacing the first gonadotropin-releasing hormone treatment in an Ovsynch protocol with human chorionic gonadotropin decreased pregnancies per AI in lactating dairy cows.

E. M. Cabrera*[1], M. R. Lauber[1], T. Valdes-Arciniega[2], M. S. El Azzi[2], J. P. N. Martins[2], T. R. Bilby[3], and P. M. Fricke[1], [1]*Department of Animal & Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [3]*Merck Animal Health, Kenilworth, NJ.*

Our objective was to compare the effect of treatment with gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) at the first treatment (G1) of the Breeding-Ovsynch portion of a Double Ovsynch (DO) protocol on pregnancies per AI (P/AI). In experiment 1, lactating Holstein-Jersey crossbred cows (n = 1,932; 527 primiparous and 1,405 multiparous) were submitted to a DO protocol for first timed AI (TAI). Cows were blocked by parity and were randomly assigned to receive 100 µg GnRH or 2,500 IU hCG at G1. Cows were inseminated with sexed dairy or conventional beef semen, and data were analyzed within semen type using the GLIMMIX procedure of SAS. The model included the fixed effects of treatment, parity, farm, and the treatment × parity interaction. Overall, P/AI 39 d after TAI for cows inseminated with sexed dairy semen was greater ($P = 0.01$) for cows treated with GnRH than for cows treated with hCG within each parity (primiparous: 42.6% vs. 38.2%; multiparous: 39.4% vs. 30.3%). Similarly, P/AI 39 d after TAI for multiparous cows inseminated with conventional beef semen tended to be greater ($P = 0.06$) for cows treated with GnRH than for cows treated with hCG (41.1% vs. 34.3%). In experiment 2, lactating Holstein cows (n = 43) were randomly assigned to the treatments described for experiment 1. Transrectal ultrasound was conducted every 4 h from 24 to 40 h after treatment to assess time to ovulation, and blood samples were collected immediately before G1, every 8 h from the first $PGF_{2\alpha}$ treatment until the second GnRH (G2) treatment and at TAI to assess luteolysis based on serum progesterone (P4) concentrations. Although mean (±SEM) time from treatment to ovulation was greater ($P = 0.02$) for cows treated with hCG than for GnRH (33.7 ± 0.6 vs. 31.5 ± 0.6 h), P4 concentrations during luteolysis and the proportion of cows with complete luteolysis (P4 < 0.4 ng/mL at G2) did not differ between treatments. We conclude that replacing GnRH with hCG at G1 of a DO protocol decreased fertility to TAI but did not affect the rate or completeness of luteolysis despite the ~2 h longer interval from treatment to ovulation.

Key Words: human chorionic gonadotropin, gonadotropin-releasing hormone, lactating dairy cow

239 Effect of human chorionic gonadotropin post-AI on time to conceptus attachment in lactating dairy cows.

A. Santos*, T. Minela, and J. Pursley, *Michigan State University, East Lansing, MI.*

Elevated levels of progesterone (P4) during embryonic elongation are crucial for successful pregnancy in dairy cattle. We developed a robust technique to estimate d of conceptus attachment (CA) based on daily within-cow changes in concentrations of pregnancy-specific protein B (PSPB; BioPRYN). Highest sensitivity and specificity were obtained with a 12.5% increase followed by 2 d of continuous increases when predicting CA. We hypothesized that increasing P4 during the elongation phase of embryonic development would reduce time to CA and early pregnancy losses in lactating dairy cows. Lactating cows (n = 375) synchronized to Double Ovsynch (first service) or Ovsynch (≥second service) were utilized. Cows received one of 4 treatments: no hCG (controls), or 3,000 IU of hCG on d 2 (D2), 2 and 5 (D2&5), or 5 (D5) after AI. Blood samples for PSBP were obtained on d 18 through 28 after AI. Data were analyzed using the MIXED and LOGISTIC procedures in SAS 9.4 for continuous and binomial variables. Average d + SEM to CA was not different between treatments (20.8 + 0.09, 20.8 + 0.12, 20.8 + 0.13, 21.2 + 0.2, in controls, D2, D2&5, and D5, respectively). However, there was a treatment × parity interaction in time to CA. Third + parity cows had increased d to CA in D5 treatment. There was an effect of treatment in the % of cows that underwent CA at any time. The d 5 treatment reduced the percentage of cows with CA. CA was observed in 62% (229/371) of all cows. There were differences in % of cows with CA determined pregnant vs. non-pregnant on d 34 post-AI (Table 1). A surprising proportion (25%) of non-pregnant cows on d 34 post-AI had CA. Of all cows with CA >23 d post-AI (n = 12), 83% had pregnancy loss before 35 d post-AI. In summary, treatment altered % of cows with CA and time to attachment in 3rd+ parity. Pregnancy losses were associated with later time to CA.

Table 1. Differences in percent of cows with conceptus attachment in pregnant (P) and non-pregnant cows (NP) diagnosed at d 34 post-AI

	Day of conceptus attachment							
Group	20	21	22	23	24	25	26	*P*-value
P (n = 183)	45	43	11	1	0	0	0	
NP (n = 188)	5	7	6	2	1	2	1	<0.001

Key Words: conceptus attachment, embryonic loss, progesterone

240 Effect of gonadotropin-releasing hormone (GnRH) dose and an additional GnRH treatment 2 days following the initiation of Resynch-25 on fertility of lactating dairy cows.

C. E. C. Consentini*[1], M. S. El Azzi[2,3], I. M. R. Leão[2], T. Valdés-Arciniega[2], E. Anta-Galván[2], L. O. Silva[1], R. Sartori[1], and J. P. N. Martins[2], [1]*Department of Animal Science, Luiz de Queiroz College of Agriculture of University of São Paulo (ESALQ/USP), Piracicaba, SP, Brazil,* [2]*Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [3]*Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, MG, Brazil.*

This study evaluated 2 strategies to improve fertility of cows submitted to a Resynch-25 program for 2nd+ AI: (1) a higher GnRH (gonadorelin hydrochloride) dose, and (2) an extra GnRH 2d after the initiation of Resynch-25. Weekly cohorts of cows on d25 after a prior timed AI (TAI; d0) were randomly assigned into 4 groups (G25, G25/27, DG25 and DG25/27) in a 2x2 factorial design. Cows in G25 and G25/27 received 100µg GnRH on d25, and cows in G25/27 received an extra 100µg GnRH on d27 (p.m.). Groups DG25 and DG25/27 initiated Resynch-25 with 200µg GnRH, and cows in DG25/27 received an additional 200µg GnRH on d27 (p.m.). At pregnancy diagnosis on d32 by ultrasound, only nonpregnant cows with CL (primiparous, n = 192; multiparous, n = 573) continued the Resynch-25 protocol [d32 = $PGF_{2\alpha}$; d33 = $PGF_{2\alpha}$; d34 (p.m.) = 100µg GnRH, d35 = TAI]. Data were analyzed by logistic regression using PROC GLIMMIX of SAS with significance declared at $P \leq 0.05$. There was no interaction between the 2 factors (dose and extra GnRH) on pregnancy per AI (P/AI) on d32. Pregnancy/AI on d32 did not differ among treatments (G25 = 45.6%; G25/27 = 41.2%; DG25 = 43.5%; and DG25/27 = 38.9%). The main effects of dose and extra GnRH also did not affect P/AI. Pregnancy/AI on d32 did not differ for cows receiving 100 vs. 200 µg GnRH [100µg (G25 + G25/27) = 43.7% vs. 200µg (DG25 + DG25/27) = 41.5%], and an extra GnRH on d27 did not improve fertility (G25 + DG25 = 44.6% vs. G25/27 + DG25/27 = 40.1%). Moreover, P/AI on d46 (G25 = 42.2%, G25/27 = 37.3%, DG25 = 39.6% and DG25/27 = 37.0%) and pregnancy loss (G25 = 6.9%, G25/27 = 8.7%, DG25 = 8.9% and DG25/27 = 4.8%) did not differ among groups. There was no interaction between treatments and parity or milk production (<53.1 or ≥ 53.1 kg/d). Primiparous and multiparous had similar P/AI on d32 (45.3 vs. 41.7%), and cows with milk production below the median had greater P/AI on d32 (46.9 vs. 38.1%). The present study was the first to determine the effect of an additional GnRH 2d after the beginning of the Resynch-25. Still, neither this strategy nor a higher GnRH dose improved P/AI for 2nd+ AI after Resynch-25 in high-producing Holstein cows.

Key Words: Resynch, gonadotropin-releasing hormone (GnRH), fertility

241 Effect of human chorionic gonadotropin on d 0 and 5 of the estrous cycle on pregnancy per embryo transfer in recipient dairy heifers.

M. S. El Azzi*[1,2], R. V. Sala[3], I. M. R. Leão[1], E. Anta-Galván[1], V. C. Fricke[3], V. A. Absalon-Medina[3,4], M. Dominguez[3], J. C. de Souza[2], and J. P. N. Martins[1], [1]*Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [2]*Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, MG, Brazil,* [3]*ST-Genetics, South Charleston, OH,* [4]*Department of Animal Sciences, Ohio State University, Columbus, OH.*

The objective of this study was to determine the effect of human chorionic gonadotropin (hCG; 3,300 IU) administered on d 0 and/or 5 of the estrous cycle on reproductive parameters in recipient dairy heifers. Dairy heifers (n = 1,297) were synchronized with a 5d-CIDR-Synch program (d-8: CIDR inserted; d-3: CIDR removed + $PGF_{2\alpha}$; d0: GnRH or hCG) and were randomly assigned to 1 of 4 treatments: CON, hCGd0, hCGd5, and hCGd0&5. CON heifers were treated with 100µg GnRH on d0, and heifers in hCGd0 received hCG on d0. Heifers treated with hCGd5 received GnRH on d0 and hCG on d5, while hCGd0&5 heifers received hCG on both d 0 and 5. Ovaries were scanned by ultrasound in a subset of heifers (n = 830) on d 5 and 12 to determine ovulatory response. Blood samples (n = 202) were collected on d 5, 7 and 12 to measure serum P4. Recipient heifers received embryos (n = 1,107) on d 7 and 8 [n = 581 in vitro produced embryos (IVF); n = 243 in vivo embryos (CONV); and n = 283 in vivo frozen embryos (FZ)]. Pregnancy diagnosis was performed on d 32, 46 and 60. Data were analyzed by PROC MIXED and GLIMMIX of SAS. Fixed effects included treatment, embryo type and 2-way interaction. Ovulatory response to d5 hCG did not differ ($P = 0.61$) between hCGd5 (79.4%) and hCGd0&5 (74.0%). Table 1 shows the effect of treatment on serum P4 and total luteal area. hCGd5 heifers had greater ($P \leq 0.06$) 60d P/ET than CON when CONV embryos were used (63.3% vs. 43.6%). In contrast, CON (53.2%) and hCGd0&5 (51.4%) heifers had greater ($P \leq 0.05$) 60d P/ET than hCGd0 (33.3%) using FZ embryos. hCGd0 (24.1%) heifers also had lower ($P < 0.05$) 60d P/ET than CON (39.9%) and hCGd5 (36.9%) using IVF embryos. The overall pregnancy loss (32 to 60 d) tended ($P < 0.10$) to be greater on hCGd0 and hCGd0&5 compared with hCGd5 (17.9, 17.6, and 11.5%, respectively). In summary, hCGd5 only improved P/ET in CONV embryos but did not affect P/ET in FZ or IVF embryos compared with CON. Also, hCG on d0 appears to be detrimental to pregnancy maintenance in FZ or IVF embryos.

Table 1. Means ± SEM showing effect of treatment on serum P4 and total luteal area

	Treatment			
Item	CON	hCGd0	hCGd5	hCGd0&5
Serum P4, ng/mL				
Day 5	3.17 ± 0.21^{a}	4.10 ± 0.47^{ab}	3.15 ± 0.22^{a}	4.84 ± 0.46^{b}
Day 7	5.38 ± 0.29^{aA}	6.89 ± 0.58^{aB}	8.10 ± 0.47^{b}	10.96 ± 0.87^{c}
Day 12	7.12 ± 0.35^{a}	7.36 ± 0.47^{a}	12.39 ± 0.54^{b}	13.00 ± 0.98^{b}
Total luteal area, mm^2				
Day 5	269 ± 6^{a}	313 ± 8^{b}	271 ± 6^{a}	326 ± 8^{b}
12	392 ± 11^{aA}	420 ± 12^{aB}	766 ± 18^{b}	772 ± 19^{b}

$^{a\text{-}c}P \leq 0.05$; $^{A,B}P < 0.10$;.

Key Words: hCG, heifer, embryo transfer

242 Pregnancy outcomes of nulliparous Holstein heifers inseminated with sexed semen after submission to 5-d or 6-d CIDR protocols and timed AI or once-daily detection of estrus and AI after treatment with prostaglandin $F_{2\alpha}$.

M. R. Lauber*, E. M. Cabrera, J. J. Parrish, and P. M. Fricke, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Our objective was to compare expression of estrus and pregnancy outcomes among 3 strategies for submitting dairy heifers for first service using sexed semen. Nulliparous Holstein heifers (n = 736) from 3 commercial farms were randomized within each farm to the following treatments for first service: 1) a 5-d CIDR protocol (n = 255; d −6, GnRH +CIDR; d −1, $PGF_{2\alpha}$ -CIDR; d 0, $PGF_{2\alpha}$; d 2, GnRH$^+$TAI), 2) a 6-d CIDR protocol (n = 254; d −6, GnRH +CIDR; d −1, $PGF_{2\alpha}$; d 0, $PGF_{2\alpha}$ -CIDR; d 2, GnRH$^+$TAI); and 3) EDAI (n = 227; $PGF_{2\alpha}$ on d 0 followed by once-daily estrus detection and AI). Heifers detected in estrus 24 h before TAI (d 1) were inseminated and the protocol was discontinued. All heifers were inseminated with sexed semen (ABS Sexcel Sexed Genetics, DeForest, WI) from sires that were randomly allocated between treatments within each farm, and AI technicians were blind to treatment at AI. Pregnancy outcomes were analyzed using the GLIMMIX procedure of SAS with farm included as a random effect in the model. The proportion of heifers detected in estrus 24 h before TAI on d 1 was greater ($P < 0.001$) for 5-d than for 6-d heifers (28% vs. 0.004%), whereas the proportion of heifers in estrus at TAI did not differ between CIDR treatments (76% vs. 73% for 5-d vs. 6-d heifers, respectively). Overall, 5-d heifers tended to have more P/AI than 6-d and EDAI heifers at 35 ± 5 (53%, 45%, and 46%, respectively; $P = 0.09$) and 64 ± 5 (52%, 45%, and 45%, respectively; $P = 0.10$) d after AI. The fertility advantage for 5-d heifers was due to more ($P = 0.04$) P/AI 64 ± 5 d after AI for heifers inseminated to early estrus on d 1 (62%; n = 71) than TAI (48%; n = 184). In conclusion, although delaying CIDR removal by 24 h suppressed early estrous expression, it tended to decrease ($P = 0.06$) P/AI (52% vs. 45%). Further, heifers inseminated with sexed semen after submission to a 5-d CIDR protocol tended to have more P/AI than heifers submitted to a 6-d CIDR protocol or EDAI. Supported by NIFA USDA Hatch project 1019532 and ABS Global, Inc.

Key Words: sexed semen, dairy heifer, timing of AI

243 Evaluation of a rapid resynchronization program that allows timed embryo transfer every 21 days.

J. P. Andrade*[1], V. Gomez-León[1,2], G. Madureira[1], L. C. Sartori[1], G. F. Grillo[1,3], P. L.

Monteiro[1], M. Fosado[4], R. V. Sala[4], R. Sartori[5], and M. C. Wiltbank[1], [1]*University of Wisconsin, Madison, WI,* [2]*Kansas State University, Manhattan, KS,* [3]*Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil,* [4]*ST Genetics, Kewaskum, WI,* [5]*University of São Paulo, Piracicaba, SP Brazil.*

This study compared a Resynch program for timed embryo transfer (TET) every 35 d (Resy35) to a program for rapid Resynch allowing TET every 21 d (RB21) in recipient heifers (RH) of embryos produced by in vitro fertilization (IVF). Nulliparous Holstein RH were used during a 105d breeding season (BS) synchronized with a modified 5-d Co-Synch (mCo-Synch: CIDR for 5d; prostaglandin treatment at CIDR removal and 3d later GnRH = d0), on d5 if CL ≥ 15 mm then on d7 RH receive fresh or frozen IVF embryo (first ET). After first ET, RH were randomized to 2 groups: Resy35 (n = 264) or RB21 (n = 246). RB21: d14 to d19, CIDR; d21, GnRH; d28, pregnancy diagnosis (PD) a.m., if nonpregnant (NP) and CL ≥ 18 mm, ET on d28 p.m. Resy35: d28 to d33, CIDR; d33, PD and PGF if NP; GnRH-d35.5, on d40, if CL ≥ 15 mm then ET on d42. Additional PDs were performed on d47 and d63 to detect pregnancy losses (PL). NP recipients that failed to ovulate or had PL were resynchronized with mCo-Synch and maintained in the same resynch program. Data analyzed with SAS; Pregnancy/ET and PL by ProcGlimmix; average day of pregnancy by ProcLifetest. Results are summarized in Table 1. During 105d BS RB21 increased rate of ET 1.5-fold for NP heifers with up to 5 ET compared with up to 3 times in the Resy35. Pregnancy/ET and PL were similar for the different resynch strategies. However, PL occurred at different times with less loss in Resy35 from d28–33 (Resynch CIDR present) and greater PL from d33–47 (after CIDR removal). RB21 reduced time to pregnancy and produced ~18% more pregnancies at the end of the BS. Thus, RB21 increased the reproductive performance of IVF embryo recipients during a 105d BS.

Table 1. Reproductive outcomes

Resynch outcomes, % (n)	Resy35	RB21 (ET at day 28)	RB21 (mCo-Synch)
Pregnancy/ET	32.5 (240)	32.0 (141)	33.9 (112)
	Resy35	RB21	
Cumulative pregnancy, %	(n = 264)	(n = 246)	
Day 0	34.5^{A}	41.5^{B}	
Day 21	34.5^{b}	50.4^{a}	
Day 42	50.7	54.9	
Day 63	53.4^{b}	63.4^{a}	
Day 84	61.7^{b}	69.9^{a}	
Day 105	64^{b}	75.2^{a}	
Average day of pregnancy	53^{b}	45^{a}	
Pregnancy loss, % (n)			
Day 28 to 33	3.5 (7)^{b}	10.1 (23)^{a}	
Day 33 to 47	10.2 (20)^{a}	4.9 (10)^{b}	
Day 47 to 63	3.4 (6)	4.6 (9)	
Day 28 to 63	16.3 (33)	18.4 (42)	

$^{a,b,\,A,B}$Lowercase ($P \le 0.05$) and uppercase letters ($P > 0.05 \le 0.1$) represent differences within rows.

Key Words: embryo transfer, ReBreed21, Resynch

Ruminant Nutrition: General II

244 Meta-analysis to determine the impact of trace mineral source on nutrient digestibility in dairy and beef animals. M. Ibraheem*[1], S. Kvidera[2], and B. Bradford[1], [1]*Michigan State University, East Lansing, MI,* [2]*Micronutrient USA LLC, Indianapolis, IN.*

Trace mineral (TM) source can potentially alter nutrient digestibility through effects on microbial populations. A meta-analysis was conducted to determine whether sulfate vs. hydroxy (IntelliBond) sources of supplemental Cu, Zu and Mn had any effect on DMI, DM and NDF digestibility. All available cattle studies (7 studies, 11 comparisons) were used to assess responses (hydroxy mean - sulfate mean). Factors included in the analysis were method of digestibility analysis (total collection, marker-based total-tract, or 24-h in situ), study design (randomized design or Latin square), beef (n = 5) vs. dairy (n = 6) cattle, and days on treatment; these factors were retained when $P < 0.05$. DM digestibility was increased by hydroxy TM in beef (+1.72 ± 0.40 units, $P = 0.01$) but not in dairy models (+0.07 ± 0.26 units, $P = 0.79$). NDF digestibility increased significantly with hydroxy TM ($P = 0.02$), but digestibility assessment method influenced this response. Studies using total collection showed a significant increase (+2.81 ± 0.44 units, $P < 0.01$) in NDF digestibility; studies using $uNDF_{240}$ as a marker tended (+1.70 ± 0.67 units, $P = 0.06$) to show an increase; but studies utilizing 24-h in situ incubation did not show any change (+0.02 ± 0.46 units, $P = 0.97$). These observations may reveal differences in precision of measurement or may indicate mineral impacts beyond the rumen. DMI was not affected by TM source, when expressed as intake per day or as a proportion of body weight ($P > 0.50$). In conclusion, depending on type of cattle and method of measurement, TM source can influence DM and NDF digestibility, which may be explained by differences in solubility of the TM sources in rumen, differentially affecting fermentation.

Key Words: copper, zinc, manganese

245 Supplementing ruminally protected methionine or lysine improved milk production in transition cows. S. Elsaadawy[1], Z. Wu[1], H. Wang[1], M. Hanigan[4], and D. Bu*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Joint Laboratory on Integrated Crop-Tree-Livestock Systems of the Chinese Academy of Agricultural Sciences (CAAS), Ethiopian Institute of Agricultural Research (EIAR) and World Agroforestry Centre (ICRAF), Beijing, China,* [3]*Hunan Co-Innovation Center of Safety Animal Production, Hunan, China,* [4]*Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

The objectives of this study were to evaluate the effects of feeding ruminally protected Met (RPM), ruminally protected Lys (RPL), or their combination (RPML) on performance and blood β-hydroxybutyrate concentrations of transition cows. One hundred-20 prepartum multiparous Holstein cows were allocated to 8 groups and assigned to 4 treatments based on expected calving date, previous 305 d milk yield, parity, and body condition score. Cows were group fed a basal diet (prepartum at 1.53 Mcal/kg DM and postpartum at 1.70 Mcal/kg DM) with or without ruminally protected amino acids. Treatments were the basal diet without supplemental amino acids (CON, n = 30), with supplemental RPM (RPM, pre-calving at 0.16% DM and post-calving at 0.12% DM, n = 30), with supplemental RPL (RPL, pre-calving at 0.33% DM and post-calving at 0.24% DM, n = 30), or the combination (RPML, pre-calving at 0.16% RPM+0.33%RPL DM and post-calving at 0.12% RPM+0.24%RPL DM, n = 30). The prepartum period was 21 d, and the postpartum period was 21d. Individual milk yield and group DMI were measured daily; milk samples were taken 7, 14, and 21 d relative to calving (DRC). Tail vessel samples were taken on −21, −14, −7, 0, 7, 14 and 21 DRC. The experiment was conducted as a completely randomized design with treatments arranged in a 2 × 2 factorial. Pre and post-calving data were analyzed separately using a model containing the main effects of RPM and RPL and time and their interaction using PROC MIXED of SASv9.4. Compared with CON, RPM, RPL and RPML cows had greater DMI pre-calving (11.2 vs 13.6, 13.0, 13.2 kg/d; $P < 0.001$), post-calving (17.4 vs 19.4, 19.8, 22.6 kg/d; $P < 0.001$), and throughout the transition period (14.3 vs 16.5, 16.4,17.9 kg/d; $P < 0.001$). Energy-corrected milk (ECM, 41.3, 41.7, 50.2 vs 36.5 kg/d; $P < 0.001$), milk fat (3.94, 3.93, 4.11 vs 3.74%; $P < 0.001$), protein (3.43, 3.33, 3.53 vs 3.26%; $P = 0.0019$), lactose (5.02, 5.0, 5.10 vs 4.83%; $P < 0.001$), and total solids (13.23, 13.20, 13.60 vs 12.88%; $P < 0.001$) percentage were greater for supplemented cows compared with controls. Blood concentrations of β-hydroxybutyrate decreased with RPM or RPL or their combination pre-calving (0.66, 0.74, 0.66 vs 0.91 mmol/L; $P < 0.001$) and a trend for a decrease post-calving (1.28, 1.27, 1.08 vs 1.50 mmol/L; $P = 0.13$). In conclusion, RPM and RPL supplemented cows had increased DMI pre-and post-calving and increased energy-corrected milk and milk composition post-calving.

Key Words: transition cow, methionine, metabolizable protein

246 Plasma bioavailability of choline and markers of choline absorption in lactating dairy cows provided a ruminal bolus of rumen-protected choline. T. L. France*, W. A. Myers, A. Javaid, and J. W. McFadden, *Department of Animal Science, Cornell University, Ithaca, NY.*

In dairy cattle, choline is needed for methylation, hepatic phospholipid synthesis and triglyceride secretion, and milk production. Ruminal and post-ruminal degradation of choline may limit its intestinal absorption. Our objective was to characterize the plasma bioavailability of choline and markers of choline absorption in response to increasing spot-doses of lipid-encapsulated rumen-protected choline (RPC) delivered directly into the rumen. Four mid-lactation multiparous Holstein cows (120 ± 15 d in milk; 2.8 ± 0.5 parity) were randomly assigned to a sequence of 4 doses in a 4 × 4 Latin square study design: 0 (control), 32, 64, 96 g of RPC to provide 0, 13, 27, and 40 g of choline ion, respectively. Treatments were conditioned with ground corn and administered in gelatin capsules via an esophageal feeding tube 1 h post-feeding. Plasma was collected via a jugular catheter at 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 h relative to bolus administration. Milk was collected in alignment with thrice-daily milking schedule. Alternative treatments were administered every 7-d. Plasma and milk metabolites were quantified using liquid chromatography and mass spectrometry. Data were analyzed under a mixed model including the fixed effects of period, cow, dose, and time. Plasma and milk choline concentrations increased linearly with dose ($P \leq 0.01$). Plasma betaine concentrations were modified by treatment, being greatest for the highest dose ($P = 0.02$). We observed a robust increase in plasma and milk concentrations of trimethylamine *N*-oxide (TMAO; dose, $P < 0.01$; linear, $P < 0.01$), which is a marker of gut microbial choline degradation. Circulating TMAO concentrations were highest at h 4. Treatment also increased plasma total sphingomyelin and phosphocholine concentrations but decreased total phosphatidylcholine concentrations ($P < 0.05$). We conclude that the spot-dose of RPC to the rumen increases choline and choline metabolite concentrations in plasma and milk; however, the observed increase in plasma and milk TMAO indicate that gastrointestinal choline degradation limits bioavailability.

Key Words: bioavailability, choline, methyl donor

247 Effects of prepartum choline supplementation on health and performance in Holstein cows.

M. B. Poindexter*[1], G. Negro[1], M. J. Granados[1], P. G. L. Lima[1], D. C. Ramos[1], D. M. Burgos[1], F. X. Amaro[1], K. Estes[2], M. G. Zenobi[2], C. Zimmerman[2], J. E. P. Santos[1], and C. D. Nelson[1], [1]*University of Florida, Gainesville, FL,* [2]*Balchem Corp., New Hampton, NY.*

Objective was to determine if rumen-protected choline supplementation in the last 3 wk of gestation affects incidence of clinical disease and production of Holstein cows. Holstein cows (n = 2,171) were assigned randomly to 1 of 21 prepartum pens at 252 d of gestation and treatments were assigned to pens with 10 pens receiving control (11 pens, 1,103 cows) and 11 pens receiving rumen-protected choline (RPC, 10 pens, 1,068 cows) as a top dress at a rate of 12.9 g of choline ion per cow per day. All cows were moved to a common fresh pen after parturition where RPC was supplemented in the daily ration so that each cow received approximately 12.9 g/d of choline ion. Pen was the experimental unit and data were analyzed by Mixed and Glimmix procedures of SAS. The model included the fixed effects of treatment, parity, and the interaction between treatment and parity and the random effects of pen within treatment. Colostrum and blood were sampled at parturition. Data were collected by the farm up to 60 DIM for disease and 70 DIM for production. Number of days in the close-up pen, gestation length and body weight of Holstein heifers born to these cows was not different. Colostrum yield, quality, and total yields of components were not different between treatments. Incidence of disease, morbidity, and multiple diseases were similar between treatments except for clinical milk fever where RPC prepartum tended to decrease ($P = 0.09$) incidence. Milk yield in the first 70 DIM was similar between treatments. RPC showed a tendency for reduction in clinical milk fever, however, the data did not show significant reduction in other health parameters measured or increase milk yield in the first 70 DIM.

Table 1. Effect of prepartum choline supplementation (CHOL) on disease and production

Variable	Incidence, % (no./no.)	Treatment		*P*-value
		CHOL	CON	
Milk yield, kg/d		46.6	46.5	0.98
Colostrum yield, kg		6.5	6.8	0.38
Disease				
Calving problems	7.6 (165/2,171)	8.9	6.3	0.31
Retained placenta	4.7 (102/2,171)	4.7	4.7	0.97
Metritis	21.2 (460/2,171)	21.9	20.5	0.45
Mastitis	5.6 (121/2,171)	5.6	5.5	0.67
Milk fever	1.7 (37/2,171)	0.9	2.4	0.09
Morbidity	32.9 (715/2,171)	33.2	32.6	0.52
Multiple disease	6.6 (144/2,171)	5.5	7.7	0.39
Subclinical hypocalcemia	55.8 (967/1,733)	56.0	55.6	0.51

Key Words: choline, health, production

248 Assessment of trace mineral bioavailability: Successes, challenges, and opportunities.

O. Genther-Schroeder*[1] and S. Hansen[2], [1]*Purina Animal Nutrition Center, Gray Summit, MO,* [2]*Department of Animal Science, Iowa State University, College of Agriculture and Life Sciences, Ames, IA.*

Trace mineral (TM) bioavailability has been a topic of research for well over 50 years, and is critical for understanding of TM sources, supplementation, and metabolism. Historically, TM bioavailability was evaluated using radiolabeled isotopes, providing fascinating information about TM distribution and availability to the animal. However, safety concerns eventually limited the use of radiolabeled isotopes. More recently, apparent absorption and slope-ratio methodologies have become commonplace, evaluating mineral absorption through total collection, or careful measurement of TM intake, and subsequent appearance in tissues of interest. Poultry species have often served as models, likely due to affordability and high TM requirements. Interestingly, it appears future evaluation of TM bioavailability may be revisiting previous methods, using stable radio isotopes to evaluate bioavailability more safely. Throughout history and still today, lack of sensitive biomarkers for TM is the major hurdle in the understanding of TM nutrition and bioavailability. However, as metabolomics and bioinformatics methods develop in the animal sciences, these methods may be applied in the search for biomarkers and an improved understanding of the impact of TM on biochemical pathways. Typical methods compare organic trace mineral sources to commonly available inorganic sources, such as sulfates, oxides and chlorides. But application is key, as some sources may only appear more bioavailable when dietary antagonists are present. Challenges and opportunities include on-farm application of bioavailability data, as experimental dietary TM (basal) concentrations, semi-purified diets, animal species or even breed, and lack of antagonists may limit extrapolation of TM bioavailability research to other species and production stages. This is likely especially true for ruminants, who often receive wide ranging diet types. Critical considerations for future research include biomarker development, improved measurement sensitivity, on-farm applicability, and potential external pressures wanting to limit excess TM supplementation in dairy cattle diets.

Key Words: trace mineral, bioavailability

249 The effects of a zinc-methionine complex on blood zinc status and production performance of high-producing dairy cows.

M. J. Oconitrillo*, H. K. J. P. Wickramasinghe, N. Stepanchenko, C. J. Siberski, J. E. Koltes, and J. A. D. R. N. Appuhamy, *Department of Animal Science, Iowa State University, Ames, IA.*

Zinc (Zn) controls many enzymes involved in protein synthesis, energy metabolism, and the antioxidant defense system. The use of Zn methionine complexes (ZMC) have been shown to increase bioavailability of Zn. The objective of this study was to examine the effects of a ZMC in the diet on milk yield, milk composition, somatic cell count (SCC), milk and blood Zn, and antioxidative markers in blood of lactating cows. Twelve Holstein cows (67 ± 2.5 d in milk) were randomly assigned to 1) TMR diet with Zn sulfate at 74 mg/kg of DM (CTL, n = 6); and 2) CTL plus a ZMC (Zn-Met, Debon, Shanghai, China, n = 6) providing additional 20 mg/kg of Zn for 70 d. Milk yield was recorded daily and was analyzed for components and SCC biweekly. Concentration of Zn in blood and milk were analyzed on d 0, 35, and 70. Treatment effects on all response variables except SCC were analyzed using MIXED procedure in SAS with fixed effects of treatment, time (T), and treatment and T interaction (Trt × T), and random effect of cow. The SCC were analyzed using GLIMMIX procedure with Poisson distribution. Dry matter intake decreased by 0.8 kg/d for Zn-Met compared with CTL throughout the study. CTL had greater milk yield than Zn-Met only during first 35 d ($P = 0.010$). Milk protein and fat percentages, and fat yield ($P > 0.240$) were not different between treatments. There was a Trt × T effect on milk protein yield (MPY, $P = 0.049$) as Zn-Met increased MPY during the last 35 d (87 g/d, $P = 0.096$). Similar interaction effect ($P = 0.108$) was also observed for energy-corrected milk (ECM). The Zn-Met tended to have lower SCC ($P = 0.065$) and greater Zn concentration in milk (4.48 vs 4.06 ppm, $P = 0.075$). Blood Zn concentration was higher in Zn-Met on d 70 (1.06 vs 0.81 ppm, $P = 0.051$). The Zn-Met did not affect antioxidative markers in blood including catalase, malondialdehyde, and the ratio between reduced and oxidized glutathione ($P > 0.30$) but superoxide dismutase was lower in Zn-Met than CTL on d 70 ($P = 0.040$). The present ZMC supplement tended to increase Zn in blood, MPY, and ECM, and decrease SCC and SOD more prominently as feeding progressed.

Key Words: antioxidants, organic zinc, milk protein

250 Bioavailability of water-soluble and fat-soluble vitamins during mycotoxin binder supplementation in dairy cows.

A. Kihal*[1], C. Marques[1], M. Rodriguez-Prado[1], E. J. Cunilleras[2], and S. Calsamiglia[1], [1]*Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain,* [2]*Dept. Medicina i Cirurgia Animal, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

The objective of this study was to determine the effect of mycotoxin binders (MTB) on the bioavailability of fat-soluble (FSV) and water-soluble vitamins (WSV) in dairy cows. Six multiparous Holstein-Friesian cows (32 kg/d of milk and 175 DIM) were used. The experimental design was a 2 × 2 crossover, where main factors were the MTB [with 1.2% montmorillonite, DM basis vs. control (without MTB)] and vitamins (A, 160 mg/cow; D, 4 mg/cow; E, 160 mg/cow; B1, 240 mg/cow and B6, 240 mg/cow). Doses were calculated so that the MTB:Vitamins ratio was maintained similar to physiological conditions in vivo. Each period consisted of 7-d adaptation to the MTB, 7-d MTB and vitamin treatment and sampling, and 2 d washout. The MTB was mixed with the diet and fed during the whole period. Vitamins were dissolved in ethanol on the same day of supplementation and administered as a single dose directly into the abomasum by infusion through the ruminal cannula. Blood samples were collected from the jugular vein at 0, 1, 2, 3, 4, 6, 9, 12, 24, 48 h after vitamin administration. Results of plasma levels of vitamins were used to determine the basal concentration (0 h), the maximum concentration (Cmax), the time to reach the Cmax (Tmax), and the area under the curve (AUC) for each vitamin. Results were analyzed with the PROC MIXED of SAS (SAS Inst. Inc., Cary, NC) and differences between treatments declared significant at $P < 0.05$. No differences were observed in Cbasal, Tmax, Cmax and AUC between control and MTB supplemented cows for any of the vitamins, suggesting that MTB did not adsorbed any of the tested vitamins in vivo. Vitamin A reached the Tmax at 5.4 ± 0.89 h with a Cmax at 348 ± 36.9 ng/mL and Cbasal at 247 ± 33.4 ng/mL. Vitamin B6 reached the Tmax at 13.3 ± 3.1 h with a Cmax at 43.6 ± 5.9 ng/mL and a Cbasal at 30.8 ± 4.3 ng/mL. Plasma concentrations of vitamins D (66.6 ± µg/mL), E (5.2 ± 0.9 µg/mL) and B1 (42.2 ± 0.13 µg/mL) were constant over time with no peaks identified. Results of this study do not show evidence that MTB affect the bioavailability of vitamins in vivo.

Key Words: mycotoxin binder, vitamin adsorption, in vivo.

251 Effects of probiotics supplementation on production performance of early-lactation dairy cows.

A. Oyebade*, S. Lee, K. Arriola, H. Sultana, E. Duvalsaint, F. Amaro, I. F. Marenchino, C. Nino De Guzman, L. M. Pacheco, L. Mu, H. Guan, K. V. de Almeida, L. G. Ghizzi, A. Adesogan, D. Vyas, *University of Florida, Gainesville, FL.*

We aimed to evaluate the effect of 2 probiotic supplements on lactation performance, nutrient digestibility and rumen fermentation in early-lactation dairy cows. We used multiparous Holstein dairy cows (n = 114) with an average (mean ± SD) 27 ± 4 DIM, 42 ± 8 kg milk/d, 675 ± 68 kg BW. Study duration comprised of 14-d covariate, 7-d adaptation, and 84-d treatment and data collection period. Cows were assigned to blocks (38 blocks; 3 cows per block) based on the covariate energy-corrected milk yield. Cows within each block were randomly assigned to one of 3 treatments: 1) Control (CON): Corn silage-based TMR without probiotics, 2) PRO-A: Basal diet top-dressed with mixture of *Lactobacillus animalis* and *Propionibacterium freudenreichii* at 3×10^9 cfu/day; and 3) PRO-B: Basal diet top-dressed with a mixture of *L. animalis*, *P. freudenreichii, Bacillus subtilis* and *B. licheniformis* at 11.8×10^9 cfu/day. Milk yield, DMI, and BW were measured daily while milk samples for component analysis were taken on 2 consecutive days of each week of data collection period. Fecal grab samples were taken during covariate, wk 3, 6, 9, and 12 for nutrient digestibility estimations. Data were analyzed using GLIMMIX procedure of SAS and differences were considered significant at $P \leq 0.05$. Model included treatment, week, and treatment × week as fixed effects while cow nested within treatment was considered random factor. Fat-corrected milk (3.5% FCM) and milk fat yield was improved with PRO-B while milk fat percent and feed efficiency (ECM/DMI) showed tendency to increase with PRO-B compared with PRO-A and CON. Ether extract digestibility was greater with PRO-B (73.6 vs 68.8%) compared with the CON. Percentage neutrophils showed tendency to reduce with PRO-A compared with CON and PRO-B. The expression of CD62L in CD8+ cells showed tendency to be higher in PRO-B compared with CON. In conclusion, supplementing PRO-B in early lactation has potential to improve fat-corrected milk yield, feed efficiency and milk fat yield in dairy cows, without inducing any negative effect on digestibility and immune status.

Key Words: direct-fed microbes, fat-corrected milk, feed efficiency

252 Effects of probiotics supplementation on untargeted ruminal and plasma metabolome of early-lactation dairy cows: Untargeted metabolomics approach.

A. Oyebade*[1], A. Adesogan[1], D. Vyas[1], and I. Ogunade[2], [1]*University of Florida, Gainesville, FL,* [2]*West Virginia University, Morgantown, WV.*

This study applied an untargeted metabolomics approach to evaluate the effect of 2 probiotic supplements on rumen and plasma metabolome in lactating Holstein dairy cows. Study duration comprised a 14-d covariate and 84-d treatment period. Forty-five cows were assigned to 15 blocks based on covariate energy-corrected milk yield, and randomly assigned, within each block, to 1 of 3 treatments; 1. Corn silage-based diet without probiotics (Control; CON), 2. Basal diet top-dressed with mixture of *Lactobacillus animalis* and *Propionibacterium freudenreichii* at 3×10^9 cfu/day (PRO-A), and 3. basal diet top-dressed with a mixture of *L. animalis*, *P. freudenreichii*, *Bacillus subtilis*, and *B. licheniformis* at 11.8×10^9 cfu/day (PRO-B). Rumen fluid and blood samples were taken every 21 d during the treatment period. Collected samples were frozen and stored at −20°C. Composite plasma and rumen fluid samples were analyzed using a chemical isotope labeling-LC/MS-based untargeted metabolomics technique to detect and identify metabolites containing amine/phenol, carboxyl, hydroxyl, or carbonyl chemical groups. Differentially abundant metabolites were determined using a volcano plot in Metaboanalyst 4.0 with a false discovery rate (FDR)-adjusted *P*-value ≤0.05. In the rumen, compared with CON, PRO-A increased (FDR ≤0.05) relative concentrations of 9 metabolites including glutaric and shikimic acids, and reduced those of 14 metabolites including proline and phenylalanine. Supplemental PRO-B increased relative concentrations of 16 metabolites including propionate precursors such as succinic and methylsuccinic acids. Compared with CON, relative concentrations of 13 plasma metabolites including propionate and glucose were increased (FDR ≤0.05) by PRO-A while 9 metabolites including cysteine were increased by PRO-B. Pathway analysis of all differentially abundant metabolites revealed important roles of these metabolites in starch and sucrose metabolism, propanoate metabolism, lysine degradation and amino acid biosynthesis. This study demonstrated altered ruminal and plasma metabolome in dairy cows in response to supplemental PRO-A or PRO-B.

Key Words: metabolites, probiotics, rumen

253 Effects of nitrate sources on in vitro methane production, dry matter degradability, and ruminal fermentation parameters in diets differing in starch fermentability.

K. V. Almeida*[1,2], C. N. Guzman[1], I. F. Marenchino[1], P. H. C. Cardoso[1], B. A. R. Andrade[1], L. Mu[1], F. X. Amaro[1], K. G. Arriola[1], H. Sultana[1], G. T. Santos[2], J. L. P. Daniel[2], R. C. Araujo[3], and D. Vyas[1], [1]*University of Florida, Gainesville, FL,* [2]*Universidade Estadual de Maringa, Maringa, PR, Brazil,* [3]*GRASP, Curitiba, PR, Brazil.*

We aimed to evaluate the effects of nitrate (NO_3^-) sources supplemented in diets differing in starch fermentability on in vitro dry matter (IVDMD) and neutral detergent fiber (IVNDFD) degradability, methane (CH_4) and nitrous oxide (N_2O) production, and ruminal fermentation parameters. We hypothesized that supplemental NO_3^- would be effective at reducing CH_4 production regardless of the source used and its efficacy will be greater in diets with rapidly fermentable starch source. The experiment followed a randomized complete block design with a 5 (NO_3^- sources) × 2 (starch sources) factorial arrangement of treatments. Treatments were urea (URE) as a control, potassium NO_3^- (PON), calcium NO_3^- (CAN), dolomite NO_3^- (DON), ammonium NO_3^- (AMN), and diets containing dry rolled corn (DRC) or high moisture corn (HMC). Diets were isonitrogenous and all treatments were set at 20 g/kg DM of NO_3^-. Data were analyzed using GLIMMIX procedure of SAS. Statistical model included treatments as fixed factors while runs were considered as random factors. Data were analyzed separately for 12 and 24 h incubation duration and differences were declared significant at $P \leq 0.05$. In vitro DM and NDF degradability were not affected by NO_3^- sources while HMC improved IVDMD and IVNDFD, increased propionate production, and reduced acetate: propionate ratio. Gas production was reduced by all NO_3^- sources compared with URE regardless of the incubation time. Diets containing HMC decreased CH_4 production by 34% compared with DRC diets. Supplemental NO_3^- decreased CH_4 mitigation compared with URE regardless of the source without major effects on in vitro degradability and ruminal fermentation parameters. Concomitantly, N_2O was greater in all NO_3^- sources compared with URE, while ammonia-N concentration was lower in CAN and DON diets compared with URE. In conclusion, no interaction was observed between NO_3^- and starch sources on the evaluated parameters, and supplemental NO_3^- sources reduced CH_4 production, regardless of the rate of ruminal starch degradation.

Key Words: methane, nitrate, starch

254 Exploring the threshold of non-protein nitrogen in dairy cattle diets by postruminal urea delivery.

K. Nichols*[1], L. Lippens[2], D. Seymour[1], R. Rauch[1], and J. Martín-Tereso[1], [1]*Trouw Nutrition R&D, Amersfoort, the Netherlands,* [2]*Trouw Nutrition R&D, Burford, ON, Canada.*

Inclusion of urea in dairy cattle rations is limited by efficiency of rumen N utilization, hypophagia, and the risk for ammonia toxicity. Post-ruminal delivery of urea reduces ruminal ammonia and may mitigate these limitations and sustain ruminal microbial protein synthesis if the urea is efficiently recycled. Using 4 rumen-fistulated Holstein dairy cows (110 ± 30.8 d in milk) in a 4 × 4 Latin square design, we examined dry matter intake (DMI), milk production and composition, and N balance in response to 4 levels of continuous abomasal infusion of urea (0, 163, 325, and 488 g/d; corresponding to 0, 0.7, 1.4, and 2.1% of expected DMI, respectively). Each 28-d infusion period consisted of a 7-d dose step-up period, 14 d of adaptation, and a 7-d measurement period including 72-h of total urine and fecal collection. A corn silage-based total mixed ration (10.9% CP) meeting 50% of estimated rumen-degradable protein requirements was fed ad libitum. Linear and quadratic orthogonal contrasts of urea dose were estimated by ANOVA assuming fixed effects of period, treatment, and the random effect of cow. Dry matter intake increased with urea dose from 0 to 163 g/d and decreased at increasing doses thereafter (quadratic response; $P = 0.01$). Yield of milk, protein, fat, and lactose followed the same quadratic response ($P \leq 0.04$). Milk protein and urea content increased linearly with increasing urea dose ($P \leq 0.04$). Milk N efficiency decreased linearly with increasing urea dose ($P = 0.02$). Total N excretion (urine + feces) and the proportion of urine N in total excreta N increased linearly with increasing urea dose ($P < 0.01$). Nitrogen balance tended to increase linearly ($P = 0.10$) with increasing urea dose. Results suggest that 163 g/d of post-ruminal urea added to a low CP diet stimulates DMI and milk protein production. Above this level, hypophagic effects reduce milk production and N efficiency. Increasing post-ruminal urea shifts N excretion into urine, increases milk urea content, and may support N retention. Importantly, post-ruminal delivery may expand the threshold of urea inclusion in diets of lactating ruminants.

Key Words: non-protein nitrogen, postruminal, dairy cow

255 Effects of the brown seaweed *Chondrus crispus* on milk yield and enteric methane emissions in dairy cows.

D. C. Reyes*[1], J. P. Sacramento[1], Y. Geng[1,2], L. H. Silva[1,3], N. Price[4], C. Quigley[4], and A. F. Brito[1], [1]*Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH,* [2]*Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*Department of Agriculture and Food Science, Western Kentucky University, Bowling Green, KY,* [4]*Bigelow Laboratory for Ocean Sciences, Boothbay, ME.*

This study investigated the effects of the brown seaweed *Chondrus crispus* on milk yield and composition and enteric CH_4 emissions in dairy cows. Six primiparous and 12 multiparous organically certified Jersey cows averaging 94 ± 45 DIM and 485 ± 54 kg of BW were fed (DM basis) incremental amounts of *C. crispus* (0, 3, and 6%) in a replicated 3 × 3 Latin square design. Experimental periods lasted 24 d, with 14 d for diet adaptation and 10 d for data and sample collection. Diets were fed as TMR, with the control treatment (0% *C. crispus*) consisting of (DM basis) 65% grass-legume baleage and 35% concentrate mix; baleage was replaced with 3 or 6% *C. crispus* in the respective treatments. Diets averaged 16% CP, 35% NDF, and 19% starch (DM basis). Gaseous emissions were measured using the GreenFeed system. Data were analyzed using the PROC MIXED of SAS with treatment and period as fixed effects, and cow within square as random effect. Orthogonal polynomial contrasts were used to test linear and quadratic effects in response to incremental dietary levels of *C. crispus*. While increasing *C. crispus* linearly decreased DMI (19.7, 18.5, and 17.0 kg/d; $P < 0.01$), feed efficiency, calculated as 4% FCM/DMI (1.19, 1.22, 1.37; $P < 0.01$) and ECM/DMI (1.28, 1.31, 1.47; $P < 0.01$), increased linearly. In contrast, yields of milk, 4% FCM, and ECM, and concentrations and yields of milk fat and protein were not affected by treatments. Feeding incremental amounts of *C. crispus* decreased emissions of CH_4 (383, 352, and 351 g/d; $P < 0.01$), CO_2 (10.5, 10.0, and 9.9 kg/d; $P < 0.01$), and H_2 (1.62, 1.44, and 1.35 g/d; $P < 0.01$). Methane yield increased linearly ($P = 0.03$) with increasing dietary levels of *C. crispus* (19.8, 19.6, and 21.5 g/kg of DMI), but no change was observed for CH_4 intensity (mean = 15.3 g/kg of ECM). In summary, CH_4 production decreased linearly in cows fed varying amounts of *C. crispus*. However, the observed drop in DMI resulted in elevated CH_4 yield, particularly at 6% dietary inclusion of *C. crispus*. Collectively, our results suggest no benefits of feeding *C. crispus* at 6% of the diet DM.

Key Words: dairy cow, methane, seaweed

256 Feeding alfalfa- or red clover-grass mixtures: Effects on production and milk fatty acids in dairy cows.

M. J. Lange[2,1], L. H. P. Silva[3], M. Ghelichkhan[1], M. A. Zambom[1], and A. F. Brito*[1], [1]*University of New Hampshire, Durham, NH,* [2]*Universidade Estadual do Oeste do Parana, Marechal Candido Rondon, PR, Brazil,* [3]*Western Kentucky University, Bowling Green, KY.*

Eighteen multiparous and 2 primiparous mid-lactation organic-certified Jersey cows were blocked in pairs by DIM or parity and, within pair, assigned to treatments in a randomized complete block design to investigate the effects of 2 legume-grass mixtures on DMI, milk yield and composition, and milk fatty acid (FA). Two fields were planted with alfalfa (ALF-G)- or red clover (RC-G)-grass mixture with a 79:14:7 legume:meadow fescue:timothy seeding rate (% total). Forages were harvested as baleage, with second and third cuttings used in the study. The botanical composition (DM basis) of second-cut ALF-G or RC-G swards averaged 65 vs. 80% legume, 17 vs. 15% grasses, and 18 vs. 5% weeds, while that of third-cut ALF-G or RC-G averaged 84 vs. 96.5% legume, 3 vs. 2.3% grasses, and 13 vs. 1.2% weeds, respectively. Diets, fed as TMR, were formulated to contain (DM basis) 65% second- and third-cut ALF-G or RC-G (32.5% of each cut) and 35% concentrate. The study lasted 9 wk (2-wk covariate) with sample collection done at wk 4 and 7. Data were analyzed with repeated measures in SAS. Diets averaged 18.8 vs. 18.1% CP for ALF-G vs. RC-G, respectively. No treatments differences were observed for DMI, yields of milk and milk protein, and concentrations of milk fat and protein. In contrast, 4% FCM ($P = 0.05$) and ECM ($P = 0.06$) yields increased with feeding ALF-G. Similarly, milk fat yield was greater ($P = 0.05$) in cows fed ALF-G than RC-G. Significant diet by wk interactions were observed for MUN and proportions of total n-3 FA and 16C FA in milk fat. Specifically, MUN (14.4 to 11.6 mg/dL) and total n-3 FA (0.73 to 0.67%) decreased from wk 4 to wk 6 with the ALG-G diet and did not vary in the RC-G counterpart (mean = 10.3 mg/dL and 0.94%, respectively). Milk proportion of total 16C FA increased from wk 4 to wk 6 with feeding ALF-G (33.1 to 33.9%) without a change in the RC-G diet (mean = 31.3%). No differences were detected for total < 16C FA in milk fat but feeding RC-grass increased total n-6 FA and 18C FA. In brief, ALF-G improved yields of FCM and milk fat, and RC-G decreased MUN and elevated FA with potential human health benefits.

Key Words: baleage, dairy cow, milk yield

257 Feeding alfalfa- or red clover-grass mixtures: Effects on methane emissions and plasma amino acids in dairy cows.

M. J. Lange[2,1], L. H. P. Silva[3], M. Ghelichkhan[1], M. A. Zambom[1], and A. F. Brito*[1], [1]*University of New Hampshire, Durham, NH,* [2]*Universidade Estadual do Oeste do Parana, Marechal Candido Rondon, PR, Brazil,* [3]*Western Kentucky University, Bowling Green, KY.*

Eighteen multiparous and 2 primiparous mid-lactation organic-certified Jersey cows were blocked in pairs by milk yield or parity and, within pair, assigned to treatments in a randomized complete block design to investigate the effects of 2 legume-grass mixtures on CH_4 emissions and plasma AA concentration. Two fields were planted with alfalfa (ALF-G)- or red clover (RC-G)-grass mixture with a 79:14:7 legume:meadow fescue:timothy seeding rate (% total). Forages were harvested as baleage, with second- and third cuttings used in the study. The botanical composition (DM basis) of second-cut ALF-G or RC-G swards averaged 65 vs. 80% legume, 17 vs. 15% grasses, and 18 vs. 5% weeds, while that of third-cut ALF-G or RC-G averaged 84 vs. 97% legume, 3 vs. 2% grasses, and 13 vs. 1% weeds, respectively. Diets contained (DM basis) 65% second- and third-cut ALF-G or RC-G (32.5% of each cut) and 35% concentrate. The study lasted 9 wk (2-wk covariate) with sample collection done at wk 4 and 7. Data were analyzed with repeated measures in SAS. Diets averaged 18.8 vs. 18.1% CP for ALF-G vs. RC-G, respectively. Methane was measured using the GreenFeed system. A significant diet by wk interaction was observed for CH_4 production, with cows fed RC-grass showing lower CH_4 (378 vs. 424 g/d) in wk 4 but no change in wk 7 (mean = 416 g/d). No diet differences were found for CH_4 yield (mean = 21.4 g/kg DMI) and CH_4 intensity (mean = 15.2 g/kg ECM). Diets did not affect the plasma concentrations of Lys and Met. However, plasma Leu increased with feeding RC-G ($P = 0.03$; 178 vs. 142 μM). Significant diet by wk interactions were found for the plasma concentrations of Arg, His, Phe, Trp, Val, and total EAA. Feeding RC-G increased ($P \leq 0.05$) plasma Arg, Phe, Val, and total EAA in wk 7 but not in wk 4. Further, RC-G elevated plasma His more noticeably in wk 7 (+62%) than wk 4 (+38%). Compared with ALF-G, plasma Trp decreased in cows fed RC-grass in wk 4 and increased in wk 7. In brief, while the impact of forage sources on CH_4 emissions was small, RC-grass appeared to be more effective than ALF-grass to elevate plasma EAA concentrations when fed as baleage.

Key Words: amino acid, dairy cow, forage

Ruminant Nutrition: Calves and Heifers II

258 Effects of live yeast supplementation on calf intake and growth under stressful conditions. S. J. Davies*[1], G. Esposito[1,2], C. Villot[3], E. Chevaux[3], and E. Raffrenato[1,2], [1]*Stellenbosch University, Stellenbosch, WC, South Africa,* [2]*RUM&N Consulting, Reggio Emilia, Italy,* [3]*Lallemand SAS, Blagnac, France.*

Incorrect management practices affect the incidence of calves' morbidity and mortality. Strategies aiming at mitigating the negative effects of stressful practices should therefore be investigated. Direct-fed microbials, consisting of living microorganisms, such as bacteria and fungi, may comply with this role by balancing the microflora in the intestine. The aim of this study was to determine the effects of a live yeast (*Saccharomyces cerevisiae boulardii* CNCM I-1079, Lallemand SAS, France) on calf performance in the presence or absence of a nutritional stress. Eighty Holstein heifer calves, with blood total protein > 5.5 g/dl at 3 d of age (indicating a successful passive transfer) were enrolled from d 5 of age until weaning (90 dd) in a randomized block design and randomly assigned to one of the 4 treatment groups devised according to the presence or absence of a nutritional stress and the inclusion or exclusion of yeast. The trial was conducted at a commercial dairy farm in Klipheuwel, Western Cape, South Africa. The inclusion rate of the yeast supplement was 1 billion cfu/calf/day, equally divided between the 2 milk replacer feedings. The nutritional stress was implemented every other day by delaying one feeding by 2 h, knowing that all calves were housed in the same barn. All calves were fed milk replacer and a pelleted starter was offered ad libitum. Intake of the starter was measured daily and body weight (BW) was recorded weekly. A repeated-measures (i.e., day) mixed model was used with yeast and stress and their interaction as fixed components, and calves as random component. Individual BW at enrollment was included as covariate. Calves in the stress group had a lower intake of starter ($P < 0.01$) at 724 g/d vs. 830 g/d for the no-stress group and the inclusion of yeast tended to result in higher intake ($P = 0.08$) at 799 g/d vs. 754 g/d of for the no-yeast group. The stress resulted in lower average BW when pooling days ($P = 0.03$; 66.8 vs. 64.1 kg) and the yeast tended to increase BW ($P = 0.07$; 66.6 vs. 64.3 kg). The interaction between stress and yeast tended to be significant ($P = 0.08$), with the yeast resulting in a larger effect when stress was present. The inclusion of the yeast in the milk replacer was therefore able to decrease the negative effect of the stressful condition.

Key Words: *Saccharomyces cerevisiae boulardii*, weaning, calf management

259 The effect of feeding acidified colostrum on fecal microbiota in Holstein dairy calves throughout the weaning transition. M. Hennessy*, N. Indugu, B. Smith, S. Cady, M. Kristula, and D. Pitta, *Department of Clinical Studies, New Bolton Center, University of Pennsylvania-School of Veterinary Medicine, Kennett Square, PA.*

Calf diarrhea is a leading cause of death in pre-weaned calves and causes major economic losses to producers. Acidified milk has been shown to reduce the prevalence of diarrhea in calves but the effect on microbiota is unknown. The purpose of this study was to compare how feeding acidified colostrum to calves at birth influences fecal microbiota from birth through 8 weeks of age compared with calves fed non-acidified colostrum. In this study, 5 calves received acidified colostrum (treated group) and 5 calves received non-acidified colostrum (control group). Both groups received 10 quarts at birth and 2 quarts at 12 h of age of their respective colostrum. All calves were subsequently fed acidified whole milk until weaning at 8 weeks of age and had access to starter grain starting at d 7 and throughout the study. Fecal samples were collected at 24 h, 48 h, and at 1, 2, 3, 4, 5, 6, 7, and 8 weeks of age. Samples were extracted for genomic DNA, PCR-amplified for the V1-V2 region of the 16S rRNA bacteria gene, sequenced on the Illumina MiSeq platform, and analyzed using QIIME2. Bacterial richness, estimated by number of observed species, and bacterial diversity, estimated by Shannon diversity index, differed between time points but not between treatment groups and both increased over time ($P < 0.05$), with the largest increases occurring during the weaning transition. Weighted and unweighted UniFrac analysis showed differences ($P < 0.05$) between bacterial communities across time points but not between treatments. Six bacterial genera were different ($P < 0.05$) between treatments: *Fecalibacterium* and unclassified *Clostridiaceae* were more abundant while *Atopobium, Collinsella, CF*231, and unclassified *Veillonellaceae* were less abundant in treated versus control calves. *Fecalibacterium* is a butyrate-producing bacterium that has been linked to decreased prevalence of diarrhea in calves. Our results indicate that there is considerable flux in the calf fecal microbiome through the weaning transition but that feeding acidified colostrum followed by acidified whole milk allowed beneficial bacteria such as *Fecalibacterium* to thrive and potentially out-compete pathogenic bacteria.

Key Words: acidified milk, calf diarrhea, calf weaning

260 The effect of tributyrin supplementation to milk replacer on growth performance and microbiota of dairy bull calves. S. Liu*[1], J. Wu[2], J. Zhou[1], J. Ma[1], and Z. Cao[1], [1]*State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*Institute of Animal Science, Guizhou Academy of Agricultural Sciences, Guizhou, China.*

The aim of this study was to evaluate the effect of rumen-unprotected tributyrin supplementation on performance and microbiota of dairy bull calves during the preweaning period. Thirty newborn Holstein dairy bull calves (40.4 ± 2.65 kg of bodyweight; mean ± SD) were equally assigned to 1 of 3 treatments: a) calves were fed unaltered milk replacer (fat: 3.12%; protein: 3.51%; CON); b) calves were fed milk replacer supplemented with commercial tributyrin products (unprotected solid powder; 35% tributyrin adsorbed on silicon dioxide) at 8 g/kg of DM (B1); C) calves were supplemented with commercial tributyrin products at 16 g/kg of DM (B2). The study ended when all calves reached d 56 of age, and 18 of them (6 per treatment) were slaughtered 2 h after the morning feeding. The colon digesta was collected for microbiota analysis. Starter intake, fecal consistency scores and body weight were also recorded. All repeated, continuous data were analyzed using the MIXED procedure of SAS. The model included the fixed effects of time, treatment, and time × treatment interaction, and calf within treatment as a random effect. Differences in gut bacterial abundance were analyzed by LDA LEfSe. LEfSe analysis used the Kruskal-Wallis rank sum test to detect significantly different abundances and perform LDA scores to estimate the effect size (threshold: = 4). All treatments had similar average daily gain (518.5 g/d; $P = 0.97$) and starter intake (348.6 g/d; $P = 0.82$). Increasing tributyrin level was associated with a negative trend response in diarrhea frequency ($P = 0.02$). The results related to microbiota showed that Chao and OTU in the colon were lower in treatment B1 and B2 than in the CON group ($P < 0.05$). The principal coordinate analysis showed separations among treatments ($R^2 = 0.43$, $P = 0.001$) in colon. LEfSe analysis showed CON group had higher

Betaproteobacteria while B1 group had higher *Bacteroidia* and B2 group had higher *Firmicutes* and *Prevotella* in colon. In conclusion, rumen-unprotected tributyrin supplementation reduced diarrhea frequency of calves and altered a diversity and β diversity of microbiota in colon.

Key Words: tributyrin, calf, microbiota

261 The effects of a phytogenic feed additive on stress markers in calves subjected to a diurnal heat stress bout.

H. K. J. P. Wickramasinghe*[1], N. Stepanchenko[1], M. J. Oconitrillo[1], J. V. V. Silva[1], B. M. Goetz[1], M. A. Abeyta[1], S. M. Ramirez[2], G. R. Murugesan[2,3], A. Tacconi[3], L. H. Baumgard[1], and J. A. D. R. N. Appuhamy[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*BIOMIN America Inc, Overland Park, KS,* [3]*BIOMIN Holding GmbH, Getzersdorf, Austria.*

Dietary supplementation of plant-based (phytogenic) feed additives (PFA) is postulated to alleviate oxidative stress (OS) and intense immune activation during heat stress (HS). The study objective was to determine the effects of a proprietary blend of PFA (Digestarom, BIOMIN America Inc., Overland Park, KS) on markers of stress in calves experiencing diurnal heat stress (dHS). Holstein (18) and Jersey (4) heifer calves (5–6 mo of age) were assigned randomly to 1 of 2 dietary treatments: 1) basal diet (CTL; n = 11), or 2) CTL supplemented with 0.25 g/d of PFA (TRT; n = 11). Calves were housed individually and fed ad libitum for 14 d. Body weight was recorded and jugular blood was drawn on d 14. Calves were then subjected to 7 d of dHS by setting barn temperature to 33°C from 0900 to 2100 h and allowing it to equilibrate with outside temperature at night (24°C). Calves were followed for 4 d after dHS was ceased. Rectal (Tr) and skin (Ts) temperature were recorded, and blood was drawn for OS markers including protein carbonyl, thiobarbituric acid reactive substances (TBARS), and acute phase proteins such as haptoglobin (HPT) and lipopolysaccharide-binding protein (LBP) during and 4 d after dHS. Stress indicators, cortisol (CO) and L-lactate (LA), were also measured. Total DMI (tDMI) and DMI during 0900 and 2100 h (hsDMI) were recorded daily. Body weight was recorded on the last day of dHS and 4 d after. Treatment effects were analyzed using the MIXED procedure in SAS with treatment and time as fixed effects and calf as a random effect. Overall, dHS decreased tDMI and weight gain ($P < 0.01$), and increased Tr (40.3 vs 39.4°C), Ts, serum HPT, plasma LA and LBP ($P < 0.04$) relative to baseline. However, TRT had greater hsDMI and decreased serum HPT, plasma LBP and LA than CTL during dHS (7, 34, 17, and 11%, respectively; $P \leq 0.05$). Moreover, plasma CO tended to increase from baseline during dHS ($P = 0.07$) but both plasma CO (16%, $P = 0.11$) and TBARS (9%, $P = 0.14$) were numerically lower in TRT than CTL. Regardless of treatments, serum HPT decreased to baseline ($P = 0.02$) while tDMI and weight gain increased even above baseline values ($P < 0.01$) 4 d after dHS. Overall, PFA supplementation appeared to mitigate metabolic and inflammatory stress in calves subjected to intense diurnal heat stress conditions.

Key Words: cortisol, inflammation, oxidative stress

262 Effects of preweaning L-glutamine supplementation on growth performance and health measures in Holstein heifer calves.

G. Ceja*[1], J. P. Boerman[1], R. C. Neves[2], J. P. Schoonmaker[1], M. W. Jorgensen[3], R. N. Klopp[1], A. T. Richards[1], and J. S. Johnson[3], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN,* [3]*USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN.*

L-Glutamine (GLN) supplementation improves immune function, intestinal health, and growth performance in dairy calves subjected to health and stress challenges under controlled conditions. However, it is unknown whether supplementing dairy calves with GLN will improve growth and health outcomes under production conditions. The study objective was to determine whether feeding dairy calves GLN supplemented milk replacer (MR) throughout the preweaning period would improve growth and health measures. Thirty Holstein heifer calves [1.5 ± 0.5 d old; 37.1 ± 0.86 kg body weight (BW)] were blocked by serum total protein, BW, and age, and randomly assigned to 1 of 2 treatments: GLN [24% crude protein (CP)], 17% fat MR +10 g GLN/kg MR powder) or NS (24% CP, 17% fat MR). Milk replacer was reconstituted to 12.5% solids with warm water and fed 3.8 L/calf/d until weaning (56.4 ± 0.5 d). Calves had ad libitum grain (17% CP, 2% fat) and water access. Preweaning, calves were individually housed in hutches and respiratory and fecal scores were assessed daily, BW was measured weekly, and grain and MR intake was assessed daily to calculate average daily gain (ADG), average daily feed intake [ADFI; grain intake (DM basis) + MR intake (DM basis)], and feed efficiency (ADG:ADFI). Two NS calves died from enteric disease, and 1 GLN calf was euthanized due to a joint infection. At weaning, calves were weighed, moved to pens (n = 3 pens/treatment, 4–5 calves/pen), provided free access to grain and grass hay, and then weighed at 2 wk postweaning. Data were analyzed with PROC MIXED in SAS 9.4 and treatment, week, and their interactions were included as fixed effects. No preweaning ADG or ADFI differences were observed ($P > 0.10$), but feed efficiency tended to be greater ($P < 0.07$) for GLN (0.17 ± 0.01) vs NS (0.15 ± 0.01) calves. No treatment-related fecal or respiratory score differences were observed ($P > 0.10$). Postweaning ADG tended to be greater ($P < 0.10$) for GLN (0.89 ± 0.06 kg/d) vs NS (0.76 ± 0.06 kg/d) calves. In summary, preweaning GLN supplementation provided some growth benefits, but no significant improvements to respiratory and fecal scores were observed.

Key Words: dairy calf, growth, L-glutamine

263 Nucleotide dietary supplementation in calves: Effects on calf immune response.

P. De Palo*[1], F. R. Dinardo[1], G. Elia[1], M. F. Sgarro[1], G. M. Liuzzi[2], G. Calzaretti[1], and E. Casalino[1], [1]*Department of Veterinary Medicine, University of Bari A. Moro, Valenzano, BA, Italy,* [2]*Department of Biosciences, Biotechnologies and Biopharmaceutics, University of Bari A. Moro, Bari, BA, Italy.*

Mammals may recover nucleotides through the diet, by the salvage pathway, or by de novo synthesis. The latter one is a metabolically costly process, thus, the salvage pathway to recycle dietary nucleotides is more favorable, especially during the growth phase. This study aimed to evaluate the effects of dietary nucleotides supplementation on the immune response of calves and on their ability to counteract the oxidative stress. Twenty male Holstein Friesian calves were randomly divided in 2 groups: one was individually and orally supplemented from birth to 25 d with 5 g/head/day of an additive containing 40% of yeast free nucleotides 5′monophosphate (NG); the other group represented the control (CG), supplemented with 20 mL/head/day of fresh water. All the animals were fed with the same natural colostrum, milk replacer, calf starter and hay, and with the same standardized protocols. Blood samples were collected at 0, 3, 7, 15 and 25 d. All data were processed according to a repeteated measures ANOVA, setting dietary treatment, samples timing and their binary interaction as independent variables, each single calf as random effect. In plasma from NG, superoxide dismutase and glutathione peroxidase activities showed an increasing ($P < 0.01$) trend over time, whereas their activity remained almost stable in CG. The total antioxidant capacity of plasma (ABTS radical scav-

enging assay and Ferric Reducing Ability of Plasma, FRAP assay) was higher in NG compared with CG ($P < 0.01$). Overall, peripheral blood mononuclear cell (PBMC) viability did not show differences according to dietary treatment, even after stimulation with H_2O_2. Untreated PBMC showed the lowest levels of intracellular reactive oxygen species (ROS) in NG ($P < 0.05$). Under H_2O_2 stimulus, intracellular ROS levels significantly rose ($P < 0.05$) in PBMC from CG, while in NG there was a slight increase. The levels of ROS and of reactive nitrogen species (RNS) in PBMC supernatants were found at the lowest levels in NG ($P < 0.05$). The serum levels of matrix metalloproteinases-9 (MMP-9) were influenced by feeding supplementation. In particular, lower levels ($P < 0.05$) of MMP-9 were observed in NG compared with CG. The feeding treatments did not affect the serum concentrations of MMP-2. We concluded that dietary supplementation with nucleotides exerted a beneficial role on calves' oxidative imbalance and on immune function modulation.

Key Words: dietary nucleotides, calf, immune response

Ruminant Nutrition: Carbohydrates and Lipids II

264 Palmitic and oleic acids modulate lipogenesis in dairy cow adipocytes. U. Abou-Rjeileh*, A. L. Lock, and G. A. Contreras, *Michigan State University, East Lansing, MI.*

Targeted fatty acid (FA) supplementation in dairy cows has beneficial effects on health and production. For example, palmitic acid (C16:0; PA) increases energy output in milk while oleic acid (*cis*-9 C18:1; OA) minimizes bodyweight loss by reducing FA trafficking and enhancing insulin sensitivity. In monogastrics, OA is a potent promoter of adipocyte lipogenesis, however, it is unknown if this effect is observed in dairy cow adipocytes. Thus, the objective of our study was to evaluate the role of PA and OA on lipogenesis in dairy cow adipocytes. Subcutaneous (SAT) and visceral (VAT) adipose tissue were collected from multiparous, nonlactating, non-gestating Holstein cows (n = 8). Stromal vascular fraction was obtained via collagenase digestion. Pre-adipocytes were isolated following the outgrowth of plastic adherent cells and induced to differentiate. After induction, adipocytes were cultured with standard differentiation medium (CON) supplemented with PA and OA at 100, 200, and 300 μM, or mixed PA:OA ratios at 60:40, 50:50, and 40:60 at 300 μM for 14 d. Lipogenesis was evaluated by lipid accumulation (Adipored, RFU/ng DNA). Expression of adipogenic and lipogenic gene networks was evaluated using RT-qPCR. Statistical analyses were performed using a mixed effect linear model in JMP. Overall, SAT adipocytes accumulated more lipids compared with VAT (3,908 ± 552 vs 1,884 ± 554; $P < 0.01$). FA supplementation enhanced lipogenesis in SAT adipocytes with 300OA (4,825 ± 1,315), 300PA (6,624 ± 1,315), 50:50 (5,792 ± 1,609), and the 60:40 mix (7,753 ± 1,609) accumulating more lipids compared with CON (1,237 ± 1,315; $P < 0.05$). There was no effect of treatments in VAT adipocytes. The expression of the insulin-sensitizing *ADIPOQ* decreased in 200PA compared with CON ($P < 0.05$). 200PA also decreased the expression of the lipogenic genes *PLIN1* ($P < 0.01$) and *FABP4* ($P < 0.001$) compared with 300OA and 60:40, and the expression of *DGAT1* compared with 60:40 ($P < 0.05$). Conversely, 300OA and 60:40 decreased the expression of *PPARA* compared with CON ($P < 0.01$). Our results show that OA and PA at 300 μM enhance lipogenesis in dairy cow adipocytes. PA alone suppresses the expression of key genes involved in adipogenesis and lipogenesis, however, when combined with OA, the effect is attenuated. Together these results provide mechanistic evidence for the use of OA alone, or in combination with PA, in dairy cow diets where fat accumulation is needed to minimize lipolysis.

Key Words: palmitic acid, oleic acid, lipogenesis

265 Effects of high oleic expeller-pressed soybean meal on milk fat and fatty acid digestibility in dairy cows. S. L. Bennett*, R. Bomberger, and K. Harvatine, *Pennsylvania State University, State College, PA.*

Abomasal infusion of oleic acid has been shown to increase fatty acid digestibility. Oleic acid also has a lower risk for diet-induced milk fat depression compared with linoleic acid and thus can increase milk fat yield. The objective of this study was to evaluate the effect of high oleic compared with conventional expeller soybean meal in lactating dairy cows. The hypothesis was that the high-oleic expeller soybean meal would increase milk fat by reducing the risk for milk fat depression. Eighteen multiparous Holstein cows were used in a crossover design with 24 d periods. Each period consisted of 2 dietary phases that differed in risk for diet-induced milk fat depression. During the first 14 d, a diet with low-risk for milk fat depression (32% NDF, no added oil) was fed and during the final 10 d a moderate-risk diet was fed (28.5% NDF, 0.5% corn oil). The treatments were conventional expeller soybean meal and expeller meal from high oleic soybeans fed at 7.5% of diet DM. All cows were fed the low-risk diet with conventional soybeans for a 10-d pretrial and a 7-d washout period. Milk samples were taken on d 13, 14, 23 and 24. Data were analyzed using the MIXED procedure of SAS. The model included the random effects of cow and period and the fixed effects of treatment, day, and their interaction. There were no treatment or treatment by day interactions on milk yield (49.6 kg ± 2.23; mean and SEM) or milk fat and protein yield or composition. High oleic soybean meal increased MUN concentration by 0.64 mg/dL ($P < 0.01$) and there was no treatment by time interaction. There was an effect of experimental day on yield and concentration of milk fat and protein. Milk fat averaged 3.99% during the low-risk phase and 3.47% during the moderate-risk phase ($P < 0.01$), while milk protein averaged 2.89% and 3.01% during these phases ($P < 0.01$), respectively. There were no effects of high oleic soybean on major milk production parameters and no interaction of high oleic expeller meal and the moderate milk fat depression induced during the high-risk phase.

Key Words: milk fat depression, oleic acid

266 Altering the ratio of palmitic and stearic acids in supplemental fatty acid blends impacts production responses of mid-lactation dairy cows. A. M. Burch*, M. E. Kloboves, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We determined the effects of altering the ratio of palmitic (C16:0; PA) and stearic (C18:0; SA) acids in supplemental fatty acid (FA) blends on production responses of mid-lactation dairy cows. Twenty-four multiparous Holstein cows (47.1 ± 5.83 kg/d of milk; 109 ± 23 DIM) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 21-d periods. Treatments were a non-FA-supplemented control diet (CON), and 3 diets incorporating 1.5% DM FA supplement blends containing 80% PA and 10% SA (H-PA), 50% PA and 30% SA (M-PA), or 30% PA and 50% SA (L-PA). FA blends were balanced to contain 10% oleic acid. The FA blends replaced soyhulls in the CON diet. Diets were formulated to contain (% DM) 31.0% NDF, 27.0% starch, and 16.9% CP. The statistical model included the random effect of cow within square and the fixed effects of period, treatment, and their interaction. Pre-planned contrasts included CON vs. overall effect of FA supplementation (FAT) and the linear and quadratic effects of decreasing PA in the FA blends. Results in the text are presented in the following order: CON, H-PA, M-PA, and L-PA. There was no effect of treatment on DMI ($P = 0.37$). Compared with CON, overall FAT increased the yields of milk (44.5, 45.6, 45.5, 45.5 kg/d; $P = 0.01$), 3.5% FCM (46.1, 48.6, 48.1, 47.8 kg/d; $P < 0.01$), ECM (46.1, 48.2, 47.7, 47.5 kg/d; $P < 0.01$), and milk fat (1.66, 1.77, 1.75, 1.73 kg/d; $P < 0.01$), but did not affect milk protein yield ($P = 0.54$). Decreasing PA linearly decreased milk fat yield ($P = 0.03$) and tended to linearly decrease the yields of 3.5% FCM and ECM (both $P = 0.10$). In summary, feeding FA supplements containing PA and SA increased yields of milk, milk fat, 3.5% FCM, and ECM with no effect on DMI compared with a non-FA supplemented control diet. Decreasing the level of PA linearly decreased milk fat yield and tended to decrease 3.5% FCM and ECM yield demonstrating that FA supplements higher in PA, compared with SA, increase production responses of mid-lactation dairy cows.

Key Words: fat supplementation, palmitic acid, stearic acid

267 Milk production responses to altering the ratio of palmitic and oleic acids in diets with high or low metabolizable protein with supplemental methionine and lysine.

A. N. Negreiro* and A. L. Lock, *Michigan State University, East Lansing, MI.*

Our objective was to determine the effects of fatty acid (FA) supplements with different ratios of palmitic and oleic acids in diets formulated for high metabolizable protein (MP) or low MP with supplemental methionine (Met) and lysine (Lys) on yields of milk and milk components of mid-lactation cows. Thirty-six Holstein cows (53 ± 14 kg milk/d; 107 ± 49 DIM) at the start of the study were equally allocated to a split plot receiving either a high protein (HP) basal diet containing 18.1% CP (MP 11.6% diet DM, Lys 6.23% MP, Met 1.73% MP) or a low protein (LP) basal diet containing 16.6% CP (MP 10.4% diet DM, Lys 6.68% MP, Met 2.19% MP). Diets were balanced (% diet DM) for similar NDF (28.5%) starch (28.0%), and RDP (10.2%). Within each plot a 3 × 3 Latin square arrangement of treatments was used with 3 21 d periods, with sample and data collection during the final 5 d of each period. Treatments were a control diet with no supplemental fat (CON) and 2 diets incorporating 1.5% of DM with FA supplement blends containing 80% C16:0 + 10% C18:1 (PA) or 60% C16:0 + 30% C18:1 (OA). The statistical model included the random effect of cow nested within basal diet and fixed effects of basal diet, treatment, period, and their interactions. Compared with HP, LP increased N use efficiency (NUE; 0.29 vs 0.27%; $P < 0.01$) and had no effect on DMI or yield of milk and milk components (all $P > 0.10$). Results for FA treatment effects are presented in the following sequence: CON, PA, and OA. Compared with CON, FA treatments decreased DMI (33.6, 33.3, 32.7 kg/d; $P < 0.01$) and increased milk fat yield (1.94, 2.01, 2.00 kg/d; $P < 0.01$), ECM yield (54.1, 55.2, 55.1 kg/d; $P < 0.01$), and feed efficiency (ECM/DMI; 1.61, 1.66, 1.68 kg/d; $P < 0.01$). We did not observe treatment by basal diet interactions for the yields of milk or milk components but there was an interaction for milk protein content where FA treatments decreased protein content more in HP than LP (interaction $P < 0.09$). In conclusion, reducing dietary MP content with supplemental Met and Lys increased NUE and the addition of FA supplements increased milk fat and ECM yields regardless of basal diet.

Key Words: amino acid, fatty acid, milk production

268 Altering the ratio of dietary palmitic, stearic, and oleic acids during the immediate postpartum affects production responses of early-lactation dairy cows.

J. M. dos Santos Neto*, C. M. Prom, and A. L. Lock, *Michigan State University, East Lansing, MI.*

Our objective was to determine the effects of varying the ratio of dietary palmitic (C16:0), stearic (C18:0), and oleic (C18:1) acids on production responses of early-lactation cows. Fifty-six multiparous cows were used in a randomized complete block design and assigned to one of 4 treatments fed from 1 to 24 DIM. The treatments were a control diet (CON; non-fatty acid [FA] supplemented diet) and diets supplemented at 1.5% DM with FA blends containing different ratios of C16:0, C18:0, and C18:1. FA treatment diets were: a FA blend containing 80% C16:0 (PA); a FA blend containing 40% C16:0 and 40% C18:0 (PA+SA); and a FA blend containing 45% C16:0 and 35% C18:1 (PA+OA). FA blends replaced soyhulls in CON. Overall, diets (%DM) contained 29.8% NDF, 24.6% starch, 16.7% CP, and 2.49% FA for CON, and 4.00% FA for the FA treatments. The statistical model included the random effect of block, cow within block and treatment, and Julian date, and the fixed effects of treatment, time, and their interactions. Results are presented in the following sequence: CON, PA, PA+SA, and PA+OA. Compared with CON, PA+OA decreased DMI, while PA and PA+SA had no effect (23.1, 23.7, 23.1, 21.6 kg/d; $P < 0.05$). Compared with CON, FA treatments did not alter milk yield, but PA and PA+SA increased milk yield compared with PA+OA (41.8, 43.4, 43.1, 40.8 kg/d; $P < 0.01$). PA increased milk fat yield compared with CON and PA+OA and tended to increase it compared with PA+SA (1.78, 1.92, 1.83, 1.74 kg/d; $P < 0.01$). Compared with the other treatments, PA increased 3.5% FCM (47.6, 51.2, 49.0, 47.3 kg/d; $P < 0.01$) and ECM (46.9, 49.4, 47.2, 45.8 kg/d; $P < 0.01$). A treatment by time interaction was observed for 3.5% FCM ($P = 0.04$) because compared with CON, both PA and PA+SA increased 3.5% FCM in wk 1 and 2 postpartum, but only PA increased 3.5% FCM in wk 3. There were no effects of treatment on body weight or body weight change ($P \geq 0.25$). In conclusion, feeding a FA supplement containing 80% C16:0 during the immediate postpartum increased ECM without changes in DMI or body weight.

Key Words: fat supplementation, fatty acid profile, postpartum

269 Lysophosphatidylcholine enhances the oxidative burst response, inflammatory cytokine secretion, and *Escherichia coli* killing in polymorphonuclear neutrophils derived from Holsten heifer calves.

B. N. Tate* and J. W. McFadden, *Department of Animal Science, Cornell University, Ithaca, NY.*

The dairy calf is a vulnerable host to pathogen infection due to declining passive immunity and premature active immunity; therefore, the calf is highly susceptible to disease. Antibiotics are often used to treat disease in calves; however, their use is often mismanaged, subject to bacterial resistance, and discouraged by consumers. Alternative non-antibiotic therapies are needed. Because lysophosphatidylcholine (LPC) modifies innate immune function in non-ruminants, our objective was to determine whether LPC influences the oxidative burst response, inflammatory cytokine secretion, and *Escherichia coli* killing in bovine polymorphonuclear neutrophils (PMN). Blood was collected from 2 to 5 wk old Holstein heifer calves. PMN were isolated using density gradient centrifugation and cultured for 2 to 6 h in media. The effects of LPC, including stearoyl-LPC (LPC-18:0) at various concentrations (0 to 100 μM), were evaluated on H_2O_2 production, tumor necrosis factor-α (TNFα) and interleukin-6 (IL6) secretion, and *E. coli* killing. A minimum of 3 independent experiments (calves) with a minimum of 2 replicates per calf experiment were utilized. Data were analyzed using a mixed linear model including the fixed effect of treatment and the random effect of calf and replicate within treatment. In viable PMN, LPC-16:0, −18:0 and −18:1 enhanced phorbol myristate acetate-stimulated H_2O_2 production ($P < 0.01$). Additionally, LPC-18:0 potentiated the ability of O55:B5 lipopolysaccharide to stimulate TNFα and IL6 secretion ($P < 0.01$). We were also able to demonstrate the ability of LPC-18:0 to enhance neutrophil-mediated *E. coli* killing ($P < 0.01$). We conclude that LPC enhances neutrophil bactericidal and inflammatory mechanisms. Future work should consider whether LPC enhances innate immune function in calves exposed to pathogen infection.

Key Words: calf, lysophosphatidylcholine, immunity

270 Interaction of parity and sodium acetate supplementation on milk production.

C. Matamoros* and K. Harvatine, *The Pennsylvania State University, University Park, PA.*

Interaction between cow factors, including parity, and sodium acetate (NaAcet) supplementation are not known. The objective of this study was to characterize the response of milk production to NaAcet supplementation in primiparous and multiparous cows. Briefly, 47 primiparous and 49 multiparous cows were arranged in 2 blocks of 48 cows each in a crossover design with 14-d periods. Treatments were a no supplement control (TMR formulated to have a low risk of diet-induced milk fat depression) and acetate fed at a dosage of 10 mol/d. Acetate was supplemented as anhydrous sodium acetate and mixed in the TMR. Milk yield and composition was measured during the last 3 d of each experimental period and averaged for analysis. Data were analyzed in JMP Pro 15.0.0 with a mixed model that included the fixed effect of treatment, parity, block, and all their interactions and the random effect of cow(block) and period(block). There was no effect of acetate, parity, or interaction of parity and acetate on milk yield ($P = 0.59$, 0.47, and 0.58, respectively). There was an effect of acetate on milk fat percentage and milk fat yield ($P < 0.001$ for both), but no effect of parity (except for milk fat yield, $P = 0.02$) or interaction of parity and acetate. In both cases, NaAcet supplementation increased milk fat percentage and yield approximately 7% compared with the no supplement control. There was an interaction of acetate and parity for milk protein concentration ($P = 0.03$), where primiparous cows supplemented with NaAcet had lower milk protein percentage but there was no effect of acetate in multiparous cows. However, there were no effects of acetate or interaction of parity and acetate for milk protein yield ($P = 0.81$ and 0.25, respectively). In conclusion, NaAcet supplementation increases milk fat production similarly in both primiparous and multiparous cows.

Key Words: milk fat, lipogenesis, cow factors

Teaching/Undergraduate and Graduate Education

271 The effect of case-based teaching methods compared with lecture-based teaching methods on students' knowledge in a senior dairy management course. L. Papinchak*[1], N. Roman-Muniz[1], L. Edwards-Callaway[1], T. Buchan[2], J. Todd[2], and M. C. Cramer[1], [1]*Colorado State University, Department of Animal Sciences, Fort Collins, CO,* [2]*Colorado State University, The Institute for Learning and Teaching, Fort Collins, CO.*

Little research exists comparing the efficacy of case-based (CB) or lecture-based (LB) teaching methods in dairy science courses in higher education. The objective of this study was to determine the effect of CB teaching methods compared with LB teaching methods on student knowledge in a senior dairy management course at a Land-Grant University. Informed consent was obtained and a 1.5% final grade bonus to participate was offered; students who opted out were offered an equivalent assignment. This study utilized a crossover design conducted over 2 content modules (module 1 = 'calf health' and module 2 = 'lameness') with a washout period of 2 weeks. Students (n = 25) were randomly assigned to either CB (material presented as a case study) or LB (material presented as a lecture), and then received the other teaching method for module 2. The same instructor presented both modules. Students completed quizzes before and after each module: multiple-choice quiz (MCQ; 10 questions) and free response quiz (FRQ; 3 questions). A person not involved with assigning course grades deidentified data; one person graded all quizzes and was blinded to students' group assignment. Changes in scores were calculated (post-quiz score – pre-quiz score) and used for analysis. A 2-sample *t*-test was used to compare the change in MCQ scores between CB and LB groups. A Wilcoxon-Mann-Whitney test was used to compare the change in FRQ scores between CB and LB groups. There was no difference in the change in MCQ score ($P = 0.68$) or FRQ scores ($P = 0.56$) between CB and LB groups for module 1. The change in FRQ scores for module 2 was also not different between CB and LB ($P = 0.71$). However, students in the CB group had a greater average (mean ± SD) change in MCQ scores compared with students in the LB group (1.31 ± 1.75 vs. -0.09 ± 1.45) for module 2 ($P = 0.04$). On average, students in the CB group performed better after completing module 2 compared with students in the LB group. Next steps include identifying students' perceptions to either CB or LB teaching methods and how these vary based on module content.

Key Words: student learning, scholarship of teaching

Animal Behavior and Well-Being III

272 Estimating dry matter intake of transition dairy cows through multiple on-cow accelerometer sensors. T. C. Michelotti*, S. Encarnación, and J. S. Osorio, *South Dakota State University, Brookings, SD.*

Decreased dry matter intake (DMI) around calving is attributed to postpartal health disorders in dairy cows. Therefore, the objective of this study was to evaluate the feasibility of using 3D accelerometer sensors to estimate individual intakes of transition dairy cows. Eighteen multiparous Holstein cows housed in bedded pack pens during close-up were fitted with 3 sensors (Onset; Pocasset, MA) that record acceleration in the 3-axis (i.e., x, y, and z), one placed on the lateral side of the left hind leg and 2 attached to a halter directly superpose over the jaw and nose. After calving, cows were moved to a freestall barn bedded with straw. Cows were assigned 2 groups, a calibration group (A; n = 9) and a validation group (B; n = 9). Accelerations and individual intakes were collected from −7 to 7 d relative to parturition. Sensors were set to record accelerations at 1 min intervals. Acceleration models highly associated with DMI determined in a previous study (Carpinelli et al., 2019; J Dairy Sci, 102:11483) were used to cross-reference accelerometer data and DMI in group A. Six additional variables were derived from jaw and nose accelerations by measuring the change in acceleration between 2 consecutive time points (Lag-time). The REG procedure of SAS was used in group A to generate an intercept (B0) and slope (B1). Then, these were used in group B to derive DMI from previous acceleration models (DMIA) and compared this against the actual DMI using the CORR and MIXED procedures of SAS. DMIA was closest ($P = 0.58$) to the actual DMI using the models LagNoseZ+LagJawZ (14.0 vs 13.6 kg/d ± 0.5 kg/d) and JawX+JawZ+JawY+LagJawY (13.8 vs 13.6 kg/d ± 0.5 kg/d). Moreover, the LagNoseZ+LagJawZ model was able to produce a DMIA that exhibited a rapid decrease ($P = 0.03$) commonly observed around calving in actual DMI, while JawX+JawZ+JawY+LagJawY model did not ($P > 0.05$). Overall, DMI and DMIA were not correlated ($P \geq 0.05$; $r \leq 0.20$) in all tested models. Peripartal DMI is highly variable and challenging to estimate with sensor-based methods. However, the similar DMI and DMIA observed in this study is encouraging and demonstrates a great potential for this approach to estimate DMI around calving, which could help in the future to flag cows at risk of developing a postpartal disease.

Key Words: accelerometer, intake, sensor technology

273 Differences in dry matter intake of primiparous and multiparous lactating dairy cows assessed through multiple on-cow accelerometer sensors. T. C. Michelotti*[1], S. Encarnación[1], J. Halfen[1,2], T. Fernandes[3], M. Suazo[1], J. Bonilla[1], A. Paz[1], M. Bulnes[1], and J. S. Osorio[1], [1]*South Dakota State University, Brookings, SD,* [2]*Universidade Federal de Pelotas, Pelotas, Brazil,* [3]*Universidade de Lisboa, Lisboa, Portugal.*

The objective of this study was to evaluate the use of 3-dimensional accelerometer sensors to estimate dry matter intake (DMI) in primiparous (PP) and multiparous (MP) lactating dairy cows. Forty mid- to late-lactation Holstein dairy cows (20 PP, 136 ± 21 DMI and 20 MP, 170 ± 51 DMI) housed in a freestall barn were fitted with 3 sensors that record acceleration in the 3-axis (i.e., x, y, and z), one sensor on the lateral side of the left hind leg and 2 attached to a halter directly superpose over the jaw and nose. Cows were assigned to either a collection (A; n = 20) or validation group (B; n = 20), and each group was comprised of 10 PP and 10 MP cows. Sensors were set to record the 3D accelerations at 10-s intervals. Cows were trained to use Calan gates during a 7-d period followed by a 10-d period of data collection of acceleration and individual intakes. Acceleration models highly associated with DMI determined in a previous study (Carpinelli et al., 2019; *J Dairy Sci*, 102:11483) were used to cross-reference accelerometer data, and DMI in group A. Six additional variables were derived from jaw and nose accelerations by measuring the change in acceleration between 2 consecutive time points (i.e., lag time). The REG procedure of SAS was used in group A to obtain the intercept (B0) and slope (B1) for each acceleration model. Then, in group B, B0 and B1 were used in the respective acceleration models to derive an acceleration-based DMI (DMIA). The DMIA generated for MP and PP in group B was tested using the MIXED procedure of SAS to confirm parity differences in DMI. As expected, MP cows had a greater ($P < 0.01$) DMI than PP (27.2 *vs* 23.1 ± 0.6 kg/d). Similarly, the LegZ+JawX+LagNoseY model was able to capture the difference in DMIA between MP and PP cows ($P < 0.05$; 26.1 vs. 25.5 ± 0.2 kg/d), while there was a trend ($P = 0.06$) for differences in DMIA between groups when applying the JawX+LagNoseY (26.0 *vs* 25.7 ± 0.1 kg/d) and LegZ+JawX (26.1 *vs* 25.6 ± 0.2 kg/d) models. The contrasting underestimation and overestimation of intake in MP and PP cows via acceleration underscores the need for future refinements to this approach. However, results from this study suggest a great potential of using accelerometer sensors to estimate feed intake in dairy cows.

Key Words: accelerometer, intake, sensor technology

274 Defining physiological indicators of heat stress and their relationship to health scores of preweaned dairy calves housed individually in outdoor hutches. K. N. Brost*, M. L. Pister, C. A. Hayes, and J. K. Drackley, *University of Illinois, Urbana, IL.*

The objectives of this study were to determine physiological indicators of heat stress and their relationships with health scores of dairy calves from 7 to 56 d of age. The experiment included 26 female Holstein calves housed in individual hutches bedded with straw. Feedings and medical treatments were provided per farm protocols. Seven observation weeks were used over a 133-d period, from July to December 2020. Observation weeks took place every 14 d. Calves were observed 3 times daily at 0700, 1300, and 1900 h. Heart rate (HR), respiratory rate (RR), rectal temperature (RT), and respiratory score was recorded at each observation time. All other health scores were recorded once daily at 0700 h. Temperature and relative humidity were recorded hourly by on-site data loggers. From those data, temperature-humidity index (THI) was calculated. Potential heat stress was defined by a THI $\geq$89. Measurements were divided into non-heat-stressed calves (NHS) identified as days with 0 h/d of heat stress, and heat-stressed calves (HS) identified as days with $\geq$1 h/d of heat stress. Data were analyzed using MIXED and FREQ procedures in SAS. Rectal temperatures were significantly higher ($P < 0.05$) for HS at 1300- and 1900-h observations. Heart rates were greater or tended to be greater ($P < 0.05$ and $P = 0.058$, respectively) at 1300 and 1900 h observations for NHS. Respiratory rates were greater ($P < 0.05$) at each time point for the HS group. Ocular scores were greater ($P < 0.05$) for the HS group, and nasal scores were greater ($P = 0.02$) for the NHS group. There were no differences in fecal and ear scores between groups. Respiratory scores did not differ between groups at any time point. Illness, defined by a combination of medication use, signs, and veterinary diagnosis did not vary between groups. Based on the results, RT and RR show strong correlation to heat stress in preweaned dairy calves. Heat stress had minimal relationship with health scores during this trial, potentially due to duration of heat stress (h/d) or on-farm treatment protocols.

Key Words: heat stress, temperature-humidity index (THI), calves

275 Development and validation of an individual heat abatement tool for dairy cattle.

G. Mazon*[1], P. D. Montgomery[2], J. Jackson[2], M. Hayes[2], and J. H. C. Costa[1], [1]*Dairy Science Program, University of Kentucky, Lexington, KY,* [2]*Biosystems Engineering, University of Kentucky, Lexington, KY.*

This study aimed to develop and validate an autonomous soaking system for dairy cattle utilizing radio-frequency identification (RFID). The autonomous soaking system integrated a microcomputer (Raspberry Pi Foundation, Cambridge, United Kingdom), a temperature and humidity sensor (Aosong Electronics Co., Ltd., Guangzhou, China), an ultra-high frequency (UHF) RFID tag reader (Alien Technology, San Jose, CA), and 2 soaker nozzles (Cool Sense, Edstrom Industries, Waterford, WI). The system was programmed to activate the soaker nozzles upon detection of a UHF-RFID tag and was validated both statically and dynamically. During the static validation, 5 UHF-RFID tags were statically placed 5 times each in 11 points of interest that ranged from 0 to 1.5 m of the reader using a wooden rod. Then, during the dynamic validation, 10 tags were moved 10 times each from 1.5 m to the right of the reader to 1.5m to the right of the reader using a wooden rod at a steady speed to mimic the walking speed of a dairy cow (1.2 m/s). Tags were always held at 1.52 m from the ground to mimic the height of a dairy cow. Successful activations were defined when the soaker was activated in the presence of a UHF-RFID tag. The NPAR1WAY procedure in SAS (SAS Institute Inc., Cary, NC) was used to assess the effects of distance from the reader and UHF_RFID tag on the percentage of successful readings. Tag did not affect the percentage of successful activations ($P = 0.46$). During the static validation, the percentage of successful activations was moderate (Mean ± SD; 77.8 ± 30.1%) and significantly affected by distance ($P < 0.01$). Distances that were equal or greater than 1.2 m in the horizontal plane from the center of the UHF-RFID reader had significantly less successful activations ($P \leq 0.01$). However, during the dynamic validation, the percentage of successful readings was very high (96.0 ± 7.0%). These results suggest that the autonomous soaking system seems to be able to detect cows standing still or moving underneath the system and that could be used as a potential heat stress management tool. Thus, future research should validate the system using dairy cows.

Key Words: heat stress, technology, automation

276 Effect of concentrate allowance and cow personality on the behavior and production of dairy cows after training to use a free-traffic automated milking system.

A. J. Schwanke*[1], K. M. Dancy[1], G. B. Penner[2], R. Bergeron[1], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

Concentrated feed is typically used within an automated milking system (AMS) to motivate cows to voluntarily visit the milking unit. However, individual differences in personality traits between cows may also influence their willingness to milk voluntarily. The objective of this study was to determine the effects of concentrate allowance and the trait of boldness on the behavior and production of dairy cows after being trained to use an AMS. Thirty-two Holstein cows (218 ± 47 DIM; 1.6 ± 0.8 lactations), with no previous exposure to an AMS, were assigned a Boldness Score (BS; range = 3 to 29) based on previous observations of feed bunk displacements. Cows were randomly allocated to an AMS concentrate provision of either 6 (High) or 2 (Low) kg/d of DM. Cows were trained to use the AMS over 72 h, after which data on milking activity and production were then recorded for 9 wk, summarized by cow and day, and analyzed in repeated-measures mixed-effect linear regression models. As designed, High cows were provided more AMS concentrate (4.6 vs. 2.0 kg/d; $P < 0.01$), but had lesser PMR DMI (21.4 vs. 23.5 kg/d; $P = 0.04$). Total DMI was similar between treatments (High = 26.0 vs. Low = 25.5 kg/d; $P = 0.6$). No difference in milking frequency was detected (High = 2.6 vs. Low = 2.2 milkings/d; $P = 0.15$), however, bolder cows tended to have greater milking frequency (+0.06 milkings/d per 1-unit increase in BS; $P = 0.06$). High cows had greater milk yield than Low cows (35.0 vs. 30.2 kg/d; $P < 0.01$), although, within the Low cows there was greater milk yield with increasing boldness (+0.4 kg/d per 1-unit increase in BS; $P = 0.04$). High cows had lesser milk fat % (4.2 vs. 4.6%; $P = 0.03$) and greater protein yield (1.1 vs. 1.0 kg/d; $P = 0.04$), despite having comparable fat yield (1.3 vs. 1.2 kg/d; $P = 0.6$) and milk protein % (3.6 vs. 3.7%; $P = 0.2$). High cows also had lesser SCC (4.8 vs. 5.1 $\log_{10}$ cells/mL; $P = 0.02$). These data indicate that allocating a greater amount of concentrate in a free-traffic AMS, may promote voluntary milking for bolder cows and greater milk yield across the herd, regardless of personality.

Key Words: robotic milking, concentrate, personality

277 Impact of concentrate allowance and cow personality on the adaptability of dairy cows introduced to a free-traffic automated milking system.

A. J. Schwanke*[1], K. M. Dancy[1], G. B. Penner[2], R. Bergeron[1], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

The success of transitioning dairy cows from conventional parlor systems to an automated milking system (AMS) may be influenced by several factors. Two such factors are the amount of concentrated feed offered to cows, as well as individual dairy cow personality traits, both of which contribute to a cow's motivation to voluntarily milk. The objective of this study was to determine the effects of concentrate allowance and the trait of boldness on the adaptability of dairy cows introduced to an AMS. Thirty-two Holstein cows (218 ± 47 DIM; 1.6 ± 0.8 lactations), with no previous exposure to an AMS, were assigned a Boldness Score (BS; ranging from 3 to 29) based on previous observations of competitive feeding behavior (displacements). Cows were randomly assigned to an AMS concentrate allocation of either 6 (High) or 2 (Low) kg/d of DM. Cows were trained to use the AMS over 72 h, by being brought to the milking unit and encouraged to enter. After 72 h, cows were fetched to be milked when a minimum of 10 h had elapsed since the last milking. Milking activity and production data were recorded for the 3 d before and following the 72-h training period, then summarized by cow and day and analyzed in repeated-measures mixed-effect linear regression models. During the training period, High cows spent more time per day milking compared with Low cows (19.7 vs. 15.4 min/d; SE = 1.44; $P = 0.04$); however, for High cows, daily time spent milking tended to increase with greater boldness (+0.36 min/d per 1-unit increase in BS; $P = 0.08$). In the 3 d after training compared with 3 d before introduction to the AMS, bolder cows tended to have a lesser decrease in milk yield (+0.23 kg/d less decrease in yield per 1-unit increase in BS; $P = 0.08$). Bolder cows tended to have their first voluntary milking visit earlier (0.13 d earlier per 1 unit increase in BS; $P = 0.07$). Overall, these data indicate that allocating a greater amount of concentrate in the AMS, in a free-traffic setup, may promote quicker adaptation to voluntary milking. The results also suggest that bolder cows are more adaptable to an AMS.

Key Words: robotic milking, personality, concentrate

278 Mind the queue: A case study in identifying heterogeneous behavioral patterns in high-dimensional livestock sensor data using unsupervised machine learning tools.

C. McVey*[1], F. Hsieh[1], D. M. Alvarez[2], P. Pinedo[2], and K. Horback[1], [1]*University of California-Davis, Davis, CA,* [2]*Colorado State University, Fort Collins, CO.*

Sensor technologies create new opportunities to study cattle behavior, but extracting nuanced ethological insights from these data streams also gives rise to novel analytical challenges. The goal of this research was to assess the utility of data mining approaches that pair the flexibility of information theory with the power of modern unsupervised machine learning algorithms to better identify and characterize the complex stochastic patterns that can arise in densely sampled sensor data from large heterogeneous social groups. This methodological case study explored patterns in milking order, or the sequence in which cows arrange themselves as they enter the milking parlor, collected at the morning milking over a 6-mo period from a closed group of 200 mixed-parity Holstein cattle on an organic dairy. Cows at the front and rear of the queue proved more consistent in their entry position than animals at the center of the herd. This systematic pattern of heterogeneity was seen more clearly using entropy estimates, a discrete alternative to variance that is scale-free and robust to outliers. Permutation tests using both variance estimators further revealed all but a handful of animals were significantly ($\alpha = 0.05$) more consistent in entry position than a purely random queuing process. Temporal stationarity was then assessed. Median parlor entry positions from pre- and post- spring pasture access subperiods proved highly correlated ($R = 0.91$). Data mechanics visualizations were then used to simultaneously explore patterns in entry order on the temporal and social axes, revealing that only select subgroups of cows demonstrated temporal heterogeneity in queue positions in response to pasture access. Finally, queuing patterns were related back to behaviors recorded between milkings using HerdManager accelerometers. Significant day-by-hour interaction effects and nonindependence between animals confounded inferences from conventional repeated-measures models. By discretizing both data sets via hierarchical clustering, however, a simple permutation test based on conditional entropy estimates revealed significant associations ($\alpha = 0.05$) between queuing patterns and individual variations in activity, high activity, nonactivity, and skin temperature, which were readily characterized using interactive tubeplot visualizations.

Key Words: milking order, unsupervised machine learning, precision livestock farming

Animal Health III

279 Development of a sole ulcer induction model in Holstein heifers: The next step in lameness research. G. Cramer*[1], L. Caixeta[1], W. Knauer[1], B. Crooker[1], E. Shepley[1], L. Solano[2], and S. Wagner[3], [1]*University of Minnesota, St Paul, MN,* [2]*Farm Animal Care Associates, Calgary, AB, Canada,* [3]*Texas Tech University, Amarillo, TX.*

Sole ulcers (SU) are one of the most prevalent lesion-based causes of lameness and are a source of persistent pain in cows and economic losses for farms. Studies have thus far been unable to explain the SU pathogenesis, requiring a new approach to unravel the recurrent, multifactorial nature of SU for use in developing new preventive and therapeutic treatment strategies. The project aimed to further develop a SU induction model in heifers during the transition period and to evaluate physiological, production and behavioral changes during the induction process. We randomly assigned 12 pregnant tie-stall Holstein heifers to one of 3 groups: BLK-R, BLK-LPS, or CON. The BLK-R heifers had hoof blocks on the lateral hoof of the right hind leg for 42 d, from −14 to +28 (±7) DIM, and had lying time restricted 2x/wk during the same time period. Lying restriction occurred in 2 3-h time periods with heifers moved to a holding pen with access to water. Two days before lying time restriction days, DMI was also restricted to 70%. The same protocol was applied to BLK-LPS and also added 1–3 lipopolysaccharide (LPS) challenges between 3 and 28 DIM. The LSP challenge was derived from diluted *E. coli* (serotype O111:B4), with dosages of 0.03125, 0.0625, and 0.125 µg/kg BW given intravenously via the jugular vein in wk 1, 2, and 3, respectively. Control heifers (CON) had no interventions applied. Lying time was taken as daily measurements from −21 to +84 DIM. Hoof evaluations and weight distribution were recorded at various time periods between −21 and +84 DIM. Descriptive results of this pilot study include that 3/4, 2/4, 0/4 cows in BLK-R, BLK-LPS, and CON, respectively, developed hemorrhages in the lateral hoof of their right leg. Interestingly, 2/4, 4/4 and 3/4 cows in BLK-R, BLK-LPS, and CON, respectively, developed hemorrhages in their left hind leg. Across all time periods, average lying time was 9.3(0.11) for BLK-R, 10.6(0.11) for BLK-LPS and 11.5(0.11) for CON cows. From block removal to the final evaluation, weight distribution as a % of total weight equalized between right and left hind legs. In summary, the current protocol was unable to induce SU, even with considerable changes in weight distribution and lying time.

Key Words: sole ulcer, induction, lameness

280 Identification of key gene networks and pathways associated with sole ulcers in lactating dairy cows. F. Rosa*[1,2], A. K. Sharma[3], R. Mohan[1], N. A. Carpinelli[1], J. T.R. Carvalho[1], A. Gomez[3], K. Mjoun[4], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*University of Arkansas for Medical Sciences, Little Rock, AR,* [3]*Department of Animal Science, University of Minnesota, Minneapolis, MN,* [4]*Alltech Inc., Brookings, SD.*

This study aimed to evaluate the transcriptomic profile of the corium tissue of lactating dairy cows experiencing sole ulcers disease. Multiparous Holstein dairy cows clinically healthy or diagnosed with sole ulcers (n = 7/group) were selected for the hoof biopsy procedure. Hoof samples were collected under local anesthesia from the sole of the hoof in claw zone 4. All RNA samples were sequenced using Illumina, NovaSeq S4 at the University of Minnesota Genomics Center. Principal component analysis (PCA) and ANOVA for each gene was calculated using the DESeq2 package in R to determine the differentially expressed (DE) genes, fold-change (FC), and *P*-value based on the comparison of sole ulcer (SU) over healthy cows (HC). The DE genes with at least ± 2-fold change were retained for an enrichment pathway analysis using gene ontology (GO) terms and the enrichGO function in the Clusterprofiler package in R. The FDR cut-off value was set to <0.01. The PCA analysis clearly discriminated the hoof transcriptomes between HC and SU cows. The number of DE genes in SU and HC was 10,274 ± 21.6 and 10,289 ± 36.4 (mean ± SD), respectively. Several genes coding for keratins (e.g., *KRT34* and *KRT85*) were downregulated (FC < −15) in SU cows, while the highest upregulated (FC = 10.9) gene in SU was *IL6*. The GO analysis showed significant downregulation of GO terms in SU cows compared with HC in the Cellular Component category, including intermediate and keratin filaments, and intermediate filament cytoskeleton. Upregulated genes in SU cows resulted in a significant enrichment of GO terms in the Biological Process category, including extracellular matrix organization and vasculature development, as well as GO terms in the Cellular Component category such as extracellular matrix and cell surface. The GO terms enrichment across biological process, cellular components, and molecular function categories were highly involved in the keratinization process, inflammation, and transcriptional regulation. These processes are crucial for the development of sole ulcers causing lameness in dairy cows. This study provides an in-depth molecular and functional foundation for future nutritional interventions to reduce sole ulcer incidence in dairy herds.

Key Words: RNA-seq, lameness, sole ulcer

281 Skin transcriptome revealed key cellular functions affected by digital dermatitis in lactating dairy cows. R. Mohan*[1], F. Rosa[1,2], A. K. Sharma[3], N. A. Carpinelli[1], J. T.R. Carvalho[1], G. Cramer[4], A. Gomez[3], K. Mjoun[5], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*University of Arkansas for Medical Sciences, Little Rock, AR,* [3]*Department of Animal Science, University of Minnesota, Minneapolis, MN,* [4]*Department of Veterinary Population Medicine, University of Minnesota, Minneapolis, MN,* [5]*Alltech Inc., Brookings, SD.*

Bovine digital dermatitis (DD) is a complex multibacterial disease that is a major cause of lameness in cattle. In this study, skin tissue biopsies from the center of active (M2/M4.1) DD lesions or non-active (M0/M4/M1) skin of multiparous Holstein dairy cows (n = 7/group) were performed to assess the impact of DD on the skin transcriptome via RNA-seq analysis. The skin biopsies were performed using a sterile biopsy punch, and the RNA samples were sequenced using Illumina, NovaSeq S4 at the University of Minnesota Genomics Center. Principal component analysis (PCA) and the differentially expressed (DE) genes based on the comparison of DD over non-active cows (NA) were calculated using the DESeq2 package in R. The final DE were 4,769 and 4,463 genes upregulated and downregulated, respectively. To further investigate the biological function of the DE genes (FDR <0.05) with at least ± 2-fold change were retained for an enrichment pathway analysis using gene ontology (GO) terms and the enrichGO function in the Clusterprofiler package in R. The FDR cut-off value was set to <0.01. The PCA analysis clearly discriminated the skin transcriptome between DD cows and NA. Genes coding for several keratins-associated proteins, including *KRT34*, *KRTAP21–1*, and *KRTAP4–9* were downregulated in DD cows. The GO terms associated with downregulated genes in the

Cellular Component category were mainly correlated with collagen-containing extracellular matrix and keratin filament, while GO terms in the Biological Process category were related to humoral immune response and biomineral tissue development. Upregulated genes were highly enriched in the Molecular Function GO terms of cell adhesion and skin development as well as intermediate filament cytoskeleton in the Cellular Component category. Overall, this skin transcriptome evaluation comparing NA and DD cows identified key biological pathways in which there was a dysregulation in the skin extracellular matrix, encompassing the keratinization process, collagen binding, and immune response caused by DD lesions. This transcriptomic data set captures the molecular adaptations in the development of bovine DD, which can be further utilized to target suitable management and nutritional strategies to reduce this infectious disease.

Key Words: bovine digital dermatitis, pathway analysis, RNA-seq

282 Prognostic factors examined for association with diagnosis, treatment, and rehabilitation of nonambulatory dairy cattle: A scoping review.

W. McFarlane*, D. Renaud, C. Reedman, and C. Winder, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Nonambulatory dairy cattle represent a welfare concern and financial burden for the dairy industry. This concern is further compounded by the variation in prognostic factors used to inform treatment and culling of down cattle. The objective of this scoping review was to characterize the literature investigating prognostic factors associated with diagnosis, treatment, and culling of non-ambulatory dairy cattle. A literature search was conducted using a string of search terms developed to capture literature pertaining to nonambulatory dairy cattle in 6 databases and 2 conference proceedings. Abstracts from the literature search were deduplicated and screened for relevance. Data were then extracted and charted to allow characterization of the literature. The literature search yielded 12,239 articles and 40 conference proceedings. After removal of duplicates, 7,587 articles remained for abstract screening, of which 1,562 were reviewed as full-text documents. A total of 400 articles met criteria for data extraction, which included 162 controlled trails and 238 observational studies (154 cohort studies, 54 case-control studies, and 30 cross-sectional studies). Of the intervention studies, 91% specified non-ambulatory cattle due to metabolic conditions, primarily hypocalcemia. Of these, 73% evaluated prevention and 16% treatment. Four percent of intervention studies did not specify the mechanism for recumbency other than being 'peri-parturient'. Of the observational studies, 32% evaluated upstream risk factors, 25% downstream risk factors, 23% both, and 47% diagnostic or prognostic indicators, of which the majority were blood calcium. Studies ranged in publication date from 1932 to 2020, with half published 1996 or later. While a plethora of literature surrounding prevention of hypocalcemia exists, definitions of outcomes pertaining to non-ambulatory cattle, including time at risk were rarely given.

Key Words: milk fever, downer cow, down cow

283 Factors influencing attitudes of producers in a downer cow scenario.

W. McFarlane*, C. Winder, D. Kelton, and D. Renaud, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Exploring variability in how producers respond to down cattle is an important step toward understanding how to manage down cows. The objective of this study was to investigate factors that contribute to culling decisions and prognostic indicators used by Canadian dairy producers when responding to non-ambulatory cattle. Data were obtained from the on-farm phase of the 2015 National Dairy Study. A total of 371 farms were visited and were verbally asked questions about a downer cow scenario. This scenario involved a description of a downer cow that the producer wants to keep in the herd but could not treat in her current location. The scenario was followed by questions asking how the producer would handle the situation. Using univariable logistic regression models, the association between producer demographics and responses to the downer cow scenario were evaluated. Producers from Quebec were more likely [odds ratio (OR): 1.8; 95% confidence interval (CI): 1.02, 3.17; $P = 0.04$] to involve a veterinarian in the decision to cull a down cow than producers from Ontario. Additionally, producers with tie-stall barns take 1 d (95% CI: 0.337, 1.41; $P = 0.002$) longer to decide to cull a down cow compared with producers with freestall barns. Furthermore, producers aged 50 to 59 years were more likely (OR: 3.5; 95% CI: 1.12, 11.09; $P = 0.03$) to use whether a cow is eating as a prognostic factor in their decision to cull a down cow compared with producers under 30 years of age. Lastly, producers with a university or graduate education were much more likely (OR: 12; 95% CI: 3.21, 45.35; $P < 0.001$) to use pain as a prognostic indicator in their decision to cull a down cow compared with producers whose highest level of education was elementary school. Understanding which demographic factors are associated with attitudes and actions of Canadian dairy producers when responding to non-ambulatory dairy cattle could be valuable in the design of educational workshops for producers, improving the care these animals receive.

Key Words: milk fever, parturient paresis, Canada

284 Cannabinoids and adipose tissue lipolysis in periparturient dairy cows.

U. Abou-Rjeileh*[1], M. Chirivi[1], J. P. Giron[1], A. L. Lock[1], M. Zachut[2], J. Tam[3], and G. A. Contreras[1], [1]*Michigan State University, East Lansing, MI,* [2]*Volcani Center, Rishon Lezion, Israel,* [3]*The Hebrew University of Jerusalem, Jerusalem, Israel.*

Excessive lipolysis in adipose tissue (AT) can increase the risk of inflammatory and metabolic diseases in transition dairy cows. The endocannabinoid system is a central regulator of metabolism and energy homeostasis in mammals. In monogastric AT, cannabinoid (CB) signaling through the cannabinoid receptor 1 (CB1) can reduce lipolysis, but CB impact on AT lipid mobilization in dairy cows is unknown. We evaluated the effect of a CB on AT lipolysis in periparturient dairy cows. Subcutaneous adipose tissue (SCAT) samples were collected from multiparous Holstein dairy cows (n = 17) at 13 ± 5d prepartum (PreP), and 14 ± 2d (PP1) and 21 ± 2d (PP2) postpartum. Adipose lipolysis was determined using a short-term in vitro explant culture (3h) by measuring glycerol release in the medium (nmol glycerol/mg AT). Basal lipolysis (BAS) was determined without the addition of any reagents. Stimulated lipolysis was determined by β-adrenergic stimulation with isoproterenol (ISO, 1 μM). The effect of CB1 stimulation was determined in the presence of CB1 agonist arachidonyl-2'-chloroethylamide (AC, 0.7ug/mg) and antagonist rimonabant (RIM, 2.7 μM) in (AC+ISO) and (RIM+AC+ISO). AC and RIM concentrations were based on a preliminary dose titration study performed with SCAT from nonlactating cows. Statistical analyses were performed in JMP using a mixed effect linear model. Results are presented as LSM ± SEM. Overall, SCAT lipolysis was higher at PreP (3.46 ± 0.15) compared with PP1 (1.70 ± 0.14) and PP2 (1.76 ± 0.18; $P < 0.0001$). As expected, BAS (0.45 ± 0.18; $P < 0.001$) was lower than all other treatments. Across all time points, CB1 stimulation with AC did not reduce lipolysis ISO (2.79 ± 0.18) vs AC+ISO (2.80 ± 0.18; $P = 0.99$). Furthermore, inhibition of CB1 signaling did not affect the intensity of lipolysis RIM+ISO (2.67 ± 0.19) and RIM+AC+ISO (2.86

± 0.19; $P = 0.89$). Our results coincide with previous studies showing that SCAT lipolysis intensity is reduced as lactation progresses. In contrast to observations in monogastrics, targeting CB1 had no effect on the lipolytic response of SCAT in periparturient dairy cows. Further research is required to examine the effect of CB on AT lipogenesis and adipogenesis in dairy cows.

Key Words: cannabinoids, periparturient cow, lipolysis

285 Lipopolysaccharide induces lipolysis and insulin resistance in adipose tissue from dairy cows.

M. Chirivi*, M. Smith, C. J. Rendon, M. Runin, and G. A. Contreras, *Michigan State University, East Lansing, MI.*

Intense and protracted adipose tissue (AT) lipolysis increases the risk of metabolic and inflammatory periparturient diseases in dairy cows. This vulnerability increases when cows have endotoxemia, but the mechanisms are unknown. We hypothesized that exposure to endotoxin (ET) increases AT lipolytic responses by activation of inflammatory lipolytic pathways and reduction of insulin sensitivity (IS). Subcutaneous AT (SCAT) explants were collected from 24 nonlactating non-gestating multiparous Holstein dairy cows and incubated in the presence of ET Lipopolysaccharide (LPS = 20 µg/mL; CON = 0 µg/mL) for 3 and 7 h. The effect of LPS on stimulated lipolysis was determined by using isoproterenol (ISO = 1uM; LPS-ISO). The impact of LPS on the antilipolytic responses induced by insulin (IN = 1µL/L, LPS-IN) was determined during ISO stimulation (ISO-IN) and (LPSISO-IN). Lipolysis was quantified by glycerol release. Protein expression of pHSL (Ser563), HSL, pAKT, and AKT as indicators of lipolysis and insulin pathways activation respectively were quantified by capillary western immunoassay. Inflammatory gene networks were evaluated by RT-qPCR. LPS was an effective lipolytic agent at 3h and 7h increasing glycerol release by 115 ± 18% ($P < 0.001$) and 68.7 ± 16% ($P < 0.001$) respectively compared with CON. As expected, IN reduced the lipolytic effect of ISO-IN (−63 ± 18%) and LPS-IN (−45.2 ± 18%) at 3 h ($P < 0.05$). However, the antilipolytic effect of IN was lost in LPSISO-IN at 3h (−3.84 ± 23.6% $P = 0.9$), and 7h LPS-INS (−16.3 ± 16% $P = 0.56$). Compared with basal at 3 (0.95 ± 0.3) and 7h (2.1 ± 0.4), ISO increased pHSL (Ser563):HSL (2.53 ± 0.66, 3h) and (4.17. ± 0.64, 7h; $P = 0.04$). At 7h, LPS increased pHSL (Ser563):HSL 4.58. ± ?1.35 ($P = 0.01$). Compared with basal (0.18 ± 0.05), IN at 7h, increased the pAKT:AKT (2.63 ± 0.89, $P < 0.0001$) and LPS reduced the pAKT:AKT, 0.11 ± ?0.07 ($P < 0.0005$). LPS at 7 h enhanced expression of proinflammatory cytokines (CCL2, IL6, and SOCS1) compared with basal expression. Our results indicate that exposure to ET, reduces IS and increases lipolysis in SCAT. Together these data indicate that dairy cows with endotoxemia could be predisposed to lipolysis dysregulation, which is enhanced by loss of IS, and AT inflammation during extended exposure to ET.

Key Words: endotoxin, lipolysis, insulin sensitivity

286 Within-herd prevalence and associated factors with subclinical ketosis on Canadian dairy farms utilizing automated milking systems as diagnosed by test-day concentration of milk β-hydroxybutyrate.

B. J. Van Soest*[1], R. D. Matson[1], T. F. Duffield[2], D. E. Santschi[3], K. Orsel[4], E. A. Pajor[4], G. B. Penner[5], T. Mutsvangwa[5], 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,* [3]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada,* [4]*Faculty of Veterinary Medicine, Calgary, AB, Canada,* [5]*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

The objective of this study was to describe the within-herd prevalence of subclinical ketosis on Canadian AMS dairy farms using test-day concentration of β-hydroxybutyrate (BHB) in milk and identify associations with farm-level factors. Milk recording test data were collected October 2018 until September 2020 for 184 Canadian AMS farms (Atlantic Canada: n = 12, Ontario; n = 75, Quebec: n = 52, and Western Canada: n = 45). Management practices and housing were recorded upon visiting the farms in the summer of 2019 and updated with a survey in the summer of 2020. Farms averaged (±SD) 115 ± 71 lactating cows, 2.2 ± 1.4 robot units/farm, 39.9 ± 4.8 kg/d of milk, and first test (within first 45 DIM) milk BHB concentration of 0.11 ± 0.019 mmol/L (range = 0.05–0.19 mmol/L). SCK was defined as a first test milk BHB level ≥0.15 mmol/L. Herd-average prevalence of primiparous (PP) cows with SCK was 12.3 ± 9.4% (range = 0–50%), while the herd-average prevalence for multiparous (MP) cows was 26.7 ± 11.3% (range = 6.5–75%). Univariable models were used to screen independent variables in mixed-effect linear regression models and variables with $P < 0.25$ were offered to multivariable models (built separate for PP and MP cows). Each additional hour of light (mean = 20.4 ± 2.9 h, range = 10–22 h) at the AMS was associated ($P = 0.04$) with a 0.52 percentage point decrease in herd-average prevalence of PP cows with SCK. Prevalence of SCK in PP also decreased ($P = 0.02$) as target PMR refusal level increased: 0% (18.4 ± 2.3%; mean ± SE; n = 21), 0–3% (12.5 ± 1.7%; n = 37), 3–5% (11.8 ± 1.0%; n = 100), and >5% (8.8 ± 2.0%; n = 26). Similarly, SCK prevalence in MP decreased ($P = 0.05$) with greater target refusal level: 0% (31.8 ± 3.2%), 0–3% (29.2 ± 2.2%), 3–5% (27.2 ± 1.4%), and >5% (21.7 ± 2.6%). SCK prevalence in MP also varied with flooring type around the AMS ($P = 0.04$), averaging 29.4 ± 1.5% for concrete (n = 92), 28.6 ± 3.1% for slats (n = 18), and 24.5 ± 1.6% for rubber (n = 69). The results indicate that environmental and feeding management may be associated with decreased herd prevalence of SCK in AMS farms.

Key Words: robotic milking, negative energy balance, ketosis

287 Hyperketonemia predictions provide an on-farm management tool with epidemiological insights.

R. Pralle*[1], J. Amdall[2], R. Fourdraine[2,4], G. Oetzel[3], and H. White[4], [1]*School of Agriculture, University of Wisconsin-Platteville, Platteville, WI,* [2]*VAS, Madison, WI,* [3]*School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [4]*Department of Animal & Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Prediction of hyperketonemia (HYK) cases during routine milk sampling is an increasingly common practice for monitoring HYK on dairy farms in the upper Midwest. Our objective was to associate predicted HYK status at the cow and herd level with production and health outcomes. Data were retrieved from consenting dairy farm DHI milk tests to produce lactation records (240,714 lactations; 335 herds) and 12 mo RHA data sets (11,640 RHA mo records; 1,167 herds). Cows were predicted HYK (pHYK) or not (pNOT) based on the published equations used to generate the KetoMonitor report (AgSource Laboratories, WI). Generalized estimating equations (SAS v.9.4; GENMOD) were used to associate predicted HYK status (lactation record data) or 12 mo pHYK prevalence (RHA data) with cow milk production, reproduction, health, and genetic evaluation variables. Reported P-values represent Bonferroni corrected simple-effects of pHYK vs pNOT within parity group (1, 2, 3, 4, or 5+). Previous lactation mature equivalent (ME) milk fat, protein, and total yield were lower ($P < 0.001$) for pHYK cows. First-test ME fat yield was greater for pHYK cows, but ME protein and total yields were lower ($P < 0.001$). Peak milk yield was lower ($P < 0.001$)

for culled pHYK cows but similar ($P > 0.05$) for retained pHYK cows. Preformed and mixed fatty acids (FA; % of fat) were greater ($P \leq 0.10$) for pHYK cows while de novo FA were lower ($P \leq 0.10$). Differential somatic cell count (DSCC) was greater ($P \leq 0.06$) for pHYK from parity 2 to 4. Days open and insemination services were greater ($P \leq 0.002$) for pHYK. Greater incidence ($P \leq 0.02$; pHYK fixed effect) of culling, displaced abomasum, milk fever, and retain placenta was observed for pHYK. Herds with greater RHA milk protein or total yield had lower 12 mo pHYK prevalence ($P \leq 0.01$). As expected, frequently cited unfavorable outcomes of HYK were associated with pHYK. Contrary to common perception, higher-yielding (RHA milk) herds had a lower risk for pHYK. Additionally, novel associations to pHYK status were found (i.e., DSCC), suggesting prediction tools are viable for preliminary discovery as well as HYK monitoring and management.

Key Words: subclinical ketosis, DHI testing, precision agriculture

288 Early postpartum intravenous infusion with carnosic acid, a rosemary (*Salvia rosmarinus*) antioxidant compound, improves milk performance in transition dairy cows.

T. C. Michelotti*, S. Encarnación, M. Suazo, and J. S. Osorio, *South Dakota State University, Brookings, SD.*

One of the greatest challenges associated with the transition period of dairy cows is the high occurrence of oxidative stress due to the rapid increase in milk production in the first days of lactation. Providing effective antioxidants to cows during this period is an important strategy to improve cows' overall health and performance. Hence, the objective of this experiment was to evaluate the effects of a rosemary (*Salvia rosmarinus*) antioxidant compound (carnosic acid) on transition dairy cows. Sixteen multiparous Holstein cows were enrolled in a randomized complete block design from −21 to 21 d in milk (DIM) and blocked according to expected calving day, parity, and previous lactation milk yield. During 1 to 3 DIM, cows received a daily intravenous infusion of 500 mL of saline (CON, n = 8) or carnosic acid at a rate of 0.3 mg/kg of BW (CA, n = 8). Carnosic acid (Combi Blocks, QC-4383) was diluted with alcohol to 0.5 mg/mL, and the dose according to the cow's respective BW was supplied in 500 mL of saline. Milk yield and dry matter intake were measured daily, while BW and BCS were recorded weekly. Milk samples were collected weekly for determination of fat, protein, SCC, and MUN concentrations. Data were analyzed using PROC MIXED of SAS with treatment, time, and their interaction as fixed effects and cow within treatment as random effect. CA cows had greater milk yield ($P = 0.03$; 40.6 *vs*. 34.5 kg/d) and energy-corrected milk ($P = 0.04$; 51.2 *vs*. 43.0 kg/d) compared with CON cows until 21 DIM. Moreover, milk efficiency (milk yield/feed intake) tended to be greater ($P < 0.09$; 2.2 vs. 2.0) for CA cows than CON. Feed intake, BW, BCS, and milk composition were not affected ($P > 0.10$) by treatment. These results indicate that an adequate supply of antioxidants such as carnosic acid can have a substantial positive effect on milk performance in early lactation in dairy cows.

Key Words: peripartum period, oxidative stress, natural plant extract

289 The hidden cost of disease: Analyzing the economic losses due to the first instance of mastitis or lameness in multiparous cows.

E. Shepley*[1,3], M. A. Puerto[1], R. I. Cue[1], D. Warner[1,2], and E. Vasseur[1], [1]*McGill University, Ste-Anne-de-Bellevue, QC, Canada,* [2]*Lactanet Inc., Ste-Anne-de-Bellevue, QC, Canada,* [3]*University of Minnesota, St. Paul, MN.*

Mastitis and lameness are painful diseases commonly found on dairy farms, affecting cow welfare, performance and economic viability. Identifying when in the cow's life the impact of these diseases is most consequential may help producers make informed management decisions. The study aimed to determine the effect of the first instance of mastitis or lameness, at different lactation stages, on economic indicators of multiparous cows. A retrospective longitudinal study was conducted using DHI (Lactanet Inc.) data for second, third and fourth parity Holstein cows that calved between 2000 and 2015. After editing and identifying cows in each parity with a first instance of mastitis or lameness, cows were categorized as Transition (−21–21 DIM), Early (22–100 DIM), Mid (101–200 DIM) or Late (201+ DIM) based on when in the lactation the first instance of the disease occurred. To account for production differences, Transition and Early cows and comparable Healthy (Control) cows were stratified by previous lactation cumulative milk yield and Mid and Late cows and Healthy counterparts were stratified by cumulative milk yield before the health event. Total cows used in the analyses, across parities, were 14,108, 11,553, and 13,138 for Transition and Early (analyzed together), Mid, and Late, respectively, for mastitis and 14,109, 13,780, and 12,987, in the same respective stages, for lameness. Lactation milk value, margin over feed cost (MOFC), and gross profit were analyzed using a mixed model with herd as the random effect. Mastitic Late cows had a lower milk value than their healthy counterparts (Parity 2: −\$218 CAD, $P < 0.05$) with differences increasing with parity (Parity 3: −\$780, $P < 0.001$). MOFC ranged from \$18 to \$568 for mastitic cows, differing from healthy cows for parity 2 Transition ($P < 0.001$), Early ($P < 0.001$) and Late ($P < 0.05$) cows and for parity 4 Transition and Mid cows ($P < 0.01$). For each parity, all lactation levels had significant losses in gross profit for mastitis (−\$450 to −\$1,064) and lameness (−\$536 to −\$1,345), with the greatest losses in Transitional and Early cows ($P < 0.01$). This study shows the deleterious impact that an instance of mastitis or lameness can have, regardless of when in the cow's life the instance occurs, emphasizing the importance of including the full economic cost of a disease when making culling decisions.

Key Words: mastitis, lameness, lifetime profitability

290 The use of blood myeloid and lymphoid cell profiles to predict metritis in dairy cows.

S. Casaro*[1], J. G. Prim[1], T. D. Gonzalez[1], J. Driver[1], J. E. P. Santos[1], C. D. Nelson[1], A. C. M. Silva[1], M. G. Marrero[1], J. Laporta[2], and K. N. Galvão[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Wisconsin, Madison, WI.*

The objective was to evaluate the peripheral blood myeloid and lymphoid cell profile of cows at calving as predictors of metritis. Holstein cows (n = 108) were housed at the University of Florida dairy unit. Blood was collected at calving using sodium heparin evacuated tubes. Parity, gestation length (GL), days in close-up pen (DCU), body condition score (BCS) and rectal temperature (RT) were recorded. Cows having dystocia, twins, stillbirth, vaginal laceration, or retained placenta were classified as having a risk factor (RF) for metritis. Cows were examined for signs of metritis at 3 ± 1, 7 ± 1, 10 ± 1 and 13 ± 1 d in milk, and cows with a red-brownish, watery, fetid vaginal discharge were diagnosed with metritis. Flow cytometry was used to evaluate the percentage of myeloid and lymphoid cells, and extracellular markers of myeloid and lymphoid cell adhesion and activation. Cell markers for monocytes (CD172a+/CD14+), granulocytes (CD172a+/CD14-), B cells (MHC2+/CD21+), T-helper cells (CD4+), cytotoxic T cells (CD8+), and gamma delta T cells (γδTCR+) were evaluated. The absence of L-selectin (CD62L) and the presence of CD11b on cells were used as markers of lymphoid and myeloid cell activation. Data were analyzed by logistic regression, and the model included parity, GL, DCU, BCS, RT, RF, and immune markers with $P \leq 0.2$ in the univariate analysis. The proportion of activated B cells and the proportion of monocytes were predictors of metritis, in addition to parity, RF, GL, and BCS. Each 1-unit increase in the percentage of CD62L- B cells increased the odds of metritis by 13% (OR = 1.13; 95% CI = 1.1–1.2; $P < 0.01$). Each 1-unit increase in monocyte proportion increased the odds of metritis by 34% (OR = 1.34; 95% CI = 1–1.9; $P = 0.07$). The model with parity, RF, GL, BCS, and immune markers had a sensitivity of 85%, specificity of 67%, positive predictive value of 71%, negative predictive value of 81%, and area under the curve of 83%. In summary, markers of B cell activation and monocyte proportion were significant predictors of metritis.

Key Words: dairy cow, immune markers, metritis prediction

291 Formation of blood neutrophil extracellular traps increases the mastitis risk of dairy cows during the peripartum period.

L. Y. Jiang*, H. Z. Sun, R. W. Guan, and J. X. Liu, *Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China.*

Dairy cows have higher risk to develop a severe mastitis during the peripartum period, which results in high somatic cell count (SCC) in milk. The SCC is related to the number and function of blood neutrophils in peripartum dairy cows, but its mechanism is still unclear. The neutrophil extracellular traps (NETs) are formed by the external stimulus of neutrophils, exerting extracellular neutrophils function. Serum myeloperoxidase and neutrophils-elastase indicate the main components of NETs function, while serum DnaseI represents the NETs degradation level within host body. Here, the blood neutrophils of peripartum dairy cows were analyzed and related to the postpartum mastitis risk. Seventy-six Holstein dairy cows at wk 4 before calving were selected to collect blood samples at wk −1 and wk 1 relative to calving, and milk composition was recorded weekly. Five lower SCC (38 ± 6.0, 10^3/mL, mean ± SEM) and 5 higher SCC (3,753 ± 570.0, 10^3/mL, mean ± SEM) dairy cows were selected based on the average of 3-week SCC post-calving. The blood neutrophils were used for the transcriptome sequencing by the Illumina HiSeq X 10 platform. Serum concentrations of NETs, DnaseI, and neutrophils-elastase were measured by enzyme-linked immunosorbent assay, and serum myeloperoxidase was tested by colorimetry. The data were analyzed using the MIXED procedure with covariance type AR (1). The transcriptome analysis revealed that the change of neutrophils in higher SCC cows were mainly involved in cell cycle-related pathways in comparison to the lower SCC cows, and these pathways were essential in NETs formation. The concentrations of NETs, myeloperoxidase, and neutrophils-elastase were higher in dairy cows with higher SCC ($P < 0.05$) than those with lower SCC at both wk −1 and wk 1 relative to calving. Serum DnaseI level was lower ($P < 0.05$) in higher SCC cows than in lower SCC cows. These results indicate that formation of NETs in blood of peripartum dairy cows is different between cows with low vs. high SCC.

Key Words: peripartum dairy cow, mastitis risk, neutrophil extracellular traps

292 Effects of palmitic acid on the formation of neutrophils extracellular traps in dairy cows during the postpartum period.

L. Xie[1], Y. Guan[1], C. Shen[1], J. Lu[1], G. Opsomer[2], O. Pascottini[2,3], and Q. Dong*[1], [1]*College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China,* [2]*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium,* [3]*Veterinary Physiology and Biochemistry, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Belgium.*

We aimed to determine whether the formation of neutrophil extracellular traps (NETs) is a mechanism of palmitic acid (PA)-induced inflammation in transition cows. Peripheral blood samples of cows (n = 33, DIM 7–12) were taken to isolate polymorphonuclear cells (PMN). In total, 1×10^6 cells were cultured with or without 200 µM PA, 20 nM phorbol 12-myristate 13-acetate (PMA) and 100 mM L-epicatechin for 4 h. The experimental groups were divided into control, PA, PMA, PA+PMA, L-epicatechin, and PA+ L-epicatechin supplemented PMN. The intracellular reactive oxygen species (ROS) and H_2O_2 levels, the activity of lactate dehydrogenase (LDH) and myeloperoxidase (MPO), the extracellular amount of double-stranded DNA, the electron microscopic morphology of NETs, and the expression of proteins related to the PI3K/Akt pathway were determined by DCFH-DA, HRPO colorimetry, diaphorase colorimetry, TMB colorimetry, picogreen staining, field emission scanning electron microscopy, and western blot, respectively. Tests were run in triplicate in 3 biological replicates. Statistical evaluations were performed using one-way ANOVA with Bonferroni correction. 100–500 µM PA supplementation induced the release of extracellular DNA in a dose-response manner. Compared with the control group, PA and PMA induced a decrease in the activity of LDH, an increase in the release of extracellular DNA and double-stranded DNA, and a rise in the number of NET structures formed ($P < 0.05$). Meanwhile, PA induced an increase in intracellular ROS and H2O2 levels in PMN, which was reduced by the addition of L-epicatechin ($P < 0.05$). Furthermore, PA or PMA induced an increase in the level of MPO activity and L-Epicatechin inhibited these PA-induced extracellular MPO levels ($P < 0.05$). Additionally, PA induced an increase in the expression levels of Nox2, Akt, p-Akt and PI3K proteins, while L-epicatechin inhibited this increase ($P < 0.05$). We conclude that PA can induce the formation of NETs activated via the PI3K/Akt and NOX2 pathways. This may contribute to exacerbated oxidative stress and dysregulation of inflammation in transition dairy cows.

Key Words: palmitic acid, neutrophil extracellular traps, oxidative stress

293 High-risk management practices for Johne's disease infection in Ontario dairy herds.

J. Imada*[1], S. Roche[1,2], C. Bauman[1], and D. Kelton[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*ACER Consulting, Guelph, ON, Canada.*

Johne's disease (JD) is a chronic progressive gastrointestinal disease of ruminants caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP). Considered an untreatable disease, the most common method of control is through test and cull programs, youngstock husbandry management and biosecurity. Minimizing exposure of young calves to infective cow manure is a common management strategy in JD control programs. In 2010, the province of Ontario, Canada instituted a voluntary JD control program. The program consisted of whole herd individual animal tests using serum or milk ELISA with subsequent permanent removal of all high-test positive cows. The participant herds also completed a risk assessment and management plan with their herd veterinarian to identify high-risk practices for introducing or transmitting JD. Herds were given recommendations for management practices they could utilize to reduce their JD risk. Since the completion of the program in 2013 there has been an increase in the number of bulk tanks testing positive for JD within the province from 40% in 2013 to 70% in 2017. To investigate this increase and identify any management practices that may have changed over time, repeat risk assessments were completed in 2019 on 180 farms which had been assessed in the original program. Original and follow-up risk assessment responses were used in a logistic model where JD control was represented by a bulk tank milk ELISA result below 0.089 OD in 2017. Herds with multiple cows in the maternity pen at the same time were roughly 3 times less likely to have JD control than those that calved cows individually. Herds reporting that their cows calved outside of designated maternity areas were 2 to 4 times more likely to achieve JD control than those that only had cows calving in designated calving areas. Herds with more contaminated heifer environments were less likely to achieve JD control compared with those with cleaner heifers and heifer environments. The study provides evidence supporting the need to minimize contamination of calf environments. Further research is needed to understand the importance of young heifer cleanliness in JD control.

Key Words: Johne's, biosecurity, dairy

294 *Lactobacillus animalis* LA51 confer protection from the damaging effects of pathogenic *Salmonella* Dublin, *Salmonella* Newport, and *Salmonella* Heidelberg on the intestinal barrier.

G. Copani*, O. C. M. Queiroz, and E. J. Boll, *Chr. Hansen A/S, Animal Health and Nutrition, Hoersholm, Denmark.*

Salmonella spp. are important human pathogens that can be isolated from feces of ruminants and they can constitute a potential source of human infection via multiple types of contaminations. Additionally, these pathogens can cause disorders in the gut of young animals, such as calves and heifers. The gut plays a key role in the digestion and absorption of nutrients and constitutes an initial organ exposed to external factors influencing the health of animals. Intestinal dysbiosis can promote overgrowth of different pathogens, which can cause intestinal barrier damage (leaky gut), which in turn may facility passage of potential toxins to the bloodstream. The objective of this study was to evaluate in vitro beneficial effects of *Lactobacillus animalis* (LA51) on gut health in the presence of pathogens. The "leaky gut" assay was performed by evaluating transepithelial electrical resistance (TEER) across Caco-2 monolayers exposed to one of 3 *Salmonella enterica* serovars isolated from ruminants [*Salmonella* Dublin (SD) CHCC41286, 1×10^{8}cfu/transwell (Exp1), *Salmonella* Newport (SN) CHCC403337, 2×10^{7}cfu/transwell (Exp 2) and *Salmonella* Heidelberg (SH) CHCC403338 6×10^{7}cfu/transwell (Exp 3)] in the absence or presence of LA51. FITC-dextran-20kDa (FD) was added to the apical side of the Caco-2 cells at the same time as the *Salmonella* (2 h after the addition of LA51). The amount of FD translocated to the basolateral side was quantified upon termination of the TEER measurements (after 1 h for Exp1 and after 7 h for Exp2 and Exp3) by measuring the fluorescent signal. In Exp 1, SD caused a relative TEER decrease to 15% compared with unstimulated Caco-2 cells (100%) over the 11 h ($P < 0.0001$; SEM 1.589), while LA51 significantly reduced the TEER decrease to 60% ($P < 0.0001$; SEM 2.196). In Exp 2 and 3, the relative TEER after the 7 h in the absence or presence of LA51 was 43% vs. 91% ($P < 0.0001$; SEM 2.952) for SN and 33% vs. 81% ($P < 0.0001$; SEM 3.009) for SH. The addition of LA51 significantly reduced the amount of FD translocation in all 3 experiments (Exp 1 with SD (1% vs. 0.27%, $P < 0.001$; SEM 0.076) Exp 2 with SN (0.26% vs. 0.14%, $P < 0.0001$; SEM 0.007) and in Exp 3 with SH (0.30% vs. 0.13%, $P < 0.001$; SEM 0.019). In conclusion, LA51 confers protection against all 3 tested *Salmonella enterica* serotypes by counteracting its damaging effect on the intestinal integrity.

Key Words: *Lactobacillus animalis, Salmonella* Dublin*, Salmonella* Newport

Breeding and Genetics III

295 Estimation of genomic breed composition and effect on performance traits in crossbred dairy cattle.

M. Jaafar*[1], B. Heins[2], C. Dechow[3], and H. Huson[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Minnesota, Morris, MN,* [3]*Penn State University, University Park, PA.*

Breed composition plays an important role in cattle usage, adaptation, and production. Understanding breed composition gives us insight into population structure and breeding history, correction for population stratification in across-breed genetic evaluations and genome-wide association studies, and informing crossbreeding programs. Thus, this study examined variation in breed composition using Illumina BovineSNP50 genotypes relative to estimation from pedigree information and correlated breed composition to key performance traits. Two rotational crossbred populations, referenced as Procross and Grazecross were assessed. Procross are a product of rotational crossbreeding of Viking Red (VKR), Holstein (HOL), and Montbéliarde (MON). In contrast, Grazecross consists of VKR, Normande (NOR), and Jersey (JER). Both breeding programs were aimed at capitalizing on the positive effect of heterosis. To start, pedigree and genomic breed composition were generated on 610 crossbred cattle incorporating genotypes of the respective purebred breeds for genomic ancestry estimation, which is shown in Table 1. Accuracies of breed composition were assessed as Pearson correlation between pedigree and genome-based predictions. In the final analysis, both estimations were correlated with the performance traits by comparing extreme performance groups to identify the relationship between breed ancestry and 4 performance traits. Further analysis showed that both MON and HOL breed composition plays a significant role in higher milk and fat production in Procross while VKR and NOR are related to improved health performance in Grazecross. In all, genomic breed composition highlighted variation in the inheritance and selection of breed composition in Procross and Grazecross cattle compared with pedigree estimates. Within these rotational crossbreeds, specific ancestry was correlated with improved trait performance.

Table 1. Breed composition of Procross and Grazecross cattle

Breed composition	Procross		Grazecross	
	Pedigree	Genomic	Pedigree	Genomic
Viking Red	30%	22%	32%	21%
Montbéliarde	35%	45%	—	—
Normande	—	—	28%	49%
Holstein	35%	33%	—	—
Jersey	—	—	40%	30%

Key Words: genomic breed composition, crossbred dairy cattle, pedigree estimation

297 Fatty acid profiles of Holstein, Grazecross, and ProCROSS cows in an organic and low-input conventional dairy herd.

G. M. Pereira* and B. J. Heins, *University of Minnesota, West Central Research and Outreach Center, Morris, MN.*

The fatty acid (FA) profiles of milk vary due to nutrition and breed of dairy cattle. Milk that is higher in short-chain FA is of interest to consumers who are concerned about their health. The objective of this study was to compare FA profiles from DHIA of Holstein cows (HO; n = 54) with Grazecross crossbred cows (n = 60) composed of the Normande, Jersey, and Viking Red breeds and ProCROSS crossbred cows (n = 120) composed of the Montbéliarde, Viking Red, and HO breeds. The West Central Research and Outreach Center, Morris, MN, has a dairy herd that is managed separately as an organic and low-input conventional herd. Total FA, De novo FA (C4 to C14), Mixed FA (C16, C16:1, C17), Preformed FA ($\geq$C18) concentrations were provided by Minnesota DHIA (Buffalo, MN) from monthly DHIA test-day data from the morning milking. Independent variables for statistical analysis with PROC GLM were the fixed effects of DIM on test day, herd, parity (primiparous and multiparous), breed group and sire breed nested within breed group. There were no differences ($P > 0.10$) for FA concentrations for the organic and conventional herd. Holstein cows had lower ($P < 0.01$) (3.6 g/100 g) Total FA than Grazecross cows (4.1 g/100 g), but similar Total FA to ProCROSS cows (3.7 g/100 g). Furthermore, Grazecross cows had higher ($P < 0.01$) concentrations of De novo FA (1.1 g/100 g) than that of HO cows (0.9 g/100 g), and ProCROSS cows (0.9 g/100 g). Higher concentrations ($P < 0.01$) of Mixed FA were observed for Grazecross cows (1.8 g/100 g compared with HO cows (1.5 g/100 g), and ProCROSS cows (1.6 g/100 g). Grazecross cows had higher concentrations ($P < 0.05$) of Preformed FA (1.3 g/100 g) than HO cows (1.2 g/100 g), and ProCROSS cows (1.2 g/100 g). The FA profiles did not differ between HO and ProCROSS cows. Milk fat from Grazecross cows had +12.2% more Total FA than the other breed groups. Dairy producers interested in marketing milk with higher FA concentrations may implement the Grazecross 3-breed rotational crossbreeding system.

Key Words: milk fatty acids, crossbreeding, organic

298 Characterizing growth and carcass traits in beef × dairy crossbred animals.

K. M. Lucas*[1], M. Saatchi[1,2], and J. E. Koltes[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Top Genomics, LLC, West Des Moines, IA.*

Use of genomics and sexed semen in the dairy industry has provided opportunities for selective use of beef semen to improve beef production efficiency from dairies; however, growth and carcass data are unavailable to evaluate optimal mating strategies. The objectives of this study were to compare and provide benchmark values for growth and carcass traits of beef × dairy crosses. Data (n = 81,039) from crosses of Brown Swiss (BS), Holstein (HO) or Jersey (JE) dams bred to Limousin (LM) or Simmental (SM) sires containing >25% and <75% of beef and dairy breed(s) were used. Contemporary groups (CG) were composed of breeder ID, herd ID and animal sex. Data were analyzed in SAS9.4 for 205-d adjusted weaning weights (*WWadj*), 160d adjusted postweaning gain (*PWGadj*), and 365-d adjusted yearling weight *(YWadj*). A fixed effects model of cross and CG was used to estimate least squares means for each growth trait. The effect of cross was significant ($P < 0.0001$) only for *WWadj*; however, no significant differences ($P > 0.8$) were found between crosses within each dairy breed. Further analysis of growth traits considered breed percentages as covariates. SM percentage (SM%) had the greatest influence on *WWadj* (+0.65 lbs./SM%; $P < 0.0001$), *PWGadj* (+0.31 lbs./SM%; $P < 0.07$), and *YWadj* (+0.97 lbs./SM%; $P < 0.0001$). Least squares means for carcass weight (CW), backfat (BF), marbling score (MARB), and ribeye area (REA) from HO × LM, JE × LM, HO × LA (Limousin-Angus) and JE × LA crosses (n = 73,183) were estimated using a fixed effects model of cross, sex, and CG. Cross was significant ($P < 0.0001$) for all carcass traits. Within HO crosses, there was no difference ($P > 0.9$) between beef sire breeds for CW, while JE

× LA crosses had heavier carcasses than JE × LM ($P < 0.0001$). Across both HO and JE crosses, LA-sired animals had more BF ($P < 0.0001$) and higher MARB ($P < 0.0001$) when compared with their respective LM crosses. However, LM-sired crosses had larger REA measurements than LA-sired crosses ($P < 0.0001$). These results indicate selection of beef sires should be dependent on the age producers intend to sell their calf crop and the market grid which cattle are sold.

Key Words: beef × dairy cross, carcass traits, dairy beef

299 Accuracy of single-step genomic breeding values for milk production traits of Canadian Alpine and Saanen dairy goats.

E. Massender*[1], L. F. Brito[1,2], L. Maignel[3], H. R. Oliveira[1,2], M. Jafarikia[1,3], C. F. Baes[1,4], B. Sullivan[3], and F. S. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [3]*Canadian Centre for Swine Improvement Inc., Ottawa, ON, Canada,* [4]*Institute of Genetics, University of Bern, Bern, Switzerland.*

Genomic evaluations have been shown to substantially increase selection accuracy for young breeding candidates in a growing number of livestock genetic improvement programs, leading to increased rates of genetic gain for economically important traits. The objective of this research was to evaluate the expected gain in accuracy from the implementation of single-step genomic evaluations (GEBV) for milk production traits of Canadian Alpine and Saanen dairy goats. A total of 1,650 50K genotypes (789 Alpine, 861 Saanen) and adjusted 305-d phenotypes for milk, protein, and fat yields, and protein and fat percentages in first and later lactations were used. For each trait there were 12,024 and 7,427 first-lactation records and 21,409 and 10,418 later lactation records for Alpine and Saanen, respectively, in the full data sets. Standard errors of prediction (SEP) were obtained from pedigree-based evaluations (EBV) and GEBV predicted using within-breed multiple-trait animal models and either full or validation data sets. The SEP were used to calculate theoretical EBV and GEBV accuracies for individual animals and traits and then averaged across various groups to estimate the gains to selection accuracy that could be achieved. Trait specific gains in theoretical accuracy of GEBV relative to EBV for the validation animals (selection candidates) ranged from 8 to 31% in Alpine and 4 to 35% in Saanen, with average gains across traits of 22% and 21%, respectively. Averaged across traits within each lactation, GEBV predicted from the full data set were 10 to 12% more accurate than EBV for genotyped animals, but no gains were observed for non-genotyped animals, likely due to the small number of genotypes. The largest accuracy gains in the full data sets were found for does without lactation records (28 to 42%) and bucks without daughter records (22 to 61%), which have the least information contributing to their EBV. Consequently, the implementation of genomic selection in the Canadian dairy goat population would increase selection accuracy for young breeding candidates.

Key Words: accuracy, genomic selection, milk production

300 Calf health recording protocols on Canadian dairy farms.

E. Hyland*[1], C. Lynch[1], N. van Staaveren[1], D. Kelton[2], S. Schenkel[1], and C. Baes[1,3], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

The use of producer-recorded health data can enhance genetic evaluation programs. The availability of calf health records compared with data recorded on adult cows is still limited on most dairy farms. In addition, management protocols and codes used to identify calf health issues differ between farms, making industry wide comparisons difficult. Calf mortality is a concern on dairy farms, with diarrhea and pneumonia representing the most frequent causes of death. Calf diarrhea can be caused by various pathogens and treatment of severe cases include electrolytes, antibacterial drugs, and anti-inflammatories. The pathogenesis of calf respiratory illness and pneumonia is also multifactorial and requires treatment in almost all cases. Occurrence of these illnesses are costly, impede the welfare of the affected animals, and can reduce the animal's future performance, all of which negatively affect profitability of the farm. Objectives of this study are to develop standardized recording protocols for calf pneumonia and diarrhea in Canada. To understand current on-farm recording, a survey was distributed to gather information on overall calf management, calf disease protocol, and calf health records. The survey was sent to 13 dairy farmers in Ontario that participated in a previous project (100% response rate). Of the 13 farms, 12 utilized a combination of computer based recording and written records, while one farm exclusively used written records. Rectal temperature (3/13), alertness of the calf (13/13), and fecal score (10/13) were considered for recording cases of diarrhea. Rectal temperature (10/13), coughing (13/13), and nasal discharge (11/13) were considered for recording cases of pneumonia. Health records from all farms that participated in the survey will be analyzed in conjunction with the animal genotypes to create the foundation for calf diseases as novel traits for genetic selection. The development of a pipeline for standardized, early-recorded health data collected by producers has the potential to further improve genetic evaluation programs for calf health and mortality.

Key Words: calf, health, genetics

301 Identifying calf management practices and health events that affect cow longevity.

E. D. McFarland*[1], G. Keefe[1], I. Elsohaby[1], G. A. Oliveira Jr[2], C. F. Baes[2,3], H. Stryhn[1], R. S. Fraser[4], and J. T. McClure[1], [1]*Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada,* [2]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland,* [4]*Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, Canada.*

The primary goal of the dairy industry is to create a well-balanced and profitable cow. Closely linked with production, longevity has the greatest economic value of all non-production traits and is key to dairy profitability. The economic impact of longevity in a farm is related to 3 main elements: 1) the cost of raising heifers; 2) the profit made during lactation; and 3) the reduction of premature/involuntary culling. In the last 20 years, longevity has been included in the official genetic evaluations performed in several countries including Canada. However, most studies and breeding programs have not focused on evaluating the impact of calf health and management practices on cow longevity. Challenges in assessing calfhood factors on longevity include highly variable heifer management practices among farms, with limited records related to calf health. Determining how environmental factors related to calf health affect longevity is crucial. The objective of this study was to evaluate the impact that calf management practices and health have on the cow achieving its genetic potential for longevity and production. To assess the impact of calf management and health on longevity and produc-

tion, a cohort study was performed on 225 calves from 8 herds in the province of New Brunswick. Preweaning records on calf management and disease events were collected from 2014 to 2015. Adult production records were collected on these calves from their first lactation to 2020. Estimated breeding values (EBV) will be used to determine each calf's genetic potential for production, health, and longevity. Phenotypic and EBV correlations for longevity and production traits will be estimated. Multivariate regression will be performed to identify any calfhood environmental and health factors that interfere with a calf's ability to reach its genetic potential for production and longevity traits. Understanding the impact of these factors can help to optimize health management practices on farms, improve profitability, and enhance the genetic potential for increased longevity.

Key Words: calf, health management, genetics

302 Genomic evaluations for Feed Saved in Holsteins.

K. L. Parker Gaddis*[1], P. M. VanRaden[2], R. J. Tempelman[3], K. A. Weigel[4], H. M. White[4], F. Peñagaricano[4], J. E. Koltes[5], J. E. P. Santos[6], R. L. Baldwin[2], J. F. Burchard[1], J. W. Dürr[1], and M. J. VandeHaar[3], [1]*Council on Dairy Cattle Breeding, Bowie, MD,* [2]*Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD,* [3]*Michigan State University, East Lansing, MI,* [4]*University of Wisconsin, Madison, WI,* [5]*Iowa State University, Ames, IA,* [6]*University of Florida, Gainesville, FL.*

Official predicted transmitting abilities (PTA) for Feed Saved in Holsteins were released by the Council on Dairy Cattle Breeding (CDCB; Bowie, MD) in December 2020. Feed intake was measured mid-lactation (50 to 200 d in milk) for 4 to 6 wk in 9 US research herds. As of the December 2020 evaluation, 6,221 phenotypes of residual feed intake (RFI) were included from 5,023 Holsteins born from 1999 to 2017. A phenotypic measure of RFI was estimated by fitting dry matter intake as a linear function of milk energy, metabolic body weight, change in body weight, and cohort effects including parity and days in milk. RFI phenotypes were used to estimate traditional PTAs in an animal repeatability model including effects of herd management group, age by parity group, trial date, herd-sire interaction, and permanent environment. Regressions on genomic evaluations for energy-corrected milk and body weight composite (BWC) were also included in the model to remove genomic correlations that were present after removing phenotypic correlations. Genomic PTAs for RFI were calculated from deregressed traditional PTA using 79,060 SNP for 3.65 million genotyped Holsteins. Feed Saved PTA are provided to the industry and calculated as a combination of RFI PTA and BWC PTA (138 pounds dry matter annual intake per unit BWC PTA) to provide expected pounds of feed saved per lactation. Genomic evaluations of Feed Saved have an average PTA of approximately 10 pounds dry matter saved per lactation, ranging from −738 to 613 pounds among all genotyped animals. Young genomic bulls have an average Feed Saved reliability (REL) of 28%, while progeny-tested bulls average 38%, where REL is calculated as 35% BWC REL and 65% RFI REL. Given the limited reference population, collection of additional phenotypes is a primary goal. Studies are continuing that add data to the CDCB feed efficiency database, and international collaborations also will increase the number of available phenotypes. Feed Saved is currently provided to the industry as an individual trait evaluation, along with a proposal to incorporate it into an economic selection index.

Key Words: feed efficiency, genomic evaluation, Holstein

303 Multiple-trait random regression modeling of feed efficiency in dairy cattle.

P. Khanal*[1], K. L. Parker Gaddis[2], P. M. VanRaden[3], K. A. Weigel[4], H. M. White[4], F. Peñagaricano[4], J. E. Koltes[5], J. E. P. Santos[6], R. L. Baldwin[3], J. F. Burchard[2], J. W. Dürr[2], M. J. VandeHaar[1], and R. J. Tempelman[1], [1]*Michigan State University, East Lansing, MI,* [2]*Council on Dairy Cattle Breeding, Bowie, MD,* [3]*Animal Genomics and Improvement Laboratory, ARS, USDA, Beltsville, MD,* [4]*University of Wisconsin, Madison, WI,* [5]*Iowa State University, Ames, IA,* [6]*University of Florida, Gainesville, FL.*

Genetic improvement of feed efficiency (FE) is an increasing priority in dairy cattle breeding programs. Currently, popular traits to characterize FE include residual feed intake (RFI) and feed saved (FS). These traits are often measured or defined over short and fixed intervals of 4 or 6 weeks at various day in milk (DIM). Random regression (RR) models flexibly allow recording intervals of variable lengths at various stages of lactation which may genetically differ from each other. Multiple-trait extensions to RR models to jointly model dry matter intake (DMI), milk energy (MILKE) and metabolic body weight (MBW) facilitate estimation of genetic parameters that are specific to DIM not only for these 3 traits but also indirectly for change in body weight (dBW), RFI, and FS. We adapted a Bayesian multiple-trait random regression approach using 17,633 weekly records from 50 to 150 DIM on 1,756 cows from 5 different research herds to more flexibly model genetic parameters for FE component traits across DIM. For computational tractability, only pedigree information, rather than genomic data, was used to specify genetic relationships. The heritability estimates of MBW, DMI, MILKE, genetic RFI and genetic FS ranged within the respective intervals of [0.59,0.72], [0.22, 0.30], [0.17, 0.38], [0.16, 0.20], and [0.25,0.31] across DIM whereas the estimated heritability of dBW never exceeded 0.0005. Across DIM, the estimated genetic regressions of DMI on MBW and MILKE ranged within [0.09, 0.14 $\text{kg/kg}^{0.75}$] and [0.21, 0.39 kg/Mcal], respectively, whereas the corresponding phenotypic regressions ranged within [0.10, 0.13 $\text{kg/kg}^{0.75}$] and [0.30, 0.40 kg/Mcal], respectively. Based on this small study, we conclude that there is generally moderate heterogeneity in the genetic parameters characterizing FE across 50 to 150 DIM. We resolve to continue to address the computational challenges of this model using genomic information on our much larger reference population of over 5,000 cows.

Key Words: feed efficiency, multiple-trait analysis, random regression

304 Use of milk spectral data as set of environmental covariates to inform genomic predictions in Canadian Holstein.

F. Tiezzi*[1], A. Fleming[2], and F. Malchiodi[3], [1]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [2]*Lactanet Canada, Guelph, ON, Canada,* [3]*The Semex Alliance, Guelph, ON, Canada.*

Genomic selection models aim at predicting the phenotypic performance of a new, unobserved genotype. In animal breeding, these models are seldom tested for their ability to predict new genotypes' performance under different environmental conditions, despite of the changes in management and diet that the industry undergoes. In this study, milk spectral information was included into genomic prediction models as a set of environmental covariates. The first step involved extracting the environmental component out of the milk spectral wavenumbers (1,060 wavenumbers recorded for 1,540,935 records) by fitting a linear mixed model that included the fixed effects herd-year-season, lactation number and lactation stage as well as the additive genetic, permanent environmental and residual random effects. Variance components estimates for all the spectral wavenumbers showed a similar pattern as found in literature. The second step involved fitting a model with the additive genetic and environmental effects, using calculated herd-year-season daughter-yield-deviations for milk yield and somatic cell score as dependent variables (n = 16,981). The additive genetic effect was modeled through a genomic relationship matrix, containing 483 sires. The environmental effect was modeled as an uncorrelated random effect or with the inclusion of an environmental covariance matrix (i.e., Kernel). The environmental effect included 3,316 herd-year season classes from 406 herds. The environmental kernels were built on BLUE solutions for the herd-year-season effect for different groups of wavenumbers as well as for milk yield and somatic cell score, as obtained from the first step. Results were split between predictions to new or known environments. The inclusion of the spectral-informed environmental effect allowed to outperform the other models in predicting performance to new environments, while prediction accuracy showed a slight decrease when predicting known environments. Fourier-transformed infrared spectral data can be used as a source of information for the calculation of the ‘environmental coordinates’ of a given farm in a given time, extrapolating genomic predictions to new environments.

Key Words: genomic selection, infrared spectra, kernel regression

306 Underlying genetic architecture of mastitis: A systematic review, meta-enrichment, and gene prioritization analysis of genome-wide association study results.

S. G. Narayana*[1,2], E. de Jong[1], P. A. S. Fonseca[2], F. S. Schenkel[2], T. C. S. Chud[2], D. Powel[1], G. Wachoski-Dark[1], P. Ronskley[3], F. Miglior[2], K. Orsel[1], and H. W. Barkema[1,3], [1]*Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, Canada,* [2]*Center for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada.*

Genome-wide association studies (GWAS) have been widely used to identify genomic variants associated with complex traits, such as mastitis, aiming to understand the underlying genetic architecture of the trait. However, no systematic review and gene prioritization analysis have been conducted to date on GWAS results for mastitis. Hence, the objective of this study was to perform a systematic review, meta-enrichment and gene prioritization analysis of GWAS studies to identify key genetic markers and genes associated with mastitis-related traits in dairy cattle. The systematic review was guided using the PPO framework (population: dairy cattle; prognostic tool: GWAS; outcome: mastitis/SCS). Four electronic databases and gray literature were searched and data were extracted from suitable articles. Meta-enrichment analysis was conducted using GALLO package. Gene prioritization analysis was performed using GUILDify and ToppGene. Out of 53 full-text articles identified from systematic review, 40 articles were used for meta-enrichment analysis. Gene and QTL annotation resulted in 9,125 and 43,646 unique genes and QTL, respectively. Nearly 7% of annotated QTL were related to “health” QTL class. Seventy-four genes were found to be common to Holstein, Jersey and Ayrshire breeds. The prioritization analysis resulted in 45 significant genes after Bonferroni correction. These genes were mainly associated with immune response, regulation of secretion and locomotion, and reproduction. Therefore, this study provides a fine mapping of the previously identified genomic regions associated with mastitis and pinpoints key candidate genes for mastitis.

Key Words: mastitis, gene prioritization, meta-enrichment

307 A comprehensive catalog of regulatory variants in the cattle transcriptome - a prototype for the FarmGTEx Project.

G. Liu*, *Animal Genomics and Improvement Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, USDA, Beltsville, MD.*

The systematic characterization of genetic regulatory variants on the transcriptome of livestock is essential for interpreting the molecular mechanisms underlying traits of economic value, and to increase the rate of genetic gain through artificial selection. The Farm Animal Genotype-Tissue Expression (FarmGTEx) Consortium is a collaborative endeavor to provide a comprehensive atlas of tissue-specific gene expression and genetic regulation in livestock species. In its pilot phase, by uniformly analyzing publicly available sequence data using our newly developed transcriptome pipeline as described in the cattle GTEx preprint (https://www.biorxiv.org/content/10.1101/2020.12.01.406280v1), we build a cattle Genotype-Tissue Expression atlas (cGTEx, http://cgtex.roslin.ed.ac.uk/) for the research community, based on 11,642 RNA-seq data sets from over 100 cattle tissues. We describe the landscape of bovine transcriptome across tissues and report thousands of *cis* and *trans* genetic variants (like expression QTL, or eQTL), associated with gene expression and alternative splicing for 24 tissues. We evaluate the specificity/similarity of these genetic regulatory effects across tissues, and functionally annotate them using a combination of multi-omics data. Finally, we link gene expression in different tissues to 43 economically important traits using a large transcriptome-wide association (TWAS) study to provide novel biological insights into the molecular regulatory mechanisms underpinning agronomic traits in cattle. This study provides a prototype for the FarmGTEx Project in other major farm animal species including pigs, chicken, sheep, and goats. This is a presentation about Cattle Genotype-Tissue Expression Atlas from the FarmGTEx Project Pilot phase. The contributors include Shuli Liu, Yahui Gao, Oriol Canela-Xandri, Sheng Wang, Ying Yu, Wentao Cai, Bingjie Li, Erola Pairo-Castineira, Kenton D’Mellow, Konrad Rawlik, Charley Xia, Yuelin Yao, Xiujin Li, Ze Yan, Congjun Li, Benjamin D. Rosen, Curtis P. Van Tassell, Paul M. Vanraden, Shengli Zhang, Li Ma, John B. Cole, George E. Liu, Albert Tenesa, and Lingzhao Fang from multiple international institutions.

Key Words: expression QTL, transcriptome-wide association (TWAS), RNA-seq

308 Signatures of selection as a tool to identify past selection criteria in Holstein cattle.

F. J. Ruiz-Lopez*, A. Garcia-Ruiz, and J. G. Cortes-Hernandez, *CENIDFyMA-INIFAP, Queretaro, México.*

The small holder dairy production system in Mexico does not have an established genetic improvement program and therefore the selection criteria used by farmers to identify the genetic material to use in their dairies are unknown. Signatures of selection are regions conserved in the genome for generations due to selection processes, and one method to identify them is through runs of homozygosity (ROH). The aim of the present study was to investigate which characteristics have been improved over the years by farmers’ mating decisions through signatures of selection in the genome of Holstein cattle in the small holder system in Mexico. After quality controls the study included 214 GeneSeek Genomic Profiler Bovine GGP 50K chip genotypes of Holstein cows from 4 small holder dairy herds located in the region of Tepatitlán Jalisco, Mexico. Only runs of homozygosity that had a minimum length of 500 kb and a minimum number of 25 SNPs were included, allowing up to 1 heterozygous SNP and 5 lost genotypes per run. The analyses were carried out with the bioinformatics platform SNP & Variation Suite v7.6.8.

The most frequent ROHs were identified and according to their physical position in the genome, annotations that have been made in other populations were searched in the Animal QTLdb Release 43 database and possible genes in the regions or selection traces in the BGVD database. As a result, 10,798 ROHs were detected with an average length of 4.79 Mb, suggesting common ancestors about 16 generations back. Of the total ROH identified, 70.65% were unique and 29.35% were repeated. In the region where the most frequent ROHs were found in the population (≥20 animals), numerous associations have been reported with QTL and genes that are related to milk production and composition, fertility parameters, susceptibility to diseases, body conformation, feed efficiency and some characteristics of carcass composition. The conserved regions show the population selection objectives and, for the most part, reflect a trend related to milk production, milk composition, fertility and health, characteristics generally selected for by the foreign AI companies. In some cases, associations with improvement characteristics of the carcass are also observed.

Key Words: signatures of selection, smallholder dairy production system

Dairy Foods: Chemistry

309 Effect of heat-induced, pH-dependent micellar changes on the sol-gel transition in skim milk.

S. Tieu* and F. Harte, *The Pennsylvania State University, State College, PA.*

The manufacture of cheese and yogurt is based on casein's ability to form a gel. Stabilizers, thickeners, and emulsifiers are often used to improve gel firmness. These ingredients add cost and may not meet consumers' expectations of clean label, which has become the industry standard. The present work aims to increase gel strength with mild alkaline-thermal pretreatment below the denaturation temperature of whey protein. We hypothesized heat-induced, pH-dependent micellar changes, namely the shifts in casein and calcium between the colloidal and serum phases, result in firmer milk gels. Homogenized, pasteurized milk was adjusted to pH values in the range 6.4 to 7.3 in 0.3 ± 0.05 increments with 2 *M* NaOH or 2 *M* HCl, heated at 60°C for 15 min, cooled to refrigeration temperature, and restored to native pH (pH 6.7) at refrigeration temperature. Then, acid gels were induced by 3.33 wt% glucono-δ-lactone and rennet gels by 0.1% of 1:9 diluted chymosin. We monitored storage modulus (G′, Pa) during gel formation with small-amplitude oscillatory shear at a fixed strain of 0.1%. We ran full factorial experiments with 2 replications using randomized complete block design. Each batch of milk receiving all temperature and pH treatment combinations constituted a block. Analysis of variance and Tukey's test were used for means and pairwise comparisons (α = 0.05). Mean maximum G′ of acid and rennet gels prepared from milk heated at 60°C, measured at 3 and 2 h, respectively, are shown in Table 1 with standard deviations. Means that do not share a letter are significantly different. Increasing the pH at heating from pH 6.7 to 7.3 increased G′ 2-fold and 3-fold in acid and rennet gels, respectively. We attributed these observations to the corresponding levels of soluble casein and soluble calcium, as determined by gel electrophoresis and atomic emission spectroscopy. Our work provides a cost-effective, scalable way to increase gel firmness in acid and rennet gels. The ability to enhance milk functional properties with mild pretreatment allows for expression of novel functionality and opens avenues to manufacture clean label dairy products.

Table 1. Firmness of milk gels prepared from milk heated at 60°C

Gel	pH at heating	G′ (Pa)
Acid	6.4	84 ± 3^{a}
	6.7	105 ± 12^{a}
	7.0	125 ± 14^{ab}
	7.3	201 ± 19^{c}
Rennet	6.4	36 ± 8^{A}
	6.7	24 ± 5^{A}
	7.0	38 ± 3^{AB}
	7.3	77 ± 0^{C}

Key Words: casein micelle, milk gel, heat treatment

310 A novel method of determining critical gel-sol transition point of micellar casein concentrate using multiple waveform rheological technique.

F. Z. B. Seighalani, D. J. McMahon, and P. Sharma*, *Utah State University, Logan, UT.*

Gelation is an important functional phenomenon and essential process in preparation of food gels and structured dairy systems such as cheese and yogurt. On the other hand, in certain operations it needs to be avoided to ensure processability of liquid streams. Therefore, providing an accurate and reliable method for gel-sol transition point measurement is critical for the developing new products, optimizing processability of dairy products and designing and scaling up dairy processing equipment and operations. In this study we developed a novel method of accurately and precisely measuring gel-sol transition point of micellar casein concentrate (MCC) during heating using multiple waveform rheological technique in combination with Winter–Chambon criterion. Measurement of the crossover temperature at which storage modulus (*G′*) and loss modulus (*G″*) crosses over is a conventional rheological method widely used for determination of gelling point. All the experiments were performed in triplicate. We found that the conventional method indicates a pseudo-gelling point which is frequency dependent and does not represent true physical state of the material. Gel-sol transition temperature obtained with new method (11.1°C) was significantly ($P < 0.05$) different to the conventional method (21–27°C). While using the convergent point of loss tangent (tan δ) from different frequencies extracted from standard temperature sweep and multiwave test showed about the same gelling point (11.1°C). The low frequency dependence (n = 0.05) of the sample at 5 and 11.1°C also confirmed the sample is a strong gel at these temperatures, while the high frequency dependence at 25°C (n = 0.7) demonstrated a viscoelastic liquid behavior. These findings demonstrated that employing Winter–Chambon criterion can be an effective way for determining of true gel-sol transition point of MCC, and multiwave oscillation was a quick measurement for analyzing the sample. The technique employed in this study can be used as a reliable alternative by other researchers to evaluate gelling point of other food gels. Furthermore, findings of this study can help food and dairy industries to better design their products, processes and equipment considering the true gelling point obtained using this novel technique.

Key Words: rheology, gel-sol transition point, micellar casein concentrate

311 Nanoencapsulation of mixture of docosahexaenoic acid algae oil and chia seed oil in whey protein concentrate and gum arabic complex coacervates.

N. Chaudhary* and V. Sharma, *National Dairy Research Institute, Karnal, Haryana, India.*

Nanoencapsulation is a technology to encapsulate substances in a miniature form (10–1000 nm) and enhance bioavailability and enable precision targeting of the bioactive compounds such as omega-3 fatty acids, essential oils, vitamins, etc. In the present study, complex coacervation approach was used to encapsulate ω-3 rich docosahexaenoic acid (DHA) algae oil and chia seed oil using wall material of whey protein concentrate (WPC) and gum arabic (GA) complex coacervates. The complex coacervation process between WPC and GA was optimized in terms of pH in the range of 3.5–4.5 and WPC-to-GA ratio of 1:1, 2:1, 3:1 and 4:1 respectively by monitoring the turbidity of the solution. Turbidity test was carried out using UV-visible spectrophotometer at 750 nm by measuring the absorbance of the solution at a particular pH and ratio and the optimum pH and ratio of WPC-to-GA was found to be 3.9 and 3:1, respectively. Confocal Laser Scanning Microscopy was used to visualize the complex formation between WPC and GA and further to corroborate the data obtained by UV-visible spectrophotometer. Nanocapsules of mixture of DHA and chia seed oil was encapsulated in WPC-GA complex using a high shear homogenizer and ultrasonicator. The nanocapsules thus obtained had 20% oil content, spherical shape with a Z-average of 49.07 ± 6.46 nm, Polydispersity index (PDI) of

0.406 ± 0.07 and an encapsulation efficiency of 98.94 ± 0.26%. Confocal laser scanning microscopy showed that WPC-GA complex formation had occurred at optimum pH and ratio and the oil droplets were clearly nanoencapsulated in this WPC-GA matrix. All the experiments were performed in triplicate and the values shown above are mean ± standard error mean and 1-way and 2-way ANOVA was used for data analysis. Thus, the present study provides a process for the nanoencapsulation of ω-3 fatty acids using complex coacervation of WPC and GA which could be incorporated into various food matrices and further research could be explored using newer wall materials and core combinations.

Key Words: nanoencapsulation, complex coacervation, chia seed oil

312 Preparation of nanocapsules of curcumin using casein micelles and their characterization. A. Hooda*[1,2], B. Mann[1], R. Sharma[1], R. Bajaj[1], S. Singh[1], and N. Das[1], [1]*National Dairy Research Institute, Karnal, Haryana, India,* [2]*Banaras Hindu University, Varanasi, India.*

Global demand for functional food is increasing and nanoencapsulation has wide scope in enhancing value of foods by incorporating various nutraceuticals into food matrices. Casein (80% of total milk protein) is responsible for delivering nutrients to neonates and has excellent functional properties, good digestibility, high nutritional value, shielding ability, is inexpensive and readily available which makes it an excellent wall material for nanoencapsulation. These biological and functional properties can only be maintained if size and morphology of casein micelle are preserved. Hence, in the present study casein micelles from skim milk were used as wall material for nanoencapsulation of model bioactive compound curcumin. Modification in pH to basic and neutral sides was used as a tool to open up casein micelles for encapsulation. The encapsulation efficiency (EE) was observed as 99 ± 0.23% using HPLC based method. The formulation was then spray dried at an inlet temperature of 180°C to obtain skim milk powder with nanoencapsulated curcumin. All measurements were done for at least 3 replicates. Results are presented as means ± standard error of means (SEM). The powder properties, analyzed by reconstituting it, showed that particle size and zeta potential of powder solution (236 ± 14.1 nm, −19.9 ± 0.85 mV) were similar to skim milk (229 ± 6.38nm, −20.6 ± 0.67 mV). The morphology and size characteristics were also verified by transmission electron microscopy. The powder showed good EE of 98 ± 0.12% at normal processing conditions of pH, ionic strength, sugar and temperature. Antioxidant activity of curcumin was preserved and in vitro studies showed the encapsulated curcumin was released in the intestinal phase of digestion. This powder is highly dispersible in water and contains 0.8–1.0% curcumin and 34.0–37.0% milk protein and was shelf stable for 6 mo at ambient temperature. This type of formulations of different bioactive compounds can serve as a ready to disperse powder, which can be solubilized in liquid dairy/food beverages.

Key Words: nanoencapsulation, spray drying, native casein micelles

Dairy Foods: Dairy Products

313 Shelf life stability and physicochemical and sensory characteristics of acidophilus yogurt supplemented with marjoram oil extract.

H. N. T. Mohamed*[1], A. R. A. Hammam[1,2], and D. G. Kamel[1], [1]*Dairy Science Department, Assiut University, Assiut, Egypt,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

The natural antimicrobials extracted from herbs have been utilized recently in many dairy products. This work was aimed to develop a process to produce acidophilus yogurt (AY) using marjoram oil extract (MOE) at rates of 0.3 and 0.5% and study the characteristics of AY during storage for 16 d at 4°C. The hydrodistillation method was utilized to extract the MOE, and this process took around 2.5 h at 100°C until no more essential oil was obtained. Subsequently, the extracted oil was collected, dried over sodium sulfate, filtered, and stored at −20°C. The extracted oil before being utilized in AY manufacture was analyzed for antimicrobial activity and total phenolic content (676.894 mg/kg). The AY was manufactured using pasteurized buffalo milk. The milk was supplemented with 0.3 and 0.5% of MOE. Another treatment was also manufactured with no MOE as a control. All treatments were inoculated with 7.30 log cfu of *Lactobacillus acidophilus* (LA) per kg of milk at 40°C for 4 h. The yogurt was then cooled and stored at 4°C. This work was performed in triplicates. The acidity, total solids (TS), total protein (TP), fat, LA count, total bacterial count (TBC), yeast count, and mold count were determined in the AY at 0, 4, 8, 12, and 16 d. The sensory characteristics of AY were also examined using 10–15 trained panelists. The AY had approximately 0.6, 16, 4.6, and 7% for acidity, TS, TP, and fat, respectively. The acidity had a significant increase ($P < 0.05$) during storage from around 0.6 to 0.9%. MOE promoted the growth of LA in AY. The antimicrobial activity against *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* was found with the addition of MOE. The growth of yeast was postponed up to 12 d when MOE was added, while yeast showed up in control after 8 d. The addition of MOE in AY did not show any molds, while control presented molds after 8 d. The panelists accepted the addition of MOE and this acceptability resulted in higher scores ($P < 0.05$) as compared with control. These results showed the possibility of using MOE as a functional ingredient in the manufacture of AY.

Key Words: Acidophilus yogurt, marjoram oil extract, antimicrobial activity

314 Effect of rosemary oil extract on the shelf stability and chemical, microbiological, and sensory characteristics of drinkable yogurt.

D. G. Kamel*[1] and A. R. A. Hammam[1,2], [1]*Dairy Science Department, Assiut University, Assiut, Egypt,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Rosemary oil extract (ROE) has attracted many consumers as alternatives for artificial additives. The objective of this study was to investigate the impact of ROE at 0.5 and 0.7% on the chemical, microbiological, sensory properties, and shelf stability of drinkable yogurt (DY) during storage for 16 d at 4°C. The ROE was extracted (using the hydrodistillation method) and added to pasteurized buffalo milk at a rate of 0.5 and 0.7%, while another portion of milk had no MOE as control. Every kg of each treatment was inoculated at 40°C for 6 h with *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* at a rate of 6.47 and 6.54 log cfu, respectively. Then, the DY was stirred using a sterilized racket and stored at 4°C until further analyses. The DY was examined at 0, 4, 8, 12, and 16 d for acidity, total solids (TS), total protein (TP), fat, lactic acid bacteria (LAB), total bacterial count (TBC), coliform, yeast count, mold count, and sensory characteristics (10–15 trained panelists). This experiment was repeated 3 times. The DY had approximately 0.5% acidity, 15% TS, 4.5% TP, and 7% fat. The storage for 16 d showed a significant effect ($P < 0.05$) on the acidity of DY from around 0.5 to 0.7%. Elevating the level of ROE to 0.7% resulted in a significant increase ($P < 0.05$) in the growth of LAB in DY to approximately 8.3 log cfu/g compared with 7.9 log cfu/g in control. ROE presented antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*. That antimicrobial activity of ROE delayed the growth of yeast up to 12 d, which was more shelf-stable as compared with control (8 d). Additionally, no molds and coliform were detected in the DY made with ROE; while they were detected in control after 8 and 12 d, respectively. The overall acceptability of DY was significantly improved ($P < 0.05$) by the addition of ROE relative to control. This study revealed that ROE can serve as an antimicrobial agent in DY manufacture to extend the shelf life and to promote the growth of LAB.

Key Words: drinkable yogurt, rosemary oil extract, antimicrobial activity

315 Manufacture of set-type yogurt using transglutaminase: Texture, rheology, and structural properties.

M. Enteshari[1] and S. I. Martinez-Monteagudo*[2], [1]*South Dakota State University, Brookings, SD,* [2]*New Mexico State University, Las Cruces, NM.*

The protein network of the yogurt is mainly composed of casein micelles and plays a crucial role in the development of the structure. Transglutaminase (TGase) is an enzyme with the ability to crosslink caseins, improving the texture and mouthfeel of the resulting yogurt. In this work, we study the effect of TGase during the manufacture of skim milk set-type yogurt. Different concentrations of TGase (0, 2, 3, 4, and 6 g L^{-1}) were evaluated before the fortification of the milk base. The enzymatic treatment consisted of 3 h of incubation at 50°C before the addition of skim milk powder (SMP) at different concentrations (0, 1, 2, and 3%). An additional set of experiments was conducted in which the enzyme was added simultaneously with the starter cultures. After fermentation, yogurts were analyzed in terms of composition, syneresis, texture, rheological behavior, and microstructure. The highest firmness and hardness (6.55 ± 0.32 N and 96.43 ± 4.82 N s) were obtained in those samples containing 1% SMP and 6 g L^{-1} TGase added before fermentation. Interestingly, the minimum syneresis was observed in the yogurts containing 3% SMP and 6 g L^{-1} TGase (30.94 ± 3.9%). Dynamic rheology showed improved stability of the protein network, displaying a distinctive viscoelastic region and behavior of weak gel. Images obtained through scanning electron microscopic displayed a grainy network made of caseins aggregates linked in clusters, forming a 3-dimensional network of regular voids. Yogurts treated with TGase exhibited desirable quality parameters. The outcomes of this investigation suggest that TGase may be used during the manufacturing of set-type yogurt with improved textural properties.

Key Words: yogurt, transglutaminase, dynamic rheology

316 Effect of temperature on the rheological behavior of high-protein ice cream mix: Steady, dynamic, and creep-recovery.

H. Ranaweera*[1], P. Krishnan[1], and S. Monteagudo[2], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,*

[2]*Department of Chemical and Materials Engineering New Mexico State University, Las Cruces, NM.*

Over the last few years, milk proteins have become a popular ingredient due to numerous health benefits associated with their consumption, including promoting satiety, appetite control, and exercise recovery. As a result, concentrates and isolates of milk proteins are commonly used to formulate beverages, snacks, dietary supplements, and desserts. Consumers hold a special appeal for frozen desserts as they provide a suitable source of protein. Currently, the manufacture of high-protein ice cream, an increment of 4- to 6-fold, is an area of industrial interest. However, the formulation of such ice cream is not a trivial task. Applying the same concepts of Greek-style frozen desserts result in technical challenges such as compatibility of ingredients, mixing, formulation, freezing, and handling the changes in flow characteristics. This research project aims at studying the effect of temperature (5, 15, 25, and 35°C) on the rheological behavior of the high-protein ice cream mix (HP-ICM). The rheological behavior was evaluated in terms of steady, dynamic, and creep-recovery. Milk protein concentrate (MPC-80) was used to formulate a HP-ICM containing 12% of protein. A regular ice cream mix (4% protein) was used for comparison. The flow behavior index (n) for HP-ICM was 0.65 ± 0.08, while the regular mix was 0.75 ± 0.13, meaning that all samples exhibited shear-thinning behavior. The consistency coefficient (K) was significantly higher ($P < 0.005$) in HP-ICM (4.01 ± 0.25 Pa s^n) than regular mix (0.27 ± 0.01 Pa·s^n). Interestingly, the addition of MPC-80 resulted in a 14.5-fold viscosity increment within the high spectrum of shear rate (50 s^{-1}) compared with the regular mix. Strain sweep analysis revealed that all HP-ICM displayed a distinctive viscoelastic region. Also, increase in temperature from 5°C to 35°C has impacted the decrease of storage modulus(G') and loss modulus (G''), reduction of shear stress(pa) with respect to shear rate (1/*s*) from 83 to 25 and increase of maximum creep compliance *j*(*t*)[1/*pa*] from 3.6 to 5.7. The outcomes of this investigation provide opportunities for designing freezing strategies for high-protein frozen desserts.

Key Words: rheology, protein, temperature

317 Impact of hydrodynamic cavitation on rheological and quality aspects of ice cream.

J. Y. Sim[1], M. Enteshari[1], K. Rathnakumar[1], and S. I. Martinez-Monteagudo*[2], [1]*South Dakota State University, Brookings, SD,* [2]*New Mexico State University, Las Cruces, NM.*

Ice cream is one of the most popular desserts with more than 10 kg of the product being consumed per person each year in the US alone. Over the past few years, consumer-to-consumer persuasion through social media has created a new set of desirable attributes for processed foods, where the perception of natural, healthy, and sustainable plays a significant role in the purchase decision. A popular theme within such trends is the removal of stabilizers from the formulation, which are perceived as unnatural and considered less acceptable by consumers. We report the viability of hydrodynamic cavitation (HC, 2400–3600 rpm/100–200 L h^{-1}) to manufacture ice cream with reduced stabilizer. The study was carried out at a pilot-scale level and consisted of determining the impact on particle size and rheological behavior of ice cream mixes (ICM) and evaluating the resulting melting and meltdown behavior of ice cream. Ice cream manufactured with 2-stage homogenization was used as a control treatment. Overall, HC resulted in larger mean particle size than the control mixes (3.52 ± 0.28 and 0.34 ± 0.02 μm, respectively). Additionally, HC (3600 rpm/100 L h^{-1}) changed the flow behavior of ICM from viscoelastic solid to viscoelastic liquid. Cavitated mixes resulted in a 2.2-fold viscosity increment within the high spectrum of shear rate (30–50 s^{-1}) compared with the control mixes. These modifications altered the melting and meltdown characteristics of the resulting ice cream. The highest rate of the meltdown was observed for reduced stabilizer ice cream (1.59 ± 0.25 mL min^{-1}), followed by the cavitated ice cream (1.22 ± 0.15 mL min^{-1}) and control ice cream (1.09 ± 0.09 mL min^{-1}). The analysis of the particle size and gross microstructure of dripped melted ice-creams suggested a significant collapse of the ice cream structure, allowing clumps of fat to migrate into the melted phase. This study showed that HC might have the potential to develop novel structures for frozen desserts.

Key Words: ice cream mix, dynamic rheology, hydrodynamic cavitation

Forages and Pastures II

318 Diversity of the ruminal microbiota in Jersey cows grazing forage canola. L. H. P. Silva*[1,2], Y. Zang[1,3], M. Ghelichkhan[1], Y. Geng[1,4], S. L. Dillard[5], K. J. Soder[6], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH,* [2]*Western Kentucky University, Bowling Green, KY,* [3]*William H. Miner Agricultural Research Institute, Chazy, NY,* [4]*Chinese Academy of Agricultural Sciences, Beijing, China,* [5]*Auburn University, Auburn, AL,* [6]*USDA-Agricultural Research Service, Pasture Systems and Watershed Management Research Unit, University Park, PA.*

Forage canola (CAN) has high concentrations of sugars and soluble CP, which can alter the ruminal microbiota population and methanogenesis. In fact, our research revealed that dairy cows grazing CAN showed decreased CH_4 production. We aimed to evaluate the effect of CAN, as grazed herbage, on diversity of ruminal microbes in lactating dairy cows, and investigate the correlation between microbiota and CH_4 emissions (measured with 2 GreenFeed units). Twelve multiparous and 6 primiparous mid-lactating organic-certified Jersey cows were paired by parity, milk yield, or DIM and, within pair, randomly assigned to control (CTRL) or grazed CAN in a randomized complete block design. Cows in the CTRL group were kept in confinement, while CAN cows stayed in the barn during the day and had access to pasture from 1800 to 0500 h. Diets were formulated to yield a 60:40 forage:concentrate ratio, with 50% of the baleage replaced by herbage in the CAN diet. The experiment lasted 7 wk (2-wk covariate) with sample collection done during wk 3 and wk 5. Daily herbage allowance was set at 12 kg of DM/cow, and ruminal fluid was collected using a stomach tube. Canola biomass averaged 4,423 kg of DM/ha, with CAN herbage averaging 15.5% aNDFom, 21.6% ESC, 24.8% CP, and 13.6% soluble CP. Methane yield was 16% lower ($P < 0.001$) in CAN than CTRL (18.3 vs. 21.7 g/kg of DMI). There were no treatment effects on the eukaryote α diversity indices Shannon and Faith, and number of amplicon sequence variants (ASV). Similarly, principal coordinate analysis (PCoA) of ruminal eukaryotes showed no clusters. The prokaryote α diversity indices Shannon and Faith were lower ($P < 0.05$) in CAN than CTRL, and number of prokaryote ASV tended ($P = 0.06$) to decrease with CAN. In addition, PCoA showed cluster formation for the prokaryote community for both treatments. While the bacteria:archaea ratio was not affected by diet, CH_4 yield was negatively correlated with this ratio (−0.36; $P = 0.01$). Cows fed CAN had a lower relative abundance of the archaeal phylum *Thermoplasmatota* ($P = 0.01$) compared with CTRL, with uncultured *Prevotella* being negatively associated with CH_4 yield. In brief, CAN decreased prokaryote community diversity but did not affect the eukaryote population.

Key Words: brassica, grazing, ruminal microbiota

319 Ruminal fermentation and methane emissions in Jersey cows grazing forage canola. L. H. P. Silva*[1,2], J. P. Sacramento[1,3], D. C. Reyes[1], Y. Geng[1,4], M. Ghelichkhan[1], S. L. Dillard[5], K. J. Soder[6], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH,* [2]*Western Kentucky University, Bowling Green, KY,* [3]*Universidade Federal de Sao Joao del Rei, Sao Joao del Rei, MG, Brazil,* [4]*Chinese Academy of Agricultural Sciences, Beijing, China,* [5]*Auburn University, Auburn, AL,* [6]*USDA-Agricultural Research Service, Pasture Systems and Watershed Management Research Unit, University Park, PA.*

Canola (CAN) can be used as an annual forage crop to extend the grazing season later in the fall. Forage CAN is an exceptional source of rapidly fermentable carbohydrates, which can alter ruminal fermentation and enteric CH_4 production. We aimed to evaluate the effect of partially replacing baleage with CAN herbage on ruminal fermentation profile and CH_4 production. Twelve multiparous and 8 primiparous mid-lactation organic-certified Jersey cows were paired by parity, milk yield, or DIM and, within pair, randomly assigned to control (CTRL) or CAN diet in a randomized complete block design. Cows in the CTRL group were kept in confinement, while CAN cows stayed in the barn during the day and had access to pasture from 1800 to 0500 h. Diets were formulated to yield (DM basis) 60:40 forage:concentrate ratio, with 67% of the baleage replaced by grazed CAN in the diet DM. The experiment lasted 7 wk (2-wk covariate) with sample collection done during wk 3 and wk 5. Ruminal fluid was collected using a stomach tube, and gaseous fluxes were measured using 2 GreenFeed units. Daily herbage allowance averaged 19 kg of DM/cow. Data were analyzed with repeated measures in SAS. Canola herbage yield averaged 6,662 kg of DM/ha, with 17.6% ESC and 24.1% CP. There was no treatment effect on DMI (mean = 21.1 kg/d; $P = 0.11$), but cows in the CRTL group tended to have a greater milk yield (21.2 vs. 20.1 kg/d; $P = 0.10$) than those in the CAN group. Enteric CH_4 production (436 vs. 295 g/d; $P < 0.01$), CH_4 yield (20 vs. 14.1 g/kg DMI; $P < 0.01$), and CH_4 intensity (16.0 vs 11.5 g/kg ECM; $P < 0.01$) decreased significantly with feeding CAN vs. CTRL. While ruminal total VFA concentration and the molar proportions of propionate and butyrate increased, those of acetate, isobutyrate, and isovalerate, and the acetate:propionate ratio decreased in CAN vs. CTRL ($P < 0.01$). These shifts in ruminal fermentation likely explains the reduction in methanogenesis in cows grazing CAN as metabolic pathways favoring propionate formation decrease H_2 used for CH_4 synthesis. Glucosinolates present in CAN may also have been involved on CH_4 suppression. In summary, cows grazing CAN herbage produced up to 32% less CH_4, which can be linked to the shift in ruminal fermentation from acetate to propionate.

Key Words: brassica, grazing, methane

320 Performance of lactating dairy cows fed mechanically processed alfalfa silage. D. A. Pintens*[1], K. F. Kalscheur[2], K. J. Shinners[1], J. C. Friede[1], and M. F. Digman[1], [1]*Department of Biological Systems Engineering, University of Wisconsin–Madison, Madison, WI,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Increasing the cutting interval of alfalfa leads to greater yield, however nutritional value suffers. Extreme mechanical processing (ExMP) has been explored as a method to recover lost feed value. Mechanical processing ruptures cells and increases surface area for microbial attachment in the rumen, facilitating greater fiber digestion. The objective of this study was to evaluate the effect of mechanical processing of alfalfa before ensiling on the performance of lactating dairy cows. Thirty-six Holstein cows (119 ± 38 DIM) were assigned to one of 2 diets in a randomized complete block design with a 2-wk common feeding period, followed by a 6-wk experimental period. The 2 diets were identical, except for alfalfa harvest techniques. Both diets contained 30% alfalfa haylage, 30% BMR corn silage, and 40% concentrate (DM basis). Alfalfa in the control (CON) diet was conventionally harvested with a forage harvester set to 9.5mm theoretical-length-of-cut (TLOC). The ExMP diet was chopped with the same harvester at 22.2mm TLOC and then processed with an experimental shredding-impact processor. The forage processing was quantified by a processing level index (PLI). The CON alfalfa had a PLI of 38%, while the ExMP alfalfa PLI was 73%. Data were analyzed using MIXED procedures of SAS. Cows fed the ExMP diet tended to have less DMI (27.3 vs 28.0 kg/d; $P = 0.09$) than cows fed the CON diet. Milk yield was not significantly different between treatments ($P = 0.22$), however, milk fat percentage for cows fed the ExMP diet was higher than cows fed the CON diet (3.94 vs 3.81% fat; $P = 0.02$). Protein percentage did not differ between diets ($P = 0.40$). Because of greater fat percentage, both fat-corrected milk (FCM) and energy-corrected milk (ECM) for cows fed the ExMP diet was greater than cows fed the CON diet [46.2 vs. 44.8 kg/d ($P = 0.03$) and 49.5 vs. 48.1 kg/d ($P = 0.04$), respectively]. Feed conversion efficiency, defined as either FCM/DMI or ECM/DMI, was greater for cows fed the ExMP diet compared with cows fed the CON diet [1.69 vs 1.60 ($P = 0.003$) and 1.81 vs 1.72 ($P = 0.003$), respectively]. Overall, cows fed the ExMP diet were more efficient and out-performed cows fed the CON diet.

Key Words: alfalfa silage, processing

321 Inoculant effects on fermentation of grass-clover silage with or without Clostridia challenge.

V. Jenkins[1], J. Kesselring[1], I. Mueller[1], R. Nylund[2], and H. Gonda*[2], [1]*BIOMIN Holding GmbH, Getzersdorf, Austria,* [2]*Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden.*

The goal of silage production is to maintain feed value. Clostridia in silage challenges this with protein and sugar breakdown and production of butyric acid. We examined the effects of a mixed homo- and heterofermentative inoculant on grass-clover silage quality with or without Clostridia challenge. First cut grass and clover (73% grass on DM basis) was cut at 8 cm height in early June, wilted to 28% DM and chopped to 30 mm. With 2.63% water-soluble carbohydrate (FM basis) and a fermentation coefficient of 28.7 [$FC = DM\% + 8 \times (WSC\% / \text{buffer capacity})$], the silage was classified as difficult to ensile. The experiment was a 2 (inoculant or no inoculant) × 2 (Clostridia or no Clostridia) factorial design with 4 replicates. The inoculant (*Lactobacillus plantarum* DSM 19457, *L. brevis* DSM 23231 and, *L. kefiri* DSM 19455) was applied at 2×10^5 cfu/g, and the Clostridia challenge was with *Clostridium tyrobutyricum* at 10^4 spores/g. The forage was ensiled in 1.75-L glass jars at a density of 153 kg DM/m^3. Fermentation profile and aerobic stability were tested after 14, 21, 42 and 90 d. The Clostridia challenged silage was tested at d 90. Data were analyzed by Proc GLM and Wilcoxon Scores (Proc Npar1way, SAS). At d 14, pH of the inoculated silage was 2.1 units below control. The inoculant improved fermentation profile at all openings with increased lactic acid, acetic acid and 1,2-propandiol and reduced ammonia-N, ethanol, 2,3-butandiol and butyric acid ($P < 0.001$). With the inoculant, DM losses were 48% and 54% lower on d 14 and 90 respectively and NEL was 15% higher at 90 d compared with control ($P < 0.001$). Aerobic stability was improved at all openings. With Clostridia challenge, the inoculant increased NEL by 6% and reduced DM losses by 57% ($P < 0.001$). Clostridia did not proliferate in inoculant-treated silos and butyric acid and ethanol were reduced by 86% and 43% respectively ($P < 0.001$). A 61% reduction in ammonia-N indicated improved protein preservation ($P < 0.001$). In conclusion, a commercial inoculant significantly improved fermentation quality of difficult to ensile grass-clover silage even under Clostridia challenge.

Key Words: grass-clover silage, Clostridia, aerobic stability

322 Effect of wilting, microbial inoculation, and storage length on the fermentation profile, nutrient composition, and aerobic stability of Bermuda grass silage.

E. C. Diepersloot*[1], M. R. Pupo[1], C. Heinzen Jr.[1], C. L. McCary[2], L. G. Ghizzi[3,2], J. O. Gusmão[4,2], and L. F. Ferraretto[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*University of Florida, Gainesville, FL,* [3]*University of São Paulo, Pirassununga, SP, Brazil,* [4]*Federal University of Lavras, Lavras, MG, Brazil.*

The objective of this experiment was to determine the effects of wilting, microbial inoculation, and storage length on the fermentation profile, nutrient composition, and aerobic stability of Bermuda grass silage. Unwilted (UN; 30% DM) or wilted (WT; 45% DM) Tifton 85 Bermuda grass was inoculated with one of 4 inoculants CL (*Lactobacillus plantarum* and *Pediococcus pentosaceus*; 200,000 cfu/g), FT (*L. plantarum*, *Pediococcus acidilactici*, and *Lactococcus lactis*; 300,000 cfu/g), FD (*L. plantarum*, *P. acidilactici*, and *L. lactis*; 600,000 cfu/g) or CON (50 mL distilled water) and packed into 20 L experimental silos before being allowed to ferment for 30 or 90 d of storage length. Data were analyzed as a completely randomized design with a 2 × 4 × 2 factorial arrangement of treatments using Proc Glimmix of SAS and significance was declared at $P < 0.05$. A 3-way interaction was detected ($P = 0.01$) for total acid production, with UN silage inoculated with FD at 90 d of storage being greatest (7.7% DM), and WT silage inoculated with FD at 30 d of storage lowest (2.0% DM). A 2-way interaction between wilting and inoculation was observed for acetic acid ($P = 0.04$), with FD inoculated silage being greater (5.2% DM) than other treatments in UN, while WT treatments were all lower (0.9% DM). Similarly, interactions between wilting and storage length were observed for lactic acid ($P < 0.001$), ammonia ($P < 0.001$), and effluent production ($P < 0.001$). Lactic acid concentration was greatest (2.7% DM) in UN silage after 30 d, while other treatments were similar (1.5% DM). In contrast ammonia concentration and effluent production increased from 30 to 90 d for UN, but not WT silage. Aerobic stability was similar ($P > 0.10$) across all treatments. Microbial inoculation of grass silage can positively influence grass silage fermentation. Additionally, wilted Bermuda grass silage may limit fermentation, as evidenced by lower total acid concentration, but may improve silage quality by decreasing ammonia concentration and effluent production.

Key Words: Tifton 85, homofermentative, ammonia

323 Effect of a genetically modified corn hybrid containing alpha-amylase and storage length on fermentation profile and starch degradability of whole-plant corn silage and earlage.

C. Heinzen Jr.*[1], M. Pupo[1], L. G. Ghizzi[2], E. C. Diepersloot[1], B. A. Saylor[1], H. Sultana[2], and L. F. Ferraretto[1], [1]*University of Wisconsin, Madison, WI,* [2]*University of Florida, Gainesville, FL.*

Two experiments were conducted to evaluate the effects of a genetically modified corn hybrid containing α-amylase expressed in the kernel (AMY) on fermentation profile, nutrient composition, and ruminal effective starch degradability of WPCS (experiment 1) and earlage (experiment 2). Both hybrids AMY and its isogenic counterpart (ISO) were grown in 10 replicated plots (5 for WPCS and 5 for earlage) at the University of Florida – Plant Science Research and Education Center (Citra, FL). Samples of each plot were collected at harvest, homogenized, and within plot divided into 5 subsamples which were randomly allocated into 5 storage lengths (0, 30, 60, 90 and 120 d). For both experiments (WPCS and earlage), data were analyzed as a completely randomized design in a 2 × 5 factorial arrangement of treatments using PROC GLIMMIX of SAS, with a model including the fixed effects of hybrid, storage length and their interaction. Fermentation profile of WPCS and earlage samples were adequate and similar between AMY and ISO. When averaged across all ensiling times, AMY had greater starch concentration than ISO in WPCS (40.9 vs. 38.3%, respectively; *P*

< 0.01) and earlage (60.7 vs. 59.6%, respectively; $P < 0.10$). In addition, AMY had 1%-unit greater water-soluble carbohydrates concentration ($P < 0.01$) in earlage, but not WPCS. Although ammonia-N concentration was greater ($P < 0.01$) for AMY than ISO WPCS (10.1 vs. 3.82% of CP, respectively) and earlage (12.0 vs. 6.2% of CP, respectively), after ensiling, ISO had greater ($P < 0.01$) starch effective ruminal degradability (76.0 vs. 73.6% of starch, respectively, in WPCS and 84.8 vs. 81.7% of starch, respectively, in earlage). Under the conditions of this study, a hybrid containing amylase expressed in the kernel had similar silage fermentation profile to its isogenic counterpart. Contrary to our hypothesis, however, AMY had lower starch degradability, and further research is warranted to elucidate the mechanism affecting this response.

Key Words: amylase, starch degradability, hybrid

324 Effect of planting population and maturity at harvest on yield and nutritional composition of conventional and brown midrib (BMR) genotype of corn silage.

O. M. Peña*[1], C. Velasquez[1], G. Ferreira[2], and M. J. Aguerre[1], [1]*Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC,* [2]*Department of Dairy Science, Virginia Tech, Blacksburg, VA*

Previous studies have shown that increasing planting population may increase corn silage yield without compromising nutrient composition. However, this effect may depend on the maturity of the crop at harvesting time. We hypothesized that increasing planting population increases dry matter (DM) yield when corn is harvested at a late (L) but not at early (E) stage of maturity. The objective of this study was to evaluate the effects of corn planting population and maturity stage at harvest on DM yield of conventional and brown midrib corn genotypes. The study was conducted in field plots as a randomized complete block design with a split-plot arrangement of treatments (4 replicates) in 2 locations (Loc1 and Loc2) with contrasting yield potential. Two brown midrib and 2 conventional genotypes of corn were planted at a theoretical planting population of 60,000, 80,000, and 100,000 seeds/ha (60K, 80K, and 100K, respectively). Plots were harvested at either early-dent or 3/4 milk-line stages (E and L, respectively). Samples were stored for 60 d in polyethylene embossed pouches before nutrient analyses. A location by density interaction existed for DM yield. On Loc1, planting corn at 80K resulted in the highest DM yield, whereas DM yield was similar for 100K and 60K. On Loc2, DM yield increased linearly from 14,203 to 17.027 kg/ha when density was increased from 60K to 100K. No differences between corn genotypes existed for DM yield (12,780 kg/ha; $P < 0.12$). Yield of E harvest corn was lower compared with L harvest corn (11,915 vs. 13,624 kg/ha, $P < 0.01$), no interaction existed between maturity and planting density ($P < 0.52$). Silage ADF was higher at 80K than 60k and 100k (25.5 vs. 24.4%; $P < 0.01$). Corn genotype had a small effect on NDF and ADF content. Compared with E harvest corn, silage DM, NDF and ADF were higher ($P < 0.01$) in L harvest corn (27.0 vs. 38.9%, 39.8 vs. 41.6%, and 24.0 vs. 25.6%, respectively). This study suggests that the response to planting population was not affected by maturity at harvesting.

Key Words: corn silage, yield, maturity.

Physiology and Endocrinology III

325 Effects of 2 different prepartum diets for improvement of calcium homeostasis on postpartum reproductive outcomes. P. L. J. Monteiro*, W. S. Frizzarini, J. Campolina, A. Vang, and L. L. Hernandez, *University of Wisconsin–Madison, Madison, WI.*

This study aimed to evaluate the effect of 2 different prepartum strategies to increase circulating ionized calcium concentrations at parturition of lactating dairy cows on reproductive outcomes. Twenty-one days before expected parturition date, multiparous Holstein cows (n = 122; 3.2 [2 to 6] lactation with average BCS 3.34 [2.75 to 4.25]) were randomly assigned into one of 3 different diets with low potassium corn silage: control (CON; +190.24 mEq/kg; n = 41), negative dietary cation-anion difference (DCAD; −64.71 mEq/kg; n = 41), and a diet with sodium aluminum silicate (XZ; +277.40 mEq/kg with 500 g/day X-Zelit, Protekta Inc.; n = 40). Ionized calcium was measured daily, from −9d to d 0 (parturition), and subsequently evaluated on d 1, 2, 3, 6, 9, 12, 15, 18, and 21 postpartum. Endometrial tissue was sampled and ovaries scanned by ultrasound on 35 ± 3 and 49 ± 3 DIM. On 50 ± 3 DIM, cows were enrolled on a Double Ovsynch program. After first AI, nonpregnant cows were enrolled on an Ovsynch program until they become pregnant. Pregnancy diagnosis was determined by ultrasound on d 32 and 67 after AI. Data were analyzed using the GLIMMIX and LIFETEST procedure of SAS. There was no difference ($P = 0.69$) in incidence of anovulation between treatments (14.6 ± 5.6 [6/41], 21.9 ± 6.6 [9/41], and 20.0 ± 6.4 [8/32], for CON, DCAD, and XZ, respectively). The incidence of subclinical endometritis on d35 ($P = 0.32$) was 12.2 ± 5.1 (5/41), 21.9 ± 6.5 (9/41), and 25.6 ± 7.1 (10/39), and on d49 ($P = 0.33$) was 12.5 ± 5.3 (5/40), 17.1 ± 5.9 (7/41), 25.6 ± 7.1 (10/39), for CON, DCAD, and XZ, respectively. At first service, the pregnancy/AI on d32 ($P = 0.61$) was 40.5 ± 8.2 (15/37), 51.4 ± 8.3 (19/37), and 50.0 ± 8.2 (19/38), and on d67 ($P = 64$) was 32.4 ± 7.8 (12/37), 43.2 ± 8.3 (16/37), and 36.8 ± 7.9 (14/38), for CON, DCAD, and XZ, respectively. Treatment did not affect ($P = 0.74$) pregnancy loss between d32 and 67 (19.9 ± 10.6, 15.7 ± 8.5, and 26.2 ± 10.4, for CON, DCAD, and XZ, respectively). By 300 DIM, there was no difference ($P = 0.17$) in average days open between treatments (117 ± 8.1, 109.4 ± 5.6, and 127.0 ± 10.2, for CON, DCAD, and XZ, respectively). Prepartum diet did not affect reproductive outcomes.

Key Words: fertility, pregnancy, DCAD

326 Body condition prepartum and its association with placentome nutrient transport, one-carbon metabolism, and amino acid utilization in Holstein cows. A. Aboragah*[1], A. Alharthi[1,2], V. Lopreiato[2], Y. Liang[1], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*King Saud University, Riyadh, Saudi Arabia,* [3]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

We investigated how prepartal body condition score (BCS) alters uteroplacental tissue amino acid and glucose transporters, one-carbon metabolism, and protein synthesis regulatory enzymes. Twenty-one multiparous Holstein dairy cows were retrospectively selected according to BCS at 4 wk prepartum and divided into high (HighBCS, BCS ≥3.50, n = 10) and normal (NormBCS, BCS ≤3.25, n = 11) BCS groups. Placentome samples (n = 4) collected from the central area of the placenta immediately after parturition were frozen in liquid nitrogen and subsequently stored at −80°C. Samples were subsequently used for Western blotting and one-carbon metabolism enzyme activities. Data were analyzed using PROC MIXED in SAS. Compared with NormBCS, cows in HighBCS had greater BW ($P < 0.05$; 857 versus 730 kg) and tended ($P = 0.09$) to have lower dry matter intake before calving. Calf BW at birth did not differ ($P > 0.05$) and averaged 44.5 ± 1.5 kg. Among proteins associated with amino acid utilization, the activity of eukaryotic translation initiation factor 2A (p-EIF2A/total EIF2A) was greater ($P < 0.05$), and abundance of branched-chain keto acid dehydrogenase kinase lower in HighBCS cows. Activity of cystathionine β-synthase and betaine homocysteine S-methyltransferase did not differ ($P > 0.05$) and averaged 9.45 ± 2.6 and 4.86 ± 1.9 nmol/h/mg protein. Concentration of the antioxidant glutathione also did not differ ($P > 0.05$) due to BCS and averaged 4.86 ± 0.92 µM. Overall, preliminary observations suggested that high BCS before calving is associated with some alterations in abundance of enzymes associated with amino acid utilization.

Key Words: nutrition, pregnancy, methyl donor

327 Randomized clinical trial to evaluate the effect of a prepartum cholecalciferol injection on postpartum calcium dynamics and health and performance in multiparous dairy cows. P. L. Venjakob*[1], L. Bauerfeind[2], R. Staufenbiel[2], M. R. Wilkens[4], C. Weber[3], W. Heuwieser[1], and S. Borchardt[1], [1]*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany,* [2]*Ruminant Clinic, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany,* [3]*Laboklin GmbH & Co. KG, Laboratory for Clinical Diagnostics, Bad Kissingen, Germany,* [4]*Department of Animal Sciences, Animal Nutrition Physiology, University of Goettingen, Goettingen, Germany.*

The objectives of the present study were (1) to evaluate the effect of cholecalciferol treatment on Ca concentration during the first 10 DIM and (2) to evaluate the effect of treatment on subsequent health and performance. Multiparous Holstein cows (n = 375) were fed a negative DCAD diet (−31 mEq/kg DM) for the last 21 d of gestation. On d 275, the animals were randomly assigned to a control (CON) or a treatment group (VIT D). The latter ones received an injection of 12×10^6 IU cholecalciferol intramuscularly on the day of enrollment. Serum samples were taken on 1, 2, 3, 5, 7 and 10 DIM and analyzed for Ca, P, and Mg concentrations. In a subsample of cows (n = 100) concentrations of haptoglobin, NEFA, BHB and 25-OH vitamin D were determined on 1, 5 and 10 DIM. Repeated-measures ANOVA was performed to evaluate the effect of treatment on blood metabolites, rectal temperature and milk yield. Binary data such as retained placenta and clinical metritis were analyzed using logistic regression models. Cox proportional hazards were used to model time-to-event outcomes (i.e., time to pregnancy and culling). Results were considered significant when $P < 0.05$. Treatment increased serum concentrations of 25-OH vitamin D on d 1, 5 and 10 after calving. While Ca and P concentrations on 1 to 10 DIM were significantly increased in VIT D cows, Mg concentrations were significantly lower in comparison to CON cows. Interestingly, haptoglobin concentrations were significantly increased on 5 DIM in VIT D cows. In comparison to the CON group, VIT D cows showed elevated NEFA levels, a numerically higher incidence of retained fetal membranes and an increased the risk of incurring clinical metritis. In addition, treated cows produced 3.97 kg and 2.55 kg less energy-corrected milk on first and second test day, respectively, compared with CON cows. While there was no effect of treatment on culling until 300 DIM, time to pregnancy was delayed by 27 d in treated cows. In the present study, VIT D treatment had detrimental effects on health status and the subsequent lactation despite the beneficial effects on Ca homeostasis. Since this experiment was conducted on a single dairy farm, further research including more herds is warranted to evaluate whether the negative effects were herd specific.

Key Words: vitamin D, hypocalcemia, calcium dynamics

328 Association of genomic prediction of daughter pregnancy rate and ovulatory responses in Holstein cows receiving a Double OvSynch program.

R. Martinez*[1,2], R. G. S. Bruno[3], G. Pena[3], R. Cleale[3], G. H. Choi[1], and F. S. Lima[1], [1]*1Department of Population Health and Reproduction, University of California, Davis, Davis, CA,* [2]*California State University, Fresno, CA,* [3]*Zoetis Inc., Parsippany, NJ.*

Genomic prediction of daughter pregnancy rate (GDPR) has been associated with improved pregnancy outcomes in dairy cows. However, the mechanism by which GDPR leads to improved reproductive performance remains unclear. This study's objectives were to determine the ovulatory response at first GnRH of the Breeding OvSynch in Holstein cows receiving a Double OvSynch program. A subset of 523 Holstein cows genotyped with Clarifide Plus had their first AI synchronized with Double OvSynch program starting at 61 ± 3 d in lactation. Ovaries were scanned at the first GnRH of the breeding OvSynch and at PGF$_{2\alpha}$ given 7 d later to determine the ovulatory response. Cows were ranked by GDPR and then blocked by parity and sequential ascending GDPR to receive 2 mL or 4 mL of GnRH (gonadorelin hydrochloride, 50 µg/mL, Factrel Zoetis Inc.). The GDPR data were categorized in quartiles (Q1 = lowest vs. Q4 = highest). Data analysis was performed using logistic regression on JMP. The final model included effects of GDPR, treatment, parity, and their interactions. There were no differences in GDPR between treatments (2 mL = 0.03 ± 0.07 vs. 4 mL = 0.09 ± 0.08, $P = 0.56$) or parities (primiparous = 0.18 ± 0.09 vs. multiparous = 0.005 ± 0.06, $P = 0.12$). There was a tendency ($P = 0.06$) for GDPR to affect ovulatory response and a tendency for interaction between GDPR and parity ($P = 0.09$). Multiparous cows in Q1 (89.5 ± 5.3%) had greater ovulatory rate than multiparous cows in Q3 (59.6 ± 5.3%) and Q4 (65.4 ± 4.4%). Conversely, no effects of GDPR in ovulatory responses were present among primiparous cows. There were effects of treatment on ovulatory response (2 mL = 69.4 ± 3.7% vs. 4 mL = 81.2 ± 3.7%, $P = 0.02$), but no treatment by GDPR ($P = 0.89$) or parity ($P = 0.63$) interactions were present. In summary, GDPR and treatment were associated with an altered ovulatory response, but further studies are needed to understand factors influencing positive associations of GDPR with fertility in dairy cows.

Key Words: genomic prediction of daughter pregnancy rate (GDPR), ovulatory response, dairy cows

329 Ability of rumination time in the first week after calving to detect Simmental dairy cows with different metabolic status and milk performance in early lactation: A cluster-analytic approach.

V. Lopreiato*[1], A. Minuti[1], M. H. Ghaffari[2], J. J. Loor[3], and E. Trevisi[1], [1]*Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, Germany,* [3]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL.*

An unsupervised machine learning approach (ML) of rumination time (RT) data were used to evaluate metabolic and lactation performance in a cohort of Simmental dairy cows [multiparous (Mu) and primiparous (Pr)]. The RT data were recorded with Hr-Tags (SCR by Allflex) in the first week after calving. The k-means clustering procedure in SAS was used to generate clusters based on RT through the entire day from 1 to 7 d post calving. The Elbow method was applied for k-means clustering and 2 large clusters were obtained: High-RT and Low-RT. Milk production was recorded until 42 d in lactation and milk samples collected weekly. Blood samples were collected at −21, −7, 1, 3, 7, 14, and 28 d relative to calving. Liver functionality index (LFI) was also calculated. After identifying clusters, data were subjected to PROC MIXED of SAS with cluster, parity, day, and their interaction as fixed effects. Compared with Low-RT, High-RT cows had lower plasma BHB (3, 7, and 14 d), haptoglobin (3 and 7 d), and nonesterified fatty acids (NEFA; 1 and 3 d). Compared with Low-RT, High-RT cows had greater tocopherol after calving, fructosamine and albumin at 7 d, and retinol at 3 and 7 d. Compared with Low-RT Mu, milk production was greater in High-RT Mu cows, but no differences were detected for primiparous. Milk urea was greater in High-RT compared with Low-RT, and BHB was greater only in Mu High-RT than Low-RT. Compared with Low-RT, only multiparous High-RT cows displayed a greater LFI. Overall, the analyses indicated that the unsupervised ML applied to RT data from the first week of lactation was able to identify cows with a different metabolic state (energy metabolism, inflammatory response, and liver functionality) in the first month of lactation, mainly in multiparous cows. A quicker increase in RT after calving was associated with a lower inflammatory response and lipid mobilization, and greater milk production. At the farm level, these outcomes provide information to farmers that can be helpful in management decisions for cow health.

Key Words: rumination time, machine learning, transition cow

330 Late gestational muscle and adipose diameter affect nutrient partitioning and production performance of periparturient cattle.

C. J. McCabe*, A. Suarez-Trujillo, T. M. Casey, and J. P. Boerman, *Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Due to insufficient dry matter intake and heightened nutrient requirements in early-lactation, periparturient dairy cows mobilize adipose and muscle tissues to bridge energy and amino acid gaps, respectively. Our objective was to evaluate the relationship between late pregnancy muscle diameter and early-lactation performance. At 35 d before expected calving (BEC) *longissimus dorsi* muscle diameter (LDD) was measured in 41 multiparous Holstein cows via ultrasound. All animals were enrolled to a control (CON; n = 8) or 6 h forward phase shift (PS, n = 8) of the light-dark cycle every 3 d. CON timing of light was given at calving through 60 d in milk (DIM). Tissue mobilization was evaluated via ultrasound images of LDD and backfat depth (BFD) at 21 and 7 d BEC as well as at 0, 10, 30, and 60 DIM. Plasma concentrations of 3-methylhistidine (3-MH), creatinine (CRE), nonesterified fatty acids (NEFA), and β-hydroxybutyrate (BHB) were evaluated weekly. Milk yield and milk component data were collected through 60 DIM. Cattle were assigned post-hoc to high muscle (HM; n = 20; LDD > 4.49 cm) or low muscle (LM; n = 21; ≤ 4.37 cm) groups with no cows between these groups. HM cows had greater LDD at 35 d BEC. Between 35 and 21 d BEC, LM cows gained LDD, whereas HM cows gained BFD. At all time points except for 7 d BEC, HM had greater ($P < 0.05$) LDD than LM cows. HM cows mobilized more muscle from 21 d BEC to 60 DIM, as reflected in greater loss of LDD, greater 3-MH concentrations (532 vs. 438 ± 30 ng/mL; $P < 0.05$), and a greater 3-MH:CRE ratio (0.164 vs. 0.131 ± 0.008; $P < 0.05$) in the first 3 wks postpartum. The LDD and BFD at 21 d BEC were related to the amount of respective tissue mobilized through 60 DIM ($R^2 = 0.34$ and 0.89, respectively).

Although calves born to HM cattle were larger (45.2 vs. 41.8 ± 0.7 kg; $P < 0.05$), HM cows produced less milk (38.8 vs. 41.6 ± 0.8 kg/d; $P < 0.05$) with a tendency for higher fat content (4.33 vs. 4.05 ± 0.12%; $P = 0.09$), which was related to mobilization of more back fat from 0 to 60 DIM (1.78 vs. 0.68 ± 0.34 mm; $P < 0.05$). These findings suggest a cow's metabolic status as measured by LDD and BFD prepartum, determines the metabolic strategy the animal uses to meet energy and amino acid requirements in late gestation and early lactation.

Key Words: tissue mobilization, ultrasound, late gestation

Production, Management, and the Environment III

331 The effect of feeding a blend of artificial and natural flavors to dairy cows during periods of heat stress and effect of reducing milk yield depression.

G. Acetoze*[1] and C. Ribeiro-Filho[2], [1]*Archer Daniels Midland (ADM), Decatur, IL,* [2]*Western Milling LLC, Goshen, CA.*

Heat stress costs US dairy producers $1.2 billion each year. Recent studies suggest that some phytomolecules such as eugenol, cinnamaldehyde and capsicum are able to modify biological response, alter rumen fermentation and encourage feeding behavior which are important factors to consider when mitigating milk production losses due to heat stress. The objective of this study was to evaluate the impact of feeding combined ratios of artificial and natural flavors (ANF; RumeNext Beef, ADM Animal Nutrition, Quincy, Illinois) to lactating dairy cows as a strategy to reduce milk yield depression during periods of heat stress on a commercial dairy in Visalia, CA. Three hundred fifty lactating Holstein dairy cows (130 ± 20 DIM) were randomly assigned to 2 treatments: Control (no additive) and Treatment (ANF at 14g/hd/d), with 2 replications per treatment. Feed additive was provided in the complete grain premix manufactured by a commercial feed mill and incorporated into the TMR at the dairy. Supplementation started May 19, 2019, and ended September 1, 2019. Variables such as baseline milk yield (before supplementation), monthly milk yield and somatic cell count (SCC) were collected in this study. Statistical analysis was performed using the Fit model procedure of JMP (SAS Institute Inc., Cary, NC). Although no differences were observed for SCC ($P = 0.91$) among treatments, the average monthly milk yield depression calculated using monthly milk yield difference (monthly milk yield – milk yield baseline) did differ among treatments ($P < 0.01$) and we smaller for ANF treated cows. The average milk yield depression (lbs/hd/d) were −1.57, −4.03 and −5.16 for the ANF treated cows and −7.68, −7.92, −10.81 for the control cows during the months of June, July and August, respectively. These results show that feeding ANF to lactating cows affected by heat stress on a commercial dairy during the summer months can reduce production and economic losses by over 50%.

Key Words: heat stress, dairy cattle, phytomolecules

332 Modeling the profitability of investing in cooling systems in the Mediterranean area with different intensities of heat load.

O. R. Espinoza-Sandoval and S. Calsamiglia*, *Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Barcelona, Spain.*

The evaluation of investing in cooling systems (CS) in dairy farms is complex because the effects of heat stress (HS) on dry matter intake, milk production, reproduction and culling interact, resulting in seasonal fluctuations. These interactions are difficult to incorporate into traditional economic evaluation methods. The objective of the present study was to model and evaluate the effect of HS and CS on profitability under different intensities of HS and economic scenarios. A 300-dairy cow farm with average performance indexes in Spain was created using a stochastic dairy farm simulator (www.dairyfarm.com). The biological effect of HS ($THI_{Threshold}$ of >68 units) and CS were evaluated from the temperature and relative humidity in 21 zones in the Mediterranean using the model proposed by St. Pierre et al. (2003). Fluctuation in fat and protein content were evaluated using the model proposed by Salfer et al. (2019). Simulation were conducted for each zone at 3 milk prices (€0.28, €0.32 and €0.36/L), 2 investment costs in fans and sprinklers (€100 and €200/cow) with 6 yr pay-off, and an operation cost of €7/cow/month of operation. The model simulated only milking cows. The impact of the different scenarios of HS and CS simulated were analyzed using the PROC GLM, NLN, and REG in SAS v.9.2. The THI_{Load} of the 21 zones ranged from 6,908 to 31,424 units/yr. The implementation of CS improved average milk production average in 327 L/cow/yr (range of 171 to 850); increased the average feeding costs by €52/cow/yr (range of 26 to 139); increased the average pregnancy rate by 0.3 percentage units/yr (range of 0.1 to 1.0); and reduced the average culling rate by 1.7 percentage units/yr (range of 1 to 3.9). When THI_{Load} was ≤7,000 units/yr, CS were never profitable. When THI_{Load} was between 7,000 to 10,000 units/yr, the profitability of CS depends on the investment cost and milk price. When THI_{Load} was >11,000 units/yr, the investiment in CS was always profitable. Based on CS cost and milk price, the increased net margin (€/cow/yr) was from €15 to €62 in Seville (Spain), from €32 to €88 in Trapani (Italy) and from €159 to €277 for Tel Aviv (Israel). The profitability of CS depends on the THI_{Load}, milk price, feeding costs, and the cost of investment in CS.

Key Words: heat stress, cooling systems, cost-analysis

333 Late-gestation heat stress alters placental morphology in multiparous cows.

L. T. Casarotto*[1], V. Ouellet[3], B. Dado-Senn[2], J. Laporta[2], and G. E. Dahl[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Wisconsin–Madison, Madison, WI,* [3]*Laval University, Quebec City, QC, Canada.*

Late-gestation heat stress in dairy cattle alters dam productivity and offspring weight, morbidity, mortality, and future milk production. Placental dysfunction due to heat stress may drive these changes in the dam and calf, particularly if nutrient, heat, and gas exchange capacity is altered. In the present study, we hypothesized that late-gestation heat stress reduces placental development and may limit fetal growth. Eighty-two multiparous Holstein pregnant cows (blocked by parity, previous milk yield, and sire of fetus) were randomly enrolled into either cool (CL; access to shade of the barn plus forced ventilation via fans, and water soakers; n = 41) or heat stress (HT; access to shade of the barn and natural ventilation; n = 41) treatments for the duration of their dry period (54 ± 5 d). Gestation length was calculated as the date of breeding subtracted from date of calving. Calf body weight was measured immediately upon birth. At parturition, placentas were collected from a subset of cows (n = 6/treatment) (4 ± 0.07 h), and measurements of total weight, cotyledon number, weight, and surface area (n = 4/treatment) were taken within 1h of placental expulsion. Data were analyzed using the GLIMMIX and MIXED procedures in SAS. Significance was declared at $P \leq 0.05$ and tendency was declared at $0.10 \geq P > 0.05$. Gestation length was reduced in HT dams, which calved approximately 3 d earlier than CL cows (275 vs. 278.3 ± 0.7d, $P < 0.01$), and HT heifers weighed 4 kg less at birth relative to heifers from the CL dams (34.8 vs. 38.9 ± 0.7 kg, $P < 0.01$). Placentae from the HT animals tended to have lower total weight (4.1 vs. 5.2 ± 0.7 kg, $P = 0.10$) and cotyledon number was decreased (48.2 vs. 94 ± 12.5, $P < 0.01$) relative to CL cows. The surface area of total cotyledons was lower in the HT dams (323 vs. 552 ± 50.5 cm^2, $P = 0.01$) compared with CL, consistent with the greater number of cotyledons. However, no difference was found in individual cotyledon area or cotyledon weight (1.9 vs. 2.2 ± 0.4 kg). Heat stress during late gestation induced placental morphological alterations which might contribute to reduced function and impaired fetal growth and development.

Key Words: heat stress, prenatal, placenta morphology.

334 In utero effects of hyperthermia on hair length and skin morphology.

B. D. Davidson*[1], K. M. Sarlo Davila[2], R. G. Mateescu[2], 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.*

Skin performs important functions for maintaining homeostasis and hair coat plays a key role in thermoregulation for cattle which can impact all 4 mechanisms of heat loss: radiation, conduction, convection, and evaporation. Our objective was to determine whether hyperthermia experienced in utero during late gestation alters hair length and skin properties of heifer calves at birth. Pregnant dams were heat stressed (HT, shade; n = 41) or cooled (CL, shade, plus fans and soakers; n = 41) for the last 56 d of gestation and gave birth to calves that were in utero heat stressed (IUHT) or in utero cooled (IUCL), respectively. Hair samples and skin biopsies were collected from both the neck and the rump at approximately 4 h after birth, from a subset of 8 heifer calves per in utero treatment. Skin biopsies were fixed for 16 h in 10% formalin, dehydrated, and embedded in paraffin. Sectioned tissues were stained with Hematoxylin and Eosin to visualize morphology. Hair length, skin area, epidermis thickness, sweat gland number and area, sebaceous gland number and area, and sweat gland depth were assessed. Hair and skin measurements were preformed using the ImageJ software. Data were analyzed using PROC MIXED in SAS, neck and rump locations were analyzed separately and in utero treatment was used as a fixed effect. There were no differences (all $P \geq 0.12$) in either location for hair length, epidermis thickness, and sweat gland number or area. In the neck, IUHT heifers had reduced skin area (0.08 vs. 0.17 ± 0.02 mm^2, $P = 0.003$) and reduced sebaceous gland area (1,636 vs. 2,238 ± 243 µm ^{2}, $P = 0.03$) relative to IUCL heifers. In the rump, IUHT heifers had smaller (2,100 vs. 3,352 ± 379 µm ^{2}, $P = 0.007$), but more sebaceous glands relative to IUCL heifers (13 vs. 9 ± 2 glands, $P = 0.05$). Additionally, in the rump the sweat gland depth tended to be longer in the IUHT relative to the IUCL heifers (0.43 vs. 0.36 ± 0.04 mm, $P = 0.10$). Overall, in utero hyperthermia alters important skin properties with potential implications on postnatal thermoregulation.

Key Words: skin, in utero, heat stress

335 Inclusion of sugar cane condensed molasses solubles in the diet of dairy cows in the tropics.

J. S. Henao-Beltrán*, J. Loyola-Jiménez, N. L. Rosa-Padilla, E. Ruiz-Cortés, N. de la Cruz-Fontánez, E. Valencia, and G. Ortiz-Colón, *University of Puerto Rico, Mayagüez, Puerto Rico.*

This study evaluated the effect of the inclusion of condensed molasses solubles (CMS), a by-product of the rum industry, on dry matter intake (DMI), milk yield (MY), and income over feed costs (IOFC) in lactating dairy cows. Treatments consisted of totally mixed rations (TMR) with 0, 5, 10, or 15% (dry matter basis) of a 50:50 CMS/molasses mixture. Sixteen Holstein/Holstein cross milking cows were divided into 4 blocks by parity and days in milk (n = 4) in a Latin square cross over design (4 treatments × 4 periods). All animals were fed a TMR balanced for 14.33% crude protein and to provide 25.55 Mcal NE$_L$/cow/day. All dairy cows were individually fed ad libitum each of the experimental diets for 14 d (7-d adaptation period followed by 7-d data collection). Dry matter intake was recorded daily with a Calan Feeding System and milk yield per cow was recorded daily with the Afimilk system. There was no difference in MY, fat % and protein % between treatments ($P \geq 0.2053$). Average MY, fat and protein percentage were 17.39 ± 1.2 kg, 4.41 ± 0.18%, and 3.22 ± 0.11%, respectively. However, there were treatment differences ($P < 0.001$) on DMI. Cows on 0% and 5% CMS had similar DMI of 10.21 ± 0.45 and 10.7 ± 0.48 kg/cow/day, respectively. However, cows on 10 and 15% CMS had higher DMI, 15.26 ± 0.46 and 11.97 ± 0.46 kg/cow/day, respectively. Experimental diet costs were $0.29, $0.28, $0.27/kg and $0.26/kg, for treatments 0, 5,10, and 15% CMS, respectively. Feed costs per cow per day were $2.96, $3.01, $3.13 for treatments 0, 5, and 15% CMS, respectively, and were lower than the cost ($4.16) of the 10% CMS treatment ($P < 0.05$). Assuming a constant income of $0.744/kg of fluid milk, IOFC per cow per day was $13.27, $12.63, $13.34, $12.51, for treatments 0, 5,10, and 15% CMS, respectively. Consequently, the IOFC per cow per day for treatments 0, 5, 10, and 15% CMS were $10.31, $9.56, $9.18, $9.38, respectively ($P > 0.05$). In conclusion, the inclusion of CMS in the diet of dairy cows was not economically advantageous.

Key Words: vinasse, by-product, feed efficiency

336 Predictive equations to calculate pen-level dry matter intake utilizing on-farm data.

A. T. Richards*[1], P. Ousterhout[2], and J. P. Boerman[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*iYOTAH Solutions, Westminster, CO.*

Feed costs represent the largest single cost of producing milk on a dairy farm, with unconsumed feed accounting for an increase in the price of feed without resulting in marginal milk production. Our objective was to incorporate data collected on dairy farms from technology sensors and software to predict dry matter intake (DMI) on a pen level on a commercial dairy farm. This would allow daily adjustments in amount of feed offered to the pen to minimize feed waste and maximize milk production. We utilized data from a weather station installed on the commercial farm, feed management software, and milking parlor software to determine which variables impact daily pen-level DMI. These data were used to develop a predictive equation to determine daily feed delivery to reduce feed waste while also maximizing marginal milk production. Data were collected from June 17, 2020 to February 2, 2021 and was filtered, merged and analyzed in RStudio v 1.3.1073. The platform that enabled comparison of data collected from the different software programs and data validation was developed by iYOTAH Solutions. Due to discrepancies in number of cows per pen across software programs, the number of cows per pen was determined by assigning a cow to the pen she was milked with for that day using milking records. Variables that were considered from milking parlor software included number of cows per pen and milk yield; from the weather station included temperature, barometric pressure, relative humidity, wind chill, heat index, rain amount, rain rate; from feed management software included dry matter of the diet as well as the response variable of DMI. Using multiple linear regression analysis, dry matter of the diet, temperature, number of cows, the quadratic effect of milk yield, and the interaction between milk yield and the number of cows were all variables that significantly effect DMI (all $P < 0.01$, $R^2 = 0.23$). Regression analysis of individual variables showed number of cows ($P < 0.01$, $R^2 = 0.11$), the quadratic effect of milk yield ($P < 0.01$, $R^2 = 0.07$), and the interaction between milk yield and number of cows ($P < 0.01$, $R^2 = 0.14$) are all positively correlated with DMI. This suggests it is possible to use existing on-farm data to predict daily intake and alter feed delivery in near-real time.

Key Words: dry matter intake, predictive equation, feed waste

337 Using weakly supervised learning to improve performance of deep neural networks for animal identification.

R. E. P. Ferreira* and J. R. R. Dorea, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Contemporary approaches for animal identification use deep learning techniques to recognize coat color patterns and identify individual animals in a herd. However, deep learning algorithms usually require a large number of labeled images to achieve satisfactory performance, which creates the need to manually label all collected images, when automated methods are not available. In this study, we propose a method based on weakly supervised learning to automatically label images in large data sets, when only a small portion of the images is already labeled. We evaluated our approach in the challenge of identifying individual animals using top-view images of their back. Our data set consisted of 2,354 images manually labeled by humans, and 20,194 unlabeled images, all acquired at the UW-Madison Research Farm (Arlington, WI), containing 59 different cows. All images were automatically collected after milking, and the data set contained just one animal per image. Pre-processing was implemented to remove background and only keep pixels related to the animal body. The proposed method consists of 3 steps: (1) training a network based on the Xception architecture with a small data set (data set 1: 2,354 manually labeled images), (2) using this trained network to predict the probabilities that each cow appears in each of the 20,194 unlabeled images, and (3) setting the cow with the highest probability value for each image as the label to train a new network, if this value is higher than a certain threshold. Using a testing set with annotated data (n = 1,161), we compared the performance of networks trained with a small data set (data set 1), and with both manually and automatically labeled images together, using thresholds for the highest probability value of 0.90 (n = 15,532), 0.95 (n = 14,005), and 0.98 (n = 12,198). We achieved an accuracy of 67% when using data set 1, and 76%, 76%, and 78% when using the weakly supervised approach for each probability threshold (0.90, 0.95, and 0.98, respectively). These results indicate that it is possible to achieve better performing deep neural networks by using images that are automatically labeled based on a small data set of manually labeled images. Such strategy can save time and resources that would otherwise be used for labeling, and leverage well annotated small data sets.

Key Words: computer vision, deep learning, machine learning

338 Machine learning anomaly detection as an approach to detect mastitis events in lactating dairy cows.

S. Jha*[1], L. M. Campos[2], W. Feng[1], and M. D. Hanigan[2], [1]*Department of Computer Science, Virginia Tech, Blacksburg, VA,* [2]*Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

Mastitis is a costly disease affecting dairy cattle that is characterized by inflammation, abnormal milk, and lost production. It often escapes detection when animals do not exhibit primary symptoms. Automated detection systems that supplement visual observations would help identify infections sooner allowing earlier treatment and potentially better outcomes. The objective of this work was to build an anomaly detection system capable of automatically identifying mastitis events based on production loss. A simulated data set representing 500 lactating dairy cows over a 5-year period was used for code development. Normal lactation curves for primi- and multiparous (2 and 3) animals were generated with 10% genetic variation in each parameter. Random production variation was introduced ($\sim N(0{,}2.1$ kg/d), and milk loss due to random mastitis events (n = 250) of varying severity (−2.35 kg/d), and duration (2.35 d) was subtracted. Anomaly analysis consisted of 1) fitting Wood's equation [Wood, P. *Nature* 1967; 216:164–165) by animal and parity, 2) smoothing Wood's equation residuals using a sliding 3-d average. Code was written to flag milk production deviations in consecutive 3-d windows that exceeded a threshold. Different thresholds were tested to investigate how well the system was able to detect true mastitis events. Preliminary results (Table 1) show that narrower standard deviation (SD) results in greater discovery rates and greater false positives (FP). Normal variation in daily milk yield limits the value of single-point detection methods. More advanced methods of anomaly detection and pattern recognition including timestamp-based machine learning were explored to improve sensitivity and reduce FP rates.

Table 1. Number of detected anomalies and false positives

Threshold (SD)	Anomalies detected	Undetected	False positives
1	239	11	1,943
2	203	47	657
3	164	86	193
4	125	125	67

Key Words: machine learning, health, management

339 Cloud-based artificial intelligence infrastructure for automated real-time integration of dairy data in support of predictive analytics.

M. M. Perez*, W. Song, Y. Yang, M. Liu, K. P. Birman, and J. O. Giordano, *Cornell University, Ithaca, NY.*

We present a cloud-based infrastructure that integrates real-time numeric and imaging data into a database optimized for machine learning (ML). The system captures data from a diverse collection of cow wearable and non-wearable sensors, herd management software, climate sensors, and real-time streaming image data from cameras. Data are first staged at a local server (PC) on the farm that automatically performs a variety of preprocessing tasks, and then uploads processed data to a Microsoft Azure platform using Azure CLI authentication and security. On Azure, data are stored as files in the Azure Blob Store (ABS), which offers scalable storage for both structured data like Excel files (.xlsx-.csv) as well as unstructured data like web pages, images (.jpg), and video (MP4). As new data arrives in the cloud, an Azure Function runs, triggering an "aggregation" computation that can draw on current and historical data from individual cows for summarization and ML-based analytics. We employ the Pandas platform for computational tasks, such as AI predictions of cow health status, reproductive outcomes, and profitability. The output is then stored back in ABS and combined with historical data to deploy a comprehensive record in which the evolution of metrics could be visualized. To minimize ABS storage costs and improve responsiveness with large data objects (e.g., images or video clips) our platform implemented a pipeline in Cascade, a new high-performance IoT cloud platform for real-time visual-data analytics and classification. Data are uploaded directly into Cascade and analyzed instantly, enabling ML-based predictive analytics and automated determination of data storage needs in Azure. Our work establishes that a cloud-based infrastructure can integrate dairy data from multiple sources and types, can support comprehensive and immediate analysis using ML tools, and as such can serve as an effective tool for herd management by supporting AI predictive analytics for health, reproductive and financial management in dairy farms. Supported by USDA-NIFA 2017–67015–26772

Key Words: integration, analytics, computer vision

340 Giving calves a head start: A machine learning approach to analyze offspring longevity based on birth conditions.

G. M. Dallago*, K. M. Wade, R. I. Cue, and E. Vaseur, *McGill University, Sainte-Anne-de-Bellevue, QC, Canada.*

Studies of dairy cow longevity usually begin only after first calving, with few studies focusing on early-life conditions and their effect on longevity. Our objective was to evaluate the effect of birth conditions routinely collect by DHI agencies on offspring longevity. Lactanet Inc. provided information on 886,051 Holstein animals born from 6,409 Quebec dairy herds between 1997 and 2015. Birth conditions used in this study were birth ease (unassisted, pull, surgery, or mal-presentation), birth size (small, medium, or large), and twinning (yes or no). Observations were considered censored if the culling reason was “exported,” “sold for dairy production” or “rented out.” If animals were not yet culled when the data were extracted, the last test date was considered the censoring date. Both offspring longevity (birth to culling/censoring) and length of productive life (LPL) were measured. Conditional inference survival trees were used as they are less prone to overfitting and more reliable than other survival tree algorithms. The resulting subset of individuals was used as a categorical variable to estimate the hazard ratio of culling ($\alpha < 0.05$). The subset with the lowest culling risk was used as the reference. Six subsets were identified with different offspring longevity based on their birth condition ($P < 0.001$). Calves with the highest longevity were those that did not have a twin birth, had a birth size of medium or large, and had an unassisted calving (median = 4.5 yr; $P < 0.001$). Animals with a twin birth had the lowest longevity (3.3 yr; $P < 0.001$) and were 1.7 times more likely to be culled ($P < 0.001$). Seven subsets were identified with different LPL ($P < 0.001$). Unassisted or surgery birth of a large or medium calf was the subset with the highest LPL (3.1 yr; $P < 0.001$). Having a mal-presentation or pull in a twin birth was the subset with the lowest LPL (1.7 yr; $P < 0.001$) and was 2.5 times more likely to be culled ($P < 0.001$). In conclusion, birth condition greatly affects the ability of offspring to remain in the herd, providing new insight on early selection of best candidates for replacement.

Key Words: survival tree, culling, precision dairy farming

341 A non-time-series approach to predict milk lactation curves for primiparous cows relying on dam and siblings’ production information.

F. Zhang*, K. Weigel, and V. E. Cabrera, *Department of Animal and Dairy Science, University of Wisconsin, Madison, WI.*

Predictions of the shape of the lactation curve, which are currently unavailable for primiparous cows, are paramount for herd management and decision-making. Therefore, the aim of this study was to develop and illustrate a machine learning approach based on K-medoids clustering and support vector regression, for predicting lactation curves of individual primiparous cows using data from their dams and half-siblings. This approach can provide expected test-day yields, and therefore lactation curves, at an early age. The analysis used filtered test-day milk yield and pedigree records sourced from 26,500 newborn calves from Wisconsin dairy herds (n = 260) collected over a 7-year period (2010–2016). Model training used 95% of the data set, with the remainder saved for model validation. Accuracy of predicted daily milk yield observations for randomly selected cows in the validation set are shown below in Table 1. Given the accuracy of predictions observed in this study, coupled with lack of a suitable alternative for predicting lactation curves of primiparous cows, this machine learning approach could provide a valuable source of information for herd management decision support tools.

Table 1. Performance of prediction for first-lactation milk yield for 33 randomly selected animals from the reserved dataset for validation

	Aggregated value of 10 test-days (kg/animal)				
Item[1]	Mean	Minimum	Maximum	SD	Median
Total actual MY	376.17	271.25	459.04	57.05	391
Variance in actual MY	35.46	6.91	138.14	27.55	28.17
Total predicted MY	400.02	370.92	416.32	13.82	400.42
Variance in predicted MY	20.39	13.08	32.06	5.42	17.96
MAE of predicted MY	5.76	1.5	12.55	2.77	5.22
MSE of predicted MY	53.01	3.22	164.96	44.15	38.38
RMSE of predicted MY	6.69	1.79	12.84	2.92	6.19

[1] MY = milk yield, MAE = mean absolute error, MSE = mean squared error, RMSE = root mean squared error of prediction.

Key Words: dairy production, dairy modeling, lactation forecasting

342 Exploring integrated data as a tool for better understanding health-associated issues in dairy farms.

L. Fadul-Pacheco* and V.E. Cabrera, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

The aim of this study was to distinguish healthy from sick cows using integrated data streams. Data from lactating Holstein dairy cows in a farm during 2020 were used for this analysis. Records from 3 different data streams (management software (BoviSync), DHI and genetics) were integrated. Only complete records were used (n = 6,912 records from n = 1,079 cows). Selected health issues were clinical mastitis (CM), ketosis (KT) and lameness (LM). Cows were divided into positive and negative for each disease, and the average of each genetic trait and DHI variables were calculated for each group. Positive animals were those that had at least one episode of the disease. Statistical differences between positive and negative groups for each disease were evaluated using a *t*-test. Results showed that 305-d mature-equivalent milk production was lower for the KT+ and CM+ groups (595 and 1,797 kg, respectively; $P = 0.01$). Milk fat percentage was lower for the CM+ (0.26%; $P = 0.01$), and higher for KT+ cows (0.66%; $P = 0.01$). As expected, the SCC and linear score were higher for the CM+ cows ($P < 0.01$). Among the genetic variables, TPI was higher for the KT− and CM− groups ($P < 0.001$), however it tended to be higher for the LM+ group ($P = 0.07$). Milk trait was higher for the LM+ than for the LM- cows ($P = 0.01$), whereas for the livability trait, it was the opposite. The ketosis and metritis traits were lower for the CM+ and KT+ groups ($P < 0.01$). The traits for milk fever and displaced abomasum were lower for the KT+ group ($P = 0.01$). This exploratory analysis showed that there are differences in genetic traits and DHI records between positive and negative cows that could be used to detect anomalies and likely anticipate health issues when aggregated data are available. We are exploring the same analysis in a longer period of time to better understand the implications of some traits such as livability and health traits in the overall herd management. This work is supported by the Food and Agriculture Cyberinformatics and Tools grant no.2019–68017–29935/project accession no.1019780 from the USDA-NIFA.

Key Words: data integration, dairy brain, decision management

343 Assessment of smallholder dairy farmers' willingness for performance recording in response to different incentive approaches in Pakistan.

M. Q. Shahid*[1], S. H. Magsi[1], N. Khosa[1], and H. Mustafa[2], [1]*Department of Livestock Management, University of Veterinary and Animal Sciences, Lahore, Pakistan,* [2]*Department of Animal Breeding and Genetics, University of Veterinary and Animal Sciences, Lahore, Pakistan.*

Sustainable performance recording is the first step in breed improvement programs. Smallholder buffalo farmers are less interested in maintaining production records. They are more interested in records with immediate response and economic benefits. The objective of current study was to assess the farmers' willingness for performance recording in response to different incentive approaches. For this purpose, 30 farms having at least 10 adult buffaloes (lactating and dry) were enrolled at a convenient distance from the University of Veterinary and Animal Sciences, Lahore, Pakistan. Four milk testing approaches; 1) LT, laboratory milk testing and feedback; 2) FT, on-farm milk testing and immediate feedback; 3) FTM, on-farm milk testing and immediate feedback along with on-farm California mastitis test, and 4) FTI, on-farm milk testing and immediate feedback along with deworming of animals as an incentive. Every farm was visited 4 times to test one approach per visit. The duration between the successive visits was at least 30 d. The order of testing approaches was LT, FT, FTI, and FTM. At each visit after following the specific milk testing approach, the farmers were asked if they wanted to continue the recording process as "yes" or "no." The logistic regression was applied to assess the farmers' willingness to performance recording. The data on farmers' demographics, herd structure and management practices were also collected. The results showed that the odds of willingness for performance recording were similar between LT, FT and FTM approaches. The FTI approach had the highest odds of willingness for performance recording compared with LT and FT approach. It was also observed that farmers with 12 years or more education had higher odds of willingness for performance recoding compared with those without any formal education. In conclusion, monetary incentive in the form of deworming animals and education were associated with the farmers' willingness for performance recording.

Key Words: performance recording, smallholder buffalo farmers, monetary incentives

344 Perspectives of dairy farm employees on the COVID-19 pandemic.

F. C. Ferreira*[1], M. Rovai[2], N. Silva-del-Rio[1], M. Chanine[3], M. De Haro Martí[4], J. Wenz[5], and J. Dalton[6], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare, CA,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [3]*Animal, Veterinary and Food Sciences Department, University of Idaho Twin Falls Research and Extension Center, Twin Falls, ID,* [4]*University of Idaho Extension Gooding County, Gooding, ID,* [5]*Field Disease Investigation Unit, College of Veterinary Medicine, Washington State University, Pullman, WA,* [6]*Animal, Veterinary and Food Sciences Department, University of Idaho, Caldwell Research and Extension Center, Caldwell, ID.*

Our objective was to understand dairy employees' perceptions and educational needs at the onset of the COVID-19 pandemic. A bilingual (English and Spanish), anonymous survey targeted at dairy employees was circulated nationwide via university and allied industry media outlets. Responses (n = 63) from 11 states were received (May to Sep 2020). Respondents worked in herds ranging from 50 to 35,000 animals in size. Dairy managers (33%) responded mostly to the English survey (52%), whereas dairy workers (67%) chose the Spanish format (76%). Overall, 83% of the respondents were somewhat concerned or very concerned about the COVID-19 pandemic, but the Spanish respondents (93%) showed greater concern than English respondents (62%). Respondents (51%) indicated that their main concern was "to bring the virus from work to home and make my family sick." Most dairy workers (83%) perceived that their employers were somewhat or very concerned about the pandemic. Respondents (66%) indicated that COVID-19 informative training was provided at the workplace; but training was more frequent among dairy managers (90%) than dairy workers (51%). Most trainings (75%) were limited to posters on walls. The preferred means of information delivery was through videos (64%). A good understanding of COVID-19 symptoms was reported among 85% of dairy workers trained at work vs. 71% for those not trained. English respondents (50%) indicated that television was their preferred source of information on COVID-19 (50%), whereas Spanish respondents favored social media (63%). Hand sanitizer use (60%), limiting agglomeration in break rooms (63%), frequent handwashing (76%), and limiting on-farm visits (66%) were the most common safety measures implemented at the workplace. Few respondents (38%) indicated that face-covering was required at work. Successful emergency plans on dairies should consider the outreach needs and preferences of dairy workers. These survey results highlighted different perspectives, educational needs, and preferred sources of information between English and Spanish speaking dairy workers.

Key Words: COVID-19, dairy employees, training

345 An investigation into the perceptions of farmers toward dairy cow-calf rearing.

O. F. D. Bolton*, G. L. Charlton, and E. C. L. Bleach, *Harper Adams University, Newport, Shropshire, UK.*

Assessing farmers perception toward cow-calf rearing is important to understand uptake of alternative rearing strategies, and the obstacles to doing so. It is common practice to separate the dairy cow and calf after birth (and rear calves artificially) to facilitate the collection of saleable milk, however, the practice is receiving increasing criticism on grounds of impaired animal welfare. Cow-calf rearing systems, where calves are reared entirely or in part by the dam or foster cow, are gaining interest, however uptake is minimal and there is no consensus of the benefits or challenges of these systems. Therefore, the aim of the current study was to gain insight into why farmers may or may not adopt these rearing strategies and what they thought were the key benefits or challenges of these management options. Telephone interviews were conducted with 11 UK dairy farmers (6 cow-calf rearers, 5 conventional) and transcripts analyzed thematically using Nvivo software (QSR International Pty Ltd. (2018) NVivo Version 12). Farmers rearing calves on cows spoke positively about the method, stating that calves had better growth, health, and welfare, and that "the calf reared by its mother is twice the calf reared by even the best calf rearer." They emphasized that it required a change in attitude and that it was necessary for the farmer to relinquish control during the milk feeding period; "it is actually a whole system change, you really need a complete mindset change, you need a change of management." However conventional farmers expressed concern with the perceived lack of control during the milk feeding period; "How can you assess problems…in calf rearing, of which there are many, if you don't understand the beginning"? stating that cow-calf rearing could not offer the consistent and uniform rearing environment that was important to them. They also expressed concerns about the impact on calf health, the potential to increase separation stress and the financial impacts of cow-calf rearing. Nevertheless, conventional farmers stated that foster

cow rearing was a preferred option for cow-calf rearing providing uptake was incentivized and that colostrum delivery was still farmer led.

Key Words: cow-calf, rearing, calf

346 Self-organizing map to segment Canadian herds based on animal welfare responses and their relationship with productivity, profitability and longevity.

G. M. Dallago*[1], D. Warner[2], K. M. Wade[1], R. I. Cue[1], R. Lacroix[2], A. B. Diallo[3], and E. Vasseur[1], [1]*McGill University, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada,* [3]*Université du Québec à Montréal, Montreal, QC, Canada.*

Animal welfare (AW) is part of the sustainability certification program. However, the implementation of AW solutions is influenced by its economic impact and the relationships between herd welfare and productivity, profitability and longevity are unclear. Our objective was to describe the welfare status of Quebec, Canada dairy herds and assess its relation to performance and economic metrics. Five AW responses, collected at herd level between 2016 and 2019, were extracted from the proAction database (Dairy Farmers of Canada) from 2,696 herds: prevalence of lameness, hock, neck and knee lesions and animals with a body condition score (BCS) ≤ 2. Lactanet provided productivity (energy-corrected milk; ECM), profitability (milk value), longevity (length of productive life and percentage of animals on third or greater lactation; 3+ Lact) and composite herd status (i.e., welfare and health remote monitoring tool; HIS) metrics on the same herds. Self-organizing maps and hierarchical clustering were used to segment farms based on welfare responses, HIS, season and barn type. Longevity, profitability and productivity were then compared between the 4 identified clusters using a decision tree. Cluster 2 (n = 1,377) was composed of herds with the lowest probability of animals with BCS ≤2 (≤3.03%), lameness (≤14.81%) and neck lesions (≤6.90%). Cluster 1 (n = 456) had the highest probability of animals with BCS ≤2 (>14.29%). Clusters 3 (n = 366) and 4 (497) both had the highest probability of lameness (>14.81%). However, cluster 4 had a higher probability of animals having neck lesions (>16.70%) than cluster 3 (≤16.70%). Cluster 3 was also found to have a higher probability of herds producing ≤8,972 kg ECM and 3+ Lact (≤26.8%) compared with the others. In contrast, cluster 2 had the highest probability of >8,972 kg ECM and 3+ Lact (>38.3%). In summary, we were able to segment herds based on their animal welfare responses and good welfare status was associated with better performance and economic metrics.

Key Words: dairy cow, machine learning, artificial neural network

347 Environment, nutrition, and management practices for far-off, close-up, and fresh cows on Canadian dairy farms: A retrospective descriptive study.

R. C. Serrenho*[1], C. Church[2], and T. F. Duffield[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Elanco Animal Health, Guelph, ON, Canada.*

The complex and interrelated management components of dairy farming are associated with health, production, and profitability of the herd. Yet, there is limited objective data on current management practices of the far-off (FO), close-up (CU), and fresh (FR) periods across farms. We aimed to describe management practices of Canadian farms by using a pre-existing risk assessment tool and outline management opportunities. Upon veterinarians' or producers' request, a transition management risk assessment (The Vital 90^{TM}, Elanco) was performed by trained observers (n = 10) during farm visits (n = 79; between August 2014 and March 2018). Most farms were in Ontario (n = 65), while the remaining were in Alberta (n = 5), British Colombia (n = 4), Manitoba (n = 1), Prince Edward Island (n = 2), Newfoundland (n = 1), and Saskatchewan (n = 1). The tool included 78 questions about comfort, nutrition, and pen management of the dry (FO, −60 to −20DIM; CU, −20 to 0DIM) and FR (0 to 30DIM) periods. The herds averaged 125 milking cows and most had 2 defined dry groups (81%). Freestall (54%) and bedded pack (81%) were the most common housing systems observed in the FO and CU periods, respectively. Heifers and cows were housed together in 55, 80, and 60% of the FO, CU, and FR pens, respectively. A large proportion of the FO (40%), CU (51%), and FR (48%) pens were overstocked. Poor water access was observed across all periods (65, 57, and 25% of the FO, CU, and FR, respectively). Only a few farms had proper heat abatement systems in place (<10% in the dry and 15% in the FR period). Cows were able to sort their ration in 60% of the dry period pens and 30% of the FR pens. In 73% of the farms, fresh-cow health monitoring protocols were not well defined. Colostrum cows and sick cows were housed together in 40% of the farms; 60% separated the newborn from the dam within 2 to 12h of birth. This work describes prevalent management practices in the dry and fresh periods and highlights areas with greater potential for improvement. Future research should focus on the associations of the studied practices with health and performance of dairy farms.

Key Words: transition period, management, risk assessment

Reproduction III

348 The use of automated infrared thermography and tail movement to detect in estrus Holstein cows. H. J. Perez Marquez*[1], A. L. Schaefer[2,1], H. von Gaza[3], D. J. Ambrose[4,1], N. J. Cook[4,1], and C. J. Bench[1], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*Animal Inframetrics, Lacombe, AB, Canada,* [3]*HVG Software Solutions, Tofield, AB, Canada,* [4]*Livestock Research and Extension Branch, Edmonton, AB, Canada.*

The objectives were to develop an automated infrared platform (Estrus Benchmark; EBM) that can measure skin temperature and tail movements to flag cows in estrus and to evaluate its accuracy compared with milk progesterone (P_4) analysis (Herd Navigator, DeLaval; HN) and an accelerometer system (CowManager SensOor tags; CSTags). Cows (n = 46) were housed in a voluntary milking freestall barn (VMS) where EBM captured (20 frames/milking) skin temperature and tail movements when the cows were leaving the VMS robot (45–120 post-calving). Progesterone was determined automatically (at set intervals) throughout the study period and cows were flagged in estrus when P_4 fell below 5 ng/mL. The CSTags and EBM true positive estrus alerts (sensitivity) were compared with HN estrus alerts at different time windows (Same-day, ± 24 h, ± 48 h, and ± 72 h). The EBM was able to collect skin temperature and tail movements (left tail movements; LTail, non-defined direction of tail movement; NTail, and right tail movement; RTail). Skin temperature increases were associated with the decrease in milk P_4 concentration (LSMeans ± SE) at d 0 (P_4; 3.51 ± 0.05 ng/mL, Skin temperature; 33.31 ± 2.38°C) compared with d −14 (P_4; 20.22 ± 0.73 pg/mL, Skin temperature; 32.05 ± 3.77°C) and d 4 (P_4; 10.98 ± 0.66 pg/mL, Skin temperature; 31.67 ± 3.48°C). The percentage of tail movement (no. cows with vulva exposed/total no. cows) was higher at d 0 (LTail; 62.50%, NTail; 68.75%, and RTail; 56.25%) compared with d −14 (LTail;15.62, NTail; 6.25%, and RTail;15.62%), and d 4 (LTail; 9.37%, NTail; 9.37%, and RTail; 12.5%). The sensitivity (%) of EBM was then compared with CSTags using HN estrus alerts as a standard for each time window, Same-day (CSTags; 6, EBM; 42), ± 24 h (CSTags; 23, EBM; 50), ± 48 h (CSTags; 43, EBM; 58), and ± 72 h (CSTags; 44, EBM; 56). The highest EBM Youden index (0.45), diagnostic odds ratio (9.04), and efficiency (0.77) was achieved by estrus alerts in a ± 48 h window relative to HN estrus alerts. The EBM was found effective to measure skin temperature, tail movements, and to create estrus alerts with an accuracy comparable to CSTags systems.

Key Words: thermography, vulva exposure, robotic milking

349 Effect of first-service management programs including AI at detected estrus based on automated estrus alerts and estrus occurrence during the voluntary waiting period on reproductive outcomes of dairy cows. C. Rial*, A. L. Laplacette, and J. O. Giordano, *Deparment of Animal Science, Cornell University, Ithaca, NY.*

Our primary objective was to evaluate first-service management programs that prioritized AI at detected estrus (AIE) based on automated estrus alerts (AEA) for dairy cows. Secondary objective was to evaluate the effect of different AIE period duration for cows with or without AEA during a voluntary waiting period (VWP) of 50 d. All cows (n = 1,172) were fitted with an ear-attached sensor (Smartbow, Zoetis) and blocked by parity and semen type used for first AI (sexed vs beef). Cows were randomized to a control group (ALL-TAI; n = 395) for which timed AI (TAI) occurred at 83 ± 3 DIM after a Double Ovsynch protocol, a group (AIE+TAI; n = 388) in which cows were AIE for up to 21 d past the VWP and if not AIE received TAI at 83 ± 3 DIM after Ovsynch with progesterone supplementation (P4-Ov), or a group (AEA/NoAEA-AIE+TAI; n = 388) for which cows were AIE for 28 or 14 d past the VWP if AEA were or were not recorded during the VWP, respectively. Cows not AIE received TAI at 88 ± 3 (AEA) or 74 ± 3 DIM (NoAEA), respectively after P4-Ov. All data were analyzed by logistic regression. The proprotion of cows AIE did not differ ($P = 0.60$) for AIE-TAI (73.9%) and AEA/NoAEA-AIE+TAI (70.0%). More cows ($P < 0.01$) received AIE if AEA were (87.2%) than if AEA were not recorded (51.6%) during the VWP and cows with AEA (46%) had greater ($P = 0.02$) pregnancy per AI (P/AI) than cows without AEA (37.9%). Overall P/AI did not differ for all ($P = 0.29$; ALL-TAI 44.5%, AIE+TAI 40.7%, AEA/NoAEA-AIE+TAI 38.7%) or for AIE services only (AIE+TAI 41.7%, AEA/NoAEA-AIE+TAI 43.3%). For TAI services, P/AI tended to differ ($P = 0.06$; ALL-TAI 44.8%, AIE+TAI 42.0%, AEA/NoAEA-AIE+TAI 29.0%). For a subgroup of cows that completed a 150 DIM at-risk period, percent pregnant did not differ [$P = 0.30$; ALL-TAI 79.9% (n = 163), AIE+TAI 85.8% (n = 143), AEA/NoAEA-AIE+TAI 80.3% (n = 148)]. We conclude that reproductive programs that used AIE after AEA for first service led to similar reproductive performance than an all-TAI program, and using AEA data helps identify cows more likely to be AIE and become pregnant to first service.

Key Words: dairy cow, reproduction, automated

350 Effect of reproductive programs that prioritized timed AI or AI at detected estrus on cash flow of primiparous dairy cows. E. Sitko*, M. M. Pérez, and J. O. Giordano, *Cornell University, Ithaca, NY.*

Our objective was to evaluate the effect of reproductive management programs that prioritized timed AI (TAI) or AI at detected estrus (AIE) during first lactation on the cash flow of dairy cows. Primiparous cows (n = 6 farms) were randomly assigned to a program that prioritized TAI (P-TAI; n = 1,279) or a program that prioritized AIE and used TAI for cows not AIE (P-AIE; n = 1,387). Cows in P-AIE received AIE after a PGF2α (PGF) treatment at 53 ± 3 DIM. Cows not AIE 21 d later received TAI after Ovsynch with progesterone supplementation (P4-Ovsynch). Thereafter, cows were AIE and if not pregnant 32 ± 3 d later received P4-Ovsynch for TAI 10 d later. Cows in P-TAI received TAI at 84 ± 3 DIM after a Double Ovsynch protocol. All cows received GnRH treatment 25 ± 3 d after AI if not AIE. Non-pregnant cows with a corpus luteum 32 ± 3 d after AI completed Ovsynch. The remaining cows received P4-Ovsynch. Cash flow was evaluated per slot occupied by each cow enrolled for 18-mo after calving in the experimental lactation (EL). Slots remained filled for the 18-mo period by replacing cows sold and dead with a first-lactation cow from the same group. Total cash flow was the aggregation of daily income over feed cost (IOFC), calf value (CV), replacement cost (RC), reproductive cost, and operating expenses. Binary and quantitative data were analyzed using logistic regression and ANOVA, respectively. Farm was a random effect and age at first calving offered as confounder. No differences ($P > 0.10$) were observed for IOFC ($3,071 ± 55 P-TAI vs $3,080 ± 55 P-AIE), RC (-$98 ± 12 P-TAI vs -$102 ± 12 P-AIE), cows that left the herd (13 ± 1.6% P-TAI and 13 ± 1.6% P-AIE), and CV ($72 ± 1.8 P-TAI vs $72 ± 1.8 P-AIE). Reproductive costs were greater ($P = 0.04$; $52 ± 1.8 P-TAI vs 49 ± 1.8 P-AIE) and lactation length was longer during the EL for P-TAI ($P < 0.01$; 315 ± 5.6 d P-TAI vs 306 ± 5.6 d P-AIE). Total cash flow did not differ ($P = 0.60$; $1,228 ± 49 P-TAI vs $1,212 ± 49 P-AIE). We conclude that primiparous cows managed with reproductive program that prioritized TAI or a program that prioritized AIE had similar cash flow for up to 18-mo after calving in their first lactation.

Key Words: reproduction, profitability, cow

351 Economic impact of adding a second prostaglandin $F_{2\alpha}$ treatment during an Ovsynch protocol using a meta-analytical assessment and a stochastic simulation model.

S. Borchardt*[1], C. Tippenhauer[1], P. Fricke[2], and W. Heuwieser[1], [1]*Clinic of Animal Reproduction, Free University Berlin, Berlin, Germany,* [2]*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI.*

Incomplete luteal regression after treatment with a single dose of prostaglandin $F_{2\alpha}$ during an Ovsynch protocol decreases fertility to timed artificial insemination (TAI). To increase the proportion of cows with complete luteal regression and subsequently pregnancy per AI (P/AI), an additional treatment with prostaglandin $F_{2\alpha}$ (PGF) 24 h after the first has been recommended. This is associated with additional costs due to additional labor required to administer the second PGF treatment and hormones. The objective of this study was to develop a stochastic partial budget model to estimate the economic impact of an additional PGF treatment on d 8 during an Ovsynch protocol. A systematic review of the literature and a meta-analytical assessment was performed to evaluate the effects of adding a second PGF treatment during the Ovsynch protocol on P/AI in lactating dairy cows. Eleven randomized controlled experiments from 10 published manuscripts including 9,432 cows were used. Adding a second PGF treatment during the Ovsynch protocol increased the relative risk for pregnancy (RR = 1.17; 95% CI = 1.11 to 1.23) in lactating dairy cows 32 d after TAI using the fixed effects model. An additional treatment with PGF yielded a 5.73 risk difference in P/AI (95% CI = 3.79 to 7.68) using a fixed effects model. Revenue was based on the associated improvement in reproductive performance due to an increase in P/AI multiplied by the value of a pregnancy (PGVAL). Median PGVAL was €252 (5% percentile €42 and 95% percentile €623) with a minimum of −€138 and a maximum of €1,198. The median PGVAL varied among the 10 farms ranging from €192 to €462. Using a stochastic simulation model with 10,000 iterations adding a second PGF dose on d 8 during an Ovsynch protocol was more profitable (€7.98/cow; 5% percentile €0.25 and 95% percentile €30.31) compared with a single PGF administration on d 7. The median return on investment was €2.17. In conclusion, there was a clear benefit of an additional PGF treatment during the Ovsynch protocol on P/AI (+5.7 percentage units). Despite additional costs for hormones and labor an additional treatment with prostaglandin $F_{2\alpha}$ on d 8 was more profitable in 95% of all scenarios due to the associated increase in fertility.

Key Words: timed AI, economic impact, stochastic simulation

352 Economic analysis of 3 strategies for submission of nulliparous Holstein heifers for first insemination using sexed semen.

M. R. Lauber*, V. E. Cabrera, and P. M. Fricke, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Our objective was to use a partial budget analysis to compare costs among 3 reproductive management strategies for dairy heifers inseminated with sexed semen. Nulliparous Holstein heifers (n = 736) from 3 commercial farms were randomized to 3 treatments for first AI: 1) 5-d CIDR protocol (n = 255; d −6, GnRH +CIDR; d −1, $PGF_{2\alpha}$ -CIDR; d 0, $PGF_{2\alpha}$; d 2, $GnRH^{+}TAI$); 2) 6-d CIDR protocol (n = 254; d −6, GnRH +CIDR; d −1, $PGF_{2\alpha}$; d 0, $PGF_{2\alpha}$ -CIDR; d 2, $GnRH^{+}TAI$); and 3) EDAI (n = 227; $PGF_{2\alpha}$ on d 0 followed by once-daily estrus detection and AI). Heifers were followed for 84 d after first service to determine days to AI and pregnancy. Feed costs for nonpregnant heifers or heifers moved to a bull pen during the 84-d breeding period (n = 112) were allocated to the feed costs for heifers that became pregnant during the 84-d breeding period. Actual farm costs (US$) were used for hormonal treatments, detection of estrus, semen and AI, pregnancy diagnosis, and feed ($1.70/heifer/d) to calculate cost per pregnancy. Costs were analyzed using the MIXED procedure of SAS with treatment as a fixed effect and farm as a random effect in the model. For 5-d, 6-d, and EDAI heifers, respectively, although CIDR heifers had more ($P < 0.01$) hormonal treatment costs than EDAI heifers ($22.29 ± 0.36, $21.85 ± 0.36, $4.05 ± 0.38) they had less ($P < 0.01$) feed costs ($50.10 ± 2.73, $56.84 ± 2.56, $82.79 ± 3.01). Thus, total costs per pregnancy were less ($P = 0.04$) for 5-d CIDR than for EDAI heifers ($153.26 ± 5.36, $169.92 ± 5.55). Based on a sensitivity analysis of feed costs ($1.25, $1.50, $1.75, $2.00, and $2.25 per heifer/d), when feed costs were greater than or equal to $1.75 per heifer/d, the difference in costs per pregnancy was greater ($P < 0.05$) for EDAI than for 5-d CIDR heifers by $17.62, $22.43, and $27.24 when feed costs were $1.75, $2.00, and $2.25 per heifer/d, respectively. In conclusion, submission of heifers to a 5-d CIDR-Synch protocol for first AI resulted in the lowest total costs per pregnancy due to the most pregnancies per AI which resulted in fewer days to AI and pregnancy which decreased overall feed costs.

Key Words: dairy heifer, sexed semen, economics

Ruminant Nutrition: General III

353 Assessment of the effects of intrauterine dextrose infusion on clinical cure rate, daily milk yield, daily rumination, metabolic stress, systemic inflammation, and reproductive performance in postpartum dairy cows diagnosed with clinical metritis. J. Hamilton*[1], A. A. Barragan[1], E. Hovingh[1], M. Martinez[1], S. Bas[2], J. Zug[3], and S. Hann[3], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [3]*Zugstead Farm, Mifflintown, PA.*

The objective of this study was to assess the effects of intrauterine dextrose infusion on clinical metritis cure rate, daily milk yield and rumination time, metabolic stress, systemic inflammation, and reproductive performance in dairy cows diagnosed with clinical metritis (CM). Cows (n = 641) from a farm located in central Pennsylvania were screened at 7 ± 3 DIM to assess vaginal discharge. Cows that presented a fetid red-brownish watery vaginal discharge (n = 74) were classified as CM cows, blocked by parity and randomly assigned to one of 2 groups: 1) CONV (n = 37): 2 injections of ceftiofur (per label; 6.6 mg/kg) 72 h apart; and 2) DEX (n = 37): 3 intrauterine infusions of a 50% dextrose solution (1 L/cow) every 24 h. Cows that presented a normal vaginal discharge at 7 ± 3 DIM (NOCM; n = 37) were randomly selected and matched by parity to CONV and DEX cows. Cows were re-screened at 14 ± 3 and 21 ± 3 DIM to assess CM cure rate. Daily milk yield and rumination time for the first 150 DIM and reproductive performance data were collected from on-farm computer records. Body condition score was assessed, and blood samples were collected at enrollment, and at 9 ± 3, 14 ± 3 and 21 ± 3 DIM. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized complete block design. There was no difference in CM cure rate between treatment groups at 14 ± 3 DIM (CONV = 68.59 ± 7.95%; DEX = 61.12 ± 8.25%) and 21 ± 3 DIM (CONV = 88.26 ± 5.586%; DEX = 85.74 ± 5.994%). CONV and DEX cows had higher concentration of haptoglobin at 7 ± 3 DIM and 9 ± 3 DIM, but there was no difference between study groups at 14 and 21 ± 3 DIM. Overall, cows in the CONV and NOCM groups produced 3.67 kg/d and 3.68 kg/d more milk, respectively, compared with cows in the DEX group during the first 150 DIM. There was no difference in reproductive performance between study groups. These results suggest that, although intrauterine infusion of dextrose may achieve similar clinical cure rates as ceftiofur treatment, dextrose may not be as effective at restoring milk production in CM cows.

Key Words: clinical metritis, intrauterine dextrose, dairy cattle

354 Production performance and metabolism of Holstein cows fed low protein diets balanced for lysine, methionine, and histidine during heat stress. A. Ruiz-González*[1,2], Y. H. Leung[3], A. Celimin[2], A. Kenez[3], P. Y. Chouinard[1], R. Gervais[1], H. Lapierre[4], D. R. Ouellet[4], and D. E. Rico[2], [1]*Université Laval, Quebec, Canada,* [2]*CRSAD, Deschambault, QC, Canada,* [3]*Department of Infectious Diseases and Public Health, City University of Hong Kong, Hong Kong,* [4]*Agriculture et Agroalimentaire Canada/Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Decreasing dietary protein may reduce the impact of heat stress on dairy cows, but supplementation of Lys, Met, and His may be needed to meet lactation requirements. Six multiparous (42.2 ± 10.6 kg milk/d; 86.9 ± 10 d in milk) and 6 primiparous (29.5 ± 1.2 kg milk/d; 95.2 ± 16 d in milk) Holstein cows were used in a split-plot design to evaluate the effect of decreasing metabolizable protein (MP) supply, but meeting Lys, Met, and His requirements) on animal performance and metabolism. Within each plot (parity), cows were randomly assigned to 1) heat stress (HS; 16% CP), 2) thermoneutral pair-feeding (TNPF; 16% CP) or 3) HS with a low MP supply (14% CP, −300 g/d of MP), but balanced for Lys 115, Met 35 and His 60 g/d HS+AA (AA supply was increased to compensate for DMI decline) in a Latin square design with 14-d periods. Lactation performance was determined on d 0, 3, 7, 10, and 14. The statistical model included the random effects of cow and period, and the fixed effects of parity, treatment, day, and their interactions. No parity × treatment interactions were detected for any variable. Peak rectal temperature was 1.5°C greater in HS than in TNPF cows from d 1 to 14 ($P < 0.001$), whereas it tended to be 0.4°C lower in HS+AA relative to HS on d 12 and 13 ($P = 0.07$). Respiration rate was 2.6-fold greater over the 14-d period in HS relative to TNPF ($P < 0.001$), but was not different between HS and HS+AA. Heat stress progressively reduced DMI by 34% (Time $P < 0.05$) irrespectively of treatment. Milk yield decreased over time in all groups, but was 14% lower in HS and HS+AA compared with TNPF ($P < 0.05$) from d 3 to 14. Relative to the TNPF group, HS and HS+AA reduced milk component yields, resulting on an average decrease of 180, 186, and 205 g/d for fat, protein, and lactose, respectively, from d 3 to 14 ($P < 0.05$). Heat stress increased insulin concentrations by over 30% relative to TNPF cows on d 7 and 14 ($P < 0.05$). Relative to HS, the HS+AA group exhibited a reduction of 14% insulin concentrations ($P < 0.05$). Plasma free fatty acids were reduced in HS by over 30% relative to TNPF ($P < 0.05$), but were not different between HS and HS+AA. Balancing for Lys, Met, and His while reducing MP moderately modulated the metabolic response of cows to heat stress, but did not affect milk yield and components.

Key Words: heat stress, dairy cows, protein supply

355 The impact of a *Saccharomyces cerevisiae* fermentation product on performance and metabolic and immunological responses to a feed restriction challenge of mid-lactation dairy cows. T. N. Marins*[1], F. A. Gutierez Oviedo[1], M. L. G. F. Costa[1], Y.-C. Chen[1], H. Goodnight[1], M. Garrick[1], D. J. Hurley[2], J. K. Bernard[1], I. Yoon[3], and S. Tao[1], [1]*Department of Animal and Dairy Science, University of Georgia, Tifton, GA,* [2]*Food Animal Health and Management Program, Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA,* [3]*Diamond V, Cedar Rapids, IA.*

To examine the effect of a *Saccharomyces cerevisiae* fermentation product on performance and metabolic, inflammatory and immunological responses to a feed restriction challenge (FR) in mid-lactation dairy cows, 60 Holstein cows blocked by parity, days in milk and milk yield (MY) were randomly assigned to 1 of the 2 supplements (19 g/d/cow): NutriTek (NT, Diamond V, Cedar Rapids, IA) or placebo (CTL). Intake and MY were assessed daily and milk composition weekly during the 12 wk production trial. Then, a subset of cows (n = 32) were enrolled in a 5-d FR (40% of ad libitum intake) followed by a 5-d realimentation. Relative to FR, DMI and MY and composition were measured daily from d −2 to 10, blood drawn on d −2, −1, 1, 2, 3, 4, 5, 6, 8, and 10 to analyze metabolites, interleukin 10, tumor necrosis factor-α, LPS binding protein and haptoglobin, and peripheral mononuclear cells proliferation and neutrophil function assessed on d −3 and 4. No differences were observed in DMI, MY or concentrations and yield of components ($P \geq 0.10$), except for fat yield. Milk fat yield was reduced for primiparous cows fed CTL compared with NT, whereas, no difference was observed for multiparous cows (diet by parity interaction, $P = 0.05$). Cows fed NT had similar decrease in MY but had greater reduction in plasma glucose and higher β-hydroxybutyrate concentrations during FR than CTL ($P \leq 0.07$). FR did not induce systemic inflammation but reduced neutrophil function. Compared with CTL, feeding NT tended to improve neutrophil reactive oxygen species production after stimulation by extracellular antigens ($P = 0.08$). In conclusion, feeding NT increased milk fat yield of primiparous cows but did not affect overall productive performance. Importantly, actual energy intake of cows was 12–22% higher than combined energy cost for maintenance and milk synthesis and cows were exposed to environments with minimal stress. NT improved induced neutrophil oxidative burst possibly related to the greater glucose utilization compared with CTL during FR.

Key Words: *Saccharomyces cerevisiae* fermentation product, feed restriction, immunity

356 Transition diet starch content impacts colostrum and transition milk composition and immunoglobulin G and insulin concentrations in Holstein dairy cattle.

A. Fischer-Tlustos*[1], K. Hare[1], J. Haisan[2], W. Shi[2], J. Cant[1], M. Oba[2], and M. Steele[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to evaluate how prepartum dietary starch content of dairy cows affects colostrum composition, immunoglobulin G (IgG), and insulin concentrations and how pre- and postpartum dietary starch content affects the aforementioned variables in colostrum and transition milk (TM). Multiparous (n = 51) and primiparous (n = 36) Holstein cattle were randomly assigned to a prepartum diet containing moderate (CON; 14.0% starch, %DM) or high (HI; 26.1% starch, %DM) levels of dietary starch from 28 ± 3 d before calving, and randomly assigned within prepartum treatment to a high fiber (HF; 33.8% NDF, 25.1% starch, %DM) or high starch (HS; 27.2% NDF, 32.8% starch, %DM) diet after calving. Colostrum (milking 1, n = 87) and TM (milkings 2 to 6, n = 51 per milking) were collected twice daily and yield was recorded. Fat, CP, lactose, SCC, urea-N and TS concentrations were determined by infrared spectroscopy, IgG was quantified by radial immunodiffusion, and insulin was quantified by enzymatic immunoassay. Interactions between parity and treatment diets were non-significant and were not included in the statistical model; thus, data were analyzed using PROC GLIMMIX with the fixed effects of parity, milking, pre- and postpartum diet, and milking × pre- and postpartum diet, and the random effect of cow. In colostrum, CON cows had lower ($P = 0.005$) insulin concentrations (30.5 ± 3.48 vs. 54.4 ± 3.98 μg/L) and higher ($P = 0.032$) IgG concentrations (106.3 ± 4.83 vs. 91.1 ± 5.03 g/L) than HI cows; however, colostral IgG yield (g) was not different ($P = 0.51$). Fat yield increased ($P < 0.012$) 57.1, 78.0, and 65.0% at milkings 4, 5 and 6, respectively, for HI cows compared with CON cows but did not differ ($P > 0.35$) in milkings 1–3. Prepartum diet did not affect ($P \geq 0.21$) CP or lactose yield. From milkings 1 to 6, HI-HF (965.4 ± 71.72 g) cows had greater ($P = 0.013$) CP yield than HI-HS (581.8 ± 83.29 g), CON-HS (676.7 ± 77.35 g), and CON-HF (677.0 ± 73.99 g) cows. These results indicate that maternal nutrition can impact colostrum and TM composition and suggest that transition rations could be managed to ensure that the cow produces high-quality colostrum and TM for her offspring.

Key Words: colostrum, immunoglobulin G, insulin

357 Derivation of the maintenance energy requirements and efficiency of metabolizable energy utilization for dry and lactating Jersey cows.

D. L. Morris*[1,2] and P. J. Kononoff[1], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*Perdue AgriBusiness, Salisbury, MD.*

Maintenance energy is the energy required to conserve the state of an animal when no work is completed. The objectives of the current experiment were to quantify maintenance energy requirement and energy efficiency of Jersey cows when lactating or dry. Data were collected on 8 non-pregnant Jersey cows and evaluated across 3 physiological phases and nutritional planes: lactation, dry cows fed at maintenance, and fasted dry cows. Through total collection of feces and urine as well as using headbox-style indirect calorimeters, energy balance and heat production (HP) were measured across all phases. Lactation data were collected across four 28-d periods, maintenance data were collected after 14 d, and fasting HP was measured during the last 24 h of a 96-h fast. Net energy for maintenance (NE_M), and the efficiency of converting metabolizable energy (ME) into net energy were compared between lactating and dry cows using regression techniques. HP of dry cows fed at maintenance, which represents ME for maintenance, was 0.146 ± 0.0087 Mcal per unit of metabolic body weight ($\text{BW}^{0.75}$, MBW). Fasting HP, which represents NE_M, was 0.102 ± 0.0071 Mcal/MBW. When estimated via regressing energy balance (tissue energy plus milk energy) on ME intake, NE_M was not different between dry and lactating cows (0.120 ± 0.32 vs. 0.103 ± 0.0052 Mcal/MBW). The slope of the regression of energy balance on ME intake was greater for dry compared with lactating cows (0.714 ± 0.046 vs. 0.685 ± 0.010) when evaluated with a single intercept for each group. This suggests that dry cows were more efficient at converting ME into net energy and that the efficiency of utilizing ME for maintenance may be greater than for milk. Our measurements of NE_M and the slope of ME on energy balance were greater than the value used by NRC (2001), which are 0.080 Mcal/MBW for NE_M and approximately 0.64 for the slope. Results of this study suggest that NE_M and the efficiency of converting ME into NE_M of modern lactating Jersey cows is greater than NRC, 2001 and similar to recent measurement on Holstein cows.

Key Words: fasting, heat production, energy utilization

358 The dose responsive induction of acute hyperketonemia by ruminal infusion of calcium butyrate in dairy cows.

D. E. Rico[1], A. Celemín-Sarmiento[1], and J. E. Rico*[2], [1]*Centre de recherche en sciences animales de Deschambault, Québec, Canada,* [2]*University of Maryland, College Park, MD.*

In dairy cows, hyperketonemia is sometimes associated with peripartal disease and reduced milk production; however, ketones are known to exert anti-inflammatory and insulin-sensitizing effects in non-ruminants. We aimed to evaluate the dose-response effects on Ca-butyrate (CB) on the acute induction of hyperketonemia, and its effects on rumen fermentation, and cow performance and health status, in the absence of feed restriction or negative energy balance. Four multiparous, lactating, and ruminally cannulated cows (290 ± 51 DIM; 25 ± 5 kg of milk/d) were randomly allocated to 1 of 4 ruminally administered single bolus treatments in a 4 × 4 Latin square design with 24-h periods and 5-d washouts. Ca-butyrate was dosed at 0, 2, 4 or 6% of ration DM. Treatments were balanced for Ca content. Blood samples were collected at 0, 2, 4, 8, and 12 h relative to bolus administration. Ruminal fluid was sampled at 0, 2, 4, and 8h relative to bolus administration, and analyzed for pH and VFA concentrations. Cow behavior and health status were monitored including general attitude, ear position, standing posture, lying position, panting and shivering. Data were analyzed under a mixed model with fixed effects of dose and time relative to infusion. Blood B-hydroxybutyrate (BHB) increased linearly with dose, peaking at ~4.9 mM, and reaching nadir of 1.03 mM, at 2 and 12 h post infusion, respectively, in the 6% CB dose ($P < 0.05$). No clinical signs of disease were observed upon CB infusion. Similarly, no differences were observed in skin, vaginal, or rectal temperatures, nor respiration rates (all $P > 0.50$). Molar concentrations of butyrate in ruminal fluid increased linearly with increasing CB dose ($P < 0.001$). No differences were observed in ruminal pH ($P = 0.84$). DMI and milk yield were reduced at the 6% CB dose ($P < 0.05$). Milk fat content increased with CB infusion ($P < 0.05$), concomitantly with increased milk de novo FA concentrations ($P < 0.01$). Acute nutritional induction of hyperketonemia had no impact on short-term behavior or health status of dairy cows, but reduced milk yield and DMI at the highest CB dose.

Key Words: ketone, calcium butyrate, dairy cow

359 Effect of a specific blend of flavonoids on lactation performance when fed to Holstein dairy cows during the transition period and beyond.

S. E. Schuling*[1], D. E. Schimek[1], G. Camacho[2], D. Ruiz[2], and A. Lago[2], [1]*NutriQuest, Mason City, IA,* [2]*DairyExperts, Tulare, CA.*

Sixty-six primiparous and 297 multiparous Holstein cows were used in a randomized complete block design to determine the effect of a specific blend of flavonoids (FLV) on milk and ECM yields through 150 DIM. Cows were blocked before calving by parity and expected calving date. Cows within block were randomly assigned to one of 4 dietary treatments (TRT): 1) control diet (CON), 2) CON + 10 g/d FLV from −21 to 21 DIM (FLV42), 3) CON + 10 g/d FLV from −21 to 90 DIM (FLV111), and 4) CON + 10 g/d FLV from 1 to 90 DIM (FLV90). Lactating cows were housed in a freestall barn (Pixley, CA) and milked 3 times/d in early lactation and 2 times/d thereafter. The lactation CON TMR was fed once daily and contained 34% forage, 16% almond hulls, and 50% concentrate. Cows were restrained in headlocks for 30 min once daily for delivery of a pellet (113.5 g/d) containing 10 g/d of FLV to individual cows on FLV TRT. Milk yield was measured at each milking by individual milk meters. Milk was sampled biweekly for analysis of components, and BCS were determined at −21, 1, 21 and 90 DIM. Data were analyzed as repeated measures using the GLIMMIX procedure of SAS. Fixed effects were TRT, parity, week and their interactions. Random effect was cow(block). Cows fed FLV111 had increased milk yield (1–150 DIM) compared with cows fed CON (mean ± SE; 41.4 and 39.7 ± 0.58 kg/d, respectively). Cows fed FLV90 had intermediate milk production (40.4 kg/d), and milk yield for cows fed FLV42 was similar to CON (39.5 kg/d). There was no effect of TRT on milk protein content (3.18 ± 0.02%), milk fat content (3.33 ± 0.04%), milk fat yield (1.37 ± 0.03 kg/d), MUN (11.72 ± 0.15 mg/dL), somatic cell linear score (1.98 ± 0.09), BCS (2.84 ± 0.02), or BCS at 90 DIM (2.75 ± 0.02). Feeding FLV111 and FLV90 tended to increase ECM yield (41.8 and 41.4 ± 0.61 kg/d) and milk protein yield (1.32 and 1.30 ± 0.01 kg/d, respectively) compared with control (40.5 and 1.28 kg/d, respectively). Feeding FLV111 increased milk production by 1.68 kg/d from 1 to 150 DIM. The improvement in milk production did not result in increased BCS loss.

Key Words: flavonoids, milk production, transition cow

360 Effect of dietary supplementation of polyunsaturated fatty acids on intake, digestibility, milk production, and milk fatty acids of dairy goats: A meta-analysis.

A. A. Pech-Cervantes*[1], I. M. Ogunade[2], Z. M. Estrada-Reyes[1], C. A. Sandoval-Castro[3], A. Oliveira[4], D. Vyas[5], and A. T. Adesogan[5], [1]*Agricultural Research Station, Fort Valley State University, Fort Valley, GA,* [2]*Division of Animal and Nutritional Sciences, West Virginia University, Morgantown, WV,* [3]*Faculty of Veterinary Medicine and Animal Science, Autonomous University of Yucatan, Merida, Yucatan, Mexico,* [4]*Institute of Agriculture and Environmental Sciences, Federal University of Mato Grosso, Mato Grosso, Sinop, Brazil,* [5]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

We conducted a meta-analysis to determine the effects of dietary supplementation of polyunsaturated fatty acids (PUFA) on intake, digestibility, milk production, and milk fatty acids of lactating dairy goats. A systematic search in the literature from 1990 to 2019 was conducted and data from 46 peer review papers with 119 treatments and 1,262 goats were used for the analysis of raw mean differences (RMD) between PUFA supplementation and control. The means were weighted by inverse variance in a mixed model and the heterogeneity was calculated by meta-regression analysis and subgroup analysis using forage (hay, silage, and grass), breed (Alpine, Saanen, crossbreed and others), source (oils, by-products, mixtures), and level of supplementation as covariates (51.7 to 127.4 g/day). Compared with control, PUFA supplementation had no effects ($P > 0.05$) on DMI (RMD = −0.01 kg/d [-0.03, 0.01]) and total-tract DM digestibility (RMD = 0.03% [-0.79, 0.86]); however, intake of NDF was increased (RMD = 0.03 kg/d [0.02, 0.04]) by PUFA supplementation. Milk yield (RMD = 0.03 kg/d [0.01, 0.05]), milk fat (RMD = 0.18% [0.08, 0.28]), and milk lactose (RMD = 0.04% [0.02, 0.07]) were increased ($P < 0.01$) by PUFA supplementation. Concentrations of C18:1, C18:2, C18:3 in milk were increased ($P < 0.01$) by PUFA supplementation (RMD = 1.47 g/100 g Fatty Acid Methyl Esters (FAME) [0.66, 2.27], 0.08 g/100 g FAME [0.02, 0.14]), and 0.15 g/100 g FAME [0.10, 0.19], respectively). Compared with control, PUFA supplementation decreased ($P < 0.01$) saturated fat in milk by 9.3% (RMD = −6.2 g/100 g FAME [-7.56, −4.84]). The subgroup analysis showed that oils alone or a mixture of by-products and oils increased ($P < 0.05$) milk yield and concentrations of fat and lactose in milk, compared with by-products alone. Despite the high heterogeneity ($I > 95\%$) observed in this study, these results demonstrated that PUFA supplementation improved the performance and milk quality (increased concentrations of unsaturated fatty acids, fat, and lactose) of lactating dairy goats.

Key Words: dairy goat, fatty acid, meta-analysis

361 Meta-analysis of production, efficiency, and digestibility differences between Holstein and Jersey cows.

G. I. Zanton*, *USDA-ARS, U. S. Dairy Forage Research Center, Madison, WI.*

The national and international dairy herd is composed of cows from multiple breeds that have varied genotypic and phenotypic traits. Two very distinctive breeds of considerable economic relevance are cows of the Jersey and Holstein breeds. Due to readily observable differences in traits such as body size and milk components, these breeds of cows are perceived to have different productive efficiencies although the experimental evidence for this perception is equivocal. Therefore, the objective of this study was to evaluate the production, efficiency, and digestibility differences between cows of the Holstein and Jersey breeds through meta-analysis. Literature search was conducted to include as many published papers as possible comparing both Holstein and Jersey cows; crossbreed or other breeds of cows were outside the scope of this analysis. The production (digestibility) databases included 30 (8) publications, 67 (14) treatment groups, and 1,669 (168) Holstein and 1,354 (159) Jersey observations. Breeds were compared within treatment groups as a random effect, weighted by the square root of the number of experimental units in each treatment group, and where breed differences with $P < 0.05$ are discussed as significant. As expected, Holstein cows had greater body weight (BW) than Jersey cows and consumed more feed dry matter (DM). Production of fluid milk and milk components were higher in Holstein compared with Jersey cows. Concentrations of fat, protein, lactose, and energy were greater for Jersey than Holstein cows. Likewise, DM intake (DMI) and milk energy output per unit BW were greater for Jersey than Holstein cows. Milk energy output per DMI was greater in Jersey than Holstein cows (1.12 vs 1.05 ± 0.023 Mcal/kg, $P < 0.01$), indicating higher feed efficiency in Jersey than Holstein cows. Likewise, analysis of the subset of studies including digestibility measurements showed increased apparent total-tract digestibility in Jersey cows for DM, neutral detergent fiber, and crude protein. From these results, it is inferred that Jersey cows were more efficient that Holstein cows in part due to differences in BW, milk composition, and digestibility.

Key Words: Holstein, Jersey, efficiency

362 Effects of lifetime calcidiol supplementation combined with prepartum acidogenic diet on first-lactation blood calcium and performance of Holstein cows.

A. C. M. Silva*[1], D. C. Ramos[1], L. V. Drehmer[1], A. Vieira-Neto[2], P. Celi[3], C. Cortinas[3], M. Engstrom[3], L. Tamassia[3], J. E. P. Santos[1], and C. D. Nelson[1], [1]*University of Florida, Gainesville, FL,* [2]*Kansas State University, Manhattan, KS,* [3]*DSM Nutritional Produts, Parsippany, NJ.*

Objectives were to evaluate the effects of lifetime calcidiol and prepartum DCAD on blood ionized Ca (iCa) and production of Holstein cows. Nulliparous cows (n = 95) that had been supplemented with 0 (CTRL) or 1.7 µg/kg BW calcidiol (CAL) in addition to cholecalciferol since birth were assigned to receive a diet with DCAD of −50 (-50) or +200 (+200) mEq/kg of DM from 248 d of gestation until calving resulting in 4 treatments: CTRL+200, CAL+200, CTRL-50, and CAL-50. After parturition, cows received the same lactation diet and continued to receive CTRL or CAL until 100 DIM. Pre- and postpartum diets included cholecalciferol at 2,600 IU/kg DM. The CTRL and CAL treatments were designed to achieve normal (50 ng/mL) and elevated (120 ng/mL) serum calcidiol, respectively. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS. Cows fed −50 had greater blood iCa prepartum than +200. The −50 DCAD increased postpartum blood iCa for CA cows, whereas DCAD did not affect iCa for CTRL cows. Feeding −50 decreased prepartum DMI compared with +200. Compared with +200, −50 increased postpartum DMI for CAL cows but decreased DMI for CTRL cows. Calcidiol decreased energy-corrected milk yield. In conclusion, CAL with prepartum negative DCAD increased postpartum blood iCa, but neither long-term CAL nor prepartum negative DCAD were beneficial for first-lactation milk yield.

Key Words: vitamin D, acidogenic diet, calcium

363 Effects of feeding a supplemental antioxidant during summer on milk and component yields in Holstein cows.

A. Gilbert[1], M. Carabeau*[1], J. Durkin[2], K. Luchterhand[3], M. Tetreault[1], and W. Seymour[3], [1]*Poulin Grain, Inc., Newport, VT,* [2]*Cornerstone Nutrition, East Aurora, NY,* [3]*Novus International, St. Charles, MO.*

The effect of feeding an antioxidant blend[1] during summer was tested on 3 commercial dairy farms, each with a control and treatment pen of multiparous cows, averaging 49.5 +/ 3.4 (mean +/ SD) kg of ECM and 133 +/ 71 d in milk. Cows were milked 3 times per day and fed 1 time per d. The herd ration (56% forage, 16% CP, 30% NDF) was fed with or without 10g/d supplemental antioxidant (AOX) for 90 d starting in mid-June. Individual daily milk yields (14-d average) and pen milk composition data (pen sample, mid-day milking at 0, 30, 60 and 90d) were analyzed with a mixed model (SAS) with farm as a random variable and pre-trial covariates. Significance was at $P < 0.05$ and $P < 0.15$ for a trend. Data were tested for unequal variances by F-Test (Excel). Temperature-humidity index (THI) averaged 69 +/ 6 (range 48- 82) over the study. Milk production (45.9+/ 2.0 kg, LS mean+/ SD), milk protein percent (2.9+/ 0.05%) and yield (1.34+/ 0.05 kg) were not different ($P > 0.15$). Cows fed AOX maintained higher milk fat percent (0.1% unit) and greater milk fat yield (0.045 kg/d) than controls ($P = 0.04$). Milk de novo fatty acids (g/100g milk) tended ($P = 0.12$) to be greater for cows fed AOX vs. control (0.87 vs 0.85). Variance of weekly ECM was reduced for AOX vs control ($P < 0.05$). ECM at 90d was reduced vs pre-trial for control (−2.0 kg, $P = 0.05$) but not for AOX (−0.4 kg). Fat yield of control cows was reduced at 60d and 90d vs 0d, declining 0.08 kg in control cows vs. 0.01 kg in cows fed AOX (Table 1). In conclusion, feeding an antioxidant during summer months supported higher milk fat yield in high-producing multiparous Holstein cows.

Table 1 (Abstract 362). Effects of feeding diets differing in DCAD with lifetime supplementation of calcidiol

	Treatment LSM					P-value[1]		
Variable	CTRL+200	CAL+200	CTRL-50	CAL-50	SEM	DCAD	CAL	DCAD × CAL
iCa, mM[2]								
Prepartum	1.24	1.23	1.26	1.27	0.01	0.05	0.83	0.32
Postpartum	1.15	1.12	1.15	1.16	0.01	0.02	0.50	0.03
DMI, kg/d								
Prepartum	9.6	9.0	8.8	8.6	0.3	0.02	0.14	0.34
Postpartum	18.9^{a}	17.4^{b}	17.4^{b}	18.5^{a}	0.4	0.57	0.63	0.001
ECM, kg/d[3]	36.4	33.0	34.5	33.3	0.9	0.36	0.01	0.25

[1]Effects of DCAD (−50 vs. 200), calcidiol (CTRL vs. CAL) and interaction between DCAD and calcidiol (DCAD × CAL).

[2]Blood ionized Ca (iCa), measured at 268 d of gestation and 0, 1, 2, and 4 d postpartum.

[3]ECM = [(0.3246 × kg milk) + (12.86 × kg fat) + (7.04 × kg protein)] for first 100 DIM.

Table 1. Fat yield (kg/d) in cows fed control versus AOX diet

	Control			Supplemental antioxidant (AOX)		
Trial day	LSM	Change vs baseline	P =	LSM	Change vs. baseline	P =
0	1.84	Baseline	NS	1.85	Baseline	NS
30	1.83	−0.02	NS	1.87	0.02	NS
60	1.76	−0.09	0.04	1.81	−0.04	NS
90	1.77	−0.08	0.07	1.85	0.01	NS

[1] Agrado Plus 2.0, EW Nutrition, Adel, IA.

Key Words: dairy, antioxidant, heat stress

364 Effects of lactic acid bacteria in a silage inoculant on ruminal nutrient digestibility, N metabolism, and lactation performance of high-producing dairy cows. H. F. Monteiro*[1], E. M. Paula[2], R. E. Muck[3], G. A. Broderick[3], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Instituto de Zootecnia, Centro APTA Bovinos de Corte, Sertãozinho, SP, Brazil,* [3]*USDA-ARS, US Dairy Forage Research Center, Madison, WI.*

We tested if alfalfa silage inoculated with *Lactobacillus plantarum* MTD/1 would improve ruminal N metabolism and increase milk production in high-producing dairy cows. Twenty-eight Holstein cows (8 ruminally cannulated) were used in the study in a double crossover design totaling 4, 28-d periods. There were 2 treatments: a diet containing uninoculated alfalfa silage (Control) and a diet containing alfalfa silage inoculated with *L. plantarum* MTD/1 (LP). Diets were formulated to contain 50% of alfalfa silage, 16% CP and 25% NDF [dry matter (DM) basis]. Milk production and DM intake were recorded in the last 14 d of each period. Milk samples were collected twice at both daily milkings on d 20, 21, 27, and 28 of each period. On d 22, omasal samples were collected from the cannulated animals over a period of 3 d to quantify ruminal digestibility and nutrient flows. Data were analyzed using PROC MIXED in SAS. Compared with control, cows receiving the LP treatment had greater milk production (40.4 vs. 39.6 kg/d) and lower milk urea nitrogen concentration (11.6 vs. 12.7 mg/dL), despite minor changes in ECM. Milk lactose concentration was greater in the milk produced by cows fed the LP treatment which reflected a tendency for increased milk lactose yield. Although milk true protein concentration was lower for cows in the LP treatment, milk true protein yield was the same on both control and LP treatments. Improvements in milk production on animals under the LP treatment were associated with greater OM truly digested in the rumen (16.2 vs. 14.6 kg/d), especially ruminal NDF digestion (41.0 vs. 35.9%). Minor changes were observed in total omasal microbial nonammonia N flow in cows receiving the LP treatment. Therefore, alfalfa silage treated with *L. plantarum* MTD/1 may improve ruminal fermentation and milk production, but because of a lack of greater responses in ruminal N metabolism these changes may not be translated into greater ECM in high-producing dairy cows.

Key Words: additives, ensiling, *Lactobacillus plantarum* MTD/1

365 Effect of native rumen microbe supplementation in feed on milk yield, composition, and feed efficiency in lactating dairy cows. A. Valldecabres*[1], S. Gilmore[2], I. Zhelev[2], J. Embree[2], S. Quinlan[2], C. Martino[2], M. Embree[2], G. Camacho[1], and A. Lago[1], [1]*DairyExperts Inc., Tulare, CA,* [2]*Native Microbials Inc., San Diego, CA.*

The objective of this study was to evaluate the effect of native rumen microbial supplementation on milk production and feed efficiency (FE). A total of 90 multiparous cows between 41 to 61 d in milk were blocked by milk yield and randomly assigned to 3 study groups. Treatments added for 150 d at 0.33 g/kg of the total mixed ration dry matter contained: *Clostridium beijerinckii* at 2x10^6 cfu/g and *Pichia kudriavzevii* at 2x10^7 cfu/g (Galaxis; Native Microbials Inc., San Diego, CA); *C. beijerinckii* at 2x10^6 cfu/g and *P. kudriavzevii*, *Ruminococcus bovis* and *Butyrivibrio fibrisolvens* each at 2x10^7 cfu/g (Galaxis Frontier; Native Microbials Inc.); or Control. Cows were fed ad libitum in a single group with a system that controls access to feed mangers and measures individual feed intake from which dry matter intake (DMI) was calculated. Individual milk yield was recorded using electronic milk meters, and milk fat and protein were measured using optical in-line analyzers at each of 2 daily milkings. Energy-corrected milk (ECM) and FE (ECM/DMI) were calculated using these records. Treatment and treatment by time effects were assessed by multiple linear regression. Least squares means (LSM) comparisons were Bonferroni adjusted. Treatment and treatment by time effects were observed for all outcomes ($P < 0.05$). All milk and components yields were higher for Galaxis Frontier vs. Galaxis and Control (Table 1). Also, yields of ECM, fat and protein were higher for Galaxis than Control. Both treatments lead to higher FE compared with Control. In conclusion, supplementation of native rumen microbes effectively improved economically important outcomes such as milk yield, solids and FE with effects increasing over time.

Table 1. LSM and standard error of the means for the treatment effect

Item	Control	Galaxis	Galaxis Frontier
Yield (kg/d)	40.7 ± 0.2^a	41.2 ± 0.2^a	42.5 ± 0.2^b
ECM (kg/d)	40.4 ± 0.2^a	41.6 ± 0.2^b	43.1 ± 0.2^c
Fat (kg/d)	1.47 ± 0.01^a	1.51 ± 0.01^b	1.55 ± 0.007^c
Protein (kg/d)	1.18 ± 0.006^a	1.22 ± 0.006^b	1.25 ± 0.006^c
FE	1.52 ± 0.006^a	1.54 ± 0.005^b	1.55 ± 0.005^b

[a-c] Within row different superscripts indicate LSM difference at $P < 0.05$.

Key Words: microbiome, feed additive, rumen

366 Supplementation of chromium-L-Met benefits lactation performance, and antioxidative capacity in early-lactating dairy cows. Z. Z. Wu*[1], W. C. Peng[1], J. X. Liu[1], G. Z. Xu[2], and D. M. Wang[1], [1]*Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China,* [2]*Zinpro Sci. & Tech. Co. Ltd., Shanghai, China.*

The objective of this study was to investigate the effect of chromium methionine (Cr-Met) supplementation on lactation performance, hepatic respiratory rate and antioxidative capacity in early-lactating Holstein dairy cows. Sixty-four multiparous cows were equally grouped into 16 blocks based on parity, days in milk and milk production, and then were assigned randomly to one of 4 dietary treatments with 0, 4, 8 or 16 g/d of Cr-Met per cow supplemented to a basal TMR. The experiment lasted for 14 weeks, with the first 2 weeks as adaptation period. Milk yield and composition were recorded weekly. Dry matter intake was measured on an individual cow basis every other week. The plasma variables were measured in wk 4, 8 and 12 of the experiment. The data were analyzed using PROC MIXED of SAS, with covariance type AR (1) for repeated-measures analysis. Supplementation of Cr-Met did not affect dry matter intake of early-lactating dairy cows. With the increased level of supplemental Cr-Met, yields of milk, fat, lactose and energy-corrected milk increased in a linear manner ($P < 0.01$). Plasma

concentration of insulin decreased in a linear manner (*P* = 0.04), while plasma concentrations of pyruvate and nicotinamide adenine dinucleotide reflective of hepatic respitatory rate increased in linear (*P* < 0.01) and quadratic manners (*P* < 0.01), respectively, and lactic dehydrogenase activity increased linearly as the supplemental level of Cr-Met increased (*P* < 0.01). Moreover, plasma glutathione peroxidase and superoxide dismutase activity increased in a linear manner (*P* < 0.01). In summary, our study suggested that Cr-Met supplementation may improve lactation performance of early-lactating dairy cows through enhancing antioxidant capacity and hepatic cellular respiration.

Key Words: chromium-L-methionine, early-lactating cow, lactation performance

Ruminant Nutrition: Carbohydrates and Lipids III

367 Effects of Enogen corn silage and grain on ruminal fermentation and nutrient flows and bacterial protein synthesis in lactating cows.

L. R. Rebelo*[1], W. P. Weiss[1], M. Eastridge[2], and C. Lee[1], [1]*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Ruminal starch digestibility (RSD) affects microbial protein synthesis and production of lactating cows. Enogen corn for feed (Syngenta Seeds, LLC) expresses high α-amylase activity and we evaluated Enogen corn silage (CS) and grain (CG) on ruminal fermentation and nutrient digestibility and bacterial protein synthesis (BPS). A replicated 3 × 3 Latin square design was used with 6 ruminally cannulated cows. Treatments were isoline CS and isoline CG (CON), Enogen CS and isoline CG (ECS), and Enogen CS and Enogen CG (ECSCG). Isoline is a regular corn hybrid. The diets included 3.5% alfalfa silage, 50% CS and 18% CG (15.5% CP, 31% NDF, and 31% starch on a DM basis). Dry matter (30%), starch content (35%), and particle size distribution of the silages were similar. However, the mean particle size of Enogen CG was larger (1.05 vs. 0.65 mm) than isoline. Reticulum sampling was conducted to estimate nutrient flows and BPS using triple markers (Cr, Yb, and indigestible NDF) and ^{15}N-(NH4)2SO4. Data were analyzed using PROC MIXED (fixed effects: diet, time (repeated), their interaction, and period; random effects: cow and cow within square). Dry matter intake was not different among CON, ECS, and ECSCG (24.8, 25.7, and 25.9 kg/d, respectively). True ruminal digestibility of DM (64%), OM (71%), NDF (41%), and starch (82%) were not different. True digestibility of CP tended to be greater (85.2 vs. 78.4 and 74.9%; $P = 0.08$) for ECS compared with CON and ECSCG. Flows of bacterial DM and OM tended to be greater ($P < 0.10$) for ECS vs. CON and ECSCG. Efficiency of BPS tended to be greater (34.1 vs. 31.6 and 30.6 g N/kg OM truly digested; $P = 0.08$) for ECS vs. CON and ECSCG. Rumen pH and concentrations of acetate and propionate were not different. Concentration of NH_3 for ECS tended to be lower (10.4 vs. 13.4 mmol/L; $P = 0.07$) compared with CON. In conclusion, ECS did not affect RSD but likely altered starch digestion rates in favor of more efficient BPS. However, these effects were not observed for ECSCG, likely due to the larger particle size of Enogen CG vs. isoline.

Key Words: Enogen corn, bacterial protein synthesis, ruminal digestibility

368 Milk metabolome from dairy goats fed with sunflower and rapeseed oil supplements.

E. Vargas-Bello-Pérez*[1], J. Khushvakov[2], Y. Ye[2], N. C. Pedersen[1], H. H. Hansen[1], L. Ahrné[2], and B. Khakimov[2], [1]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark,* [2]*Department of Food Science, Faculty of Science, University of Copenhagen, Frederiksberg C, Denmark.*

The objective of this study was to determine the effect of 2 different sources of unsaturated fatty acids (rapeseed oil or sunflower oil) as a feeding supplement on milk metabolome profiles in dairy goats. Nine Danish Landrace goats at 42 ± 5 d in milk at the start of the study were allocated to 1 of 3 treatment groups (3 goats per group). All animals received a basal diet formulated with a 85:15 forage:concentrate ratio for 42 d. The basal diet was based on (% of dry matter) 21% lucerne + clover hay, 23% clover haylage, 42% lucerne + grass hay, and 14% straw. The control concentrate consisted (% of dry matter) of 93% of a grain mix (rolled barley, rolled oats, rolled wheat and peas), 6% of molasses and 1% of a premix of vitamins and minerals. Dietary treatments consisted of the basal diet with no lipid supplement (control), and the basal diet supplemented with either rapeseed oil or sunflower oil at 4% of dry matter. Milk samples were collected on d 14, 21 and 42, and subjected to metabolomics using untargeted GC-MS and ^{1}H NMR spectroscopy. ANOVA, principal component analysis, ANOVA-simultaneous component analysis, with permutation test, were employed to determine effects of oil supplementation on milk metabolome measured by GC-MS and ^{1}H NMR. All data analysis was performed in MATLAB (MathWorks Inc., Massachusetts, USA) using customized scripts written by the authors. A total of 69 metabolites were identified from the ^{1}H NMR spectra of all milk samples. Milk levels of ethanol, 2-oxoglutaric acid, and glucose-1-phosphate were higher ($P < 0.05$) in rapeseed oil supplemented group compared with control, while, oxaloacetic acid, taurine, tyrosine and tyrosine were higher ($P < 0.05$) with sunflower group compared with control. GC-MS analysis allowed level 2 identification of 97 milk metabolites and revealed that 2-hydroxyisovaleric acid was higher ($P < 0.05$) in milk from sunflower oil. Overall, lipid supplements had a modest effect on milk metabolome whereby sunflower oil increased metabolites related to amino acids and organic acids while rapeseed oil increased aliphatic alcohols and organic acids. Also, results showed that the unbiased ^{1}H NMR based approach identified more milk metabolite variations compared with GC-MS, which is concluded to be a more suitable approach to quantify volatile and semi-volatile metabolites.

Key Words: goats, metabolome, unsaturated fatty acids

369 Effect of whole cottonseed on production responses of lactating dairy cows: A meta-analysis.

J. M. dos Santos Neto*, A. M. Burch, M. Kloboves, and A. L. Lock, *Michigan State University, East Lansing, MI.*

Our objective was to perform a meta-analysis to evaluate the effects of feeding whole cottonseed supplemented diets (WCS) to non-cottonseed supplemented control diets (CON) on production responses of lactating dairy cows. The data set was formed from 19 peer-reviewed publications that at least contained a comparison between CON and WCS. CON diets (% DM) contained (mean ± SD) 36.8 ± 8.32 NDF, 17.7 ± 1.80 CP, and 4.62 ± 1.59 fat. WCS diets (% DM) diets contained 38.7 ± 8.34 NDF, 18.4 ± 2.09 CP, and 5.91 ± 1.21 fat. The inclusion rate of whole cottonseed in WCS diets ranged from 4.60 to 33.2% DM. The data set was comprised of 13 change-over and 6 continuous design publications. There were 112 production observations, 81 were from change-over and 31 from continuous designs. The meta-analysis was performed using PROC MIXED of SAS and included the fixed effects of treatment (CON and WCS), the random effects of study, and its interaction with treatment. Studies were weighted by the inverse of the squares of their standard errors. We did not test the effect of experimental design and its interaction with treatment because change-over and continuous designs had an unbalanced number of observations. Compared with CON, WCS tended to increase DMI (0.80 ± 0.49 kg/d [±SED]; $P = 0.10$), increased the yields of milk (1.13 ± 0.42 kg/d; $P = 0.01$) and milk protein (0.07 ± 0.01 kg/d; $P < 0.01$), and tended to reduce milk fat content (0.11 ± 0.06 percentage units; $P = 0.09$). There was no effect of treatment on milk fat yield ($P = 0.60$), milk protein content ($P = 0.53$), body weight ($P = 0.53$), body weight change ($P = 0.93$), or BCS ($P = 0.58$). Given the wide range in whole cottonseed inclusion in diets in the data set, meta-regression is required to evaluate possible quadratic relationships and determine dose-response effects of whole cottonseed on production responses of lactating dairy cows. Further analyses are also needed to evaluate the effects of whole cottonseed feeding on nutrient digestibility and milk fat composition. In conclusion, the inclusion of whole cottonseed in lactating dairy cow diets increased the yields of milk and milk protein and had no effect on milk fat yield and body weight.

Key Words: meta-analysis, milk production, oilseeds

370 Supplemental palmitic acid and chromium propionate impact production responses during the immediate postpartum period in multiparous dairy cows.

J. Parales-Giron*, J. M. dos Santos Neto, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of supplemental palmitic acid (C16:0) and chromium (Cr) on production responses of early-lactation cows. Thirty-six multiparous cows were used in a randomized complete block design and assigned to one of 4 treatments in a 2x2 factorial arrangement of treatments fed from 1 to 24 DIM. Treatments were: 1) a control diet containing no supplemental C16:0 or Cr (CON); 2) diet supplemented with an 85% C16:0-enriched supplement at 1.5% diet DM (PA); 3) diet supplemented with Cr-propionate at 0.45 ppm Cr/kg diet DM (CR); 4) diet supplemented with an 85% C16:0-enriched supplement and Cr-propionate (PA+CR). The diets contained (% DM) 22.5 forage NDF, 26.3 starch, and 17.3 CP. The C16:0-enriched supplement replaced soyhulls in CON. The statistical model included the random effect of block, cow within block and treatment, and Julian date, and the fixed effects of PA, CR, time, and their interactions. Significance was declared at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions. Results are presented in the following order: CON, PA, CR, and PA+CR. Overall, CR increased the yields of milk (45.1, 46.5, 49.3, 49.4 kg/d; $P < 0.05$) and milk protein (1.49, 1.51, 1.63, 1.58 kg/d; $P < 0.01$), and tended to increase milk fat content (4.48, 5.03, 4.48, 4.60%; $P = 0.07$). Overall, PA increased the yields of milk fat (1.98, 2.36, 2.16, 2.23 kg/d; $P < 0.01$), 3.5% FCM (51.2, 58.4, 55.6, 57.6 kg/d; $P < 0.01$), and ECM (50.8, 56.6, 55.2, 56.3 kg/d; $P < 0.05$), decreased milk protein content (3.43, 3.28, 3.44, 3.25%: $P < 0.01$) and tended to increase milk fat content ($P = 0.06$). We observed interactions between PA and CR for milk fat content (interaction $P = 0.08$) and milk fat yield (interaction $P = 0.05$) because the responses for milk fat content and yield were lower in PA+CR than they were in PA. In conclusion, feeding Cr-propionate during the immediate postpartum increased the yields of milk and milk protein, while feeding a C16:0-enriched supplement increased the yields of milk fat and ECM.

Key Words: chromium, early lactation, palmitic acid

371 Physical characterization of milk fat from dairy cows fed supplemental palmitic or stearic acid.

M. Kiser[1], R. Shepardson*[1,2], and K. Harvatine[1], [1]*The Pennsylvania State University, State College, PA,* [2]*Milk Specialties Global, Eden Prairie, MN.*

Fatty acid (FA) supplements including palmitic and stearic acid are commonly fed to lactating dairy cows and are known to cause changes in milk FA profile. Specific changes in the physical properties of milk fat caused by feeding palmitic and stearic are not well characterized in published literature. The purpose of this experiment was to compare effects of feeding palmitic versus stearic acid on the melting temperature of milk fat samples using direct scanning calorimetry (DSC). Twelve high-producing Holstein cows (mean pretrial milk yield = 53.4 ± 8.7 kg/d; mean ± SD) were arranged in a replicated 4x4 Latin square design with 21-d periods. Treatments were (1) Control (CON; no supplemental fat), (2) high palmitic supplement (HP; 91% C16:0), (3) an intermediate blend of palmitic and stearic acid (INT; 45% C16:0 and 49% C18:0), and (4) a high stearic supplement (SA; 93% C18:0) and were included at 1.95% of diet DM. Milk samples were collected the last 3 d of each period. Fatty acid profile of milk samples was analyzed by gas chromatography. Direct scanning calorimetry was used to obtain melting temperatures and enthalpies for 2 melting processes of each individual milk fat sample. Data from DSC was gathered on a Discovery DSC auto-sampler (TA Instruments). Briefly, thermal analysis was conducted using a 5 to 10 mg sample. After the DSC had cooled to −60°C, the temperature was increased 10°C/min to 60°C, and this process was repeated a second time to allow for even distribution of heat to the entire sample. Discovery DSC @ Lab software was utilized for data collection, and statistics were analyzed using JMP Pro 14 using the "Fit Model" procedure. During the second melting sequence, the melting temperature of milk fat from HP cows was 8.1°C (62.5%) and INT was 6.5°C (50.3%) higher than CON. Enthalpy values were not changed. Both supplements containing notable amounts of palmitic acid increased melting temperature compared with non-supplemented diets, but a high stearic supplement did not alter physical characteristics.

Key Words: milk fat, physical characterization, palmitic acid

372 Interaction of dietary fiber level and sodium acetate supplementation on milk production and total-tract digestibility.

C. Matamoros* and K. Harvatine, *The Pennsylvania State University, University Park, PA.*

The interaction between dietary factors and sodium acetate (NaAcet) supplementation is not known. The objective of this study was to characterize the response of milk production to NaAcet supplementation at 2 dietary fiber levels. Briefly, 12 multiparous Holstein cows were arranged in a 4 × 4 Latin square with 21-d periods. Treatments were a 2 × 2 factorial arrangement of dietary fiber level (32% and 28% NDF) and NaAcet supplementation. Acetate was fed to provide 10 mol/d of acetate mixed in the TMR. Milk yield and composition was observed on d 10 and the last 3 d of each period. Fecal samples were collected every 9 h during the last 3 d of each period and apparent total-tract digestibility was estimated using undigestible NDF as an internal marker. Time course data were analyzed in PROC MIXED of SAS 9.4 with repeated measures with a model that included the fixed effect of fiber, acetate, day, and their interactions, and the random effect of cow and period. Other data were analyzed in JMP PRO 15.0.0 with a similar reduced model. There was no effect of acetate on milk yield ($P = 0.36$). There was no interaction of acetate and fiber level on milk fat production. Acetate increased milk fat concentration and yield by approximately 0.3 percentage units and 167 g/d, respectively, ($P < 0.001$, for both) and decreasing NDF decreased milk fat percent but did not change milk fat yield ($P < 0.001$ and 0.44, respectively). Acetate also increased the concentration of milk fatty acids of mixed origin ($P < 0.001$) suggesting that the increase in milk fat synthesis is most likely due to an increase in mammary de novo lipogenesis. Acetate and the low NDF diets both increased DM digestibility ($P < 0.001$ and $P = 0.01$, respectively). In conclusion, NaAcet supplementation increased milk fat production regardless of dietary fiber level in a similar mechanism as previous reports.

Key Words: lipogenesis, milk fat, de novo

Ruminant Nutrition: Protein/Amino Acids II

373 Abomasal amino acid infusions in postpartum dairy cows. L. Bahloul[1], L. E. Hernández Castellano[2], H. Lapierre[3], C. Schwab[4], and M. Larsen*[2], [1]*Centre of Expertise and Research in Nutrition, Adisseo, Commentry, France,* [2]*Aarhus University, Foulum, Denmark,* [3]*Agriculture and Agri-Food Canada, Sherbrooke, Canada,* [4]*Schwab Consulting LLC, Boscobel, Wisconsin.*

Our objective was to investigate the effect of additional supply of all AA (TAA) or only essential AA (EAA) in early postpartum dairy cows on milk production and composition. Nine multiparous Holstein cows were used in a randomized block design with repeated measurements. At the day of calving, continuous abomasal infusion of TAA (n = 4) or only EAA (n = 5) was initiated. The TAA infusion was graduated with half of full dose at 1 d in milk (DIM), full dose (805 g/d) at 2 to 5 DIM, and followed by daily reductions until 0 g/d at 35 DIM. The TAA infusate was based on casein profile and EAA infusate had only the EAA portion of TAA. Cows received the same TMR diet (NE: 6.85 MJ/kg DM, MP: 102 g/kg DM, NorFor 2011) from calving to 50 DIM. Feed intake and milk yield were recorded daily; milk was sampled and analyzed on DIM 5, 15, 29 and 50. Data were analyzed using MIXED procedure of SAS for repeated data. The DMI did not differ between treatments ($P = 0.55$) but increased with DIM ($P < 0.01$). Milk yield was greater with TAA ($P < 0.01$; +8.6 ± 1.9 kg/d from 1 to 50 DIM), averaging 40.4, 50.0, 51.8 and 49.5 (SEM = 2.42) kg/d for TAA, and 31.3, 39.0, 44.3 and 42.6 (SEM = 2.18) kg/d for EAA, at 4, 15, 29, and 50 DIM, respectively. Milk protein yield was greater ($P = 0.01$; 1,635 vs. 1,393 g/d, SEM = 49.6) although milk protein content tended to be lower ($P = 0.06$; 41.1 vs. 47.0 g/kg from 1 to 50 DIM), with TAA compared with EAA. Milk fat content was lower with TAA compared with EAA ($P = 0.02$; −5.85 ± 2.01 g/kg from 1 to 50 DIM), but treatments did not affect milk fat yield. Although milk lactose content was not affected, milk lactose yield increased with TAA compared with EAA ($P = 0.02$; +422 ± 97.3 g/d from 1 to 50 DIM). Overall, ECM yield increased with TAA ($P = 0.02$; +5.97 ± 2.16 kg/d from 1 to 50 DIM). Results indicate that some of or all NEAA are as important as EAA in the early postpartum period and further research is needed to delineate their role during this critical period. Further, continued higher milk yield through 50 DIM with TAA 15 d after ceasing infusions indicate early postpartum AA supply influences subsequent lactational performance.

Key Words: amino acid, early lactation, cow

374 Effect of abomasal amino acid infusions on metabolism in postpartum dairy cows. L. Bahloul[1], L. E. Hernández Castellano[2], H. Lapierre[3], C. Schwab[4], and M. Larsen*[2], [1]*Centre of Expertise and Research in Nutrition, Adisseo, Commentry, France,* [2]*Aarhus University, Foulum, Denmark,* [3]*Agriculture and Agri-Food Canada, Sherbrooke, Canada,* [4]*Schwab Consulting LLC, Boscobel, WI.*

Our objective was to investigate the effect of continuous abomasal infusion of total AA (TAAi) or only essential AA (EAAi) in early postpartum dairy cows on metabolism. Nine multiparous Holstein cows were used in a randomized block design with repeated measurements at 5, 15, 29, and 50 d in milk (DIM). At the day of calving, TAAi (n = 4; casein profile) or only EAAi (n = 5; EAA portion of TAAi) was initiated. The TAAi was graduated with half of full dose at 1 d in milk (DIM), full dose (805 g/d) at 2 to 5 DIM, and followed by daily reductions until 0 g/d at 35 DIM. Cows received the same TMR (NE: 6.85 MJ/kg DM, MP: 102 g/kg DM). Feed intake and milk yield were recorded daily. Milk samples and 6 sets of tail and mammary venous plasma samples were obtained at sampling days. The coccygeal vessel was considered equivalent to an artery due to limited metabolism in the tail. Data were analyzed using the MIXED procedure of SAS for repeated data. The DMI did not differ between treatments ($P = 0.55$). Overall, with no treatment × DIM interaction (Trt × d), milk yield was greater with TAAi compared with EAAi ($P < 0.01$; 47.9 vs 39.3 kg/d, SEM = 1.4) as was milk protein yield ($P = 0.01$; 1,635 vs. 1,393 g/d, SEM = 50). Milk fat content was lower with TAAi compared with EAAi ($P = 0.02$; 41 vs. 47 g/kg, SEM = 1.5), but treatments did not affect milk fat yield ($P = 0.20$). The arterial total EAA concentration was lower with TAAi compared with EAAi at 5 (0.97 vs. 1.24 m*M*) and 15 DIM (0.97 vs 1.13 m*M*), but did not differ afterward (Trt × d, $P = 0.01$; SEM = 0.07). The arterial total non-EAA concentration was higher with TAAi compared with EAAi at 5 DIM (1.35 vs. 1.17m*M*), but did not differ afterward (Trt × d, $P < 0.01$; SEM = 0.04). Yet, plasma concentration differences across the udder of EAA and non-EAA did not differ between treatments ($P > 0.88$), indicating that the intramammary utilization of both EAA and non-EAA was changed. Arterial urea concentration was greater with TAAi compared with EAAi at 5 (2.87 vs. 1.75 m*M*) and 15 DIM (3.06 vs. 1.90 m*M*), but did not differ afterward (Trt × d, $P = 0.01$; SEM = 0.21) indicating catabolism of some of the AA supplied with TAAi in early but not later lactation. These results indicate that in early postpartum cows, EAAi could not be utilized for milk protein synthesis without non-EAA.

Key Words: amino acid, early lactation, cow

375 Effects of rumen-protected glutamate supplementation during the periparturient period on performance and metabolic responses of dairy cows. S. Hisadomi*[1], A. Haruno[2], T. Fujieda[2], T. Sugino[3], and M. Oba[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Ajinomoto Co. Inc., Tokyo, Japan,* [3]*Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan.*

The aim of the study was to evaluate the effects of supplementing Glu during the calving transition period in dairy cows. As Glu is a preferred metabolic fuel in the small intestine and a glucogenic amino acid in the liver, we hypothesized that supplementing Glu would increase milk yield and reduce serum β-hydroxybutyrate (BHB) concentration. Fifty-two multiparous Holstein cows (parity, 2.4 ± 1.3; body condition score (BCS), 3.20 ± 0.34; mean ± SD) were blocked by parity, BCS and expected calving date, and randomly assigned to one of the experimental diets with rumen-protected monosodium Glu prototype (RP-MSG; intestinally available Glu = 8.8%) or without (CON) at 3 weeks before expected calving date. The RP-MSG was fed at 4% and 3% of dietary dry matter, respectively before and after calving. Close-up diets contained 17.1% and 16.5% crude protein, 2.2% and 2.3% Met/metabolizable protein (MP), 6.6% and 6.7% Lys/MP, and 13.1% and 13.3% starch, and postpartum diets contained 18.8% and 18.3% crude protein, 2.2% and 2.2% Met/MP, 6.2% and 6.3% Lys/MP, and 22.5% and 22.7% starch on a DM basis, respectively for RP-MSG and CON treatments. Contrary to our hypothesis, cows fed Glu did not decrease serum BHB concentrations ($P = 0.56$) and did not increase milk yield ($P = 0.12$) during the first 3 weeks after calving. However, dry matter intake of cows fed Glu tended to be higher on d −1 (13.9 vs 12.7 kg/d; $P = 0.10$) and was higher on d 1 (15.7 vs 13.7 kg/d; $P = 0.03$) relative to calving. Cows fed Glu increased plasma concentrations of Glu (4.60

vs 3.89 µmol/dL; $P < 0.01$) and insulin-like growth factor-1 (44.2 vs 30.1 mg/mL; $P < 0.01$), and decreased serum concentrations of free fatty acids (670 vs 981 µEq/L; $P < 0.01$) and total bilirubin (0.22 vs 0.34 mg/dL; $P < 0.01$), and plasma 3-methylhistidine concentration (1.28 vs 1.50 µmol/dL; $P = 0.03$) on d4 after calving. These results suggest that Glu supplementation may neither decrease ketosis nor increase milk production, but it may increase feed intake and decrease mobilization of body fat and protein immediately after calving.

Key Words: glutamate, calving transition, dry matter intake

376 Determining bioavailability of rumen-protected lysine and its utilization and excretion after absorption by lactating cows.

L. R. Rebelo*, W. P. Weiss, and C. Lee, *Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH.*

A method was developed to estimate bioavailability of rumen-protected Lys (RPLys) and its utilization and excretion after intestinal absorption using a stable isotope technique. Four ruminally cannulated cows (average ± SD; 176 ± 16 DIM and 33 ± 2 kg/d MY) were used in a 4 × 4 Latin square (17-d periods). Cows fed a typical diet received continuous infusion of Lys into the abomasum at 0, 25, or 50 g/d during the last 5 d or 105 g/d (42 g/d Lys) of a commercial RPLys for 17 d in each period (L0, L25, or L50, or RP, respectively). 15N-Lys (epsilon) was infused into the abomasum of all cows for the last 5 d during which total collection of milk, urine, and feces were conducted. 15N enrichment data indicated that steady state occurred on d 4 for all samples. To estimate digestible Lys (dLys), the amount of 15N absorbed (infused – fecal excretion) and 15N enrichment of milk Lys (representing 15N enrichment of body labile Lys pool) at steady state were used (15N-Lys absorbed divided by 15N enrichment of milk Lys). 15N transferred from absorption into milk and urine was used to calculate Lys utilization and excretion after absorption. Additional Lys supply from RPLys for RP was calculated by the difference in dLys between L0 and RP. Data were analyzed using PROC MIXED of SAS (cow as random and treatment as fixed effects). Digestible Lys for L0 was estimated as 110 g which is comparable to the estimate (120 g/d) by NRC (2001). Increasing Lys supply from L25 to L50 decreased ($P = 0.04$) 15N enrichment of milk Lys and increased ($P = 0.05$) dLys supply by 20 g which was close to the expected (24 g), indicating successful estimation of dLys. For cows on RP, 136 g/d of dLys was estimated with 27 g of dLys from RPLys and estimated bioavailability of RPLys was 66.5%. This is close to the bioavailability value (64%) provided by the manufacturer. Furthermore, 47% of bioavailable Lys from RPLys was secreted in milk and 19% of that was excreted in urine. In conclusion, the current method successfully estimated bioavailability of RPLys and provided further information about the fate of bioavailable Lys.

Key Words: rumen-protected lysine, bioavailability, Lys utilization

377 Dietary fatty acid and starch content and supplemental Lys supply affect energy and nitrogen utilization in lactating Jersey cows.

D. L. Morris*[1,2] and P. J. Kononoff[1], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*Perdue AgriBusiness, Salisbury, MD.*

The effects of dietary fatty acid (FA) and starch content as well as supplemental digestible Lys (sdLys) on energy and N utilization were evaluated using a central composite design. Each factor was feed at 5 levels. Factor limits were as follows: 3.0 to 6.2% for FA (57% C16:0 and 21% C18:0); 20.2 to 31.3% for starch, and 0 to 17.8 g/d of sdLys from rumen-protected Lys. Fifteen unique treatments were fed to 25 Jersey cows across 3 blocks with 4 28-d periods each. Total collection of feces and urine and headbox-style indirect calorimetry were used to determine N utilization, and energy utilization. The statistical model included linear and quadratic effects, all 2-way interactions and the random effect of cow, period, and block. All significant responses ($P < 0.05$) are reported. Increasing dietary FA decreased intake and milk protein yield. A starch by sdLys interaction occurred for milk true protein concentration. When dietary starch was less than 24%, milk protein concentration increased with increasing sdLys, but when dietary starch was greater than 26% milk protein concentration decreased with increasing sdLys. Digestibility of FA increased when dietary FA increased from 3.0 to 4.2% and decreased as FA increased beyond 4.2%. AAs dietary FA increased, metabolizable energy (ME) content quadratically increased, and ME supply increased as dietary FA increased from 3.0 to 4.2% and decrease as FA increased beyond 4.2%. Increasing dietary FA and starch increased conversion of DE into ME due to decreased CH_4 and urinary energy. Increasing dietary starch increased the efficiency of utilizing dietary N for milk N. Increasing sdLys quadratically decreased N balance as sdLys increased from 0 to 8 g/d and increased N balance as sdLys increased from 8 to 18 g/d. Increasing dietary FA can increase ME content, however, at high dietary FA, decreased intake and nutrient digestibility resulted in a plateau in ME content and a decrease in ME supply. Increasing sdLys supply is important for milk protein in cows fed low dietary starch.

Key Words: indirect calorimetry, energy metabolism, net energy system

378 A field application model for optimizing postruminal amino acid supply to lactating dairy cows.

J. M. Prestegaard*, L. M. Campos, A. C. Hruby, and M. D. Hanigan, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Updated nutrient requirement models for lactating dairy cows may be used in conjunction with optimizer functions to provide greater return to dairy producers while maximizing nitrogen (N) efficiency. The objective of this study was to leverage existing knowledge of postabsorptive AA metabolism through application of a mathematical ration-balancing model to predict N efficiency through more accurate postabsorptive amino acid (AA) delivery. After a 3 wk adjustment period, lactating Holstein cows (n = 48) were fed 1x/d in Calan gates for 12 wk. Three treatments were developed to test proof of concept of the requirement models and optimizer (n = 16 cows/treatment). A control ration was balanced to fulfill all nutrient needs of lactating dairy cows producing 45 kg milk/d using the NRC (2001) dairy model (NRC). Two rations were developed using an updated copy of the NRC model and a nonlinear optimizer (NlcOptim library) in RStudio. Both diets were balanced to fulfill requirements according to the revised NRC model (NRC, 2021) to either: 1) maximize N efficiency through tailored post-ruminal AA supply (NEFF), or 2) maximize income over feed cost (IOFC). A forage base of corn silage, triticale silage and legume hay was held constant across treatments. Cows were milked 2x/d and milk volume and milk composition were recorded. Nutrient and DM intake, milk yield, milk composition, feed efficiency (FE = kg milk/kg DMI) and N efficiency (kg milk protein/kg CP consumed) were analyzed as repeated measures (days) in a mixed model in RStudio to test for differences in treatment means. Pairwise differences are based upon least squares means and significance was declared at $P < 0.05$. No differences were observed between treatments for milk yield (kg/d; $P = 0.30$), milk components, or FE ($P < 0.99$). Cows consuming NEFF had lesser DMI (kg/d; 21.7) than both NRC (24.6) and IOFC (24.7; $P < 0.01$). Nitrogen efficiency (%) was greatest for NEFF (32.8; $P < 0.01$); no pairwise difference for N efficiency was observed between NRC (26.1) and IOFC (22.3). Reducing N excretion of dairy cows will mitigate environmental N waste of dairy farms. Rations that more precisely balance postruminal AA supply provide an opportunity to increase efficiency of dietary protein use without compromising FE in lactating dairy cows.

Key Words: nitrogen efficiency, amino acids, nutrient requirements

379 Feeding rumen-protected lysine alters transcriptome indicators of liver composition in Holstein cows during the transition period.

L. K. Fehlberg*[1], A. R. Guadagnin[1], B. L. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*Ajinomoto Co Inc, Tokyo, Japan.*

This experiment was conducted to determine the effects of feeding rumen-protected lysine (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) from 27 ± 5 d prepartum (0.54%DM of TMR) to 28 d postpartum (0.39%DM of TMR) on liver composition. Seventy-five multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and body condition score during the far-off dry period were assigned to 1 of 4 dietary treatments in a randomized, complete block design with a 2 × 2 factorial arrangement. Treatments consisted of TMR top-dressed prepartum with RPL (PRE-L) or without (PRE-C) and with RPL prepartum and postpartum (PRE-L POST-L), with RPL prepartum and without RPL postpartum (PRE-L POST-C), without RPL prepartum and with RPL postpartum (PRE-C POST-L), and without RPL prepartum and postpartum (PRE-C POST-C). Liver samples were collected via biopsy on −14 ± 0.8 and 14 ± 0.10 d relative to calving and analyzed for liver composition, mRNA gene expression, and protein abundance. Statistical analysis was performed using MIXED procedure of SAS. Triacylglyceride and carnitine concentrations did not differ among treatments ($P > 0.10$). Expression of carnitine palmitoyltransferase 1 (*CPT1A*) was greater ($P = 0.05$) for cows in PRE-C POST-L compared with others. Cows in PRE-L had lesser ($P = 0.02$) expression of gamma butyrobetaine hydroxylase 1 (*BBOX1*) the terminal enzyme in carnitine synthesis, and trimethyllysine dioxygenase (*TMLHE*) postpartum, compared with cows in PRE-C. Cows in PRE-L POST-L had decreased ($P = 0.05$) expression of carnitine transporter (*SLC22A5*) compared with cows in PRE-C POST-L. There was a tendency for greater protein abundance of SLC7A7 and lesser BBOX1 prepartum and postpartum in cows that consumed RPL prepartum compared with those that did not ($P \leq 0.10$). In conclusion, liver carnitine concentrations did not differ but upregulation of carnitine transporters for cows consuming RPL postpartum but not prepartum could indicate improved carnitine utilization. The association of carnitine and lysine transporters still needs to be explored.

Key Words: carnitine, lysine, liver composition

380 Production and temporal plasma metabolite effects of soybean meal versus canola meal fed to dairy cows during the transition period and early lactation.

J. Kuehnl*[1] and K. Kalscheur[2], [1]*University of Wisconsin–Madison, Department of Animal and Dairy Science, Madison, WI,* [2]*US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Replacement of soybean meal (SBM) with canola meal (CM) in diets fed to early-lactation dairy cows increases milk production. Our objective was to determine production and temporal plasma metabolite effects of dairy cows fed isonitrogenous diets formulated with SBM or CM during the transition period and early lactation. Multiparous Holstein cows (n = 79), blocked by calving date, were enrolled in a 2 × 2 factorial arrangement of treatments in a randomized complete block design. Cows were fed diets with SBM or CM as the main protein source from wk −3 to +16 relative to calving. Half of the cows consumed each diet pre- (PRE) and postpartum (POST), with half of each PRE group switching protein source at calving. Canola meal was included at 19.4% and 13.5% and SBM was included at 14.2% and 9.9% (% DM) for the PRE and POST diets, respectively. Milk production, components, dry matter intake (DMI), and body weight (BW) were determined weekly. Plasma was collected twice weekly from wk −3 to +8, composited by cow, and evaluated using ELISA. Data were analyzed using the MIXED procedure of SAS. Cows fed CM compared with SBM increased DMI both PRE and POST (15.3 vs. 14.5 ± 0.3 kg/d; 26.2 vs. 25.0 ± 0.4 kg/d; $P < 0.05$). Milk production tended to increase for cows fed POST CM compared with POST SBM (52.8 vs. 50.9 ± 0.7 kg/d; $P = 0.09$). The PRE and wk interaction was significant for ECM ($P < 0.05$) and FCM ($P = 0.05$). Cows fed POST CM decreased in MUN compared with POST SBM (12.9 vs. 13.7 ± 0.2 mg/dL; $P < 0.05$). Diet did not affect BW, BW change, feed efficiency, components, plasma insulin, insulin-like growth factor 1, or glucose ($P > 0.10$). Plasma triiodothyronine decreased both PRE and POST for cows fed CM compared with SBM (1.38 vs. 1.54 ± 0.03 ng/mL; 1.14 vs. 1.35 ± 0.03 ng/mL; $P < 0.01$). Plasma growth hormone decreased for cows fed PRE CM compared with PRE SBM (5,050 vs. 6,230 ± 382 pg/mL; $P < 0.05$). Altogether, our data demonstrates that the early-lactation dairy cow fed a diet formulated with CM, in substitution of SBM, increased production and DMI and altered physiological status.

Key Words: canola meal, transition period, early lactation

381 Dietary supplementation with *N*-carbamoylglutamate initiated during the prepartum stage improves the lactation performance of postpartum dairy cows.

F. F. Gu*, M. C. Miao, L. Y. Jiang, D. M. Wang, H. Y. Liu, and J. X. Liu, *Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China.*

The objective of this study was to investigate the effects of supplementing *N*-carbamoylglutamate (NCG, an arginine precursor) on AA utilization and productive performance of the early-lactating dairy cows. Thirty multiparous Holstein dairy cows were randomly divided into a CON (control, n=15) and NCG (control plus 20 g NCG/d, n=15) groups at wk 4 before calving. The experiment lasted until wk 10 postpartum. Milk production was recorded and milk samples were collected weekly; and dry matter intake (DMI) was recorded for 2 consecutive days every other week after calving. Blood samples were collected from the coccygeal vein and deposited into 10-mL tubes containing an anticoagulant (heparin lithium) 3 h after the morning feeding at wk −3, −1, 0 (the day of calving), 1, 3, 7, and 10 relative to calving, and blood samples were also taken from the coccygeal artery and superficial epigastric vein at wk 3 and 10 postpartum. The data were analyzed using SAS software with covariance type AR (1) for repeated measures. Compared with the CON cows, DMI tended to be higher ($P = 0.06$), and yields of milk ($P < 0.01$), protein ($P < 0.01$), and fat ($P < 0.01$) were higher in the NCG-cows. The activity of aspartate aminotransferase ($P < 0.01$) and alanine aminotransferase ($P = 0.03$), and level of β-hydroxybutyrate ($P = 0.04$) were lower in NCG-cows than in CON cows, whereas plasma glucose ($P = 0.05$) and nitric oxide (NO, $P < 0.01$) were higher. No significant differences were observed in the coccygeal vein concentrations of Thr and His between 2 groups, but other essential AA ($P < 0.01$), Cys ($P < 0.01$), Pro ($P < 0.01$), Tyr ($P < 0.01$), total essential AA ($P < 0.01$), and total AA ($P < 0.01$) were higher in the NCG than in the control. The arterial supply of all AA was greater in cows with NCG. The NCG-cows had higher mammary plasma flow ($P = 0.04$) and clearance rate in Cys ($P < 0.01$), Pro ($P < 0.01$) and Asp ($P < 0.01$), and higher ratio of uptake to output in Met ($P = 0.01$), Lys ($P < 0.01$), Cys ($P = 0.01$), Pro ($P = 0.03$), and Asp ($P = 0.01$). In summary, addition of NCG initiated during the prepartum period improved the lactation performance of postpartum cows, which may be attributed to increased supply of Arg and NO, improved AA utilization, and enhanced DMI in these cows.

Key Words: *N*-carbamoylglutamate, lactation performance, transition cow

382 Effect of source and frequency of metabolizable protein supplementation on milk production and nitrogen efficiency.

K. Nichols*[1], C. Cirot[2], M. Rolland[2], and J. Dijkstra[1], [1]*Animal Nutrition Group, Wageningen University and Research, Wageningen, the Netherlands,* [2]*Ajinomoto Animal Nutrition Europe, Paris, France.*

Replacing soybean and rapeseed meals with tailored amino acid profiles can improve N efficiency of dairy cattle. Further, the frequency of protein supplementation may influence N recycling in favor of reduced N excretion. We investigated the effect of source and frequency of metabolizable protein (MP) supplementation on milk production and N efficiency. Twenty-eight Holstein-Friesian cows (93 ± 27 DIM) were blocked (4 cows/block) by DIM and parity and fed a basal ration (15.5% CP; formulated to 95 and 100% of MP and NE_L requirements) in tie stalls for 16 d at a fixed amount according to the mean ad libitum intake of each block during a 7-d diet adaptation period. Cows within block were randomly assigned to the basal ration, or 1 of 3 iso-MP rumen-protected (RP) supplements mixed into the basal ration. Supplements were 1) a 384-g mixture of RP His (Ajinomoto Co, Japan), Lys (AjiPro-L; Ajinomoto Health & Nutrition, USA), and Met (Smartamine; Adisseo, France) fed daily (RPAA), 2) a 768-g mixture of RP His, Lys, and Met fed every other day (Osc-RPAA), and 3) a 315-g mixture of RP soybean meal and RP rapeseed meal (MervoBest; Agrifirm, the Netherlands) fed daily (RPSR). The basal ration + supplements were designed to deliver 100% of required MP per 48-h period. The RPAA mixture was formulated to deliver AA in amounts relative to a casein profile. Dry matter intake, milk yield, and milk composition averaged over the final 4 d of each period was subjected to ANOVA with treatment as a fixed effect and block as a random effect. Dry matter intake and milk yield were not affected by treatment ($P > 0.10$). Milk protein content increased with RPAA over all treatments ($P \leq 0.01$). Milk fat content decreased with RPSR compared with RPAA and Osc-RPAA ($P \leq 0.05$), and milk fat yield decreased with RPSR compared with Osc-RPAA ($P = 0.04$). Milk nitrogen efficiency was numerically higher with RPAA compared with RPSR (33.6 vs. 32.4%). Findings suggest that supplementing RP His, Lys, and Met in a casein profile can increase the transfer of dietary N into milk N, and that there is no benefit of supplementing RP amino acids in an oscillating pattern.

Key Words: amino acid, oscillation

383 Comparing rumen-degradable protein source (urea versus peptides) and supplementation level in continuous culture.

B. A. Wenner*[1], X. Huang[2], P. S. Yoder[3], and N. R. St-Pierre[3], [1]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [2]*Cumberland Valley Analytical Services, Waynesboro, PA,* [3]*Perdue AgriBusiness, Salisbury, MD.*

Dairy nutrition models credit urea and peptides equally as rumen-degradable protein (RDP) sources for cellulolytic bacteria. Dual-flow continuous culture (DFCC) fermentation affords investigation into this relationship without interaction of the cow. The present study investigated the effects of both increasing RDP and shifting source from urea to peptides on the fermentative activity within DFCC fermenters. Our hypothesis was that increasing RDP supply would stimulate microbial fermentation and growth and supplementation of peptides would further stimulate cellulolytic activity within the microbial population compared with urea. DFCC fermenters (n = 8) were randomized to 1 of 8 treatments for 5 periods: negative control (CON, 94 g DM/d, 50:50 orchardgrass:concentrate pellets, 36% NDF, 25% starch, 11.1% CP), CON with supplemental urea (UL, UM, and UH at +1.5%, +3.0%, and +4.5% CP), CON with supplemental peptides (PL, PM, and PH at +1.5%, +3.1%, and +4.6% CP), and positive control (SOY, +8.4% CP from soy protein powder). Experimental periods lasted 11 d with 7 d of adaptation and 4 d of sampling. Total dilution was 7%/h, solids dilution 5%/h, and urea was excluded from the buffer. The statistical model included the fixed effect of treatment, and random effects of fermentor and period; contrasting the effect of CP source, level, and source × level. NH_3-N concentration scaled linearly with increasing CP level, regardless of source ($P < 0.001$) while urea increased NH_3-N (+61%) more than peptides ($P < 0.001$). While NDF digestibility was not affected by CP level ($P = 0.11$) nor source ($P = 0.93$), ADF digestibility was increased by CP level ($P = 0.05$) from 43.5% to 47.9% in low to high CP level, respectively. Propionate, isobutyrate (IsoBut), isovalerate (IsoVal), and valerate (Val) production were all increased ($P = 0.05$) by peptides compared with urea. IsoBut, IsoVal, and Val production were all increased by increasing CP regardless of source ($P = 0.02$). The increases in branched-chain VFA yield indicate a potential cellulolytic bacterial response to peptide supplementation versus urea despite a lack of quantifiable improvements in fiber digestibility, which may translate to improved microbial energy efficiency. Microbial growth and relative sequence abundance will be investigated to further describe the effect of peptide versus urea provision as RDP sources.

Key Words: continuous culture, peptides, rumen-degradable protein

384 Performance of dairy cows fed 2 levels of digestible histidine and metabolizable protein.

S. E. Räisänen*[1], C. F. A. Lage[1,2], C. Zhou[1,3], A. Melgar[1,4], T. Silvestre[1], D. E. Wasson[1], S. F. Cueva[1], T. Takagi[5], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of California, Davis, Davis, CA,* [3]*University of Chinese Academy of Sciences, Beijing, P. R. China,* [4]*Instituto de Innovation Agropecuaria de Panama, City of Knowledge, Panama,* [5]*Ajinomoto Nutrition North America, Chicago, IL.*

The objective of this experiment was to investigate the effect of dietary levels of digestible (d)His and metabolizable protein (MP) on lactational performance of dairy cows. A randomized block design experiment was conducted with 48 Holstein cows, including 20 primiparous, averaging (±SD) 103 ± 22 d in milk and 51 ± 11 kg/d milk yield (MY) at the beginning of the experiment. A 2-wk covariate period preceded 12 experimental wks, of which 10 wks were for data and sample collection. Experimental treatments were (as estimated based on NRC, 2001): (1) MP-adequate diet (MPA) with 2.1% dHis of MP (MPALH), (2) MPA with 3.0% dHis (MPAHH), (3) MP-deficient diet (MPD) with 2.1% dHis (MPDLH), and (4) MPD with 3.0% dHis (MPDHH). Actual dHis supply was estimated at 65, 96, 56 and 86 g/d, respectively. Diets supplied MP at 110% (MPA) and 95% (MPD) of NRC (2001) requirements calculated based on production data during the experiment. All data were analyzed using PROC MIXED of SAS as repeated measures with dHis and MP levels, wk and the dHis × MP and dHis × wk interactions in the model. The overall DMI was not affected by dHis or MP (averaging 27.3 kg/d, SEM = 0.87), but HH had 1.7 kg/d greater ($P = 0.003$) DMI on experimental wk 7 compared with LH. Milk yield tended to be increased ($P = 0.10$) by HH compared with LH (41.9 vs. 39.7 kg/d, respectively). Milk true protein concentration and yield were not affected ($P \geq 0.42$) by treatment, while MUN concentration was lower ($P < 0.001$) for MPD vs. MPA. Milk fat concentration and yield were lower ($P \leq 0.05$) and energy-corrected milk yield (ECM) tended to be lower ($P = 0.08$) for MPD vs. MPA. There was a MP × dHis interaction ($P \leq 0.01$) for milk fat yield and ECM: milk fat was lower for MPDHH vs. MPDLH, but not different between MPALH and MPAHH, whereas ECM was greater for MPAHH vs. MPALH but tended to be lower for MPDHH vs. MPDLH. Body weight and BW change were similar ($P \geq 0.20$) across treatments. Overall, cows fed MPD had similar MY and milk protein yield to cows fed the MPA diet. Greater dHis supply increased MY but had minor or no effects on other production variables in dairy cows.

Key Words: histidine, metabolizable protein, milk production

385 Dietary protein oscillation: Effects on production, digestibility and nitrogen metabolism in lactating dairy cows. R. Rauch*[1,2], J. Martín-Tereso[1], J.-B. Daniel[1], K. Nichols[1], and J. Dijkstra[2], [1]*Trouw Nutrition R&D, Amersfoort, the Netherlands,* [2]*Animal Nutrition Group, Wageningen University and Research, Wageningen, the Netherlands.*

Limited research shows that oscillating (OS) dietary crude protein (CP) content may improve nitrogen (N) efficiency in growing ruminants. We hypothesized that milk N efficiency in dairy cows would be increased by feeding a total mixed ration delivering CP according to an OS (48 h phases of 13.4% and 17.1% CP, respectively) as compared with a static (ST; 15.3% CP) pattern. We evaluated nutrient intake, milk production, and N metabolism using total urine and fecal collection in 12 multiparous cows (172 ± 39 d in milk) during d 9–16 of a 16 d study. The experiment was a randomized complete block design with preceding covariate period during which cows were blocked by parity, days in milk and milk protein yield, and randomly assigned to ST or OS. The statistical model used fixed effects of treatment, period (repeated; 2 periods of 4 d each) and the associated covariate value. Block was included as random effect. Dry matter intake, N intake, milk composition and yield of milk and components did not differ between OS and ST. Feed and milk N efficiency, and changes in body weight and body condition score were not affected by OS. Apparent total-tract digestibility (ATTD) of dry matter (70.2 vs 69.1%), organic matter (71.9 vs 71.0%) and gross energy (69.8 vs 68.7%) increased for OS vs ST. Crude fat and starch ATTD tended to increase in response to OS, but CP ATTD was similar for OS and ST (63.6 vs 62.8%). Daily excretion of uric acid tended to increase with OS, but daily allantoin and total purine derivative excretion, predicted microbial N flow, and estimated efficiency of microbial protein synthesis were not affected by treatment. Nitrogen balance was unaffected, but urinary N excretion increased (124 vs. 109 g/d), urinary urea excretion tended to increase (2.4 vs. 1.9 mol/d), and milk N as proportion digested N tended to decrease (46.7 vs. 51.2%) with OS, indicating increased post-absorptive N losses in OS cows. Overall, performance and efficiency of total feed nutrient use for milk synthesis was unaffected, but OS increased ATTD and reduced efficiency of post-absorptive N use for milk synthesis.

Key Words: nitrogen efficiency, post-absorptive metabolism

386 Comparison of hemp meal and canola meal as protein supplements for non-lactating dairy cows. F. Addo*, K. Ominski, C. Yang, and J. C. Plaizier, *University of Manitoba, Winnipeg, MB, Canada.*

Hemp meal (HM) may be a suitable protein supplement for dairy cows, but its use as a ruminant feed has not yet been approved in Canada. To obtain information regarding the potential to utilize hemp meal as a cattle feed, dry matter intake (DMI), rumen fermentation, blood metabolites, and total-tract digestibility were compared between nonlactating dairy cows fed HM and those fed canola meal (CM). Six nonlactating, nonpregnant Holstein cows were used in a 3 × 3 Latin square design feed trail with 3, 3-week experimental periods. The first 2 weeks served as an adaptation period, and sample collection occurred during the third week of each period. Cows were fed a basal partial mixed ration (PMR) diet that included either 10.2% DM HM, 13.5% DM CM or a combination of 6.25% DM HM and 6.16% DM CM (HC). All diets were isoenergetic and isonitrogenous. Total-tract digestibility of dry matter (DM) and neutral detergent fiber (NDF) were determined using acid insoluble ash as an internal marker. Data were analyzed using the PROC MIXED procedure of SAS 9.4 with treatment, time, week and their interaction declared as fixed effects and cow and period declared as random effects. Treatment did not affect DMI, pH, VFA or ammonia concentrations in the rumen, total-tract DM or crude protein digestibility, nor concentration of glucose, urea, β-hydroxybutyrate (BHB) and total fatty acids in blood serum. However, the total-tract NDF digestibility of the CM diet (43%) was significantly higher ($P < 0.05$) than that of HM (38%). Our data show that hemp meal may be a good alternative for canola meal as a protein supplement for nonlactating dairy cows.

Table 1. DMI, pH, rumen VFA and ammonia nitrogen concentrations, serum BHB, total fatty acids, glucose and urea concentrations, and total-tract DM, and NDF digestibility of experimental diets

Parameter	CM	HC	HM	SEM	*P*-value
DMI, kg/d	14.9	14.5	14.8	0.97	0.56
Acetate, mmol/L	71.0	73.3	69.5	1.93	0.41
Propionate, mmol/L	21.4	22.9	21.3	1.09	0.29
Butyrate, mmol/L	13.6	13.7	12.7	0.54	0.27
NH_3N, mg/dL	8.48	10.4	9.24	0.99	0.15
Urea, mmol/L	4.71	4.90	4.86	0.24	0.84
Glucose, mmol/L	4.38	4.47	4.43	0.16	0.74
BHB, mmol/L	0.39	0.42	0.38	0.02	0.25
Total fatty acids, mmol/L	0.07	0.07	0.07	0.02	0.97
DM digestibility, %	55.0	54.0	54.0	0.01	0.28
NDF digestibility, %	43.0	39.0	38.0	0.01	0.03

Key Words: hemp, canola, protein supplementation

387 Association between plasma essential amino acid profile, cow performance, and plasma urea nitrogen in lactating dairy cows revealed through meta-analysis and hierarchical clustering. P. Letelier*[1], G. I. Zanton[2], J. R. R. Dorea[1], and M. A. Wattiaux[1], [1]*Department of Dairy Science, University of Wisconsin–Madison, Madison, WI,* [2]*USDA-ARS, U.S. Dairy Forage Research Center, Madison, WI.*

We hypothesized that plasma essential AA (EAA) profile reflect (im) balances of EAA supply to the mammary gland and cow performance. Thus, we studied the association between plasma EAA profile, cow performance, feed efficiency (FE) and plasma urea-N (PUN). The data set included 22 feeding trials and 96 dietary treatments. Multivariate regression analysis was conducted on EAA plasma concentration (p[EAA]) in response to the fixed effect covariates of NRC predicted metabolizable protein (MP) supply, days in milk (DIM), body weight (BW), and random effect of study, and weighted by the square root of number of observations per treatment. The residuals of the multivariate model were clustered using hierarchical method resulting in 2 clusters. The plasma EAA profiles were determined for the 2 clusters as the proportion of individual p[EAA] relative to the total p[EAA]. A fixed effect model specifying heterogeneity in the covariance structure was used to evaluate the association between cluster for plasma EAA profile and, cow performance, FE, and PUN. Significance was declared at $P < 0.05$ and tendency at $0.05 < P < 0.10$. Individual and total p[EAA] were lower in cluster 1 compared with cluster 2. Cluster 1 had lower molar proportions of Arg, Leu, Lys, greater proportions of Met, Ile and Thr, and no differences for His, Phe and Val compared with cluster 2. Compared with cluster 2, cluster 1 had greater milk energy (26.7 vs 22.8 Mcal/d, SE = 0.47), fat yield (1.35 vs 1.16 kg/d, SE = 0.03), milk Mcal:dry matter intake (1.09 vs 1.01, SE = 0.02), lower PUN (11.8 vs 14.8 mg/dL, SE = 0.59), and tended to have greater true protein yield (1.11 vs 1.06 kg/d, SE = 0.02). Due to the use of residual p[EAA] in the multivariate model, no difference in MP supply, energy supply, BW and DIM was found between clusters. The association between cluster 1 and cow performance may suggest greater utilization of EAA for milk protein and less AA catabolism in part due to a balanced profile of EAA reaching the mammary gland compared with cluster 2.

Key Words: plasma amino acid profile, milk protein, plasma urea nitrogen

388 A network meta-analysis of the impact of feed-grade urea and slow-release urea on lactating dairy cattle metabolism and production.

M. Simoni*[1], G. Fernandez-Turren[2], F. Righi[1], M. Rodriguez-Prado[3], and S. Calsamiglia[3], [1]*Department of Veterinary Science, University of Parma, Parma, Italy,* [2]*Departamento de Producción Animal y Salud de los Sistemas Productivos, Instituto de Producción Animal, Facultad de Veterinaria, Universidad de la República, San José, Uruguay,* [3]*Animal Nutrition and Welfare Service (SNiBA), Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Spain.*

A network meta-analysis was conducted to determine the effects of feeding feed-grade urea (FGU) or slow-release urea (SRU) as replacement for plant protein (PP) in high-producing dairy cattle diets. Research papers were selected (n = 16) from experiments published between 2006 and 2019 based on the following criteria: dairy breed, detailed description of the isonitrogenous diets fed, provision of FGU or SRU, in vivo trials involving high yielding cows and results that included at least milk yield and composition; data on nutrient intake, digestibility, ruminal fermentation profile, and N utilization were also considered. Most studies compared 2 treatments only, and a network meta-analysis approach was adopted to compare the effects among PP, FGU and SRU. Data were analyzed through a generalized linear mixed model network meta-analysis with SAS/STAT (SAS Institute Inc., Cary, NC, USA), using treatment as a fixed effect and the study as a random effect. Differences were declared at $P < 0.05$. Average diet contained (DM basis) 1.64 ± 0.09 Mcal NE_L, 16.8 ± 1.05% CP, 31.9 ± 3.85% NDF, and 22.1 ± 3.76% starch. The average supply of urea was 167 g/cow/d. The FGU and SRU did not affect nutrient intake and N utilization. The SRU reduced total-tract apparent digestibility of DM (69.6 vs 69.7%, respectively) and increased the proportion of acetate (62.3 vs 62.2 mol/100mol, respectively) in the rumen compared with FGU; and increased ruminal ammonia concentration compared with PP (7.53 vs 6.63 mg/dL, respectively). However, all these differences were numerically small. The DMI (23.1 ± 3.00 kg), milk yield (34.4 ± 6.0 L/d), and milk fat (3.59 ± 4.3%) and protein (3.10 ± 2.2%) content were not affected by treatments. However, cows supplemented with FGU and SRU had lower lactose concentration compared with PP (average of 47.1 vs 47.4 g/kg, respectively). Despite the frequent use of urea in dairy diets, only a limited number of papers met the criteria adopted in the present meta-analysis. Neither the use of FGU or SRU modified milk yield nor composition. Thus, feeding FGU to high-producing dairy cows can be justified by its lower cost in comparison to SRU and PP.

Key Words: feed-grade urea, milk production, meta-analysis

389 Effects of dietary protein on microbial protein flow from the rumen in dry cows.

A. C. Hruby*, D. J. Gravley, L. M. Campos, J. M. Prestegaard, C. Campbell, and M. D. Hanigan, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

Microbial protein (MiP) often contributes over half of the absorbed amino acids (AA) in dairy cattle; thus, MiP flow can indicate nitrogen (N) efficiency. Crude protein (CP) is often overfed because specific AA needs are not well understood, resulting in poor N efficiency. Accurate predictions of MiP flow would provide a better representation of AA absorption in the small intestine. The objective of this study was to investigate the accuracy of a revised MiP flow prediction equation relative to observed flows when animals were fed diets formulated for cows producing 37 kg of milk/d to: 1) meet requirements according to the 2001 Dairy NRC model (NRC; 16.6% CP); 2) maximize N efficiency while meeting essential AA requirements (NEFF; 13.2% CP); and 3) maximize income over feed cost (IOFC; 19.1% CP); all diets had maintained rumen degraded protein (RDP) levels. Eight Holstein dry cows were randomly assigned to each treatment sequence, in a Youden square design, containing 8 treatments arranged in 7 periods of 10 d each. Between d1 and d7 cows were fed 1x/d ad libitum in Calan gates targeting 5% refusals. On d8 cows were moved to metabolism stalls and fed 4x/d for 48 h followed by 12x/d for 24 h. Urine spot samples were collected 4x/d from d 8–10 and stored at −20°C. Samples were pooled by treatment and cow and assessed for creatinine and purine derivatives (PD) (Young and Conway, 1942, Fujihara et al., 1987); then, urine volume and MiP flow were calculated (Chen and Gomez, 1992, Valadares et al., 1999). Equation inputs were dry matter intakes of 1) rumen degraded starch, 2) rumen degraded NDF, 3) RDP, and 4) residual organic matter (White et al., 2016; Hanigan, *personal communication*). Data were analyzed using a mixed linear model (Table 1). Observed and predicted MiP flows did not differ by diet; although it was numerically greater for the NEFF and IOFC diets, but not for the model prediction. Importantly, the results suggest that a low CP diet (NEFF) can be fed without sacrificing MiP flow if adequate RDP levels are present.

Table 1. Least squares means of urinary output of purine derivates and estimates of MiP flow

	NRC	NEFF	IOFC		
	16.6% CP	13.2% CP	19.1% CP		
Item	11.6% RDP	9.45% RDP	12.9% RDP	SEM	*P*-value
Urine output (L)	24.1^{b}	20.5^{a}	27.2^{c}	2.54	0.04
Allantoin (mM)	13.5	17.1	13.0	1.79	1.96
Uric acid (mM)	2.49	2.74	2.46	0.48	0.84
PD (mmol/d)	390	421	425	72.2	0.70
Microbial N from PD (g/d)	246	272	270	52.3	0.68
Microbial N from model (g/d)	253	239	240	14.4	0.27

Key Words: creatinine, purine derivatives

Animal Behavior and Well-Being IV

390 Outcome-based welfare measures of high-producing Holstein drylot–housed dairy cattle across regional benchmarks in the United States and Mexico. K. M. Luchterhand*, *Novus International Inc., St. Charles, MO.*

The objective of this observation study was to describe animal welfare in dry lot housed cattle across 2 regions in the United States and 1 region in northern Mexico. The data were collected from 42 commercial dry lot dairy farms from March 2012–2020. Herd size averaged 3,174 (range 560–10,200) lactating cows. The high production Holstein pen was used and benchmarks were created by geographical region: Northwest USA (NW; n = 6), Southwest USA (SW; n = 28) and Northern Mexico (MEX; n = 8). The MIXED procedure of SAS was used to analyze lameness, injuries, lying times and milk yield. The fixed effect was region with the covariates of average temperature-humidity index and time away from pen offered to the model. Prevalence of clinical lameness (%) was determined by locomotion scoring (LS; 1 = normal, 5 = severely lame; LS ≥ 3 considered lame). Lameness prevalence were (lsmean ± SE):38.2 ± 3.3, 24.0 ± 3.7 and 18.6 ± 1.7 for MEX, NW, and SW, respectively. Lameness prevalence was greater in MEX than NW ($P = 0.01$) and SW ($P < 0.01$) with no differences between NW and SW. Hock injury prevalence (%; hair loss and/or swollen) were: 6.9 ± 2.8, 4.3 ± 3.2 and 11.2 ± 1.5 for MEX, NW, and SW, respectively. Hock injury prevalence tended to be greater for SW than NW ($P = 0.06$) and not different from MEX. Knee injury prevalence (%, swollen knees) were: 1.5 ± 0.5, 1.2 ± 0.5 and 0.5 ± 0.2 for MEX, NW, and SW. Knee injury prevalence in MEX tend to be greater than SW ($P = 0.08$) and not different from NW. Daily lying times (h/d) were 10.13 ± 0.41, 9.70 ± 0.49 and 9.48 ± 0.22 for MEX, NW and SW. Lying time did not differ among regions. Milk yield (kg/h/d) were greater in MEX 45.0 ± 1.6 than NW 37.4 ± 2.5 ($P = 0.02$) and not different from SW 42.8 ± 1.3. Milk yield tended to be greater for SW than NW ($P = 0.07$). Based on these results each region assessed has opportunities to improve welfare by reducing lameness or injuries.

Key Words: dry lot, welfare, lameness

391 Behavioral changes in grazing dairy cows with metritis during 2 calving seasons. R. Held*[1], D. Cartes[1], and P. Sepúlveda-Varas[2], [1]*Escuela de Graduados, Facultad de Ciencias Veterinarias, Universidad Austral de Chile (UACh), Valdivia, Región de Los Ríos, Chile,* [2]*Instituto de Ciencias Clínicas Veterinarias, UACh, Valdivia, Región de Los Ríos, Chile.*

Cows may display behavioral changes based on metritis status, yet no work to date has evaluated this association in pasture-based systems. The objective of this observational study was to evaluate the effect of metritis on the lying and rumination time of grazing dairy cows during 2 different calving seasons. The sample included 74 multiparous cows that calved during the spring (July to October; n = 38) and autumn calving season (February to April; n = 36) at the Universidad Austral de Chile Dairy Unit (Valdivia, Chile). In all animals, electronic data loggers continuously recorded lying (Hobo Pendant G) and rumination (Hi-Tag) behavior. Metritis was diagnosed by inspection of the vaginal discharge evaluated every 2 or 3 d during the first 10 d in milk. Cows were retrospectively categorized into 2 health categories: (1) healthy, no signs of clinical postpartum disorders [autumn season, (n = 25); spring season, (n = 25)]; and (2) metritic, diagnosed as having metritis only and with no other signs of clinical postpartum disorders with the exception of retained placenta [autumn season, (n = 11); spring season, (n = 13)]. Data were analyzed using mixed-effects linear models with repeated measures over cow. The mean (±SE) postpartum days when the cows developed metritis was 6.8 ± 0.7 and 7.2 ± 0.8 during the spring and autumn calving season, respectively. Cows that developed metritis during the spring calving season spent more time lying (~1.4 h/d; $P < 0.05$) during d 4, 5 and 6 after calving compared with healthy cows, but we did not observe this effect during the autumn calving season. Rumination time was reduced during the first week after calving (~ ≤ 40 min/d; $P < 0.05$) in cows with metritis that calved only during the autumn season. In summary, changes in lying and rumination time after calving were associated with metritis in grazing dairy cows, but the differences observed depended on the calving season. Project funded by FONDECYT #11170820.

Key Words: rumination time, lying time, metritis

392 The impact of the social environment on the behavior of late pregnant dairy cattle. K. Creutzinger*[1], H. Dann[2], P. Krawczel[3], and K. Proudfoot[4], [1]*University of Guelph, Guelph, ON, Canada,* [2]*William H. Miner Agricultural Institute, Chazy, NY,* [3]*University of Helsinki, Helsinki, Finland,* [4]*University of Prince Edward Island, Charlottetown, PEI, Canada.*

Late pregnant dairy cattle are commonly housed in loose housing systems, such as bedded packs; however, there is limited information regarding the design of these environments for cows as they approach calving. The objective of this study was to determine the impact of lying space allowance and a physical space to separate from other cows (“blind”) in bedded pack pens on the social behavior of dairy cattle during the 3 weeks before calving. A total of 374 Holstein dairy cattle were enrolled into treatment pens once weekly −21 ± 3 d relative to expected calving. Animals were assigned randomly to 1 of 4 treatments using a 2 × 2 factorial arrangement including: 1) high vs. low stocking density (9.7 to 12.9 m^2 vs. 19.4 to 25.8 m^2 lying space /animal), and 2) presence of a blind (yes vs. no). The blind was created using 2 plastic road barriers and plywood (3.6 × 0.6 × 1.5 m). A subset of focal animals (n = 48; nulliparous heifers = 16 and parous cows = 32) was selected randomly while balancing across treatments and parity for detailed social behavior. Social behaviors in the bedded pack (agonistic and allogrooming bouts recorded as both initiator and receiver) were observed using continuous observation for 3 24 h-periods for each animal during the 2 weeks before calving. Linear mixed models were constructed with SAS to determine treatment effects on social behaviors. Animals in low stocking density pens experienced more agonistic (26 vs. 18 ± 2; $P = 0.04$) and allogrooming bouts per day (5 vs. 2 ± 0.7; $P = 0.02$) than animals in high stocking density pens. There was an interaction between parity, blind, and initiator vs. receiver of agonistic bouts ($P = 0.03$). For animals in pens with a blind, heifers received more agonistic bouts per day than they initiated (11 vs. 3 ± 2; $P = 0.04$) and heifers initiated fewer agonistic bouts per day than cows (3 vs. 15 ± 2; $P = 0.002$). Also, heifers initiated fewer agonistic bouts per day in pens with a blind than without (3 vs. 13 ± 2; $P = 0.01$). Results from this study suggest that dairy cattle perform more behaviors, both agonistic and allogrooming, when given more space. Additionally, providing a resource in the pen such as a blind may alter the social dynamic between members of a group, depending on their age.

Key Words: stocking density, maternity pen, calving

393 The use of proximity loggers for estimation of affiliative and agnostic traits in Holstein calves' social network.

Y. A. Ben Meir*[1], I. Izhaki[2], M. C. Zinder[1], and A. Shabtay[1], [1]*Department of Ruminant Science, Institute of Animal Science, Agricultural Research Organization (ARO), Rishon Lezion, Israel,* [2]*Department of Evolutionary and Environmental Biology, The Faculty of Natural Science, University of Haifa, Haifa, Israel.*

Due to animal welfare concerns, rearing suckling dairy calves in groups, rather than in individual housing, has recently become more common. However, in addition to the higher risk of diseases infection, this practice may uncover negative factors of social network dynamics, which impose stress on less dominant individuals in the group, thus impacting their health status, nutritional state and development; Developing tools to estimate affiliative and agnostic traits within the social network dynamics is therefore prerequisite to successfully manage calves reared in groups. To this end, we used proximity loggers (E2C 181C 900 - 1400, Lotek, NZ), to map the social network of 15 suckling Holstein calves during 70 d. Calves were provided with milk replacer (23% CP, 18% fat) and had free access to a mixed ration (16% CP, 4.8% fat, 19.8% NDF) and drinking water. Data collected from the proximity loggers included tag ID, encounter tag ID, and encounter start time and length. Using 3D matrix and trend lines, enabled the identification of 3 distinct encounter types: "Rapid decline" (up to ~120 s) distribution, "moderate decline" (~120 to ~600 s) and "exponential decline" (longer than ~600 s). We hypothesized that random agnostic encounter between any 2 calves would terminate quickly, while encounter with affiliative relation would be of lingering characteristic. Therefore, a negative correlation between short ("agnostic") and long ("affiliative") encounters, was expected. Indeed, a quadratic assignment procedures (QAP) with 5,000 random permutations (UCINET 6, Analytic Technologies, Lexington, KY), revealed a significant negative correlation between "agnostic" and "affiliative" sum of encounters between dyads ($R^2 = 0.15–0.57$, $P < 0.001$). Based on these results we suggest this procedure as a useful tool to assess group social network dynamics in following studies.

Key Words: Israeli Holstein male calves, social network dynamics, group housing

394 New adventures are easier with a buddy: Postweaning behavioral differences in individual and pair-housed dairy calves.

L. Patton*[2], B. Ventura[2], and W. Knauer[1], [1]*Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, MN,* [2]*Department of Animal Science, College of Food, Agricultural and Natural Resource Sciences, University of Minnesota, Saint Paul, MN.*

Pair housing of pre-weaned dairy calves confers several benefits to calves, but effects on social behavior upon social grouping at weaning are poorly understood. Our objective was to assess the behavior of previously pair- (PP) or individually- (PI) housed calves after movement into group pens at weaning. We observed video footage of 5 calves up to 78hr following entry into weaned group pens (n = 2 PP, n = 3 PI; housed with their treatment group). We focused on synchrony of behaviors with other calves. Five min scan sampling was conducted in 6hr increments (0hr, 24hr, and 72hr after pen entry) to capture the following behaviors: lying, standing, or walking by self; and social lying, standing, or walking. Additionally, continuous sampling [frequency (n) or time (s)] was conducted for 6hr directly after pen entry to describe the following behaviors: self-groom, self-explore, self-locomotor play, cross-suck, social explore, kick, mock fight, mount, allogroom, social sniff, and social locomotor play. Data were aggregated for each calf over the observation periods; data were non-normally distributed and so the Kruskal-Wallis rank sum test was used to assess differences in behavior between PP and PI calves. Results are reported as $\mu \pm$ SD. In general, scans did not reveal substantive differences in recorded behavior categories, with the following exception: PP calves were more frequently observed to lay near a companion compared with PI calves (65 ± 9 vs. 40 ± 8 scans). Continuous sampling showed that PI calves spent more time engaged in self (383 ± 233 vs. 106 ± 45 s) and social locomotor play (266 ± 69 vs. 96 ± 22 s) and mock fighting (31 ± 16 vs. 7 ± 2 bouts) but less time self-grooming (29 ± 17 vs. 79 ± 11 s) and exploring socially (71 ± 3 vs. 118 ± 12 s) compared with PP calves. Only PP calves were observed to allogroom. Though limited on sample size, these results suggest PP calves may be more socially adjusted and able to cope with novel stimuli after movement to weaned calf pens as compared with PI calves, lending further support to social housing for pre-weaned dairy calves.

Key Words: animal welfare, group housing, social facilitation

500 Comparison of fan type and cow temperatures during summer conditions on a California dairy.

S. T. Smith*, K. C. Dhuyvetter, and P. W. Jardon, *Elanco Animal Health*, *Greenfield, IN.*

Heat stress in dairy cattle is an economically important factor on dairy farms. Factors affecting the effectiveness of cow cooling include shade/housing, soakers, and fans. Fan type has not been extensively evaluated as to effects on dairy cow heat abatement and was the objective of this study. A cow-cooling evaluation was done on a commercial Holstein dairy in central California August 19-26, 2020. Cows (n=9-10 per group) from 4 different freestall pens (615-679 head per pen) were selected for temperature monitoring. Group 1 was primiparous cows and groups 2-4 were multiparous cows. Cows were selected to be similar in DIM (170-186), gestation (67-86 DCC), and milk production within parity. Cow temperatures were monitored by attaching an iButton (Embedded Data Systems DS1921H-F5 for Lactation=1 and DS1922L for Lactation>1) to a Controlled Internal Drug Release (CIDR) device inserted intravaginally with temperatures taken every 5 min. Cow-level temperatures were aggregated to 15-minute intervals for analyses. Temperature/humidity monitors (HOBO proV2) were placed in 3 of the 4 pens (no monitor in L = 1 pen) and in milking parlor holding area to capture temperature and humidity every 5 minutes. During the evaluation 92% of all pen-level THI readings were above the heat stress threshold THI of 68. Each freestall pen differed in fan type. Group 1 had no fans in the pen, group 2 had 2.13 m high-capacity fans spaced at 30.48 m intervals, group 3 had 1.83 m louvered fans spaced at 18.29 m intervals and group 4 had 1.32 m panel fans spaced at 8.23 m intervals Mean cow temperatures averaged across days, in 15-min increments (n = 96 per pen), were compared across pens using Tukey-Kramer HSD test with a = 0.05. The mean for group 1 was statistically higher than the other groups (39.4 ± 0.03°C). The means of groups 2 and 4 were similar (39.1 ± 0.02°C and 39.0 ± 0.03°C). The mean of group 3 was statistically lower than the other groups (38.8 ± 0.02°C). In addition, cumulative time of temperatures over 39.17°C varied by group, with group 3 having only 9.4% readings above 39.0°C compared to group 1 (78.5%), group 2 (35.5%) and group 4 (34.0%). These results suggest fan type may be an important factor when designing cow cooling strategies.

Key Words: heat abatement, fans, cow cooling

Animal Health IV

395 Pegbovigrastim injections altered the immunometabolic state of Holstein cows when administered at the time of dry-off.

J. M. Strickland*[1], J. L. de Campos[2], P. L. Ruegg[1], and L. M. Sordillo[1], [1]*Large Animal Clinical Sciences, Michigan State University, East Lansing, MI,* [2]*Animal Science, Michigan State University, East Lansing, MI.*

The early dry period is a critical juncture in determining mammary gland health and milk production for the subsequent lactation. Mammary involution in the early dry period is characterized by dramatic alterations in metabolism, highly orchestrated immune responses, and changes to oxidant status. One of the initial immune responses to involution include recruitment of neutrophils to the mammary gland. We hypothesized that pegbovigrastim would optimize mammary involution due to its capacity to increase circulating neutrophils. However, it is not known how pegbovigrastim affects metabolic, immunologic, and redox changes that occur during the early dry period. The objective of this study was to evaluate physiological changes in healthy early dry-off cows. Late lactation cows (n = 20) were matched by parity, milk production, BLV status, and SCC and randomly assigned to receive either 15 mg pegbovigrastim or saline 1 week before and on the day of dry-off. Blood samples were taken −7, −2, −1, 0, +1, +2, +4, +7, and +14 d relative to dry-off as well as +5, +10, and +14 d post-parturition. Samples were analyzed for number of neutrophils, mononucleocytes, eosinophils, total calcium, BHB, NEFA, albumin, glucose, haptoglobin, reactive oxygen species (ROS), and antioxidant potential. Repeated-measures models using PROC MIXED were used to assess the effects of treatment and means were separated using Bonferroni correction (SAS ver.9.4). Pegbovigrastim increased serum concentrations of neutrophils and mononucleocytes compared with control cows ($P < 0.001$). There was a significant treatment and time by treatment effect of pegbovigrastim depressing serum glucose concentrations the 4 d post dry-off ($P < 0.001$). Pegbovigrastim tended to increase serum ROS concentrations while reducing serum calcium and haptoglobin concentrations ($P < 0.10$) during the early dry period. Control cows had elevated BHB 14 d post-parturition ($P < 0.01$). This study demonstrated pegbovigrastim injection at dry-off had broad ranging effects on early dry cows which could influence health and production in the subsequent lactation.

Key Words: pegbovigrastim, involution, dry-off

396 Bovine mammary involution measured histologically after dry treatment with casein hydrolysate alone or combined with other treatments.

D. J. Wilson*[1], J. E. Britten[2], K. A. Rood[1], and C. S. Clancy[3], [1]*Utah State University, Logan, UT,* [2]*Udder Health Systems, Meridian, UT,* [3]*National Institutes of Health Rocky Mountain Laboratories, Hamilton, MT.*

The dairy industry is interested in alternatives to antibiotic dry treatments. The objective was to evaluate mammary gland involution following intramammary casein hydrolysate (CH), alone or combined with cloxacillin dry-cow treatment (DCT) and/or teat sealant (TS) at dry-off (FDA and animal care sanctioned). Non-pregnant cows > 300 d in milk (n = 16) from 6 dairy farms were studied; normal milk, CBC and clinical chemistry results were required. A split udder design was used, with each udder half assigned a treatment and the contralateral half administered DCT+TS as a control. There were 4 treatments: CH, CH$^+$DCT, CH$^+$TS, CH$^+$DCT+TS. Cows were euthanized 2 d (n = 8) or 7 d (n = 8) after dry treatment. Mammary tissue was sampled from ventral, mid, and dorsal regions of each quarter. Fixed tissue slides were stained and CellSens software measured epithelial cell height (E), alveolar lumen diameter (L), and interalveolar stroma thickness (S). A general linear mixed model (SAS Studio) tested for days dry, mammary gland region, and treatment effects on E, L and S sizes. Marked differences in secretory structure sizes existed between 5 large and 11 small structure sized cows; thus to avoid confounding, measurements were analyzed as % of control udder halves' tissues within each cow. Analysis of variance was used to test for significant differences in tissue sizes ($\alpha = 0.05$). Dry treatment group was significantly associated with E, L and S sizes. At 2 d dry, CH, CH$^+$DCT, and CH$^+$DCT+TS were associated with increased involution compared with the control; at 7 d dry, only CH$^+$DCT was (Table 1). CH was associated with increased early involution and may be useful as an adjunct or a non-antibiotic treatment at dry-off of dairy cows.

Table 1. Mammary gland structure sizes relative to control udder half

Item	CH	CH+DCT	CH+TS	CH+DCT+TS	Control (DCT+TS)
2 d dry					
Epithelia	102%	120%**	102%	103%	100%
Lumina	92%	73%**	103%	75%**	100%
Stroma	115%**	136%**	115%**	148%**	100%
7 d dry					
Epithelia	90%*	106%	106%	111%	100%
Lumina	96%	76%**	96%	104%	100%
Stroma	89%**	126%**	115%*	101%	100%

*$P < 0.03$, **$P < 0.00001$, all comparisons versus control (dry-cow treatment + teat sealant).

Key Words: casein hydrolysate, dry cow, involution

397 Association between dry-cow therapy and antimicrobial resistance of mastitis pathogens after calving on California dairies.

W. ElAshmawy*[1,2], E. Okello[1,3], D. Williams[1], T. Lehenbauer[1,3], and S. Aly[1,3], [1]*Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California-Davis, Tulare, CA,* [2]*Department of Internal Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt,* [3]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California-Davis, Davis, CA.*

Dry-cow therapy (DCT) is part of the National Mastitis Council 10-point plan for control of mastitis in dairy cows. In the US, 93% of dairy cows received intramammary (IMM) antimicrobial drugs (AMD) at dry-off in 80.3% of herds (USDA, 2016). Our study objective was to evaluate the effect of DCT on the antimicrobial resistance (AMR) profile of mastitis pathogens. Bacterial isolates from 382 cows were tested for AMR, a subset of 1,106 cows randomized to receive IMM AMD or not at dry-off. Milk samples were collected at dry-off (S1), within 2 weeks post-calving (S2) and at first mastitis event within 150 d in milk. All milk samples were submitted to the Milk Quality Lab (Tulare, CA) for bacterial isolation. Antimicrobial susceptibility was determined using broth microdilution minimum inhibitory concentration method against a panel of 10 AMD. Interval regression models provided estimates of association between DCT and AMR phenotype. Only coagulase-negative *Staphylococcus* species (CNS) were modeled, as they were the most prevalent species isolated at S1 and S2. AMR was increased in CNS isolated from AMD treated cows between S1 and S2, with the greatest increase observed for penicillin (14.95%) followed by erythromycin (8.41%) and pirlimycin (4.17%). In contrast, the smallest increase in resistance was observed for penicillin/novobiocin (0.23%). Models for cows with CNS isolated at both S1 and S2 showed an increase in resistance against penicillin, ampicillin, oxacillin, cephalothin and ceftiofur in cows that received DCT from the same drug class, or a class with a shared resistance mechanism. In contrast, resistance to tetracycline, sulphadimethoxine, and erythromycin were associated with any AMD therapy at dry-off. Pirlimycin resistance decreased for cows received cephalosporins at dry-off. However, resistance to pirlimycin increased for cows received non-cephalosporin DCT in comparison to cows that did not receive DCT. Compared with winter, CNS resistance to ceftiofur post-calving increased in the summer. Our study provided evidence that AMD DCT is associated with AMR of CNS post-calving.

Key Words: dry-cow therapy, coagulase-negative staphylococci, interval regression model

398 Results from a negatively controlled randomized clinical trial evaluating antimicrobial treatments for mastitis caused by gram-positive pathogens.

Q. K. Kolar*[1], S. M. Godden[2], and P. L. Ruegg[3], [1]*Department of Animal Science, Michigan State University, East Lansing MI,* [2]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul MN,* [3]*College of Veterinary Medicine, Michigan State University, East Lansing, MI.*

Mastitis is the most common bacterial disease of dairy cows and accounts for a large proportion of antimicrobial usage. Improving antimicrobial stewardship on dairy farms includes selective treatment of clinical mastitis cases using culture-guided treatment protocols. Antimicrobials are recommended for treatment of gram-positive mastitis, but few trials have compared duration or drug choice. The objective of this trial was to compare outcomes of antimicrobial treatments of non-severe mastitis caused by gram-positive pathogens identified using on-farm culture. Non-severe cases of clinical mastitis (n = 239) were enrolled from 4 farms based on diagnosis of a gram-positive etiology using a tri-plate based on-farm culture system. After confirmation of gram-positive growth, cases were randomly assigned to: No treatment (NT, n = 32), or 1 of 3 intramammary treatments: 3 d hetacillin potassium (3PM, n = 68, Polymast, Boehringer Ingelheim), 3 d (3SM, n = 70) or 8 d of ceftiofur hydrochloride (8SM, n = 69, SpectraMastLC, Zoetis). Outcomes included: days of abnormal milk (evaluated visually for 10 d); bacteriological cure (evaluated based on quarter milk samples collected at 28 d); quarter somatic cell count (QSCC, d 14, 21, 28) and daily milk yield and retention in the herd for 90d. Statistical analysis was performed using SAS v 9.4. Days to normal milk and recurrence were analyzed using, time-to-event analysis (Proc Phreg), while the remaining outcomes were analyzed using Proc Mixed to generate multiple linear regression models. All models were adjusted for potential confounding variables including parity, days in milk, and lactation. Mean days to normal milk were 5.5 (3PM), 5.3 (3SM), 5.5 (8SM), and 4.9 (NT) and did not vary by group ($P = 0.52$). Bacteriological cure at d 28 was not associated with treatment ($P = 0.43$) and was 73% (3PM), 76% (3SM), 83% (8SM), and 61% (NT). No differences in QSCC ($P = 0.40$) over 4 weeks or daily milk yields over 90 d ($P = 0.64$) were found among groups. Intramammary treatment of non-severe clinical mastitis caused by pathogens identified as gram-positive using on-farm culture-based systems was not associated with the outcomes that were measured in this study.

Key Words: mastitis, antimicrobial treatment, management

399 Description of *Staphylococcus* spp. intramammary infections in early-lactation primiparous cows on organic dairy farms.

F. P. Mosca*[1], C. J. Dean[1], B. J. Heins[2], V. S. Machado[3], P. J. Pinedo[4], N. R. Noyes[1], 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,* [3]*Department of Veterinary Sciences, Texas Tech University, Lubbock, TX,* [4]*Department of Animal Sciences, Colorado State University, Fort Collins, CO.*

Staphylococcus spp. are frequently associated with intramammary infections (IMI) in dairy cows. *Staphylococcus aureus* (SAU) is recognized as an important cause of mastitis in conventional and organic dairy herds. However, the role of non-aureus staphylococci (NAS) in mastitis development is unclear. Our aim was to describe IMI caused by *Staphylococcus* spp. in early-lactation primiparous cows on organic dairy farms. In this prospective cohort study, we enrolled 501 primiparous cows from 5 organic dairy farms. Quarter milk samples were taken weekly during the first 5 weeks of lactation. Milk samples were combined into a single composite sample in the laboratory inside a sterile laminar hood. Composite milk samples were sent to the Udder Health Laboratory at the University of Minnesota for bacterial culture. Bacterial isolates were identified using MALDI-TOF MS. Contaminated samples (>3 different isolates) and cows with results from only 1 or 2 samples were excluded from the analysis. In total, 1,697 samples (n = 430, 549, 525, 59, 134 for herds A, B, C, D and E, respectively) from 397 cows (n = 111, 117, 116, 16, 37 for herds A, B, C, D and E, respectively) were used in the analysis. The association between bacterial group and risk of chronic IMI (defined as harboring the same microorganism for ≥3 weeks postpartum) was analyzed using tabular methods. Risk ratios (RR) and Wald 95% confidence intervals (CI) were calculated using SAU as the reference group. The cumulative incidence of SAU and NAS was 19.1% and 52.1%, respectively. Among cows with SAU-IMI, 63.2% had chronic SAU-IMI (referent, RR = 1), whereas 13.2% had SAU in only one week. Results varied among the NAS species, *Staphylococcus chromogenes* (SCH), the most prevalent NAS, was associated predominantly with chronic SCH-IMI (52.0%, RR (95%CI) = 0.82 (0.65–1.04)). Other NAS species demonstrated a small proportion of chronic IMI (11.24%, RR (95%CI) = 0.18 (0.10–0.32)) and a predominance of short-lasting (1 week) IMI (74.15%). These results show that a large proportion of the early-lactation primiparous cows in this study harbored NAS. Within the NAS-IMI cases, different NAS species demonstrated divergent behavior in terms of chronicity.

Key Words: *Staphylococcus* spp., mastitis, organic dairy farms

400 Effect of extended voluntary waiting period from calving until first insemination on udder health.

J. Ma*[1], A. Kok[1], R. M. A. Goselink[2], T. J. G. M. Lam[3], B. Kemp[1], and A. T. M. van Knegsel[1], [1]*Adaptation Physiology group, Wageningen University & Research, Wageningen, the Netherlands,* [2]*Wageningen Livestock Research, Wageningen University & Research, Wageningen, the Netherlands,* [3]*Department of Population Health Sciences, Utrecht University, Utrecht, the Netherlands.*

One possible solution to reduce health disorders in peripartum dairy cows is to reduce the frequency of calving and associated critical transitions by extending the voluntary waiting period until first insemination (VWP). The aim of the present study was to get insight into the effect of an extended VWP on somatic cell count (SCC), SCC elevations and clinical mastitis incidence during the complete lactation and start of the next lactation. Holstein-Friesian dairy cows (n = 154) were blocked for parity, expected milk yield and calving season and randomly distributed across 3 VWP (50, 125 or 200 d: VWP-50, VWP-125, VWP-200). Cows were monitored from calving until 6 weeks after the second calving in experiment, or until culling. The natural logarithm of SCC (LnSCC) was used for analyses to approximate normality and were analyzed weekly. An elevation of SCC was defined as SCC ≥ 200,000 cells/mL after 2 previous weeks with SCC < 200,000 cells/mL. A case of clinical mastitis was defined as a case of visibly abnormal milk, visible changes in the udder due to inflammation, or both. Statistical analyses were performed using a repeated measurements model in SAS version 9.4. As a result of the VWP treatment, lactation length was 363, 445 and 481 d for cows in VWP-50, VWP-125 and VWP-200. Extended VWP did not have an effect on the occurrence of elevation of SCC or clinical mastitis per lactation or per year. In the complete lactation, multiparous cows in VWP-125 tended to have higher average SCC compared with cows in VWP-50 ($P = 0.09$), primiparous cows had no difference in SCC after different VWP. At dry-off, SCC was 191, 211 and 142 $*10^3$ cells/mL and selective dry-cow antibiotic therapy was used for 43% vs 32% vs 35% for cows in VWP-50, VWP-125 and VWP-200. Within 6 weeks in the second lactation of the experiment, cows with VWP-200 had higher SCC compared with cows with VWP-50 ($P = 0.04$). In conclusion, extending the VWP did not affect elevations of SCC and occurrence of mastitis per lactation. Extending the VWP may be used to reduce the frequency of transition periods and associated dry-cow antibiotics without a negative impact on udder health.

Key Words: extended calving interval, somatic cell count, clinical mastitis

401 Modifiable management practices to improve udder health in dairy cattle during the dry period and early lactation: A scoping review.

C. K. McMullen, J. M. Sargeant, D. F. Kelton, K. J. Churchill, K. S. Cousins, and C. B. Winder*, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

With increasing scrutiny over preventive use of antimicrobial in livestock, the objective of this scoping review was to characterize the literature on modifiable management practices used during the dry period that have been evaluated for their impact on udder health. Five databases and 2 conference proceedings were searched for relevant literature, including controlled trials and analytic observational studies. Articles published in or after 1990 were eligible, and must have evaluated a modifiable management practice. Antimicrobials and teat sealants were enumerated but not further characterized as published systematic reviews on these topics exist. Other modifiable management practices were reported in 229 articles. Nurtition (n = 79) which included ration formulation and delivery (n = 44) and vitamin and mineral additives (n = 35) were the most commonly evaluated, followed by vaccines (n = 40), and modification of dry period length (n = 27). Risk of clinical mastitis (CM) was the most common udder health outcome (n = 151), although risk period length varied. Prevention of new intramammary infections (IMI) over the dry period (n = 54) and cure of existing IMI (n = 40) were also reported. Future quantitative synthesis could target practices such as nutrition, vaccination, and dry period length. Establishment of core outcomes for evaluating the impact of interventions or exposures on udder health over the dry period and early lactation could improve the ability to quantify and connect different intervention areas to establish efficacy of multiple different management strategies at dry-off.

Key Words: dry cow, management, mastitis

403 Evaluation of heat and pH treatments on the degradation of ceftiofur-spiked cow milk.

A. Garzon*[1], P. Pandey[1], L. A. Tell[2], S. Aly[1,3], S. Biswas[1], R. Poppenga[4], and R. Pereira[1], [1]*Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, Davis, CA,* [2]*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, CA,* [3]*Veterinary Medicine Teaching and Research Center, University of California, Davis, Tulare, CA,* [4]*California Animal Health and Food Safety Laboratory, Davis, CA*

Waste milk feeding practices have been implicated as a potential source for disseminating antimicrobial resistant bacteria among animals and the environment. Two potential interventions to degrade antimicrobial drugs in cow's milk are heat and pH treatment. The aim of this study was to evaluate the effect of heat and pH treatments on the degradation of ceftiofur and ceftiofur free acid equivalents (CFAE) in milk at concentrations previously found in waste milk on dairy farms. Saleable whole milk was spiked with ceftiofur sodium to a final concentration of 200 or 400 ppb for heat or pH trials, respectively. Four replicates were performed for each set of treatments. Three temperature treatments were evaluated: low-temperature long-time (LTLT, 63°C for 30 min), high temperature short time (HTST, 72°C for 15 s) and high-temperature long-time (HTLT, 92°C for 20 min). A control group stored at room temperature was used. Two pH treatments were evaluated: low pH group (LpH), using formic acid to achieve a pH of 4.0, and high pH group (HpH), by adding sodium hydroxide until a pH of 10.0 was achieved. A control milk group was also used. Ceftiofur was quantified using liquid chromatography mass spectrometry (LC-MS/MS). The concentration of CFAE was measured using high-performance liquid chromatography (HPLC). Dunn All Pairs for joint ranks test was used to evaluate significant differences in the ceftiofur concentrations. There was a significant effect on the degradation of ceftiofur for HTLT milk treatment, which represented a 35.24% degradation of the initial concentration of ceftiofur. The degradation of ceftiofur did not differ between the control and the remaining 2 heat treatment groups. Concentration of ceftiofur remained stable at normal and low pH but declined significantly immediately after sodium hydroxide was added and pH 10 was achieved. Initial concentration of CFAE in samples decreased after milk was alkalized to pH 10. Increasing milk pH resulted in the degradation of the 95.72 and 96.28% of the initial concentration of ceftiofur and CFAE, respectively. Heat and pH treatments are cost effective on-farm strategies that could decrease antimicrobial concentrations in waste milk below FDA-approved tolerances. Further studies to evaluate the possibility of using these approaches on farms are needed.

Key Words: antibiotics, drug residues, waste milk

404 Occurrence of mycotoxins in US total mixed rations.

P. N. Gott*[1], E. F. Schwandt[1], S. M. Ramirez[1], U. Hofstetter[2], and G. R. Murugesan[1], [1]*BIOMIN America Inc, Overland Park, KS,* [2]*BIOMIN Holding GmbH, Getzersdorf, Lower Austria, Austria.*

Mycotoxins are fungal metabolites found in a variety of feedstuffs. Due to the inclusion of multiple ingredients in a total mixed ration (TMR), the potential for mycotoxin contamination may be increased. Ruminants are generally considered less susceptible to mycotoxins due to natural detoxification in the rumen; however, health, productivity, and reproduction can still be compromised depending on mycotoxin type and contamination level. This study investigated mycotoxin occurrence and contamination levels in TMR samples from across the US. Samples were screened via LC-MS/MS for 6 major mycotoxin groups: aflatoxins, type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A. Information collected included state of origin and presence of clinical health or performance concerns. Contamination levels were analyzed using GLIMMIX procedure (SAS

9.4, Cary, NC) to investigate the interaction of clinical concern (yes/no) and harvest year. No interaction was significant ($P > 0.05$) for each toxin group, so only main effects of harvest year are presented. To date, 231 samples from 21 states were analyzed (Table 1). Detectable levels of B-Trich have been identified in 97% of samples with ZEN and FUM detected less frequently. Mean contamination levels (parts per billion, ppb) are presented on dry matter basis and were similar in 2020 compared to 2019 for B-Trich, FUM, and ZEN (P>0.05). Although TMR formulations vary widely, preliminary results indicate B-Trich occur frequently in US TMR samples. Co-contamination in diets is an additional consideration with ZEN detected in over half of the samples analyzed.

Table 1. Mycotoxin contamination in US TMR samples (dry matter basis)

	Year						
Parameter	2016	2017	2018	2019	2020	SEM[1]	P-value[1]
Number	37	109	211	258	231		
B-Trich							
Prevalence, %	84	92	95	100	97		
Mean, ppb	430[b]	729[b]	882[b]	2425[a]	2607[a]	153.4	<0.001
FUM							
Prevalence, %	46	25	11	26	23		
Mean, ppb	335	296	880	857	715	280.7	0.513
ZEN							
Prevalence, %	27	41	29	65	52		
Mean, ppb	79	144	315	368	329	78.6	0.081

[a–c]Values across rows with different letters are different $P<0.05$.

[1]SEM and P-values were generated using GLIMMIX procedure of SAS to test the effect of harvest year on mean contamination level of each mycotoxin group when samples were above respective limit of detection.

Key Words: mycotoxins

405 Occurrence of mycotoxins in 2020 US forages.

P. N. Gott*[1], E. F. Schwandt[1], S. M. Ramirez[1], U. Hofstetter[2], and G. R. Murugesan[1], [1]*BIOMIN America Inc, Overland Park, KS,* [2]*BIOMIN Holding GmbH, Getzersdorf, Lower Austria, Austria.*

Mycotoxins are fungal metabolites found in feedstuffs that can limit animal health and performance. This study monitored mycotoxin occurrence and contamination levels in US corn silage, haylage, and straw. Sample pools differ year-to-year and by ingredient, but primarily originate from Midwest and Northeast regions. Samples were screened for 6 major mycotoxin groups: aflatoxins, type A trichothecenes (A-Trich), type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A via LC-MS/MS. Information collected included harvest year, state of origin, and presence of clinical health or performance concerns. Effect of clinical concern (yes/no) and harvest year on contamination levels were analyzed using LSMeans statement with Tukey's adjustment in the GLIMMIX procedure (SAS 9.4, Cary, NC). No interaction was significant ($P > 0.05$) for each toxin group, so main effects of harvest year are presented. Among 106 corn silage samples from 2020, B-Trich were most prevalent (Table 1). Mean contamination levels (parts per billion, ppb) are on dry matter basis and were similar in 2020 compared with 2019 for B-Trich, ZEN, FUM, and A-Trich ($P > 0.05$). B-Trich were most prevalent in both 2020 haylage (n = 19; 95% positive) and 2020 straw (n = 12; 83% positive). Haylage B-Trich levels were similar in 2020 (2456 ppb) vs. 2019 (3147 ppb; $P > 0.05$), while mean B-Trich in 2020 straw (772 ppb) was reduced vs. 2019 straw (2055 ppb; $P < 0.05$). Preliminary results indicate B-Trich are the most frequently detected mycotoxin group in 2020 US corn silage, haylage, and straw.

Table 1. Mycotoxin contamination in US corn silage (dry matter basis)

	Year						
Parameter	2016	2017	2018	2019	2020	SEM[1]	P-value[1]
Number	133	214	183	183	106		
A-Trich							
Prevalence, %	2	0	0	2	11		
Mean, ppb	383	—	—	235	557	277.2	0.754
B-Trich							
Prevalence, %	85	85	92	93	97		
Mean, ppb	1283[b]	727[b]	3340[a]	3378[a]	2804[a]	190.6	<0.001
FUM							
Prevalence, %	22	12	28	12	18		
Mean, ppb	1072	598	1500	780	929	411.7	0.190
ZEN							
Prevalence, %	59	27	60	44	33		
Mean, ppb	313[b]	221[b]	950[a]	454[b]	434[b]	39.8	<0.001

[a–c]Values across rows with different letters are different $P < 0.05$.

[1]SEM and P-values were generated using GLIMMIX procedure of SAS to test the effect of harvest year on mean contamination level of each mycotoxin group when samples were above respective limit of detection.

Key Words: mycotoxins

406 Occurrence of mycotoxins in 2020 US corn and corn by-product feed ingredients.

E. F. Schwandt*[1], P. N. Gott[1], S. Ramirez[1], U. Hofstetter[2], and G. R. Murugesan[1], [1]*BIOMIN America, Overland Park, KS,* [2]*BIOMIN Holding GmbH, Getzerdorf, Lower Austria, Austria.*

Mycotoxins are harmful secondary fungal metabolites that are detrimental to animal health and performance. This study investigated occurrence and contamination levels of mycotoxins in the 2020 US corn harvest including corn grain and corn by-product feed ingredients. Corn samples marked as 2020 harvest and corn by-products submitted from October 2020 through February 2021 were screened for: aflatoxins, type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A. Samples that were above the limit of detection for each mycotoxin group were analyzed using LSMeans statement with Tukey's adjustment in GLIMMIX procedure of SAS (SAS 9.4, Cary, NC) to test the effect of harvest year on average contamination level. Results are presented in Table 1. Mean B-Trich level (parts per billion, ppb) for corn grain samples were similar in 2020 as 2019. Fumonisin and ZEN contamination was similar in 2020 compared with previous years. Prevalence of mycotoxin contamination remained high in corn by-products and were 99, 85, and 98% for B-Trich, ZEN, and FUM, respectively. Mean B-Trich and ZEN levels in 2020 by-products were similar to levels seen in 2018 and lower than 2019. Mean FUM contamination was similar in 2020 and 2019. Preliminary results of the 2020 corn-based feed ingredients survey suggest mycotoxin occurrence and contamination levels are approaching those observed in the 2018 and 2019 crop in corn grain and corn by-product feeds.

Table 1. ***Fusarium*** **toxin contamination in US corn-based feed ingredients**

	Year				
Parameter	2018	2019	2020	SEM	*P*-value
Corn Grain					
Number	447	329	222		
B-Trich					
Prevalence, %	76	81	57		
Mean, ppb	1,162^{ab}	883^{bc}	847^{bc}	95.4	<0.001
ZEN					
Prevalence, %	45	46	20		
Mean, ppb	310	415	418	109.7	0.273
FUM					
Prevalence, %	79	78	64		
Mean, ppb	3,305	3,348	2,733	375.3	0.503
By-Product Feeds					
N	67	54	79		
B-Trich					
Prevalence, %	94	100	99		
Mean, ppb	2,202^{b}	2,819^{a}	2,116^{b}	226.7	<0.001
ZEN					
Prevalence, %	78	94	85		
Mean, ppb	326^{b}	635^{a}	319^{b}	39.8	<0.001
FUM					
Prevalence, %	90	98	98		
Mean, ppb	1,093^{b}	2,467^{ab}	2,098^{ab}	412.3	0.004

$^{a–c}$Values across rows with different letters are different $P<0.05$.

Key Words: by-products, corn, mycotoxins

Breeding and Genetics IV

407 Genetic aspects of colostrum quality traits including immunoglobulin G in Holstein cows. A. Costa*[1], A. Goi[1], G. Visentin[2], M. De Marchi[1], and M. Penasa[1], [1]*University of Padova, Legnaro, Padova, Italy,* [2]*University of Bologna, Bologna, Italy.*

Immunoglobulins G (IgG), A (IgA), and M (IgM) account for 80% of total proteins in bovine colostrum and are important for the passive transfer of antibodies from the dam to the calf. The practical difficulties of samples collection and the high cost of gold standard analysis have so far hampered the estimation of genetic parameters of both bovine colostrum and mature milk IgG, IgA, and IgM concentrations (g/L). In the present study, 672 colostrum samples from the same number of Holstein cows were collected within 6 h after calving. The concentration of IgG, IgA, and IgM was determined through radial immunodiffusion. The pedigree included 6,714 animals, i.e., cows with phenotypes and up to 6 generations of ancestors. Linear models were used to estimate variance and covariance components through univariate and bivariate analyses, respectively. The model included the random animal additive genetic effect and the fixed effects of parity (5 levels: 1, 2, 3, 4, and ≥5), season of calving (4 levels), year of calving (2 levels), and herd (9 levels). Colostrum IgG, IgA, and IgM averaged 93.24, 4.89, and 5.16 g/L, respectively, and were normally distributed. Heritabilities (±standard error) of IgG, IgA, and IgM were 0.14 ± 0.14, 0.33 ± 0.16, and 0.19 ± 0.14, with coefficients of genetic variation of 13.1, 30.8, and 19.7%, respectively. The IgG correlated phenotypically with IgA (0.52 ± 0.04) and IgM (0.59 ± 0.03), and IgA correlated with IgM (0.48 ± 0.04). Overall, genetic correlations were weak (<0.10) except for the association between IgG and IgA (0.37 ± 0.41). Results agreed with the literature and highlighted that the most important colostrum quality trait, i.e., the IgG, tended to be the least heritable Ig. Further efforts will allow to improve data size and variability and will be focused on coupling Ig data with other information to estimate the association between Ig of the colostrum administered to the calf and health and productivity in the subsequent lactations.

Key Words: colostrum quality, dairy cattle, genetic improvement

408 Gene mapping, gene-set analysis, and genomic prediction of postpartum blood calcium in Holstein cows. L. Cavani*[1], M. B. Poindexter[2], H. A. Pacheco[1], C. D. Nelson[2], J. E. P. Santos[2], and F. Peñagaricano[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*University of Florida, Gainesville, FL.*

The onset of lactation results in a sudden irreversible loss of calcium for colostrum and milk synthesis. Some cows are unable to quickly adapt to this demand and succumb to clinical hypocalcemia. Our goal was to perform a comprehensive genomic analysis of blood total calcium concentration (Ca) in periparturient Holstein cows. We first performed a genomic scan and a gene-set analysis to identify major genes, pathways and mechanisms affecting postpartum Ca. Then, we assessed the prediction of postpartum Ca using genomic data. Data consisted of 7,691 records of plasma or serum Ca measured in the first, second, and third day after parturition (DAP) of 959 primiparous and 1,615 multiparous cows that calved between Dec-2015 and Jun-2020 in 2 dairy herds. All cows were genotyped with 80k SNPs. The statistical models included lactation (1 to 5+), calf category (male, female, twins), and DAP as fixed effects, and season-treatment-experiment, animal, and permanent environmental as random effects. Model predictive ability was evaluated using 10-fold cross-validation. Heritability and repeatability estimates were 0.083 (SE = 0.017) and 0.444 (SE = 0.028), respectively. The association mapping identified 2 major regions located on BTA6 and BTA16 that explained 1.2% and 0.7% of additive genetic variance of Ca, respectively. Interestingly, the region on BTA6 harbors the gene GC, which encodes the vitamin D binding protein, and the region on BTA16 harbors LRRC38, which is actively involved in potassium transport. Other sizable peaks were identified on BTA5, BTA2, BTA7, BTA14, and BTA9. These regions harbor genes associated with calcium and potassium channels (*CATSPER4*, *CACNA1S*, *CRACR2A*, *CDKN1B*, *KCNK9*). The gene-set analysis revealed terms related to calcium ion transport, calcium ion binding, and calcium signaling. Genomic predictions of yet-to-be observed Ca yielded predictive correlations equal to 0.73 using both fixed and random effects, and 0.21 using only genomic breeding values. Overall, our results can contribute to the development of novel genomic strategies for reducing periparturient hypocalcemia in dairy cattle.

Key Words: calcium homeostasis, postpartum hypocalcemia, whole-genome scan

409 Estimates of genetic parameters for health traits in US organic Holstein cows. L. C. Hardie*[1], I. W. Haagen[1], B. J. Heins[2], and C. D. Dechow[1], [1]*Pennsylvania State University, University Park, PA,* [2]*University of Minnesota, St. Paul, MN.*

The objectives of this study were to estimate genetic parameters for health traits measured in US organic Holstein cows and establish genetic relationships with national evaluations for corresponding traits. Data were gathered from 38,949 lactations from 19,139 Holstein cows on 16 USDA certified organic farms. Health traits analyzed on these cows included mastitis, lameness, any metabolic disease (METAB; ketosis, displaced abomasum, and milk fever), any reproductive disease (REPRO; retained placenta and metritis), died, transition cow diseases (defined as any disease occurring ± 21d of parturition), and any health event. Stayability through a given lactation was also analyzed. All traits were analyzed as binary variables within each lactation (1 = diseased, 0 = otherwise). Genotypes on 2,347 cows with breed representation of at least 87.5% Holstein were combined with a minimum of 4-generation pedigree. Using the BLUPF90 family of programs, multivariate threshold models were employed to estimate genetic parameters with fixed effects of herd and lactation and random effects of herd-year-season of birth (stayability), herd-year-season of calving (health traits), animal, and permanent environment. Heritabilities ranged from 0.03 ± 0.01 (REPRO) to 0.11 ± 0.03 (METAB). Genetic correlations among health traits ranged from −0.42 ± 0.17 (mastitis and METAB) to 0.93 ± 0.03 (transition and any health event). When evaluating traits in terms of resistance to disease, genetic correlations between stayability and health traits ranged from 0.21 ± 0.22 (lameness) to 0.69 ± 0.12 (any health event). Approximate genetic correlations with corresponding national evaluations for health traits were 0.72 for mastitis; 0.42, 0.33, and −0.05 between METAB and displaced abomasum, ketosis, and milk fever, respectively; 0.51 and 0.44 between REPRO and metritis and retained placenta, respectively; and 0.62 between died and livability. In conclusion, there is a heritable component to health traits in US organic cows and a favorable relationship with health traits evaluated nationally, such that organic dairy producers will be able to achieve genetic improvement in the health of their cows.

Key Words: health, organic, genomic

410 Genome-wide association study of horn fly resistance in organic Holstein cows.

B. L. Basiel*[1], L. C. Hardie[1], B. J. Heins[2], and C. D. Dechow[1], [1]*Pennsylvania State University, University Park, PA,* [2]*University of Minnesota, St. Paul, MN.*

The objective of this research was to identify genomic regions and candidate genes for horn fly resistance in organic Holstein cattle. Observations of fly load were recorded on 640 genotyped and 506 additional pastured Holstein cows with known pedigrees on 13 organic dairies. Fly load was determined using a 0 to 4 scale based on fly coverage on one side of the body where 0 indicated few to no flies and 4 indicated high infestation. Proportions of black and white coat coloration were observed on 390 individuals from photos taken during scoring. A multi-trait model of fly load, stayability, and proportion of coat color was used in single-step genomic analysis with the BLUPF90 family of programs. Stayability was included to account for bias if fly score was correlated with early-life survival and color was included because coat color was associated with fly load. Models varied by trait and included the random effect of cow. The color model included the fixed effect of herd-year-season of birth as did the stayability model in addition to the effect of parity and the random effects of herd-year-season of parity and permanent environment. The fly score model had fixed effects of scorer, the interaction of date scored and herd, stage of lactation by week, and parity. Estimated breeding values of the traits were decomposed by GWAS into SNP effects. The association between fly score (n = 435 observations of fly score from 233 cows with color evaluated) and a SNP variant of interest detected by GWAS was evaluated by a mixed linear model containing fixed effects of SNP variant, fly scorer, the interaction of date scored and herd, and a random effect of cow. The same model was run with percentage of black or white coat color as a covariate. A 1 MB region on BTA 6 explained the most variation (1.7%) in fly score and contained most of the significant SNP identified by GWAS. The only significant SNP located on a gene in this region, Hapmap27516-BTC-042465, was located on *KIT*, a gene associated with piebaldism. Variants of the SNP significantly influenced fly score where animals with 0 copies of the variant had the lowest fly score while those with 2 copies had the highest score. The SNP effect was nullified when color was added to the model and coloration tended to influence fly score with black cows having more flies than white cows. The results suggest that *KIT* is a likely candidate gene for horn fly resistance in Holstein cattle, potentially by altering coat color.

Key Words: fly, organic, *KIT*

411 Genomic analysis of visceral fat accumulation in Holstein cows.

L. C. Novo*[1], L. Cavani[1], P. Pinedo[2], P. Melendez[3], and F. Peñagaricano[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Colorado State University, Fort Collins, CO,* [3]*Texas Tech University, Amarillo, TX.*

Visceral fat is related to important metabolic processes, including insulin sensitivity and lipid mobilization. The goal of this study was to identify individual genes, pathways, and molecular processes implicated in visceral fat deposition in dairy cows. Data from 172 genotyped Holstein cows classified at slaughterhouse as having low (n = 77; omental fold <5mm in thickness and minimum fat deposition in omentum) or high (n = 95; omental fold >20mm in thickness and marked fat deposition in omentum) omental fat were analyzed. The identification of regions with significant additive and non-additive genetic effects was performed using a 2-step mixed model-based approach. Genomic scans were followed by gene-set analyses to reveal the genetic mechanisms controlling abdominal obesity. The association mapping revealed 4 regions located on BTA13, BTA19, BTA20 and BTA24 with significant additive effects. These regions harbor genes, such as *SMAD7*, *ANKRD55*, and the *HOXB* family, that are implicated in lipolysis and insulin tolerance. Two regions located on BTA1 and BTA6 showed marked non-additive effects. The region on BTA1 harbors genes *MRAP* and *MIS18A*, that are involved in energy balance and obesity. The gene-set analysis revealed functional terms, such as tyrosine-kinase receptors and negative regulation of fibroblast growth factor receptor 1 and 2, that could be implicated in visceral fat deposition. We further evaluated the genetic link between visceral fat and 2 metabolic diseases, ketosis and displaced abomasum. For this, we analyzed 28k records of incidence of diseases from 14k cows across lactations using a single-step genomic BLUP approach. Notably, the region on BTA20 significantly associated with visceral fat deposition was also associated with the incidence of displaced abomasum. However, the correlations between SNP effects among visceral fat and metabolic diseases were negligible. Overall, our findings suggest that visceral fat deposition in dairy cows is controlled by both additive and non-additive effects. We detected at least one region with marked pleiotropic effects affecting both visceral fat and displaced abomasum.

Key Words: abdominal fat, genomic scan, metabolic diseases

412 Hepatic mitochondrial function in 2 Holstein genotypes during early and mid-lactation in a pasture-based system.

M. Garcia-Roche*[1,2], D. Talmón[1], A. Mendoza[3], C. Quijano[2], A. Cassina[2], and M. Carriquiry[1], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay,* [2]*Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay,* [3]*INIA La Estanzuela, Colonia, Uruguay.*

To assess differences in hepatic mitochondrial function between Holstein genotypes, multiparous cows of New Zealand (NZH, n = 10) and North American (NAH, n = 10) origin (512 ± 19 vs. 563 ± 29 kg body weight, 3.1 ± 0.1 body condition score (BCS), fall calving) were used. Cows grazed a mixed pasture of *Medicago sativa* and *Dactylis glomerata* (8.7 ± 0.7 kg DM/ha in one grazing session during early lactation and 16.3 ± 1.1 kg DM/ha in 2 sessions during mid-lactation) and were supplemented with 7.3 ± 0.4 kg DM/d of concentrate and 4.4 ± 0.5 kg DM/d of corn silage and forage reserves during early lactation or 6.0 ± 0.4 kg DM/d of concentrate during mid-lactation. Liver biopsies were collected and cryopreserved at 21 and 180 ± 17 d postpartum (DPP) and mitochondrial function was assessed measuring oxygen consumption rates using: glutamate/malate (G/M), succinate (succ), palmitoyl-CoA/carnitine (p-CoA/car) and palmitoyl-carnitine (p-car). Data were analyzed using a mixed model that included DPP, genotype and their interaction as fixed effects. Milk yield was 5.3 kg/d higher ($P < 0.01$) for NAH vs. NZH cows but solid corrected milk yield did not differ ($P = 0.49$). Cow BCS was higher for NZH than NAH (2.74 vs. 2.67 ± 0.03, $P < 0.05$). Milk, fat and protein yield decreased from early to mid-lactation. State 3 respiration increased with the progress of lactation for all substrates (11 vs. 14 ± 1 for G/M, 34 vs. 45 ± 6 for succ, 6.2 vs. 10.3 ± 0.8 for p-CoA/car and 7 vs. 12 ± 1 pmolO_2/min/mg for p-car $P < 0.05$). Although maximum respiratory rate was not affected by DPP when G/M was used, it had a 1.7-fold increase ($P < 0.01$) from early to mid-lactation when p-car and p-CoA/car were used. State 3 and maximum respiratory rate in succinate-driven respiration were affected by the interaction between DPP and genotype ($P < 0.05$) as it was 2-fold greater for NZH vs. NAH cows at 180 DPP. Also, maximum respiratory rate when p-CoA/car was used tended to be higher for NAH than NZH (12 vs. 9 ± 1.4 pmolO_2/min/mg, $P = 0.09$). Our results point out to early-lactation mitochondrial function impairment and a preference

for gluconeogenic vs. fatty acids as ATP synthesis precursors for NZH and NAH, respectively.

Key Words: grazing, dairy

413 The effect of the interleukin-10 receptor alpha gene on bovine mammary epithelial cells infected with *Mycobacterium avium* ssp. *paratuberculosis*.

A. Fong*[1], M. M. M. Muniz[1], U. K. Shandilya[1], A. Sharma[1], F. S. Schenkel[1], N. A. Karrow[1], and C. F. Baes[1,2], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Johne's disease is a chronic wasting disease caused by the bacterium *Mycobacterium avium* subspecies *paratuberculosis* (MAP). It is highly contagious and eventually leads to death. Testing for Johne's disease in dairy cattle is often done with a milk ELISA test, as it is non-invasive, has a quick turn-around time, and is low-cost. However, it has low sensitivity (~30%), which can lead to false-negative results and it may not detect early stages of infection. There is currently no cure for Johne's disease and no vaccine is available in Canada. A vaccine has been approved for use in the United States of America however, it does not provide complete protection against the disease. In combination with improvements in management and vaccine development, genetic selection can further enhance control of this disease, as heritability for resistance to Johne's disease has been estimated to be around 6%. One candidate gene of particular interest for this disease is the gene encoding for the interleukin-10 receptor subunit α (IL10Rα). This is an anti-inflammatory cytokine that aids in tissue repair by regulating the antimicrobial activity of macrophages. The expression of IL10Rα is upregulated in response to MAP bacteria and this is suspected to help the survival of the bacteria. A bovine mammary epithelial cell line (MAC-T) was created with the gene encoding IL10Rα knocked out (IL10RαKO), using the CRISPR/cas9 method. This cell line was exposed to live MAP bacteria for 48 h, and thereafter mRNA was extracted from both infected and uninfected cells. The differentially expressed genes (DE) have been compared between the IL10RαKO cell line and the wild type MAC-T cell line. There are 561 DE genes between infected and uninfected IL10RαKO cells, 1613 DE genes between infected IL10RαKO cells and infected wild type MAC-T cells, as well as 1487 DE genes between uninfected IL10RαKO cells and uninfected wild type MAC-T cells. Gene ontology for the DE genes, as well as copy number variant detection will be performed. The results of this study will help to better understand how Johne's disease is affected by the IL10Rα gene, how it affects the animal, and how to incorporate resistance into the genomic evaluation.

Key Words: Johne's, IL10Rα, genetics

Dairy Foods: Processing

414 Stability, temperature dependency, and microstructure of high-pressure jet-treated dairy foams. G. L. Voronin*, J. N. Coupland, and F. M. Harte, *The Pennsylvania State University, University Park, PA.*

High pressure jet (HPJ) technology has recently been shown to improve foam stability in skim and whole milks. This improved functionality was related to the disruption of innate casein micelle structure at pressures ≥400 MPa, resulting in the release of surface-active casein protein monomers. The objective of the current work was to further investigate the exceptional foam stability of HPJ-treated whole milk by focusing on foam microstructure, temperature dependency, and composition. The ultimate goal is to create dairy foam products with improved foam rigidity and foam shelf life. HPJ-treated whole milk (400 MPa) was foamed at 5, 20, 40, and 50°C using a custom-made air injection device to determine the foam expansion and stability in comparison to a non-HPJ-treated control. In addition, aliquots of the HPJ-treated and non-HPJ-treated foams (at 5 and 50°C) were extracted for proximate analysis and visualization using confocal scanning laser microscopy (CSLM). All quantitative results were analyzed using one-way ANOVA to identify significance at a 0.05 level (n = 3, $P < 0.05$) with Tukey's test applied for mean comparisons. The foam expansion volumes for the non-HPJ-treated sample at 5, 20, 40, and 50°C were 35, 0, 64, and 70 mL, respectively. Notably, the non-HPJ-treated sample did not form a foam at 20°C, likely due to the partially crystalline milkfat disrupting foam formation. The HPJ-treated sample was able to develop a stable foam regardless of temperature with foam expansion volumes of 22, 48, 40, and 60 mL at 5, 20, 40, and 50°C, respectively. Furthermore, the HPJ-treated foam was >10-fold more stable than the non-HPJ-treated foam at all foaming temperatures. CSLM of the non-HPJ-treated sample revealed that there was more fat present in the foam formed at 50°C than at 5°C, likely due to the ability of liquid fat (at 50°C) to migrate to the air-water interface during air incorporation. In contrast, the 400 MPa-treated foam had fat and protein present at both 5 and 50°C as well as micron-sized bubbles. A HPJ-treated foam has many potential industry applications including the formation of stable dairy foams in a range of products including milk froths and whipped cream.

Key Words: high pressure jet, whole milk, foam

415 Understanding the effect of homogenization pressure on shelf life and foam properties of cream foamed by effervescence. M. Blankart* and J. Hinrichs, *University of Hohenheim, Stuttgart, Germany.*

Aerosol whipping cream (AWC), a dairy product which is homogenized in the presence of an emulsifier to achieve shelf lives of up to 6 mo, offers the possibility to generate a foam with minimal effort in a matter of seconds by effervescence. Homogenization was shown to affect foam build up and foam stability of dairy products such as mechanically whipped cream and ice cream, but no such studies have been conducted for AWC. We hypothesize, that too low or too high homogenization pressures negatively affect the emulsion and foam properties of AWC. Skim milk and cream were mixed to a fat content of 30% (wt/wt), heated in a water bath and 0.4 g saturated monoacylglyceride per 100 g was added. After equilibration at 80°C for 5 min, a pre-emulsion was generated with a ring gear dispersion unit. Then, homogenization at pressure settings between 0 and 24 MPa in single stage and double stage homogenization was conducted to generate AWC. Particle size distribution, viscosity and foam properties were measured. For all analysis, a one-way ANOVA with subsequent Tukey test was performed ($P < 0.05$). The diameter of fat globules decreased significantly ($P < 0.05$) with increasing homogenization pressure, until a homogenization pressure of 6/1 MPa (first stage/second stage) was exceeded. Homogenization pressures above the determined threshold led to a decrease in foam stability ($P < 0.05$). Microscopic images revealed that excessive homogenization led to flocculation of fat globules, which reduced the foam stability of AWC due to slower adsorption onto the gas-liquid interface. The results of the study provide insight into the stabilization mechanism of AWC and may help to adapt the processing and the composition of AWC to meet different demands.

Key Words: aerosol whipping cream, homogenization pressure, emulsion/foam stability

416 Vacuum microwave drying of concentrated milk: Process optimization and powder functionality. J. Dumpler* and C. I. Moraru, *Department of Food Science, Cornell University, Ithaca, NY.*

Vacuum microwave drying (VMD) has evolved as an alternative to traditional drying methods. VMD is a relatively gentle drying process that leads to low Maillard browning, heat damage, or off-flavors in the dried product. The interaction of multiple processing parameters (microwave power, vacuum level, dielectric properties, shape, size, product structure, and texture) have contributed to the limited application of this promising technology in the dairy industry to date. To explore its potential for dairy applications, we investigated the use of VMD (NutraREV, Enwave, BC, Canada) for drying concentrated skim milk, obtained by reconstituting nonfat dried milk to 37.5% total solids. A custom factorial design was used to optimize drying parameters with respect to product properties and drying efficiency. Vacuum level (33 - 133 mbar), specific power input (0.87 – 1.75 W g^{-1}), and product layer thickness (1 – 4 mm) were studied as factors. Drying time, product foaming, maximum product temperature, browning, solubility, and product yield were used to identify optimal VMD processing parameters. Drying conditions that maximized yield and minimized drying time while maintaining a good product quality were identified as 2 mm layer thickness, 60 mbar, and specific microwave power input of 1.29 W g^{-1}. Regression analysis indicated that layer thickness was the most important parameter ($P < 0.01$) to limit the product temperature to below 55°C in the final drying stage. This allowed good solubility indices of the powder (<0.3 mL) to be achieved. Specific energy input and vacuum level did not affect solubility ($P > 0.1$), but significantly affected foaming, as did layer thickness ($P \ll 0.01$). The average whey protein nitrogen index (WPNI) of the VMD powder was only 2.5% lower than of the initial skim milk powder ($P < 0.01$) and no correlation between WPNI and VMD drying conditions could be established. These findings can be useful for dairy processors interested in adopting VMD as a versatile drying process for low heat milk powders, starter cultures, products that contain probiotics, or infant formula.

Key Words: vacuum microwave drying, concentrated skim milk, milk powder

417 Impact of concentration and temperature on flow behavior of lactose and protein-rich reconstituted dairy powders. F. Z. B. Seighalani and P. Sharma*, *Utah State University, Logan, UT.*

Increasing solid content in the dryer feed could be an effective strategy for minimizing the energy consumption and reducing cost associated with spray drying of various dairy powders. For optimal dryer performance, it is important to understand flow behavior of high solids loadings to the dryer. In this study, we investigated flow behavior of reconstituted high-lactose and high-protein dairy powders at varying concentrations and temperatures. Lactose-rich samples including skim milk (SM), whey protein concentrate (WPC35), and sweet whey (SW) powders were reconstituted in triplicate to the maximum possible concentrations i.e., 50%, 60%, and 50% solids respectively. Protein-rich powders, micellar casein concentrate (MCC), whey protein concentrate (WPC80), and whey protein isolate (WPI) powders were reconstituted to range of 30–40% solids, 30–50% solids, and 40–60% solids, respectively. Optical microscopy was used to observe state of lactose crystal in the reconstituted samples. Steady shear flow behavior of the samples was investigated using Anton Paar MCR302 rheometer equipped with parallel plate (d = 25 mm) using shear rate sweep ranging from 0.01 to 100 s^{-1} at 25 and 50°C. One-way ANOVA was used to determine significant differences between samples. Herschel–Bulkley model was fitted on rheological data to obtain yield stress, consistency coefficient (K), flow behavior index (n), and apparent viscosity of the reconstituted samples. Herschel–Bulkley model could not be fitted on lactose-rich samples because of presence of big lactose crystals as observed in the microscopic images. Protein-rich dairy powders showed shear-thinning behavior fitting well to Herschel–Bulkley model (R^2~0.99). With increasing concentration and decreasing temperature, WPC and WPI solutions exhibited relatively more shear-thinning behavior (n = 0.32 at 60% WPC and n = 0.23 at 50% WPI), while it was vice versa for MCC samples (n = 0.76 in 35% MCC), indicating shear induced structuring. K value of MCC solution (632 $Pa.s^n$) even at 30% solids was higher than both 60% WPC80 (170 $Pa.s^n$) and 50% WPI (79 $Pa.s^n$). With increase in concentration and decrease in temperature, yield stress increased significantly ($P < 0.05$) in all samples except 60% WPC80 solution. Overall, the finding provides a fundamental knowledge on flow behavior of lactose-rich and protein-rich dairy powders useful for both academic and industrial dairy powder research.

Key Words: dairy powders, flow behavior, dairy proteins

418 Customized phase treatment for shelf-stable milk using microfiltration and microwave heating: Effects of processing and storage.

B. Graf*, M. Rosenberger, J. Schaefer, and J. Hinrichs, *University of Hohenheim, Stuttgart, Germany.*

Indirect ultra-high temperature (UHT) heating of milk involves limited plant running times due to fouling, whey protein denaturation ≥95%, and a characteristic cooked flavor. However, indirect pre-heating at 95°C for 128 s ensures a shelf life of milk of ≥6 mo at room temperature as it reduces the activity of plasmin, an indigenous, protein degrading milk enzyme. Contrarily, direct heating through steam injection involves less whey protein denaturation but has a limited shelf life due to residual plasmin activity. We hypothesize that customized phase treatment (CPT) of milk, involving microfiltration and separate heating of retentate and permeate, results in a shelf-stable milk with low plasmin activity and decreased whey protein denaturation. Skim milk was microfiltered (pore size 0.1 µm) and retentate, containing caseins and plasmin was indirectly UHT heated (140°C, 5 s) with long pre-heating. Permeate, containing whey proteins, was directly heated by microwave technology (120°C, 5 s). Finally, both phases were sterilely mixed and filled. Directly (steam injection) and indirectly heated UHT milk served as references. Whey protein denaturation was analyzed after production while particle sizes, viscosity, and apparent plasmin activity were monitored during storage at 20 and 30°C for 6 mo. For all analysis, a one-way ANOVA with subsequent Tukey test was performed ($P < 0.05$). CPT milk showed whey protein denaturation in-between direct ($P = 0.005$) and indirect ($P = 0.003$) UHT milk. Direct UHT milk showed particle formation (after 2 mo at 30°C) and age gelation (after 3 mo at 20°C) during storage. Apparent plasmin activity was elevated in direct UHT milk (0.31–0.37 pkat/mL) compared with indirect ($P < 0.001$) and CPT milk (0.03–0.04 pkat/mL, $P < 0.001$), while the last 2 were comparable ($P = 0.933$). Finally, the new process presents an alternative to produce shelf-stable milk with low plasmin activity and reduced whey protein denaturation.

Key Words: direct heating, long-life milk, microwave technology

419 EAAP Exchange Symposium Speaker Presentation: Enteric methane mitigation in dairy cattle via feeding strategies and the impact on the carbon footprint D. Van Wesemael*[1], L. Vandaele[1], J. Van Mullem[1], S. De Campeneere[1], V. Peiren[2], and N. Peiren[1], [1]*Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Animal Sciences Unit, Melle, Belgium,* [2]*Ghent University, Department of Animal Sciences and Aquatic Ecology, Laboratory for Animal Nutrition and Animal Product Quality, Gent, Belgium.*

On a global level, cattle (beef and dairy) generate 65% of the total livestock greenhouse gas emissions, mainly due to the emission of enteric methane (CH_4) (Gerber et al., 2013). The enteric CH_4 production from ruminants is mainly driven by the feed composition and by the level of feed intake, and thus feeding strategies are considered most promising for CH_4 mitigation. Examples are the use of feed additives, lipids in the diet, increasing starch content, reducing fiber content, etc. However, the production of feeds for (dairy) cattle is the largest or second largest source of GHG emissions from the dairy sector depending on the applied diet. Therefore, this should be evaluated together with the CH_4 mitigating potential of feeding strategies. The aim of the presented research was to assess the potential of different feeding strategies to reduce overall environmental impact of dairy production, by reducing enteric CH_4 emissions and/or the carbon footprint of feed production, without losing sight of performance parameters. Feeding strategies that were evaluated were: 1) the use of the feed additive 3-NOP in 2 different ways of supplementation; 2) the replacement of grass silage with maize silage and 3) the (combined) use of brewers' grains and rapeseed meal as a replacement of soybean meal. The first strategy is proven to be effective in reducing enteric CH_4 emissions and the impact on the carbon footprint of feed production is small. In our experiment the replacement of grass silage with maize silage, in diets balanced for net energy and true protein digestible in the small intestine, did not influence enteric CH_4 emissions nor did it affect the carbon footprint of feed production. Finally, the (combined) use of brewers' grains and rapeseed meal showed very promising in reducing the carbon footprint of feed production, because of the replacement of soybean meal. But there was also an effect on the enteric CH_4 emissions when rapeseed meal was used.

Growth and Development

420 Gradual weaning based on dry matter intake: Effects on feeding behavior and weight gains. A. Welk*, H. W. Neave, M. A. G. von Keyserlingk, and D. M. Weary, *University of British Columbia, Vancouver, BC, Canada.*

The aim of this study was to compare feeding behavior and performance of dairy calves (n = 108) weaned using 3 methods: based on age (WA), individual DMI (WI), or a combination of individual DMI and age (WIA). All calves were offered 12 L/d of milk until d 30; milk was then gradually reduced, over a 3-d period, by 25% of the individual's average milk intake. For WA calves, milk allowance then remained stable until d 62; allowance was then reduced over 8 d such that calves were fully weaned by d 70. For WI calves, milk allowance declined when calves reached different targets for solid feed intake; milk was reduced by 25% (each time over a 3-d period) once calves consumed a 3-d rolling average of 200 g/d, 600 g/d, and (finally) 1150 g/d (DMI of forage and starter combined). WIA calves were only required to reach the first target (a 3-d rolling average of 200 g/d DM); milk allowance was then reduced linearly until weaning at d 70. WI and WIA calves that failed to reach the final DMI target by d 62 (FI calves, n = 10) were force weaned over 8 d to complete weaning at d 70. Milk and starter intake, and unrewarded visits at milk feeder were calculated over the total experimental period (d 2 - 84). WI and WIA calves had greater final BW than WA calves, while FI calves had the lowest final BW (123.1 vs 121.5 vs 117.2 vs 100.5 ± 3.1 kg, respectively). WI calves ate more total starter and consumed less total milk than WIA, WA and FI calves (starter: 86.3 vs 72.9 vs 51.5 vs 37.4 ± 5.7 kg, respectively; milk: 362.9 vs 428.4 vs 497.7 vs 472.7 ± 16.1 L, respectively). Total unrewarded visits were greatest for WI and WIA calves compared with WA and FI calves (456 vs 498 vs 373 vs 359 ± 53 visits, respectively). These results suggest that weaning using a DMI target can improve starter intake and final BW, but the high unrewarded visits to the milk feeder suggest that refinements to these milk reduction methods are required to reduce signs of hunger during weaning. Specialized weaning methods should be developed for calves that fail to consume sufficient starter by 62 d of age.

Key Words: individualized weaning, performance, precision dairy

421 Do different types of prolonged cow-calf contact affect dairy calves' health and growth? M. L. Wenker*[1], C. M. Verwer[2], E. A. M. Bokkers[1], and C. G. van Reenen[3], [1]*Wageningen University & Research, Wageningen, the Netherlands,* [2]*Louis Bolk Institute, Bunnik, the Netherlands,* [3]*Wageningen Livestock Reseach, Wageningen, the Netherlands.*

The dairy industry is faced with the challenge of sometimes high morbidity levels in young calves. As maternal care is usually essential for the survival of mammalian offspring, it has been suggested that prolonged contact with the dam may be beneficial for health and growth of calves. However, knowledge on the effects of different types of cow-calf contact on dairy calves' health and development is limited. The objective was to evaluate health and growth of dairy calves reared in 3 different systems. Therefore, cows either: i) had no contact with their calf (NC); the calf was removed from the dam directly after birth (n = 10), ii) were allowed to have partial contact (PC) with their calf; the calf was housed in a calf pen adjacent to the cow area allowing physical contact on initiative of the dam but no suckling (n = 18), or iii) were allowed to have full contact (FC) with their calf including suckling; calves were housed together with their dams in a freestall barn (n = 20). Weekly, calves were weighed and their health was assessed using a standardized clinical health protocol (scoring intensity of neonatal diarrhea, respiratory issues, pyrexia, and navel inflammation from 0 to 3) for each calf until 48 ± 1 d of age. A total health score (ranging from 0 to a maximum of 22) was calculated based on these clinical assessments, summarizing length and intensity of detected health problems. Health and weight data were analyzed using a generalized linear model. The total health score of NC calves (mean ± SE, 2.9 ± 0.4) did not significantly differ from that of PC (4.1 ± 0.5) or FC calves (4.9 ± 0.7) ($P > 0.05$). However, a 3-d antimicrobial treatment was needed for 5 PC and 6 FC calves whereas none of the NC calves were treated with antimicrobials (non-significant differences, $P > 0.05$, Fisher's exact test). FC calves had a greater average daily gain (kg) (mean ± SE, 0.97 ± 0.04 kg) compared with both NC (0.73 ± 0.02 kg) and PC calves (0.71 ± 0.05 kg) ($P = 0.001$). These results indicate that cow-calf contact did not reduce morbidity in comparison with no contact. Full contact with the dam improved the growth rate of calves.

Key Words: calf rearing, clinical assessment, suckling

422 Maternal Cu, Mn, and Zn supplementation alters muscle morphometry and innate immune response in lambs. H. Tucker*, J. Chen, S. Bettis, K. Cook, D. Hancock, and M. Vazquez-Anon, *Novus International, St Charles, MO.*

Trace minerals, Cu, Mn, and Zn, play a key role in fetal development, being required for immunity and muscle development. However, little is known about the effects of maternal Cu, Mn, and Zn nutrition on muscle and immune development of offspring. The objective of this trial was to evaluate effects of trace mineral source during gestation on changes to muscle and immunity of progeny lambs. Ewes were allocated by body weight to receive: no supplemental Cu, Mn, or Zn (CON); supplying 150% of NRC requirement of Cu, Mn, and Zn as sulfates (ITM) or as chelates (MINTREX trace mineral, Novus International; MMHAC). Treatments were fed 37 d before ram introduction and through parturition, totaling 190 d (±10.5 d) of supplementation. Following parturition, one lamb per ewe was weighed and harvested. From each lamb, longissimus dorsi (LD) and thymus were weighed and sampled for gene expression measures. Statistical analysis was done using MIXED procedure of SAS 9.4. Supplementation of MMHAC resulted in greater ewe body weight ($P < 0.01$) than CON and ITM. Lamb body weight ($P = 0.79$), and weight of thymus ($P = 0.87$) and LD ($P = 0.30$) did not differ. Though LD weight did not differ, MMHAC altered LD morphometry resulting in greater ($P < 0.01$) fiber area, ellipse major, and ellipse minor. Moreover, gene expression in LD resulted in FoxO3 tending to be greater ($P < 0.06$) with MMHAC than CON and ITM, while IGF1 was greater ($P < 0.05$) in MMHAC than ITM. Increased IGF1 and FoxO3 reflect upregulation of synthesis and degradation in muscle. This suggests a role for maternal supplementation of MMHAC to improve morphometry while upregulating LD metabolism. In thymus of lambs, expression of IκBKB ($P < 0.05$) and A20 ($P < 0.07$) were greater in MMHAC than CON, suggesting MMHAC reduces inflammation in thymus. Moreover, TLR2 ($P < 0.05$) and TLR4 ($P < 0.01$) were greater with MMHAC than CON and ITM indicating an improved innate immune response. Together these data suggest supplementing MMHAC through gestation increases maternal BW while reducing progeny thymic inflammation and improving innate immunity response and muscle fiber size of resulting progeny.

Key Words: trace mineral, muscle, immunity

423 Effect of a whey-based starter on calf performance when fed different milk levels.

S. D. Parsons*[1], K. E. Leslie[2], M. A. Steele[1], D. L. Renaud[2], 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.*

The objective of this study was to investigate how a high lactose, whey-based starter affects dairy calf feed intakes, growth, and rumen development compared with a grain-based starter, when fed 1 of 2 levels of milk replacer. A total of 120 Holstein heifer calves were individually housed and randomly assigned preweaning (d 1–42) to 1 of 4 treatment combinations in a factorial design: high milk replacer (HM; 15 L/d (150g/L)) or low milk (LM; 6 L/d (150g/L)) and either a grain-based ration (GF; 95% starter pellet (20% CP, 2% CF), 5% chopped wheat straw) or a whey-based starter pellet (WF; 21% CP, 12% CF). The WF treatment calves were offered 150g/d of the whey-based pellet until they consumed the entire 150g on 2 of 3 consecutive d, then the whey-based pellet was top-dressed onto the grain-based ration to allow for ad libitum consumption. After gradual weaning (d 43–63), all calves were offered the same grain-based ration for the postweaning period (d 64–77). Feed intakes were recorded daily, BW and size was measured weekly, and rumen fluid sampled on d 36, 57, 70, and 77. Data were analyzed with repeated-measures mixed-effect linear models. Irrespective of milk treatment, the WF calves tended to have greater solid feed DMI than GF calves during the preweaning phase (0.2 vs. 0.1 kg/d, SE = 0.01; $P = 0.06$). Feed intake did not differ between feed treatments during weaning (WF = 1.3 kg/d, GF = 1.3 kg/d; SE = 0.03; $P = 0.9$) or postweaning (WF = 3.5 kg/d, GF = 3.4 kg/d; SE = 0.09; $P = 0.6$). Growth did not differ by feed treatment either preweaning ($P = 0.9$), weaning ($P = 0.7$), or postweaning ($P = 0.8$). Heart girth gain tended to be greater preweaning for LM and WF treatment calves compared with LM and GF treatment calves (0.42 vs. 0.38 cm/d, SE = 0.02; $P = 0.08$). Total ruminal VFA concentrations were similar for both feed treatments ($P = 0.6$), but WF calves had a greater proportion of valeric acid than GF calves on d 36 (2.5 vs. 1.7%, SE = 0.17, $P < 0.01$) and d 57 (3.4 vs. 2.7%, SE = 0.18, $P < 0.01$). The results indicate the WF treatment, regardless of milk allowance, may encourage greater early feed consumption, which may increase certain ruminal VFA proportions before weaning.

Key Words: dairy calf, concentrate, lactose

424 Effects of feeding medium-chain fatty acids on the adaptive immune response of Holstein dairy calves during the first 60 days of life.

R. N. Klopp*[1], J. R. Hernandez Franco[2], H. Hogen-Esch[2], T. S. Dennis[3], K. E. Cowles[3], and J. P. Boerman[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Department of Comparative Pathology, Purdue University, West Lafayette, IN,* [3]*Nurture Research Center, Cargill Animal Nutrition, Lewisburg, OH.*

Medium-chain fatty acids (MCFA) have been shown to have antimicrobial and antiviral effects, which may improve calf health. The objective of this study was to determine if feeding C8:0-C10:0 to dairy calves improves their health status by targeting the systemic adaptive immune system. Twenty-two Holstein bull calves (3–4 d of age) were assigned to either CON (24% CP:17% fat milk replacer (MR) and 18% CP calf starter) or MCFA (24% CP:17% fat MR with 0.5% MCFA oil added and 18% CP calf starter with 0.75% MCFA oil added). A blood sample was collected on d 1 for baseline antibody levels. On d 16, nasal and fecal samples were collected to determine viral and bacterial shedding and calves were injected intramuscularly with 1 mL of ovalbumin (OVA; 0.2 mg) combined with aluminum hydroxide adjuvant (1 mg Al^{3+}) with a second dose on d 37. On d 37 and 51, fecal samples, nasal swabs, and blood samples were collected. Blood samples collected on d 37 were used to measure serum anti-OVA IgG1 and IgG2; samples collected on d 51 were used to analyze serum antibodies and determine IFN-γ and IL-4 secretion by peripheral blood mononuclear cells (PBMCs) upon stimulation with OVA or phytohemagglutinin (PHA). Data were analyzed as a completely randomized design in SAS v.9.4. No differences were observed for nasal viral and bacterial shedding but MCFA had an increased incidence of *Salmonella* in fecal samples at d 37 ($P = 0.04$) compared with CON calves. Incubation of PBMCs with OVA or PHA increased IFN-γ and IL-4 secretion ($P < 0.05$), but there were no differences between treatment groups. Serum anti-OVA IgG1 increased at d 37 compared with baseline ($P < 0.01$), and was further increased on d 51 ($P < 0.01$) with no treatment differences observed. Serum anti-OVA IgG2 was increased at d 37 compared with baseline for CON ($P < 0.01$) but not for MCFA, however, by d 51, IgG2 for both groups were increased compared with baseline ($P < 0.01$). In conclusion, we observed increases in cytokine and antibody production in response to the vaccine challenge but supplementation with MCFA did not significantly affect the response.

Key Words: calf, immune status, medium-chain fatty acids

425 Effects of feeding medium-chain fatty acids on the growth performance and health of Holstein dairy calves through 60 days of age.

R. N. Klopp*[1], T. S. Dennis[2], K. E. Cowles[2], and J. P. Boerman[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Nurture Research Center, Cargill Animal Nutrition, Lewisburg, OH.*

Prior research shows that medium-chain fatty acids (MCFA) can improve health status and growth in dairy calves. The objective of this study was to determine the effect of feeding C8:0-C10:0 to dairy calves on growth performance and health. Thirty-two Holstein bull calves were assigned to one of 2 treatments; CON (24% CP: 17% fat milk replacer (MR) and 18% CP calf starter) or MCFA (24% CP: 17% fat MR with 0.5% C8:0-C10:0 oil added and 18% CP calf starter with 0.75% C8:0-C10:0 oil added). Blood samples were collected on d 1 to estimate serum total protein (STP). Calves were offered 2.84 L (13% solids) of MR twice daily from d 1 to 7, 3.79 L of MR twice daily from d 8 to 42, and 3.79 L of MR once daily from d 43 to 49. Individual body weight (BW) was measured weekly and used to calculate average daily gain (ADG). We calculated change in body condition score (BCS), hip width (HW), hip height (HH), heart girth (HG), and paunch girth (PG) based on d 1 and d 57 measurements. Intake and fecal score were recorded daily and all medical treatments were documented. A blood sample was collected from each calf 4 h post AM feeding on d 42, 49, and 56, and NEFA, BHBA, insulin, and glucose were measured. Data were analyzed as a completely randomized design in SAS v.9.4 with repeated measures when applicable. We observed a tendency for a treatment effect on BW at d 57 ($P = 0.08$), where MCFA calves weighed more than CON (82.2 vs. 78.3 kg). During wk 8, ADG was significantly increased ($P = 0.02$) for MCFA calves (1.20 vs. 0.95 kg/d). No differences were observed for change in BCS, HW, HH, HG, or PG. There were no differences between treatments for intake or feed efficiency. No differences were observed for initial STP, medical days, treatments for respiratory disease, or scouring days (fecal score >2). At d 42, NEFA was reduced ($P = 0.001$) for MCFA calves. This data suggests that feeding MCFA to calves can improve ADG and energy status before weaning.

Key Words: calf, growth, medium-chain fatty acids

426 Peripheral blood mononuclear cell mitochondrial enzyme activity in dairy heifers from birth to lactation.

A. M. Niesen* and H. A. Rossow, *University of California-Davis, Davis, CA.*

Mitochondria are central to metabolism, nutrition, health and are the primary energy producers for all biosynthesis. The objective of this study was to determine if mitochondrial enzyme activity rates of peripheral blood mononuclear cells (PBMC) changed over time and if calf enzymatic activity indicated survivability as an adult. Twenty-three Holstein and 23 Jersey heifers were enrolled, and data were collected from age 4 d to 2 yr on surviving heifers. Blood samples were collected at 1, 2, 9, 36, 52 wk and 2 yr of age to determine complete blood counts as an indicator of health, plasma total protein (TP, g/dl) and to isolate the PBMC fraction. Mitochondrial isolation from PBMC and enzyme activities for citrate synthase, complex I, complex IV, and complex V were determined using kits from Abcam (Cambridge, MA). Activity rates were compared by time point, breed, reproductive success, and survivability over the 2 yr period. Data were analyzed using the MIXED procedure of SAS (v.9.4) with breed as a fixed effect and time point as a random effect. Age significantly affected activity in all complexes ($P < 0.01$) with a general trend of increased activity from birth to early lactation (65 ± 10 DIM) but breed was not different. Heifers that were culled for breeding, health events or died before their first lactation had lower complex V activity in the first week of life ($P = 0.001$) and lower citrate synthase activity during the second week of life ($P < 0.02$). When comparing heifers culled between 12 mo and 2 yr, heifers that failed to conceive tended to have greater citrate synthase activity compared with those culled for health events. These findings suggest that differences in heifer calf PBMC mitochondrial enzyme activity during the first 2 weeks of life predicts survivability and could identify heifers for early culling, ultimately reducing costs of raising animals that will fail to thrive.

Key Words: mitochondria, PBMC, survival

427 Morphological remodeling of adipocytes in mammary glands of dry period of dairy goats.

N. Jiang*, J. Liu, and H. Shi, *Institute of Dairy Science, College of Animal Science, Zhejiang University, Hangzhou, China.*

The objective of this study was to investigate the morphological remodeling of adipocytes to underscore its role in the remodeling of ruminant mammary gland. Adipose tissues are major functional mammary components and play an important role in the development of mammary gland. However, it is not well known on how the adipose tissues exhibit morphological and functional changes during the turnover of involution to lactation in dairy livestock. In the current study, the mammary tissues were biopsied from 3 pregnant and dry dairy goat (Saanen) at wk −8 (beginning of dry period), −4, −1, +1, and +4 related to lambing. These tissues were fixed for immunofluorescent staining and transmission electron microscopy. Adipose and epithelial cells were stained with polyclonal antibodies against adiponectin and cytokeratin 18, respectively. Cell nucleus and cellular lipid droplets were stained by DAPI and Bodipy, respectively. The numbers of adipose and epithelial cells were counted using the image J software. One-way ANOVA was used for statistical comparisons among the different periods by SPSS Statistics. The adipose cells accounted for low proportion of the mammary tissues (average total ratio <0.05), but its proportion (about 0.07) was the highest at wk −4 than at other times with no difference among wk −8, −1, +1, and +4. The ratio of the epithelial cells was the lowest at wk −4, and increased significantly at wk −1($P < 0.05$), +1($P < 0.05$), and +4 ($P < 0.01$). The results of Bodipy staining showed that huge lipid droplets were found in the cinar cavity at wk −8, +1, and +4. However, the expected Bodipy staining for adipose cells was negligible for most of the tissues, and only showed a few lipid droplets. Combated with the immunofluorescent staining against adiponectin, the data of Bodipy staining suggested an immature precursor status of adipocytes during the turnover of involution and lactation in dairy goats. This result was confirmed by the observation of transmission electron microscopy. Endoplasmic reticulum structure was still abundant at wk −4, and vesicle-like secretions were found in the immature adipose cell. Collectively, our data provided direct evidence that mammary adipocytes undergo morphological changes in mammary gland of the dry dairy goats, suggesting a role of adipocytes in the remodeling of mammary gland.

Key Words: adipocytes, morphological alternation, mammary gland remodeling

Lactation Biology II

428 Milk metabolites: A novel noninvasive approach to assess energy balance in dairy cattle.

A. Leduc*[1,3], S. Souchet[1,3], F. Le Provost[2], A. Fischer[1,3], P. Faverdin[1], J. Pires[4], M. Gelé[3], and M. Boutinaud[1], [1]*INRAE, Institut Agro, PEGASE, Saint-Gilles, France,* [2]*Université Paris-Saclay, INRAE, AgroParisTech, GABI, Jouy-en-Josas, France,* [3]*Institut de l'Elevage, Beaucouzé, France,* [4]*INRAE, Université Clermont Auvergne, VetAgro Sup, UMR H, Saint-Genès-Champanelle, France.*

In dairy cattle, negative energy balance (NEB) can occur in early lactation or during feed restriction. It can negatively impact animal health and milk production. The aim of this study was to identify NEB biomarkers in milk. We hypothesized that mammary gland metabolism, which is impacted by feeding, can be evaluated through the measure of selected milk metabolite concentrations. Thirty Holstein dairy cows were studied both in early lactation (21 DIM), and in mid-lactation (173 DIM), during a 5-week feed restriction experiment. The feed restriction protocol aimed to reduce milk production by 20%, and to induce body reserves mobilization, i.e., a NEB. Thus, the diet was diluted with wheat hay to reduce the energy density and the diet digestibility by about 10%. Milk samples were collected at 21 DIM, before and during the first week of feed restriction. Data were analyzed by mixed models that included time, parity, and their interaction as fixed effects, and cow as random effect. NEB was observed in early lactation (−5.3 Mcal/d) and several milk metabolite concentrations varied: galactose (−52%, $P < 0.001$), glucose (−25%, $P < 0.001$) and glucose-6-phosphate (+291% $P < 0.001$) compared with during mid-lactation. The accumulation of glucose-6-phosphate may be due to a blockage of the pentose phosphate and isocitrate dehydrogenase pathways. Feed restriction at mid-lactation did not lead to a NEB. Milk production decreased by 10% ($P < 0.001$). Several milk metabolite concentrations also decreased as early as the first week of feed restriction: galactose (−24%, $P < 0.001$), glucose (−17%, $P < 0.001$), glucose-6-phosphate (−9%, $P < 0.005$), glutamate (−19%, $P < 0.001$) and isocitrate (−13%, $P < 0.001$). These variations suggest a global slowdown of mammary epithelial cell metabolism. The feed restriction model used in this study was not as severe as expected because feed intake remained high despite the reduced digestibility. Nonetheless, milk metabolite concentrations were still affected, showing their sensibility to energy intake variations. This study shows that milk metabolite concentration vary both with NEB at early lactation and feed level. Glucose-6-phosphate concentration varies differently between conditions suggesting that its increase may reflect a NEB state. These results need to be validated, and suggest new ways of using milk metabolites as markers of dairy cow energy state.

Key Words: biomarker, feed restriction, bovine milk

429 In utero hyperthermia impacts early-life dairy calf mammary development.

B. Dado-Senn*[1], S. L. Field[1], B. D. Davidson[1], A. J. Geiger[2], G. E. Dahl[3], and J. Laporta[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Research and Nutritional Services Department, Zinpro Corporation, Eden Prairie, MN,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Dairy heifers born to dams heat-stressed in late gestation produce less milk across multiple lactations. Herein, the objective was to characterize differences in early-life mammary gland morphology and microstructure of in utero heat-stressed or cooled heifers. Calves were born to dams that were either heat-stressed (IUHT, shade, n = 41) or cooled (IUCL, shade plus fans and soakers, n = 41) for the last ~54 d of gestation where THI remained > 68. Upon birth, calves were weighed (BW) and fed 3.78 L high-quality colostrum. Calves were reared identically from birth to weaning, fed 0.90 kg/d milk replacer and ad libitum grain and water with half-milk weaning from 49 to 56 d. Subsets of calves were euthanized at birth (n = 8/treatment, ~5 h post-birth, no colostrum) and after weaning (n = 8/treatment, 63 d, fasted) to harvest the mammary gland (MG). Gross MG measurements were taken, and trimmed MG, parenchyma (PAR), and fat pad (FP) portions were weighed. Histological analysis was assessed on left rear teat PAR for H&E staining. Number and area of ductal (i.e., duct with lumen surrounded by epithelium) and epithelial (i.e., epithelial cell cluster with or without a lumen) structures within the PAR were quantified in ImageJ (average of four 4× images). Data were analyzed using PROC MIXED in SAS. Despite similar BW, IUHT calves had a smaller MG at birth (103 vs. 120 ± 6 g, $P = 0.05$) and after weaning (193 vs. 247 ± 16 g, $P = 0.03$) relative to IUCL calves. At birth, IUHT calves had a shorter MG length, distance from front to rear teats, and distance between front teats ($P \leq 0.09$); MG length was also shorter after weaning ($P = 0.02$). Further, PAR and FP tissue portions weighed less in IUHT heifers at birth but only FP was lighter at weaning ($P \leq 0.03$). Histological analysis revealed that IUHT calves had reduced PAR ductal and epithelial structure number and area at birth ($P \leq 0.09$). After weaning, IUHT heifers had lesser ductal area (36 vs. 98 ± 13 μm^2; $P < 0.01$) but greater non-luminal epithelial area (13 vs. 9 ± 1 μm^2; $P = 0.06$) relative to IUCL calves. In utero heat stress impacts mammary gland size and development of epithelial structures with potential long-term consequences on milk synthesis.

Key Words: histology, heat stress

430 Mammary gland transcriptome analysis in lactating cows reveals differential mechanisms associated with milk energy efficiency and the response to high- and low-starch diets.

X. Dai*[1,3], W. Li[1], A. Fischer[2], and K. F. Kalscheur[1], [1]*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.,* [2]*Institut de l'élevage, Beaucouzé, France,* [3]*Royal Veterinary College, University of London, Hatfield, UK.*

The objectives of this study were to identify key differences in gene expression and metabolic pathways in the mammary gland associated with feed efficiency of lactating cows fed high (HS; 27% starch) or low starch (LS; 13% starch) diets. Ten lactating Holstein cows were involved in a crossover design experiment consisting of 2 56-d treatment periods. Milk energy efficiency (Rmilk) was measured by the residual of predicting net energy in milk within each experimental period. The 3 highest cows were considered high Rmilk (HRmilk), the 3 lowest Rmilk cows were considered low (LRmilk), and the remaining 4 cows were considered medium Rmilk (MRmilk). At each period, mammary gland biopsies were sampled and used for RNA-sequencing. Raw reads were mapped against the cattle genome (UMD3.1) using STAR. Significant differential gene expression (DEG) (fold-change ≥1.5 and $P \leq 0.05$) was identified using DESeq2. Gene-set enrichment analysis was performed to identify biological processes and molecular pathways using the DEGs. For the comparison between HS and LS, 715 and 487 DEGs were identified in periods 1 and 2, respectively. The pathways enriched by the DEGs in period 1 were mainly involved in immune response while the ones enriched in period 2 were mainly involved in the molecular pathways related to developmental processes and mechanisms associated with inflammation. For the comparison between HRmilk and LRmilk, the 218 DEGs identified in period 1 were enriched in biological pathways contributing to cell development. A much bigger list of DEGs (2,481) was identified in period 2. These genes were associated with the biological pathways of oxidation-reduction and carbon metabolism. Despite the significant number of DEGs identified in each of the comparisons, very little commonality was observed among the DEG lists. Our results suggested that unique sets of genes were associated with diet and Rmilk. Further functional annotation of the shared DEGs may identify novel molecular factors associated with both diets or milk energy efficiency.

Key Words: mammary gland, RNA-seq, feed efficiency

431 Mammary epithelial cell extrusion into milk is inversely related to milk yield.

H. H. Hanling*, A. J. Lengi, M. L. McGilliard, and B. A. Corl, *Virginia Tech, Blacksburg, VA.*

Milk yield (MY) naturally declines after peak lactation. One approach to improving production is to increase lactation persistency, or the length of time a cow maintains lactation. To do so, cows must retain functional mammary epithelial cells (MEC). However, for unknown reasons, viable MEC are extruded from the udder. The objective of this experiment was to determine the relationship between MY and extruded MEC into milk to determine if this type of cell loss substantially impacts milk production. Our hypothesis was that MY and extruded MEC were negatively correlated; milk from high yielding cows would contain fewer MEC. Thirty-six multiparous and 12 primiparous Holstein cows were sampled only once during lactation. We sampled cows from parities 1, 2, 3, and 4+ between 25 and 255 DIM to more accurately evaluate the effect of natural differences in MY on MEC extrusion. A single 3.8-L milk sample was obtained from each cow. Milk somatic cells were stained with an antibody specific for CD45 to exclude hematopoietic cells from analysis. An antibody against butyrophilin, a novel method, labeled MEC. Live cells were analyzed by using Hoechst to label nucleated cells and propidium iodide to label and exclude dead cells. For statistical analysis, we analyzed the effect of parity, MY, and DIM on the number and concentration (cells/mL) of both total and live extruded MEC. Milk yield was inversely related to the number ($P < 0.03$) and concentration ($P = 0.0004$) of live MEC in milk. Primiparous cows extruded less MEC ($P = 0.0004$) and had a lower concentration of live milk MEC ($P < 0.0001$) than multiparous cows. This could be caused by continued mammary growth in first lactation. Milk from primiparous cows contained 3.55×10^7 MEC of which 3.77×10^6 cells were live with a total concentration of 1,632 cells/mL. Milk from multiparous cows contained 9.55×10^7 MEC of which 1.84×10^7 cells were live with a total concentration of 4,334 cells/mL. Understanding mammary epithelial cell extrusion could improve retention to increase lactation persistency and farm profit.

Key Words: mammary epithelial cells, cell extrusion, lactation persistency

432 Modulation of mammary gland secretory activity and prolactin signaling by incomplete milking.

A.-M. Deacon*[1], R. Blouin[1], and P. Lacasse[2], [1]*Univeristé de Sherbrooke, Sherbrooke, QC, Canada,* [2]*AAFC-Sherbrooke R&D Center, Sherbrooke, QC, Canada.*

The mammary gland secretory activity is modulated by systemic and local factors; however, the relationship between these factors is unknown. The aim of this study was to determine if a local factor, such as incomplete milking, affects the mammary gland responsiveness to blood prolactin (PRL). Eight cows in mid-lactation were differentially milked, i.e., right quarters were milked at approximately 70% (incomplete) and left quarters were milked completely, twice daily for 4 weeks. On the weeks before and after the experiment, mammary gland functional capacity was evaluated by measuring the volume of milk harvested after complete filing of the gland by a 26-h milking interval (residual milk was obtained after oxytocin injection). Throughout the experiment, milk yield was measured at the quarter level. Milk samples were collected once a week to assess: milk components, epithelial cells and to isolate milk fat RNA. Milk production of quarters milked completely remained stable during the treatment period whereas that, as expected, of incompletely milked quarters declined ($P < 0.001$) representing only 58% of completely milked quarters at the end of the period. Accordingly, expression of several genes in milk fat related to milk synthesis (*CSN2*, $P = 0.05$; *LALBA*, $P = 0.05$; *ACACA*, $P = 0.08$) was lower, or tended to be lower, in the quarters that were milked incompletely. Incomplete milking decreased milk lactose content ($P < 0.001$) indicating that patency of tight junctions was increased. The concentration of epithelial cells in the milk ($P = 0.03$), the *BAX*/*BCL2* gene expression ratio ($P < 0.01$) and the lost of mammary functional capacity were greater in the quarters milked at 70%, suggesting an acceleration of involution. The expression of both short and long isoforms of the PRL receptor (*PRLR*) gene tended ($P = 0.06$ for both) to be lower and expression of *STAT5A* ($P = 0.09$) and *STAT5B* ($P = 0.08$) tended to decline in the incompletely milked quarters. In mammary gland biopsies samples, the ratio of PRLR short isoform and PRLR long isoform was lower ($P = 0.05$) in the quarters milked at 70%. Although additional evidence is needed, the results of this experiment support the hypothesis that modulation of the responsiveness of the mammary gland to PRL is part of the mechanism by which local factors, such as incomplete at milking, modulate milk synthesis.

Key Words: gene expression, apoptosis, involution

Physiology and Endocrinology IV

433 Impact of heat stress and glycemic state on plasma γ-aminobutyric acid (GABA) in lactating Holstein cows. A. G. Arneson*, J. W. Stewart, M. K. Harrod, H. M. Newberne, M. K. Burgess, J. A. Jordan, R. R. White, A. D. Ealy, S. W. El-Kadi, R. P. Rhoads, and M. L. Rhoads, *Virginia Tech, Blacksburg, VA.*

Heat stress (HS) induces hyperinsulinemia and hypoglycemia in lactating dairy cows. We hypothesized that γ-aminobutyric acid (GABA) participates in the regulation of this altered glycemic state as it is produced by pancreatic β cells and has a stimulatory effect on pancreatic secretion of insulin. Multiparous lactating Holstein cows ($n = 6$; 63.33 ± 2.35 DIM, 3.17 ± 0.40 lactations) were placed in environmentally controlled rooms for 4 experimental periods: 1) thermoneutral (TN; d 1–5; THI 67.1 ± 0.26), 2) TN + hyperinsulinemic-hypoglycemic clamp (HHC; d 6–10), 3) heat stress (HS; d 16–20; THI 72.2 ± 0.36), and 4) HS + euglycemic clamp (EC; d 21–25). Cows were milked twice daily, and blood samples were collected once daily via coccygeal venipuncture into heparinized evacuated tubes. Plasma GABA concentrations were determined using a competitive ELISA. Since plasma GABA concentrations in heat-stressed lactating dairy cows have not previously been assessed alongside a thermoneutral control, data were analyzed in 2 ways. The first analysis included data from all treatment periods and yielded no period-based differences in plasma GABA concentrations. In this analysis, however, plasma GABA was moderately correlated with milk production ($r = 0.31$, $P < 0.01$). The second excluded data from HHC and EC periods so that GABA concentrations during TN were directly compared with concentrations during HS. In this analysis, plasma GABA concentrations tended to be higher in TN than HS (16.31 ± 2.14 vs 13.80 ± 2.15 ng/mL, respectively, $P < 0.10$). Milk production was again moderately correlated with plasma GABA ($r = 0.42$, $P < 0.01$) and the average plasma GABA during TN and HS was moderately correlated with baseline glucose levels for those periods ($r = -0.57$, $P = 0.05$). Furthermore, the percent change in plasma GABA was strongly correlated with the percent change in plasma glucose from TN to HS ($r = -0.95$, $P < 0.01$). While these analyses are not indicative of causality, the results suggest that GABA is involved in the regulation of the altered glycemic state observed during HS. More research is needed to determine its precise role in heat-stressed lactating dairy cattle.

Key Words: glucose, GABA, heat stress

434 Effects of heat stress on ruminal volatile fatty acid concentrations and gastrointestinal microbiome in weaned Holstein calves supplemented with or without dietary organic acid and plant botanicals. A. B. P. Fontoura*[1], V. Sáinz de la Maza-Escolà[1,2], M. X. Rodrigues[1], A. Javaid[1], E. Grilli[2,3], M. E. Van Amburgh[1], R. C. Bicalho[1], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Bologna, Bologna, Italy,* [3]*Vetagro Inc., Chicago, IL.*

Our objectives were to evaluate the effects of heat stress on ruminal volatile fatty acid (VFA) concentrations and gastrointestinal tract microbiome in post-weaned calves subjected to heat stress and dietary organic acid and plant botanicals (OA/PB) supplementation. In a completely randomized design, 62 bull and heifer calves were fed starter grain and assigned to one of 5 groups ($n = 12$–13/group) for 19 d: thermoneutrality without OA/PB (TN-Con), heat stress (temperature-humidity index: 75 to 83) without OA/PB (HS-Con), thermoneutrality but pair-fed to HS-Con (TN-PF), heat stress supplemented with low or high dose OA-PB (75 and 150 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; AviPlusR, Vetagro, Italy). Rumen fluid VFAs (d 3 and 19) were measured using liquid chromatography and bacteria (d 19) were profiled using 16S rDNA sequencing. The statistical model included fixed effects of body weight at birth, treatment, time, and their interaction as well as the random effect of calf. Heat stress increased ruminal lactate concentrations, relative to TN-Con ($P < 0.05$). TN-PF had greater lactate and isobutyrate concentrations than TN-Con or HS-Con ($P < 0.01$). Although the major phyla in ruminal content did not differ between HS-Con and TN-Con, relative abundance (RA) of *Bacteroidetes* was higher in calves fed high-dose OA/PB, relative to TN-Con ($P < 0.05$). Duodenal *Fibrobacteres* RA tended to be reduced in heat-stressed calves, relative to TN-Con ($P = 0.06$). Jejunal *Euryaechaeota* and *Methanobacteriaceae* RA were greater in heat-stressed calves, relative to TN-Con ($P < 0.05$). The RA of ileal *Bacteroidetes* and *Bacteroidaceae* tended to be increased in heat-stressed calves ($P \leq 0.15$). Fecal *Proteobacteria* was also increased in HS-Con compared with TN-Con calves, and high dose OA/PB reduced this phylum ($P = 0.01$), relative to HS-Con. We conclude that heat stress and dietary OA/PB supplementation modified the gastrointestinal microbiome. Such changes may explain the ability of HS to modify ruminal VFA concentrations.

Key Words: heat stress, microbiome, organic acid

435 Heat stress and glycemic state alter milk production and composition in Holstein dairy cows. J. W. Stewart*, H. M. Newberne, M. K. Harrod, A. G. Arneson, V. M. Negron-Perez, R. R. White, S. W. El-Kadi, A. D. Ealy, R. P. Rhoads, and M. L. Rhoads, *Virginia Tech, Blacksburg, VA.*

During heat stress (HS), lactating dairy cows become mildly hyperinsulinemic and hypoglycemic. The objective of this experiment was to separate the effects of glycemic state from those of thermal environment on lactation. Multiparous lactating Holstein cows ($n = 23$; 58.3 ± 3.9 DIM, 3.1 ± 0.3 lactations) were housed in temperature-controlled rooms and all were subjected to 4 experimental periods: 1) thermoneutral (TN; d 1–5; THI 67.1 ± 0.26), 2) TN + hyperinsulinemic-hypoglycemic clamp (HHC; insulin infused at 0.3 ug/kg of BW/h, glucose infused to maintain 90 ± 10% of baseline blood glucose for 96h; d 6–10), 3) Heat stress (HS; d 16–20; THI 72.2 ± 0.36), and 4) HS + euglycemic clamp (EC; glucose infused to reach 100 ± 10% of TN baseline blood glucose for 96h; d 21–25). Cows were milked twice daily and milk samples were collected at the beginning and end of each period for component analyses. Feed intake was similar during TN and HHC periods (48.6 ± 1.6 and 48.1 ± 2.1 kg/d, respectively), after which it declined for HS and EC (42.8 ± 1.3 and 41.9 ± 1.9 kg/d; $P < 0.01$). Milk production was greatest during TN (50.7 ± 2.7 kg/d), intermediate during HHC (46.6 ± 2.8 kg/d) and lowest during HS and EC (41.5 ± 2.3 and 41.3 ± 2.5 kg/d, respectively; $P < 0.01$). Milk fat ($P < 0.01$) and lactose ($P < 0.01$) were affected by thermal environment but did not differ with glycemic state. Both components were higher during TN (4.2 ± 0.2 and 5.0 ± 0.1% for fat and lactose, respectively) and HHC (4.1 ± 0.2 and 5.0 ± 0.1%) than during HS (3.6 ± 0.1 and 4.9 ± 0.1%) and EC (3.7 ± 0.1 and 4.9 ± 0.1%). Milk protein appeared to be affected by both thermal and glycemic states as it was higher during TN (2.7 ± 0.1%) than during all other periods (2.6 ± 0.01, 2.6 ± 0.1 and 2.5 ± 0.1% for HHC, HS and EC, respectively; $P < 0.01$). As expected, milk urea nitrogen increased from TN (8.3 ± 0.6 mg/dL) to HS (9.9 ± 0.7 mg/dL). Interestingly, it then decreased from HS to EC (8.6 ± 0.6 mg/dL; $P < 0.05$). Somatic cell counts did not differ between experimental periods. These results indicate that milk components are differentially affected by glycemic state and thermal environment. Isolation of these effects may help to identify opportunities for improved management of heat-stressed cows.

Key Words: heat stress, hypoglycemia, milk

436 Rumen-protected methionine during heat stress alters hepatic cystathionine β-synthase and methionine adenosyltransferase activity.

D. N. Coleman*[1], Q. Jiang[1], M. Vailati-Riboni[1], R. T. Pate[1], D. Luchini[2], F. C. Cardoso[1], and J. J. Loor[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Adisseo, Alpharetta, GA.*

The objective was to investigate the effects of dietary rumen-protected Met (RPM) during a heat stress (HS) challenge on antioxidant status and enzyme activity in one-carbon metabolism. Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental treatments, and 1 of 2 dietary treatments [TMR (CON) or TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM as top dress)] in a crossover design. The study was divided into 2 periods with 2 phases. During phase 1 (9d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9d), group 1 was exposed to HS using electric heat blankets. Group 2 remained in TN, but was pair-fed to HS counterparts. After a 14d washout, the study was repeated (period 2). Environmental treatments were inverted relative to phase 2 in period 1, while dietary treatments were the same. Liver biopsies were performed at the end of each period to measure cystathionine β-synthase (CBS) and methionine adenosyltransferase (MAT) activity and glutathione (GSH) concentrations. Data were analyzed using PROC MIXED in SAS and Pearson correlations were performed between CBS, MAT, GSH, milk yield (MY) and dry matter intake (DMI) in HS cows. There was an Env × Diet effect for CBS ($P = 0.01$); activity was lower in RPM vs. CON cows during HS ($P = 0.01$) and CON cows tended to have greater activity during HS compared with TN conditions ($P = 0.09$). An Env × Diet effect was observed for MAT activity ($P = 0.02$), with RPM cows having lower activity than CON during HS ($P = 0.02$). Additionally, while MAT increased in CON cows during HS vs. TN ($P = 0.06$), RPM cows tended to have greater activity during TN vs. HS ($P = 0.10$). During HS CBS tended to be positively correlated with MY ($r = 0.31$; $P = 0.08$). Concentrations of GSH were positively correlated with DMI ($r = 0.37$; $P = 0.05$), MY ($r = 0.37$; $P = 0.04$) and MAT activity ($r = 0.46$; $P = 0.01$). Overall, preliminary observations suggest that RPM may help maintain homeostasis in one-carbon metabolism during HS. Increases in CBS activity during HS may help support antioxidant and milk production.

Key Words: amino acid, lactation

437 Liver microRNA profile in heat-stressed cows substantially differs from that in pair-fed Holstein cows.

N. Reisinger*[1], S. Rodriguez-Jimenez[2], J. Faas[1], M. Hackel[3], and L. H. Baumgard[2], [1]*BIOMIN Research Center, Tulln, Austria,* [2]*Iowa State University, Ames, IA,* [3]*TAmiRNA GmbH, Vienna, Austria.*

Heat stress negatively affects cow performance as well as metabolic pathways within the liver. Therefore, study objectives were to investigate the liver microRNA (miRNA) profile of heat-stressed and pair-fed Holstein cows in mid-lactation. The trial consisted of 2 periods. In the first period, lactating Holstein cows (n = 12; 604 ± 3 kg BW; 156 ± 5 DIM) were kept at thermoneutral conditions and fed ad libitum for 10 d. In period 2, cows were assigned to heat stress (HS) or pair-fed (PF) in thermoneutral conditions for 7 d. Heat stress was induced using electric heat blankets (Thermotex Therapy Systems Ltd., Calgary, AB, Canada) resulting in a decrease of dry matter intake by 45%. Overall, compared with PF, HS increased rectal temperature (40.5 vs. 38,3°C; $P < 0.01$), and HS cows had a more severe decrease in milk yield (34% vs. 20%). On the last day of period 2, liver biopsies were obtained from all cows. Total RNA was isolated from liver tissue, and miRNAs were sequenced using the RealSeq small RNA library prep kit (RealSeq Biosciences, US). Sequencing was performed on an Illumina NextSeq 550. Reads were mapped against miRBase using miRDeep2. Raw read counts of miRNA were processed using EdgeR and generalized linear models to perform differential expression analysis. P-values were adjusted for multiple testing using Benjamini-Hochberg's false discovery rate (FDR). Hierarchical clustering showed a strong clustering between animals in the HS or PF groups. Overall, in the HS cows, 32 miRNAs were upregulated, while 30 miRNAs were downregulated compared with the PF group (FDR <0.1). KEGG pathway enrichment analysis (miRNet 2.0) based on predicted genes indicated that affected miRNAs are involved in immune and stress signaling pathways (e.g., HIF-1 signaling pathways, MAPK signaling pathway, TGF-β signaling pathway) as well as several metabolic pathways (FDR <0.05). Three miRNAs were affected that are predicted to be involved in the regulation of heat shock proteins. Namely, in the HS group, the expression of bta-miR-2332 was downregulated −3.9-fold, while bta-miR-16a was upregulated 0.78-fold, and bta-miR-142–3p 0.62-fold compared with the PF group (FDR <0.01). Overall, our data suggest that heat stress and reduced feed intake affect the miRNA profile in Holstein cows differently.

Key Words: heat stress, liver, miRNA

438 Hepatic insulin and antioxidant signaling are altered by rumen-protected methionine during heat stress in response to lipopolysaccharide challenge.

D. N. Coleman*[1], P. Totakul[1,2], Q. Jiang[1], N. Onjai-uea[1,3], M. Vailati-Riboni[1], R. T. Pate[1], D. Luchini[4], F. C. Cardoso[1], and J. J. Loor[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand,* [3]*Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand,* [4]*Adisseo, Alpharetta, GA.*

The objective was to investigate the effects of rumen-protected Met (RPM) during a heat stress (HS) challenge on the response of liver tissue to lipopolysaccharide (LPS). Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental treatments, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases. During phase 1 (9 d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9 d), group 1 was exposed to HS using electric heat blankets (THI = 89 ± 3). Group 2 remained in TN (THI = 61 ± 4) but was pair-fed to HS counterparts. After a 14-d washout, the study was repeated (period 2). Environmental treatments were inverted relative to phase 2 in period 1, while dietary treatments were the same. Liver biopsies were performed at the end of each period. Fifty mg of tissue per cow was incubated with 0 or 3 μg/mL of LPS for 2 h and protein extracted. Data were analyzed using PROC MIXED in SAS. An Env × diet × LPS interaction was observed for phosphorylated (p) protein kinase B (AKT); when incubated with LPS, abundance was lower in explants from HS RPM cows compared with CON HS cows ($P = 0.01$). There was a tendency for an Env × LPS for insulin receptor (INSR; $P = 0.08$) where explants from HS cows incubated with or without LPS had a greater abundance of INSR than explants from TN cows ($P < 0.05$). An Env × Diet was observed for glucose transporter 4, pAKT and INSR ($P < 0.001$ for all); abundance of each protein was greatest in tissue from CON HS cows but did not differ with RPM ($P < 0.05$). The same Env × Diet was also observed for glutathione peroxidase 1 ($P = 0.02$). Lastly, there was an Env × Diet for cullin 3 ($P = 0.02$) where abundance was greater in HS CON cows compared with TN CON ($P = 0.01$) and HS RPM cows ($P = 0.03$). Overall, preliminary evaluation indicates that under HS RPM did not alter the response of liver tissue to LPS but may help maintain homeostasis in hepatic insulin and antioxidant signaling.

Key Words: amino acid, lactation

Production, Management, and the Environment IV

439 Nitrogen efficiency as potential performance indicator for commercial dairy herds.

F. X. Amaro*[1], D. Vassolo[1], J. M. Piñeiro[2], A. Vieira-Neto[3], D. R. Bruno[4], F. Ferreira[5], and D. Vyas[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Science, Texas A&M AgriLife Research and Extension Center, Amarillo, TX,* [3]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS,* [4]*University of California Cooperative Extension, Fresno, CA,* [5]*Department of Population Health and Reproduction, University of California-Davis, Tulare, CA.*

We aimed to evaluate nitrogen efficiency (NE; milk N/N intake) as a potential performance indicator for dairy cows in commercial herds. Farms were located in California (n = 13) and were visited from July to August 2020. Pen dry matter intake (DMI) was measured and individual feed ingredients were collected, dried, and pen diets were reconstituted for chemical analysis. Nitrogen intake was calculated using estimated cow DMI (based on pen DMI) and crude protein percent of each diet. Milk yield (MY) was recorded, and milk samples from individual cows were collected for component analysis. A total of 143 pens were sampled with average pen size of 213 ± 107 cows. PROC MIXED was used to develop model explaining production responses. Model included fixed effects of NE as linear (NEL) and quadratic (NEQ), parity (Par; primiparous vs. multiparous), lactation stage (LS; Early vs. Mid vs. Late), and their interaction (NEL × Par; NEQ × Par; NEL × LS, NEQ × LS). Pen nested within herd was used as random effect. A stepwise backward elimination was used to remove non-significant interactions. Nitrogen efficiency was classified as Low, Medium and High (19, 29 and 39%, respectively; Table 1). A quadratic association between MY and NE was observed suggesting that maximum MY can be achieved between Medium and High NE. Milk protein and fat percent were positively associated with NEL, while yields of protein and fat were associated with NEQ and dependent on LS. In conclusion, NE can be used as an indicator for MY and milk composition in dairy cows although some of the responses are dependent on LS.

Key Words: dairy cow, nitrogen efficiency, protein

440 Development of a globally standardized calibration system to measure enteric methane emissions from cattle using GreenFeed.

D. Pang*, S. Zimmerman, and P. Zimmerman, *C-Lock Inc., Rapid City, SD.*

The GreenFeed system (GF) is a cost-effective, non-intrusive way to monitor the enteric methane and other gas emissions such as carbon dioxide (CO_2) from the muzzles of ruminant animals. For universally comparable results, it is important to obtain accurate calibrations for both concentrations and gas fluxes. The GF absolute accuracy is maintained through globally uniform, standardized calibration methods. The GF concentration measurements are standardized through an automated onboard system that routes onboard zero and span standard gases into GF sensors every 1 to 3 d. The GF calibration gases are supplied to C-Lock Inc. in high-pressure cylinders by a certified laboratory. Each cylinder is then independently verified against a second long-term standard maintained and archived by C-Lock Inc. GF auto calibration utilizes a unique computer-controlled regulator system with pressure sensors and solenoid valves and data transmission system so calibration events can be remotely triggered and monitored. Calibration results stable within ± 3% of the average of the past 5 calibrations are deemed acceptable. The GF also includes a gravimetric CO_2 gas recovery system to verify flow rate measurements. Recovery tests are conducted monthly by injecting a known mass of CO_2 into the GF over a 9-min time period. The GF users are supplied with a verified mass scale, a release device that maintains CO_2 flow, and 90g disposable CO_2 cylinders. The online GF program then compares the predicted CO_2 against the gravimetrically measured mass of CO_2. If the predicted and measured CO_2 are not within 5%, the user is immediately notified. An analysis 1,075 CO_2 recovery tests completed for 101 GF units, showed the average recovery of CO_2 was 99.5 ± 5.5% and the correlation between the gravimetric CO_2 mass and the predicted GF CO_2 mass was ($R^2 = 0.99$). The calibration procedures ensure GF produces universally comparable results across applications and around the world.

Key Words: methane, GreenFeed, global standard program

441 Environmental assessment of United States dairy farms.

C. A. Rotz*[1], R. Stout[1], A. Leytem[2], G. Feyereisen[3], H. Waldrip[4], G. Thoma[5], M. Holly[6], D. Bjorneberg[2], J. Baker[3], P. Vadas[7], and P. Kleinman[1], [1]*USDA-ARS, University Park, PA,* [2]*USDA-ARS, Kimberly, ID,* [3]*USDA-ARS, St. Paul, MN,* [4]*USDA-ARS, Bushland, TX,* [5]*University of Arkansas, Fayetteville, AR,* [6]*University of Wisconsin-Green Bay, Green Bay, WI,* [7]*USDA-ARS, Beltsville, MD.*

Dairy farms in the United States are diverse and although regional dairy production and farm strategy has been evaluated for environmental impact, a comprehensive national assessment is important to define national priorities for sustainable intensification. We estimated important environmental footprints of dairy farm production using process-level simulation and cradle-to-farm gate life cycle assessment. Dairy farms representing the sizes and management practices found in 6 regions were simulated with the Integrated Farm System Model (IFSM). Regional and national environmental footprints were determined as an average of all simulated farms weighted by the portion of milk each contributed to the total. Nationally, dairy farms were assessed to emit 99,000 ± 8,480 Gg

Table 1 (Abstract 439). Responses to nitrogen efficiency in early, mid, and late lactation

	Early lactation			Mid lactation			Late lactation			
Item	Low	Medium	High	Low	Medium	High	Low	Medium	High	SE
Milk yield, kg/d$^{\S\P\#*}$	38.4	43.3	43.8	38.8	43.7	44.1	36.4	41.3	41.7	1.62
Protein, %†	3.05	3.10	3.27	2.97	3.01	3.19	2.96	3.00	3.17	0.07
Fat, %†	3.86	4.12	4.42	3.47	3.73	4.03	3.63	3.90	4.19	0.12
Protein yield, kg/d$^{\#*\ddagger}$	1.21	1.25	1.35	1.25	1.29	1.39	1.12	1.17	1.27	0.06
Fat yield, kg/d$^{\#*\ddagger}$	1.47	1.62	1.79	1.45	1.60	1.77	1.32	1.47	1.64	0.07

$P \le 0.05$: §NEL; ¶NEQ; $^{\#}$Par, *LS, †NEL × LS; ‡NEQ × LS.

CO_2e of greenhouse gas, equivalent to 1.5% of the estimated US total greenhouse gas emission, with a commodity-based intensity of 1.01 ± 0.09 kg CO_2e/kg of fat and protein corrected milk (FPCM) produced. Fossil energy consumption was 242,700 ± 38,400 TJ, 0.3% of the US total, or 2.48 ± 0.39 MJ/kg FPCM. Blue (non-precipitation) water consumption was 11,600 ± 2,480 Tg, roughly 3.0% of the estimated US total freshwater use, with an intensity of 119 ± 25 kg/kg FPCM. While these environmental footprints represent a relatively small portion of their respective national inventories, the dairy industry's contribution to reactive nitrogen (N) losses appears to be considerably greater. Losses of reactive N were estimated at 970 ± 133 Gg, with an intensity of 9.92 ± 1.36 g/kg FPCM. Although there are no national estimates of total reactive N emissions, dairy farms were found to contribute 19 – 24% of national inventories of ammonia (NH_3) emissions. While strategies are available to reduce NH_3 emissions, finding economical and sustainable solutions that do not result in pollution swapping remains a challenge for the dairy industry.

Key Words: dairy farm, life cycle assessment, footprint

442 Impact of feed additives on the farmgate carbon footprint of milk across regions in the United States. M. E. Uddin*[1], J. M. Tricarico[2], Y. Wang[2], and E. Kebreab[1], [1]*University of California, Davis, CA,* [2]*Innovation Center for US Dairy, Rosemont, IL.*

Enteric methane (CH_4) contributes about 70% of all livestock greenhouse gas (GHG) emissions. Feed additives such as 3-nitrooxypropanol (3-NOP) and nitrate have great potential to reduce enteric emissions. Our objective was to determine the net effects of 3-NOP and nitrate on the farmgate milk carbon footprint (CF) across various regions of the US. A cradle to farmgate life cycle assessment was performed to determine regional and national CF to produce 1 kg of fat- and protein-corrected milk (FPCM). Records from 1355 farms across 37 states included herd structure, milk production and composition, cattle diets, manure management and farm energy information. Enteric and manure CH_4 and nitrous oxide were calculated using either Intergovernmental Panel on Climate Change (IPCC) Tier 2 or best equations available in the literature. Emissions were allocated between milk and meat using biophysical allocation method. Impacts of feed additives on baseline regional and national CF were accounted for using literature-based equations adjusted for dry matter intake and neutral detergent fiber. Uncertainty analysis was performed using Monte Carlo simulations. Overall, milk CF for the base, nitrate and 3-NOP scenarios were 1.15, 1.09 (4.8% reduction) and 1.01 (12% reduction) kg CO_2-eq./kg FPCM across US regions. The greatest CF for the base scenario was in the Southeast (1.26 kg CO_2-eq./kg FPCM) and lowest for the West region (1.02 kg CO_2-eq./kg FPCM) whereas both nitrate and 3-NOP showed the lowest and greatest emission reduction potential for southeast and west regions, respectively. Enteric CH_4 reductions were 12.4 and 31% for nitrate and 3-NOP scenarios, respectively. The uncertainty analysis showed CF values ranged widely (0.88 to 1.52 and 0.56 to 1.84 kg CO_2-eq./kg FPCM within one and 2 standard deviation, respectively) suggesting the importance of site-specific estimates of CF. Considering 101 billion kg of milk produced by the US dairy industry in 2020, the potential net reductions of GHG from the base 117 billion kg CO_2-eq. were 5.6 and 13.9 billion kg CO_2-eq. for the nitrate and 3-NOP scenarios, respectively.

Key Words: 3-nitrooxypropanol, nitrate, greenhouse gas

443 Incorporating nitrogen mass balance into the Ruminant Farm Systems (RuFaS) Animal Simulation. T. L. Hansen*[1], M. Sotirova[1], K. Wang[1], J. M. Tricarico[2], and K. F. Reed[1], [1]*Cornell University, Ithaca, NY,* [2]*Dairy Management Inc., Rosemont, IL.*

Dairy farms are complex systems that have massive imports and exports of nutrients. These nutrients, like nitrogen, are critical for maintaining farm productivity; farms use nitrogen fertilizer for crop production, formulate rations to meet protein requirements, and manage manure to prevent environmental contamination. To accurately account for nitrogen in the farm system, we developed a mass balance model of individual animals in the Ruminant Farm Systems (RuFaS) model. RuFaS is a modular, object-oriented simulation model that simulates nutrient flows through soil + crop, feed storage, animal, and manure modules. The animal module incorporates animal production, ration formulation, and manure production. Nitrogen intake from the diet, nitrogen needed to meet nutrient requirements, and nitrogen excreted were used to create a nitrogen mass balance model for all animal classes (calves, heifers, and cows). Crude protein from feed was assumed to be 16% nitrogen, except for milk and milk replacer which was assumed to be 15.7%. Protein to meet growth and gestation requirements were converted to a nitrogen-equivalent value. Nitrogen excretion included nitrous oxide emissions from enteric fermentation (0.48 g N_2O/d for all animals except calves), manure, and milk. Fecal nitrogen excretion was calculated from empirical equations available in the literature. Baseline estimates for nitrogen in urine and milk urea nitrogen were estimated from maintenance requirements and the literature, respectively. If nitrogen excretion was less than dietary intake minus growth and gestational nitrogen-equivalent values, then the error term was calculated and used to adjust urine and milk urea nitrogen amounts. For calves, heifers, and dry cows, the urinary nitrogen excretion was increased to include the error term. The error term was applied to both milk urea nitrogen and urinary nitrogen for lactating cows, assuming urinary nitrogen had a constant relationship with milk urea nitrogen and bodyweight. Combined with similar individual animal mass balance models for phosphorus and potassium, RuFaS is uniquely situated as a whole-farm system model to answer research and industry challenges related to nutrient management.

Key Words: nutrient management, mass balance, simulation model

444 Rearing of organic dairy calves with cows. B. J. Heins* and K. T. Sharpe, *University of Minnesota, Morris, MN.*

The objective of this study was to determine growth and health of organic dairy calves raised on cows compared with calves fed in individual pens or calves raised on an automated calf feeder. The study was conducted at the University of Minnesota West Central Research and Outreach Center, Morris, MN, organic dairy. Forty-five Holstein and crossbred calves were assigned to treatment groups by birth order during one calving seasons from March to May 2020. Calves raised on cows (n = 14) were housed in a compost bedded pack barn and on pasture, individual calves (n = 16) were housed in single Calf-Tel hutch (Hampel Corp., Germantown, WI), and group calves (n = 15) were housed in an indoor-outdoor barn with a Holm & Laue HL100 Calf Feeder (Holm & Laue GmbH & Co KG, Westerronfeld, Germany). Calves raised on cows were allowed to bond with the dam for 3 d before grouped with other calves and cows. Individual and group calves were introduced to hutch and feeder on d 4 and were fed 8 L/d. All calves were weaned at 90 d, and were weighed weekly and scored for health and behavioral measurements. Data were analyzed using PROC MIXED of SAS. Independent variables for analyses were the fixed effects of birthweight as a covariate, treatment group, along with calf within pen as a random effect. At weaning of 90 d, calves raised on cows had higher ($P < 0.05$) weaning weight (137.6 kg vs. 120.0 kg vs. 128.4 kg, respectively) and average daily gain (1.12 kg/d vs. 0.93 kg/d vs. 1.01 kg/d, respectively) compared with individual calves and automated feeder calves. individual-fed calves and calves from the automated feeder were not different ($P > 0.05$) for average daily gain. Average daily gain at 120 d of age, was not different ($P > 0.05$) for all treatment groups. automated feeder calves had higher ($P < 0.05$) fecal scores (0.18) compared with calves raised on cows (0.04) and individual calves (0.03). calves raised on cows had higher ($P < 0.05$) hygiene scores which indicated calves had dirtier bellies and sides than other treatment groups. Results from this study indicate that there may be advantages to feeding organic dairy calves on cows during the preweaning period.

Key Words: calves, organic, calf rearing

445 Effects of supplement crude protein concentration and genotype on milk production in grazing dairy cows.

M. J. Doran*[1], F. J. Mulligan[2], M. B. Lynch[1,3], A. G. Fahey[1], N. J. Ryan[1], C. McDonnell[1], and K. M. Pierce[1], [1]*School of Agriculture and Food Science, University College Dublin Lyons Farm, Celbridge, Naas, Co. Kildare, Ireland,* [2]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland,* [3]*Teagasc Environmental Research Centre, Johnstown Castle, Wexford, Ireland.*

The objective of this study was to investigate effects of supplement crude protein (CP) concentration and cow genotype on milk production and milk composition in spring-calving grazing dairy cows. A 2 × 2 factorial experiment, with 2 feeding strategies (14% and 18% CP concentrate supplements) fed at varying levels according to pasture availability and d in milk (DIM; n = 29) was conducted over the main grazing season from April 3 to September 3, 2019, at UCD Lyons Farm. Cows were also assigned to 2 genotype groups: lower milk genotype (LM; milk kg predicted transmitting ability (PTA): 45 ± 68.6 kg, fat kg PTA: 10 ± 4.9 kg, and protein kg PTA: 7 ± 2.3 kg; n = 29) and higher milk genotype (HM; milk kg PTA: 203 ± 55.0 kg, fat kg PTA: 13 ± 3.8 kg, and protein kg PTA: 10 ± 2.4 kg; n = 29). Fifty-eight Holstein Friesian dairy cows were blocked on parity, and balanced on DIM, Economic Breeding Index, and pre-experimental milk yield. Cows grazed full time and were offered a basal diet of perennial ryegrass pasture. No interactions were observed for any milk production or milk composition parameter. Time had effects on all daily milk production and milk composition parameters measured. No effect of supplement CP concentration was observed for total accumulated milk production, daily milk production, or milk composition. The HM cows had an increased daily energy-corrected milk (+1.7 kg) and fat-corrected milk (+2.2 kg) yield compared with LM cows. Differences in milk composition between genotypes were not observed. Cows fed decreased supplement CP concentration had increased pasture dry matter intake (+0.9 kg) compared with cows fed increased supplement CP concentration. In conclusion, decreasing the supplement CP concentration offered to cows did not negatively impact on either total accumulated or daily milk production, or milk composition over the main grazing season where high pasture quality was maintained. The results are positive for farmers considering current national nitrates derogation legislation, whereby farmers are required by the Irish Department of Agriculture, Food and the Marine to reduce supplement CP fed to dairy cows during the main grazing season.

Key Words: dairy cow, supplement crude protein concentration, milk production

446 Impact of residual feed intake measured during the growing period on subsequent performance, feed intake, and feed conversion efficiency of finishing beef × dairy calves.

J. Johnson*, N. Deeb, J. Moreno, and G. Toro, *STgenetics, Navasota, TX.*

The objective of this project was to evaluate the impact of residual feed intake (RFI) measured during the growing period on feed intake, performance, and feed conversion efficiency in AN × HO steers (n = 70; Initial BW = 314 ± 33 kg) and heifers (n = 50; Initial BW = 289 ± 28 kg) fed grower and finisher diets. DMI was measured using a GrowSafe System for 225 d while calves consumed a grower (90 d; NE_g = 1.1 Mcal/kg) and finisher (135 d; 1.3 Mcal/kg) ration at STgenetics' Genetic Development Center. RFI was computed as the difference between actual and expected DMI from a linear regression of DMI on mean $BW^{0.75}$ and ADG; computed during the first 70 d of the growing (RFI_G) and finishing (RFI_F) periods. Calves were divided into low-, medium-, and high-RFI groups based on being ± 0.50 SD from mean RFI. Overall, DMI and ADG averaged 9.7 (±0.79) and 1.22 (±0.17) kg/d for heifers and 10.2 (±0.95) and 1.36 (±0.17) kg/d for steers during the growing RFI period and 13.0 (±1.29) and 1.16 (±0.22) kg/d for heifers and 13.4 (±1.11) and 1.28 (±0.22) kg/d for steers during the finishing RFI period. Across the 225-d feeding period steers consumed 4% more ($P = 0.01$) feed and had 8% greater ($P < 0.01$) ADG, but no significant sex by RFI_G class interactions ($P > 0.05$) were observed. Calves with low-RFI_G consumed 13% less ($P < 0.01$) feed during the growing period and 10% less ($P < 0.01$) feed during the finishing period, with no difference ($P > 0.26$) in ADG or final BW. The correlation between RFI_G and RFI_F was highly significant ($P < 0.01$; r = 0.50), with only 7.5% of calves changing from high-RFI_G to low-RFI_F or vice versa. During the 225-d feeding period, low-RFI_G calves consumed 305 kgs DM less than high-RFI_G calves which equated to a feed savings of $105.81 for efficient calves (Grow ration = $0.34/kg DM; Finish ration = $0.35/kg DM). These results indicate that selection for RFI during the growing period can lead to significant reductions in feed costs at the feedlot and subsequent increased profitability of terminal beef × dairy calf programs. The data presented is part of an ongoing project to evaluate the performance, feed efficiency, and carcass traits of beef × dairy progeny to develop genomic predictions for feed conversion efficiency and other economically relevant traits of beef × dairy calves.

Key Words: beef × dairy, feed efficiency, RFI

447 Validation of body condition scoring system in dairy buffaloes (*Bubalus bubalis*).

M. Q. Shahid*[1], S. H. Magsi[1], M. A. Rashid[2], N. Ahmed[1], and M. Akhter[3], [1]*Department of Livestock Management, University of Veterinary and Animal Sciences, Lahore, Pakistan,* [2]*Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan,* [3]*Buffalo Research Institute, Pattoki, Pakistan.*

Body condition scoring (BCS) is a subjective assessment of body fat reserves and is widely used for nutritional management and welfare assessment at dairy farms. Currently used BCS system for dairy buffaloes, reported in literature, requires tactile palpation and has larger increments. That limits its application at farm level. The objectives of current study were to apply the US BCS system for dairy cows on dairy buffaloes and validate it by assessing the intra- and interobserver agreement of the BCS system. For this purpose, a 5- point BCS system on a scale 1–5 with 0.25 increment developed by Elanco was applied on buffaloes. Three hundred buffaloes of varying parity, lactation stage, and pregnancy status were enrolled from the Buffalo Research Institute, Pattoki, Pakistan. Four observers independently assigned a body condition score to each buffalo at 2 different time points; 1) trained for BCS using BCS chart and videos, 2) trained on live animals. The Kappa statistics was used to determine the intra- and interobserver agreement. The results revealed that with video training the interobserver exact agreement was moderate (k_w = 0.48–0.55) and it increased to

substantial after training on live animals ($k_w = 0.63–0.87$). Similarly, with video training the intraobserver exact agreement was moderate ($k_w = 0.45–0.64$) that increased to substantial after training on live animals ($k_w = 0.63–0.64$). Furthermore, the intra- and interobserver exact agreement was higher ($k_w = 0.57- 0.58$) for buffaloes tied at the feed bunk compared with the buffaloes standing in the outdoor open area ($k_w = 0.50$). The interobserver agreements within 0.25 and 0.5 points were almost perfect ($k_w = 0.97–1.0$). The current results suggested that the 5- point BCS system on a scale 1–5 with 0.25 increment had substantial agreement for assessment and repeatability when applied on buffaloes. Therefore, that BCS system can be used for dairy buffaloes.

Key Words: BCS, dairy buffaloes, validation

448 Phenotypic evaluation of rumination time in Canadian dairy cattle.

L. S. F. Lopes*[1], H. R. Oliveira[1], T. C. S. Chud[1], F. S. Schenkel[1], and C. F. Baes[1,2], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland.*

Rumination is a natural behavior in cows, however, it produces methane (CH_4) which has a global warming potential 28 times greater than that of CO_2. Ruminal CH_4 production represents a loss of dietary energy for the animals. Thus, strategies to reduce CH_4 emissions from cows while maintaining or improving milk production levels are crucial for an efficient and sustainable dairy industry. Large-scale recording of rumination time (RT) is feasible at the commercial level through the use of rumination collars. However, patterns of RT and its relationships with milk production and methane emission traits have not yet been largely explored. The objectives of this study were: 1) investigate the phenotypic variability of RT, and 2) evaluate the environmental factors that impact RT. Daily records of total RT (in minutes) were obtained from 241 Canadian Holstein cows over 4 years. Rumination collars were used to record RT at 2h intervals for 7 d. For each day, the 12 2h records were summed to obtain the total daily RT. Statistical significance of environmental effects on RT such as year and season of measurement, day of the week (Monday to Friday), age at calving (in years), and days in milk (covariate) was assessed using ANOVA. Cows were classified as having high, medium, or low RT using the k-means clustering algorithm. Environmental effects of season and the interaction between year and season were significant ($P < 0.05$). Averages (SD) for daily RT of 363.63 (±36.31), 444.93 (±20.28), and 520.08 (±31.28) minutes were observed for the low, medium, and high groups, respectively. These results suggest that there is phenotypic variability for RT between animals and that season and year have an impact on RT. The next steps for this study are the estimation of phenotypic correlations of RT with milk production, dry matter intake, CH_4 emission, and other important economic traits. As more data accumulates, genetic parameters for RT will also be estimated.

Key Words: dairy, rumination time, methane emissions

449 Association of herd ketosis prevalence and herd productivity.

R. C. Serrenho* and T. F. Duffield, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of this observational study was to assess the relationship between herd-level prevalence of ketosis and herd milk production. We hypothesized that herds with greater daily milk yield have lower herd prevalence of ketosis. Herds were selected based on their inclusion in a concurrent herd management risk assessment study (August 2014-March 2018). Data from multiple milk recording test-days (Dairy Herd Improvement, Lactanet) from 71 Canadian dairy herds were included in the analysis. Tests performed within ± 6 mo relative to each farm's risk assessment date were included (10 ± 2SD tests per farm). The majority of the farms were located in Ontario (83%). The data set included herd-average milk yield (MY; kg/cow per day), milk fat and protein (%), somatic cell count (SCC; cells/mL), days in milk (DIM), n cows tested for ketosis and n positive tests (milk β-hydroxybutyrate ≥ 0.15 mmol/L). Prevalence of ketosis within 5–21DIM was calculated based on data available per farm (sum of all positive tests within 5–21DIM/sum of all cows tested within 5–21DIM). Each farm average was obtained by considering all test-days. A logit-transformation was applied to ketosis prevalence (outcome of interest). Univariable models and collinearity assessment were followed by multivariable linear regression models (MIXED procedure of SAS) built based on a stepwise regression. The MY (kg/cow per d), fat (%), milk (%), SCC, DIM, and the proportion of primiparous cows were the main effects considered; interaction terms were tested based on biological plausibility. Herd prevalence of ketosis was 27 ± 14% with an average herd size of 141 ± 110 cows. The MY was 34 ± 5 kg/cow per day with 3.95 ± 0.36% fat and 3.21 ± 0.26% protein. On average, the herds had 215,000 ± 67,000 SCC and 171 ± 12DIM. Herd prevalence of ketosis was negatively associated with MY (OR = 0.96[95%CI 0.92–0.99], $P = 0.03$) and proportion of primiparous cows (OR = 0.98[0.96–0.99], $P = 0.04$; $R^2 = 0.11$). In Canadian dairy herds, the herd prevalence of ketosis in early lactation was negatively associated with herd productivity. Future work should consider additional herd explanatory variables to improve the understanding of the variability of ketosis prevalence data between herds.

Key Words: hyperketolactia, milk yield, performance

450 Economics of reproductive strategies in Jersey herds.

J. M. V. Pereira*[1,2], F. H. S. Teixeira[3], D. Bruno[4], R. Bruno[5], M. I. Marcondes[6], and F. C. Ferreira[2], [1]*Universidade Federal de Vicosa, Vicosa, MG, Brazil,* [2]*Veterinary Medicine Teaching and Research Center, Department of Population Health and Reproduction. School of Veterinary Medicine. University of California-Davis, Tulare, CA,* [3]*Progressive Dairy Health Services LLC, Clovis, NM,* [4]*University of California Cooperative Extension, Fresno, CA,* [5]*Dairy Technical Service Veterinarian, Zoetis, Fresno, CA,* [6]*Washington State University, Pullman, WA.*

Jersey bull calves have little or no commercial value. The use of beef semen (BS) or purebred Angus beef embryos (ABE) may be a strategy to improve herd profit. Our objective was to estimate the effect of breeding strategies combining conventional Jersey semen (JS), sexed semen (SS), conventional BS, and ABE on annual gross profitability of a Jersey herd under a maximum 1,000 cows parlor capacity and maximum 120 calvings per mo constraints. We adapted a Markov chain model with seasonality of production due to heat stress, which impairs milk production, mortality, and conception rate (CR). To simulate a default herd, a nonlinear model was used to maximize the annual herd profit by changing the number of heifers calving each mo. We varied ABE prices ($70 or $150, ABE70 or ABE150), Angus calf premium ($50, $75, and $125 over crossbred price), the breedings in which heifers and cows received JS, SS, BS, or ABE, and the maximum number of surplus heifers per year (SurpHF, 15%). Milk price was $19/cwt, dry matter cost was $0.25/kg, Jersey bull calf was $20, and dairy-beef crossbred calf premium was $75. Only cows received ABE and no genetic gain was considered. Mean CR during cool and hot months were 0.47 and 0.33 (heifers SS), 0.38 and 0.21 (cows SS), 0.53 and 0.40 (heifers BS and JS), 0.42 and 0.25 (cows BS and JS), and 0.39 and 0.31 (cows ABE). When SS was used (up to breeding 4 in heifers, breedings 1 and 2 in first- and second-lactation cows and breeding 1 in third-lactation cows) combined with JS (all other breedings), profit/cow/yr was $571, and 325 SurpHF were produced. When JS was replaced by BS, profit/cow/yr increased to $618, with 140 SurpHF. Limiting max. SurpHF (15%) when SS and BS were used increased profit/cow/yr to $641 (61 SurpHF). When ABE70 was used, profit/cow/yr was $611, $621, and $640 for Angus premiums of $50, $75, and $125, respectively. If ABE150 was used, profit/cow/yr was $545, $555, and $575 for Angus premiums of $50, $75, and $125, respectively. Using ABE yielded 62 SurpHF. In conclusion, the use of ABE may be a profitable strategy in Jersey herds, depending on ABE cost, dairy-beef crossbred calf premium, and Angus calf premium, as it can vary greatly.

Key Words: Jersey, economics, beef on dairy

451 The macroalga *Asparagopsis taxiformis* decreases dry matter intake and milk production in dairy cows.

D. E. Wasson*[1], H. Stefenoni[1], S. Welchez[1], M. Fetter[1], C. Lage[1], A. Melgar[1], S. Räisänen[1], T. Silvestre[1], C. Yarish[2], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*The University of Connecticut, Stamford, CT.*

Studies with sheep and cattle have identified the macroalga *Asparagopsis taxiformis* (AT) as a potent methane-mitigating feed additive but have also raised concerns related to bromoforms (a group of halogenated compounds responsible for the antimethanogenic effect of AT) stability over prolonged storage. Therefore, the purpose of this experiment was to determine the effect of AT stored for over 20 mo on enteric methane emission and lactational performance of dairy cows. The study was conducted with 18 Holstein cows in a replicated 3 × 3 Latin square design with 3, 28-d periods. Treatments were control (basal diet), 0.50% AT, and 0.75% AT [feed dry matter (DM) basis]. Enteric methane emission was measured using the GreenFeed system. Data were analyzed using the MIXED procedure of SAS with treatment and period in the model, and square and cow within square as random effects. Bromoform concentration in AT at the beginning of the experiment was 4.09 mg/g DM. Daily methane emission decreased linearly ($P < 0.001$) with AT dose: 404, 338, and 283 g/d (SEM = 13.7), for control, 0.50, and 0.75% AT, respectively. AT inclusion, however, decreased linearly ($P < 0.001$; from 24.7 to 19.2 kg/d; SEM = 1.03) DM intake, which resulted in only a small decrease in methane yield ($P = 0.07$; 17.1 vs. 15.6 g/kg DM, control and AT treatments, respectively). Milk true protein and lactose concentrations and yields and energy-corrected milk yield (ECM; 37.0, 36.1, and 31.8 kg/d, respectively; SEM = 1.05) were linearly decreased ($P \leq 0.01$) by AT. Methane emission intensity was also linearly decreased ($P < 0.001$) by AT (11.0, 9.4, and 9.0 g/kg ECM, respectively). Additionally, there was a 3% decrease ($P < 0.002$) in cow body weight for 0.75% AT, compared with the control. In conclusion, *Asparagopsis taxiformis* stored over 20 mo and included at up to 0.75% (feed DM basis) in the diet of dairy cows decreased methane yield by only 9% and decreased DM intake by 22% and milk production by 8%. These data confirm previous observations for negative effect of AT on feed intake and question the feasibility of this mitigation practice for the dairy sector.

Key Words: methane, *Asparagopsis taxiformis*, dairy cow

452 Diagnosing pregnancy status of US dairy cows with milk infrared spectroscopy.

P. Khanal* and R. J. Tempelman, *Department of Animal Science, Michigan State University, East Lansing, MI.*

An accurate early diagnosis of pregnancy is important for timely reproductive management of dairy farms. Fourier-transform mid-infrared (FTMIR) milk spectral data is routinely used for determining milk components, whereas milk composition is known to change with advancing stages of pregnancy. The objectives of this study were to compare partial least squares (PLS) and Bayesian variable selection regression models for the diagnosis of pregnancy status (PS) from milk FTMIR data and to infer any spectral regions that might be highly associated with PS at various stages of pregnancy. Milk samples (47,364) from 13,225 pregnant cows were obtained from Holstein cows within 123 herds in Michigan, Ohio, and Indiana during 2018 and 2019. Milk samples taken from these pregnant cows at 7 different stages of pregnancy were case-control matched with open contemporary herd mates within the same stage (±10 d for DIM) of lactation at the same milk test date. Herd-independent cross validation-based area was used to compare PLS vs Bayesian methods using the area the receiver operating characteristic curve (AUC). Furthermore, at each stage, the Bayesian method was used to infer associations of PS with individual wavelengths and windows of adjacent wavelengths with the windows determined by a spatially constrained clustering algorithm. The Bayesian method demonstrated higher mean AUC ($P < 0.05$) compared with PLS at all stages exceeding 60 d of pregnancy. The mean Bayesian AUC at stage 1 (1–30d) was 0.58 ± 0.02 which was superior ($P < 0.05$) to a random guess (AUC = 0.50) yet too low to be of practical use. Mean AUC at stages of pregnancy beyond 1–30d was not seen to increase until beyond 151d. Various windows of wavelengths had various strengths of association with pregnancy containing some regions having posterior probabilities of association approaching 100% across stages including stage 1 of pregnancy. Our study provides new insights into spectral regions that may be associated with PS.

Key Words: dairy cattle, Fourier-transform infrared spectroscopy, pregnancy

453 Sources of forage quality variation in New York State harvest 2020.

J.A. Barrientos-Blanco* and K.F. Reed, *Department of Animal Science, Cornell University, Ithaca, New York.*

Our objective is to identify and quantify sources of quality variation in corn and alfalfa-grass silage harvested in 2020 on New York State (NYS) dairy farms. Forage samples were collected from 8 NYS dairy farms. A composite forage sample was collected for every 15–20 acres at harvest. We recorded soil type (ST), texture (STX), and organic matter (OM, %), haylage drying time (DT, d), haylage cut number, and crop maturity (CMT, d) from each field. Weather variables included growing day degrees (GDD), cumulative precipitation for growth (CPG), solar radiation for growth (SRG), and precipitation (PH) and temperature at harvest (TH). Forage quality analysis and grass (%) were evaluated at DairyOne. Haylage DM, NDF, and CP and corn silage DM, NDF, and starch were analyzed with a mixed model to identify influential management and environmental factors and quantify the extent of variation. Random effects of farm and field were included for both forages, and cut number was included for haylage. Fixed effects tested were ST and STX, CMT, GDD, PG, SRG, TH, and PH for both forages and grass (%) and DT were tested for haylage. Results highlight the influence of field variability on both forages with no significant between-farm variability for NDF and starch in corn silage. The fixed effects of ST, STX, and OM (%) were not significant for either forage. The PH and TH had some influence on haylage composition but did not affect corn silage outcomes. Haylage DM and corn silage starch and CP content were influenced by GDD. The CMT had the largest impact on both haylage and corn silage composition at harvest.

Key Words: quality variability, corn silage, alfalfa-grass haylage

Reproduction IV

454 Relationship between parturition perineal hygiene scores and postpartum uterine diseases occurrence in crossbred dairy cows. L. F. M. Silva, G. F. Moraes, B. M. Fernandes, and R. M. Santos*, *Universidade Federal de Uberlândia, Uberlândia, MG, Brazil.*

Retained placenta (RP), metritis and clinical endometritis are uterine diseases that occur in dairy cows globally and cause damage to animal health and to system profitability. It is important to investigate possible factors predisposing to such clinical conditions. In this context, our objective was to assess the relationship between perineal hygiene scores at calving and the occurrence of RP, metritis and clinical endometritis in crossbred dairy cows. The hygiene of the perineal region of 101 cows were evaluated at calving and classified into scores from 1 to 3 (Score 1 - free of feces and completely dry; Score 2 - subtle presence of feces and wet perineum in 1–10% of the surface; Score 3 - moderately wet perineum, covered with manure on more than 10% of the surface). After calving, the cows were evaluated for diagnosis of RP (incomplete elimination of the placenta within the first 12 h after fetal expulsion), metritis (an enlarged, flaccid uterus, a fetid, watery red-brown discharge concurrently or not with fever, anorexia, depression, decreased milk yield and feed intake within 21 d post-calving) and clinical endometritis (presence of purulent vaginal discharge detectable 21 d or more after parturition, or mucopurulent discharge detectable in the vagina after 26 d postpartum). The uterine disease RP, metritis and clinical endometritis rate, as binary dependent variables were assessed using logistic regression, fitted using the GLIMMIX procedure of SAS with perineal score (1 vs. ≥ 2) as independent variable. Statistical significance was defined as $P \leq 0.05$. Cows classified with perineal hygiene score 1 was 53.76% (50/93), score 2 35.48% (33/93) and score 3 10.75% (10/93). As the number of animals classified with score 3 was small, the data the cows with score 2 and 3 were grouped and analyzed together. Cows with hygiene score ≥2 had a higher incidence of RP (41.9% vs. 14.0%), metritis (32.5% vs. 14.0%) and clinical endometritis (42.5% vs. 15.8%) than cows with score 1. It is concluded that the hygiene score of the perineal region in crossbred dairy cows in the prepartum period is related to the occurrence of uterine diseases in the postpartum period.

Key Words: calving, postpartum health, metritis

455 Association of metabolic status with puerperal metritis and reproductive outcomes in lactating Holstein dairy cows. A. Hubner*[1], I. F. Canisso[1], P. M. Peixoto[1], W. M. Coelho Jr.[1], L. Ribeiro[1], B. M. Aldridge[1], and F. S. Lima[2], [1]*Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL,* [2]*Department of Population Health and Reproduction, University of California–Davis, Davis, CA.*

We hypothesized that cows with concurrent hyperketonemia (HK) and hypoglycemia (HG) would have a higher incidence of puerperal metritis (PM) and poorer reproductive outcomes than cows without concurrent HK and HG. Cows (n = 2651) from 5 dairy farms had blood samples collected between 3 and 9 d postpartum (DPP) and whole blood β-hydroxybutyrate (BHBA) and glucose were measured using a cow-side device validated in dairy cows (Precision Xtra, Abbott). Hyperketonemia was defined as BHBA ≥1.2 mmol/L and hypoglycemia was defined as glucose ≤2.2 mmol/L. Cows were categorized into 4 groups: no HK and HG (Norm, n = 1996), HK only (HK, n = 260), HG only (HG, n = 181), and concurrent HK and HG (HKHG, n = 214). Incidence of PM (watery, fetid discharge) diagnosed by researchers, first service pregnancy per AI (PAI), proportion of cows pregnant at 150 DIM (P150), and days open (DOPN) were evaluated. Parity (primiparous vs. multiparous), season (Cool vs. warm), and farm were used in all models. Statistical analysis was performed using ANOVA and logistic regression with JMP Pro 15. Considering all cows tested PM was greater ($P < 0.01$) for HK only than all other groups (HK = 16.5 ± 1.8%; HKHG = 8.9 ± 2.0%; HG = 3.9 ± 2.2%; and Norm = 8.0 ± 0.9%), and P150 was less ($P = 0.02$) for HK only than Norm (HK = 52.3 ± 3.2%; HKHG = 57.0 ± 3.6%; HG = 59.1 ± 3.8%; and Norm = 67.3 ± 1.5%). However, PAI (HK = 40.5 ± 3.6%; HKHG = 38.0 ± 4.0%; HG = 45.2 ± 4.2%; and Norm = 46.8 ± 1.7%), and DOPN (HK = 126.8 ± 4.8; HKHG = 124.6 ± 5.3; HG = 130.9 ± 5.5; and Norm = 120.6 ± 2.2) were similar ($P > 0.05$) across groups. In conclusion, concurrent HKHG was not associated with impaired uterine health and reproductive performance, but HK only cows had poorer uterine health and reproductive performance. For cows with HG an unexpected decrease in the incidence of PM was found. The relationship of HG with uterine health and fertility needs to be further investigated.

Key Words: hyperketonemia, hypoglycemia, metritis

456 Meta-analysis of the effect of antibiotics and prostaglandin $F_{2\alpha}$ for the treatment of endometritis in dairy cows. A. Husnain*[1], M. Sagheer[2], U. Arshad[1], K. N. Galvão[1], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan.*

The objective was to perform a meta-analysis to determine the effect of antibiotics (AB) and prostaglandin $F_{2\alpha}$ (PGF) on cure rate and reproductive performance of dairy cows with endometritis. The literature was systematically reviewed and 31 randomized experiments that used AB and/or PGF for endometritis treatment and had a control (CON) group were found. Endometritis was diagnosed based on either vaginal discharge, uterine content or cytology. A total of 104 treatment means and 9,558 cows were included in the meta-analysis. Data collected included parity, cyclicity, disease severity, type of antibiotic used (cephapirin vs. others), cure rate, first-service conception (FSCR), median and variance for days to first service and days to pregnancy and cumulative pregnancy rates (CPR). Mixed effects meta-analysis was conducted using the MIXED and GLIMMIX procedures of SAS and statistical models investigated the effect of treatment on cure rate and reproduction. Additionally, classical meta-analysis was used to estimate relative risk (RR) with METAN and METAREG procedures of STATA. Parity and cyclicity within each treatment were not reported in enough studies to include in final analyses. Results (LSM ± SEM) revealed that treatment affected ($P = 0.001$) the cure rate, and it was greater for AB and PGF than CON (67 ± 4 and 61 ± 5 vs. 56 ± 5%, respectively). Cure rate did not differ between AB and PGF ($P = 0.24$). Treatment did not affect ($P > 0.65$) time to first service or time to pregnancy. Treatment affected ($P < 0.001$) FSCR, and it was greater for AB (RR = 1.27; 95% CI = 1.12–1.44) and PGF (RR = 1.15; 95% CI = 1.01–1.31) than CON. We further evaluated the type of AB, and cephapirin improved ($P < 0.001$) FSCR compared with CON (RR = 1.41; 95% CI = 1.25–1.59), whereas other AB did not ($P = 0.28$) (RR = 1.12; 95% CI = 0.91–1.38). Treatment affected ($P < 0.001$) CPR, and it was greater for AB than both PGF and CON (78 ± 3 vs. 66 ± 4 and 68 ± 4%, respectively). In summary, results of the current meta-analysis support endometritis treatment with AB or PGF to improve the cure rate and reproduction in dairy cows. Nonetheless, AB was more effective than PGF.

Key Words: endometritis, treatment, reproduction

457 Rumen-protected methionine improves uterine immunometabolic status of dairy cows.

A. R. Guadagnin*[1], D. A. Velasco-Acosta[2], S. L. Stella[1], D. Luchini[3], and F. C. Cardoso[1], [1]*University of Illinois, Department of Animal Sciences, Urbana, IL,* [2]*The Colombian Corporation for Agricultural Research, Mosquera, Colombia,* [3]*Adisseo NACA, Alpharetta, GA.*

Methionine is one of the most limiting amino acids in the diet of dairy cows. We hypothesize that feeding rumen-protected methionine (RPM, SmartamineM, Adisseo, Alpharetta, GA, USA) peripartum and early lactation would decrease the susceptibility to reproductive tract inflammatory diseases through a modulation of the uterine immune defense mechanisms. Twenty multiparous Holstein cows were assigned to one of 2 dietary treatments. Treatments were fed 21 ± 1 d before calving until 73 ± 1 d in milk (DIM) and consisted of a TMR top-dressed with RPM (MET; RPM at a rate of 0.08% of DM: Lys:Met = 2.8:1; n = 11) or without (CON; Lys:Met = 3.5:1; n = 9). Uterine cytology smears and endometrial biopsy samples were collected at 15, 30, and 73 DIM and analyzed for mRNA expression of genes related to metabolism and inflammation. Data collected was analyzed using the MIXED procedure in SAS, modeling the fixed effects of treatment, time, and their interaction. Regarding cytological smear samples, cows in MET tended to increase ($P = 0.07$) the expression of MAT1A and increased ($P < 0.01$) the expression of FGF7. Additionally, cows in MET had decreased ($P = 0.05$) expression of genes involved in inflammatory processes (IL1β, IL6, IL8, PTGES3, MUC1 and SOD1) and in metabolism (GLUT4 and TSPO). With the exception of MAT1A, there was an effect of time ($P = 0.05$) for all the transcripts, which decreased expression over time. For endometrial samples, cows in MET tended to have increased ($P = 0.08$) expression of LCAT and increased ($P = 0.03$) expression of APOL3, genes involved in cholesterol metabolism. Additionally, cows in MET had increased ($P = 0.04$) expression of SAHH, which encodes the enzyme of Met cycle S-adenosyl-L-homocysteine hydrolase; as well as increased ($P = 0.05$) expression of genes involved in tissue metabolism (FGF7, GLUT4). There was a tendency ($P = 0.09$) for cows in MET to have decreased expression of IL1β. In conclusion, feeding RPM to dairy cows during peripartum and early lactation improved the expression of genes involved in uterine metabolism and immune defense mechanisms in early lactation.

Key Words: gene expression, immune function, amino acid

458 The vaginal microbiome of primiparous and multiparous Holstein and Jersey dairy cows with and without clinical endometritis.

S. G. Moore*[1], C. Feehily[2], R. Doyle[1], F. Crispie[2], F. Buckley[1], P. Lonergan[3], P. D. Cotter[2], and S. T. Butler[1], [1]*Teagasc Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Ireland,* [2]*Teagasc Food Research Centre, Moorepark, Fermoy, Ireland,* [3]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.*

The objective was to identify differences in the vaginal microbiome associated with dairy cow breed, parity, and clinical endometritis (CE). Vaginal samples were collected at wk 1 postpartum from Holstein-Friesian (HF; n = 36) and Jersey (JE; n = 29) cows [primiparous (PP; n = 17) or multiparous (MP; n = 48)]. At wk 3 postpartum, cows were diagnosed as having (CE+; n = 21) or not having (CE-; n = 44) CE. Microbial DNA was isolated for shotgun metagenomics sequencing. Differences in α- and β-diversity and relative abundance of bacterial species and gene functions were determined by fitting a linear model with breed, parity, and CE diagnosis (significant at $P \leq 0.05$). Species and gene function α-diversity was increased in JE compared with HF cows. Species β-diversity was different between breeds and between parities. α- and β-diversity were not associated with CE. The relative abundance of *UBA*2856 *sp*900318615 was greater in PP CE+ compared with PP CE- cows. The relative abundance of *Trueperella pyogenes*, *Fusobacterium C necrophorum*, and *Porphyromonas somerae* was greater whereas *Streptococcus canis* was lesser in MP CE+ compared with MP CE- cows. Nine species were associated with parity, including *T. pyogenes* and *F. necrophorum* which had lesser relative abundance in MP compared with PP cows. Twenty-nine species were associated with breed, including *Escherichia coli and Bacteroides graminisolvens,* which had lesser relative abundance in JE compared with HF cows. The relative abundance of gene functions signal transduction and malate metabolism was less in PP CE+ compared with PP CE- cows, whereas 56 gene functions, including histidine catabolism and sporulation, were greater in MP CE+ compared with MP CE- cows. Nineteen gene functions, including histidine and glucose catabolism, had lesser relative abundance in MP compared with PP cows. Nine gene functions, including sporulation, had greater relative abundance in JE compared with HF cows. In summary, previously unreported breed differences were detected in the vaginal microbiome and differences between CE+ and CE- cows were parity dependent.

Key Words: host-microbe interaction, microbiota, reproduction

459 Flow cytometric assessment of viability and functionality of uterine polymorphonuclear leukocytes in postpartum dairy cows.

L. Lietaer*[1], K. Demeyere[2], S. Heirbaut[3], E. Meyer[2], G. Opsomer[1], and O. B. Pascottini[1,4], [1]*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium,* [2]*Department of Pharmacology, Toxicology and Biochemistry, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium,* [3]*Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium,* [4]*Department of Veterinary Sciences, Laboratory of Veterinary Physiology and Biochemistry, University of Antwerp, Wilrijk, Belgium.*

Postpartum dairy cows experience impaired peripheral polymorphonuclear leukocyte (PMN) functionality. However, less is known about endometrial PMN characteristics. The objective of this study was to develop a method for endometrial PMN isolation and flow cytometric (FCM) assessment of their viability and functionality, adapting validated protocols from circulating PMN. While circulating PMN can easily be differentiated based on their morphology (size and granularity) the differentiation of endometrial PMN from debris and epithelial cells might be challenging. Consequently, we also aimed to evaluate PMN immunolabeling using CH138A, a specific bovine granulocyte marker. Seventeen randomly selected, clinically healthy dairy cows between 9 and 37 d in milk were enrolled. Endometrial cytobrush samples were collected and vortexed to dislodge endometrial cells. After endometrial cell isolation, cells were FCM sorted based on CH138A-positivity (CH138A$^+$ cells) to confirm the specificity of CH138A for endometrial PMN (n = 3). All sortings had > 85% PMN purity. The Lin's concordance correlation coefficient (CCC; *P*-value obtained by the corresponding Spearman correlation) between the proportion of PMN in endometrial cytology smears (27 ± 28% (mean ± SD); range 0–95%)

and the proportion of CH138A$^+$ cells in endometrial cell suspensions (28 ± 20.5%; range 1–68%) was 0.7 ($P < 0.001$). The proportion of viable, apoptotic, and necrotic PMN roughly ranged from 10 to 80%. To assess phagocytosis, PMN were co-incubated with fluorescent beads and stimulated with activated normal cow serum. The percentage of PMN that performed phagocytosis (PPC) was calculated for CH138A$^+$ PMN and non-immunolabeled PMN selected by size and granularity. The PPC for CH138A$^+$ PMN (50 ± 22%; range 20–92%) was positively correlated (CCC 0.65; $P < 0.001$) with the PPC for the non-immunolabeled PMN (40 ± 16%; range 15–70%). In conclusion, CH138A is a valuable marker for endometrial PMN. The endometrial PMN population in the postpartum uterus is highly dynamic and functional. Endometrial PMN phagocytosis could be assessed accurately, also in non-immunolabeled endometrial samples.

Key Words: postpartum, uterus, neutrophils

460 Association between transition health and estrus detection by activity monitors in dairy cows.

T. C. Bruinjé*[1], E. I. Morrison[1], R. C. Serrenho[1], D. Renaud[1], E. S. Ribeiro[2], and S. J. LeBlanc[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective was to investigate associations between transition health and detection of spontaneous estrus by activity monitors in 1,084 Holstein cows from 2 dairies. Body condition score (BCS) and lameness were assessed from wk −3 to 9 relative to parturition. Serum total calcium (Ca), haptoglobin (Hp), and nonesterified fatty acids (NEFA) were measured at 2 and 6 (±1) d in milk. Blood β-hydroxybutyrate (BHB) and metritis were assessed at 4, 8, 11, and 15 (±1) d. Purulent vaginal discharge (PVD) and endometritis (defined as ≥ 6% PMN) were assessed at wk 5, and additional disease data obtained from farm records. Reproductive management for first AI was based on estrus detection by activity monitors with no hormonal intervention until approximately 75 d. Metabolites were categorized based on ROC curve cutpoints associated with endometritis and PVD, and detection of estrus between 50 and 75 d was analyzed in multivariable logistic regression models including parity, BCS, BCS loss from wk −3 to 9, metabolites, and disease variables separately (model 1) or combined as uterine (RP, metritis, PVD, endometritis) and nonuterine clinical disease (milk fever, DA, mastitis, lameness; model 2). Estrus was detected in 75% of primiparous and 63% of multiparous cows. Prevalence of hypocalcemia (Ca ≤ 2.1mmol/L), high Hp (≥1.1 g/L) and high NEFA (≥0.8mmol/L) were 53, 42, and 30%, and of metritis, endometritis, and PVD, were 15, 31, and 20%. Variables associated with reduced estrus detection were endometritis (55 vs. 65%; $P = 0.01$), PVD in multiparous (52 vs. 65%; $P = 0.04$), DA (38 vs. 61%; $P = 0.04$), and ≥ 0.5-point BCS loss (56 vs. 64%; $P = 0.02$). In model 2, estrus detection was lower in cows with both high Hp (d 6) and a uterine disease (54%) compared with cows with low Hp and no uterine disease (69%; $P < 0.01$) and to cows with high Hp but neither uterine nor nonuterine clinical disease (79%; $P < 0.02$). It was also lower in cows with BCS at wk 9 ≤ 2.75 (61%), or ≥ 3.00 after a ≥ 0.5 loss (65%) compared with cows with BCS ≥ 3.00 after a < 0.5-point loss (81%; $P < 0.05$). Findings suggest that uterine inflammation and body condition loss are major risk factors for reduced estrus detection. Identifying such risk factors will allow refinement of reproductive management based on activity monitors.

Key Words: activity monitor, reproduction, transition health

461 The effect of small and large ovarian follicle fluid on oviduct cell gene expression in cattle.

K. Peterson*, M. A. Oliver, S. Bhandari, S. Moorey, and D. Mathew, *University of Tennessee, Knoxville, TN.*

Ovarian follicle fluid (FF) accrues around the cumulus-oocyte complex (COC). FF contains steroids that promote COC development. A recent study found that bovine FF can affect oviduct activity. Follicle size at ovulation, which would affect steroid concentrations within FF, may impact the oviduct. Bovine oviduct epithelial cells were isolated from the ampulla (AMP) or isthmus (ISTH) (n = 4). Small follicle fluid (SFF; follicles 1 to 5 mm) or large follicle fluid (LFF; follicles 6 to 20 mm) was aspirated to develop treatments: (1) RPMI [Control (CON)], (2) RPMI with SFF (1:5 total dilution) or (3) RPMI with LFF (1:5 total dilution). Cells were treated for 24 h before cell mRNA was isolated and reverse transcribed for RT-qPCR. mRNA for genes previously reported to be influenced by FF within bovine AMP cells (*NTS*, *CYP1A1* and *MX1*) and estradiol regulated genes (*ESR1*, *PGR* and *BAX*) were measured using the delta-delta Ct method. A GLM procedure (SAS; Cary, NC) was used to test for an effect of cell collection, FF (TRT), cell type (CT), and their interaction (INT) on log-transformed relative gene expression. Data are presented as least squares means (LSM) ± standard error of the LSM (SEM). As previously reported, compared with CON, cells treated with FF had greater and less *NTS* and *CYP1A1*, respectively (TRT; $P < 0.001$). There was an effect of TRT depending on CT for *MX1* (INT; $P < 0.05$). Compared with CON (0.74 ± 0.08) and SFF (0.54 ± 0.11), AMP cells treated with LFF (1.22 ± 0.11) had greater *MX1*. However, compared with CON (0.67 ± 0.11) and SFF (0.49 ± 0.11), ISTH cells treated with LFF (0.13 ± 0.11) had less *MX1*. For E2 regulated genes, there was an effect of TRT depending on CT for *PGR* (INT; $P = 0.05$). Compared with CON (0.56 ± 0.17) and LFF (0.84 ± 0.20), AMP cells treated with SFF (0.45 ± 0.20) tended to have less *PGR*. However, compared with CON (0.25 ± 0.20), ISTH cells treated with LFF (0.32 ± 0.20) or SFF (0.35 ± 0.20) tended to have greater *PGR*. There was no effect of TRT or CT on *BAX* or *ESR1*. In conclusion, oviduct activities may be altered by small vs. large follicle fluid, possibly effecting quality of the traversing COC and conceptus.

Key Words: oviduct, follicle

462 Relationships between modeled energy balance curve parameters and fertility traits in first-lactation cows.

E. H. Cabezas-Garcia*[1], M. Civiero[2], and C. P. Ferris[1], [1]*Agri-Food and Biosciences Institute, Hillsborough, UK,* [2]*Universidade do Estado de Santa Catarina, Lages, SC, Brazil.*

The aim of this study was to calculate phenotypic relationships between parameters of energy balance (EB) curves modeled at individual-cow level, and reproduction performance in primiparous Holstein Friesian cows (mean forage-to-concentrate ratio on a DM basis was 47:53). Data (320 cows in total) from calving until at least 90 d in milk (DIM) from 19 studies conducted in Northern Ireland over a 20-year period (1997 to 2016) was used to estimate EB (MJ of metabolizable energy; ME/d) using equations within 'Feed into Milk'. Modeling of EB curves at individual-cow level was performed using a polynomial random regression model (Ali and Schaeffer, 1987). From each individual EB curve, 4 parameters were calculated: 1. Nadir EB (MJ of ME/d), 2. Time to nadir EB (d), 3. Interval for return to positive EB (d), and 4. Total energy deficit (TED; MJ of ME). Successful model parameterization was achieved in 92 cows, with these cows included in the subsequent statistical analysis. Fertility data were collated for each individual cow, with first observed heat (FOH) based on visual observations. Start of luteal activity (SLA) was determined for 64 cows from milk progesterone data (twice weekly samples). Partial correlations between EB parameters and fertility traits were determined using MANOVA in PROC GLM of SAS, controlling for the effect of individual study within the data set. Primiparous cows reached nadir EB (−40.5 ± 27.79 MJ of ME/d) at 10.2 DIM and returned to positive EB at 54.0 DIM. The TED during early lactation was calculated to be 1115 ± 1122.6 MJ of ME. On average, age at the first calving was observed at 24.5 mo. The SLA occurred at 32.4 DIM and FOH was observed at 44.9 DIM. Considerable variation was observed for days from start breeding season until pregnancy (63.7 ± 47.69). A smaller nadir EB was associated with a reduction in days to FOH ($r = -0.263$; $P < 0.01$). Other partial correlations were not significant. For each 10 MJ/d increase in nadir EB (ME basis), FOH was delayed by 2.2 d. These results confirm nadir EB is associated with the onset of reproductive activity.

Key Words: energy balance, fertility traits, primiparous cows

463 Relationship between milk production and circulating concentration of progesterone on double ovulation in lactating Holstein cows submitted to Double Ovsynch.

T. Valdés-Arciniega*[1], E. Anta-Galván[1], I. M. R. Leão[1], M. S. El Azzi[1,2], T. O. Cunha[1], N. B. Cook[1], and J. P. N. Martins[1], [1]*Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [2]*Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, MG, Brazil.*

The aim of this study was to determine the association of circulating concentration of progesterone (P4) and milk production during the growth of the ovulatory follicle with double ovulation (DOV) in lactating Holstein cows. First-service multiparous cows (DIM = 63.5 ± 0.08) submitted to a Double Ovsynch program [DO; GnRH–7d–PGF–3d–GnRH–7d–GnRH (G1)–7d–$PGF_{2\alpha}$(PG1)–1d–$PGF_{2\alpha}$–32h–GnRH (G2)–16h–timed AI (TAI)] were used. Ovarian ultrasonography examinations were performed at G1 (n = 1,282) and G2 (n = 1,334) and 2d after each GnRH to assess ovulatory response. Average milk yield (kg/d) for the wk before G1 and PG1was recorded. Blood samples were collected at G1 (n = 1,321) and PG1 (n = 1,374) to measure serum P4. Data were analyzed using GLIMMIX and MIXED procedures of SAS. Milk yield for the 2 wk period was 56.0 ± 0.1 kg/d. Overall, ovulation to G1 was 70.9%, and DOV was 15.4%. Circulating concentrations of P4 at G1was higher in cows with single ovulation (SOV) than cows with DOV ($P < 0.01$; 4.2 ± 0.1 vs. 3.1 ± 0.1 ng/mL). Serum P4 at G1 × milk yield interaction was not significant for DOV to G1 ($P = 0.86$). Milk yield ($P = 0.01$) and parity ($P = 0.01$) were positively associated with DOV to G1. Low serum P4 at G1 was related ($P = 0.01$) to DOV to G1. Ovulation to G2 was 90.3%, and DOV to G2 was 12.9%. Cows with DOV to G2 also had lower P4 at PG1 than cows with SOV (7.1 ± 3.4 vs. 8.8 ± 4.0 ng/mL; $P < 0.01$). Serum P4 at G2 × milk yield interaction ($P = 0.76$) and fixed effect parity ($P = 0.60$) were not significant for DOV to G2. In contrast, high milk yield ($P = 0.01$) was associated with DOV to G2. Similar to G1, serum P4 at G2 also had a negative relationship ($P < 0.001$) with DOV to G2. Double ovulation to G1 was also associated ($P < 0.001$) with DOV to G2, indicating that cows with DOV have a DOV pattern throughout the timed AI program. In summary, serum concentrations of P4, milk production and previous double ovulations are highly associated with double ovulation in lactating Holstein cows. These data can contribute to future research focus on decreasing twinning in lactating dairy cows.

Key Words: double ovulation, progesterone, milk production

464 Pharmacological inhibition of lipid accumulation in bovine oocytes.

L. M. Mayo*, O. U. Bolden-Tiller, and G. G. Wirtu, *Tuskegee University, Tuskegee, AL.*

Despite progress made, the in vitro embryo production industry has not overcome low oocyte survival and blastocyst development rates, increasing costs and decreasing the application in cattle. We hypothesize that the use of lipogenic inhibitors improves bovine oocyte survival. The objective of this project was to determine the effects of 2 pharmacological lipogenic inhibitors, 1) CP630186 [inhibits the rate limiting enzyme acetyl co-A carboxylase] and 2) sulfosuccinimidyl oleate [SSO; inhibits fatty acid transport into the cytoplasm], on oocyte maturation and lipid accumulation during bovine oocyte in vitro maturation (IVM). Bovine slaughterhouse ovaries were rinsed in 0.9% NaCl thrice before follicular aspiration with an 18-gauge × 1 1/2” short-beveled needle and 6-mL syringe. The cumulus-oocyte complexes (n = 1,459) were subsequently added to recovery medium for washing and selection then randomly transferred to IVM media with the following treatments: Control; 1.5% DMSO; CP640186 at 0.02, 0.06, or 0.10 μM; or SSO at 0.1, 0.5, or 1.0 mM for a 24-h incubation at 39°C, 5% CO_2, and high humidity. Oocytes (n = 10/treatment/replicate) were denuded and stained with Hoechst to determine nuclear maturation stage and Nile Red to estimate lipid content. Statistical analysis included normality test with the UNIVARIATE procedure and log-transformation of fluorescence intensity with the GLIMMIX procedure (SAS 9.4). The model included replicate as random. Treatments (Control, + DMSO, 3 mM L-Carnitine, 0.02 μM CP640, 0.06 μM CP640, 0.1 μM CP640, 0.1 mM SSO, 0.5 mM SSO, 1.0 mM SSO) did not affect maturation rate (75 ± 9, 74 ± 10, 63 ± 9, 76 ± 9, 60 ± 9, 69 ± 9, 85 ± 10, 70 ± 9, 59 ± 10; $P > 0.05$) percent MII stage of total oocytes stained respectively or lipid abundance [(CTCF × 10^8) 3.08 ± 0.941, 2.42 ± 0.974, 2.66 ± 0.955, 2.87 ± 0.943, 2.94 ± 0.965, 2.30 ± 0.961, 2.07 ± 0.939, 2.20 ± 0.951, 2.25 ± 0.965; $P > 0.05$]; however, images indicated SSO reduced lipid content. In conclusion, the 2 inhibitors of lipid accumulation (CP630186 and SSO) are compatible with nuclear maturation of bovine oocytes.

Key Words: oocyte, in vitro maturation, bovine

465 Effect of progesterone on bovine endometrial cell expression of select transporters and growth factors.

M. A. Oliver*, K. D. Peterson, S. Bhandari, and D. J. Mathew, *The University of Tennessee, Knoxville, TN.*

Progesterone (P4) stimulates endometrial epithelial (EPI) and stroma fibroblast (SF) cells to secrete molecules that promote conceptus growth. P4 supplementation during early pregnancy in cattle can increase conceptus size by influencing the endometrium. We hypothesize that P4 stimulates specific transporters and growth factors in endometrial cells, supporting conceptus growth. Mid-luteal phase endometrium was collected from bovine uteri (n = 4) and EPI and SF cells isolated and cultured separately. The cells were then cultured with RPMI [Control (CON)] or RPMI with 1 (P4–1), 15 (P4–15), or 50 (P4–50) ng/mL of P4 for 12 h. EPI and SF mRNA were isolated, and reverse transcribed before RT-qPCR. mRNA from genes related to cell growth (*FGF2*, *FGF7*, and *CTGF*) or vitamin and amino acid transporters (*SLC1A1*, *SLC5A6* and *SLC7A1*) were calculated using the delta-delta Ct method. A GLM (SAS; Cary, NC) was used to test for an effect of animal, P4 (TRT), cell type (CT) and their interaction (INT) on log-transformed relative gene expression. Data are presented as least squares means (LSM) ± standard error of the LSM (SEM). *SLC1A1*, a transporter for glutamate, was greater in the EPI (6.48 ± 0.67) compared with SF (0.57 ± 0.64) cells (CT; $P < 0.001$). The same was true for *SLC5A6* and *SLC7A1*, multi-vitamin and arginine transporters, respectively. Compared with SF *SLC5A6* (0.93 ±

0.14) and *SLC7A1* (1.04 ± 0.08), EPI *SLC5A6* (1.31 ± 0.15) and *SLC7A1* (1.33 ± 0.08) was greater (CT; $P < 0.05$). The opposite was true *FGF2* and *CTGF*. Compared with EPI *FGF2* (0.58 ± 0.71) and *CTGF* (0.52 ± 0.28), SF *FGF2* (7.00 ± 0.69) and *CTGF* (3.50 ± 0.27) was greater (CT; $P < 0.001$). There was an effect of CT ($P < 0.001$) and TRT ($P = 0.05$) on *FGF7*. Compared with EPI (0.16 ± 0.93), SF (27.19 ± 0.89) had greater *FGF7*. Compared with CON (16.56 ± 1.33), cells treated with P4–1 (12.90 ± 1.20), P4–15 (12.32 ± 1.20) and P4–50 (12.92 ± 1.44) tended to have less *FGF7*. A greater understanding of the effects of P4 on the uterine environment and conceptus growth, may lead to technologies that improve in vitro produced bovine embryo quality.

Key Words: endometrium, progesterone

466 Evaluation of automatic or manual methods of computing blood flow indices of uterine arteries during the estrous cycle in *Bos indicus* dairy cows.

M. Hassan*[1,2], U. Arshad[2,3], H. I. Habib[2], and N. Ahmad[2], [1]*Department of Clinical Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan,* [2]*Department of Theriogenology, Faculty of Veterinary Science, University of Veterinary and Animal Sciences, Lahore, Pakistan,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective was to evaluate the automatic (AUT) or manual (MAN) methods of computing blood flow indices of uterine arteries (UA) during the estrous cycle in *Bos indicus* dairy cows. Cyclic cows (n = 9) having healthy reproductive tract were enrolled at their spontaneous estrus (Day 0) and subjected to Doppler ultrasonography throughout the estrous cycle. Blood flow indices of left and right UA were computed either using AUT or MAN methods at days −1, 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 of the estrous cycle from each cow. Data were generated either using AUT method based on the means of 3 similar and consecutive waveforms of UA, however, MAN method involved the storage of 3 images of UA for the quantification of pixels using Pixel Flux software throughout the estrous cycle. Data on continuous responses of blood flow indices including pulsatility index (PI) and resistance index (RI) were analyzed using MIXED procedures, and Pearson correlation coefficients were calculated using the PROC CORR statement in SAS. The statistical models included the fixed effects of method, day of the estrous cycle, and interaction between the method and day, and the random effect of cow nested within the method. Results revealed that PI in left (1.03 ± 0.18 *vs*. 1.37 ± 0.18; $P < 0.01$), and right (0.97 ± 0.18 vs. 1.29 ± 0.18; $P = 0.03$) UA was underestimated in MAN as compared with AUT method, respectively. Similarly, RI in left (0.75 ± 0.08 vs. 0.86 ± 0.08; $P = 0.03$), and right (0.64 ± 0.08 vs. 0.73 ± 0.08; $P = 0.05$) UA was also undervalued in MAN as compared with AUT method, respectively. A weak association for PI ($r = 0.39$: $P < 0.001$), and RI ($r = 0.35$; $P < 0.001$) existed between the methods in *Bos indicus* dairy cows. In conclusion, the AUT method seemed more efficient and robust having less chances of human error and might be used as a standard method to elucidate the blood flow hemodynamics in *Bos indicus* dairy cows. It is easier and less time consuming compared with MAN method. There are no standard values previously documented in the literature.

Key Words: automatic method, manual method, Doppler ultrasound

Ruminant Nutrition: Gut Physiology, Fermentation, and Digestion II

467 Identification of bioactive phytochemicals from 6 forest plants: Insights into the mechanistic inhibition of rumen protozoa, ammoniagenesis, and α-glucosidase. A. G. Ayemele[1], L. Ma[1], X. Li[2], P. Yang[2], J. C. Xu[1], Z. T. Yu[3], and D. P. Bu*[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P.R. China,* [2]*Feed Research Institute, Chinese Academy of Agricultural Science, Beijing, P.R. China,* [3]*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Rumen protozoa, especially *Entodinium* have a little contribution to the feed digestibility but are unfortunately culprit of the nitrogen utilization inefficiency. This study aimed at inhibiting the detrimental rumen protozoa and regulating the microbial α-glucosidase activity using bioactive compounds of 6 functional plant leaves (*Adansonia digitata*, *Brassica rapa* ssp. *chinensis*, *Calotropis gigantea*, *Flemingia macrophylla*, *Kalimeris indica* and *Portulaca oleracea*). The plants leave were analyzed for 5 major phytochemical compounds (previously reported for their antiprotozoal activity) using reversed phase-HPLC analysis then, evaluated for their in vitro suppressing effect on rumen protozoa using light and scanning electron microscopy. Rumen fluid was collected from 3 cannulated cows, and protozoa were cultured anaerobically with the dried and ground plant leaves at 4 doses: 0, 0.7, 0.9, and 1.1 mg/mL supplemented to the same TMR fed to the cows that donated the rumen fluid. There were 3 replicates per treatment. Ammoniagenesis and microbial α-glucosidase activity were followed up using colorimetric and α-glucosidase inhibitory assays. Data were analyzed in a complete randomized design with one-way ANOVA using the PROC GLM procedure of SAS 9.4. All the tested plants reduced total rumen protozoa counts, associated with an extracellular damaging known as a cell death metabolic pathway. Among the 5 targeted phytochemicals, quercetin, 3-Hydroxibenzoic acid and myricetin were found to different extent ($P < 0.05$) in all the tested plant leaves and may contribute either alone or in association to the protozoa inhibition. *C. gigantea* and *B. rapa* ssp. *chinensis* had the most inhibitory effect ($P \leq 0.05$), inhibiting the rumen protozoa by 50 ± 2.3 and 70 ± 3.1%, respectively, at the dose of 1.1 mg/mL. *C. gigantea* was the only plant that reduced the wasteful ammonia nitrogen (NH_3-N) production ($P \leq 0.05$). Besides, *A. digitata* and *F. macrophylla* showed the highest inhibition rate (70 ± 1.3%) of microbial α-glucosidase activity at the lower tested crude extract concentration (100 μg/mL). Overall, the tested plants showed promising results as potential feed additives but future research using animal feeding trials is needed to evaluate the feasibility and the effect on the whole microbiome.

Key Words: bioactive phytochemicals, nitrogen efficiency, rumen protozoa

468 The rumen microbiome of yak co-evolves with its host adding the adaptation to the harsh environments. C. Zhao[1], X. Chen[2], L. Wang[1,2], J. Yao[1], Z. Yu[3], and Y. Cao*[1,2], [1]*Northwest A&F University, Yangling, Shaanxi, China,* [2]*Harvard Medical School, Boston, MA,* [3]*The Ohio State University, Columbus, OH.*

The objective of this study was to reveal the potential mechanism of rumen microbiota contributes to yak adaptation. Ten Qaidam cattle, dzo, and plateau yak with similar body weight (200 kg) and age (5–6 years old) were selected. All animals were grazing in the grassland, where *Kobresia myosuroides* and *Phragmites communis* were the predominant pasture species. Data for volatile fatty acids profile and the abundance of genes were compared by one-way ANOVA. The relative abundance of microbiota difference was tested using the Kruskal-Wallis H test, and the differences were considered significant at $P < 0.05$. Metataxonomic analysis showed rumen bacteria and fungi composition were quite different ($P < 0.05$, ANOSIM) among 3 species. The bacteria 16S rRNA gene against Silva 128 database, and fungi ITS sequences were assigned to UNITE 7.0 database. Metagenomic analysis displayed a larger gene pool encoding a richer ($P < 0.05$) repertoire of carbohydrate-active enzymes in the rumen microbiome of yak and dzo than of cattle. Some genes encoding glycoside hydrolases (GH) that mediate the digestion of cellulose (GH48 and GH45) and hemicellulose (GH44, GH5, GH16, GH17) were significantly enriched ($P < 0.05$) in the rumen of yak than cattle, but the cattle rumen microbiome had more ($P < 0.05$) genes assigned to GH57 that primarily includes amylases. Yak had a higher molar proportion of propionate, but a lower molar proportion of acetate than cattle ($P < 0.05$). The increased relative abundance of succinate-producing and utilizing species seen in yak might be attributable to the higher propionate proportion detected. Metabolomic analysis showed differences in rumen metabolites, mainly in amino acids, carboxylic acids, sugars, and bile acids. Pearson correlation analysis found some microbial species correlated with differential rumen metabolites. Nine differential metabolites showed a positive correlation with 7 species belonging to *Bacteroides* and *Alistipes* but a negative correlation with 10 species belonging to *Prevotella* and *Ruminococcus*. This study showed that the rumen microbiome of yak and its host are co-adapted to the harsh dietary environment. In particular, the yak rumen microbiome has more genes encoding cellulase and hemicellulose, making yak better adapted to rough forage. And yak has more succinate-producing and utilizing species making more pyruvate metabolized to propionate, which provides more energy for its host body.

Key Words: yak, rumen microbiome, glycoside hydrolases

469 Effects of mycotoxin deactivator on rumen parameters: In vivo and in vitro approaches. N. L. Whitehouse*[1], B. K. Kerns[1], L. H. P. Silva[5], G. Bromfam[2], E. Pinloche[3], J. Dvorska[4], and D. P. Preveraud[4], [1]*University of New Hampshire, Durham, NH,* [2]*Adisseo USA Inc., Alpharetta, GA,* [3]*Adisseo Center of Expertise and Excellence in Nutrition, Commentry, France,* [4]*Adisseo, SAS, Antony, France,* [5]*Western Kentucky University, Bowling Green, KY.*

Ingestion of mycotoxins by dairy cows can result in many problems including decreased feed intake, production, fertility, and an impaired immune system. The objective of this study was to determine the effects of a mycotoxin deactivator on rumen parameters using rumen-fistulated cows and an in vitro intestinal fermentation model. Nine ruminally fistulated Holstein cows were used in a replicated randomized block trial for 7 wk. Treatments were 1) negative control diet; 2) positive control diet (PC) with mycotoxin contaminated dried distillers grains; and 3) PC diet plus 30 g/d of mycotoxin deactivator (UNIKE Plus (UP), Adisseo). Rumen samples were collected on the last 2 d of the covariate period, wk 3 and 6 of the study. Samples were processed for VFA and microbial DNA. Four other nonlactating Holstein cows were used as rumen fluid donors. After a 2-wk adaptation period, rumen fluid was collected for each cow and kept in an anaerobic buffer. For each inoculum (n = 4), treatments were a control treatment with 800 mg feed and a treatment with 800 mg feed + 96 mg UP. Using the APES (Automated Pressure Evaluation System), VFA production was measured after 48 h fermentation. Data were analyzed using the MIXED procedure of SAS with REPEATED measurements. Significant effect was noted at $P \leq 0.05$. The in vivo study showed a significant increase for butyrate and isobutyrate ($P < 0.02$) and isovalerate ($P < 0.01$) with supplementing the mycotoxin deactivator. Bacteria and archaea relative abundance was also affected by UP, resulting in a higher bacteria:archaea ratio ($P = 0.04$). UP also resulted in a significant increase ($P < 0.05$) of propionic, isobutyric, butyric, isovaleric and valeric acids measured in the APES model. Total branched VFA was also significantly higher ($P < 0.001$) in the treatment group while total VFA tended to increase ($P = 0.07$). This study showed that mycotoxins contamination can impair the rumen parameters. These parameters can be restored by dietary mycotoxin deactivator enable to shift the microbiota resulting in an improvement of the production of beneficial metabolites and thus, enhancing health and performance of the cows.

Key Words: mycotoxin, rumen function, microbiota

470 Reduction of leaky gut and inflammation markers through dietary antioxidants and immune modulators in lactating dairy cows during heat stress.

A. Ruiz-González*[1,2], W. Suissi[2], L. H. Baumgard[3], P. Y. Chouinard[1], R. Gervais[1], and D. E. Rico[2], [1]*Université Laval, Quebec, QC, Canada,* [2]*CRSAD (Centre de recherche en sciences animales de Deschambault), Quebec, QC, Canada,* [3]*Iowa State University, Ames, IA.*

Twelve multiparous Holstein cows (42.2 ± 5.6 kg milk/d; 83.4 ± 27.1 DIM) were used in a split-plot design testing the effects of mineral and vitamin supplementation on gut health and inflammation during heat stress. The main plot was the level of dietary vitamin E and Se (HESe: 200 IU/kg and 1.2 ppm; LESe: 20 IU/kg and 0.3 ppm; respectively). Within each plot, cows were randomly assigned to 1) heat stress (HS), 2) thermoneutral pair-feeding (TNPF), or 3) HS with vitamin D and Ca supplementation (HS+DCa; 1820 IU/kg vitamin D and 1.5% Ca) 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. The highest rectal temperature recorded was at 1700 h for HS cows (39.4°C, mean of d 1 to 14), being 1.2°C and 0.8°C higher than TNPF and HS+DCa, respectively ($P < 0.05$). Respiration rate and water intake were higher in HS ($P < 0.05$, 73/min, and 115 L) relative to TNPF (28/min and 76 L), respectively. Heat stress increased ($P < 0.05$) fecal calprotectin concentrations over time, being 14 and 17% higher relative to TNPF and 12 and 6% higher relative to HS+DCa on d 7 and 14, respectively ($P < 0.05$). A 3-way interaction was detected for C-reactive protein (CRP) plasma concentrations ($P = 0.10$). In the HESe group, HS increased CRP by 56% on average, relative to TNPF, whereas relative to HS, HS+DCa reduced CRP concentrations by 46% and 29% on d 7 and 14, respectively ($P < 0.05$). However, in the LESe block, no differences in CRP concentrations were detected between HS and HS+DCa. Plasma lipopolysaccharide-binding protein concentrations increased in HS cows by 14 and 49% on d 7 and 14, respectively, relative to TNPF ($P < 0.05$), whereas they were reduced by 32% in HS+DCa on d 14 ($P < 0.05$). Plasma tumor necrosis factor-α concentrations were 49% higher in HS, relative to TNPF cows and 33% higher relative to HS+DCa on d 14 ($P < 0.05$). Vitamin D and Ca supplementation reduced markers of leaky gut and inflammation in lactating dairy cows during heat stress, which may be dependent on the basal levels of dietary vitamin E and Se.

Key Words: heat stress, leaky gut, vitamins

471 The effect of supplementing a natural additive containing *Hibiscus sabdariffa* and a blend of essential oils to Holstein dairy cows on ruminal bacteria and archaea populations.

D. Pitta*[1], J. Ferguson[1], B. Vecchiarelli[1], C. Pappalardo[1], M. Hennessy[1], N. Indugu[1], L. Baker[1], J. Bender[1], J.-P. Ricaud[2], and M. Aoun[2], [1]*Department of Clinical Studies, New Bolton Center, University of Pennsylvania-School of Veterinary Medicine, Kennett Square, PA,* [2]*Idena Inc., Sautron, Pays de la Loire, France.*

The purpose of this study was to investigate the effects of supplementing dairy cows with a natural feed additive containing *Hibiscus sabdariffa* and a blend of essential oils (ValKalor; Idena, Inc.) on ruminal bacteria and methanogen populations. Forty Holstein cows (mean milk production [SD] and DIM, 40.8 kg/d [8.3] and 96.7 d [25.3]) were randomly assigned to 4 treatment pens. The experiment was a switchback design with 2 dietary treatments offered to 2 pens for 2 3-week periods, separated by a 2-week washout period. The treatment cows received 20 g/cow/d of the feed additive in 1.4 kg of ground corn top-dressed on the TMR while the control cows received 1.4 kg of ground corn top-dressed on the TMR. Rumen samples (obtained via orogastric tube) were extracted for DNA, PCR-amplified for the 16S rRNA bacterial and archaeal genes, sequenced on the Illumina MiSeq platform, and analyzed using QIIME2, and were additionally analyzed for volatile fatty acid (VFA) composition. Bacterial richness, species diversity, and principal coordinate analysis showed no differences between control and treatment cows at the community level; however, supplementation with the feed additive increased relative abundance of *Clostridiales* but decreased relative abundance of genera such as *Prevotella*, *RFN*20, *Treponema*, and *Anaeroplasma*. Supplementation with the feed additive tended to increase isovalerate (2.5 vs 2.2%, $P < 0.09$) and valerate (2.1 vs 1.8%, $P < 0.07$) and decreased isobutyrate (1.0 vs 1.1%, $P < 0.02$) concentrations as a percentage of rumen fluid VFA relative to the control treatment. There was no statistical difference in molar proportion of acetate, propionate, and butyrate in rumen fluid VFA. Species richness, species diversity, and principal coordinate analysis revealed differences in archaea community composition in period 1 but not period 2. At the genus level, *Methanobrevibacter* was less abundant ($P < 0.02$) and *Methanosphaera* was more abundant ($P < 0.02$) in treated cows compared with control cows over the course of the experiment. The change in methanogen populations suggests that the feed additive has a tendency toward a hydrogen-sparing effect, altering bacteria-methanogen cohorts which should favor the formation of propionate in the rumen.

Key Words: rumen microbiota, rumen fermentation, amplicon sequencing

472 Quantification of the effects of pH on rumen microbial fermentation and nitrogen metabolism using dual-flow continuous culture studies.

A. A. Alamouti[1], M. Cerrato[2], and S. Calsamiglia*[2], [1]*Department of Animal Science, University of Tehran, Tehran, Iran,* [2]*Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain.*

The objective of this experiment was to quantify the effects of low ruminal pH on rumen microbial fermentation and protein metabolism in a dual-flow continuous culture system. Data were compiled from 6 dual-flow continuous culture studies with 31 treatments. Average pH resulted from either constant pH or pH-cycles with different frequency, duration, or magnitude of reduction, and ranged from 4.9 to 7.0. All studies were conducted in the same laboratory, with a similar diet (60:40 forage:concentrate; 18.5% CP and 33% neutral detergent fiber, NDF) and inoculum (rumen fluid from dairy cattle), and same solid ($0.06/h^{-1}$) and liquid ($0.10/h^{-1}$) dilution rates. Data were analyzed with the PROC

MIXED of SAS for linear, quadratic and cubic effects. Reducing pH linearly decreased NDF degradation (y, % = −32.0 + 11.15xpH; R^2 = 0.74; $P < 0.01$), total volatile fatty acids (y, mM = 7.77 + 16.4xpH; R^2 = 0.49; $P < 0.01$) and the acetate proportion (y, mol/100mol = 9.07 + 8.68xpH; R^2 = 0.65; $P < 0.01$). The effect of pH on true dry matter fermented was quadratic (y, % = −191 + 75.9xpH - 5.91xpH^2; R^2 = 0.39; $P < 0.01$) and that on the propionate proportion was cubic (y, mol/100mol = −1322 + 711xpH - 123xpH^2 + 6.97xpH^3; R^2 = 0.44; $P < 0.01$). Reducing ruminal pH decreased rumen ammonia-N concentration (y, mg/dL = 4.260 × pH – 14.931, R^2 = 0.81; $P < 0.05$) and microbial N flow (y, g/d = 0.0699 + 0.2189 × pH; R^2 = 0.29; $P < 0.01$); and increased non-ammonia-N flow (y, g/d = 3.5834 – 0.111 × pH; R^2 = 0.45 $P < 0.01$) and dietary N flow (y, g/d = 3.5588 – 0.3378 × pH; R^2 = 0.82, $P < 0.01$). The efficiency of microbial protein synthesis (average of 29.2 g bacterial N/kg organic matter truly fermented) was not affected by pH. Reducing pH from a normal (6.50) to a subacute ruminal acidosis (5.70) conditions results in a 6, 22 and 11% reduction in true dry matter degradation, NDF degradation and total volatile fatty acids, respectively; and a 3% increase in non-ammonia-N flow, 12% reduction in microbial N flow and 20% increase in dietary N flow. The contribution of microbial N to total non-ammonia-N flow decreased from 52 to 42%. Changes in pH may affect total protein supply and the partial contribution of microbial and dietary protein available for absorption.

Key Words: dual-flow continuous culture, rumen pH, fermentation

473 Effects of supplemental source of magnesium and inclusion of buffer on ruminal fermentation in continuous culture.

J. A. Arce-Cordero*[1], A. Ravelo[1], J. R. Vinyard[1], H. F. Monteiro[1], B. C. Agustinho[2], E. Sarmikasoglou[1], S. L. Bennett[3], and A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Idaho, Moscow, ID,* [3]*Pennsylvania State University, University Park, PA.*

Magnesium oxide (MgO) is the most common supplemental source of Mg and a proven ruminal alkalizer. It is unclear if Mg supplements influence the effects of bicarbonate-based buffers on ruminal fermentation. We aimed to evaluate the effect of Mg source on ruminal fermentation with diets formulated to a final concentration of 0.25% Mg, and to determine if the effect of sodium sesquicarbonate buffer varies with the source of Mg. We used 8 fermenters in a duplicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments, by combining 2 factors: 1) Mg source: using either MgO as the common source (MgO) or a proprietary mineral formulation (PMF) of carbonates, oxides, and hydroxides of Mg; and 2) sodium sesquicarbonate buffer inclusion: at 0 or 0.6% of dry matter intake. Treatments were: 1) MgO; 2) MgO + 0.6% buffer; 3) PMF; 4) PMF + 0.6% buffer. Four 10-d periods were completed, where the last 3 d were used for pH measurements and collection of samples for: volatile fatty acids (VFA), ammonia (NH_3-N), Mg solubility, N metabolism, and nutrient digestibility. Effects of Mg source (Source), sodium sesquicarbonate inclusion (Buffer), and their interaction (Source × Buffer) were tested with the MIXED procedure of SAS. Source of Mg did not affect ruminal fermentation variables; however, concentration of soluble Mg in ruminal fluid was greater for MgO compared with PMF. Buffer supplementation increased ruminal pH and molar proportions of acetate and branched-chain VFA; tended to increase NDF digestibility; and decreased both area under the curve and time below pH 6.0. Interaction Source × Buffer was found for propionate, butyrate, and NH_3-N, the first one decreasing and the 2 others increasing only when buffer was supplemented to the PMF diet. Our results indicate that supplementing Mg with either MgO or PMF promote similar ruminal fermentation in diets with total concentration of 0.25% Mg. However, addition of buffer affected more ruminal fermentation variables when added to PMF than when added to MgO diet. Further evaluations are needed to assess Mg availability and animal performance in dairy cows fed PMF.

Key Words: alkalizer, in vitro, minerals

474 Comparative network analyses of continuous culture rumen fermentation.

S. Sujani*[1], B. Wenner[2], J. L. Firkins[2], and R. R. White[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*The Ohio State University, Columbus, OH.*

Drawing inferences from the complex, multifactorial rumen environment remains a challenge despite progress made by synthesizing knowledge through empirical and mechanistic models. An alternative synthesis approach for characterizing highly dimensional data is network analysis. The objective of this study was to leverage frequentist and Bayesian network analyses to summarize relationships among parameters measured in 4 previously published dual-flow continuous culture fermentation experiments conducted within a single laboratory. These experiments fed pelleted diets (50:50 forage: concentrate) ranging from 36.3 to 60 g DM daily and controlled at a constant 7%/h total dilution rate. Nodes included were volatile fatty acid concentrations; non-ammonia non-bacterial nitrogen (N); non-ammonia nitrogen (NAN); bacterial N (BN); ammonia nitrogen (NH_3-N); degradability of neutral detergent fiber (NDF) and organic matter (OM); dry matter intake (DMI), urea, fluid passage rate; and total protozoa count. In the frequentist network acetate (Ac) and NAN had central roles, indicated by a high number of connections (degree), short distance to other nodes (closeness), and serving as an intermediate in multiple relationships (betweenness). Urea also had high degree and closeness indices; while valerate (Va) had high closeness and betweenness. Other notable nodes included NH_3-N, which had high degree, and DMI, which had high betweenness. The Bayesian analysis differs from the frequentist in that its relationships are directional and the network is cascading, not circular. Despite this, the Bayesian network also identified Ac, NH_3N, and NAN as important drivers of dynamics. In contrast, the Bayesian network suggested the importance of Va and urea identified in the frequentist network is mostly due to these variables responding to the rumen environment, not driving it. Both analyses highlight the importance of Ac, NH_3-N, and NAN as critical indicators of rumen kinetics, and provide inference on the connectedness and directionality of relationships among fermentation parameters which can be used to drive future studies.

Key Words: acetate, model, nitrogen

475 Effects of corn kernel particle size on rumen kinetics of corn silage nutrients.

I. Ansia*[1], A. Santos[2], B. M. Tas[3], J. A. Metcalf[4], and H. van Laar[1], [1]*Trouw Nutrition, Amersfoort, the Netherlands,* [2]*Trouw Nutrition Spain, Tres Cantos, Spain,* [3]*ForFarmers, Lochem, the Netherlands,* [4]*Trouw Nutrition Canada, Guelph, ON Canada.*

This experiment assessed the effect of corn kernel particle size on in situ rumen degradation kinetics of corn silage. Treatments consisted on 3 processing roll clearances (between 1 to 14 mm) during harvesting of 15 original corn crops. Crops varied in country of origin (Spain, Canada, The Netherlands), field, harvesting date and hybrid. After ensiling, particle size was evaluated by dry (corn silage particle score; CSPS; [44–86%]) and wet sieving (WCSPS; [37–92%), and by the mean particle size (MPS; [0.23–0.46mm]) using the Penn State Particle Separator. Each of the 45 samples were incubated as received in nylon bags (6.45 ± 0.35 g DM/bag) in the rumen of 3 cannulated cows for

3, 8, 16, 32, 56, 96 and 336 h in duplicate. Residues were pooled by time point and sample, then analyzed for DM, CP and starch (ST). The washable fraction (W) was determined using a washing machine rinse cycle for 30 min at 10°C (0 h residue). The degradable fraction (D) was calculated as the difference of the undegradable fraction (U; 336 h residue) and the 0 h residue (100 - W). Degradation rate (K_d) of D was estimated using PROC NLIN in SAS according to the model: $Y = U + D * \exp(-K_d * \text{time})$ where Y is the fraction degraded over time. Effective degradability (ED) was calculated at a passage rate of 5%. Results were compared using PROC MIXED with either CSPS, WCSPS or MPS, plus NDF, ADF, CP, DM, ash and ST as covariates. The original sample, hybrid and harvesting date nested within field and location were random factors. MPS did not affect any variable. The W of DM increased (0.10% units of DM) per unit of CSPS ($P = 0.02$) or WCSPS (0.15%; $P < 0.001$). ST_W increased (0.72%; $P = 0.02$) and CP_W tended to increase (0.20%; $P = 0.1$) with WCSPS. DM_U was decreased 0.05% per unit of CSPS ($P = 0.02$). ST_Kd increased by 0.002%/h per unit of CSPS ($P = 0.05$) or WCSPS ($P = 0.02$). The ED of DM (72 ± 3%) and ST (76 ± 12%) increased ($P < 0.03$) per unit of WCSPS (0.1% and 0.74% respectively) and CSPS (0.08% and 0.51% respectively). Reducing corn kernel particle size (as estimated by WCSPS and CSPS) increases rumen degradability of DM and ST in corn silage.

Key Words: digestibility, forage, processing score.

476 Liquid marker flow: Implications of marker infiltration in ruminal solids. M. B. Hall*, *U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Liquid (Liq) passage rate (kp; h^{-1}) is used to estimate rumen outflow of Liq-associated nutrients. Liqkp estimated as the slope of the line through ln of marker (MA) μg/mL plotted against time (lnkp) assumes MA only associates with free Liq. Published work suggests existence of 2 rumen Liq pools. A likely second pool found in the present cow study was the 6 to 14% of MA dose (90 g polyethylene glycol, 3500 g/mol; PEG) in rumen digesta solids (Sol); Liq and Sol were separated with a wine press (press) at 9 h post-dose, post-feeding. We compared 1 (lnkp) and 2 (Trikp) pool methods of estimating Liq MA passage using lactating cow data (8 cows, 2 diets, 2 period reversal design). In each period rumen Liq was sampled hourly 2 to 9 h post-dose for lnkp calculation, with rumen evacuation at 9 h to measure residual PEGg in press Liq and Sol; PEGg = PEG μg/g × 10^6 × Liq g or Sol g). Trikp was calculated with PEGg pools: A = Liq and B = Sol, kp: kp1 = PEG transfer from B to A; passage from rumen are kp2 = PEG from B (SolPass) and kp3 = PEG from A (LiqPass). Variables were calculated from residual PEGg (DR) = initial PEGg (DI) × $e^{(-kt)}$; k = kp and t = hour. PEGg DR of each hour = DI of the next. At each hour, PEGg DR by pool: A = DI A – LiqPass + from B; B = DI B – B to A – SolPass. Trikp predicted PEGg in A by hour-cow (PRED) was compared with observed (OBS) using mixed model analysis with variables period, hour, OBS or PRED, 2-way interactions, and cow (random) (108 cow-hour observations). PEGg LiqPass between 2 h and 9 h for lnkp and Trikp were compared with a paired *t*-test (n = 16). Significance declared at $P < 0.05$. PEGg movement between 2 and 9 h were (mean ± standard deviation): LiqPass, lnkp = 24.8 ± 5.4, Trikp = 35.8 ± 3.7; Trikp B to A = 9.70 ± 3.5 and SolPass = 4.28 ± 0.91. PEG kp were lnkp = 0.146 ± 0.026, Trikp: kp1 = 0.066 ± 0.02, kp2 = 0.029 ± 0.01, kp3 = 0.215 ± 0.04. Trikp hourly PEGg in A PRED (25.3 g) and OBS [25.7 g; standard error (SE) = 1.30; $P = 0.29$] did not differ. PEGg LiqPass was less for lnkp than Trikp (mean difference −11.0; SE 1.92; $P < 0.01$). If the 2 pool approach is correct, current views on rumen liquid passage and liquid × solid interactions bear reconsideration.

Key Words: passage, liquid, kinetics

477 Metagenomic sequencing reveals that the ruminal resistome of dairy cattle is individualized and associated with milking traits. M. Y. Xue*[1], Y. Y. Xie[1], Y. F. Zhong[1], J. X. Liu[1], L. L. Guan[2], and H. Z. Sun[1], [1]*Institute of Dairy Science, Ministry of Education Key Laboratory of Molecular Animal Nutrition, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China,* [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Antimicrobial resistance is one of the biggest public health challenges and increases morbidity and mortality in humans and food-producing animals. The rumen microbiome carries a large number of antimicrobial resistance genes (ARGs), which could disseminate to the environment through saliva, or flow to the hindgut and release through feces. The host effect on the ruminal resistome and their interactions remain largely unknown. Here, 49 mid-lactating Holstein dairy cows (parity = 2.48 ± 0.62, mean ± SD) raised on a commercial dairy farm (Hangzhou, China) were selected to investigate the ruminal resistome and its relationship with host feed intake and milk protein yield using metagenomic sequencing. The ARGs in different groups were compared using the Wilcoxon rank- sum test, with a false discovery rate (FDR) < 0.05 considered significantly different. The ruminal resistome conferred resistance to 26 classes of antimicrobials, with genes encode resistance to tetracycline being the most predominant. The ARG-containing contigs were assigned to bacterial taxonomy, and the majority of highly abundant bacterial genera were resistant to at least one antimicrobial, while the abundances of ARG-containing bacterial genera showed distinct variations. Although the ruminal resistome is not co-varied with host feed intake, it could be potentially linked to milk protein yield in dairy cows. Host feed intake did not affect the α or β diversity of the ruminal resistome or the abundances of ARGs, while the Shannon index ($R^2 = 0.63$, $P < 0.01$) and richness ($R^2 = 0.67$, $P < 0.01$) of the ruminal resistome were highly correlated with milk protein yield. A total of 128 significantly different ARGs (FDR <0.05) were identified in the high- and low-milk protein yield dairy cows. We found 4 ruminal resistotypes that are driven by specific ARGs and associated with milk protein yield. Particularly, cows with low milk protein yield are classified into the same ruminal resistotype and featured by high-abundance ARGs, including mfd and sav1866. Our study uncovered the prevalence of ruminal ARGs in the rumen and the host-ruminal resistome interactions of dairy cows, providing fundamental knowledge and evidence for interventions to reduce AMR and regulate ARGs in ruminant livestock.

Key Words: metagenome, ruminal resistome, milk trait

478 Comparing rumen degradability characteristics of conventional and alternative grazing swards for dairy cows. K. M. McCarthy*[1,3], M. B. Lynch[2], K. M. Pierce[2], V. P. Gath[1], M. McDonald[3], A. G. Fahey[2], and F. J. Mulligan[1], [1]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland,* [2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [3]*UCD Lyons Research Farm, Celbridge, Kildare, Ireland.*

Grazing swards containing multiple plants can reduce nitrogen losses and maintain milk production in grazing dairy production systems. Our objective was to compare the rumen digestion characteristics of 3 swards;

perennial ryegrass (PRG), perennial ryegrass (64.2%) and white clover (31.3%) (PRGWC), and multispecies (MULTI) containing perennial ryegrass, timothy, white clover, red clover, chicory and plantain (11.6%, 2.5%, 4.8%, 25.4%, 16.5% and 36.7% respectively). Six dairy cows were used in a replicated 3 × 3 Latin square. Cows were acclimatized to diet for 14 d then began a 7-d sampling period. Cows were housed in individual metabolism stalls and offered 20 kg dry matter (DM) of forage, 3 kg concentrates and ad libitum water daily. Swards were cut each morning using a zero-grazer. Indwelling rumen pH probes (eCow) were used to collect rumen pH data on d 15 and 16. Ruminal degradability was assessed using in situ nylon bags. Fresh forage (4 g DM/bag) was cut with scissors to 3 cm and weighed into nylon bags. Bags were incubated in the rumen for 0, 2, 4, 6, 8, 10, 12, 24 and 72 h. All bags, including 0 h bags, were placed in ice water, rinsed in cold water, pummeled in a stomacher for 5 min in 0.9% cold saline, then washed in a washing machine for 30 min. Degradability parameters for DM and CP were estimated using PROC NLIN in SAS according to Ørskov and McDonald (1979). Effective degradability (ED) was determined using a rumen outflow rate (k_p) based on NRC (2001) equation for wet forages ($k_p = 4.55\%$/h). Degradability parameters were analyzed using PROC MIXED. Model included the fixed effect of treatment, period and their interaction, and the random effect of cow. Hour was included in the model for pH analysis. Sward type had a significant effect on rumen degradability. MULTI had the highest ED (76.52%) and *c* fraction (0.099%/h) for DM of all treatments ($P < 0.05$). All swards had a different DM *a* fraction. MULTI (39.78%) was highest, followed by PRG (35.74%) and PRGWC (30.86%) ($P < 0.05$). PRG had a higher CP *a* fraction (34.15%; $P = 0.007$) and ED (81.40%; $P = 0.056$) than PRGWC and MULTI. Sward type had no significant effect on mean, minimum or maximum rumen pH. Our experiment showed the DM fraction of multispecies swards is more degradable than perennial ryegrass and perennial ryegrass - white clover swards. This information may help inform decisions on optimal supplementation strategies for cows offered these swards.

Key Words: multispecies, in situ

479 Amla (*Phyllanthus emblica*) fresh fruit as new feed source: I. Effect on apparent digestibility, ruminal fermentation, and milk performance in lactating dairy cows.

M. T. Meselu[1], L. Zhao[1], G. Zitai[1], S. Yifan[1], L. Ma[1], T. Callaway[3], J. Xu[2,5], and D. Bu*[1,4], [1]*Institute of Animal Science, Beijing, China,* [2]*Kunming Institute of Botany, Kunming, China,* [3]*University of Georgia, Athens, GA,* [4]*Joint Laboratory on Integrated Crop-Tree-Livestock Systems of the Chinese Academy of Agricultural Sciences (CAAS), Ethiopian Institute of Agricultural Research (EIAR) and World Agroforestry Center (ICRAF), Beijing, China,* [5]*World Agroforestry Centre East and Central Asia, Kunming, China.*

Amla fruit, or indian gooseberry *(Phyllanthus emblica),* is a common deciduous fruit crop in Asia. This study sought to determine the effect of feeding 3 levels of fresh amla fruit on ruminal digestibility, fermentation parameters, and milk production of lactating dairy cows. Eight cannulated cows were evenly divided and were randomly assigned into the 2 treatment groups, 4 cows per group (control and treated). After a 14-d adaptation period, in period 1, we supplemented the first group of cows who assigned in treatment groups, TMR with sequentially increasing levels of fresh amla fruit at a 14-d interval (200, 400, then 600 g/d). Simultaneously, the remaining 4 cows assigned to the control group were provided TMR without amla fruit for the same period. Following the completion of period 1 and 2 weeks of washout period, control and treatment cows were crossed over to take part in period 2 (6-weeks). For each subperiod, the first 1–10 d for each period were adjusted to diet adaptation and the last 4 d for sampling of milk, feed, rumen, faces, and urine. Data were analyzed using PROC MIXED of SAS. Amla fruit supplementation did not affect the DMI. However, the apparent digestibility of DM ($P < 0.01$), OM ($P < 0.01$), and CP ($P = 0.02$) were increased cubically at 200 and 600g/day. Amla fruit addition quadratically decreased ($P < 0.001$) ruminal NH3- N concentrations by −54.9 and −11.9% at 200 and 400 g/d, respectively, but was increased by +29.8% at 600 g/d compared with controls. Amla fruit increased propionate proportion ($P < 0.01$), while acetate was reduced cubically ($P = 0.01$). Milk yield was quadratically increased, with the highest milk production occurring at 200 g/d amla fruit supplementation. In addition, the highest ECM, milk fat% and milk fat yield ($P = 0.01$), true milk protein yield ($P < 0.001$), milk protein% ($P < 0.01$), milk lactose yield, and milk NE_L ($P = 0.02$) occurred on the 400 g/d treatment. Nitrogen efficiency in milk was increased ($P < 0.05$) by 2.6% for 400 g/d supplementation than control cows. MUN was reduced quadratically ($P < 0.01$) by 28.8 and 20.2% compared with controls at 200 and 400 g/d amla fruit, respectively. Therefore, we suggest amla fruit can be incorporated from 200 to 400 g/d on a fresh base in the lactating dairy cow diet to improve milk performance, fermentation, digestibility, milk nitrogen efficiency, and MUN.

Key Words: amla, rumen fermentation, milk

480 Heritable and non-heritable rumen bacteria are associated with differing roles and predictability of lactation performance of dairy cows.

X. W. Zang*[1], H. Z. Sun[1], M. Y. Xue[1], G. Plastow[2], T. F. Yang[2], L. L. Guan[2], and J. X. Liu[1], [1]*Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China,* [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Recent studies have shown the evidence of rumen microbes to be heritable, but it is not known whether their functions and contributions to cattle phenotypes (microbiability) differ from taxa that are non-heritable, nor if they can be used to help predict animal performance traits. A total of 285 healthy mid-lactation Holstein dairy cows that fed the same total mixed rations under the same environment and management were used in the current study, and the samples of milk, blood and rumen fluids were collected from all the cows before morning feeding. We aimed to identify the distribution and predicted functions of heritable and non-heritable bacterial taxa in the rumen of dairy cows. We also investigated the relationship between bacterial groups and lactation performance traits including milk yield (MY), milk protein percentage (MP), and milk protein yield (MPY); and their predictive ability based on the use of random forest approaches. Linear mixed model methods and restricted maximum likelihood were used to estimate heritability, microbiability, and to identify SNP associated with rumen microbes. Forty-seven heritable and 40 non-heritable bacterial taxa were identified in the rumen of lactating Holstein dairy cows and the predicted functions differed between 2 classes of microbiota. Functional analysis found 277 differentially enriched bacterial functions between heritable and non-heritable bacteria, and 22 between high- and low-MPY dairy cows. The heritable and non-heritable bacterial microbiability explained 8 and 4%, 3 and 2%, and 14 and 8% of the variation in MY, MP, and MPY, respectively. Twenty-seven heritable microbial taxa were associated with lactation traits ($P < 0.05$); and 39 SNP were related to 14 rumen bacteria ($P_{adj} < 0.05$). The prediction accuracy of MY, MP, and MPY was 0.88, 0.54, and 0.77, and 0.79, 0.51, and 0.55, based on incorporation of heritable and non-heritable bacteria taxa, respectively. Our study suggests that heritable bacteria taxa have a high association with lactation performance of dairy cows and may be used to predict lactation traits with moderate to good accuracy. The results from this work provide novel insights into a microbe-based prediction of phenotypic traits.

Key Words: heritable microbial taxa, microbiability, random forest

481 Effects on rumen pH and milk composition of feeding either zero-grazed fresh grass or grass silage-based diets in lactating dairy cows.

O. Neville*[1], A. Fahey[2], M. Sheehy[3], and F. Mulligan[1], [1]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland,* [2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [3]*Devenish Nutrition Ltd., Belfast, United Kingdom.*

In many temperate regions of the world, grazed grass is often the predominant feed for dairy cows. However, farm fragmentation or unfavorable weather periods can limit the use of grazed grass in milk production systems, creating a need for alternative feeding systems. Two alternatives to grazed grass are zero-grazed fresh grass or grass silage. However, effects on rumen pH and performance of these 2 alternatives are not well known. Thus, the objective of this experiment was to investigate the effects of zero-grazed fresh grass and grass silage-based diets on rumen pH and performance in lactating dairy cows. Four rumen cannulated lactating dairy cows were used in a 4 × 4 Latin square design experiment consisting of 4 26-d periods with 2 replicates per treatment per period. Treatments were: 1) Zero-grazed fresh grass-based diet (ZG) and 2) Grass silage-based diet (GS). Grass used for both treatments was harvested from perennial ryegrass-based swards during summer. ZG consisted of zero-grazed fresh grass offered alone several times daily with a partial TMR fed separately once daily. GS consisted of a grass silage-based TMR. To have a comparison of fresh and ensiled grass that was not confounded by diet energy density, both diets were isoenergetic and fed ad-lib. Rumen pH was measured over 3 d, using internal pH probes. Milk yields and milk samples were collected from a.m. and p.m. milkings on 2 consecutive days in each period. Concentrations of milk fat and protein were determined using infrared analysis. Data were analyzed using a mixed model including fixed effects of treatment and period, with cow considered as the random effect. No differences were observed between treatments for mean, minimum, maximum, or standard deviation of rumen pH. ZG increased milk yield ($P < 0.05$), combined fat plus protein yield ($P < 0.05$) and protein yield ($P < 0.05$) in comparison to GS. No treatment differences were detected for fat yield, fat content or protein content. Feeding a zero-grazed fresh grass diet improves milk and protein yield in lactating dairy cows but rumen pH is not different when compared with grass silage diets.

Key Words: rumen pH, lactating cow, grass

482 Brown midrib (BMR) genotype improves neutral detergent fiber digestibility within, but not necessarily across, species.

H. Galyon*, C. Teets, R. Cockrum, and G. Ferreira, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

The objective of this study was to compare NDF digestion kinetics of brown midrib (BMR) and conventional (CONV) genotypes within and across species. Two BMR and 2 CONV varieties of corn (CO), sorghum (SG), and pearl millet (PM) were planted (3 plots/variety) in Blacksburg and Glade Spring, VA in 2019 and 2020. At harvest, 5 plants were chopped, and leaf blades and stem internodes from 5 plants were collected. Samples were incubated in the rumen of 3 cannulated cows for 0, 3, 6, 12, 24, 48, 96, and 240 h. The potentially degradable NDF (pdNDF) and the fractional rate of disappearance (*kd*) were estimated and analyzed using PROC NLIN and PROC MIXED of SAS, respectively. Effective ruminal degradability (ERD) was estimated using a 4%/h rate of passage. The statistical model included the fixed effects of site, year, species (S), and genotype (G) and residual error. In whole plants, BMR had greater pdNDF than CONV (75.5 vs. 68.1%; $P < 0.01$). CO had the greatest (78.5%), PM had the intermediate (70.8%), and SG had the least (66.2%) pdNDF ($P < 0.01$). BMR had greater *kd* than CONV (3.1 vs. 2.4%/h; $P < 0.01$), but *kd* did not differ among species ($P < 0.37$). BMR had greater ERD than CONV for whole plants (31.5 vs. 24.9%; $P < 0.01$), and CO (30.6%) had greater ERD than SG and PM (27.1%; $P < 0.02$). In leaf blades, BMR had greater pdNDF than CONV in CO (85.5 vs. 78.6%) and SG (79.8 vs. 76.7%) but not in PM (84.0%; SxG $P < 0.02$). Neither genotype ($P < 0.08$) nor species ($P < 0.86$) affected *kd* (2.8%/h). BMR had greater ERD than CONV (34.2 vs. 31.2%, $P < 0.02$). ERD did not differ among species ($P < 0.28$). In stem internodes, BMR had greater pdNDF than CONV in CO (65.9 vs. 57.0%) and PM (62.1 vs. 52.5%) but not in SG (58.8%; SxG $P < 0.03$). BMR had greater *kd* than CONV (2.5 vs. 2.0%/h, $P < 0.04$) but *kd* did not differ among species ($P < 0.57$). BMR had greater ERD than CONV for stem internodes (23.2 vs. 17.9%; $P < 0.01$), and CO (22.9%) had greater ERD than SG and PM (19.4%; $P < 0.05$). In conclusion, BMR has greater fiber digestion kinetics than CONV within species. However, when comparing across species, BMR does not always have greater fiber digestion kinetics than CONV.

Key Words: brown midrib (BMR), fiber digestibility

Small Ruminant II

483 Sheep cheese fatty acid composition: How much can we improve by dietary means? E. Vargas-Bello-Pérez*[1], B. Darabighane[2], F. E. Miccoli[3,4], and P. Gómez-Cortés[5], [1]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark,* [2]*Department of Animal Science, University of Mohaghegh Ardabili, Ardabil, Iran,* [3]*Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora, Lomas de Zamora, Argentina.,* [4]*Facultad de Agronomía, Universidad de Buenos Aires, CABA, Argentina,* [5]*Instituto de Investigación en Ciencias de la Alimentación (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain.*

Oil and seeds supplementation is a promising strategy for producing healthier fatty acid (FA) profiles in cheese. While several studies have focused on improving cheese FA composition in cows, sheep cheeses have received less attention and the connection between sheep nutrition and dairy quality is an interesting research field that needs to be further investigated. Thus, the main objective of this study was to use a meta-analysis approach to evaluate the effects of dietary plant sources rich in unsaturated fatty acids (UFA) on cheese contents of total saturated (SFA), total monounsaturated FA (MUFA) and total polyunsaturated FA (PUFA) in sheep. Another objective was to elucidate the degree of heterogeneity of responses. Data from this study will be useful to further understand cheese FA when animals are fed with vegetable oil and oilseeds. Publications from 2005 to 2019 were extracted from Web of Science and Scopus. The keywords used for the search were: fatty acids and cheese and sheep. Around 223 publications were obtained, however, the inclusion criteria required only studies with a control treatment and animals fed on diets based on either pastures or preserved roughages (n = 7). Plant sources were flaxseed, sunflower, extruded linseed and oils from olive, soybean, linseed, canola, and rice bran. In the meta-analysis, size effect for all outcomes was reported as standardized means difference (SMD) with 95% confidence intervals. Q test and I^2 were calculated to detect the heterogeneity. Eighteen comparisons were made between the plant supplements and control treatments for SFA, MUFA and PUFA. Results indicated that inclusion of rich sources of UFA to the diet of dairy ewes, can decrease SFA (SMD = −3.83 g/100g, $P < 0.001$), while significantly increasing MUFA (SMD = 3.82 g/100g, $P < 0.001$) and PUFA (SMD = 3.08 g/100g, $P < 0.001$). The I^2 statistic for SFA, MUFA and PUFA analyzed here was more than 90% with respect to heterogeneity. Results also showed that the level of inclusion of dietary lipids and the contents of C18:2 and C18:3 in the diets are some factors affecting heterogeneity. Overall, the magnitude of changes in the main groups of FA in cheese will depend on the chemical configuration of dietary lipids as they will influence the formation of biohydrogenation intermediates and saturated FA end products escaping from the rumen.

Key Words: cheese fatty acids, oil supplementation, dairy sheep

484 Predictive biometrics of hair sheep through digital image analysis. A. J. Chay-Canul[1], J. A. Tapia-González[1], J. R. Canul Solis[2], F. Casanova-Lugo[3], A. Piñeiro-Vázquez[4], R. Portillo-Salgado[5], R. G. Herrera[1], and E. Vargas-Bello-Pérez*[6], [1]*División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Tabasco, México,* [2]*Tecnológico Nacional de México, Instituto Tecnológico de Tizimín, Tizimín, Yucatán, Mexico,* [3]*Tecnológico Nacional de México, Instituto Tecnológico de la Zona Maya, Othón P. Blanco, Mexico,* [4]*Tecnológico Nacional de México, Instituto Tecnológico de Conkal, Conkal, Mexico,* [5]*Programa de Ganadería, Colegio de Postgraduados, Campus Montecillo, Texcoco, Estado de México, Mexico,* [6]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark.*

Direct collection of biometric measurements (BM) from sheep is an expensive and stressful procedure for animals; instead, indirect and novel methods have recently been used. The objective of this study was to use digital image analysis (DIA) to predict biometric measurements of Pelibuey sheep as a non-invasive approach under on-farm conditions. In vivo measurements of withers height (WH), body length (BL), body diagonal length (BDL) and rib depth (RD) were performed using a flexible fiberglass tape and a 65 cm caliper. Then, WH, BL, BDL and RD were predicted using DIA. For that, images were taken from the left flank from 65 non-pregnant and nonlactating Pelibuey ewes using a digital camera and analyzed by DIA. Three photographs were taken from each animal from the left flank. The best image was selected using the criteria that the entire body of the ewe should stand inside the rectangular area setup and that the image should be of good (clear and steady) resolution. The images were analyzed using ImageJ software 1.51. Correlation coefficients (r) between variables were estimated using the CORR procedure of SAS. The relationships between BM determined by DIA and BM determined in vivo were estimated with linear regression models using the GLM procedure of SAS. The BM determined from both in vivo and by DIA presented a positive and moderate ($P < 0.05$) correlation coefficients (r) of 0.43, 0.66, 0.73 and 0.75 for BL, BDL, WH and RD, respectively. The regression equations from BM by DIA had a determination coefficient (r2) of 0.19, 0.44, 0.54 and 0.56 for BL, BDL, WH and RD, respectively. Overall, the use of digital images analysis was able to predict biometric measurements in Pelibuey ewes with low to moderate precision (r2 > 0.18 ≤ and ≤0.55) and accuracy (>0.69). Future studies should address the relationships between digital images analysis and prediction of body weight, body mass index and body composition in animals from different physiological and production conditions. Also, to improve precision and accuracy, it is necessary to further evaluate that the equations are not population-dependent.

Key Words: biometric measurements, digital image analysis, linear regression equations

485 Rumen microbiome and efficiency parameters of dairy ewes using 16S rRNA gene amplicon sequencing. F. Miccoli*[1,2], L. Guerrero[4,3], D. Colombatto[2,3], and R. A. Palladino[1,3], [1]*School of Agriculture Science, National University of Lomas de Zamora (UNLZ), Buenos Aires, Argentina,* [2]*Department of Animal Production, University of Buenos Aires (UBA), Buenos Aires, Argentina,* [3]*Consejo Nacional de Investigaciones Cientificas (CONICET), Buenos Aires, Argentina,* [4]*Instituto de Investigaciones en Ingeniería Genética y Biología Molecular "Dr. Héctor N. Torres" (INGEBI), Buenos Aires, Argentina.*

As long as new advances were achieved since the first studies with culture-based techniques up to the Next Generation Sequencing (NGS), there is a better understanding of how to manipulate rumen microbial communities to improve feed utilization and productive parameters. The aim was to analyze the correlations between efficiency parameters and the dominant rumen taxa of dairy ewes supplemented with corn grain or soybean hulls on a fresh ryegrass-based diet. Microbiome from 18 samples of rumen liquor (solid and liquid) from 6 fistulated ewes

(30DIM; 78.2 ± 13.53kg) in a crossover design was analyzed. Briefly, The V4 region of the 16S rRNA gene in bacteria and archaea was PCR amplified to generate 18 amplicon libraries and run on an Illumina MiSeq 500. OTU identification was assessed by QIIME. Microbiome composition and taxa differences were previously reported (Miccoli et al., 2016). This analysis focused on operational taxonomic units (OTUs) over 0.1% abundance and present at 50% samples. Pearson correlations were assessed in R software. *Methanobrevibacter* was negatively correlated with feed efficiency (FE) ($R^2 = -0.48$; $P = 0.01$), possibly explained by methane emissions. FE was positively correlated with order *Bacteroidales* ($R^2 = 0.48$; $P = 0.04$). Family *Chitinophagaceae* was negatively correlated with Forage Intake ($R^2 = -0.61$, $P = 0.04$); genus *Butyrivibrio* and *Ruminococcus* were negatively correlated with neutral detergent fiber (NDF) and acid detergent fiber (ADF) intake ($P < 0.05$), with higher abundance in corn grain diet. Phylum TM7 tended to be negatively correlated with ADF intake ($R^2 = -0.43$; $P = 0.07$). Milk Production (MP) was correlated with genus *Clostridium* ($R^2 = 0.49$; $P = 0.04$) and order *Bacteroidales* ($R^2 = -0.46$; $P = 0.05$). MP tended to be associated with genus *Ruminococcus* ($R^2 = 0.46$; $P = 0.05$), and family *Chitinophagaceae* ($R^2 = -0.42$; $P = 0.08$). These correlations highlight the need of better study taxas within genus *Clostridium*, such as *Ruminococcus*, which may be positively correlated with milk production in dairy ewes.

Key Words: rumen microbiome, efficiency parameters, dairy ewes

486 How does the weaning age of dairy goat kids affect their growth and behavior?

S. Bélanger-Naud*[1], A. Zambelis[1], J. Lévesque[2], C. Julien[2], and E. Vasseur[1], [1]*McGill University, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*Centre de Recherche en Sciences Animales de Deschambault, Deschambault, QC, Canada.*

Weaning is often associated with a period of stress and poor welfare in the kids' life, leading to a decline in growth; however, little is known on the best weaning management practices. This study aimed to determine the optimal weaning age for high-fed dairy goat kids to maximize weight, feed intake, and behavioral outcome measures of welfare around weaning. 36 newborn female Alpine goat kids were randomly allocated to 1 of 3 weaning age treatments: 6 (6W; n = 12), 8 (8W; n = 12) and 10 weeks of age (10W; n = 12), blocked by weight and birth date. Kids had ad libitum access to acidified milk replacer, concentrates, hay, and water from birth. Weaning was performed progressively over 7 d (day −7 to −1). Kids were weighed weekly from birth to 12 weeks of age. Feed intake was measured daily for milk consumption, and weekly for hay and concentrate consumption. Live behavior observations were performed by a single observer before, during, and after morning feed delivery for 5, 2 and 5 non-consecutive minutes, respectively. Observations for 10 behaviors were completed on days −8, −4, 0, 6, and 12 relative to weaning. Based on Kruskal-Wallis tests, no difference in weight ($P > 0.1$) was found among the 6W (+3.0 ± 0.3 kg), 8W (+3.6 ± 0.3 kg) and 10W (+3.8 ± 0.4 kg) treatment groups over the weaning period relative to baseline. No difference was found in lying time either. Over the weaning period, 10W kids were found to have the greatest reduction in milk consumption (−2921.2 ± 378.8 mL; $P < 0.0001$) and greatest increase in concentrate consumption (421.7 ± 200.0 g; $P = 0.016$) relative to baseline, followed by 8W and 6W kids respectively. 6W kids had the greatest reduction in self-grooming time (−14.9 ± 1.3 s; $P < 0.0001$) and greatest increase in vocalization frequency (7.2 ± 3.5; $P = 0.0008$) over the weaning period relative to baseline compared with 8W and 10W kids, respectively. In conclusion, weaning kids at 6 weeks of age was found to limit experience with concentrate consumption, reduce self-grooming time, and increase vocalization frequency compared with moderate and late weaning, indicating a higher stress level in kids weaned earlier.

Key Words: weaning, dairy goat kid, behavior

487 Effects of low level of organic trace minerals on milk performance and, antioxidant and trace minerals status in dairy goats.

P. Wang*[1], Y. Weng[1], J. Luo[1], G. Lin[2], and Q. Hu[3], [1]*Northwest A&F University, Yangling, China,* [2]*Institute of Quality Standards and Testing Technology for Agricultural Products, Chinese Academy of Agricultural Sciences, Key Laboratory of Agrifood Safety and Quality, Ministry of Agriculture and Rural Affairs, Beijing, China,* [3]*Beijing Alltech Biological Products (China) Co. Ltd., Beijing, China.*

The objective was to evaluate effects of replacing inorganic trace minerals (ITM) with 50% level of organic trace minerals (OTM) on milk performance, antioxidant and trace minerals status in dairy goats. Forty Xinong Saanen dairy goats (71.55 ± 1.32 kg; 23.80 ± 3.41 d before expected parturition date) were randomly assigned to 2 treatments: ITM diet with inclusion of ITM (Fe, Cu, Zn, Mn provided as sulfate, and Se as selenite) at 120% level of NRC (2007) recommendations, while OTM diet with inclusion of OTM (Fe, Cu, Zn, Mn provided as amino acid complex, and Se provided as selenium yeast) at 50% level of ITM for Fe, Cu, Zn, Mn. Milk yield was recorded every day for each goat, and milk samples were collected on the last 2 d of each week for mo 1, 2, 4, and 8 of lactation to determine milk composition and trace minerals. Blood serum was taken on the final day of mo 1, 2, 4, and 8 of lactation. Trace minerals were determined using atomic absorption spectrometer. Data were run by GLM procedure (SPSS 18.0) and treatment means were compared by LSD test ($P < 0.05$). OTM maintained the similar milk yield and composition as ITM through lactation. OTM milk Se concentration was significantly or numerically higher than ITM, while little change was observed for other trace minerals. Compared with ITM, OTM showed higher GSH-Px activity in the first month of lactation ($P < 0.01$) and higher total antioxidant capacity in the second month of lactation ($P < 0.01$), while no difference was observed for superoxide dismutase activity between the 2 groups during lactation. In addition, malondialdehyde concentration with OTM was lower in mo 8 of lactation ($P < 0.05$). In mo 4 of lactation, serum Cu in OTM was significantly higher than that in ITM ($P < 0.05$). In mo 8 of lactation, serum Mn ($P < 0.05$) in OTM showed lower concentration than that in ITM. In conclusion, replacement ITM with 50% level of OTM in lactating dairy goats was able to maintain similar milk performance and trace minerals status, indicating potentially high bioavailability of organic trace minerals. Different antioxidative mechanisms were implicated through the 4 stages of lactation considering different responses for each trace minerals in serum.

Key Words: organic trace mineral, antioxidant status, selenium

488 Effect of weaning age on growth performance and rumen development in Alpine goat kids.

C. M. Perdomo*[1,2], J. Lévesque[1], E. Vasseur[3], D. Cinq-Mars[4], S. Buczinski[2], J. Arsenault[2], P. Hélie[2], Y. Chorfi[2], and C. Julien[1,4], [1]*Centre de recherche en sciences animales de Deschambault, Deschambault, QC, Canada,* [2]*Faculté de médecine vétérinaire de l'Université de Montréal, St-Hyacinthe, QC, Canada,* [3]*Animal Science, McGill University, Ste-Anne-de-Bellevue, QC, Canada,* [4]*Département des sciences animales, Université Laval, Québec, QC, Canada.*

The present study was aimed to determine the effect of weaning age on growth performance and rumen development in Alpine goat kids.

Thirty-six male kids were randomly assigned to one of 3 treatments: (1) early weaning at 6 weeks of age (6-wk), (2) medium weaning at 8 weeks of age (8-wk), and (3) late weaning at 10 weeks of age (10-wk). Treatments were blocked by body weight at birth and birth date, and each male kid was housed with a female companion of similar age and body weight. From birth, kids had ad libitum access to acidified milk replacer, starter feed, hay, and water. Weaning was performed progressively over 7 d. Kids were slaughtered at 12 weeks of age and rumen development was assessed by measuring rumen papillae in 4 rumen areas: ruminal atrium, ventral sac, caudodorsal blind sac, and caudoventral blind sac. Rumen bacteria population was characterized using the V4 region of the 16S rRNA gene. Results show that 10-wk had higher body weight at 12 weeks of age (27.4 kg), compared with 8-wk (25.5 kg) and 6-wk (24.1 kg) weaned kids ($P < 0.05$). Blood levels of β-hydroxybutyrate (BHB) increased at weaning time for all treatments but were higher in 6-wk compared with 8-wk (+21%) and 10-wk (+41%) weaned kids ($P < 0.05$). Blood levels of nonesterified fatty acids in 6-wk weaned kids spiked at weaning and were higher compared with 8-wk (+40%) and 10-wk (+101%) weaned kids ($P < 0.05$), suggesting a more pronounced adipose tissue mobilization in early-weaned kids at weaning. At 12 weeks of age, dual-energy x-ray absorptiometry measurements revealed a higher fat percentage in 10-wk (19.0%) compared with 8-wk (17.8%) ($P < 0.05$), but the fat percentage was not different in the 6-wk weaned kids (18.9%). The papilla surface area was greater in 10-wk, compared with 8-wk (+49%) and 6-wk (+22%) weaned kids ($P < 0.05$). Predominant bacteria taxa in rumen in 10-wk kids were *Lachnospiraceae*, *Clostridiales*, *Succinivibrionaceae, Veillonellaceae*, *Succinivibrio*, *Syntrophococcus*, *Ruminococcus* and *Prevotellaceae*, which are commonly found in adult rumen and related to ruminal fermentation and butyrate production. Overall, weaning kids at 10 weeks of age limited the negative impact of earlier weaning on growth and rumen development in the Alpine breed.

Key Words: weaning, rumen development, kids

Small Ruminant: Nutrition

489 Effect of different growth stages of canola on nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in sheep diets. L. E. Robles Jimenez[1], A. Z. Sánchez[1], O. A. Castelán Ortega[1], J. O. Avalos[1], J. G. Estrada Flores[2], M. González-Ronquillo[1], and E. Vargas-Bello-Pérez*[3], [1]*Facultad de Medicina Veterinaria y Zootecnia. Universidad Autónoma del Estado de México, Toluca, Estado de México, México,* [2]*Instituto en Ciencias Agropecuarias y Rurales. Universidad Autónoma del Estado de México, Toluca, Estado de México, México,* [3]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark.*

The objective of the present study was to determine the dietary effect of different growth stages of canola (*Brassica rapa* L.) on nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in sheep. The hypothesis was that early vegetative stages of forage canola would result in higher nutrient intake, and higher digestibility compared with late stages of growth. Four dietary treatments were elaborated. A basal diet (control) based on alfalfa hay, oat hay, soybean meal, and corn grain. Then alfalfa hay was replaced with 300 g/kg DM of canola forage at different growth stages: Vegetative, Flowering, and Pod. Crude protein for control was 156 g/kg DM and 130, 140 and 118 g/kg DM for Vegetative, Flowering, and Pod, respectively, while neutral detergent fiber was 268, 238, 269 and 294 g/kg DM, respectively. Nutrient intake and digestibility, and nitrogen balance were determined using 4 Suffolk sheep in a 4 × 4 Latin square design where fixed effects were experimental periods and diets, and the random effect was the sheep. Periods consisted of 14 d for diet adaptation and 7 d for sample collection. A completely randomized design was used to determine in vitro gas production where diet was considered as factor and the incubation series as a block. Feed intake and excretion of feces and urine were recorded. Digestibility coefficients of dry matter, organic matter, neutral detergent fiber, and acid detergent fiber were not different between treatments ($P > 0.1$). Nitrogen balance was higher ($P < 0.05$) for control and Pod, and lower for Vegetative and Flowering. In vitro gas production was not different between treatments ($P > 0.1$), DM intake was higher ($P < 0.05$) for control and Pod compared with Vegetative and Flowering. Overall, inclusion in 300 g/kg DM of canola forage pods in substitution of alfalfa hay is an alternative source of protein without affecting nutrient intake and digestibility.

Key Words: forage, nutrition, metabolism

490 Effect of ryegrass hay and ryegrass silage, cut at 2 growth stages, on nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in growing sheep. M. Gonzalez-Ronquillo[1], L. E. Robles Jimenez[1], J. Romero-Bernal[1], C. Ariciaga-Gonzalez[1], B. V. Ramirez[1], A. C. Canul[2], and E. Vargas-Bello-Pérez*[3], [1]*Departamento de Nutrición Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de Mexico, Toluca, Estado de México,* México, [2]*División Académica de Ciencias Agropecuarias, Universidad Juárez Autónoma de Tabasco, Villahermosa, Tabasco, México,* [3]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark.*

Nutritional quality of preserved forages is determined by its phenological stage and in grasses; it generally decreases with advanced growth. The objective of this study was to determine the effect of ryegrass hay (H) and ryegrass silage (S), cut at 2 growth stages (21 and 35 d), on nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics in growing sheep. The hypothesis for this study was that ryegrass cut at early stages for silage making would be of higher nutritional quality than hay cut at an earlier or late growth stage and this will be reflected in nutrient intake and digestibility, nitrogen balance, and rumen fermentation kinetics. For in vivo data, 32 Suffolk × Merino growing sheep (22 ± 2 kg LW) were used. Animals were fed on a total mixed ration (75% forage and 25% concentrate). In vitro gas production and fermentation kinetics were performed using 3 rumen-fistulated sheep (40 ± 1.0 kg LW). In vivo and in vitro gas data were analyzed using a completely randomized factorial arrangement 2 × 2. Considered factors were conservation method (n = 2), growth stage (n = 2) and their interaction. Hay-fed lambs had similar ($P > 0.05$) DM intake than those fed silage. Dry matter intake and organic matter (OM) intake were higher ($P < 0.05$) in H21 and S21. Digestibility of DM and OM were highest ($P < 0.05$) in S21 while H35 had the lowest ($P < 0.05$). Neutral detergent fiber digestiblity, N intake and N balance were not affected ($P > 0.05$) by preservation method and growth stage. Allantoin excretion was higher ($P < 0.05$) in silage than in hay, and was highest ($P < 0.05$) in S21 and lowest ($P < 0.05$) in H35. Uric acid excretion was higher ($P < 0.05$) in silage than in hay. In vitro gas production parameters were not affected ($P > 0.05$) by preservation method. Dry matter dissapearance and OMd were highest ($P < 0.05$) in S21 and lowest ($P < 0.05$) in H35. Microbial protein production was higher ($P < 0.05$) in silage than in hay and was highest ($P < 0.05$) in S21 and S35 and lowest ($P < 0.05$) in H35. Overall, compare with ryegrass hay, ryegrass silage cut at 21 d resulted to be a more suitable forage source for growing lambs fed on total mixed rations.

Key Words: allantoin, in vitro, purine derivatives

491 Effects of the level of dietary protein and methionine supplementation in dairy ewes in early lactation. G. Caja*[1], A. Elhadi[1], M. Rodriguez-Prado[2], A. Belaid[2], X. Such[1], L. Bahloul[3], and S. Calsamiglia[2], [1]*Grup de Recerca en Remugants, Universitat Autonoma de Barcelona, Bellaterra, Spain,* [2]*Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Universitat Autonoma de Barcelona, Bellaterra, Spain,* [3]*Adisseo France SAS, Malicorne, France.*

Multiparous Lacaune dairy ewes (n = 47; 77.2 ± 1.2 kg BW) in early lactation (37 ± 2 DIM), were used to assess the effects of the level of CP (14.8 vs. 16.6%; DM basis) and supplementation with rumen-protected Met (0 vs. 5 g/d; Smartamine, Adisseo, France) in the diet to cover 100% of metabolizable Met based on cow requirements (INRA 2007). Ewes were in balanced groups of 6 per pen (8 groups), milked 2× daily and fed a TMR ad libitum (44:56% forage:concentrate; DM basis). Met was supplemented mixed with 40 g of cracked corn grain fed individually at the a.m. milking. The trial was a crossover split-plot design with 2 periods of 3 wk with a 2-wk washout period. The ewe-period was the experimental unit. Intake (group) and milk yield were measured daily, and milk sampled for composition on d 16 and 17 of each period. Individual intake was assessed by using 30 g/d of PEG6000 as external marker. Blood samples were taken on d 18 of each period for metabolites analyses. BW, BCS and wool growth (shearing 200 cm^2/ewe) were recorded at the start and the end of each period. Data were analyzed by using the GLIMMIX of SAS (v.9.4). No differences in DM intake (3.50 ± 0.12 kg DM/d), milk yield (2.52 ± 0.17 kg/d), milk protein

(5.42 ± 0.07%) and casein (4.02 ± 0.06%) were detected either by CP or Met treatments. Milk urea content increased ($P < 0.01$) with CP (47.0 vs. 54.9 ± 1.2 g/L), milk fat (6.54 vs. 6.31 ± 0. 13%) and solids (17.7 vs. 17.4 ± 0.2%) decreased ($P < 0.01$) with Met. Blood urea increased (33 vs. 48 ± 2 mg/dL; $P < 0.01$) and glycemia decreased (66 vs. 62 ± 2 mg/dL; $P < 0.05$) with CP, whereas BOHB tended to increase (0.585 vs. 0.640 ± 0.042 mmol/L; $P = 0.06$) with Met. Blood NEFA, cholesterol, insulin or greasy wool growth were not affected by treatment, but BW gain tended to increase with CP ($P = 0.06$). Plasma Met concentration did not change with CP but increased with Met supplementation. CP or Met had no effect on N efficiency (24.8 ± 2.4%). Increasing CP level did not improve lactating dairy ewe performance. An excess of metabolizable protein supply could explain the poor milk protein yield responses in Met balanced diets.

Key Words: dairy sheep, dietary protein, methionine

492 Effects of low level of organic trace minerals on nutrients digestibility, rumen fermentation, and excretion of trace minerals in dairy goats.

P. Wang*[1], Y. Weng[1], J. Luo[1], G. Lin[2], and Q. Hu[3], [1]*Northwest A&F University, Yangling, China,* [2]*Institute of Quality Standards and Testing Technology for Agricultural Products, Chinese Academy of Agricultural Sciences, Key Laboratory of Agrifood Safety and Quality, Ministry of Agriculture and Rural Affairs, Beijing, China,* [3]*Beijing Alltech Biological Products (China) Co., Ltd., Beijing, China.*

The objective was to evaluate effects of replacing inorganic trace minerals (ITM) with 50% level of organic trace minerals (OTM) on nutrients digestibility, rumen fermentation, rumen trace minerals status, urine, fecal excretion of trace minerals in dairy goats. Forty Xinong Saanen dairy goats (71.55 ± 1.32 kg; 23.80 ± 3.41 d before the expected parturition date) were randomly assigned to 2 treatments: ITM diet with inclusion of ITM (Fe, Cu, Zn, Mn provided as sulfate, and Se as selenite) at 120% level of NRC (2007) recommendations; OTM diet with inclusion of OTM (Fe, Cu, Zn, Mn provided as amino acid complex, and Se provided as selenium yeast) at 50% level of ITM for Fe, Cu, Zn, Mn. Twelve of 40 goats with similar body weight and expected parturition date were assigned to a digestion and metabolism trial during the final week of mo 1, 2, 4, and 8 of lactation. Data were run by GLM procedure (SPSS 18.0) and treatment means were compared by LSD test ($P < 0.05$). For mo 4 of lactation, the apparent digestibility of CP in OTM was lower than that in ITM ($P < 0.01$), which could be related to lower digestibility of NDF ($P < 0.05$). For mo 8 of lactation, the apparent digestibility of CP in OTM was higher than that in ITM ($P < 0.05$). In other lactation months, there was no difference observed between OTM and ITM ($P > 0.05$) for DM, CP and NDF, ADF digestibility. Rumen fermentation parameters were not affected by replacement with OTM. Numerically or statistically lower concentration of Fe, Cu, Zn, Mn in rumen fluid was observed in OTM than that in ITM, presenting similar patterns for both groups through lactation. Except for the first month, rumen Se concentration with OTM was lower significantly or numerically than ITM. Urine concentration of trace minerals were not changed by the present treatments. For the first month, no difference of fecal excretion of trace minerals was found between OTM and ITM, whereas for mo 2 and 4, less fecal trace minerals were excreted in OTM. In conclusion, each trace mineral concentration in rumen was affected to different extent by the replacement of ITM with 50% level of OTM in lactating dairy goats. The types of trace minerals did not change rumen fermentation or urine mineral concentrations. Lower levels of OTM showed the potential for higher bioavailability and lower fecal excretion of trace minerals.

Key Words: organic trace minerals, trace minerals excretion, selenium

493 *Camelina sativa* cake and *Cynara cardunculus* meal into the diet of dairy goats affects the antioxidant activity and levels of total phenolic compounds in their milk.

C. Giromini[1], F. O. Zorini[1], S. Sandrini[1], G. Savoini[1], A. Buccioni[2], and G. Invernizzi*[1], [1]*Department of Health, Animal Science and Food Safety 'Carlo Cantoni', University of Milan, Lodi, Italy,* [2]*Dipartimento di Scienze e Tecnologie Agrarie Alimentari Ambientali e Forestali, University of Florence, Florence, Italy.*

The incorporation of plant by-products in the ruminants' diets may affect the composition of milk and may influence its functional activities. Nevertheless, the bioactive compounds in goat's milk have not yet received much study. Accordingly, the aim of this work was to quantify total phenolic compounds (TPC) and antioxidant capacity (AOX) in milk samples from goats fed a standard commercial diet supplemented with either *Camelina sativa* cake (Came) or *Cynara cardunculus* meal (Cardoon). The experiment lasted 3 wk. Eighteen multiparous and primiparous Alpine goats were divided in 3 homogeneous groups for parity (3 primiparous and 3 multiparous), milk production and days in milk (1.83, 2.23 kg, 273 DIM) and assigned randomly to 3 treatments: Came (n = 6) receiving 200 g/d of Came, Cardoon (n = 6) receiving 200 g/d of Cardoon and control (CT, n = 6) receiving the standard diet. Milk production, composition and live body weight were assessed weekly. Rumen content was sampled at d 0 and 21 on 3 goats per group selected by parity. Milk samples were collected at d 0, 7, 14 and 21. Milk collected at d 21 was in vitro digested with pepsin/pancreatin hydrolysis protocol. Milk digest was further analyzed for its antioxidant capacity by ABTS assay and for the total phenolic compound using Folin-Ciocalteu assay. Performance data were analyzed by PROC MIXED of SAS for repeated measures with diet, time and their interaction considered as fixed effect (LSM ± SEM). Significance was set at $P \leq 0.05$. No differences were observed in milk production, fat, protein, lactose content, live body weight and rumen pH during the trial among treatments. Milk urea decreased at d 14 in CT compared with Came and Cardoon (29.47 vs. 35.78 and 34.37 ± 1.68; $P < 0.05$) and at d 21 in CT and Cardoon compared with Came (20.35 and 20.90 vs. 26.42 ± 1.68; $P < 0.05$). Came and Cardoon milk showed higher antioxidant capacity (207.9 ± 4.25 and 195.4 ± 26.66 μmol Trolox equivalent/mL) compared with CT (115.6 ± 26.02 μmol Trolox equivalent/mL). Cardoon showed also higher TPC (32.49 ± 2.08 mg GAE/L) compared with CT and with Came. Came and Cardoon showed some interesting antioxidant activity on goat milk.

Key Words: camelina cake, cardoon meal, antioxidant activity

494 Metabolomic profile of goat milk associated with feed supplementation with *Camelina sativa* cake and *Cynara cardunculus* meal.

C. Giromini*[1], A. A.K. Salama[2], L. Bignardi Da Costa[3], A. Baldi[1], F. O. Zorini[1], S. Sandrini[1], G. Savoini[1], A. Buccioni[4], and G. Invernizzi[1], [1]*Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy,* [2]*Ruminant Research Group, Universitat Autonoma de Barcelona, Barcelona, Spain,* [3]*Department of Veterinary preventive medicine, Ohio State University, Columbus, OH,* [4]*Dipartimento di Scienze e Tecnologie Agrarie Alimentari Ambientali e Forestali, University of Florence, Florence, Italy.*

The aim of the study was to evaluate changes in milk metabolomics in response to *Camelina sativa* cake and *Cynara cardunculus* meal supplementation in dairy goats. Eighteen primiparous and multiparous Alpine lactating goats were divided into 3 balanced groups according to parity number, milk production and days in milk (1.83, 2.23 kg, 273 DIM), and assigned randomly to 3 treatment groups. The treatment groups were: 200 g/d of camelina cake (CAM; n = 6), 200 g/d of *Cynara cardunculus* meal (CAR; n = 6), and control (CON, n = 6) receiving the standard diet without supplementation. The supplementation lasted 21 d. Milk yield and composition were measured weakly; milk samples for metabolomics analysis were collected at d 0 and d 21 and frozen at −80°C. Metabolomics analysis were performed by ^{1}H NMR spectroscopy operating at 600 MHz. Productive data were analyzed by the Mixed Procedure for repeated measures of SAS. Metabolomics data were processed by the R program (ChemoSpec package) and MetaboAnalyst program. The multivariate analysis included the principal component analysis and partial least square–discriminant analysis to detect differences in the metabolome before and after the supplementation in the same animals. Feed supplementation had no effect ($P > 0.10$) on milk yield (CAM 1.19 ± 0.17, CAR 1.36 ± 0.17, CON 1.23 ± 0.17 kg/d) and composition (fat % CAM 6.21 ± 0.58, CAR 5.75 ± 0.58, CON 6.46 ± 0.58; protein % CAM 5.79 ± 0.49, CAR 5.11 ± 0.49, CON 5.55 ± 0.49) throughout the experiment. However, metabolomics profile of milk was affected by the experimental day (i.e., d 0 vs. 21). Compared with the CON diet, milk uridine, lecithin and ethanolamine decreased, whereas lactate and lactose increased when CAM and CAR were supplemented. There was no variation in milk metabolomics profile between CAM and CAR diets. In conclusion, feeding both camelina cake and *Cynara cardunculus* resulted in changes in metabolism that reflected in modified milk metabolomics profile. These findings would help in understanding the action mechanism of these feed supplements in dairy goats.

Key Words: milk metabolomics, camelina cake, *Cynara cardunculus*

495 High rumen-degradable starch diet can induce abnormal bile acid metabolism in the liver of dairy goats.

L. Zheng*[1], J. Zhang[2], X. Wei[3], and J. Yao[2], [1]*Newhope Dairy Co., Ltd., Chengdu, Sichuan, China,* [2]*Northwest A&F University, Yangling, Shannxi, China,* [3]*College of Animal Science and Technology, College of Veterinary Medicine, Zhejiang A&F University, Hangzhou, Zhejiang, China.*

Starch-enriched diets can provide sufficient energy for high-yielding dairy animals, but also pose challenges to rumen health and milk fat synthesis. The low pH value of rumen fluid caused by higher rumen-degradable starch (RDS) will increase the concentration of ruminal free-LPS which can further affect liver by absorbing into the portal vein. The increase of LPS in plasma impairs liver function, immune response and bile acid metabolism, which depress the precursors for milk fat. The object of this study was to use metabolomics and transcriptomics to reveal the effect of RDS on bile acid metabolism and liver transcription of dairy goats. Eighteen Guanzhong dairy goats (day in milk = 185 ± 12 d) with similar parity, weight, and milk yield were selected and randomly assigned into one of 3 groups (n = 6), which were fed an LRDS diet (Low RDS = 20.52%), MRDS diet (Medium RDS = 22.15%), or HRDS diet (High RDS = 24.88%) for 5 weeks. During the last week of the experiment, the milk yield of individual goats was recorded at each milking for 3 consecutive days. On the last day of experiment, all goats were anesthetized and then the peripheral blood and liver tissue were collected. Liver tissues was used for transcriptomics analysis. Peripheral blood was used for metabolomics, immune cells count and LPS content followed by one-way ANOVA. Compared with that in the LRDS group, the milk fat contents in the MRDS and HRDS groups significantly decreased ($P < 0.05$). The amount of white blood cell significantly increased in HRDS group compared with LRDS and MRDS groups ($P < 0.05$). Based on metabolomics analysis, the concentration of primary bile acids in peripheral blood was significantly reduced in the HRDS group ($P < 0.05$). The concentration of LPS was significantly increased in the HRDS group ($P < 0.05$). Based on transcriptomics analysis, genes related to immune response in the liver were significantly upregulated in the HRDS group ($P < 0.05$). Remarkably, 4 genes related to bile acid secretion (*MDR1, RXRα, AE2, SULT2A1*) were significantly down-regulated in the HRDS group ($P < 0.05$), and were consistent with the results of RT-qPCR verification. High rumen-degradable starch diets can induce LPS-mediated hepatitis in the liver of dairy goats and interfere with liver bile acid metabolism, which may be one of the reasons for the decrease in milk fat production in dairy goats.

Key Words: rumen-degradable starch, bile acid metabolism, dairy goat

Midwest Young Scholars

496 Elucidating the serotonin-calcium axis.

M. Connelly*, *Affiliation, City, country.*

Dysregulation of calcium (Ca) occurs during the peripartal period due to the rapid and robust increase in Ca demand by the mammary gland. Thus, mammary-derived signals, such as serotonin (5HT), play a role in coordinating responses to this abrupt Ca loss. Serotonin plays a diverse role in maternal circulation and mammary physiology, modulating Ca metabolism and mammary parathyroid hormone-related protein (PTHrP) production. Still, the biology underpinning the 5HT-Ca axis and timing of Ca homeostatic mechanisms in relation to Ca loss remains largely unknown. Therefore, the objective was to elucidate how 5HT modulates Ca metabolism and responds to Ca perturbations as well as delineate timing of Ca feedback in the peripartal cow. The initial aim was to uncover 5HT's action on Ca by infusing the precursor, 5-hydroxy-L-trytophan (5-HTP). Infusion of 5-HTP elicited a transient hypocalcemia and increased mammary 5HT content and Ca trafficking, resulting in increased milk Ca concentrations. These robust blood Ca changes elicited no response in blood parathyroid hormone but did increase mammary PTHrP mRNA. Next, the 5HT-Ca axis under Ca perturbations was examined. Ca chelation did not alter blood 5HT in early lactation and dry, non-pregnant cows. However, circulating 5HT, mammary 5HT and PTHrP mRNA were increased in early lactation compared to dry, non-pregnant cows, with PTHrP mRNA being robustly upregulated in chelated early lactation cows when compared to all other groups. Moreover, when cows were infused for 24h immediately after parturition with Ca to maintain normocalcemia, blood 5HT was unchanged. However, blood 5HT was increased in cows fed a prepartum negative dietary cation anion difference (-DCAD) diet. Cows fed a -DCAD diet also had higher Ca concentrations in the peripartum period and required less Ca to maintain normocalcemia in the 24h post-partum. Interestingly, an approximate 18h delay in blood Ca changes occurred in both perturbation studies, suggesting Ca homeostatic mechanisms require time to adapt to Ca change. Collectively, this work demonstrates 5HT's ability to modulate the Ca axis and improve Ca metabolism, while suggesting 5HT may drive Ca, but Ca perturbations do not drive 5HT.

Key Words: calcium, serotonin

497 Elucidating the physiological and genomic underpinnings of dairy cow lipid-related metabolic disorders and leveraging farm data streams to predict disorder cases.

R. S. Pralle*, *School of Agriculture, University of Wisconisn–Platteville, Platteville, WI.*

Hyperketonemia (HYK) and fatty liver syndrome are thought to be comorbid metabolic disorders related to endogenous lipid metabolism. These lipid-related metabolic disorders (LRMD) have subclinical prevalence of approximately 50% during the first 21 d postpartum. The frequency of LRMD cases and their association with reduced lactation performance, impaired fertility, and greater risk of comorbidities and involuntary culling cause LRMD incidence to be a significant economic burden for the dairy production system. Thus, investigations into the pathophysiology of these LRMD and innovations in the LRMD case identification have tremendous potential for beneficial impact on cow health and dairy profitability with two major hypotheses: 1) LRMD pathology is determined in part by variation in the liver expression of key regulatory genes and metabolic pathways and 2) that data streams available within dairy production systems can be leveraged to identify biological features of HYK and develop convenient, high-throughput HYK prediction tools for diagnosis. After exploring the effects of a ketosis-induction protocol on peripartum cows, we found evidence for liver PNPLA3 protein abundance to regulate peripartum liver triglyceride accumulation. Through whole-transcriptome RNA sequencing of liver samples, we discovered novel divergence in the regulation of immunometabolism was associated with LRMD risk and severity. Genetic epidemiology of a HYK phenotype determined by repetitive blood BHB sampling, identified candidate SNP and inferred genes associated with human metabolic syndrome that contribute to genetic susceptibility to HYK. Finally, using machine learning algorithms we developed prediction equations based on farm data streams as a less invasive and high-throughput option for managing HYK. Future progress in the integration of biological research into dairy herd data collection and development of data-based management tools has tremendous potential to improve dairy husbandry and will provide opportunities for the precision management of LRMD.

498 Evaluation of alpha-1-acid glycoprotein as a marker of transition cow health, metabolism, and feed intake: A potential diagnostic tool?

W. Brown*, *Department of Animal & Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Production performance of livestock is dependent upon adequate feed intake to meet metabolic demands, yet activation of the immune system reduces feed intake. Transition dairy cows suffer from inflammatory activation from multiple sources which may contribute to poor intake during this period. While feed intake reduction resulting from inflammation most classically is caused by cytokines, recent evidence has also implicated the acute-phase protein alpha-1-acid glycoprotein (AGP). Using a large dataset, we analyzed plasma AGP in 434 transition dairy cows to determine its association with dry matter intake (DMI), common blood inflammatory and metabolic biomarkers, and transition cow health disorders with an overarching goal of evaluating AGP as a diagnostic tool. The plasma AGP concentration increased after parturition, but there was no evidence of difference from d 3 to d 21 postpartum. There was a strong negative association between AGP and postpartum DMI. Conversely, there were positive associations between AGP and metritis, retained placenta, hyperketonemia and haptoglobin in the postpartum period. Overall diagnostic ability of AGP to predict depressed intake or transition disorders was marginal based on receiver operating characteristic analysis. Nonetheless, the ease of quantifying plasma AGP and the lack of association with common blood metabolic biomarkers suggest it may be a useful tool to evaluate transition status in dairy cows. Further work elucidating mechanisms and contributions of inflammation-induced hypophagia in transition dairy cows is warranted. Additionally, exploration of acute-phase proteins as biomarkers and mediators of hypophagia should also be evaluated.

499 Effects of rumen-protected methionine supply and body condition prepartum on antioxidant, inflammation, and mechanistic target of rapamycin pathways in adipose tissue during the periparturient period.

Y. Liang*[1], A. S. Alharthi[1], R. Bucktrout[1], A. A. Elolimy[1], V. Lopreiato[2], I. Martinez-Cortés[3], E. Trevisi[2], C. Parys[4], and J. J. Loor[1], [1]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL,* [2]*Department of Animal Sciences, Food and Nutrition, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [3]*Agricultural and Animal Production*

Department, UAM-Xochimilco, Mexico City, Mexico, [4]Evonik Nutrition & Care GmbH, Hanau, Germany.

In non-ruminants, adipose tissue is responsive to AA supply, can utilize them as fuels, or for protein synthesis regulated in part via insulin and mechanistic target of rapamycin (mTOR) signalling. Nuclear factor, erythroid-derived 2-like 2 (NFE2L2, formerly Nrf2) is a key transcription factor controlling cellular oxidative stress in non-ruminants. Our research examined the effects of rumen-protected methionine (RPM) supply or body condition (BCS) prepartum on pathways associated with mTOR and NFE2L2 pathways in subcutaneous adipose tissue (SAT) during the periparturient period. Multiparous Holstein cows were assigned from –28 to 60 d relative to parturition to a basal diet [control; 1.47 Mcal/kg of DM and 15.3% CP prepartum; 1.67 Mcal/kg and 17.7% CP postpartum] or the control plus ethyl-cellulose RPM. The RPM was fed individually at a rate of 0.09% of DMI prepartum and 0.10% postpartum. SAT harvested at -10, 10 and 30 d relative to parturition was used for quantitative PCR and western blotting. Enhanced Met supply led to greater overall mRNA abundance of Gln (SLC38A1), Glu (SLC1A1), L-type AA (Met, Leu, Val, Phe; SLC3A2), and neutral AA (SLC1A5) transporters along with greater gene expression of glutathione (GSH) metabolism-related genes. Furthermore, it upregulated protein abundance of insulin-responsive proteins phosphorylated (p) protein kinase B (p-AKT). A diet × day interaction was observed for mTOR protein abundance due to greater values for RPM cows at 30 d postpartum compared with controls. Additionally, supply of Met resulted in an overall upregulation of protein abundance of glutathione peroxidase 1 (GPX1), GPX3, glutathione S-transferase mu 1 (GSTM1), and glutathione S-transferase alpha 4 (GSTA4) all related to GSH metabolism. There was a diet × time effect for protein abundance of NFE2L2 and its repressor kelch like ECH associated protein 1 due to lower values at 30 d in cows fed Met versus controls. Thus, the data suggest that exogenous Met may play a role in activating GSH metabolism and the anti-oxidant NFE2L2 pathways in SAT. In our BCS studies, compared with cows with normal BCS (NBCS, BCS ≤ 3.17), cows calving at high BCS (HBCS, BCS ≥ 3.5), although HBCS cows had greater overall total protein abundance of NFE2L2 inSAT, ratio of p-NFE2L2-to-total NFE2L2 was lower suggesting a decrease in the activity of this antioxidant system. Overall mRNA abundance of the GSH metabolism-related genes along with protein abundance of GSTM1 were greater in HBCS cows. Overall mRNA abundance of the high-affinity cationic (SLC7A1), proton-coupled (SLC36A1), and sodium-coupled amino acid transporters (SLC38A2) also was greater in HBCS namely due to upregulation in the postpartum phase. Data suggest that increased abundance of mRNA and protein components of the GSH metabolism pathway in SAT of HBCS cows might help alleviate tissue oxidant status. Overall, these responses suggested that mTOR and NFE2L2 pathways in bovine SAT are responsive to nutritional status and BCS prepartum.

Key Words: amino acid, oxidative stress, periparturient period

Animal Behavior and Well-Being: Posters

P100 Individual variation and reproducibility of infrared thermography of the eye and rectal temperature in dairy calves. M. Woodrum Setser*, M. Hayes, M. Cantor, G. Mazon, and J. H. C. Costa, *University of Kentucky, Lexington, KY.*

Rectal temperature is the most common proxy for body temperature in dairy calves. However, this process is labor intensive and requires calf handling. Ocular infrared thermography (IRT) is a possible alternative and has shown potential to detect fever but its reliability and reproducibility remains uncertain. This study aimed to measure the reliability and reproducibility of an IRT camera measuring ocular temperature in weaned bull calves (n = 12) age 95 ± 16 d (mean ± SD). Calves were placed and restrained in a chute with an IRT camera placed 1.5m from their eyes and provided a 10 min acclimation period. At the end of the habituation period, rectal temperature (GLA M700 Thermometer, GLA Agriculture Elect., San Luis Obispo, CA) and ocular temperature using an IRT camera (FLIR T540sc, FLIR Systems, Inc. Wilsonville, OR) were measured simultaneously for 5 min per calf (20 ± 6 pictures; mean ± SD). The eye temperatures for each calf was recorded as the maximum temperature found within an oval drawn around the eye using the accompanying image processing software (ResearchIR Max Software, FLIR Systems, Inc. Wilsonville, OR). Mean rectal temperature was greater than ocular temperature (rectal = 39.3 ± 0.30, ocular = 37.6 ± 0.21°C; $P \leq 0.001$). Furthermore, the mean difference between the rectal temperature and IR eye temperature across calves was 2.0 ± 0.44°C. The Bland-Altman plot between the rectal and IR ocular temperatures for all calves revealed a negative bias and a positive increased error with magnitude increase for IRT temperature when compared with rectal temperatures. The Pearson correlation between the rectal temperature and ocular IRT temperature across calves was moderate ($r = 0.40$ $P \leq 0.001$). The coefficient of variation for temperatures within individual calf was greater for ocular than it was for the rectal temperature (median % CV [Q1, Q3]; ocular IRT = 1.16 [0.84,2.03], rectal = 0.10 [0,0.13]; $P \leq 0.001$). These results suggest that IR imaging was repeatable, but may not serve as proxy for rectal temperature. Future research should determine optimum fever detection thresholds for ocular temperatures measured utilizing IRT.

Key Words: precision technology, health, fever

P101 Wisconsin farmer-reported management strategies for individually vs. socially reared preweaned calves. F. L. M. Silva*[1], J. Van Os[1], C. Winder[2], M. Akins[1], T. Kohlman[3], T. Ollivett[4], H. Schlesser[3], B. Schley[3], S. Stuttgen[3], and J. Versweyveld[3], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [3]*Division of Extension, University of Wisconsin–Madison, Madison, WI,* [4]*School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI.*

Although research has demonstrated many benefits, social housing of pre-weaned dairy calves remains an uncommon farm practice. To characterize calf-rearing practices in Wisconsin, an online survey was distributed to dairy farmers and calf raisers. A total of 201 producers responded, of which 163 (81%) housed their heifer calves only individually and 38 (19%) housed at least some calves socially. Among farms using social housing, 12 (32%) kept their calves only in pairs, 11 (29%) in groups of 2–8, and 15 (39%) in groups of more than 8. Among farms using only individual housing, 10 (6%) kept their calves with no visual contact, 114 (70%) with visual contact, and 39 (24%) with visual and tactile contact. Practices on farms housing their calves individually vs. socially were compared using chi-squared or Wilcoxon tests. Compared with farms raising calves individually, farms with social rearing had more milk-fed calves (median 25 vs. 50, respectively; $P = 0.02$) and milking cows (200 vs. 400; $P = 0.04$). The daily milk feeding frequency for 4-week-old calves was greater for farms using social vs. only individual housing ($P < 0.0001$); 22 (58%) vs. 16 farms (42%) with social housing fed them ≤2 times/d vs. ≥ 3 times/d, and 146 (90%) vs. 17 farms (10%) with only individual housing fed with those respective frequencies. Milk was fed through a nipple more frequently on farms that housed calves socially vs. only individually ($P < 0.0001$); 28 farms (74%) housing calves socially used nipples vs. 10 farms (26%) which did not, whereas 38 (23%) vs. 125 farms (77%) with only individual housing fed using those respective methods. The amount of milk or milk replacer fed to 4-week-old calves did not differ between farms using social vs. only individual housing ($P = 0.40$); the most common quantity was 8 L/d on 13 (36%) vs. 54 (34%) of farms raising their calves socially vs. only individually. These results indicated that many Wisconsin farmers housing calves socially also used feeding strategies previously demonstrated to promote calf welfare, such as feeding milk several times daily at a high plane of nutrition, using nipples.

Key Words: survey, behavior, welfare

P102 Effects of airspeed from fans located above freestalls on heat stress and lying time. K. J. Reuscher*[1], N. B. Cook[2], M. R. Mondaca[3], and J. M. C. Van Os[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [3]*Honorary Fellow, The Dairyland Initiative, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI.*

Our objective was to evaluate how airspeed from variable-speed fans above the resting area affect heat stress and lying time, a key indicator of cow comfort. Lactating Holsteins (n = 8 groups of 16 cows) were exposed to 3 treatments in a replicated crossover design (3 d acclimation, 4 d data collection): control (fans off, 0.4 ± 0.2 m/s, measured 0.5 m above the stall surface to represent cow lying height) vs. 60% (1.7 ± 0.5 m/s) and 100% (2.4 ± 0.8 m/s) fan power. Vaginal temperature (VT) and lying time (LT) were recorded at 1-min intervals with data loggers. Temperature-Humidity Index (THI) in the pens was measured at 5-min intervals. Linear mixed-effects models were used to analyze fixed effects of treatment, maximum daily THI, and their interaction, with a random term for group of cows. There were main effects of treatment and treatment × THI interactions for all dependent variables. Among all treatments, LT differed in a dose-dependent fashion (100% vs. 60% fan power vs. control: 14.3 vs. 14.0 vs. 13.3 h/d, respectively, SEM = 0.17 h/d; $P < 0.036$). For every 10-unit THI increase, LT in the control decreased 0.4 h/d, whereas both fan treatments showed the opposite pattern (+0.3 and 0.7 h/d in 60% and 100% fan power treatments, respectively; $P < 0.046$). Maximum daily VT was lower in the 2 fan treatments (39.1°C in both 60% vs. 100% fan power treatments, respectively, SEM = 0.06°C) relative to the control (39.5°C; $P < 0.001$). For every 10-unit THI increase, VT increased 0.3°C in the control but was stable in both fan treatments (+0.0°C in both 60% vs. 100% fan power treatments; $P < 0.019$). Milk yield (MY) was higher in the 2 fan treatments (42.7 vs. 43.3 kg/d in 60% vs. 100% fan power treatments, respectively, SEM = 0.4 kg/d) relative to the control (41.4 kg/d, $P <$

0.001). For every 10-unit THI increase, MY in the control decreased 1.1 kg, whereas both fan treatments showed the opposite pattern (+1.1 vs. 1.9 kg in 60% vs. 100% fan power treatments, respectively; $P = 0.011$). In conclusion, higher airspeed at cow resting height was effective not only for maintaining vaginal temperature, but also for improving lying time and milk yield in heat stress conditions.

Key Words: heat abatement, lying behavior

P103 Effects of hutch ventilation on preference and heat stress in pair-housed dairy calves.

K. J. Reuscher*, C. S. Yu, R. Salter, T. Bresolin, and J. M. C. Van Os, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Our objective was to evaluate the effects of social contact and hutch ventilation on calves' responses to heat stress. Newly weaned Holstein heifers (n = 25 pairs; 55 ± 1 d of age) were pair housed in adjacent hutches with a shared outdoor area. In each pair of hutches, 1 was ventilated with 2 25.4-cm-diameter windows at the rear base and the rear bedding door propped open; the other had no rear windows and the bedding door was closed. Calves were exposed to 4 treatments for 1 h each (1100–1200 and 1230–1330 h on 2 consecutive d) in a 2 × 2 factorial design in a balanced order: individually or in pairs in the nonventilated or ventilated hutch. Respiration rate (RR) was recorded before and after each exposure. Temperature-Humidity Index (THI) was recorded in both hutches at 5-min intervals. A generalized linear mixed model was used to evaluate the fixed effects of ventilation, number of calves inside the hutch, and their interactions, on THI and change in RR after 1 h; the random effect was pair of calves. To evaluate preference, calves were observed for the subsequent 3 d with time-lapse cameras. The calves' locations were recorded at 15-min intervals by an observer blinded to hutch ventilation. The proportion of time calves spent in each hutch was averaged within pairs and across the 3 d of observation. A 1-sample t-test was used to evaluate preference for the ventilated hutch, compared with 50% (chance, no preference). After 1 h, RR decreased vs. was unchanged, respectively, when calves were in the ventilated vs. nonventilated hutch (−10.1 vs. +0.3 breaths/min, respectively, SE = 2.0 breaths/min; $P < 0.001$), regardless of how many calves were inside (no main effect or interaction with number of calves, $P \geq 0.475$). Calves preferred the ventilated hutch (72.3 ± 4.3% of the total time inside both hutches, mean ± SE; $P < 0.001$). This preference was likely driven by calves seeking heat abatement: after calves were inside for 1 h, THI was lower in the ventilated vs. nonventilated hutch (72.7 vs. 78.2 THI units, respectively, SE = 1.7; $P > 0.001$). In conclusion, newly weaned calves preferred ventilated hutches, which reduced their heat stress in outdoor pair housing.

Key Words: heat abatement, social housing

P104 Impact of stationary brush quantity on brush use in group-housed dairy heifers.

F. S. Baier*[1], A. R. Gimenez[1], K. M. Coel[1], E. K. Miller-Cushon[2], J. R. Dorea[1], and J. M. C. Van Os[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*University of Florida, Gainesville, FL.*

Grooming is an important natural behavior for cattle and can be practically facilitated with objects in the environment, such as brushes. Our objective was to evaluate the effect of the number of brushes on brush use and competition in group-housed, weaned dairy heifers naïve to brushes. We predicted providing more brushes would allow for greater use and less competition. Sixty-three Holstein heifers (96 ± 6 d old) were housed in groups of 8 (with the exception of 1 group of 7) in straw-bedded pens with either 2 or 4 stationary brushes (n = 4 groups/treatment). Behavior was recorded continuously for all heifers from video for 6-h time periods on d 1 (0–6 and 18–24 h of exposure) and 6 (120–126 and 138–144 h of exposure). We measured brush oral manipulation (contact with mouth or tongue), grooming (rubbing head, neck, or body), and displacements (physical contact between heifers that stopped brush use); values were averaged at the pen level. Linear models were used to evaluate the effect of brush quantity on brush use and displacements. Pairwise comparisons were performed using a Tukey's adjustment. Latency to use any brush after entering the pen was 4.0 ± 8.4 min (mean ± SD). All heifers used a brush at least once. Heifers provided access to 4 vs. 2 brushes displayed greater durations of oral manipulation (1.9 ± 0.2 vs. 1.5 ± 0.1 min/6 h, mean ± SE; $P = 0.047$), grooming (5.6 ± 0.4 vs. 4.0 ± 0.3 min/6 h; $P = 0.002$), and total brush use (7.5 ± 0.4 vs. 5.4 ± 0.4 min/6 h; $P = 0.001$). Regardless of treatment, duration of oral manipulation was greater in the initial 6 h of exposure than on d 6 ($P \leq 0.03$). However, the greatest brush use for grooming was during the last 6 h of d 6 ($P \leq 0.03$). Competition did not differ between treatments ($P = 0.54$). Overall, more frequent displacements were observed in the first 6 h of exposure on d 1 compared with the corresponding time period on d 6 ($P = 0.01$). In conclusion, heifers provided with more brushes used them for longer periods of time, suggesting that expression of brush-directed behavior may depend on resource availability.

Key Words: grooming, oral behavior, competition

P105 Early-life behavior of organic-certified Holstein heifer calves housed in pairs.

A. Velasquez-Munoz* and P. Pinedo, *Colorado State University, Fort Collins, CO.*

The objective was to describe the behavior of Holstein heifer calves during their first days of pair housing. Newborn calves (n = 15 pairs) were monitored from July to August 2020 in a rearing facility in northern CO, USA. Due to management constrains, a control group individually housed was not included. Calves were moved to the rearing facility at 1 d of life, and milk was offered 3 times per day. Calves were housed in pairs sharing 2 polyethylene hutches and a sand bedded front yard (4.50 m^2), enclosed by a galvanized welded wire fence. Pairs were video recorded during the first 10 d of life, and behavior was assessed during 1 h before and after each milk feeding (6:30AM, 12:30PM, 7:30PM). Behaviors included resting and active time and time alone or interacting as a pair (inside or outside the hutches). Continuous THI inside the hutches was collected during the study. Data were summarized using SAS and paired t-test was used to assess differences among feeding times within age (Table 1). Calves were less active at noon regardless of age, likely due to higher THI. Over time, calves increased their time spent interacting inside the hutch, especially in the AM and Noon periods. At noon, calves spent > 83% of the time resting and they spent more time alone or in pairs inside the hutch. In conclusion, activity and pair interactions varied depending on time of the day and calf age.

Key Words: behavior, calves, pair housing

P106 Peripartum feeding behavior is related with onset of ovarian cyclicity in dairy cows with different dry period lengths.

B. G. C. de Bruijn, A. Kok, J. Ma, and A. T. M. van Knegsel*, *Adaptation Physiology Group, Wageningen University & Research, Wageningen, the Netherlands.*

Poor reproductive performance is one of the main factors contributing to culling of dairy cows. Monitoring of feeding behavior can be valuable in early detection of reproductive problems. The objective of the

Table 1 (Abstract P105). Percentage of time that calves performed the behaviors of interest for each feeding period at 2, 5, and 9 days of age

Age (d)	Activity, %(SD)		Pair interaction, %(SD)			THI hutch
	Resting	Active	Alone	Pair outside	Pair inside	Mean (SD)
2						
AM	85.2^{a} (3.8)	14.8^{a} (3.8)	78.3^{a} (26.8)	6.6^{a} (12.2)	15.1^{a} (23.14)	60.8 (3.4)
Noon	89.9^{a} (7.6)	10.1^{a} (7.6)	57.9^{a} (31.4)	0.8^{a} (1.0)	41.3^{b} (31.22)	81.5 (1.6)
PM	65.4^{b} (12.1)	34.6^{b} (12.1)	55.3^{a} (29.8)	19.2^{b} (17.3)	25.5^{a} (22.7)	69.4 (1.8)
5						
AM	72.2^{a} (7.2)	27.8^{a} (7.2)	61.3^{a} (27.6)	11.3^{a} (8.1)	27.4^{a} (28.7)	61.2 (2.2)
Noon	88.2^{b} (10.0)	11.8^{b} (10.0)	55.8^{a} (25.2)	2.3^{b} (3.2)	41.8^{a} (25.0)	80.5 (2.1)
PM	67.1^{a} (9.8)	32.9^{a} (9.8)	45.9^{a} (31.6)	19.5^{a} (13.6)	34.6^{a} (23.5)	72.1(2.5)
9						
AM	75.3^{a} (8.2)	24.7^{a} (8.2)	44.7^{a} (30.0)	9.8^{a} (6.6)	45.5^{a} (29.6)	59.3 (3.1)
Noon	83.4^{b} (12.1)	16.6^{b} (12.1)	42.8^{a} (40.0)	2.4^{b} (3.6)	54.8^{a} (43.7)	81.6 (1.7)
PM	65.4^{c} (10.5)	34.6^{a} (10.5)	54.3^{a} (17.3)	18.6^{a} (9.2)	27.1^{b} (17.3)	72.2 (0.7)

$^{a–c}$Different letters within column and age indicate statistical differences.

current study was to investigate relations between feeding behavior and onset of luteal activity (OLA) in dairy cows subjected to no (0-d) or a short (30-d) dry period (DP). Feeding behavior was continuously recorded by computerized feeders for 123 dairy cows and analyzed from 4 weeks prepartum to wk 14 of lactation. Cows were randomly subjected to a transition treatment: 0-d DP with a low postpartum dietary energy level (0-d DP LOW) (n = 41), 0-d DP with a standard postpartum dietary energy level (0-d DP STD) (n = 40), or 30-d DP with a standard postpartum dietary energy level (30-d DP STD) (n = 42). Milk progesterone concentration was determined 3 times per week until 100 d in milk (DIM) to assess onset of luteal activity (OLA). Feeding behavior variables were analyzed using mixed models, including fixed effects of OLA class (OLA < 21 or ≥ 21 DIM), transition treatment, parity (2, or > 2), and their interactions. Cow was included as repeated subject. Before calving, visits to the feeder per day were higher in cows with OLA < 21 DIM (OLA < 21) compared with OLA ≥ 21 DIM (OLA ≥ 21), and often interactions with transition treatment were found. During the first 4 weeks postpartum, cows with OLA < 21 had a higher feed intake, meal size, feeding rate, meal duration and more visits per meal and visits per day, but fewer meals per day compared with cows with OLA ≥ 21. During the first 2 d postpartum, cows with OLA < 21 had higher cumulative feed intake, meal size, and more visits per day and visits per meal compared with OLA ≥ 21. In conclusion, the relation between prepartum feeding behavior and OLA was greatly affected by transition treatment, i.e., dry period length and postpartum dietary energy level. Cows with an early OLA had a postpartum feeding behavior that reflected a faster recovery from parturition and better adaptation to a new lactation compared with cows with a later OLA.

Key Words: fertility, behavior, dry period length

P107 Effects of eliminating head lockup in stalls during transition period in a dairy farm: A case study. S. Paudyal[1], J. Piñeiro*[1,2], and L. Papinchak[1], [1]*Department of Animal Science, Texas A&M University, College Station, TX,* [2]*Texas A&M AgriLife Research and Extension Center, Amarillo, TX.*

Self-locking feed stanchions improve labor efficiency by reducing the amount of time spent handling cattle on dairy farms. However, extended lockup times have been associated with stress and increased total daily standing time, which leads to negative impacts on milk production and health. The objective was to evaluate the association of eliminating stall lockup during the early postpartum period of dairy cows (21 d after parturition) on test-day milk yield, reproductive performance, and health disorders. A retrospective cohort case study was conducted on 400 Holstein cows from a dairy farm in northwest Texas divided into treatment (TRT; n = 200) and control group (CON; n = 200). CON received an average of 2 h per day lockup time during 21 d postpartum whereas TRT animals were not exposed to headlock restraint during this period. Farm records including monthly milk yields, monthly linear somatic cell score (SCS), days in milk to first breeding (DIMFB), days in milk to subsequent pregnancy (DIMPREG), and incidence of mastitis and lameness, were obtained from the farm management software. Herd records were analyzed using SAS ver.9.4. The continuous variables were analyzed using a mixed model using cow as a random effect and the frequencies were analyzed using Chi-squared tests. TRT demonstrated greater milk yield (LSM ± SEM) on second (86.2 ± 1.68 vs. 82.6 ± 1.29; $P < 0.05$), third (72.9 ± 2.50 vs 67.6 ± 1.19; $P = 0.06$), and fourth monthly test day (53.9 ± 3.70 vs 45.2 ± 6.20; $P = 0.05$) compared with CON cows. TRT cows had lower SCS on the third monthly test day (2.6 ± 0.24 vs 3.2 ± 0.11; $P = 0.01$). TRT cows came to estrus earlier in the lactation as indicated by smaller DIMFB (66.2 ± 3.70 vs 76.8 ± 2.90; $P = 0.02$) and became pregnant sooner as indicated by smaller PREGDIM (66.9 ± 12.32 vs 112.1 ± 5.50; $P < 0.01$). Cows in TRT group also had lower incidence of mastitis (3% vs 23%; $P < 0.001$), and lower incidence of lameness (3% vs 9.5%; $P = 0.007$). We conclude that eliminating lockup time in early lactation might provide improvements in milk production, health, and reproductive performance of lactating dairy cows.

Key Words: lockup, dairy, freestall

P108 Preferences of dairy cattle for supplemental light-emitting diode lighting in the lying area. A. M. Wilson*[1], T. C. Wright[2], J. P. Cant[1], and V. R. Osborne[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, ON, Canada.*

Supplemental lighting has been used to increase milk yield in dairy cows. Light-emitting diode (LED) lights are highly efficient and are becoming more common on farms. The objective of this experiment was to determine the effect of 3 LED light colors in the stall area on short-term preferences of dairy cows. Second-lactation cows (n = 14; 210.1 ± 35.2 DIM; 35.8 ± 5.1 kg/d) were housed in a pen with unrestricted access to 28 freestalls. The stall platform was separated into 2 sides by an opaque divider. Lights were installed as part of the stall partitions and positioned above cows' heads when resting. Two combinations of light were tested each period (P1: white light, 3000K vs. no light; P2: white light vs. green-yellow/570 nm light; P3: white light vs. blue/475 nm light). The experiment consisted of 3 periods, each with 3 d of adaptation (no light) followed by 4 d of light treatment where treatments were applied to opposite sides after 2 d. Facility lights were on from 0400 h to 2000 h and supplemental LED lighting was provided from 0400 h to 2400 h. Video data and lying behavior were recorded continuously using cameras and leg-mounted pedometers, respectively. Preference was assessed by the amount of time spent lying down and number of bouts in each treatment. Data were summarized by treatment per block of time (dark, facility+LED lights, LED only) per cow and analyzed by ANOVA with a Tukey's adjustment (PROC GLIMMIX, SAS v.9.4). Differences between treatments within each period were not significant for lying time ($P = 0.98$) or number of bouts ($P = 0.86$). There were no treatment differences between block of time within periods for lying time ($P = 0.88$) and number of bouts ($P = 0.73$). Cows spent 2.1 vs. 2.2 ± 0.13 h/block of time lying in white vs. no light, 2.0 vs. 2.0 ± 0.14 h/block of time lying in white vs. green light, and 2.1 vs. 2.1 ± 0.13 h/block of time lying in white vs. blue light. Cows did not avoid lying under white light when also given the option of no light, suggesting that supplemental light in the stall area was not aversive short-term to cows. Additionally, cows did not have a preference between white and green or blue light.

Key Words: light, behavior, stall

P109 The effect of heat stress on behavior and milk production of dairy cows.

Y. Ma*[1], C. Shen[1], K. Yang[1], K. Shen[1], D. Renaud[2], D. Kelton[2], T. Duffield[2], and Q. Dong[1], [1]*College of Veterinary Medicine, Northwest A&F University, Yangling, Shaanxi, China 712100,* [2]*Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.*

Heat stress (HS) is reported to affect the standing, feeding, drinking, and lying behavior of dairy cows. This study was to observe and summarize the effect of HS on the behavior and milk production of cows with differing levels of milk production. A self-powering meteorological station was established in a herd of about 1,500 Holstein cows to monitor temperature and humidity, and THI (temperature-humidity index) was calculated. The degree of heat stress was classified into 3 categories according to the daily average THI from 08:00 to 20:00 [no heat stress (NOHS, THI <72), mild heat stress (MIHS, $72 \le \text{THI} \le 79$), and moderate heat stress (MOHS, THI >79)]. All cows were divided into 3 groups (high productive group (HG, daily milk yield 40.1 ± 2.0 kg), middle productive group (MG, 85 – 154 DIM, daily milk yield 28.7 ± 1.4 kg), low productive group (LG, 15 – 84 DIM, daily milk yield 23.6 ± 1.2 kg)). According to the change of DIM, the cows flowed between groups. The cows were housed in a freestall equipped with fans, sprinklers and water trough. Feeding time was at 06:00, 13:00, 19:30. Cows were milked 3 times daily (07:00, 13:00, 21:00) in a milking parlor and make milk yield measurements. Changes in feeding, drinking, lying, and standing behavior were monitored visually and the proportion of the 4 behaviors were calculated every hour from 12:00 to 18:00 by manual scanning and tracking under the condition of NOHS and MOHS. A mercury thermometer was used to determine the rectal temperature of cows from 12:00 to 15:00 every day. The influence of HS on rectal temperature and behavior of dairy cows with different productive groups were analyzed by one-way ANOVA test (Tukey test). There was no significant difference in rectal temperature between HG, MG, LG when cows experiencing NOHS, MIHS, and MOHS. Under the influence of MOHS, the feeding proportion of HG and MG increased about 100%, and the standing proportion of HG and MG decreased by 51.06% and 23.33% respectively ($P < 0.05$), while these 2 behaviors of LG had no significant change. The potential reason may be that the position of the sprinklers is above the feeding stall so the cows want to cool down and eventually eat casually. There was no significant difference in daily milk production between HG, MG, LG under the conditions of NOHS and MIHS. HS can cause different degrees of behavioral changes for dairy cows with different levels of milk production.

Key Words: animal behavior, dairy cow, heat stress

P110 Assessing human-directed behavior in dairy calves reared with varying social contact.

S. B. Doyle* and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

While social contact for calves has broad effects on behavioral development, influences on human-animal relationships have been minimally studied, despite implications for longer-term calf management and welfare. We characterized human-animal interactions in 2 distinct testing contexts to examine response repeatability, effects of housing, and activity following testing. At birth, calves were randomly assigned to individual housing (IH; n = 6 calves) or pair-housing (PH; n = 6 calves; 1 focal calf/pair). A human approach test was performed in the home pen (wk 3) and within a test arena (wk 4), where a human approached, and then extended their hand, over a 5 min period. We measured latency to contact the human, frequency of human-directed oral behavior, latency to lie down following testing, and assigned a score for human approach (0 = complete avoidance of human, 4 = close proximity and human contact). Latency to contact the human was 60.3 ± 54.0 s (mean ± SD) for the home pen approach test, and 133.3 ± 121.6 s, for the arena test. Human-directed nonnutritive oral behavior occurred in 59% of calves, on average. There was no effect of housing treatment on any approach test outcomes in our preliminary data set ($P > 0.2$). For the home pen approach test, we found that calves who performed human-directed nonnutritive oral behaviors had reduced latency to lie down following testing (7.4 vs. 16.7 min; SE = 3.26; $P = 0.04$). Latency to lie down following testing was also negatively associated with approach test score (estimate = −2.79; SE = 1.36; $P = 0.04$), indicating that calves that approached the human more readily lay down faster following testing, which may reflect reduced disruption in calves that are more comfortable with human interaction. We found no association between the 2 approach tests in latency to approach the human or approach test score ($P > 0.4$). Overall, these results highlight variability in human-directed behavior between individual calves and testing contexts in response to human approach.

Key Words: human-animal interaction, social housing, nonnutritive oral behavior

P111 Effect of pair-housing on calf health, intake, and growth performance.

E. E. Lindner*[1], K. N. Gingerich[1], P. D. Krawczel[2], and E. K. Miller-Cushon[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Helsinki, Helsinki, Finland.*

Dairy calf social housing affects early-life calf behavior and performance, yet less is known about longer-term impacts of social housing. We hypothesized that pair-housed calves would consume more solid feed during the preweaning period compared with individually housed calves, which would translate into a growth benefit, potentially carrying over into the postweaning period. Calves were randomly assigned at birth to either individual (IH; n = 15 calves) or pair pens (PH; n =

10 pairs). Calves received 8 L/d of milk replacer in 2 meals, and were provided ad libitum access to calf starter and water. Calves were mingled between treatments and group-housed (5 ± 1 calves/pen) at 8 wks of age and then regrouped and moved to pens on pasture and followed for 2 mo. We monitored health (days scouring) during the first week of life. Milk and starter intake were measured 5x/wk and body weight was measured weekly until wk 9 of age, and again after 2 mo on pasture. Data were averaged for pair-housed calves, and analyzed in a general linear mixed-effects model, with week as a repeated measure for preweaning data. Duration of scours was not affected by housing (5.7 vs. 3.9 d; IH vs. PH; SE = 0.95; $P = 0.16$). Milk intake during the first 3 wks of age did not differ (3.4 L/meal; SE = 0.09; $P = 0.22$), and all calves finished their allotment beyond this time point. Calves housed in pairs tended to consume more starter during the preweaning period (96.1 vs. 62.5 g/calf/d; PH vs. IH; SE = 0.9; $P = 0.064$) and maintained greater starter intake during weaning (1.1 vs. 0.88 kg/calf/d; PH vs. IH; SE = 0.01; $P = 0.05$). Preweaning ADG did not differ between treatments (0.59 vs. 0.57; IH vs. PH; SE = 0.03; $P = 0.17$), and body weight did not differ at the time of postweaning grouping (wk 8; 69.6 kg; SE = 2.6; $P = 0.67$) or after 2 mo on pasture (120.1 kg; SE = 5.4; $P = 0.80$). In conclusion, pair-housing supported increased starter intake during the preweaning and weaning period, but we did not observe a longer-term effect on weight gain.

Key Words: social contact, feed intake, weight gain

P112 Association between personality traits and behavior in the home pen in group-housed dairy calves.

K. N. Gingerich*, E. E. Lindner, and E. K. Miller-Cushon, *University of Florida, Gainesville, FL.*

Given evidence of considerable individual variability in the expression of many behavioral traits, the objective of this study was to evaluate associations between personality and aspects of social behavior, feeding behavior, and interaction with environmental features. Holstein heifer and bull calves (n = 32) were introduced to group pens at 2 weeks of age (8 calves/pen; 7.4 × 16.0 m). Each pen included contained 2 rotating brushes and 2 shelters (3 sided; 1.2m x 1.2m) offering visual seclusion from the rest of the pen. Calves were fed a maximum of 12 L/d of milk replacer (min meal size = 2 L; max meal size = 4.5 L) via an automatic milk feeder. At 4 weeks of age, calves were tested in a series of standardized behavioral tests, including open field, novel object, unfamiliar calf, and unfamiliar human tests. Behavior was recorded continuously for 24 h in the week following behavioral testing to characterize use of the shelters and brushes, and feeding behavior data were obtained from the milk feeder for a 7-d period encompassing this focal day. Four factors were identified from principal component analyses of responses during behavioral tests and were interpreted as “exploratory active,” “inactive avoidant,” “calf-avoidant,” and “human-directed.” Association between these factor scores and behavior were analyzed using linear regression. The “calf avoidant” factor tended to be positively associated with shelter use ($P = 0.10$), lying duration within the shelter ($P = 0.07$), and milk intake ($P = 0.10$), and was negatively associated with frequency of entering an occupied shelter ($P = 0.06$). The “exploratory active” factor tended to be negatively associated with shelter use ($P = 0.10$). The “human-directed” factor was positively associated with duration of brush use ($P = 0.01$). The “inactive avoidant” factor was not associated with any focal behaviors. Our results suggest that personality is associated with behavioral expression in the home pen, including aspects of feeding behavior and use of shelters and rotating brushes, highlight the need for further study of individual differences in livestock behavior.

Key Words: personality, behavior, calf social housing

P113 Assessment of the associations of management practices during the dry period and activity patterns in dairy cattle.

A. A. Barragan*[1], M. Shabloski[1], E. Hovingh[1], and L. da Costa[2], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.*

The objective of this study was to assess the associations of dry period management practices and activity patterns (i.e., lying time, number of lying bouts, and lying bout duration) during the 14 d before calving. Heifers and cows from 4 dairy farms (700–2,800 milking cows) located in Pennsylvania were enrolled in this trial. Based on on-farm records, cows that were between 20 and 30 d from expected calving date were fitted with a HOBO accelerometer on the rear right leg. Only animals that had complete activity data for 14 d before the actual calving date were included in the study (heifers, n = 66; cows, n = 70). A 20-question multiple-choice survey was developed to collect farm management practices information (e.g., feed push-up frequency, bedding material used). The data were analyzed using the MIXED procedure of SAS. Heifers and cows that were housed in pens with sand or straw as bedding materials spent more time lying (sand = 815.7 ± 12.01 min/d; straw = 845.61 ± 21.68 min/d; sawdust/recycled manure = 650.30 ± 17.06 min/d), and had more lying bouts (sand = 11.18 ± 0.25 bouts/d; straw = 12.69 ± 0.51 bouts/d; sawdust/recycled manure = 9.45 ± 0.37 bouts/d) and of shorter duration (sand = 75.02 min, 95% CI:56.79–99.13; straw = 66.21 min, 95% CI:56.98–76.93, sawdust/recycled manure = 98.31 min, 95% CI:67.92–142.30) compared with cows that were housed in pens with either sawdust or recycled manure as bedding materials. Furthermore, heifers and cows that were fed 2 times a day spent nearly 3 h less lying down (2 times feeding = 650.36 ± 22.05 min/d; one time feeding = 823.77 ± 13.57 min/d) and tended to have longer lying bouts (2 times feeding = 98.33 min, 95% CI:65.61–147.37; one time feeding = 72.23 min, 95% CI:58.30–89.47) compared with cows that were fed one time a day. The results from this study suggest that farm practices during the dry period may alter cow behavior.

Key Words: dry period, farm practices, activity patterns

P114 Temperament and dominance in confined beef cattle: Effect on feeding behavior, intake and performance.

V. Fischer*, A. F. Bettencourt, A. T. Machado, I. D. V. Angelo, D. G. Adamich, C. S. Silva, J. A. Guimarães, L. S. Garcia, C. A. K. Ximenes, A. C. Vieira, and J. U. Tarouco, *Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.*

This study aimed to evaluate the relation between temperament and dominance and the effects on the feeding behavior, dry matter intake (DMI) and daily weight gain (ADG) of beef cattle. Two trials were conducted during 70 d (each one), with 20 Angus bulls and 21 Brangus calves, housed in drylots with automatic feeders and drinkers. The social hierarchy of the animals was evaluated at the beginning of the study, classifying them in dominant and subordinate animals based on the sucess rate in agonistic interactions (Dominance Index, DI). The temperament was evaluated at the beggining, middle and end of the trials, as the time spent by animais to exit the squeeze chute and traverse a fixed distance of 1.80 m (ET); also as the composite score

(CS) considering the movements, breathing intensity, vocalization and kicking while animal was hold at the scale. Feeding behavior and DMI were assessed using automated feeders. All measurements were averaged per animal, generating one value per bovine. The values of ID and ET were used to classify the animals in classes of dominance (DIT) and temperament (ETT). Means of ET, CS, DI, ingestive behavior, DMI and ADG within trials were submitted to correlation analysis and ANOVA (GLM procedure of SAS), considering the fixed effects of DIT, ETT and their interaction according to a complete randomized design. Dominance did not correlate with temperament in bulls. Reactive bulls (ETT ≤6.2 s) tended ($P < 0.10$) to have a higher DI. Dominant bulls (DIT >0.49) tended ($P < 0.10$) to have higher DMI rate. Calm bulls (ETT >6.2 s) tended ($P < 0.10$) to have fewer visits to the feeder and higher DMI rate, but had larger meal duration ($P < 0.05$). Dominance was moderately correlated with ET ($r = 0.45$; $P = 0.04$), indicating that dominant calves were calmer than subordinates. The DMI rate tended ($P < 0.10$) to be higher for reactive calves (ETT ≤3.5 s). Neither DIT nor ETT affected DMI and ADG of bulls and calves. The temperament and dominance, despite changing some characteristics of feeding behavior, did not influence DMI and ADG of confined beef cattle with access to automated feeders.

Key Words: beef cattle, dominance, temperament

P115 Attitudes and perspectives of Brazilian dairy farmers regarding the use of automated behavior recording and analysis systems.

V. Fischer*[1], A. C. Vieira[1], M. E. A. Canozzi[2], L. S. Garcia[1], and J. T. Morales-Piñeyrúa[2], [1]*Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil,* [2]*Instituto Nacional de Investigacion Agropecuaria (INIA), Colonia, Uruguay.*

Automated behavior recording and analysis systems (ABRS) have been developed to help in the farm management. However, the knowledge about attitudes and perspective of farmers and manager staff in relation to technology use and alerts are still scarce. The aim of this study was to investigate the motivations and perspectives of Brazilian dairy farmers who adopted (YABRS) or not (NABRS) the automated system. Thirty-eight farmers were contacted. A semi-structured interview was conducted by telephone, and the answers were transcribed and codified for further descriptive analysis. Farmers were classified in 2 groups, 22 and 16 in the NABRS and the YABRS, respectively. The data were analyzed descriptively and by ANOVA comparing groups of farmers using SAS Studio (SAS Institute, version 3.8, 2016). In both groups, the majority of farmers are less than 40 years old and have superior education level ($P > 0.10$); 68.7% of the farmers in the YABRS group are in the dairy activity for more than 20 years against 36.3% of the farmers in NABRS ($P < 0.05$). In NABRS group, 77.2% of the farms are pasture-based systems, while 100% in the YABRS group confined cows. Herds were larger in NABRS commpared with YABRS ($P < 0.05$), 165.5 ± 139.7 vs 50.6 ± 41.6 cows, respectively. The estrus detection and cow's health monitoring were the main motives to invest or desire to invest in this technology for both groups but cost is the most important factor that prevents farmers from purchasing monitoring systems. All YABRS famers observed the target cows after receiving a health or estrus alert, but less than 33% give feed back about the alerts to the company, although 75% of YABRS considered it was easy to adapt to the monitoring system and 85% of them claimed to partially trust the alerts. YABRS farmers believe that they can intervene in the evolution of the animals' health status, as the alerts give a window of 3 to 4 d before the onset of clinical signs of diseases, anticipating the start of the treatment and 75% of them stated they improved service and conception rates. All contacted farmers showed interest in health, calving, thermal comfort and especially estrus alerts, but factors as cost, difficult access to internet, impairing access to the system, to the equipment and service's suppliers, prevent ABRS acquisition or the spread of its use by farmers.

Key Words: behavior, farmer's perspectives, monitoring technology

P116 Evaluation of parity impact on social competition and feed efficiency in lactating cow.

F. S. Baier*, M. J. Martin, S. J. Erb, G. J. Combs, K. A. Weigel, H. M. White, and J. M. C. Van Os, *University of Wisconsin–Madison, Madison, WI.*

Social order can impact feeding behavior and other feed and production related outcomes in dairy cows. Our objective was to evaluate the impact of parity and grouping on feed efficiency and feed bunk social dynamics. Lactating Holstein cows (29 primiparous, 30 multiparous; 168 ± 22 DIM mid trial, mean ± SD) were housed in a freestall pen with 30 Insentec feeders. Cows were randomly assigned to 1 of 3 treatments (PRIM: 100% primiparous, MULT: 100% multiparous, MIX: 50% primiparous (MIXP), 50% multiparous (MIXM)) with 2 replicates each (10 cows/replicate). Each cow had access to 5 bins. Video was recorded continuously for 6 nonconsecutive h in wk 1 (1 h postmilking, 0450 and 1545 h; 4 h post-feed delivery, 1000 h). Competitive replacements (actor is successful or not in replacing the receiver at the feed bunk) were coded, and the ratio of successful replacements out of total attempts were calculated for each cow (excluding 6 cows uninvolved in replacements). Residual feed intake (RFI) was calculated by regressing DMI on milk energy output, median DIM, metabolic BW, and change in BW, each nested within parity. Linear models with fixed effect of parity within treatment were conducted in R (v. 3.6.3) with planned contrasts between MULT vs. PRIM, MULT vs. MIXM, and PRIM vs. MIXP. However, due to nonnormal data, a Wilcoxon rank sum test was used to evaluate replacements. Cows in MULT were more successful when initiating replacements relative to PRIM (0.96 ± 0.03 vs. 0.76 ± 0.1, mean ± SE; $P = 0.02$), but no differences in success index were found between multiparous or primiparous cows in same- vs. mixed-parity treatments (MULT vs. MIXM: 0.96 ± 0.03 vs. 0.81 ± 0.1; PRIM vs. MIXP: 0.76 ± 0.1 vs. 0.92 ± 0.1; $P \geq 0.41$). Parity within treatment did not impact RFI (PRIM vs. MIXP: −0.11 ± 0.3 vs. 0.24 ± 0.4; MULT vs. MIXM: −0.31 ± 0.3 vs. 0.58 ± 0.4; PRIM vs. MULT: −0.11 ± 0.3 vs. −0.31 ± 0.3; $P \geq 0.48$). The potential influence of parity grouping on social dynamics and RFI warrants further investigation.

Key Words: social dynamics, grouping strategies

P117 Coefficient of variation of daily lying bouts increases at parturition.

J. M. Piñeiro*[1], B. T. Menichetti[2], and G. M. Schuenemann[2], [1]*Department of Animal Science, Texas A&M AgriLife Extension, Amarillo, TX,* [2]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH.*

The objective was to assess the effect of parturition on behavior [lying time, steps, lying bouts (LB), LB duration (LBd), standard deviation (SD) of LBd and the coefficient of variation (CV) of LBd]. A total of 1,052 prepartum Holstein cows (401 heifers and 651 cows) from 3 Ohio dairy herds were enrolled in monthly cohorts of 20 to 36 cows 2 weeks before parturition. Individuals animals were fitted with electronic data loggers (IceQube, IceRobotics, Edinburgh, UK) on a rear leg to assess their behavioral activity. Lying time (min/d), number of steps (no./d), number of LB (no./d), LBd (min/d), SD of LBd and the CV of LBd were recorded. Data were analyzed using MIXED procedure of SAS using days relative to calving as repeated measures (SAS Institute Inc., Cary, NC). Cows had decreased lying time (10.6 h/d) the day of parturition compared with each day of the week before calving (over 12 h/d; $P < 0.0001$). However, heifers had decreased lying time at parturition (8.6 h/d) and day −1 (8.7 h/d) compared with days −7 to −2 (over 9.8 h/d; $P < 0.0001$) but day −1 and the day of parturition did not differ ($P = 0.75$). Heifers showed a different behavioral pattern compared with cows with the decrease of lying time starting one day before parturition compared with the decrease observed in cows occurring the day of parturition. Heifers and cows showed significantly increased steps and more LB of shorter duration the day of parturition compared with days −7 to −1 relative to parturition ($P < 0.0001$). The SD of LBd of cows and heifers at parturition decreased compared with days −7 to −2 ($P < 0.0001$). However, the CV of LBd increased at parturition compared with each day of the week before calving ($P < 0.0001$). The CV of LBd duration is standardized by the mean of LBd (CV = SD/mean × 100), and mean LBd decreased at parturition. Therefore, the CV of LB duration best measures the dispersion compared with the SD of LBd to assess the increased variation of LBd before parturition. These findings show that the CV of LBd increases at parturition.

Key Words: parturition, prediction, behavior.

P118 Predicting pre- and postweaning performance of dairy calves: An investigation using precision technology data.

M. C. Cantor*[1], H. W. Neave[2], M. M. Woodrum Setser[1], and J. H. C. Costa[1], [1]*Dairy Science Program, University of Kentucky, Lexington, KY,* [2]*Animal Behaviour and Welfare, AgResearch Ltd., Hamilton, New Zealand.*

The early identification of individual traits that are associated with performance in dairy calves is fundamental to tailor management and characterize phenotypes. We aimed to identify if feeding behavior patterns and activity in early life (first 30 d) were associated with total performance (ADG) and total starter intake from birth to 14 d postweaning. Calves (n = 42) wearing a leg accelerometer (IceRobotics, Scotland) were fed from an automated feeder (Förster-Technik, Germany) starting at 3 ± 2 d of age. Calves were weighed at birth and twice weekly on a scale. Calves had an allowance of 10 L/d of milk replacer until d 50 and ad libitum access to starter from another feeder. Calves were step-down weaned off milk from d 50 (50% reduction until d 64, additional 20% reduction until complete weaning at d 70). Calves were followed for 14 d postweaning. Total starter intake and ADG were summarized over the experimental period (d 3 to 87 ± 2 d of age). The automated feeder recorded daily feeding behavior variables: starter intake, milk intake, rewarded and unrewarded visits, and drinking speed. The accelerometer recorded daily activity variables: lying time, lying bouts, total step count, and activity index based on acceleration rate and steps. All feeding behavior and activity variables from pen entry to d 30 were input variables in a Principal Component Analysis, resulting in 3 factors explaining 70% of the variance. In a linear regression, factor 3 ("Feed motivated": high drinking speed and starter intake) was associated with greater ADG ($P < 0.01$) and total starter intake over the experiment ($P = 0.02$). Factor 2 ("Milk driven": high rewarded and unrewarded visits at milk feeder) was associated with lower total starter intake ($P = 0.03$) but not with ADG ($P = 0.45$). Factor 1 ("High activity": high steps, lying bouts and activity index; low lying time) was not associated with ADG or total starter intake ($P > 0.2$). These results suggest that feeding behavior patterns during the first 30 d of life are associated with total starter intake and weight gains up to 14 d postweaning; these indexes could serve as a proxy for later calf performance.

Key Words: growth, automated feeder, activity

P119 Rumination and activity of cows with metritis treated with chitosan microparticles.

J. G. Prim*[1], E. B. de Oliveira[2], A. Veronese[1], R. C. Chebel[1], and K. N. Galvão[1], [1]*University of Florida, Gainesville, FL,* [2]*University of California, Davis, CA.*

The objective was to compare the rumination and activity of cows with metritis treated with chitosan microparticles (CM) or ceftiofur crystalline-free acid (CEF). In addition, rumination and activity of cows that cured and did not cure from metritis were also compared. Nulliparous Holstein cows (n = 311) were fitted with an automated monitoring device from −21 to 60 DIM. Cows with metritis (d 0; watery, fetid, pink/brown uterine discharge) were assigned to: CEF (n = 47) – subcutaneous injection of 6.6 mg/kg of CEF on d 0 and 3; CM (n = 45) – intrauterine infusion of 24 g of CM dissolved in 40 mL of distilled water on d 0, 2, and 4; CON (n = 39) – no treatment. Cure was defined as a uterine discharge that was not watery, fetid, pink/brown on d 12. Data were analyzed by ANOVA for repeated measures. Pre- (d −4 to −1) and post- (d 0 to 21) diagnosis data were analyzed separately. Pre-diagnosis, treatment was not associated with rumination or activity. Postdiagnosis, CM (447.3 ± 10.6 min/d) had lower ($P \leq 0.05$) rumination than CEF (499.3 ± 9.6 min/d) and CON (491.5 ± 11.3 min/d). There was no difference between CEF and CON. Postdiagnosis, CM (653.4 ± 13.4 arbitrary unit) had ($P \leq 0.05$) lower activity than CON (713.4 ± 14.2 arbitrary unit), whereas CEF was intermediate (673.2 ± 12.3 arbitrary unit) and was not different from CON. Cure of metritis was not associated with rumination pre- and post-diagnosis. There were tendencies for an association between cure and activity. Cows that did not cure had lower activity than cows that cured pre-diagnosis (596.3 ± 18.7 vs. 638.8 ± 14.8 arbitrary units; $P = 0.07$) and post-diagnosis (634.0 ± 13.0 vs. 665.3 ± 10.2 arbitrary units; $P = 0.06$). In summary, CM decreased rumination and activity compared with CON, which indicates that CM hinders the recovery of cows with metritis. Furthermore, the pattern of activity of cows that did not cure from metritis was different from cows that cured, even before diagnosis, which could potentially be used to predict cure.

Key Words: metritis, rumination, activity

P120 Accuracy of infrared temperatures taken from different anatomical regions on neonatal dairy calves.

M. L. Pister*, K. N. Brost, C. A. Hayes, and J. K. Drackley, *University of Illinois, Urbana, IL.*

The objective of this study was to determine if skin temperatures collected via infrared thermometer were comparable to rectal temperatures of neonatal dairy calves. The experiment used 25 calves (4 male, 21 female) enrolled on trial at 4 d of life and observed until 24 d. Calves were housed in individual hutches bedded with straw. Feeding and medical treatments were performed per farm protocol. Rectal and infrared temperatures (IRT) were recorded daily at 0700 h. The anatomical regions used to collect IRT were: neck, rump, and forehead. All IRT measurements were taken from a distance of 30 cm. Infrared temperatures were recorded twice from each anatomical region. Health scores, heart rate (HR), and respiratory rate (RR) were recorded daily. Temperature and relative humidity were recorded hourly by on-site data loggers. Data were analyzed using MIXED and FREQ procedures in SAS. Variables used to determine the accuracy of IRT were: rectal temperature (RT), maximum daily temperature of the environment, maximum daily temperature of the hutch, heat stress (h/d) of the environment, and heat stress (h/d) of the hutch. Heat stress was defined as the hours/day of environmental or in-hutch temperature-humidity index (THI) ≥ 89. Regression analysis demonstrated that RT, maximum environmental temperature, and maximum hutch temperature were significant ($P < 0.05$) for all anatomical regions. Although significant, the IRT range differed greatly by season (−5.4 to 37.9°C) whereas RT was maintained closely by the calf (36.9 to 39.6°C), causing the correlation to be difficult to use on farm without further analysis. The IRT was correlated ($P < 0.05$) with RR and RT, maximum environmental temperature, and heat stress (h/d) inside the hutch, demonstrating a relationship between the heat variables and RR. No significant results were observed for HR. Incidence of pyrexia was low and in general, the calves were healthy. To conclude, the infrared thermometers provided statistically significant relationships, but do not reflect RT with the current parameters. Further research is needed on IRT collection methods using health challenged calves.

Key Words: infrared thermometer, temperature, calves

Animal Health: Posters

P121 Genetic relationship of *Escherichia coli* isolated from the reproductive and gastrointestinal tracts of dairy cows pre- and postpartum. K. L. Jones*[1], F. Cunha[1], S. J. Jeon[2], K. C. Jeong[1], Y. Yang[3], and K. N. Galvão[1], [1]*University of Florida, Gainesville, FL,* [2]*Long Island University, Brookville, NY,* [3]*Sun Yat-sen University, Guangzhou, China.*

The objective was to investigate the source of bacterial colonization of the uterus by exploring the genetic relationship among *E. coli* strains isolated from the recto-anal junction (RAJ) and the reproductive tract (RT) of dairy cows pre- and postpartum. Cows (n = 34) had a swab sample collected from the vulva, vagina, and RAJ every 3 d starting 6 d before expected calving until 9 d postpartum. A blood sample was collected at all time points. A swab sample was collected from the uterus at the same time points postpartum. All samples were cultured aerobically on CHROMagar *E. coli* selective medium for 24 h at 37°C. Isolates from cows with growth from the vulva and/or vagina in addition to the uterus (n = 8) were used for whole-genome sequencing (WGS). All RAJ samples grew and none of the blood samples grew. A total of 44 isolates were selected for WGS, which was performed using an Illumina MiSeq. PATRIC was used to annotate each genome. The Harvest Suite was used for core genome alignment, SNP identification and phylogenetic tree rendering. Clades with no branching were evaluated for SNPs. Strains were considered clonal isolates if $\leq$4 SNPs difference between strains. Clonal strains were isolated from individual cows from the RAJ and vulva (1 cow; 1 SNP), the RAJ, vulva and vagina (1 cow; 0 SNP), the vulva and uterus (1 cow; 0 SNP), the vagina and uterus (2 cows; 0 SNP between strains in each cow), and the vulva, vagina, and uterus (1 cow; 0 SNP) postpartum. Clonal strains were also isolated from different cows from the vulva prepartum, and vulva and vagina postpartum (0 SNP between strains), the RAJ prepartum, and vagina and uterus postpartum (max 1 SNPs between strains), the RAJ, vulva and vagina postpartum (max 1 SNPs between strains), and the uterus, vulva and vagina postpartum (max 2 SNP between strains). Finding clonal *E. coli* strains in the RAJ from the same cow or different cows in the vulva, vagina, and uterus postpartum indicates that the GI is a source of *E. coli* that can colonize the RT, and that *E. coli* can be transferred among cows.

Key Words: *Escherichia coli*, genetic characterization, uterus

P122 Genes associated with immune function are downregulated in blood-derived neutrophils from periparturient cows. E. Asiamah*[1], K. Ekwemalor[2], S. Adjei-Fremah[2], B. Osei[3], and M. Work[2], [1]*University of Arkansas, Pine Bluff, Pine Bluff, AR,* [2]*North Carolina Agricultural and Technical State University, Greensboro NC,* [3]*Oklahoma Medical Research Facility, Oklahoma City, OK.*

The periparturient period (3 weeks pre-calving and 3 weeks postcalving) is a challenging period for dairy cows in large part due to the dysfunction of their immune system. The compromise in the immune system puts the dairy cow at a higher risk of infections and other diseases. The impairment of neutrophil function plays a crucial role in the immunosuppression of the periparturient dairy cow. Research is emerging to better understand the molecular mechanisms underlying the impaired immune function in dairy cows during the periparturient period. This study aimed at evaluating the global gene expression of blood-derived neutrophils from periparturient cows. Blood was collected from Holstein Friesian periparturient cows (n = 3) at −14 d relative to expected calving date and 7 d relative to the actual calving date. Neutrophils were isolated based on procedures described by Abdelmegeid et al. (2017; *Journal of Dairy Science* 100:3155–3165). Isolated neutrophils were subsequently used for transcriptional profiling using the Agilent bovine (v2) 4 × 44 K array. Data normalization and statistical analysis were performed using GeneSpring GX software version 13.0. The analysis was carried out using a *t*-test unpaired statistical method with Benjamini-Hochberg FDR method. Fold changes in gene expression calculated were filtered at a cut-off of ≥ 2 ($P < 0.05$). The results showed that 249 genes were differentially expressed (FC ≥ 2, $P < 0.05$.). Eighty-seven genes were downregulated and among the top 20 downregulated genes were genes essential to neutrophil response and immunity. These included *PGLYRP1*, which is involved in pathogen recognition, and *SERPINB4*, which is a protein that inhibits neutrophil-derived proteinases to protect tissue damage at inflammatory sites. Additionally, genes associated with cellular adhesion and migration (*ADRM1* and *THY1*) were also significantly downregulated. Concurrently, the pathway analysis also revealed that the TLR, inflammation response, oxidative stress, and MAPK signaling pathways are affected in bovine neutrophils during the periparturient period ($P < 0.05$). This work sheds some light on the altered gene expression in neutrophils during the periparturient period and the knowledge generated will ultimately be used for the development of novel management strategies to combat immunosuppression and disease susceptibility during this stage in the dairy cow.

Key Words: periparturient, neutrophils, dairy

P123 Effect of Holstein milk yield genotype on ex vivo innate immune response to lipopolysaccharide and lipoteichoic acid during the periparturient period. A. A. Brink*[1], W. J. Weber[1], J. D. Lippolis[2], J. B. Cole[3], S. M. Godden[4], and B. A. Crooker[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*USDA-ARS National Animal Disease Center, Ames, IA,* [3]*USDA-ARS Animal Genomics and Improvement Laboratory, Beltsville, MD,* [4]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN.*

Our objectives were to determine effects of milk yield genotype on innate immune response to ex vivo lipopolysaccharide (LPS) and lipoteichoic acid (LTA) stimulation. Unselected (UH, stable milk yield from 1964, n = 10) and contemporary (CH, n = 11) Holsteins that differ by more than 4,500 kg milk/305 d were fed the same diet *ad lib* and milked twice daily. Cows were blocked (1/genotype) by parity and expected calving date. Heparinized blood was collected at −14, 7, 28, and 49 DIM, mixed with a low or high dose of LPS (10 and 100 µg LPS/mL blood) or LTA (0.01 and 1.0 µg LTA/mL blood), and incubated for 4 h at 37°C. Plasma concentrations (pg/mL) of IL-6 and IL-1β were quantified by ELISA (Invitrogen, Carlsbad, CA), log-transformed and analyzed by repeated measures using PROC MIXED (SAS) with DIM as the repeated effect. Means differed when $P < 0.05$. Milk yield was less in UH than CH (29.0 vs. 45.3 kg/d FCM). The response of IL-6 and IL-1β to LPS and LTA were greater ($P < 0.01$) with the high dose of antigen. The IL-6 response to LPS was greatest ($P < 0.01$) at d7 and did not differ among other days while the IL-1β response was greatest ($P < 0.01$) at d-14 and decreased postpartum (2.7, 3.2, 2.8, 2.6 ± 0.14 for IL-6 and 3.8, 3.6, 3.3, 3.3 ± 0.08 for IL-1β on d-14, 7, 28, and 49, respectively). There was a genotype by dose interaction ($P < 0.01$) for IL-1β response to LPS as the low dose was greater in UH than CH (3.2 vs. 2.9 ± 0.10) but did not differ for the high dose (4.0 vs. 3.9 ± 0.10). The IL-6 response to LTA was greatest ($P < 0.01$) at d7 and did not differ among other days while the IL-1β response was greatest ($P < 0.01$) at d-14 and decreased postpartum (2.6, 3.0, 2.8, 2.5 ± 0.14 for IL-6 and 3.5, 3.2, 3.1, 3.0 ± 0.07 for IL-1β on d-14, 7, 28, and 49, respectively). There was a trend ($P = 0.07$) for IL-6 response to LTA to be greater in UH than CH cows. The UH cows had a greater ($P < 0.02$) IL-1β response to LTA than the CH cows (3.4 vs. 3.1 ± 0.08). Cytokine profiles demonstrate postpartum alterations in the innate immune response and a more sensitive IL-1β response to both antigens by the UH cows.

Key Words: milk yield genotype, LPS, LTA

P124 Whole-blood transcriptomic signature after 17 and 35 days of feeding OmniGen AF to prepartum Holstein cows.

M. Garcia*, J. Chapman, and B. Humphrey, *Phibro Animal Health Corporation, Teaneck, NJ.*

OmniGen AF (OG; Phibro Animal Health, Teaneck, NJ) is a feed additive with demonstrated benefit on improving the immunocompetence of stressed dairy cows. This study aimed to identify an early whole-blood transcriptomic signature, focused on immunity, during the first 35 d of feeding OG to prepartum Holstein cows. Sixteen cows (60 d before expected calving date) were randomly assigned to 1 of 2 treatments at dry-off: CTL (no supplementation) or OG (56 g OG/cow/d). Cows were bled at d 17 and 35 after OG feeding began. Isolated whole-blood RNA samples with RNA integrity number >7 (n = 5 per treatment) were submitted for RNA sequencing (Novogene Co., Ltd.). Sequencing libraries were generated using NEBNext Ultra^{TM} (NEB, Illumina, USA). Processed reads were mapped to the bovine genome using HISAT2. Differentially expressed genes (DEG, $P < 0.05$) were identified using DESeq2 R package. Gene ontology (GO) analysis of DEG was performed with clusterProfiler R package, and enriched GO terms with corrected $P < 0.05$ were deemed significantly different. Cows fed OG had 1,130 and 1,574 DEG at d 17 and d 35, respectively. At d 17, 18 DEG, 17 upregulated, were part of 4 enriched GO terms. Three GO terms were ancestor terms of neutrophil chemotaxis (neutrophil migration, granulocyte chemotaxis, and granulocyte migration). At d 35, 325 DEG, 264 upregulated, were part of 46 enriched GO terms. Two GO terms enriched at d 17 were also enriched at d 35. Noticeable, 10 of the 18 DEG enriching GO terms at d 17 were also part of enriched GO terms at d 35. Furthermore, the other 44 enriched GO terms at d 35 were mostly biological processes involved in innate (e.g., inflammatory response, phagocytosis) and adaptive (e.g., regulation of T-helper 1 type immune response, positive regulation of IFNγ production) immunity. A unique set of genes were identified that are similarly regulated at d 17 and d 35 of OG feeding. These findings confirm that biomarkers of immunity, regulated by OmniGen AF, can be detected as early as 17 d, and are further enhanced after 35 d of initial day of feeding.

Key Words: OmniGen, dairy cow, immunity

P125 Effect of in vivo heat stress on respiration rate, rectal temperature, and blood mononuclear cell function of dairy cows ranked for immune response.

S. Cartwright*[1], J. Schmied[1], M. McKechnie[1], A. Livernois[1,2], and B. Mallard[1,2], [1]*Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Centre of Genetics of Livestock Improvement, University of Guelph, Guelph, ON, Canada.*

Heat stress (HS) causes disease in dairy cattle. High (H) immune response (IR) dairy cattle have reduced disease, however variations in response to HS have not been evaluated. Therefore, the objective was to evaluate the effect of in vivo HS on respiration rate (RR), rectal temperature (RT), and blood mononuclear cell (BMC) function related to heat shock protein 70 concentration (HSP70 conc) and cell proliferation (CP) in IR phenotyped cattle. Holstein cattle (n = 24), ranked for IR [8 H, 8 average (A), 8 low (L)], based on estimated breeding values, were evaluated. Cattle were HS at the same time on 2 subsequent days (HS1 = 1 HS, HS2 = 2 HS), in the tie-stall wing of the barn, by increasing temperature to 29°C for 4 h. Blood samples were taken pre and post HS1 and HS2. Manual RR and RT and barn temperature (temp) and humidity (hum) were taken pre-HC and every 30 min during HS. Temperature-humidity index (THI) was calculated for each measurement using temp and hum. From blood samples, BMC were obtained to assess HSP70 conc and CP. Repeated-measures models, run in R, evaluated differences in RR and RT and included effects of IR phenotype, THI, parity, pregnancy status and production. General linear models, run in R, evaluated differences in HSP70 conc and CP with similar models. Results showed HIR (THI 76–81 LSM from 43.2 to 55.7 breaths per min (bpm)) had lower RR at higher THI compared with AIR (THI 76–81 LSM from 53.2 to 66.1 bpm) and LIR (THI 76–81 LSM from 50.4 to 73.4 bpm). Differences in RR were observed at THI of 76 (H vs A $P = 0.0247$, H vs L $P = 0.0202$), 77 (H vs A $P = 0.0419$, H vs L $P = 0.0453$) and 81 (H vs A $P = 0.0247$, H vs L $P = 0.008$). No differences in RT was observed. Results also showed HIR had greater HSP70 conc after HS1 (H LSM HS = 12.1 ng/mL SEM = 0.5, A LSM HS1 = 9.2 ng/mL SEM = 0.8, L LSM HS1 = 7.5 ng/mL SEM = 0.5) compared with A ($P = 0.0247$) and L ($P = 0.001$) and greater CP after HS1 (H LSM = 3.36 SEM = 0.65, L LSM = 0.99 SEM = 0.3, $P = 0.0279$) and HS2 (H LSM = 2.83 SEM = 0.62, L LSM = 0.59 SEM = 0.1, $P = 0.0425$) compared with L. Therefore, results may indicate HIR are more thermotolerant compared with A and L.

Key Words: heat stress, immune response, blood mononuclear cells

P126 Persistent, transient or delayed hypocalcemia in Jersey cows: Associations with metabolites and milk production.

A. M. Fillus*[1], C. C. Baccili[2], V. Gomes[2], R. B. Navarro[3], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil,* [2]*Universidade de São Paulo, São Paulo, São Paulo, Brazil,* [3]*Capal Cooperativa Agroindustrial, Arapoti, Paraná, Brazil.*

The aim of this study was to determine the types of subclinical hypocalcemia (SCH) and relate it to milk yield (10, 30, and 60 d postpartum) and blood metabolites. Thirty-one primiparous and 85 multiparous Jersey cows were housed in a compost barn with a robotic milking system. Blood samples were collected at 1, 2, 3, and 4 d postpartum to characterize the level of plasma total Ca and diagnose cows with SCH. Primiparous cows were categorized in 4 early postpartum Ca status groups: NC (normocalcemic; > 2.15 mmo/L at 1 and 2 DIM), tSCH (transient; ≤ 2.15 mmo/L at 1 DIM and >2.15 mmol/L at 2 DIM), dSCH (delayed; > 2.15 mmol/L at 1 DIM and ≤2.15 mmol/L at 2 DIM), and pSCH (persistent; ≤ 2.15 mmol/L at 1 and 2 DIM). The plasma Ca thresholds for multiparous were the same described above, but the second blood collection was at 4 DIM. Statistical analyzes were performed by SAS procedures (v.9.4) using the GLM for single measures and MIXED for repeated measures over time. We found 52.6% NC cows (n = 61), 26.7% tSCH (n = 31), 12.1% dSCH (n = 14), and 8.6% pSCH (n = 10). Average milk yield in the first 10 d was greater ($P = 0.04$) for tSCH than pSCH cows (23.24 vs. 18.96 kg/d, respectively), while NC and dSCH cows showed intermediate yields (21.66 and 20.54 kg/d, respectively). No differences on average milk yield in the first 30 and 60 d were found ($P > 0.10$) among groups. Haptoglobin concentrations were higher ($P < 0.05$) for pSCH and dSCH cows than for tSCH ones; 24.5 ± 3.3 and 22.8 ± 2.7 vs. 14.9 ± 2.1 mg/dL, respectively. NEFA concentrations were higher ($P < 0.05$)

for pSCH cows than NC ones; 0.43 ± 0.07 vs. 0.25 ± 0.03 mmol/L, respectively. Bilirubin concentrations were higher ($P < 0.01$) for pSCH cows than NC ones; 0.33 ± 0.03 vs. 0.17 ± 0.01 µmol/L, respectively. Albumin concentrations were lower ($P < 0.05$) for pSCH and dSCH cows than for NC ones; 3.14 ± 0.06 and 3.18 ± 0.05 vs. 3.40 ± 0.03 g/L, respectively. No differences were found ($P > 0.05$) for BHB, AST, cholesterol, and glucose concentrations among groups. In conclusion, transient SCH Jersey cows produce more milk than persistent SCH and their metabolic profile are similar to normocalcemic cows.

Key Words: calcium, transition period

P127 Calcium dynamics in blood and milk in dairy cows after calving. T. Aubineau*[1,2], P. Gaignon[3], M. Pocher[1,2], R. Guatteo[2], and A. Boudon[3], [1]*GDS Bretagne, Rennes, France,* [2]*BIOEPAR, INRAE, Oniris, Nantes, France,* [3]*PEGASE, INRAE, Agrocampus Ouest, Saint-Gilles, France.*

Routine identification of cows suffering from subclinical hypocalcemia around calving has great significance in dairy cattle to adapt prevention programs. Milk and blood calcium contents may be correlated, as the calcium sensing receptor contributes to the regulation of calcium secretion into milk according to blood calcium level. Milk would be a more convenient sample to identify cows at risk. Thus, our objective was to describe milk calcium (MiCa) dynamics and plasma calcium (PlCa) one during the week after calving and to assess their correlation. Eight primiparous and 12 multiparous Holstein dairy cows, milked twice a day, were submitted to morning daily simultaneous blood and milk sampling from the day of calving to 7 d later. Plasma and milk samples were analyzed for total calcium content by atomic absorption spectrometry. Plasma and milk calcium dynamics were first described using PROC GLIMMIX in SAS, with a generalized linear mixed model with repeated values. Time, parity and the interaction between both were included as fixed effect and cow as random effect. Post hoc tests were then realized to identify sampling times which differ between them. Finally, a Spearman correlation matrix aimed to identify sampling days with best correlations between PlCa and MiCa. PlCa increased from the day of calving to d 7 (time effect, $P = 7.10^{-11}$), and decreased with parity ($P = 0.002$). Primiparous and multiparous PlCa dynamics were different (interaction time × parity, $P = 0.007$), as multiparous had lower PlCa on the day of calving (75 ± 12 mg/L) and the day after (80 ± 12 mg/L), and primiparous had lower PlCa only the day of calving (89 ± 5 mg/L). MiCa was higher in the first 2 d after calving, sharply decreased until d 3 before stabilizing over the following days (time effect, $P = 2.10^{-9}$). MiCa was not affected by parity and correlations between PlCa and MiCa greatly depended on parity. Higher correlations were observed between PlCa on d 3 and MiCa on d 4 when considering only multiparous cows ($r = -0.8$, $P = 0.002$). Our results suggest that milk calcium measurements might be effective to assess blood calcium levels after calving in multiparous dairy cows, but these results remain to be confirmed in broader experimental conditions.

Key Words: hypocalcemia, milk, dairy cow

P128 Assessment of prevalence of ketosis in dairy cattle. C. Marquès*[1], S. Calsamiglia[1], A. Jubert[2], J. Marxuach[3], and L. Castillejos[1], [1]*Servicio de Nutrición y Bienestar Animal, Dpto. Ciencia Animal y de los Alimentos, Universidad Autónoma de Barcelona, Bellaterra, Spain,* [2]*Associació Lletera Interprofessional de Catalunya (ALLIC), Passeig de Cabrils, Barcelona, Spain,* [3]*It Elazos, Barcelona, Spain.*

Ketosis (KET) is a metabolic disorder in which the prevalence of its subclinical form ranges between 6.9 and 43% during the first 2 mo postpartum. The prevalence of ketosis can be monitored by determining β-hydroxybutyrate (BHB) in milk. The objective of this study was to evaluate the prevalence of ketosis using a database of Interprofessional Milk Analysis Laboratory and to study its correlation with other biomarkers. A database of the Interprofessional Milk Analysis Laboratory in Catalonia from 2017 to 2020 containing 84,216 individual cow records collected between 5 to 25 d postpartum was used. Data were obtained by infrared spectrometry (MilkoScan). Prevalence of KET (BHB in milk ≥0.10 mM) was evaluated according to parity and season. Statistical analysis was performed using GLM adjusted by Tukey, correlation, and regression with the SAS program. A significant lower prevalence ($P < 0.01$) was observed in primiparous cows (15.0%) than in multiparous cows (20.9%). A significant lower risk of KET ($P < 0.01$) was observed in spring (15.0%), a medium risk in winter (17.1%) and autumn (20.3%), and a higher risk in summer (22.6%). Finally, a correlation of 0.62 was found between BHB and Acetone: BHB = 0.0487 + 0.2617 × Acetone; n = 43,955; Rsq = 0.3894; $P < 0.01$. No correlation was observed between BHB and fat (%), protein (%), or fat (%) / protein (%) ratio. In conclusion, the prevalence of ketosis is higher in multiparous than in primiparous cows. In summer there is a higher prevalence of ketosis. Acetone in milk could be a good alternative biomarker to BHB in milk.

Key Words: ketosis, prevalence, BHB in milk

P129 Association between transition diseases and changes in body condition score of Holstein cows. D. Manriquez*[1], A. De Vries[2], and P. Pinedo[1], [1]*Colorado State University, Fort Collins, CO,* [2]*University of Florida, Gainesville, FL.*

The objective was to quantify the impact of dystocia (DYS), clinical hypocalcemia (CHC), clinical ketosis (CKT), left displaced abomasum (LDA), and metritis (MET) on the magnitude of body condition score change (ΔBCS) at multiple time periods. Holstein cows (5,894) in one dairy located in CO, USA were enrolled at calving from April 2019 to August 2020 and daily BCS were obtained from an automated camera system (BCS$^{\text{TM}}$, DeLaval Inc.). Disease events were retrieved from DairyComp (VAS, Tulare, CA). Cows were classified in 6 health categories (HLC; Table 1) based on their first disease diagnosed within 21 DIM. Mixed models for repeated measures (SAS Institute Inc., Cary, NC) were used to calculate LSM for BCS (1, 14, 21, and 30 DIM) and ΔBCS by HLC. Models included HLC, parity (1; ≥ 2), DIM, and HLC by DIM interaction. As no significance was established for the interaction HLC by parity, this term was excluded from the models. A total of 531 (9%), 373 (6.3%), 300 (5.1%), 53 (0.9%), and 847 (14.4%) cows were diagnosed with DYS, CHC, CKT, LDA, and MET, respectively. Differences in average BCS among HLC were established only at 1 and 14 DIM (Table 1). The magnitude of the ΔBCS depended on HLC and period in analysis. Overall, small magnitude differences in BCS and ΔBCS were detected among HLC using the automated BCS system. Cows affected by ketosis and LDA evidenced the greatest losses in BCS at 21 and 60 DIM.

Key Words: body condition score, disease, transition

P130 Effects of dandelion supplements on lactation performance, antioxidative activity, and plasma metabolome in primiparous dairy cows. Y. Li*[1], J. Wang[1], B. Wang[2], J. Liu[1], and H. Liu[1], [1]*College of Animal Sciences, Zhejiang University, Hangzhou, China,* [2]*State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Table 1 (Abstract P129). LSM (± SEM) for BCS and ΔBCS measured by an automated camera system among health status categories

Item	Dystocia	Clinical hypocalcemia	Clinical ketosis	LDA	Metritis	Healthy
BCS						
1 DIM	3.35±0.01^{a}	3.35±0.01^{ab}	3.44±0.01^{c}	3.36± 0.05^{ab}	3.33± 0.07^{a}	3.36±0.003^{b}
14	3.25±0.05^{ab}	3.24±0.01^{ab}	3.32±0.004^{b}	3.24±0.014^{ab}	3.23±0.013^{a}	3.26±0.007^{ab}
21	3.15±0.05^{a}	3.12±0.01^{a}	3.19±0.01^{a}	3.10±0.05^{a}	3.12±0.01^{a}	3.16±0.003^{a}
60	3.05±0.01^{a}	3.02±0.01^{a}	3.00±0.02^{a}	2.91±0.05^{a}	3.01±0.01^{a}	3.10±0.004^{a}
ΔBCS						
DIM difference:						
21 − 1	−0.19±0.008^{c}	−0.24±0.01^{a}	−0.24±0.01^{a}	−0.25±0.02^{a}	−0.22±0.007^{ab}	−0.20±0.003^{bc}
60 − 1	−0.29±0.01^{b}	−0.32±0.01^{b}	−0.40±0.02^{a}	−0.42±0.04^{a}	−0.31±0.009^{b}	−0.29±0.004^{b}
60 − 14	−0.19±0.010^{b}	−0.20±0.01^{b}	−0.30±0.01^{a}	−0.31±0.04^{a}	−0.20±0.007^{b}	−0.19±0.003^{b}
60 − 21	−0.09±0.011^{b}	−0.09±0.01^{b}	−0.18±0.01^{a}	−0.18±0.03^{a}	−0.08±0.005^{b}	−0.09±0.003^{b}

$^{a–c}$Different letters within rows indicate significant differences between HLC at the Tukey adjusted $P < 0.05$.

Dandelion contains various biological phytochemicals that are beneficial to animal health. It is commonly used as a medicinal herb owing to its excellent antibacterial, anti-inflammatory, antiallergy, and antioxidant properties. However, only limited data are available concerning the production performance of dairy cows. In this study, we evaluated the effects of dandelion supplements on lactation performance, plasma antioxidative activity and metabolomics in primiparous dairy cows. A total of 60 mid-lactation Holstein dairy cows (days in milk = 151.72 ± 2.36 d; milk yield = 34.29 ± 0.34 kg/d) were divided into 4 treatment groups randomly, comprising the addition of dandelion at 0, 100, 200, 400 g/d per head. The whole plant part except root was used and composition was detected in the previous study. The experiment lasted for 8 weeks with a 10-d adaptation period. All data were analyzed by the mixed model (SAS 9.2, 2000) and checked for normality by the UNIVARIATE procedure. Results indicated that 200 g/d of dandelion increased the yield of milk and lactose ($P \leq 0.05$). The milk somatic cell counts ($P \leq 0.05$) were lower in all dandelion groups than that in the control group. The concentrations of glutathione peroxidase ($P \leq 0.05$) and superoxide dismutase ($P \leq 0.05$) were increased, and malondialdehyde ($P = 0.01$) was decreased in plasma when cows were fed 200 g/d dandelion. The plasma metabolomics analysis showed that 19 metabolites were upgraded and 4 metabolites were downgraded in the 200 g/d dandelion group. The hub differential metabolites such as ribose, glutamic acid, valine, and phenylalanine were enriched in phenylalanine, tyrosine and tryptophan biosynthesis ($P < 0.01$, impact = 1), phenylalanine metabolism ($P = 0.01$, impact = 0.36) and starch and sucrose metabolism ($P = 0.20$, impact = 0.42). Moreover, correlation analysis showed that plasma ribose, mannose, and glutamic acid were positively related to milk yield. In summary, dandelion supplementation improved lactation performance by elevating the plasma carbohydrate and amino acids metabolomics and antioxidative activity. Supplementation of 200 g/d dandelion is recommended for lactating dairy cows.

Key Words: dandelion, lactation performance, dairy cow

P131 Effect of injectable trace mineral supplementation on peripheral polymorphonuclear leukocyte function, oxidative stress, health, and performance in dairy cows in semi-arid conditions.

P. R. Menta*[1], T. Silva[1,2], I. Guimaraes[1], D. Paiva[1], L. Fernandes[1], M. L. Celestino[1], A. S. Netto[2], M. A. Ballou[1], and V. S. Machado[1], [1]*Texas Tech University, Lubbock, TX,* [2]*University of Sao Paulo, Pirassununga, Sao Paulo, Brazil.*

The objective of this study was to evaluate the effect of subcutaneous injections of Cu, Se, Zn, and Mn on peripheral blood neutrophil activity, on polymorphonuclear leukocyte (PMNL) function, concentrations of antioxidant enzymes, and haptoglobin (Hp) in dairy cows during summer months. This study was conducted in 2 dairy farms located in West TX. A total of 923 Holstein multiparous cows were randomly allocated to receive 3 injections of 15 mg/mL of Cu, 5 mg/mL of Se, 60 mg/mL of Zn, and 10 mg/mL of Mn (Multimin 90, Multimin North America, Fort Collins, CO) following the label dose of 1 mL/90.7 kg of body weight at 208 ± 3 and 260 ± 3 d of gestation, and at 35 ± 3 d postpartum (ITMS) or remain untreated (CON). Blood samples were collected at enrollment, and at 3 ± 1, 7 ± 1, 10 ± 1, and 35 ± 3 DIM to evaluate PMNL function; phagocytosis and oxidative burst capacity of neutrophils, and the quantification of the adhesion molecule L-selectin; glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity and thiobarbituric acid reactive substances (TBARS) and Hp. Statistical analysis was undertaken in SAS using logistic regression and mixed linear models. We observed that ITMS decreased the incidence of stillbirth ($P = 0.05$) and metritis ($P = 0.05$) compared with CON cows. Innate immunity during the postpartum period was improved in ITMS cows due to increased proportion of PMNL that performed phagocytosis ($P < 0.01$) and oxidative burst ($P < 0.01$) and also increased oxidative burst intensity ($P = 0.05$). The expression of the L-selectin in neutrophils was increased in CON cows compared with ITMS counterparts ($P = 0.04$). Injectable trace mineral supplementation did not affect the activity of the SOD ($P = 0.32$) and GPx ($P = 0.97$) and TBARS ($P = 0.56$). In contrast, CON tended to present higher serum haptoglobin concentration at 10 d after calving ($P = 0.09$). In conclusion, ITMS improved innate immunity by increasing neutrophil activity, but it did not influence the oxidative inflammatory status of dairy cows.

Key Words: trace minerals, innate immunity, oxidative stress

P132 Assessing the relationship between the metabolic health index and energetics, inflammation, and milk production in lactating Holstein cows.

E. A. Horst*, C. Sousa, and L. Rodriguez, *Innovative Liquids LLC, El Dorado Hills, CA.*

Composite indexes such as the liver functionality, liver activity, and metabolic health index (MHI) have been demonstrated to be effective strategies for depicting the magnitude of inflammation and its potential downstream consequences on health and performance in transition cows. Therefore, objectives of the study were to evaluate changes in milk production, energetics, and inflammation in cows with high or low MHI.

Multiparous Holstein cows calving on a commercial dairy were retrospectively categorized into high (positive MHI, mean = 1.74; n = 54) or low (negative MHI, mean = −1.96; n = 48) MHI groups. Blood samples were collected at 7 DIM and submitted to the Texas A&M Veterinary Medical Diagnostic Laboratory for analysis. MHI was calculated using albumin, bilirubin, and cholesterol concentrations according to previously described methods (Askel et al., 2020; *J. Dairy Sci.* 103, Suppl. 1). Milk yield was recorded daily and samples for composition analysis were obtained weekly. Milk and energy-corrected milk (ECM) yields are presented as an average of the first 4 weeks postpartum. Fixed effects of MHI group were analyzed using the mixed procedure of SAS. Fibrinogen and globulin concentrations were increased (19 and 7%, respectively; $P \leq 0.03$) whereas the albumin to globulin ratio was decreased (15%; $P < 0.01$) in low vs. high MHI cows. Relative to the high MHI group, average milk yield in the first 4 weeks postpartum was decreased (7%; $P = 0.03$). No treatment differences were observed for ECM yield in the low relative to high MHI group (48 vs. 58 kg, respectively; $P > 0.13$). Low MHI cows had decreased calcium concentrations (5%; $P < 0.01$) when compared with the high MHI group. NEFA and BHBA concentrations were increased in low relative to high MHI cows (54 and 48%, respectively; $P < 0.01$), however, BUN and glucose concentrations did not differ. Relative to the high MHI group, the NEFA to cholesterol ratio increased 2-fold in low MHI cows ($P < 0.01$). In summary, a more pronounced inflammatory response, decreased milk yield, and increased NEFA and BHBA were observed in low vs. high MHI cows.

Key Words: inflammation, MHI

P133 Transition Cow Index as a monitor of fresh cow health and performance in Brazilian dairy herds.

D. C. Silva*[1], J. K. Poncheki[1], J. H. Carneiro[1], J. A. Horst[2], K. Nordlund[3], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil,* [2]*Associação Paranaense de Criadores de Bovinos da Raça Holandesa, Curitiba, Paraná, Brazil,* [3]*University of Wisconsin, Madison, WI.*

The objective of this study was to develop and to validate an index to monitor fresh cow health and performance based upon standard milk recording data, known as Transition Cow Index (TCI). Data set from official DHIA Paraná herds, Southern Brazil, had originally 373,458 first monthly test-days (TD) from 2010 to 2019. First-lactation data were excluded and after editions only Holstein data were kept in the final data set, which had 116,264 first TD from 602 herds. Previous cumulative milk yield, DIM in prior lactation (L and Q effects), linear somatic cell score at the last TD of previous lactation, days dry (L and Q effects), lactation number, month of calving, milking frequency and DIM at first TD were the independent variables used in GLIMMIX procedure from SAS to generate an expected milk yield in the first TD after calving. The expected daily production was compared with the production in the first TD after calving, collected between 5 and 45 d, to generate TCI in kg/d. The results from the DHI data indicated that 53% had a positive TCI and 47% a negative TCI and the overall TCI average was −0.0004 kg/d. To validate TCI equation, we had used data from 4 herds which monitored health disorders on 3,326 fresh cows between 2017 and 2019. The GLM procedure from SAS was used to compare the TCI from cows with and without disease. Cows with clinical hypocalcemia (n = 30) had lower ($P < 0.01$) TCI than healthy cows (n = 3,296); −5.18 vs. +1.05 kg/d, respectively. Cows with retained placenta (n = 156) had lower ($P < 0.01$) TCI than healthy cows (n = 3,170); −4.82 vs. +1.25 kg/d. Fresh cows with metritis (n = 318) had lower ($P < 0.01$) TCI than healthy cows (n = 3,008); −1.67 vs. +1.37 kg/d. Animals with displaced abomasum (n = 114) had lower ($P < 0.01$) TCI than healthy cows (n = 3,212); −10.52 vs. +1.33 kg/d. Cows with clinical mastitis (n = 153) in the first 30 DIM had lower ($P < 0.01$) TCI than healthy cows (n = 1,996); −0.80 vs. +1.18 kg/d. Fresh cows with 1 disease (n = 655) had lower ($P < 0.01$) TCI than healthy cows (n = 2,671); −2.75 vs. +2.05 kg/d. Transition Cow Index proved to be an efficient tool for monitoring fresh cow health and performance of dairy herds.

Key Words: health, transition period

P134 Pattern of rumination time and physical activity captured by an ear-attached sensor around the time of clinical diagnosis of metritis and mastitis in dairy cows.

C. Rial*[1], A. L. Laplacette[1], M. M. Perez[1], C. C. Florentino[2], F. Pena-Mosca[2], L. Caixeta[2], and J. O. Giordano[1], [1]*Deparment of Animal Science, Cornell University, Ithaca, NY,* [2]*College of Veterinary Medicine, University of Minnesota, St. Paul, MN.*

The objective of this prospective observational study was to characterize the pattern of rumination time (RT), and physical activity (PA) as measured by an automated health monitoring system based on an ear-attached sensor (SmartBow, Zoetis) immediately before, during, and after clinical diagnosis (CD) of metritis (MET) and mastitis (MAST) in lactating dairy cows. Rumination time and PA represented by active time (AT) data were collected from 540 lactating Holstein cows. Clinical health events were monitored daily until 21 DIM. Data were daily summed from −7 to 7 d relative to CD for cows diagnosed with MET (n = 84), MAST (n = 39), and cows without clinical signs of disease (NCD; n = 417). Data for RT and AT were analyzed using ANOVA with repeated measurements. Fixed effects were group (NCD, MET, MAST), time, and their interaction whereas parity and calving season were offered as covariates. Cow was included as a random effect and was the subject of repeated measurement analysis. Compared with NCD, cows in the MET group had reduced ($P < 0.05$) RT from −7 to 3 d after CD and reduced AT from −7 to 7 d after CD. On the day of CD, RT was 413 ± 13 min/d and AT was 652 ± 17 min/d for the MET group compared with RT of 522 ± 6 min/d and AT of 828 ± 8 min/d for the NCD group. For the comparison of cows in MET and NCD, parity and season of calving affected ($P < 0.05$) RT and AT. Compared with NCD, cows in the MAST group had reduced ($P < 0.05$) RT and AT from −2 to 0 d after CD. On the day of CD, RT was 250 ± 18 min/d and AT was 403 ± 24 min/d for the MAST group compared with RT of 536 ± 6 min/d and AT of 839 ± 8 min/d for the NCD group. For the comparison of cows in MAST and NCD, parity and season of calving affected ($P < 0.05$) RT and tended ($P = 0.06$) to affect AT. We conclude that rumination time and physical activity monitoring data captured by an ear-attached sensor from an automated health monitoring system might be used to identify cows with metritis and mastitis.

Key Words: dairy cow, health, rumination

P135 Association of prepartum lying time with colostrum immunoglobulins and cortisol concentrations at calving and milk yield in Holstein dairy cows.

B. T. Menichetti*[1], A. Garcia-Guerra[2], J. Lakritz[4], W. P. Weiss[3], J. S. Velez[5], B. Bothe[5], D. Merchan[6], and G. M. Schuenemann[1], [1]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [3]*Department of Animal Sciences, The Ohio State University, Wooster, OH,* [4]*Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH,* [5]*Aurora Organic Farms, Boulder, CO,* [6]*ABS Global Inc., DeForest WI.*

The objective was to assess the association of the coefficient of variation of lying time (CV of LT) within 7 d before parturition (dpp) with colostrum immunoglobulin G (IgG), cortisol concentrations at calving and milk yield at first DHIA test in Holstein dairy cows. A prospective cohort study was conducted in 292 Holstein multiparous dairy cows from one large dairy herd in Colorado. All prepartum cows were housed in deep bedded-pack barns with access to outside patio. At 35 ± 3 dpp, a group of 43 to 53 pregnant cows were fitted with an electronic data logger (IceQube, IceRobotics, Edinburgh, UK) to assess their LT. Once calving date was known, the within cow CV of LT was computed by dividing the standard deviation of LT over the mean for the last 7 dpp and reposted as an absolute ratio. Cows were then classified into 1 of 3 groups based on their CV of LT using the 50th (0.13) and 95th (0.24) percentile: < 0.13 (n = 154), from 0.13 to 0.24 (n = 125), and ≥ 0.25 (n = 13). Colostrum samples were collected within 1 h following parturition. Data were analyzed using MIXED or CORR procedures of SAS (SAS Institute Inc., Cary, NC). Mean LT within 7dpp were significantly different among cows with a CV of LT of < 0.13 (720 min/d), 0.13 to 0.24 (657 min/d), and ≥ 0.25 (555 min/d; $P < 0.001$). The CV of LT within 7 dpp was significantly associated ($P < 0.05$) with colostrum IgG concentrations at calving and milk yield at first DHIA test but was not associated with colostral cortisol at calving ($P = 0.26$). Cows with a CV of LT of ≥ 0.25 (116 g/L) within 7 dpp had 43 g/L less colostrum IgG concentrations at calving compared with cows having a CV of LT of < 0.13 (159 g/L) or from 0.13 to 0.24 (157 g/L; $P = 0.006$). Cows having a CV of LT ≥ 0.25 (31.9 kg/d) within 7 dpp had 5.8 kg/d less milk yield compared with cows having a CV of LT < 0.13 (37.7 kg/d) or from 0.13 to 0.24 (37.6 kg/d; $P = 0.03$). These findings suggest that prepartum pregnant cows with inconsistent LT within the last 7 dpp, defined as CV of LT ≥ 0.25, was detrimental for colostrum quality at calving and milk yield in early lactation.

Key Words: lying time consistency, immunoglobulins, dairy cattle

P136 Prophylactic effect of dietary direct-fed *Bacillus subtilis* C-3102 on incidence of mastitis in dairy cows.

H. Aso[1,2], M. Urakawa[1,2], T. Zhuang[1,2], H. Sato[3], N. Takada[3], N. Ashida[4], T. Imabayashi[4], N. Otomo[4], K. Watanabe[1,2], T. Nochi[1,2], and T. Marubashi*[5], [1]*Cellular Biology Laboratory, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan,* [2]*CFAI, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan,* [3]*Miyagi Prefectural Livestock Experiment Station, Osaki, Miyagi, Japan,* [4]*Asahi Biocycle Co. Ltd., Shibuya-ku, Tokyo, Japan,* [5]*Calpis America Inc., Peachtree City, GA.*

The objective was to study the effects of dietary *Bacillus subtilis* C-3102 (Calsporin; BS) on the incidence of mastitis and on hematological and immunological changes. Holstein dairy cows (control group: 10 head, calving number = 4.3 ± 2.0; BS-fed group: 6 head, calving number = 4.5 ± 1.0) having history of mastitis in their previous lactations were fed a ration comprised of timothy hay and a commercial concentrate without or with BS (3×10^9 cfu/head) 2 times daily for 11 mo (from 1 mo before parturition). The cows with onset of mastitis were diagnosed by the on-site veterinarian's observation and by the milk quality including its somatic cell count. Plasma concentrations of glucose, urea nitrogen, NEFA, total cholesterol, cortisol, and TBARS were measured monthly. Flow cytometry determined frequencies of peripheral granulocytes, monocytes, B cells, T cell subsets, and dendritic cells (DC). Data were analyzed by nonparametric Mann-Whitney U-test using a statistical software, jSTAT. The number of cows with mastitis onset and the days of medical care required were significantly lower in BS-fed group than those in control group ($P < 0.05$). The average milk somatic cell count (SCC) in BS-fed group was 4.2×10^4 cells/mL which was significantly lower compared with 5.7×10^6 cells/mL in control group ($P < 0.02$). After parturition, the plasma total cholesterol rapidly recovered to the original level in BS-fed group, but not in control group. In mid- and late lactation, plasma urea nitrogen, cortisol, and TBARS levels were lower ($P < 0.05$) in BS-fed group than in control group. The BS-feeding increased ($P < 0.05$) the proportion of DC, surface marker expression of CD80, and production of cytokines IL-12a and IL-6 in peripheral blood. Results indicate that a ration containing direct-fed BS may reduce the incidence of mastitis by the modulation of immune metabolic functions in dairy cows, with 99.26% reduction in milk SCC that would contribute to increased wholesome milk production in the dairy industry.

Key Words: *Bacillus subtilis* C-3102, direct-fed microbial, mastitis

P137 The effects of intramammary ceftiofur hydrochloride and cephapirin benzathine antibiotic treatment at dry-off on peripheral blood mononuclear cell mitochondrial enzyme activity in Holstein cows.

T. A. Batchelder*, A. M. Niesen, and H. A. Rossow, *University of California, Davis, Davis, CA.*

Cephalosporin antibiotics are commonly used at dry-off to treat and prevent intramammary infections. Research in murine and other mammalian cell types has shown that cephalosporin antibiotics can negatively affect mitochondrial respiratory chain activity, which then impacts cellular function. The objective of this study was to evaluate the effects of intramammary cephalosporin (Cefitiofur Hydrochloride; CEFT, and cephapirin benzathine; CEP) antibiotic treatment administered at dry-off on peripheral blood mononuclear cell (PBMC) mitochondrial enzyme activity at 7 d post dry. Thirty-seven Holstein cows from a commercial dairy were enrolled at the time of dry-off and assigned to 1 of 4 treatments: 1) Low SCC control (LCON), 2) High SCC Control (HCON), 3) High SCC CEP (CB), (ToMorrow; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), and 4) High SCC CEFT (CH), (Spectramast DC; Zoetis Inc., Kalamazoo, MI). Control treatments received no antibiotics. Low and high SCC cows were defined as <100,000 cells/mL (low), and >200,000 cells/mL (high) at the day of dry-off. Whole blood and milk samples were collected at the time of dry-off and 7 d post dry. Mitochondria were isolated from the PBMC fraction of whole blood. Enzyme activities of citrate synthase (CS), complex I, complex IV, and ATP synthase were performed on crude mitochondrial extracts using kits from Abcam (Cambridge, MA). Data were analyzed using linear mixed models with repeated measures in R (Version 4.0.2). Least squares means CS activity deceased by time within treatment: LCON, 0.588 to 0.325 mOD / min per μg of crude mitochondrial protein ± 0.09 SEM; and CB, 0.614 to 0.299 mOD / min per μg of crude mitochondrial protein ± 0.11 SEM Since changes in CS activity at the end of lactation (dry-off) and at 7 d into the dry period were similar between treatments these data indicate that intramammary infusion of CEFT and CEP administered at dry-off do not affect PBMC mitochondrial enzyme activity. The decrease in CS enzyme activity were similar between treatments and may be associated with the involution of the mammary gland, or indicative of energetic changes with the cessation of lactation.

Key Words: mitochondria, enzyme, cephalosporin

P138 Randomized clinical trial evaluating effects of an alternative dosing schedule for pegbovigrastim on mammary gland health and milk production.

J. L. de Campos*[1], J. Strickland[2], J. Gandy[2], C. Robison[1], L. M. Sordillo[2], and P. L. Ruegg[2], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,*

[2]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI.*

The objective of this randomized clinical trial was to evaluate effects of an alternative dosing schedule for pegbovigrastim (PEG; Imrestor, Elanco Animal Health) on mammary gland health and milk production. Pregnant late lactation cows were randomly assigned to receive treatment with 15mg of PEG ($n = 10$ cows) or a sham injection with saline ($n = 10$) administered 7d before dry-off and on the day of dry-off (DRY). No antimicrobial therapy was administered at DRY. Quarter (QTR) milk samples were collected for bacteriological culture and somatic cell count (SCC) at 8 periods (7 and 2d before DRY, DRY, 7 and 14d after DRY, and 5, 10, and 14d after calving). Daily milk yield in the subsequent lactation were evaluated on 10, 14, 30, 60, and 120 DIM. Chi-squared analysis was used to assess the effect of treatment on incidence of intramammary infection (IMI) and multivariate modeling was used to determine effects of treatment on SCC and milk yield. The incidence of IMI was greater for QTR of cows in the control group as compared with QTR of cows that received treatment ($X^2 = 6.3$; $P = 0.006$). Compared with cows receiving treatment, the odds of IMI were 7.5 times greater (95% CI:1.5, 36.7) for QTR in the control group. While the overall effect of treatment on SCC was not significant ($P = 0.23$), significant effects were found for period and the interaction of treatment by period ($P < 0.01$). As expected based on the mode of action and administration periods, greater Log10 SCC were observed for treated cows 2d before DRY (4.09 control; 4.68 PEG) and at DRY (4.12 control; 4.62 PEG). Significant effects of sampling period ($P < 0.001$) and an interaction of treatment by sampling period ($P = 0.001$) were observed for milk yield, and there was an overall tendency for treated cows to have greater milk yield in the subsequent lactation ($P = 0.09$). Cows in the in the control group produced 45, 48, 52, 52 and 42 kg/cow/d at each sampling period. In contrast, cows in the treatment group produced 48, 51, 62, 58 and 50 kg/cow/d. Cows treated with PEG using an alternative dosing schedule had reduced incidence of IMI during the dry period and increased milk yield in the subsequent lactation.

Key Words: dairy, pegbovigrastim, mastitis

P139 Intramammary lipopolysaccharide infusion alters the fatty acid profile in blood triacylglycerides and phospholipids of lactating dairy cows. C. Lalonde*[1], J. Kraft[1], R. K. Choudhary[1], D. E. Bourne[1], E. M. Shangraw[2], T. B. McFadden[2], and F.-Q. Zhao[1], [1]*University of Vermont, Burlington, VT,* [2]*University of Missouri, Columbia, MO.*

Mastitis depresses milk yield and reduces milk quality in infected glands, but also has systemic effects that may impair function of noninfected mammary glands. There is much to learn about the impact of these systemic effects on lactation performance and health of the cow. We hypothesized that intramammary endotoxin infusion would affect the blood lipid profile of cows. This study aimed to determine the changes in concentration of fatty acids in blood of dairy cows that receive intramammary infusion of lipopolysaccharide (LPS). Ten multiparous Holstein cows were blocked into pairs by milk yield and somatic cell count. One cow of each pair was assigned to LPS treatment (T) or control (C) group ($n = 5$). In T cows, one udder half was infused with 50 μg of *Escherichia coli* LPS (serotype O55:B5) in 10 mL saline into both glands and the contralateral glands received 10 mL saline. C cows received an infusion of 10 mL saline into both glands of one udder half. Blood was collected from the coccygeal at −1h, 3, 6, 12 and 24 h postinfusion. Plasma lipid fractions (cholesterol, free fatty acids, phospholipids and triacylglycerides) were separated by solid-phase extraction, and each fraction was analyzed by gas-liquid chromatography. Statistical significance was determined using a mixed model with Tukey's test. Principal component analysis revealed that the phospholipid fatty acid profiles of T and C cows clustered together at −1 h and 3 h but diverged at 6 h, 12 h and 24 h postinfusion. Fatty acids in triacylglycerides of T and C cows diverged at 6 h and 12 h. In T vs C cows, there was an increase in concentrations of 18:0 at 6, 12 and 24 h ($P < 0.0004$) in triacylglycerides and at 12 h ($P < 0.0001$) in phospholipids, a decrease of 18:2 9*c*,12*c* at 6 h ($P = 0.0384$) and 12 h ($P = 0.0004$) in triacylglyceride and at 6, 12 and 24 h ($P < 0.0001$) in phospholipids, and an increase of 22:0 at 12 and 24 h ($P = 0.0002$) in phospholipids. These findings showed that intramammary LPS results in changes in the fatty acid profiles of plasma triacylglycerides and phospholipids, indicating a systemic effect on lipid metabolism.

Key Words: fatty acid, mastitis, systemic effect

P140 Changes in phospholipids in milk produced by dairy cows with clinical or subclinical mastitis in the first 21 days postpartum. H. K. Peterson*[1], J. E. Williams[1], S. P. Couvillion[2], T. Kelley[1], C. D. Nicora[2], K. E. Mostoller[2], B. M. Webb-Robertson[2], E. S. Nakayasu[2], T. O. Metz[2], E. L. Peterson[3], M. K. McGuire[4], and M. A. McGuire[1], [1]*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Moscow, ID,* [2]*Pacific Northwest National Laboratory, Richland, WA,* [3]*Peterson Dairy, Filer, ID,* [4]*Margaret Ritchie School of Family and Consumer Sciences, University of Idaho, Moscow, ID.*

Mastitis is inflammation of the mammary gland frequently caused by bacteria and triggering an immune response that may impact different classes of milk phospholipids (PL), including phosphatidylcholines (PC) and phosphatidylethanolamines (PE). PC and PE are the most abundant PL of animal cell membranes, and PE is the primary PL in bacterial cell membranes. As such, characterizing levels of PC and PE in milk may be useful biological markers of mastitis. The objective of this study was to quantify and compare PC and PE in milk produced by healthy cows and those with clinical or subclinical mastitis. Quarter milk samples ($n = 112$) were collected from clinical (SCC >400,000 cells/mL and abnormalities), subclinical (SCC >200,000 cells/mL and no abnormalities), and healthy animals (SCC <200,000 cells/mL) on 4 Idaho dairies in the first 21 d postpartum. We performed untargeted lipidomics using liquid chromatography coupled with tandem mass spectrometry and data were analyzed using a generalized linear mixed-effects model with health group (clinical, subclinical, healthy) and day postpartum (0–1 d, ≥5 d) as independent variables and cow nested within dairy as a random variable. Changes were considered significant with P-value <0.0001. We found no differences in PC or PE levels in milk produced by subclinical vs healthy quarters in either time postpartum; and no differences in PC or PE levels in milk produced by clinical vs healthy quarters at 0–1 d. However, at ≥5 d postpartum, levels of PE (18:1/19:0), PE (17:0/18:0), and PE (18:1/0:0) were lower and levels of PC (18:0/22:5), PC (18:1/20:4); PC (16:0/22:5), PC (16:0/O-16:0), PC (18:0/18:2); PC (18:1/18:1), and PC (18:0/20:4) were higher in clinical vs healthy quarters. Higher levels of PC in milk of cows with clinical mastitis may be due to immune cell migration into the udder. Lower levels of PE in milk of cows with clinical mastitis may be related to changes in bacterial community membership associated with mastitis. These results could be useful for monitoring udder health. Funding provided by the National Institutes of Health grant number 1R01HD092297–01A1.

Key Words: phospholipids, mastitis, milk

P141 Retinoic acid affects barrier integrity in bovine mammary endothelial cells. J. M. Strickland* and L. M. Sordillo, *Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

The barrier between systemic circulation and mammary gland tissue is composed of monolayer of endothelial cells. During coliform mastitis, however, endothelial cell barrier integrity is compromised during the acute inflammatory response. All-*trans*-retinoic acid (ATRA), the active form of vitamin A, has many important effects on inflammation such as repressing the damaging effects of oxidative stress in human and murine cell models. Previous studies showed that ATRA increased dramatically in the systemic circulation in dairy cows experimentally infected with coliform mastitis. However, it is unknown whether ATRA is cytoprotective or contributes to the pathogenesis of coliform mastitis. The objective of this research was to identify the effects of ATRA on bovine mammary endothelial cells (BMEC) viability, oxidative stress, and barrier function in vitro. An in vitro BMEC model of oxidative stress was used to evaluate barrier integrity. Cells were treated with 10 μM all-*trans*-retinoic acid or the reactive oxygen producing compound, 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH). Cell viability and reactive oxygen species were evaluated using fluorometric assays. Barrier integrity was quantified by measuring resistance using endothelial cell-substrate impedance sensing (ECIS). Statistical analysis was performed with one- or 2-way ANOVA with Graphpad Prism. The BMEC viability was unaffected by treatment with ATRA at 24 h ($P > 0.05$). ATRA reduced ROS production 75% when treated concurrently with AAPH (4.4- to 1.1-fold change compared with control, $P < 0.0001$). On ECIS analysis, ATRA+AAPH treated cells had increased resistance compared with AAPH treated BMEC ($P < 0.05$). However, ATRA treated cells had decreased resistance compared with controls ($P < 0.05$). Based on this data, ATRA has cytoprotective properties for BMEC in the face of oxidative stress. The precise method by which ATRA decreases barrier integrity in BMEC will need to be further investigated. This research indicates that ATRA may be protective against the pathologic effects of coliform mastitis.

Key Words: retinoic acid, mastitis

P142 Comparison of cow-side tests to predict individual cow quarter SCC. L. A. Jacobsen*, A. M. Niesen, and H. A. Rossow, *University of California, Davis, Davis, CA.*

Intramammary infections, such as mastitis, are an extensive problem in the dairy industry as this disease can increase treatment and labor costs, decrease milk production and affect milk quality. Cow-side tests that predict SCC could be used to make more informed dry cow therapy decisions in individual quarters. However, there are very few studies that have examined the performance of these tests using the same individual cow quarter samples under the same conditions across multiple herds. This study compares the ability of the California Mastitis Test (Dairy Research Product, Inc., IN), the DeLaval Cell Counter (DCC; DeLaval, Graiguecullen, Carlow), the RT-10 iPhone meter (Dairy Quality Inc., Newmarket, Canada), a pH meter (Hanna Instruments, RI), an electrical conductivity meter (OHAUS, NJ), a dual laser infrared temperature thermometer (Klein Tools, IL), and the Porta Check Quick Test (PC; PortaCheck, NJ) to predict SCC in individual quarters compared with a gold standard, the Chem-Spec 150 (DHIA; Bentley Instruments, MN) at Tulare County Dairy Herd Improvement Association. This study enrolled 80 cows from 2 dairy herds (40 cows per herd). Milk samples were collected on all 4 quarters of the cows at the time of dry-off. The SCC were measured in well mixed milk samples using each cow-side test and by DHIA within 4 h of being collected. The SCC predicted by meters were regressed on DHIA and dairy using PROC GLM (SAS Institute, 2021, v. 9.4). Dairy was not significant. The RT-10 iPhone meter and DCC predicted DHIA quarter SCC the best ($R^2 = 0.93$, $R^2 = 0.73$, respectively), followed by PC ($R^2 = 0.67$). These results indicate that the RT-10 iPhone meter, DCC meter, and PC have the ability to predict individual quarter SCC and could be used to identify individual mammary quarters to treat, potentially reducing antibiotic use at dry-off.

Key Words: mastitis, RT-10 iPhone, DeLaval Cell Counter

P143 Interactions of candidate gene expression and in vitro infection with *Streptococcus uberis* in bovine mammary epithelial cells. H. Malcomson*, E. Shepherd, and G. Pighetti, *University of Tennessee, Department of Animal Science, Knoxville TN.*

Streptococcus uberis can internalize into mammary epithelial cells (MECs) and evade intracellular degradation to cause clinical and chronic mastitis. Our objective was to investigate interactions between *Strep. uberis* and MEC function relative to RAB4A (Ras-related protein RAB-4a), HECTD4 (HECTD Domain E3 Ubiquitin Protein Ligase 4), and PSAP (Prosaposin coding gene) whose SNP were associated with in vivo infection phenotypes and are involved with intracellular trafficking and degradation. A common MEC-line (MACT) was inoculated with *Strep. uberis* strains isolated from a chronic (UT888 cfu = 2.15×10^7) or acute (UT366 cfu = 1.19×10^8) mastitis case. Gene expression, MACT viability and intracellular survival of bacteria at 0, 3, 9, and 27 h postinoculation were evaluated with a mixed model ANOVA using SAS 9.4 (Cary, NC). The model included fixed effects of infection status, time, and their interactions, random effect of time, and covariate of *Strep. uberis* dose. Cell viability was over 80% (SD = 3.1). Internalization of UT888 in MACT (n = 5) was lower than UT366 (n = 1). Both peaked at 3 h, with ~95% and 100% reductions at 9 h and 27 h. Both *HECTD4* and *RAB4A* gene expression hint at an interaction between infection status and time ($P = 0.12$ and 0.18, respectively). HECTD4 dropped to 0.3 - 0.7 at 3 and 9 h postinfection relative to 0 h regardless of infection status. Only the infected cells rebounded to pre-infection levels by 27 h. RAB4a stayed stagnant in control cells until increasing to 1.6 at 27 h. Infected cells *RAB4A* gene expression peaked at 4.2 in 9 h, and dropped to 2.0 at 27 h. Only *PSAP* gene expression increased consistently within infected cells ($P = 0.01$) where it slightly increased at 3 h, doubled at 9 h, and doubled again at 27 h to 5.7 relative to time 0. Control cells only increased to a max of 1.45. Additional replicate experiments are required, but early experiments suggest changes in candidate gene expression after infection and suggest MECs modify intracellular trafficking and degradation pathways in response to *Strep. uberis*.

Key Words: mastitis, gene expression, *Streptococcus uberis*

P144 Risk factors associated with intramammary infections in primiparous dairy cows in organic herds. L. Fernandes*[1], M. L. Celestino[1], P. R. Menta[1], T. H. Silva[1,2], D. Paiva[1], T. L. Ribeiro[1], L. S. Caixeta[3], N. R. Noyes[3], and V. S. Machado[1], [1]*Texas Tech University, Lubbock, TX,* [2]*School of Animal Science and Food Engineering, University of Sao Paulo, Pirassununga, Sao Paulo, Brazil,* [3]*University of Minnesota, St. Paul, MN.*

Our objective was to identify risk factors associated with intramammary infections (IMI) during the first wk of lactation in primiparous dairy cows in organic herds and evaluate their association with SCC linear scores (LS) and milk yield in early lactation. A total of 240 nulliparous cows from 2 organic dairy herds located in NM and TX were enrolled 6 wks before expected day of parturition. Risk factors considered were presence of udder edema (UE), teat edema, milk leakage (ML), and poor udder hygiene at 6, 4, 2, 1 wks before calving and within the first wk of lactation, BCS at enrollment and within the first wk of lactation, age in days at calving, gestation length, dystocia, stillbirth, and if cow gave birth to a female singleton calf. A composite milk sample was collected within the first wk of lactation for microbiological analysis, and cows with a bacterial growth in the milk sample were considered as having IMI. A total of 29 cows were excluded from the analysis due to contaminated samples or loss to follow-up. The association between risk factors and IMI was assessed through a logistic regression model using SAS. Variables retained in the logistic regression model were offered to 2 general linear models accounting for repeated measures to evaluate the association of risk factors with milk yield and LS. Milk leakage at first wk postpartum increased the odds of IMI at 3.42 times ($P < 0.01$), while UE at one wk before calving tended to increase the odds of IMI (OR = 1.86, $P = 0.09$). Cows with prepartum BCS ≥3.75 were at 3.12 greater odds of IMI than cows with BCS = 3.25 – 3.5 ($P < 0.01$), but the odds of IMI were not different between cows with BCS ≤3.0 and BCS = 3.25 – 3.5 ($P = 0.15$). Cows with ML during the first week of lactation had increased LS ($P = 0.01$) and lower milk production in the second month of lactation ($P = 0.05$), while UE diagnosed at 1 wk prepartum tended to be associated with greater LS ($P = 0.09$). In conclusion, ML within the first wk of lactation and high BCS 6 wks before calving were associated with IMI, with ML also being associated with greater LS and lower milk production within the first 2 mo postpartum.

Key Words: mastitis, organic, risk factors

P145 Characterization of clinical mastitis on Southern Brazilian dairy herds by severity score. S. T. Guerra*[1], M. Poczynek[2], G. C. Aguiar[2], L. L. Damasceno[1], C. D. Neufeldt[1], L. Goltz[1], H. P. Janssen[1], E. M. Ribas[1], G. F. M. Leão[1], and R. Almeida[2], [1]*Negócios Leite, Castrolanda Cooperativa Agroindustrial, Castro, Paraná, Brazil,* [2]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil.*

The goal of this study was to evaluate the distribution of mastitis-causing agents among the levels of clinical mastitis severity. Data from 387 cases of mastitis evaluated in a on-farm culture system based on colorimetric qualitative method were used. Twelve herds in Southern Brazil were monitored from August 2020 to February 2021. Mastitis cases were classified in 3 categories: mild when only the milk was abnormal (n = 133), moderate when abnormal milk was accompanied by swelling of mammary gland (n = 216), and severe when cow exhibited systemic signs of illness (n = 38). Data were evaluated using the FREQ, GLM, and GLIMMIX procedures of SAS (v.9.4). The occurrence of agents differed among mastitis levels. Gram-negative bacteria were most frequent in severe cases, while the gram-positive group were most frequent in the mild and moderate ones. *Escherichia coli*, *Klebsiella* spp., and the sum of *Streptococcus agalactiae* and *Streptococcus dysgalactiae* were most frequent in severe cases. In the moderate cases, *Staphylococcus non-aureus* was the most important agent, and the major agents in the mild cases were *Streptococcus uberis* and non-*aureus Staphylococcus*.

Table 1. Distribution of etiology of milk samples from clinical mastitis cases occurring in cows on Southern Brazilian dairy herds by severity score

Microbiological diagnosis	Mild		Moderate		Severe	
	n	%	n	%	n	%
Gram-positive						
Enterococcus spp.	1	0.8	3	1.4	0	0.0
Lactococcus lactis	1	0.8	2	0.9	0	0.0
Staphylococcus aureus	3	2.5	13	6.1	2	5.7
Staphylococcus non-*aureus*	32	26.9^a	41	19.4^b	5	14.2^b
Streptococcus uberis	35	29.5^a	37	17.7^b	2	5.8^c
S. agalactiae/ dysgalactiae	13	10.9^c	39	18.5^a	5	14.2^b
Other gram-positive	3	2.5^b	15	7.1^a	3	8.7^a
Gram-negative						
Escherichia coli	18	15.1^c	39	18.6^b	9	25.7^a
Klebsiella spp.	8	6.8^b	9	4.2^b	5	14.2^a
Pseudomonas	0	0.0	1	0.6	0	0.0
Serratia	1	0.8	0	0.0	1	2.9
Other gram-negative	2	1.7	4	1.8	0	0.0
Yeast						
Prototheca spp.	2	1.7	8	3.7	3	8.6
Total	119	100	211	100	35	100

The sum of agents was higher than the number of positive cultures due to the possibility of growth of more than one agent per culture.

Key Words: bovine mastitis, pathological agents

P146 Investigating the use of a 7% iodine tincture based umbilical dip as a preventive management practice for umbilical infections in neonatal calves. M. Van Camp*[1], C. Winder[1], D. Gomez[2], T. Duffield[1], and D. Renaud[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Clinical Studies, University of Guelph, Guelph, ON, Canada.*

The objective of this randomized clinical trial was to evaluate the efficacy of 7% iodine tincture based umbilical dip as a preventive method to reduce the incidence of umbilical infections in dairy calves, as compared with no treatment. Five dairy farms in southern Ontario were visited biweekly from September 2020 through March 2021where Holstein calves were randomly assigned at birth to receive either a 7% iodine tincture based umbilical dip (n = 128) or no treatment (n = 126). The umbilical dip was administered a single time by the producer shortly after birth. During researchers' first visit with each newborn calf, record of treatment group, birth difficulty, gestation length, colostrum administration, time of birth, calving pen cleanliness, and the dam ID were collected via a calving form. The calf's body weight, health score including an umbilical assessment, and a blood sample (serum total protein) were also captured at the first visit. Calves were then health scored bi-weekly using a standardized scoring system which evaluated fecal consistency score and respiratory score. Umbilical infections were defined as enlarged navel with pain, heat or malodorous discharge. Serum total protein and birth weight did not differ significantly between treatment groups. In the umbilical dip group, 25 (19.5%) calves developed umbilical infections and 27 (21.4%) developed umbilical infections in the control group. A mixed logistic model showed no effect of treatment group ($P = 0.70$), however calves born in dirtier calving pens had a tendency for increased odds of infection (OR = 2.5, 95% CI: 1.0 to 6.3, $P = 0.06$) compared with those born in cleaner pens. Farm had a significant impact on the risk of developing an umbilical infection (ICC = 0.09). These findings suggest that administering a single dose of 7% iodine tincture dip to the umbilicus around the time of birth may not be sufficient at preventing umbilical infections. Conversely, farm level management factors including calving pen cleanliness appear to be more influential on risk of disease.

Key Words: navel, morbidity, omphalitis

P147 Supplementing preweaned dairy calves with smectite improves serum IgG concentration, and reduces mortality and antimicrobial treatments. A. Correa*[1], A. Valldecabres[1], M. Abreu[1,2], and N. Silva-Del-Río[1], [1]*Veterinary Medicine Teaching and Research Centre, Tulare, CA,* [2]*Universidade Federal de Viçosa, Department of Animal Science, Viçosa, MG, Brazil.*

Our aim was to evaluate the health effects of supplementing preweaned dairy calves with smectite. A total of 200 newborn calves (Holstein, n = 88; Jersey, n = 112) were sequentially assigned to control (CON; force-fed 50 mL of water; n = 100) or smectite [SME; force-fed 2.5 g of smectite (calcium montmorillonite bentonite clay, Redmond Inc., UT) and 50 mL of water; n = 100] at 1 d of age. Calves received treatments 2 h before the afternoon milk feeding for the first 10 d of age. Health evaluations (fecal consistency, signs of respiratory disease, and milk refusals) were performed by researchers daily from 1 to 20 d. Mortality and antimicrobial treatments administered by farm personnel were recorded until 20 d of age. Jugular blood samples (SME, n = 21; CON, n = 23) were collected at 1, 4, 8, 12, and 16 d for IgG determination and at 1 and 8 d for trace serum minerals determination. Statistical analyses were performed in SAS. Risk of health events, antimicrobial treatments, and mortality were analyzed using log binomial regression. Linear regression models were used to evaluate serum IgG and trace serum minerals concentration. Compared with CON, SME calves tended to have a 63% lower risk of mortality ($P = 0.09$) and a 45% lower risk for antimicrobial treatments ($P = 0.04$). Health events recorded by researchers were similar for SME and CON calves. Compared with CON, SME calves had higher serum IgG at 4 d (23.4 vs. 19.3 g/L; $P = 0.07$) and at 8 d (22.1 vs. 15.9 g/L; $P = 0.01$). However, after treatments ended, IgG were similar for SME and CON at 12 and 16 d Serum concentrations of Ca ($P = 0.03$), Mg ($P = 0.02$), Na ($P = 0.01$) were higher for SME compared with CON calves; P ($P = 0.06$), and Zn ($P = 0.08$) were higher for SME compared with CON calves. The positive effects of SME on mortality, antimicrobial treatments, and serum IgG of preweaned dairy calves warrant future research.

Key Words: preweaned calves, smectite, antimicrobial

P148 The effect of fish and canola oil on body weight, dry matter intake, serum haptoglobin and protectin on Holstein dairy calves. P. Melendez*[1,2], C. Roeschmann[3], A. Baudo[1], S. Tao[1], J. Bernard[1], P. Pinedo[4], F. Farcey[1], A. Kalantari[1], M. Coarsey[1], Y.-Y. Mosley[1], and H. Naikare[1], [1]*University of Georgia, Tifton, GA,* [2]*Texas Tech University, Amarillo, TX,* [3]*University of Chile, Santiago, Chile,* [4]*Colorado State University, Fort Collins, CO.*

The aim was to assess the effect of a commercial fish oil product as a source of N-3 fatty acids, compared with canola oil on body weight (BW), dry matter intake (DMI), serum haptoglobin and protectin CD59 in Holstein calves. The study was conducted at the Dairy Research Center, University of Georgia Tifton Campus. Between September and November 2019, 30 calves were randomly assigned by sex at birth to 2 groups. Calves were fed a milk replacer (26% CP, 20% fat, 12.5% solution) at rate of 2 L AM and 2 L PM during wk 1. Then, 3 L AM and 3 L PM between wk 2 and 7, and 3 L AM during wk 8. Starter and water were offered from d 3 until weaning. A group received 30 mL of canola oil 98% pure (CAN) and another received 60 g of a fish oil-based product providing 30 g of fish oil (FO), daily, at the AM feeding until weaning. Products were mixed with the milk solution in the feeding bucket. At 7, 14, 21, 28, 35, 42, 49 and 56 d of age a blood sample, and BW were taken. DMI was assessed daily (MR + Starter). In serum sample, haptoglobin, as an indicator of an inflammation process, and protectin as an indicator of inflammation resolution was measured through ELISA commercial kits. BW, DMI, haptoglobin and protectin were analyzed by ANOVA for repeated measures. There was higher haptoglobin concentration in the CAN than the FO at d 7 ($P \leq 0.1$), and 14 ($P \leq 0.05$), but lower at d 49 ($P \leq 0.1$; Table 1). There was higher protectin concentration in the FO than the CAN at d 14 ($P \leq 0.1$), but lower at d 28 ($P \leq 0.1$), 35 ($P \leq 0.05$), and 49 ($P \leq 0.05$). DMI was higher at d 42, 49, and 56 for FO than CAN ($P \leq 0.05$). There was a trend for higher weaning weight in FO than CAN (65.4 vs 61.9 kg). It is concluded that FO improved slightly DMI and weaning weight of dairy calves, perhaps due to better health and immunological status.

Table 1.

	Haptoglobin (ng/mL)		Protectin CD59 (ng/mL)		BW (kg)		DMI (g)	
Day	CAN	FO	CAN	FO	CAN	FO	CAN	FO
1	—	—	—	—	36.1	36.5	—	—
7	622.4	465.4**	49.1	50.1	39.3	39.4	359.8	376.5
14	410.5	212.8*	37.3	44.8**	38.7	40.1	362.9	369.6
21	365.0	357.6	44.0	41.2	40.9	41.4	439.0	426.0
28	295.7	314.3	48.8	40.8**	43.7	45.5	490.6	555.8
35	155.4	187.0	50.3	40.3*	48.9	50.2	738.7	791.4
42	203.5	159.9	41.5	39.4	53.4	55.5	971.9	1,072.0*
49	520.6	735.4**	45.5	33.0*	56.4	59.1	1,354.9	1,481.6*
56	53.8	91.7	41.7	39.9	61.9	65.4**	2,005.3	2,130.3*

LSM are reported.

*$P \leq 0.05$, **$P \leq 0.1$: Differences within parameter and day between treatment groups.

Key Words: n-3 fatty acids, calves, diseases

P149 Evaluation of a probiotic program on prevention of diarrhea in organic-certified Holstein heifer calves. A. Velasquez-Munoz*[1], N. Meza-Correa[2], D. Manriquez[1], and P. Pinedo[1], [1]*Colorado State University, Fort Collins, CO,* [2]*Universidad Tecnologica de Pereira, Manizales, Colombia.*

The objective was to evaluate the effect of a 2-steps probiotic program (Establish Calfbel, Perdue AgriBusiness LLC) on diarrhea incidence in preweaned Holstein heifer calves. A randomized clinical trial was completed from July to October 2020 in one calf rearing facility in CO, USA. Calves were housed in pairs sharing 2 polyethylene hutches and a sand bedded front yard (4.50 m^2), enclosed by a galvanized welded wire fence. A total of 116 pairs of calves were enrolled at birth (control [CTR] = 58; treatment [TRT] = 58) and followed until weaning (65 ± 1 d). Treatment consisted of 2 formulations of a bacterial-based probiotic to be added into colostrum (F1) and milk (F2). Treatment calves received 2 g of F1 added into each colostrum feeding and 1 g of F2 added into milk at the morning feeding 3 times per week up to weaning. Calves were weighted at birth and moved to the rearing facility, housed in pairs sharing the same treatment. Serum total protein (STP) was measured at age 3 ± 1d and a complete health assessment was performed 3 times per week. Calves were weighted at weaning and temperature-humidity index

(THI) was calculated for the whole study period. Statistical analyses were performed in SAS. Time to event analysis was used to evaluate time to the first diarrhea event and recovery time. Poisson regression was used to evaluate the total number of diarrhea events. Logistic regression was used to calculate the odds of culling and ANOVA was used for comparison of continuous outcomes (STP, average daily gain [ADG]). Calves were continuously exposed to heat stress in the day hours (July to September, mean (±SE) THI = 75 ± 0.44 units). No treatment difference was observed in STP ($P = 0.06$). At least 1 calf in each pair presented diarrhea during the study period and no differences were determined in the median time to a first diarrhea event (CTR [10d], TRT [11d]; $P = 0.78$), or in the median time to recovery from diarrhea (CTR [5d], TRT [7d]; $P = 0.1$). Likewise, treatment was not associated with the number of diarrhea events ($P = 0.28$), ADG ($P = 0.67$), or the odds of culling ($P = 0.73$). In conclusion, the probiotic program was tested in challenging environmental conditions and it did not impact the incidence of diarrhea in preweaned heifer calves.

Key Words: calf, diarrhea, probiotic

P150 Histological and ultrasound analysis of Holstein calf mammary gland development.

A. Vang*, T. Bresolin, W. Frizzarini, J. Campolina, G. Rosa, L. Hernandea, and J. Dorea, *University of Wisconsin–Madison, Madison, WI.*

Although the bovine mammary gland is an important focus area in bovine research, not many studies focus on development in the period between weaning and the first gestation. Milk production is highly dependent on the development of the mammary epithelium, which occurs during this time. Because of this, longitudinal histological mammary gland analysis is needed to better understand growth and development, as well as the effects of environmental factors such as diet. Holstein calves were pair-fed on high (HI; n = 15) and low (LOW; n = 15) nutrition diets for 8 weeks. The LOW diet contained 0.45 kg of milk replacer per day with 22% crude protein and 15% fat, whereas the HI diet contained 1.12 kg of milk replacer with 27% crude protein and 20% fat. Starter was available at 7 d and weaning began on d 42. The starter amount given to the LOW diet calves were based on the amounts the paired HI diet calves consumed. Mammary gland ultrasound images were taken twice weekly. Mammary gland biopsies were performed at 8 weeks on 15 of the 30 calves, and slides were prepared using hematoxylin and eosin stain for 8 calves. The parenchyma (PAR) tissue of 1,414 ultrasound mammary gland images were manually segmented, and the parenchyma area was extracted. Images were analyzed using Matlab 2021b and statistical analysis was conducted using a mixed model including the fixed effect of time (16 time points), diet (HI and LOW) the interaction time × diet, and birth weight, and the random effect of animal. At time point 1 and 2 of wk 8, animals receiving HI diet presented PAR area of 56 and 81 mm^2 compared with 46 and 58 mm^2 ($P < 0.05$). There was no statistical difference in PAR circularity, but the PAR perimeter and maximum length was greater for HI diets (10.1 and 290 mm, respectively) compared with LOW (8.5 and 243 mm, respectively) at wk 8 (time point 2). The average number of ducts for HI and LOW diet was 8.2 and 6.6 ducts, respectively. The number of ducts is purely descriptive in this preliminary study. These results come from a pilot study with only 30 calves, and we were only able to biopsy enough tissue for H&E slides from 8 of those calves. Our preliminary data suggests that features extracted from ultrasound mammary gland images can be a powerful tool to monitor the development of parenchymal tissue in preweaned dairy calves.

Key Words: mamamary gland, Holstein, calf

P151 Factors associated with body weight of young dairy calves at arrival to veal facilities.

C. Rot*, K. Creutzinger, H. Goetz, A. Bajus, M. Conboy, J. Morrison, C. Winder, and D. Renaud, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Body weight at arrival to veal facilities (BWA) of surplus dairy calves has been demonstrated to be a significant predictor of morbidity and mortality; however, factors influencing BWA have not been well explored. The objective of this study was to determine factors associated with BWA of young dairy calves upon arrival to a veal facility. Calves were enrolled at birth on 4 dairy farms in southern Ontario, Canada and followed until immediately after transportation. Dairy farms were visited daily before transport to record calving difficulty, birthweight, health status, and serum total protein at 24 h after birth. On the day of transport, calves were randomly assigned to 1 of 3 transportation groups: 6, 12, or 16 h of time in transit. Age on the day of transport was recorded and BWA was measured immediately at arrival to the veal facility following transport. A mixed linear regression model was constructed to identify the effect of exposure variables on BWA. Statistical significance was declared at $P \leq 0.05$. A total of 60 male and female dairy calves were enrolled. The mean (±SD) birthweight and BWA were 46.27 ± 6.91 kg and 46.85 ± 6.56 kg, respectively, and age on the day of transport ranged from 2 to 22 d. In the final model, birthweight and age at transport were significant. Specifically, for every 1 kg increase in birthweight, there was a 0.86 kg increase in BWA (95% CI: 0.76–0.95; $P = 0.001$). With respect to calf age on the day of transport, calves that were 5 to 9 d, 10 to 12 d, and ≥13 d at transport were 2.46 kg (95% CI: 0.68–4.23; $P = 0.007$), 5.94 kg (95% CI: 4.12–7.76; $P = 0.001$), and 5.93 kg (CI: 4.11–7.75; $P = 0.001$) heavier, respectively, than calves that were <5 d of age. Interestingly, health status of the calves before transport and duration of transport were not associated with BWA. The initial results of this study suggest that birthweight and age at transport have a significant effect on calf BWA.

Key Words: transport, age, health

P152 Characterizing the literature about transportation of young dairy calves: A scoping review.

H. Goetz*, C. Winder, K. Creutzinger, T. Uyama, J. Dunn, D. Kelton, and D. Renaud, *Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Transportation is a stressful event for cattle due to handling, commingling, deprivation of food and water, and fluctuating temperatures. Calves are particularly susceptible to these stressors as their physiological and immune systems are still developing. However, there has been no formal synthesis of published studies evaluating transportation of calves. The aim of this scoping review is to characterize the literature about transportation of young dairy calves. This review targeted descriptive and analytic studies examining transport of calves, including how the impact of transport has been evaluated, and identify knowledge gaps. Eight databases were searched for relevant articles with eligible studies being primary research articles investigating transportation of calves of any sex, younger than 60 d of age or weighing less than 100 kg. Two reviewers independently screened the title and abstracts of 6,862 articles with 351 eligible for full text screening. Of these 351 articles, 54 examined transportation of young calves and had data extracted. The majority of studies did not report where they were completed (n = 23), but of those which reported location, many were conducted in the USA (n = 5), Australia (n = 3), Japan (n = 3), and New Zealand (n = 3). Studies most frequently described transport of male calves (n = 19), while others included a mixture (n = 13), females (n = 2), or did not report the sex (n = 17). Common variables collected included time in transit (n = 15), distance of transportation (n = 8), vehicle-related factors (n = 6), and age at time of transportation (n = 5). Outcome measures described in these articles varied greatly with blood parameters most frequently assessed (n = 29), followed by behavioral parameters (n = 16), health scoring (n = 11), weight (n = 7), mortality (n = 7), and morbidity (n = 4). A diverse range of risk factors and outcomes were evaluated in the studies included in this scoping review, making it difficult to compare findings. Further research should focus on consistent and clear reporting of the impact of transportation on young calves and development of a core outcome set for calf transport studies.

Key Words: bull calf, veal industry

P153 Early detection of anaplasmosis in dairy calves using activity and rumination data from commercial devices.

V. A. Teixeira[1], A. M. Q. Lana[1], G. M. Souza[2], T. R. Tomich[3], T. Bresolin[4], J. P. P. Rodrigues[5], L. C. Gonçalves[1], S. G. Coelho[1], L. D. Ferreira[1], J. A. G. Silveira[1], M. M. Campos[2], E. J. Facury Filho[1], L. G. R. Pereira*[2,4], and J. R. R. Dorea[4], [1]*Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [2]*Universidade Federal de Lavras, Lavras, MG, Brazil,* [3]*Brazilian Agricultural Research Corporation-Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil,* [4]*Department of Animal and Dairy Sciences, University of Wisconsin, Madiosn, WI,* [5]*Faculty of Animal Science, Federal University of the Southern and Southeastern Pará, Xinguara, PA, Brazil.*

The annual losses caused by anaplasmosis in cattle are approximately 300 and 800 million dollars for USA and Latin America, respectively. The use of wearable devices to monitor animal behavior could be a powerful tool to early predict diseases, as anaplasmosis. The objective of this study was to compare the predictive ability of Recurrent Neural Network to detect anaplasmosis on day zero (0d, The lowest packed cell volume - PCV value, 14 ± 1.8% was used to assume that the animals were sick against 32 ± 2.4% for healthy animals) or 3 d in advance (−3d), based on rumination (RUM) and activity (ACT) data retrieved from 2 devices: collar (Heatime HR) and ear tag (eSense Flex). Fourteen Holstein female dairy heifer calves (119 ± 15 d old) were fitted with both devices (21 d before challenge) and were challenged with 2×10^7 erythrocytes infected with UFMG1 strain (GenBank no. EU676176) isolated from *A. marginale*. A 7-d sequence was randomly selected from a time window varying from −50 to −15d before d0. This time-series was used to classify a healthy pattern (sequence of days that does not result in a positive case: Anaplasmosis). The sick time-series consisted of a sequence of days that resulted in a positive case (from −7 to 0d) or a sequence of days that ended 3 d before a positive case (−10 to −3d). A Long Short-term Memory was used as a predictive approach to detect sick events and was implemented in Keras R package. To validate the trained algorithms, a leave-one-animal-out cross-validation was implemented. The accuracy (ACC), sensitivity (SEN), and specificity (SPE) for predictions at 0d were similar for collar and ear tag (100%) using ACT data. The predictions based on ACT for −3d in the advance decrease 21% (ear tag) and 29% (collar) the ACC compared with 0d models. When RUM data were used, the ACC, SEN, and SPE were lower compared with ACT data. RNN models based on RUM or ACT data from wearable sensors can be used to detect anaplasmosis in dairy calves. For predictions in advance, the models based on RUM had the best performance compared with ACT. The models based on data from ear tag had better performance compared with collar.

Table 1. Model performance to predict anaplasmosis

Item	Accuracy	Sensitivity	Specificity
ACT			
Ear tag (0 d)	100	100	100
Collar (0 d)	100	100	100
Ear tag (−3 d)	79	71	86
Collar (−3 d)	71	57	86
RUM			
Ear tag (0 d)	96	93	100
Collar (0 d)	93	86	100
Ear tag (−3 d)	86	79	93
Collar (−3 d)	82	79	86

Key Words: artificial intelligence, machine learning, tick fever

P154 Impacts of Clostridia populations on dairy cows observed on a Wisconsin dairy.

V. G. Bretl*, J. S. Thompson, R. F. Teal, A. H. Smith, and T. G. Rehberger, *Arm & Hammer Animal and Food Production, Waukesha, WI.*

Clostridium is a gram-positive, spore-forming, obligate anaerobe that is commonly found in the gastrointestinal tract and feed of dairy cows. *C. perfringens* type A has been associated with enteric diseases such as hemorrhagic bowel syndrome in ruminants. These types of *Clostridium* challenges are often acute and occur rapidly. The objective of this experiment was to better understand the variation and impact of clostridia populations on the performance of dairy cows. Clostridia populations were observed in fecal samples from 11 dairy cows on a single farm, daily, for a 3-week period. Total mixed ration (TMR) in front of each cow and fermented feeds were collected daily to identify incoming clostridia loads. Clostridia was enumerated from fecal samples on tryptose sulfite cycloserine (TSC) agar, incubated at 37°C for 24 h, isolates were picked, and DNA was isolated. *Clostridium* spp. mPCR was used to specifically identify *C. perfringens*, *C. beijerinckii*, *P. bifermentans*, and *C. butyricum*. Milk production and SCR monitoring was used to gain performance data including daily and rumination activity, measurements were collected for 4 weeks, each day of the experiment and the week after. Total clostridia counts ranged from 74 to 1,800 cfu/g in fecal samples and 32 to 990 cfu/g in feed samples. The average clostridia levels increased during each week of this observation in both fecal (300, 390, 590 cfu/g) and feed samples (160, 240 and 510 cfu/g). Clostridia loads in the TMR samples increased comparing wk 1 to both wk 2 and 3 ($P < 0.05$) and trended higher in the fecal samples, comparing wk 1 to 3 ($P = 0.07$). The source of haylage changed during the second and third week of observation. During the first 2 weeks the average level of clostridia in the haylage was 350 cfu/g which increased to 600 cfu/g during wk 3. Average milk weight/day decreased each week (158.0, 156.6, 153.6 and 146.5 lbs) with wk 1 and 2 being significantly different than wk 4 ($P < 0.05$). General activity and rumination were not significantly different across the weeks. These results could provide insight on how the level of clostridia might impact the performance of dairy cows.

Breeding and Genetics: Posters

P155 Machine learning algorithms for prediction of insemination outcome in historical data of Holstein cattle. L. Alcantara*[1], D. Tulpan[1], C. Baes[1,2], and F. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Dairy cattle fertility is a long-standing concern in the dairy industry given its huge financial impact. Knowing the insemination outcome is key to investigate what factors might have an effect on fertility at breeding. Therefore, this study aimed to classify insemination outcome in historical data of Holstein cattle as open, pregnant, or aborted using machine learning algorithms. Data (Lactanet, Guelph) consisted of on-farm insemination records (2015–2020) and their corresponding fertility records used for genetic evaluations. Final data set contained 54,048 records from 20,484 animals (parity 0 to 11). Pairwise combinations of sums and differences between features were used to construct new features, e.g., difference between calving and insemination date. Linear discriminant analysis, k-nearest neighbors, decision tree (DT), Naïve Bayes, random forest (RF), AdaBoost, and support vector machine were used. A stratified 10-fold cross-validation was performed 5 times on 70% of the data. Accuracy of models ranged from 0.93 (DT) to 0.96 (RF). Hyperparameter optimization of DT produced the best model in overall accuracy (0.97), precision (0.96), sensitivity (0.97), and Matthews Correlation Coefficient (0.93). Tree depth was set to 4 to avoid overfitting. Choosing Gini impurity over information gain as the criteria to measure the quality of splits helped increase precision of abortion predictions by 10% (0.71). However, regardless of the criteria, sensitivity was low (0.10) when classifying abortions. Having more abortion records (2.3%) is expected to improve its prediction. The binary feature *"date of insemination equal to last insemination date reported"* was found to be the most important. As conception is delayed and calving interval lengthens due to unsuccessful services, cows spend a significant portion of their lactations at low production levels. Therefore, the proposed classification model will have a positive impact on the dairy industry by enabling researchers to accurately predict insemination outcome from historical data and better understand the underlying mechanisms of fertility.

Key Words: machine learning, classification, insemination outcome

P156 Preliminary analysis for the genetic evaluation of leukosis in Canadian dairy cattle. R. Bongers*[1], F. Miglior[1], C. Lynch[1], H. Oliveira[1], N. van Staaveren[1], and C. F. Baes[1,2], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Enhanced disease resistance is an important goal in dairy breeding. One of the major global diseases in cattle is enzootic bovine leukosis. This chronic disease is caused by bovine leukemia virus (BLV) and is spread through the transmission of infected lymphocytes. Clinical leukosis takes approximately 5 years to manifest and results in persistent lymphocytosis in 30% of cases and fatal malignant lymphoma in 5% of cases. Before these symptoms developing, infected animals can display a lower immune response, decreased fertility and milk production, and experience adverse effects on welfare, thus, making the economic impact challenging to quantify. Infection with BLV is not preventable through vaccination and is currently not treatable, however; breeding for resistance against leukosis is a potential method to mitigate these negative effects. To do so, a clear understanding of leukosis in the Canadian dairy population is paramount. The objective of this analysis was to understand the frequency of leukosis in Canadian herds and assess the potential for genetic improvement of this trait. Data on 122,111 milk ELISA test records for leukosis, from 101,183 cows on 988 farms across Canada, between 2007 and 2021 were investigated. Of these farms, 855 had at least one positive case of leukosis. A total of 37,657 (30.8%) cows tested positive for leukosis, 78,588 (64.4%) tested negative, and 6,868 (5.6%) were suspect. The average prevalence in infected herds was 51.5% (±27.5%). The relatively high prevalence of leukosis highlights the importance of this disease in the Canadian population. Descriptive statistics show that the incorporation of leukosis data into a national genetic resilience evaluation is a necessary step and could benefit the industry while improving the health and welfare of Canadian dairy cattle. Further steps will be performed to estimate genetic parameters and genetic and phenotypic correlations between leukosis and other traits. This study will lay the foundation for the inclusion of leukosis resistance into a new index for resilience.

Key Words: leukosis, BLV, genetics

P157 Characterization of copy number variants identified in Canadian Holstein dairy cattle using genotype array data. H. R. Oliveira*[1], T. C. S. Chud[1], G. A. Oliveira Júnior[1], C. F. Baes[1,2], and F. S. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Genomic breeding values (GEBVs) predicted based solely on single-nucleotide polymorphism (SNP) markers may represent only a part of the true breeding values (TBVs) of animals. To increase the correlation between GEBVs and TBVs, copy number variations (CNVs) might be useful, as they can capture part of the genetic variation not accounted for by SNPs. In brief, CNVs are deletions or duplications of DNA segments that can affect gene structure and dosage. The objective of this study was to characterize CNVs identified in Canadian Holstein dairy cattle using medium-density genotype array data. A total of 5,211 animals were genotyped using the GGP Bovine 100K array, and the corresponding intensity signal files (containing the log R ratio and B allele frequency; LRR and BAF, respectively) were provided. The detection of CNVs was performed using the PennCNV software. To reduce false-positive results, the LRR values were corrected for genomic waves based on the guanine-cytosine content of the genomic regions 500Kb upstream and downstream of each SNP. After the CNV detection, quality control was performed to remove CNVs with less than 3 SNPs, LRR standard deviation above 0.30, BAF drift above 0.01, and wave factor above 0.05. In total, 14,451 deletions and 13,001 duplications were detected. Of the deletions, 10,809 (74.8%) and 3,642 (25.2%) were heterozygous and homozygous copies, respectively. Of the duplications, 11,768 (90.5%) and 1,233 (9.5%) were heterozygous and homozygous copies, respectively. These CNVs will be validated using other genotype arrays and sequence data to provide high-confidence CNV regions. Further studies will investigate the functional impact of CNVs on several economically important traits for the Canadian dairy industry and evaluate the performance of genomic predictions incorporating both SNPs and CNV.

Key Words: copy number variations (CNVs), PennCNV, structural variants

P158 Contrast of immune cell lineage, hair, dam, and pooled colostrum genotypes in a newborn calf.

T. Muratori*[1], T. Ott[1], A. Shabtay[2], M. Cohen-Zinder[2], E. Lipkin[3], and C. Dechow[1], [1]*Penn State University, University Park, PA,* [2]*Newe Ya'ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel,* [3]*The Hebrew University of Jerusalem, Israel.*

Immune cells in a newborn calf could be produced by the calf, maternally derived, or absorbed from colostrum which has implications for genotyping and telomere length determination. Our objective was to contrast immune cell genotypes from a day-old calf with genotypes from a hair sample, dam hair sample, and colostrum that was pooled from multiple cows before feeding. Polymorphonuclear cells (PMN) were separated from peripheral blood mononuclear cells (PBMC) over a Ficoll gradient. The PBMC were separated by flow cytometry into T-cell, B-cell, and monocyte populations, and samples were genotyped for 139,376 SNP. We assumed the calf hair sample was the true genotype and tested for differences in frequencies of the number of called heterozygous and homozygous loci with chi-squared tests. The call rate for calf genotypes ranged from 96.2% for T-cell to 98.6% for hair and PMN. Cell lineage genotypes mostly returned high concordance with the assumed true genotype, with 2, 37, 56, and 656 mismatches of hair genotype with PMN, B-cell, monocyte, and T-cells, respectively. Calf heterozygosity was higher for the T-cell genotype (33.9%) than for the other cell types (33%), and only the T-cell genotype was different from the hair genotype ($P < 0.05$). The colostrum sample also had a low call rate (78.3%) and a high degree of heterozygosity (65.4%). Analysis of the 656 nonconcordant T-cell genotypes indicated that 82 matched the dam, 147 matched colostrum, and 105 matched both. Of the 322 that matched neither, 70 had no colostrum genotype. For the remaining 252, 241 were instances where the T-cell genotype was heterozygous, and the dam and colostrum genotypes were homozygous for opposite SNP. Dam and colostrum derived cells contribute to building passive immunity, and T-cell genotypes suggested some degree of DNA contamination with elevated non-call rate, heterozygosity, and mismatches with the true genotype. Nevertheless, immune cells isolated from a newborn calf's blood provided largely accurate genotypes.

Key Words: calf, genotype, T cell

P159 The Resilient Dairy Genome Project: A project overview.

N. van Staaveren*[1], F. Schenkel[1], E. Goddard[2], G. Kistemaker[3], M. De Pauw[4], R. Cerri[5], M. A. Sirard[6], P. Stothard[4], and C. F. Baes1,7, [1]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Resource Economics and Environmental Sociology, University of Alberta, Edmonton, AB, Canada,* [3]*Lactanet, Guelph, ON, Canada,* [4]*Department of Agriculture, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [5]*Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada,* [6]*Département des Sciences Animals, Faculté des Sciences de l'agriculture et de l'alimentation, Université Laval, Quebec, QB, Canada,* [7]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Dairy farming is one of Canada's most important and dynamic industries. The global demand for dairy products is set to expand further, however, at the same time, the dairy industry is facing several emerging issues related to human and animal health, environmental impacts, sustainability, and social acceptability. The Resilient Dairy Genome Project (RDGP) is a large-scale collaboration between various international research and industry partners, which aims to integrate genomic approaches to enhance dairy cattle resilience. We define dairy cow resilience as the capacity of the 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. This project will develop and collect 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). We will evaluate the genomic and environmental relationships between both novel and existing traits using multigenerational analyses and epigenetic information. Our goal is to integrate these traits into a new resilience index, which can be incorporated in the Canadian dairy cattle genomic evaluation program. Furthermore, we will investigate producers, market, and public perspectives to optimize traits for maximizing resilience and societal acceptance. This strong foundation together with close collaboration with end-users will ensure rapid and meaningful translation and implementation of project results. We would like to acknowledge the support and funding from all the partners involved in the RDGP project through the Large Scale Applied Research Project program from Genome Canada and Genome Alberta.

Key Words: resilience, dairy, genomics

P160 Estimates of genetic parameters for total lactational health costs in US organic Holstein cows.

L. C. Hardie*[1], I. W. Haagen[1], B. J. Heins[2], and C. D. Dechow[1], [1]*Pennsylvania State University, University Park, PA,* [2]*University of Minnesota, St Paul, MN.*

The objective of this study was to estimate genetic parameters for total lactational health costs (TCOSTS) in US organic Holstein cows. Health records were collected from 39,347 lactations from 19,249 Holstein cows on 16 USDA certified organic farms from across the United States. A subset of producers provided costs for a case of each of the following health issues (median cost): mastitis ($19.48), milk fever ($21.17), ketosis ($26.91), retained placenta ($28.14), displaced abomasum ($250.00), lameness ($16.81), cystic ovaries ($4.74), respiratory disease ($39.91), death ($80.37), and digestive issues ($19.57). Costs reflected veterinary, on-farm treatment, and labor costs but not discarded milk because of the absence of antibiotic usage in concert with feeding nonsaleable milk to calves. New cases were assigned after meeting a minimum threshold of days based on producer-reported days per treatment. Total lactational health cost was calculated by summing across each health issue the product of the median cost and number of cases. Stayability through a given lactation (1 = stayed, 0 = otherwise) was included as a threshold trait with TCOSTS in a bivariate animal model to mitigate potential impacts of selection bias. Animal relationships were established by combining genotypes on 2,347 cows with breed representation of at least 87.5% Holstein and a minimum of 4-generation pedigree in which phenotyped animals were required to have a known Holstein sire and no other identified breeds in the lineage. The model included the fixed effects of herd and lactation and the random effects of herd-year-season of birth (stayability), herd-year-season of calving (TCOSTS), animal, and permanent environment. For TCOSTS, heritability was 0.017 ± 0.003 and repeatability was 0.058 ± 0.008. The genetic correlation between stayability and TCOSTS was -0.49 ± 0.14. The most significant genetic correlations were approximated between TCOSTS with the following nationally evaluated traits: productive life (−0.76), cow livability (−0.61), heifer livability (−0.56), displaced abomasum (−0.52), net merit dollars (−0.51), and mastitis (−0.50). In conclusion, genetic selection for reduced cow health costs is possible among organic cows and afforded by current national genetic evaluations for cow health, longevity, and profitability traits.

Key Words: health costs, genomics, organic

P161 Phenotypic parameters for Johne's disease in Canadian dairy cattle.

C. Lynch*[1], H. R. Oliviera[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, Switzerland.*

Johne's disease (JD) is an enteric infection caused by *Mycobacterium paratuberculosis* (MAP), which is often transmitted to calves through the ingestion of contaminated feces or via consumption of colostrum or milk from an infected cow. However, clinical signs of JD (i.e., diarrhea, weight loss and eventual death) are not generally expressed until 3 to 5 years postexposure. There is no cure for JD, and currently there are no available vaccines. Consequently, control JD must be achieved through management practices and genetic selection. Addressing this issue through genetic selection has the major advantage that effects are permanent and cumulative over generations. Several studies have reported genetic variability for resistance to MAP infection, indicating that genetic selection for JD can be performed. As part of a larger project aiming to add JD to genetic evaluations in Canadian dairy cattle, this study provides the groundwork by describing the current situation of JD on Canadian farms. Data were provided by Lactanet Canada and included records on 405,569 milk ELISA tests, which measure the amount of MAP-specific antibody as optical density values. These records were collected from 313,005 cows raised in 3,120 herds distributed across Canada, recorded between 2007 and 2021. From these records, 8,210 (2.0%) were classified as positive, 395,338 (97.5%) were negative, while 2,021 (0.5%) were suspect. In total, 1,146 (36.7%) herds had at least one positive test, while the overall herd median infection rate among positive herds was 3.5%, with a median absolute deviation of 3.05, and a range between 0.3% and 100% of total animals within herds. This highlights the potentially widespread impact of JD and the importance of addressing JD through genetic selection. The descriptive statistics produced in this study will be used to help optimally fit JD into a novel resiliency index for use in national genetic evaluations in Canada.

Key Words: Johne's disease, health

P162 Genetic correlations among milk yield, electric conductivity, and flow in Holstein cattle in a robotic milking system in Santiago de Querétaro, México.

N. L. Cornejo García*[1], M. D. Aguilar[1], J. L. Romano Muñoz[2], F. J. Ruíz López[2], G. J. Cantó Alarcón[1], and S. V. Eduardo[1], [1]*Facultad de Ciencias Naturales - UAQ, Santiago de Querétaro, Querétaro, México,* [2]*Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal - INIFAP, Colón, Querétaro, México.*

The goal of the study was the estimation of variance components and genetic correlations for milk production (MP), mean milk flow (MFm), maximum milk flow (MFx) and electrical conductivity (EC) in a robotic milking system and its comparison with parameters calculated in other milking systems. A total of 174 lactations (complete and incomplete subjects) of 110 primiparous and multiparous Holstein cows, with a total of 42,006 observations (2–3 per day), from 2017 to 2020 were analyzed. Multitrait animal model was used, where the fixed effects were test day, days in milk and lactation number, while as random effects animal and permanent environment were included. To estimate heritability, the restricted maximum likelihood algorithm was used to calculate the additive genetic variance components of the animal, the permanent environment and the residual. The heritability estimated for MP (0.62) was significantly higher than those estimated for MFm (0.44), MFx (0.33) and EC (0.28). Genetic correlations for MP were negative and large with MFm (−0.6117) and MFx (−0.7666), while for the EC trait (−0.1669) was considerably lower. Instead, the estimated genetic correlations for MFx were positive and large for MFm (0.7422) and EC (0.5351) and moderate for MFm and EC (0.3546). These results show that higher yields are attained in slower flow cows and suggest the possibility of including only some of the traits in a genetic improvement program. Given the negative correlation between EC and MP, the positive one with flow traits, and the reported relationship between EC and udder health, EC should be used in improvement programs along with milk yield; however, it is important to increase the number of evaluated animals to obtain more accurate results.

Key Words: heritability, correlation, Holstein

P163 The association between calf birth weight and postcalving cow performance in dairy cows.

T. Condon*[1], C. Murphy[2], R. D. Slater[2], S. Ring[3], and D. P. Berry[1], [1]*Animal and Grassland Research and Innovation Center, Cork, Ireland,* [2]*Munster Technology University, Cork, Ireland,* [3]*Irish Cattle Breeding Federation, Cork, Ireland.*

The objective of the present study was to quantify the association between calf birth weight and its dam's subsequent performance. A total of 11,112 lactation records with no recorded dystocia at calving from 4,350 spring-calving cows were used. Holstein-Friesian represented the main breed of cow with the remainder comprising Jersey, Norwegian Reds or Montbeliarde (or some combination of such). The association between a series of cow performance metrics (milk and fertility performance, body weight and body condition score) (dependent variables) and calf birth weight (independent variable) was determined using linear mixed models. Nuisance factors included in all models were calf sex, cow live-weight between 100 and 200 d of lactation relative to the mean cow weight per parity, heterosis coefficient of the cow and calf separately, previous dry period length, an interaction between parity (1, 2, 3+) and calf weight, an interaction between parity and age at calving relative to the median age at calving per parity, breed proportion of the dam, and contemporary group of herd-year-season-treatment; cow was included as a random effect in all the models. Mean birth weight was 36.2 kg with a standard deviation of 6.8 kg. Total 60 and 305-d milk yield was 60.35 and 189.5 kg more, respectively in multiparous cows that had heavier calves compared with those that had average weighed calves. Concentrations of milk fat and protein were also significantly increased in multiparous cows that had heavier calves. There was no significant effect of calf birth weight on pregnancy rates to first service and throughout the 12-wk breeding season, but clinical mastitis increased in multiparous cows that had heavier calves. The adoption of new management methods and breeding strategies like the inclusion of calf birth weight in future breeding programs will help maximize performance metrics of dairy cows, ensuring the long-term sustainability of the agricultural sector.

Key Words: calf birth weight, multiparous, milk

P164 Connecting cattle and human traits using genome-wide association study statistics.

V. Iqbal* and L. Ma, *University of Maryland, College Park, MD.*

Genome-wide association studies (GWAS) are used to reveal insights into the genetic basis of complex traits by identifying associations between genetic variations and traits. Using data from existing human and cattle GWAS and orthologous genes across species, we extracted GWAS summary statistics for 58 human traits and 43 cattle traits. The objective of this study was to find the pairwise correlations between cattle and human traits based on GWAS statistics. First, we calculated the z-score for each SNP for both human and cattle traits. Then, we found the gene level z-score statistic by calculating the mean z-score for all the SNPs inside of each gene for each trait. Additionally, we repeated the analysis using a different method, (tTs) to create gene-level *P*-value statistics that are less than a cut-off value (0.05). After creating all the gene level z-scores and gene-level *P*-value statistics for each trait, we did a pairwise correlation analysis for all the cattle to cattle traits, human to human traits, and finally human to cattle traits to investigate the genetic correlation of traits within and between species. There were several cattle production traits highly correlated with one another, several human disease traits correlated with each other, and a few cattle and human traits correlated. By using gene-level statistics and GWAS statistics we can further our knowledge of complex traits across species and help future GWAS research to pinpoint genetic variations underlying these traits.

Key Words: genome-wide association studies

P165 Identification of long noncoding RNA in *Bos taurus* calf rumen before and after weaning.

A. Marceau*[1], R. Baldwin[2], C. Li[2], G. Liu[2], and L. Ma[1], [1]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD,* [2]*Animal Genomics and Improvement Laboratory, Henry A. Wallace Beltsville Agricultural Research Center, Agricultural Research Service, Agricultural Research ServiceDA, Beltsville, MD.*

Although it has long been known that genes produce gene products, recent technological advancements have revealed that this is likely not always the case. Noncoding regions of the genome can also influence gene products; and a subcategory of these noncoding regions includes long noncoding RNAs (lncRNA) which lack an open reading frame and exceed 200 base pairs in length. This study aims to identify RNA transcripts that meet the long noncoding RNA criteria, and identify lncRNA transcripts that are differentially expressed before and after Bos taurus calves are weaned. Rumen development, which is triggered by weaning and requires changes to the gene expression profile, is crucial for healthy calf development and, when done successfully, leads to a healthy digestive system for the growing animal. To identify these transcripts, tissue samples were taken from 6 Bos taurus calf rumens before and after weaning. These samples were sequenced, and the resulting transcripts were filtered based on size, coding potential, sequence homology, and known protein domains. A Student *t*-test was then used to identify those transcripts that were differentially expressed at a statistically significant level. Beginning with over 21 million reads per sample, filtering steps lead to the identification of 2,026 and 2,074 long noncoding transcripts in Bos taurus rumen tissue before and after weaning, respectively. 2,025 transcripts were shared in both tissue conditions, 30 transcripts were found only in preweaning tissues, 78 transcripts were found only in postweaning tissues, and 336 transcripts were differentially expressed at a significant level between tissue conditions. These results suggest some connection between long noncoding RNA and rumen development in response to calf weaning, in both what is transcribed and at what levels. These relationships should be further investigated to uncover the specific roles these lncRNA transcripts are playing in rumen development.

Key Words: lncRNA, rumen, RNA-seq

P166 Identifying genetic variants and pathways influencing twinning rate in North American Holstein cattle.

B. M. Lett* and B. W. Kirkpatrick, *University of Wisconsin–Madison, Madison, WI.*

Multiple birth in dairy cattle is a detrimental trait both economically for producers and animal health. Genetics of twinning is complex and has led to several quantitative trait loci regions being associated with increased twinning. To identify variants associated with this trait, calving records from 2 time periods were used to estimate twinning rate for Holstein bulls. Multiple analyses were conducted and compared including genome-wide association studies, genomic prediction, and gene set enrichment analysis for pathway detection. While pathway analysis did not yield a congruent pathway between data sets, genome-wide association analysis across data sets and methods showed 2 strong candidate regions on BTA11. Neither region overlaps with previously identified quantitative trait loci regions for twinning or ovulation rate in cattle. The strongest associated SNPs were upstream from 2 candidate genes *LHCGR* and *FSHR* which are involved in folliculogenesis. Using high-density genotype data, the most significant SNP in a combined analysis across data sets had a *P*-value of 1.9082×10^{-9}. Genomic prediction showed a moderate correlation accuracy (0.4263) when predicting genomic breeding values for bulls with estimates from calving records from 2010 to 2016. Additionally, 5-fold cross-validation genomic prediction in the 2 data sets showed moderate accuracy (0.3816 older calving records and 0.5628 newer calving records). Future analysis of the region on BTA11 and the relation of the candidate genes could improve this accuracy.

Key Words: twin, folliculogenesis, genomic prediction

P167 EPIHAP: A computing tool for genomic estimation and prediction using global epistasis effects and haplotype effects.

Z. Liang*, D. Prakapenka, and Y. Da, *Department of Animal Science, University of Minnesota, Saint Paul, MN.*

Epistasis effects are interaction effects and haplotype effects may contain locally high order epistasis effects. To facilitate research on these effects, we developed EPIHAP computing program to integrate global epistasis effects and haplotype effects for genomic estimation and prediction. The full mixed model implemented by EPIHAP includes SNP additive (A) and dominance (D) effects, 3 pairwise epistasis effects (A × A, A × D, D × D), 4 third-order epistasis effects (A × A × A, A × A × D, A × D × D, D × D × D), and haplotype additive effects, for a total of 10 types of genetic effects. All or any or a combination of the 10 effects may be included in the mixed model. Limited tests showed that fourth-order epistasis effects virtually had no contribution to the phenotypic variance but generated considerable computing difficulty. Consequently, epistasis effects higher than the third order are not implemented by EPIHAP. For genomic estimation, EPIHAP estimates variance components and heritability for each type of genetic effect using genomic restricted maximum likelihood estimation (GREML). For genomic prediction, EPIHAP calculates genomic best linear unbiased prediction (GBLUP) and reliability for each type of genetic values, and for the total genetic values as summation of all types of genetic values in the prediction model at the end of GREML iterations. In addition, the epistasis heritability for each SNP pair can be calculated for A × A, A × D and D × D as a user option. This step is extremely demanding for memory space. We developed a triangular memory storage technique that eliminated this memory requirement. Estimates for additive and dominance heritabilities of each SNP and for haplotype additive heritability of haplotype blocks are also available. These heritability estimates can be used for identifying SNPs, SNP pairs and haplotype blocks with high heritability estimates. With the many capabilities under highly complex models for genomic estimation and prediction, the EPIHAP program offers a computing capability to investigate and utilize complex genetic mechanisms.

Key Words: epistasis, haplotype, genomic prediction

P168 Genomic heritability and prediction accuracy of additive and nonadditive effects for daughter pregnancy rate in crossbred dairy cows.

Z. Liang*[1], D. Prakapenka[1], P. VanRaden[2], and Y. Da[1], [1]*Department of Animal Science, University of Minnesota, Saint Paul, MN,* [2]*Animal Genomics and Improvement LaboratoryDA/ARS, Beltsville, MD.*

Genomic heritability and prediction accuracy of epistasis effects for daughter pregnancy rate (DPR) were evaluated using 79,294 SNPs and 9,565 crossbred dairy cows. Heritability was estimated under the model with SNP additive effects (A), SNP dominance effects (D), and second and third order epistasis effects of A × A, A × D, D × D, A × A × A, A × A × D, A × D × D and D × D × D. Heritability estimate was 0.162 for additive effects, 0.283 for dominance effects, 0.555 for A × A effects, and zero or nearly zero for A × D, D × D and the third-order epistasis effects. Genomic prediction included all SNP and epistasis effects with heritability greater than one percent, resulting in the prediction model with A, D and A × A effects only. Prediction accuracy as correlation between the genomic best linear unbiased prediction and the phenotypic values from a 10-fold validation study for each model was 0.268 for A-model, 0.363 for D-model, 0.438 for A × A, 0.446 for A+D, 0.455 for A+(A × A), 0.467 for D+(A × A), and 0.475 for A+D+(A × A). Relative to the A-model, the D-model increased the prediction accuracy by 35.6%, A × A by 63.6%, A+D by 66.4%, A+(A × A) by 69.8%, D+(A × A) by 74.3%, and A+D+(A × A) by 77.2%. The heritability estimates and prediction accuracies showed that A × A effects were the largest contributor to DPR heterosis, followed by dominance and additive effects. The high additive heritability (0.16) in crossbred dairy cows relative to the low additive heritability in Holstein cows (0.025 according to our own estimate) indicated that a larger collection of favorable alleles from different breeds in crossbreds than in purebreds was part of the genetic mechanism underlying DPR heterosis. The total heritability was almost 100%, indicating likely overestimates somewhere for unknown reasons. However, the prediction accuracies did support the conclusion that A × A effects were the largest contributor to DPR heterosis, followed by dominance and additive effects, and that prediction accuracy of DPR can be high in crossbred dairy cows. Combined with dominance and A × A effects, the results in this study support our GWAS finding in a separate study that genome-wide additive and nonadditive effects were the genetic mechanism of reproductive heterosis.

Key Words: heterosis, genomic prediction, heritability

P169 Genetic mechanisms of reproductive heterosis in crossbred dairy cows involve genome-wide additive and nonadditive effects.

D. Prakapenka*[1], Z. Liang[1], P. VanRaden[2], J. Jiang[4], L. Ma[3], J. Garbe[5], C. Maltecca[4], P. Hansen[6], and Y. Da[1], [1]*Department of Animal Science, University of Minnesota, Saint Paul, MN,* [2]*Animal Genomics and Improvement LaboratoryDA/ARS, Beltsville, MD,* [3]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD,* [4]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [5]*Genomics Center, University of Minnesota, Minneapolis, MN,* [6]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Reproduction is one of the biological processes with strong heterosis in crossbred dairy cows. To identify the genetic mechanism for reproductive heterosis, we conducted a genome-wide association study for daughter pregnancy rate (DPR) using 79,294 SNPs and 9,565 crossbred dairy cows. Using a significance level of log(1/p) = 8 with the Bonferroni correction, the number of significant SNP effects was 16,449 for additive effects and 10,449 for dominance effects. This compares to only 112 additive and 2 dominance effects for DPR from a large-scale Holstein GWAS. The most significant additive effect had a log(1/p) of 53, whereas the most significant dominance effect had a log(1/p) of 203; 481 dominance effects had log(1/p) > 53. For pairwise epistasis effects, the cut-off significance level with the Bonferroni correction was log(1/p) = 12. This study only focused on the top 50,000 pairwise effects with minimal log(1/p) value of 29. Of these 50,000 effects, 50.2% were A × A effects, 37.6% A × D and D × A, and 12.2% D × D. Epistasis effects were 30% intra-chromosome and 70% inter-chromosome effects. Of the A × A effects, 82% were inter-chromosome and 18% were intra-chromosome effects. These results indicated that the genetic mechanism of reproductive heterosis involved both additive and nonadditive effects, and inter-chromosome A × A effects had a major role in reproductive heterosis. The large number of significant additive effects indicated a larger collection of favorable alleles for DPR in crossbred cows than in purebred Holsteins in comparison with previous GWAS results for Holsteins. The large numbers of significant dominance and epistasis effects indicated a major role of nonadditive effects underlying reproductive heterosis in crossbred dairy cows. The intra-chromosome and inter-chromosome epistasis effects as well as the significant additive and dominance effects involved all chromosomes, indicating that the entire genome contributed to reproductive heterosis.

Key Words: heterosis, GWAS, genetic mechanism

P170 Genomic heritability and prediction accuracy of epistasis effects for production and fertility traits in US Holstein cattle.

Z. Liang*, D. Prakapenka, and Y. Da, *Department of Animal Science, University of Minnesota, Saint Paul, MN.*

Genomic heritability and prediction accuracy of epistasis effects were evaluated using 60,671 SNPs and 22,009 first-lactation Holstein cows for 5 production traits and 3 fertility traits, milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FPC), protein percentage (PPC), daughter pregnancy rate (DPR), cow conception rate (CCR), and heifer conception rate (HCR). Heritability was estimated for SNP additive effects (A), SNP dominance effects (D), and second and third-order epistasis effects. The additive heritability was 0.28 for MY, 0.24 for FY, 0.21 for PY, 0.51 for FPC, 0.56 for PPC, 0.025 for DPR, 0.038 for CCR, and 0.006 for HCR. Dominance heritability was 0.037 for MY, 0.035 for FY, 0.04 for PY, 0.016 for HCR, and was negligible the other traits. The A × A heritability estimate was 0.14 for MY, 0.16 for FY, 0.18 for PY, 0.08 for FPC, 0.07 for PPC, 0.19 for DPR, 0.07 for CCR and 0.08 for HCR. Other than HCR, A × D, D × D and third-order effects had low heritability estimates of 0–0.03. For HCR, epistasis heritability estimate was 0.10 for A × D, 0.03 for D × D, 0.10 for A × A × A, 0.04 for A × A × D, 0.02 for A × D × D, and 0.01 for D × D × D. These heritability estimates indicated that HCR had the most complex genetic mechanism among all 8 traits. For genomic prediction accuracy, the A+D model

increased the accuracy over the A-model by 7.9% (0.047 vs 0.043) for HCR, 1.16% for MY, 1% for FY, and 1.49% for PY, and had negligible accuracy increases for FPC, PPC, DPR and CCR. The 3 fertility traits benefited most from epistasis effects for prediction accuracy, with accuracy increase of 38.1% (0.065 vs 0.047) for HCR, 13.3% (0.153 vs 0.135) for DPR, and 2.7% (0.112 vs. 0.109) for CCR over the best SNP model. The production traits had little accuracy increase due to epistasis, 0.8% (0.477 vs 0.473) for MY, 1.5% (0.454 vs 0.447) for FY, 1.8% (0.445 vs 0.438) for PY, 0.3% (0.640 vs 0.638) for FPC and 0.2% (0.688 vs 0.687) for PPC. These results showed epistasis effects could result in substantial increases in the accuracy of genomic prediction for the 3 fertility traits and dominance should also be considered for HCR.

Key Words: epistasis, genomic prediction, Holstein

P171 Genome-wide association study of epistasis effects associated with production and fertility traits in US Holstein cattle.

D. Prakapenka*[1], Z. Liang[1], J. Jiang[2], L. Ma[3], and Y. Da[1], [1]*Department of Animal Science, University of Minnesota, Saint Paul, MN,* [2]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [3]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD.*

Genome-wide association study (GWAS) using 60,671 SNPs and 294,079 first-lactation Holstein cows was conducted for testing pairwise epistasis effects of 5 production traits and 3 fertility traits, milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FPC), protein percentage (PPC), daughter pregnancy rate (DPR), cow conception rate (CCR), and heifer conception rate (HCR). For each SNP pair, 4 types of epistasis effects were tested, additive × additive (A × A), additive × dominance (A × D), dominance × additive (D × A), and dominance × dominance effects. The results showed that A × A effects were the main epistasis effects except CCR and HCR, for which A × D and D × A were the main epitasis effects (43% for CCR and 45% for HCR). The 5 production traits each had strong intra-chromosome epistasis effects, particularly FPC and MY. Intra-chromosome epistasis effects were the primary epistasis effects for FPC, PPC and MY, whereas FY and PY involved both intra- and inter-chromosome epistasis effects. The 3 fertility traits lacked intra-chromosome epistasis effects, but a Chr05 region with the PKP2 gene had the most significant epistasis effects for DPR. Among the 50,000 most significant pairwise epistasis effects of each trait, the percentage of inter-chromosome epistasis effects was 6% for FPC, 12% for PPC, 22% for MY, 45% for FY, 53% for PY, 87% for DPR, 94% for CCR, and 96% for HCR. Majority of the inter-chromosome epistasis effects of FPC across all chromosomes involved a Chr14 region containing DGAT1, indicating potential regulatory role of this Chr14 region affecting all chromosomes for FPC. The largest percentages of A × D and D × A effects as well as inter-chromosome epistasis effects of HCR indicated that HCR had the most complex genetic mechanism among all 8 traits. The epistasis results provided new understanding about the genetic mechanism underlying production and fertility traits in Holstein cattle and provided evidence for the need to consider epistasis effects in genomic prediction for the 3 fertility traits.

Key Words: epistasis, GWAS, Holstein

P172 Chromosomal partitioning of genetic parameters in Canadian Holstein bulls.

G. Oliveira Junior*[1], M. Spehar[2], B. Perez[3], I. Pocrnic[5], L. Lara[5], F. Schenkel[1], C. Baes[1,4], and G. Gorjanc[5], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Croatian Agricultural Agency, Zagreb, Croatia,* [3]*University of Sao Paulo, Pirassununga, SP, Brazil,* [4]*Institute of Genetics, University of Bern, Bern, Switzerland,* [5]*University of Edinburgh, Edinburgh, Scotland.*

Understanding genetic correlations among traits is important for multitrait evaluation and breeding. In this study, we partition correlations among dairy traits by chromosomes in Canadian Holstein cattle. The data comprised 9,204 progeny-tested bulls genotyped with the Illumina BovineSNP50K array. De-regressed EBV of fat yield (FY), age at first service (AFS), calving to first service (CTFS), and body condition score (BCS) were analyzed. We first estimated allele substitution effects of SNP markers with a multivariate marker model (Ridge Regression) under Bayesian approach. We then used these MCMC samples to obtain posterior distributions of breeding values, which were used to obtain samples from a posterior distribution of genetic (co)variances and correlations among the analyzed traits. We partitioned these posterior genetic (co)variances in genic, within- and between-chromosome linkage disequilibrium (co)variance contribution. This partitioning step was also performed over time to observe each component's trends. Overall correlations based on allele substitution effects ranged from −0.31 (AFS-BCS) to 0.49 (AFS-CTFS), and correlations of breeding values were between −0.71 (AFS-BCS) and 0.81 (AFS-CTFS). All the time trends, except FY (genetic variance), had a regression slope different than zero, suggesting that the (co)variance components have changed over time. The genetic variances were greatly influenced by genic and between-chromosome linkage disequilibrium components, with the latter contributing to the most of genetic correlations. The relationship between the number of SNP and the proportion of additive genetic variance attributed to each chromosome was close to linear, suggesting a polygenic structure of the traits, where, given an evenly spaced SNP chip, the bigger the chromosome, the bigger its influence on genetic variance. Fat yield was the exception to this pattern, with BTA 14 standing out the regression line.

Key Words: dairy cattle, genomics, time trend

P173 Identifying loci associated with foot warts and sole ulcers in Holstein cattle.

E. Lai*, A. L. Danner, T. R. Famula, and A. M. Oberbauer, *Department of Animal Science, University of California, Davis, Davis, CA.*

Lameness is the second most prevalent disease in dairy cattle and is commonly caused by foot warts (FW) and sole ulcers (SU). Foot warts are infectious lesions, whereas SU noninfectious lesions resulting from compromised horn production. Genomic selection against these claw lesions requires the identification of loci associated with susceptibility. To detect susceptibility loci, a genome-wide association study (GWAS) was performed using SNP genotypes from the high-density SNP array (777K SNPs) and case/control phenotypes for FW (controls n = 129, FW n = 85) and SU (controls n = 102, SU n = 152). GWAS was performed using linear mixed model (LMM) and random forest (RF) approaches, and effect sizes of top SNPs were estimated using Bayesian regression. For the LMM GWAS, the number of effective SNPs (NES) was calculated as the number of SNPs that were not in linkage disequilibrium and used as the denominator to define Bonferroni-corrected thresholds of significance ($P \leq 0.05$/NES) and suggestive significance ($P \leq 0.2$/NES). Top SNPs identified in the GWAS were in or near genes that were functionally relevant to claw lesion etiology. The FW LMM and RF GWAS both identified regions of association on *Bos taurus* autosome (BTA) 1 and 2, and one of the regions on BTA2 contained candidate genes related to immune function. The LMM GWAS for SU

revealed an associated region on BTA 8 containing genes related to wound healing, skin lesions, bone growth and mineralization, adipose tissue, and keratinization. Furthermore, the region on BTA8 included a SNP previously associated with SU susceptibility. The RF GWAS for SU was overfitted, suggesting that the SNP effects were very small and prevented detection of susceptibility loci using this approach. For both FW and SU, the estimated effect sizes of top SNPs were small, reinforcing that the environment plays a nontrivial role in susceptibility and the remaining genetic component is likely governed by many loci. Larger sample sizes are necessary to identify small effect loci amidst a strong environmental effect.

Key Words: foot warts, sole ulcers, GWAS

Dairy Foods: Posters

P174 Utilization of lactose-6-phosphate as an alternative to disodium phosphate for process cheese manufacture. K. A. Alsaleem*[1,2], A. R. A. Hammam[1], and L. E. Metzger[1], [1]*South Dakota State University, Brookings, SD*, [2]*Food Science and Human Nutrition Department, Qassim University, Buraydah, Saudi Arabia.*

Process cheese (PC) is a dairy product prepared by blending dairy and nondairy ingredients then heating the blend with agitation to produce a homogeneous product with an extended shelf life. Emulsifying salts (ES) have a critical effect on the emulsification characteristics of casein by sequestering the calcium from the calcium-paracaseinate phosphate complex in natural cheese. Lactose-6-phosphate (LP) is an organic compound produced from lactose that has the potential to function as ES. The objective of this study was to develop a process to produce PC with LP instead of disodium phosphate (DSP). LP was prepared by mixing 1 mol of a-lactose with 0.5 mol of sodium cyclo-triphosphate. The pH of the solution was adjusted using sodium hydroxide to get a pH of 12. The solution was stirred for 3 d at room temperature, and then it was concentrated to 52% total solids (TS). The ingredients in PC formulations were Cheddar cheese, butter, water, milk permeate, and LP (2, 2.4, 2.8, 3.2, 4, 5, and 6%) that were formulated to contain 17% protein, 25% fat, 56% TS, and 2% salt. PC with 2.0% DSP was also produced as a control. The PC was prepared by mixing all ingredients in a kitchenaid. A 25 g 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 analyzed for TS, pH, cooked viscosity, hardness, melted diameter, and melting temperature. The experiment was repeated 3 times using different batches of LP. No significant differences ($P > 0.05$) were detected in the TS of PC; however, the pH of PC made with LP (5.6–5.7) was lower than control (5.8). The cooked viscosity increased ($P < 0.05$) from 818 to 2,060 cP as the percentage of LP increased from 2 to 6%, while it was 660 cP in control. The hardness of PC made with LP was not different ($P > 0.05$) compared with control. The melted diameter decreased ($P < 0.05$) from 43 mm in control to 29 mm in 6% LP, while the melting temperature of PC increased ($P < 0.05$) from 37.7°C in control to 59.0°C in 6% LP. We conclude that LP can be utilized as a substitute for DSP in PC manufacture.

Key Words: processed cheese, lactose-6-phosphate, emulsifying salts

P175 Textural properties of acid-set Queso Blanco cheese made with microfiltered milk products. K. J. Blake*, M. R. Sainani, and K. A. Schmidt, *Kansas State University, Manhattan, KS.*

As the demand for native whey protein ingredients continues to rise outside of the dairy industry, microfiltered milk products like micellar casein concentrate (MCC) are becoming increasingly accessible for cheese production. This increase in microfiltered milk and milk products could cause potential issues within the acid-set cheese industry, as whey proteins are often included within the cheese protein matrix. Thus, this research investigated the impact on the textural properties of acid-set cheese produced with MCC using Queso Blanco as a model system. Three trials were completed where cheesemilk was standardized to a 1.2:1 casein to fat ratio using rehydrated milk protein concentrate 80 (MPC), milk protein isolate 90 (MPI) or MCC. Data were analyzed with univariate ANOVA methodology ($P < 0.05$) and significant means differentiated with Bonferroni comparisons ($P < 0.05$). Proximate analysis results showed that cheeses exhibited similar moisture (51.18%) and fat (15.13%) contents, but the cheeses made from MPC and MPI had a greater protein (27.05%) content compared with the MCC cheese (25.49%) likely due to the lower whey protein content in MCC. Cheese texture was assessed under 50% strain with a standard double-bite texture profile analysis (TPA) at 10, 30, 50, 70 and 90 d of cheese age. TPA results for springiness, cohesiveness and chewiness differed at 10 d compared with the other days. Cheeses differed in TPA hardness and resilience, although neither attribute was impacted by time. TPA resilience, springiness and cohesiveness showed differences between the MCC cheese vs. the MPC or MPI cheeses, whereas hardness and chewiness differed among the MPC, MPI and MCC cheeses. A trained descriptive panel assessed cheeses at 30 and 70 d in a monadic sequential design on a standard 15-point line scale. No significant differences were found in organoleptic textural properties over time. These results suggest that removing whey proteins via microfiltration before producing acid-set cheese would not cause noticeable texture differences for consumers; however, there could be implications when converting the cheese to reduced formats like shreds or crumbles.

Key Words: acid-set cheese, Queso Blanco, micellar casein concentrate

P176 Effect of fat, moisture, storage temperature, and time on shreddability of mozzarella cheese and their relation to other textural properties. G. Rathod*[1], S. Sutariya[2], R. Kumar[2], P. Upreti[3], and J. Amamcharla[1], [1]*Kansas State Univerisity, Manhattan, KS*, [2]*Agropur, Appleton, WI*, [3]*Nestlé Development Centre, Solon, OH.*

Mozzarella cheese (MC) is one of the popular cheeses available in different forms such as ball, loaves, and shreds. Shredded form is more convenient for consumers. It is important to understand the effect of fat content and also variations in storage temperature and age on the shreddability of MC. As per the experimental design, 3 batches of MC (pH-5.14 to 5.46) with different fat content (3 levels; average - 45.5, 40.8, and 29.1%) were procured directly from a commercial supplier and stored at 2 different temperatures (35 and 40°F) and evaluated in terms of shreddability, texture profile analysis (TPA), and wire cutting after storing for 2 and 3 weeks. Shreddability was analyzed using a cheese grating rig (A/GR) on a horizontal friction system base assembly (A/HFS) with coarse grater insert on the texture analyzer. MC ($8 \times 6 \times 4.5$ cm^3) was grated for 3 cycles by applying 1.8 kg dead weight. Shreddability parameters such as stiction force and work of grating were extracted from force-time curve. After every cycle, the cheese shreds were collected and weighed in grams. Stiction force for MC samples ranged from 6.43 to 32.25 N and work of grating ranged from 59.20 to 159.42 N·s. The weight of shreds was in the range of 1.93 to 5.54 g. Fat content of MC had a significant ($P < 0.05$) effect on stiction force, work of grating, and weight of shreds. Storage temperature had significant ($P < 0.05$) effect on the weight of shreds. Storage time had significant ($P < 0.05$) effect on stiction force and work of grating. It was observed that the stiction force and work of grating were positively correlated with the area and peak force extracted from wire cutting force-time curve and negatively correlated with weight of shreds. Similarly, TPA parameters such as hardness and cohesiveness were positively correlated with stiction force and work of grating, and negatively correlated with weight of shreds. It can be concluded that shredding was mostly affected by fat content followed by storage time, and least affected by storage temperature.

Key Words: mozzarella cheese, shreddability, cheese texture

P177 The effect of whey protein as a fat replacer on the physicochemical, microbiological, textural properties of Mozzarella cheese D. Zhang* and N. P. Shah, *Hong Kong University, Hong Kong, China.*

The influence of 2 whey protein-based fat replacers, CH-4560 (FR1) and YO-8075 (FR2), on physicochemical, textural and functional characteristics of low-fat Mozzarella cheeses (LFC, 12.5% fat) using starter culture consisting of *Streptococcus thermophilus* (ST) strain 1275, *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB) strain 859 and with or without *Lactobacillus casei* strain 290 were studied after 0, 7, 14, 21 and 28 d of storage at 4°C. CH-4560 and YO-8075 were microparticulate whey protein and were added directly into cheese milk at a rate of 0.5 g whey protein concentrates per 100 mL milk. The color of Mozzarella cheeses was closely linked to their composition and storage time, especially moisture and fat content. LFC was darker in color and had lower cheese yield (9.24%), and higher protein content (19.87%), and lactose (0.298%) and galactose (0.156%) as compared with other cheeses ($P < 0.05$). The yield of Mozzarella cheese increased to 10.82% and 10.66, respectively, when FR1 or FR2 was added. The average protein, fat and moisture content of whey protein-containing Mozzarella cheese were 18.3%, 10.58% and 60.98%, respectively. The pH value of Mozzarella cheese increased slightly after an initial decrease ($P < 0.05$) with LFC had the highest pH value (pH = 5.74), while FR2 containing Mozzarella cheese was the lowest (pH = 5.50). Serum expulsion in low-fat Mozzarella cheeses decreased significantly when supplied with whey protein due to the great water-holding capacity and high viscosity of whey protein concentrates. The addition of whey protein improved the textural characteristics (softer and stickier texture) of low-fat Mozzarella cheeses ($P < 0.05$). In addition, whey protein-containing cheese showed better pizza bake performance with more complete shred melt, less scorching and lower browning on the cheese surface. The grated cheese was placed horizontally in a glass tube, heating to 110°C for 10 min, then the meltability of the cheese was taken by the length of the melted cheese minus the length of the cheese before heating. The meltability of low-fat control cheeses increased by 3.33 mm to a maximum distance of 69.00 mm after 28 d of storage. Overall, adding whey protein as a fat replacer improved the pizza bake, texture, lightness and meltability of LFC, showing its potential as a fat replacer in dairy products.

Key Words: mozzarella cheese, dairy, lactic acid

P178 Evaluation of fatty acid profiles of domestic and imported varieties of cow, goat and sheep milk cheeses stored under different treatment regimens. Y. W. Park* and R. Paswan, *Fort Valley State University, Fort Valley, GA.*

Fatty acid profiles of cheeses are dependent on those of the original cheese milk. Goat milk contains significantly higher short and medium-chain fatty acids (MCT) (C4:0 - C14:0) than cow milk does. Caprine milk fat also has a unique characteristic of lower lauric:capric acid (C12:C10) ratio than bovine milk (0.46 vs. 1.16), which may affect flavor characteristics of 2 species milk cheeses. The objective of this study was to evaluate fatty acid profiles of 3 different species milk cheeses, including goat milk Gouda (GMG), cow milk Gouda (CMG), cow milk Cheddar (CMC), cow milk Monterey Jack (CMMJ) and sheep milk Manchego (SMM) cheeses, stored under 2 temperatures (4°C and −18°C) and 5 storage periods (0, 1, 2, 4 and 6 mon). Fats were extracted from all the experimental cheeses, and fatty acid methyl esters were derivatized and extracted in hexane solutions for fatty acid analysis. Fatty acid profiles of all experimental cheeses were quantified using a gas chromatograph (GC- 2010 Plus; Shimadzu Scientific, Canby, Oregon, USA), equipped with a fused silica capillary column (100 m x 0.25 mm x 0.2 μm film thickness), flame ionization detector and AOC-20s auto sampler. Results showed that the level of C18:0 was highest, followed by C16:0, C18:1, C14:0, C8:00 acids among all tested fatty acids in CMG. However, GMG and SMM had significantly ($P < 0.01$) higher levels of the corresponding fatty acids compared with those of CMG. With respect to C12:C10 ratio, CMG, GMG and SMM cheeses showed 0.68, 1.49, 0.85, respectively, indicating the commercial GMG had higher ratio than CMG, which revealed somewhat different ratio reported in the previous studies. ANOVA showed that the main factors, cheese type (CT) and storage period (SP) had significant effects on all fatty acid concentrations, whereas storage temperature (ST) had minimal influence on fatty acid contents. The 2-way interactions of CT × SP were significant ($P < 0.01$), while CT × ST and SP × ST effects were not significant for most of fatty acids. It was concluded that fatty acid contents of all varieties of commercial cow, goat and sheep milk cheeses were significantly affected by cheese type, storage temperature and periods.

Key Words: cow goat sheep cheeses, fatty acid composition, storage

P179 Comparison of lipolytic and proteolytic patterns of commercial cow, goat and sheep milk cheeses during 6 months of refrigerated and frozen storage. R. Paswan*, A. Singh, and Y. W. Park, *Fort Valley State University, Fort Valley, GA.*

Cheese quality is highly affected by lipolysis and proteolysis, where levels of free fatty acids, volatile compounds, peptides, amino acids and organic acids tend to be elevated in cheeses during storage and marketing. Commercial Gouda cheeses from cow milk (CMG) and goat milk (GMG), and an imported sheep milk Manchego cheese (SMM) as third species semi-hard reference cheese were purchased at a local retail outlet. Upon purchasing, the 3 types of cheeses were subdivided into equal portions and subjected to 2 temperatures (4°C and −18°C) and 5 storage period (0, 1, 2, 4 and 6 mon) treatments. All the experimental cheese samples were evaluated for basic nutrient contents, pH, acid degree value (ADV) and water-soluble nitrogen (WSN) contents. The experimental design was replicated twice, and data were analyzed using 2-way ANOVA. Results showed that mean protein, fat, ash and moisture contents (%) for CMG, GMG and SMM at 4°C storage were: 24.7, 24.1, 23.1; 25.7, 25.6, 29.4; 3.72, 3.51, 3.78; 42.7, 36.1, 31.8, respectively, indicating moisture content of GMG was lower ($P < 0.05$) than CMG, while all other basic nutrients were similar in both species cheeses. SMM contained even lower moisture than GMG. The respective ADV values of CMG, GMG and SMM at 4°C for 0, 2 and 6 mon storage were: 0.67, 0.71, 0.78; 1.25, 1.28, 2.03; 1.11, 1.25, 1.79, suggesting that significant lipolysis occurred in all cheeses as storage period advanced, but some ADV increases were not consistent in all cheeses. The pH changes in the 3 species cheeses throughout 6 mon storage period were also significant with some variations. In proteolytic patterns of CMG, GMG and SMM, all 3 cheeses showed increases in WSN with extended storage time, where the elevations of WSN in frozen cheeses were lower than at 4°C stored samples. The lower ADVs were also observed in frozen samples compared with the refrigerated ones, suggesting that less lipolysis occurred in the frozen samples than refrigerated chesses for the same storage period. It was concluded that lipolytic and proteolytic patterns of the commercial cow, goat and sheep milk cheeses were significantly ($P < 0.05$) influenced by storage temperature and period treatments.

Key Words: three-species cheeses, lipolysis, proteolysis

P180 Chemical and microbiological properties of traditional Egyptian cheeses. S. Awad* and A. Ibrahium, *Faculty of Agriculture, Alexandria University, Alexandria, Egypt.*

The National Food Safety Agency of Egypt (NFSA) was established by law number 1/2017 as an independent authority. NFSA started auditing food industries to ensure that food is produced under good conditions to meet the standards of food composition, safety and hygiene. NFSA does not cover all food sectors in Egypt until now, especially small sectors. About 80% of traditional Egyptian cheeses are produced by small sectors. This research was aimed to determine the chemical composition and undesirable microorganisms in traditional Egyptian cheeses that were produced in small sectors to assist cheesemakers in improving their products and meet the Egyptian Standards for chemical composition and microbiological quality. Forty samples of Karish cheese, 50 samples of soft white cheese, and 40 samples of Ras cheese were analyzed for chemical and microbiological properties. All analyses were carried out using ISO methods in accredited laboratory (ISO 17025/2017). Egyptian standards (ES) recommended that the moisture content should not exceed 62% and 58% in fresh and ripened soft cheeses respectively. Moisture was higher in 28% of soft cheese samples. Salt was higher in 7%, and protein content was lower in 10% of traditional white soft cheese samples. ES recommended that the salt should not exceed 9% and protein not less than 10%. In Karish cheese (low-fat cheese), moisture contents above the recommended levels were seen in 36% of samples and 68% of samples had a high level of fat in the dry matter, ES recommended the moisture should not exceed 75%, and fat in the dry matter not exceed 10%. Most of Ras cheese samples were within the Standard, there were only 2 samples of Ras cheese that contained more moisture, as the moisture should not exceed 40% in Ras cheese. Microbiological analysis of the cheese samples showed that only one Karish sample was Staphylococci-coagulase positive. The coliform count in the Ras cheese ranged from <1 to 5.20 log cfu/g with an average of 1.70 log cfu/g. Anaerobic spore-forming bacteria (*Clostridium* spp.) were found in 29 of the 40 Ras cheese samples. All the Egyptian cheese samples had higher numbers of yeasts and mold. All cheese samples were free of *Listeria monocytogenes*. The results obtained from this work will be used to develop specific procedures of risk management along with the traditional Egyptian cheeses production chain, as well as to establish a production procedure to produce high-quality cheese within Egyptian Standards.

Key Words: Karish cheese, soft white cheese, Ras cheese

P181 Bacterial isolation and identification from Appenzeller swiss cheese surface with antibacterial and antifungal activities.

R. D. Melendrez-Alvarez*, I. Garcia-Cano, A. C. Mayta-Apaza, J. Ortega-Anaya, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Analysis of the bacterial diversity in fermented foods is the first step in studying their microbial ecology, which can be influenced by the packaging conditions, moisture, ripening time, and other environmental factors. Fermented dairy foods such as cheese harbor a rich diversity of microorganisms with growth-specific requirements. Moreover, some of these microorganisms in fermented foods are known to secrete antibacterial (AB) and antifungal (AF) components that play a role in controlling pathogens and spoilage bacteria. The present study evaluated the AB and AF activities of isolated bacteria from the surface of an Appenzeller Swiss Cheese, including the bacterial identification using conventional and 16S rRNA methods and the metagenomic analysis because it presented non-mold development during a long time of storage. Phase 1: Phenotyping isolation yielded the primary result of the identification of 33 strains. Phase 2: The results from the 16S rRNA sequence, at a genus and species level, showed a relative abundance dominated by *Staphylococcus*; the results match with the taxonomic metagenome sequencing analysis showing 49.70% of abundance in the original sample of this genus, specifically of *S. equorum*, 37.5% of uncultured bacterium, 6.3% of *Brachybacterium ginsengisoli*, 4.5% of *Brevibacterium* sp., and 2% of others. Phase 3: Focuses on testing AB and AF activity performed in agar plates. Our results show that 4 strains had inhibition against *Aspergillus fumigatus*, 2 strains against *Bacillus amyloliquefaciens*, *Listeria innocua,* and *A. fumigatus*, and the last 2 showed activity against *L. innocua*. These results have led us to hypothesize that an antagonistic effect (inhibition halo) observed could be attributed to chitinase, protease, and volatile organic compounds production (butan-1-ol and 3-methylbutan-1-ol); these are common extracellular metabolites from *Staphylococcus*. Future work will be assessing the isolated collection against *Penicillium,* which commonly affects the cheese industry. Also, the nature of the metabolites with AB and AF activities must be identified.

Key Words: antagonism activity, inhibition, metabolites.

P182 Evaluation of lactose oxidase as an inhibitor of *Listeria monocytogenes* in a laboratory-scale fresh cheese model.

B. Flynn*, D. deRiancho, M. Lawton, and S. Alcaine, *Cornell University, Ithaca, NY.*

Listeria monocytogenes is a ubiquitous pathogen that can cause morbidity and mortality in the elderly, immune compromised, and fetuses of pregnant women. The intrinsic properties of fresh cheese—high aW, low salt content, and near-neutral pH—make it susceptible to *L. monocytogenes* contamination at various points in production. The aim of this study was to investigate the effect of lactose oxidase (LO), a naturally derived enzyme, on inhibition of *L. monocytogenes* growth in fresh cheese during various points of the production process. Lab-scale queso fresco was produced and inoculated with *L. monocytogenes* at final concentrations of 1 log cfu/mL and 1 cfu/100 mL through inoculation of the milk during the cheese make. Lactose oxidase was incorporated into the milk at final concentrations of 0.12 and 0.6 g/L. A positive control inoculated with *L. monocytogenes* and no lactose oxidase treatment and an uninoculated negative control were included. Cheeses were incubated at 6°C and enumerated for microbial counts on Modified Oxford Agar on d 0, 1, 2, 4, 7, 14, 21 and 28. Each experiment was performed in triplicate and an ANOVA and Tukey's Honest Significant Difference test were used individually for each time point to compare log differences in *Listeria* counts between the treatments and positive control. The higher inoculum exhibited significant differences ($P < 0.05$) between the 2 treatments and positive control by day 1 of storage and lasted through the entire trial. By day 28, the positive control grew to above 7 log cfu/g while the 2 treatments fell below the limit of detection (LOD) of 1.3 log cfu/g. With the lower inoculum, significant differences between the positive control and the treatment groups were also found by day 1 of storage. The positive control grew to above 7 log cfu/mL and the treatment groups fell below the LOD by day 21 and continued through day 28 of storage. These results suggest that LO is an effective inhibitor of *L. monocytogenes* in a lab-scale fresh cheese model. Different applications of LO will be explored to investigate its efficacy in different *L. monocytogenes* contamination scenarios.

Key Words: *Listeria*, cheese, lactose oxidase

P183 Long production days without intermittent cleaning and sanitation events alter the final microbial composition of aged Cheddar cheese.

J. Johnson*, C. Curtin, and J. Waite-Cusic, *Oregon State University, Corvallis, OR.*

The use of continuous production schedules in modern cheese manufacturing (>18 h without intermittent cleaning and sanitation) can create circumstances which support the growth and accumulation of microorganisms in the cheese production environment. This phenomenon has been observed at several stages of cheese production, including within the pasteurizer, in milk storage and transportation vessels, and more recently in the draining-matting conveyor (DMC) at an industrial Cheddar cheese manufacturer. Selover et al. (2021) demonstrated that coliforms growing on the surfaces of DMC belts were a primary source of intermittent coliform contamination in fresh Cheddar cheese. The current study aimed to continue this work by examining changes in microbial communities in aged Cheddar cheese produced at the start or end of the production day in the same cheesemaking facility. Our hypothesis was that cheeses produced at the end of the day would contain greater concentrations of nonstarter bacteria associated with the cheese production environment. Microbial communities in aged Cheddar cheese, produced at the start and end of 30 production days ($n_{start} = 30$, $n_{end} = 30$), were investigated using 16S rRNA metabarcoding and by plating on selective growth media (MRS, M17, m-Enterococcus, and MacConkey agars). Microbiome analysis revealed that cheeses produced at the end of the production day had significantly greater concentrations of 3 sequence variants (SVs), identified as *Streptococcus thermophilus* (2 SVs) and Streptococcaceae (1 SV) (upper-tailed *t*-test with Bonferroni correction, $a = 0.0045$). Comparing these sequences to those previously observed on the DMC belt surfaces revealed that both *S. thermophilus* SVs observed in the cheese were present at high relative sequence abundance on the belt surfaces at the end of the production day. These findings provide strong evidence that nonstarter bacteria contributing to microbial time-of-day differences in the aged cheese were the result of microbial growth in the food production environment, thus supporting our hypothesis. In addition, cheeses produced at the end of the day were found to have significantly greater moisture content, as compared with those produced at the start of the day (2-sample *t*-test, $a = 0.05$), therefore indicating that time-of-day differences can have direct consequences for cheese quality.

Key Words: cheese, quality, microbiome

P184 Comparison of starter and nonstarter lactic acid bacteria of 15 aged Cheddar cheeses from different regions using next-generation sequencing. S. L. Overbeck*[1], T. S. Oberg[1], C. J. Oberg[2,1], M. Lefevre[1], and D. J. McMahon[1], [1]*Department of Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT,* [2]*Department of Microbiology, Weber State University, Ogden, UT.*

The objective of this research was to test whether there were regional difference in the bacterial community in aged Cheddar cheeses. Retail packs of cheese were purchased that had been manufactured in different USA states or in Ireland or Australia. DNA was extracted followed by PCR using primers that amplify 460 bp in the V4 region of 16S rRNA. The DNA was sequenced and data processed using QIIME 2 software with raw sequence data demultiplexed, joined, and quality filtered followed by denoising using DADA2 and Deblur. Amplicon sequence variants (ASVs) were aligned and phylogenic trees created. For all cheeses, the microbiota was dominated by starter lactic acid bacteria (SLAB) at ~98% relative abundance (RA). The samples clustered into 2 major groups separated by SLAB with one cluster being dominated by *Streptococcus thermophilus* (52 to 99% RA) along with *Lactococcus lactis* while the other cluster was dominated by *Lc. lactis* at > 95% RA. Among the lactococci, using DADA2 provided more ASVs than that obtained using Deblur although the majority of lactococci still had the same ASV. This suggests that in all of the lactococcal cheeses the strains being used have a common sequence in their 16S rRNA with other traits, such as phage-hardening, not producing difference in this region. *Streptococcus thermophilus* is added to starter cultures for Cheddar cheese to shorten the manufacturing time. For cheeses in which *Lc. lactis* was the dominant SLAB, there was a subcluster in which a lactobacilli adjunct had presumably been added. Using Deblur or DADA2 produced similar results for lactobacilli with DADA2 yielding a few more operational taxonomic units. The commonly occurring nonstarter lactobacilli, *Lactocaseibacillus casei, Lactocaseibacillus paracasei*, *Lactocaseibacillus rhamnosus*, *Lactiplantibacillus plantarum* and *Latilactobacillus curvatus*, were present in some of the cheeses but not all. Other heterofermentative lactobacilli such as *Lentilactobacillus kefiri*, *Lentilactobacillus buchneii*, *Paucilactobacillus wasatchensis* and *Secundilactobacillus malfermentans* were also present. Differences in microbiome of the cheese appeared dependent on the individual manufacturer's choice of starter and adjunct cultures as well as the plant environment that provides a source for lactobacilli as well as very low levels of non-lactic acid bacteria in the cheese.

Key Words: cheese, starter culture, nonstarter lactic acid bacteria

P185 Selective media for the isolation of *Paucilactobacillus wasatchensis*. C. Wahlstrom*[1], M. Domek[1], M. Culumber[1], D. McMahon[2], and C. Oberg[1], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

The obligate heterofermentative bacteria *Paucilactobacillus wasatchensis* has been shown to cause late gas blowing in aged cheeses, which results in defects such as splitting and crumbling of the cheese block. The ability to quickly and accurately isolate *Plb. wasatchensis*, especially when it is present at low concentrations compared with other bacteria in a cheese sample, could be beneficial to the dairy industry. However, the current protocol for isolating *Plb. wasatchensis* is time intensive and imprecise. The goal of this study was to accurately detect *Plb. wasatchensis* when as few as 10^3 cfu/g are present within 72 h, as well as to inhibit competing starter lactic acid bacteria (SLAB) and nonstarter lactic acid bacteria (NSLAB) with one media. Testing was conducted using 24 well plates in a Tecan Infinite 2000 plate reader, in which 7 SLAB and NSLAB strains were tested in triplicate along with *Plb. wasatchensis* WDC04. Each well was filled with carbohydrate-restricted MRS (CR-MRS) broth containing 1% ribose, 2% Oxyrase and 0.01% 2-deoxyglucose, a glucose analog. Results showed that under these conditions *Plb. wasatchensis* WDC04 could complete its logarithmic growth phase in 28 h while the glycolysis inhibitor, 2-deoxyglucose, limited growth of the 7 SLAB and NSLAB strains. Growth of SLAB and NSLAB strains tested were significanly reduced as compared with WDC04 growth when 01% 2-deoxyglucose was added to the CR-MRS broth (*t*-test, $P < 0.05$%). *Lacticaseibacillus casei* and *Lacticaseibacillus paracasei*, 2 common NSLAB strains, showed the greatest level of inhibition between MRS broth (OD_{600} 1.28) and CR-MRS+2-deoxyglucose (OD_{600} 0.60 and 0.54, respectively) after 28 h. Results for the incorporation of 2-deoxyglucose into CR-MRS agar as a selective plating media for *Plb. wasatchensis* shows promise. This method could be used to determine the presence of *Plb. wasatchensis* in cheese when it is low concentrations (10^3 cfu/g) versus the high concentration of SLAB (10^8 cfu/g) that obscure its detection with current isolation techniques.

Key Words: lactic acid bacteria, nonstarter lactic acid bacteria (NSLAB), media

P186 *Paucilactobacillus wasatchensis* WCD04 biofilm formation and adherence to stainless steel. L. Mejias*[1], M. Culumber[1],

C. Oberg[1], and D. McMahon[2], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

Paucilactobacillus wasatchsensis WCD04 is a nonstarter lactic acid bacteria (NSLAB) that can cause gas production during cheese aging. This NSLAB is thought to be an environmental contaminant, but its reservoir is unknown. Since *Plb. wasatchensis* does not survive pasteurization, its persistence in cheese manufacturing could be the result of post-pasteurization contamination due to biofilm formation on processing equipment. We investigated the physiological conditions in which *Plb. wasatchsensis* reaches optimal growth for biofilm formation on stainless steel. Biofilms were grown on sterilized stainless steel washers (5.92 cm^2) in the wells of sterile, 24-well polystyrene culture plates. Washers were added to 2 mL of MRS supplemented with 1% ribose (MRS+R) broth amended independently for each experiment. Wells were inoculated with either 10 or 100 mL of a 4 d *Plb. wasatchensis* WDC04 culture. Variables tested were pH (4, 5, 6 or 7), galactose concentration (0.5%,1%, or 2%), lactose (1% or 2%), glucose (1% or 2%), and oxyrase (1%) in triplicate. Biofilms developed for 7 d at 30°C. Washers were aseptically removed and rinsed with sterile DW to remove planktonic cells. Attached biofilm was removed by vortexing in sterile saline for 1 min. The solution was serially diluted and plated in triplicate on MRS+R agar, then incubated at 30°C for 5 d. Biofilm growth was not detected at pH 4 and 7. Biofilm formation was observed minimally at pH 5.0 but the greatest in MRS+R broth (pH 6.0) with 2% galactose (6.8×10^4 cfu/cm^2). Similar growth was observed in MRS+R broth (pH 6.0) with 1% lactose (5.2×10^4 cfu/cm^2). Cell recovery was the greatest overall (5.8×10^6 cfu/cm^2) in MRS+R broth (pH 6.0) with 1% oxyrase (*t*-test, $P > 0.05$). Low-oxygen conditions appear to increase *Plb. wasatchensis* exopolysaccharide synthesis. Understanding optimal growth conditions for biofilm formation will provide opportunities to test methods for the prevention or removal of *Plb. wasatchensis* biofilms that may occur in dairy processing facilities.

Key Words: lactic acid bacteria, biofilm, exopolysaccharide

P187 Gluconate metabolism by *Paucilactobacillus wasatchensis* is another risk factor for late gas production in aging cheese.

K. Sorensen*[1], C. Oberg[1], T. Oberg[2], M. Domek[1], M. Culumber[1], and D. McMahon[2], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

Paucilactobacillus wasatchensis, a nonstarter lactic acid bacterium, can cause late gas production, and splits and cracks in aging cheese when it metabolizes a 6-carbon sugar, particularly galactose, in cheese to a 5-carbon sugar, resulting in the release of CO_2. Previous studies have not explained late gas production in aging cheese when galactose is not present. Based on the genome sequence of *Lb. wasatchensis* WDC04, genes for potential metabolic pathways were mapped using Knowledgebase Predictive Biology software (KBase). Modeling predicted *Plb. wasatchensis* WDC04 could metabolize gluconate. Gluconate contains 6 carbons and *Plb. wasatchensis* WDC04 contains genes to decarboxylate it to ribose-5-P and CO_2 using phosphogluconate dehydrogenase. This study's purpose was to determine if sodium gluconate, often added to cheese to reduce calcium lactate crystal formation, could result in gas production when metabolized by *Plb. wasatchensis*. Carbohydrate-restricted MRS (CR-MRS) was mixed with varying ratios of ribose, sodium gluconate and/or D-galactose (1% total sugar content) in triplicate. Oxyrase (1.8%) was also added to create an anaerobic environment in the CR-MRS tubes similar to aging cheese. Tubes were inoculated with a 4-d culture of *Plb. wasatchensis* WDCO4, incubated at 30°C and results recorded over 8 d. Gas production was measured by determining gas volume in Durham tubes in the CR-MRS tubes. Of the 10 ratios used, gas was produced in 6 with the highest gas production resulting from 1% sodium gluconate with no added ribose or galactose followed by the ratio of 0.3% ribose/0.7% gluconate (1% total sugar concentration). Assuming other strains of *Plb. wasatchensis* have the same genes for metabolizing gluconate and producing CO_2 gas, adding sodium gluconate during manufacture of Cheddar cheese is another risk factor for growth of *Plb. wasatchensis* during cheese aging and subsequent unwanted gas production resulting in the formation of splits and cracks in cheese.

Key Words: lactic acid bacteria, gluconate, cheese

P188 Depletion of galactose by galactose-positive lactose-negative protective cultures prevents gas formation by *Paucilactobacillus wasatchensis* in a model gas production test.

D. McMahon*[2], C. Oberg[1], I. Green[2], J. Broadbent[2], and R. Thunell[2], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

Gas production by obligatory heterofermentative lactobacilli such as *Paucilactobacillus wasatchensis* is a sporadic problem in Cheddar cheese and can cause slits and cracks in the cheese. Growth and gas production by *Plb. wasatchensis*is not as rapid as in facultative heterofermentative lactobacilli which makes observation of gas production difficult to observe on a consistent basis. Our objective was to develop a model system that could be used as a gas production test. This test would account for co-utilization of galactose with ribose during exponential growth of *Plb. wasatchensis,* and then test if galactose could be removed by using a galactose-utilizing adjunct culture to prevent gas formation. *Paucilactobacillus wasatchensis* WDC04 was inoculated into carbohydrate-restricted MRS broth containing various ratios of ribose and galactose with growth monitored during incubation at 23°C. Gas production was detected using a Durham tube inverted on a 6-cm long capillary tube to increase the volume from which gas could be captured. Gas production was sporadic except when 10^5 cfu/mL of *Plb. wasatchensis* WDC04 was inoculated into broth containing 0.3% ribose and 0.7% galactose, incubated at 23°C, then gas bubbles were observed in 8 out of 9 replicates. This was designated as gas production broth and used for further testing. Gas production was observed after 8 d incubation by which time galactose levels had decreased to 0.15%. It was presumed that ribose was exhausted before this and the cells switch to using galactose for energy. When either of 3 lactose-negative galactose-positive cultures (*Pediococcus acidilactici* 23F, *Lacticaseibacillus casei* UW4 or *Lactobacillus helveticus* 7995) was inoculated along with *Plb. wasatchensis* WDC04 into the gas production broth, all of the galactose was depleted within 4 d with no gas production observed. Such protective cultures have the potential for preventing unwanted gas production in cheese. The gas production test has application in the study of CO_2 production by other obligatory heterofermentative lactobacilli.

Key Words: lactic acid bacteria, cheese, gas production

P189 Metabolic profiling of *Lactilactobacillus curvatus* WSU1 for antimicrobial metabolites.

D. Leatham*[1], T. Oberg[2], R. Ward[2], C. Oberg[1], and D. McMahon[2], [1]*Weber State University, Ogden, UT,* [2]*Utah State University, Logan, UT.*

Lactilactobacillus curvatus is a common nonstarter lactic acid bacteria (NSLAB) in cheese. If *L. curvatus* is induced to produce antimicrobial compounds it would be important to the food industry, since it could be used to inhibit pathogens and/or spoilage organisms in cheese. Using the *L. curvatus* WSU1 genome, bioinformatics analysis revealed possible genes for a metabolic pathway which utilized lactic acid to produce propanol and propionic acid, 2 antimicrobial compounds. Among these genes were 3 encoding for the enzyme diol dehydratase. This enzyme is present in *Limosilactobacillus reuteri*, a NSLAB that can utilize glycerol to produce 3-hydroxypropionic acid and 3-hydroxypropionaldehyde (reuterin), 2 antimicrobials. It is hypothesized that *L. curvatus* could also produce these compounds. Since these metabolic processes are anaerobic, cultures were grown in septum-sealed medicine bottles with the headspace purged using 95% N_2/5% CO_2. Cultures were grown in carbohydrate-restricted MRS media containing either lactate, 1,2-propanediol, hydroxyacetate, acetate, or glycerol at an 80 mM concentration in triplicate. They were grown in duplicates with half containing B12 because diol dehydratase is B12-dependent. A GC analysis was done to test for the presence of 3-hydroxypropionaldehyde, propionic acid, propanol, and 3-hydroxypropionic acid at d 0, 1 and 7. GC measured presence or absence of specific products based on identification standards, but peaks were not quantified. Peaks had to be detected in all 3 replicates for product confirmation. GC results show that *L. curvatus* WSU1 cannot produce propionate from lactate but can produce propionate when given 1,2-propanediol. This indicates the genome is lacking the gene to convert lactate to lactaldehyde, but does have functioning genes to convert 1,2-propanediol to propionate. When grown on glycerol, small amounts of 3-hydroxyproprionate were produced, indicating either fermentation conditions were not ideal for the pathway or the diol dehydratase had less affinity for glycerol than for 1,2-propanediol. These results indicate the potential for *L. curvatus* WSU1 to be used as a bioprotective adjunct in dairy fermentations.

Key Words: lactic acid bacteria, metabolites, cheese

P190 Screening of a food-grade antimicrobial from *Lactococcus* species of raw milk origin, and optimization of the antimicrobial activity.

A. Ichinomiya*, S. Anand, R. Nauth, and V. Mistry, *Dairy and Food Science, South Dakota State University, Brookings, SD.*

Antimicrobials produced by *Lactococcus* species of milk origin are valuable as natural inhibitors against food spoilage and pathogenic microorganisms. An example is nisin, which has diverse food preservation applications. This study was performed to isolate and identify *Lactococcus* species from raw milk having antimicrobial activity against selected food spoilage organisms, with the potential to optimize conditions for improved antimicrobial production. During the initial screening, lactic acid bacteria were isolated on MRS agar from 47 raw milk samples from the University dairy farm. Based on colony morphologies on the selective medium, Gram staining, and catalase test, 5 isolates were selected, and screened for antimicrobial activity using agar well assay technique. All the isolates were observed to secrete antimicrobial compounds, active against a test strains *Micrococcus luteus* (ATCC 10240) and *Lactococcus lactis* ssp. *cremoris* (ATCC 19257). The diameter of clear zone around the well of 5 isolates corresponded to an antimicrobial activity of 48.90, 265.41, 213.15, 371.95, and 505.00 IU/mL respectively. Two larger isolates, N40615 and N50615 ($P < 0.05$), were chosen for further experiments but N50615 produced less antimicrobial activity against *Lactococcus lactis* ssp. *cremoris* (ATCC 19257). As a result, N40615 was selected for further studies. On the evaluations for identification and characterization, the inhibitory substance was not identified to be either hydrogen peroxide or bacteriophage. As the inhibitory activity was inactivated by α-chymotrypsin, proteinase, and trypsin, it was concluded that the isolate produced nisin-like antimicrobial activity. N40615 in autoclaved skim milk medium heat treated at 85°C for 15 min supplemented with 0.50% peptone, 0.25% yeast extract, and 0.3% dipotassium phosphate (W/W), without pH control, had 1,571.0 IU mL^{-1} of antimicrobial activity, and the one with pH control, had 3,896.7 IU/mL of antimicrobial activity ($P < 0.05$). This nisin-like bacteriocin is inhibitory to salad dressing spoilage organisms such as *Lactobacillus brevis* (ATCC 367), *Lactobacillus buchneri* (ATCC11305), and *Lactobacillus plantarum* (ATCC 8014). In conclusion, the results demonstrated that the antimicrobial activity of the isolated culture N40615 from raw milk was comparable to that of nisin in Nisaplin.

Key Words: *Lactococcus lactis* ssp. *lactis*, nisin, optimization

P191 Use of acid whey and bovine colostrum as potential sources of antimicrobial compounds.

I. Garcia-Cano, A. Krentz, S. Badiger, D. Rocha-Mendoza*, E. Kosmerl, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Acid whey (AW) is a by-product generated from dairy processing that the industry has long struggled to use in an economically and environmentally sustainable application. Due to its high biological oxygen demand, AW cannot be easily disposed of into the environment. However, AW contains valuable proteins such as lactoferrin that has antimicrobial activity. Lactoferrin is also a component in bovine colostrum. The objective of this study is to utilize AW as a culture media, in combination with colostrum and *Lacticaseibacillus rhamnosus* (*Lbrh*) to generate proteins with antimicrobial activity against *Escherichia coli and Listeria innocua.* Seven fermentations were performed with different combinations of AW, colostrum, and *Lbrh* to determine the optimal mixture that produces the highest antibacterial activity. After 48 h fermentation, each batch was centrifuged to remove bacteria cells. The supernatant was ultrafiltered using a 10- and 3-kDa cut-off membranes. The minimum inhibitory concentration (MIC) against *E. coli* and *L. innocua* was determined by agar diffusion. The bioavailability and cytotoxicity of 10- and 3-kDa samples were evaluated over Caco-2 cell line. The results from the agar diffusions indicate that antimicrobial activity was observed in 3 of the 7 fermentations. The specific activity of AL (AW + *Lbrh*) and ACL (AW + colostrum + *Lbrh*) were 4 times greater than ML (MRS media + *Lbrh*). The MIC for ACL 10-kDa was 200 and 300 mg/mL against *L. innocua* and *E. coli*, respectively. ACL 3-kDa had a MIC of 100 mg/ml against both strains and a zone of inhibition 2.5 times greater than ACL 10-kDa. ACL 10- and 3-kDa samples did not have an effect over Caco-2 cell line, as they showed low cytotoxic effect and the viability of the cells was not compromised, indicating no damage to model intestinal epithelial cells. Acid whey in combination of bovine colostrum can be used as potential sources for low molecular weight proteins with high antimicrobial activity that can provide future uses of a natural preservative from underutilized dairy by-product

Key Words: acid whey, *Lactobacillus rhamnosus*, antimicrobial activity

P192 Antibacterial activity produced by *Bacillus velezensis* GF 610 against common milk spores.

X. Liu, I. Garcia-Cano*, D. Rocha-Mendoza, R. Jiménez-Flores, and A. Yousef, *Department of Food Science and Technology, The Ohio State University, Columbus, OH.*

Spoilage due to contamination of spores in milk and dairy products represents a great challenge for the dairy industry, in particular to the milk powder producers. Peptidoglycan hydrolases (PGHs) are a new generation of antimicrobials, which show promise to inhibit sporulated microorganisms. PGHs with different molecular weights (90, 50, 34 and 30 kDa) produced during growth of *B. subtilis* have been reported. In previous studies, 48 strains of *Bacillus* spp. produced extracellular antimicrobials compounds; however, identities of the antibacterial compounds were unknown. The aim of this work was to find the strain with highest antibacterial activity and identify the nature of the protein responsible for this activity. The bacteria were grown in tryptic soy broth and harvested by centrifugation at the end of the logarithmic phase. Such as a first approach *Micrococcus lysodeikticus* and *Bacillus subtilis* cells were used as a substrate to evaluate the antibacterial activity by agar diffusion, turbidimetric and zymogram techniques. The zymogram assay allowed us to detect the bands responsible of the antibacterial activity. LC-MS/MS was used for the protein identification. Thirty of the 48 *Bacillus* strains showed lytic activity against *M. lysodeikticus*. From the 30 positive strains, *B. velezensis* GF 610 showed the highest activity, resulting in a 76.9% turbidity reduction. Zymogram analysis of the culture supernatant of this strain revealed 2 lytic bands around 30- and 16-kDa. In the 30 kDa band, a PGH sequence was identified by LC-MS/MS. No PGH was detected in the 16 kDa band. Both bands retained partial activities after heating at 90°C for 30 min. *B. velezensis* GF 610 showed effective bactericidal activity during the germination and propagation of *B. subtilis* spores isolated from powdered milk, within the tested 10 h. The lytic bands against germinated *B. subtilis* spores were identical to those detected against *M. lysodeikticus* by the zymogram. This study screened a strong PGH-producer, *B. velezensis* GF 610, and revealed that its PGH may be a potential candidate to control *B. subtilis* spores in milk. Further experiments using *Bacillus licheniformis* and *Bacillus pumilus* that are the predominant spore species in milk powder, will be conducted. As well, biochemical characterization of the antibacterial protein will be carried out

Key Words: peptidoglycan hydrolases, *Bacillus velezensis* GF 610, *Bacillus subtilis* spores

P193 Characterization of the fermentation and sensory profiles of novel yeast-fermented acid whey beverages.

S. Luo*, D. deRiancho, T. Demarsh, and S. Alcaine, *Cornell University, Ithaca, NY.*

Acid whey, a substantial by-product of dairy processing, has become a major sustainability obstacle due to a lack of safe and reliable disposal pathways. This study proposes a solution to this issue by transforming yogurt acid whey (YAW) into potentially palatable and marketable beverages through yeast fermentation. In this study, 5 prototypes were developed and fermented by *Kluyveromyces marxianus*, *Brettanomyces bruxellensis*, *Brettanomyces claussenii*, *Saccharomyces cerevisiae* (strain: Hornindal kveik), and IOC Be Fruits (IOCBF) *S. cerevisiae* yeasts. Their fermentation profiles were characterized by changes in density, pH, and concentrations of cells and organic acids. The prototypes were also evaluated on 26 sensory attributes, which were generated through a focus group of 14 participants. Analysis of variance and Tukey's test were used to compare the differences in sensory ratings. While *S. cerevisiae* (IOCBF) exhibited the fastest fermentation (8 d) and *B. claussenii* the slowest (21 d), *K. marxianus* and *S. cerevisiae* (Hornindal kveik) exhibited similar fermentation rates; finishing on d 20. The change in pH was similar for all 5 strains (from around 4.45 to between 4.25 and 4.31). Cell concentrations remained stable throughout the fermentation for all 5 strains (at around 6 log cfu/mL) except in the case of *S. cerevisiae* (Hornindal kveik), which ultimately decreased by 1.6 log cfu/mL. *B. bruxellensis* was the only strain unable to utilize all of the sugars in the substrate, with residual galactose remaining after fermentation. While both *S. cerevisiae* (IOCBF)- and *B. claussenii*-fermented samples were characterized by a fruity apple aroma, the former also had a significant lactic acid/dairy ($P = 0.01$) and yeasty aroma ($P = 0.04$). A chemical (petrol/gasoline) aroma was perceived in samples fermented by *B. bruxellensis* ($P = 0.01$) and *K. marxianus*. A poorly aged/rancid cheese aroma also resulted from *B. bruxellensis* fermentation. In terms of appearance and mouthfeel, the *S. cerevisiae* (IOCBF)-fermented sample was rated the cloudiest ($P < 0.01$), with the heaviest body. This study provides a toolkit for product development in a potential dairy-based category of fermented alcoholic beverages, which can increase revenue for the dairy industry by upcycling the common waste product YAW.

Key Words: acid whey, novel fermented beverage

P194 Production and sensory properties of fermented milk by *Lactobacillus reuteri* J1.

Y. Zhang[1], J. Zhao[1], H. Zheng[2], C. Man[1], and Y. Jiang*[1], [1]*Key Laboratory of Dairy Science, Ministry of Education, Department of Food Science, Northeast Agricultural University, Harbin, Heilongjiang, China,* [2]*Department of Food Bioprocessing and Nutrition Sciences, Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC.*

Lactic acid bacteria are widely used in fermented dairy products, which not only enhance the flavor, but also improve the functional quality. Some of *Lactobacillus reuteri* with great functional properties and fermentation characteristics have caught significant interests. *L. reuteri* J1 as culture starter in milk matrix was studied in this work. After 3 h in artificial gastric juice and 8 h in artificial intestinal juice, the survival rate of the strain (dilution coating method) was 89.98% and 79.95%, respectively. It showed an excellent tolerance in simulated gastrointestinal juices. Adhesion rate is one of the important indicators for the development and application of lactic acid bacteria. In addition, higher viable counts of *L. reuteri* could improve the nutritional value of fermented milk. High adhesion and viable counts could be the feature points of concern. Caco-2 cells were co-incubated with *L. reuteri* J1 for 2 h, results showed that it had a great capacity of adhesion with 7.56 log cfu/mL. The viable count was >7.87 log cfu/mL in the milk system. The fermentation process using *L. reuteri* J1 was optimized. Water-holding capacity, viable count, sensory characteristics and antioxidant capacity were tested for the fermented dairy food samples. Results were expressed as mean ± standard deviation, and statistical significance was determined at $P < 0.05$. Results showed that the optimum inoculation rate was 4% (wt/wt) at 37°C for 24 h, and the end point pH was 4.85. We also used *Streptococcus thermophilus* and *Lactobacillus bulgaricus* to ferment milk as the control sample. Under these conditions, the final viable cell count of *L. reuteri* reached to $>1 \times 10^9$ cfu/mL and the water-holding capacity significantly increased ($P < 0.05$) compared with the control sample. The antioxidant capacity of fermented milk was significantly enhanced ($P < 0.05$) by fermenting with *L. reuteri* J1, the scavenging rates of OH and DPPH were 91.38% and 90.32%, respectively. Viscosity and gel firmness were measured as textural sensory attribute indicators and the results were 1,472.59 cps, 46.51 g, respectively. The viscosity and gel firmness were all significantly increased ($P < 0.05$) compared with the control sample. The results of this study suggested that *L. reuteri* J1 possessed a prospect to be applied in fermented dairy foods. It provides a practical insight of using *L. reuteri* J1 as a culture to develop a novel functional fermented milk.

Key Words: fermented milk, *Lactobacillus reuteri*, sensory properties

P195 Optimization of protoplast formation for the probiotic *Lactobacillus acidophilus*.

R. Page* and K. Aryana, *Louisiana State University, Baton Rouge, LA.*

Transformation of specific *Lactobacillus acidophilus*, a known probiotic, strains into protoplast are not known or fully explored. The aim of this study was to determine the optimal temperature, incubation time, and buffer molarity when using an optimized concentration of lysozyme for the formation of protoplast. Freeze-dried *L. acidophilus* cells were suspended in 20 m*M* of HEPES buffer (pH 7) at different sucrose osmotic conditions of 1, 1.5, or 2 *M* to yield a 10^8 cell concentration. Reagents and cell suspension before enzyme addition were incubated at either 37°C or 22°C for 10 min. After lysozyme (125 µg/mL) addition the cell suspensions were incubated for 30 min, 1 h, or 2 h at 37°C. Three fields (3 replicates) were studied by phase-contrast microscopy. Cell wall digestion were seen in all treatments after 30mins of incubation at 37°C at a fixed lysozyme concentration of 125 µg/mL. Cell lysis and aggregation of *L. acidophilus* cells increased as incubation time increased. Increasing molarity had no significant effect on protoplast production and produced the highest yield of protoplast for all treatments after 30 min. Cells suspended in 2 *M* HEPES buffer were observed to yield a higher % of protoplasts and reduced cell lysis when incubated for 2 h compared with the cells treated with 1 and 1.5 *M L. acidophilus* cell suspension, lysozyme and buffer solutions adjusted to 37°C before incubation displayed a higher yield of protoplast after 2 h compared with solutions adjusted to 22°C. A lysozyme concentration of 125 µg/mL incubated at 37°C for 30 min was sufficient in producing a high yield ($> = 90\%$) of protoplast. A 2-way ANOVA was preformed to determine differences in treatments. A *P*-value <0.05 was noted as significantly different. Buffer molarity and temperature both had significant effects on protoplast yield when incubated for 2 h.

Key Words: probiotic, protoplast

P196 *Paenibacillus odorifer* spoilage of fluid milk products and the potential impact of rework practices.

C. Rush, S. Burroughs, J. Johnson, L. Meunier-Goddik, and J. Waite-Cusic*, *Oregon State University, Corvallis, OR.*

Rework is a common practice in the dairy industry as a way to help minimize waste while recovering cost from products that might otherwise be unsaleable. However, the quality implications of reworking dairy products have not been investigated and processors have speculated that these practices could contribute to an increase in premature spoilage due to microbial activity causing flavor defects. *Paenibacillus odorifer,* a psychrotrophic spore-former, has been observed to cause flavor defects (“solventy,” “rubbery,” “athletic tape”) in both fluid and chocolate milk before the end of shelf life. The objective of this study was to characterize spoilage defects, determine growth rates, and sporulation status of *P. odorifer* strains in fluid and chocolate milk to support model development to predict spoilage rates of reworked products. Commercial UHT-pasteurized fluid milk and chocolate milk samples were independently inoculated with 4 strains of *P. odorifer* isolated from pasteurized fluid milk (n = 2) and chocolate milk (n = 2) to achieve an initial cell density of 1–2 log cfu/mL. Samples were stored at 4°C and 7°C and aerobic plate counts, and organoleptic observations (visual, aroma) were determined throughout a 21-d shelf life. Aroma deviations were confirmed across all 4 strains on d 15 for fluid milk (6.6–6.8 log cfu/mL) and on d 10 for chocolate milk (5.8–6.1 log cfu/mL) at 4°C and d 6 for both fluid milk (6.3–7.1 log cfu/mL) and chocolate milk (6.4–7.2 log cfu/mL) at 7°C. Gram stains confirmed no sporulation in milk through the shelf life. Plate count data were fit to a 3-phase linear model to ascertain growth parameters for each strain. Strain-specific growth rates ranged from 0.65 to 0.82 log cfu/mL/day and increased to 0.90–0.94 log cfu/mL/day in chocolate milk at 4°C. At 7°C, the growth rates in fluid milk increased to 1.4–2.2 log cfu/mL/day and 1.9–2.2 log cfu/mL/day in fluid and chocolate milk, respectively. Next steps will be to develop and conduct a Monte Carlo simulation to predict the impact of various rework practices on product shelf life.

Key Words: rework, spoilage, *Paenibacillus odorifer*

P197 Microbiological comparisons between milk sock filters and raw milk.

B. Riesgaard*[1], A. Torres[1], J. Johnson[1], C. Rush[1], Z. Atamer[1,2], and J. Waite-Cusic[1], [1]*Oregon State University, Corvallis, OR,* [2]*University of Hohenheim, Stuttgart, Germany.*

Dairies use milk sock filters to capture large debris (hay, soil, clots), before passage through the cooling heat exchanger, on the way to the bulk milk tank. Milk leaves the udder at 38°C and travels through the milk sock filter at warm temperatures that can support microbial growth. According to the Pasteurized Milk Ordinance, milk sock filters should be changed after each milking session or once every 24 h in continuous milking operations. The objective of this study was to characterize and compare the microbial diversity and load of milk sock filters and raw milk on a single dairy farm. Samples were collected from the Oregon State University Dairy (~80 milking head) after the morning and evening milkings for 5 d. Microbial populations on milk filters (n = 10) and in raw milk (n = 10) were characterized by 16S metabarcoding and enumerated by standard plating methods on tryptic soy agar (TSA), MacConkey (MAC) agar, and MRS agar. Plates were enumerated after 48 h of incubation at 30°C (MRS, hypoxic chamber) or 37°C (TSA, MAC). The accumulation of material on milk sock filters were visualized by scanning electron microscopy. After 2 h of use, milk sock filters harbored loads of 7.4 log cfu/g aerobic plate count (APC), 5.9 log cfu/g lactic acid bacteria (LAB), and 5.3 log cfu/g coliforms. These cell densities were significantly higher than the bulk raw milk (APC: 3.9 log cfu/mL, LAB: 3.0 log cfu/mL, coliforms: 2.5 log cfu/mL). Species richness (α diversity) was significantly lower on milk sock filters (300 ± 115 sequence variants (SVs)) compared with bulk milk (507 ± 73 SVs). *Acinetobacter* SV680, Peptostreptococcaceae SV3537, and *Brumimicrobium* SV2017 were present at significantly higher relative sequence abundance in raw milk. None of the individual SVs were consistently responsible for increased bacterial loads in milk sock filters. Increased bacterial loads on milk sock filters demonstrate the ability of the filter to capture bacteria. Further studies will evaluate whether extended milking sessions (up to 8 h) would support the growth of these captured bacteria and if these long periods of use may have a negative impact on raw milk quality.

Key Words: raw milk, milk sock filter, bacteria

P198 Determination of an effective sanitizing procedure for *Listeria innocua* in personal protective equipment used in dairy facilities.

K. A. Nieto*[1], L. Sabillón[1,2], B. Martínez[1,2], J. Stratton[1,2], and A. Bianchini[1,2], [1]*Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE,* [2]*Food Processing Center, University of Nebraska-Lincoln, Lincoln, NE.*

Listeria monocytogenes can survive and grow under wet environmental conditions often encountered in dairy processing facilities. The source of microbial contamination may include employees and their personal protective equipment (PPE), which often contact product and food contact surfaces. This study investigates the effectiveness of chlorine, quaternary ammonia, and peroxyacetic acid (PAA) in reducing *Listeria innocua* contamination from different types of gloves, aprons, and boots. The PPE was inoculated with a 2-strain cocktail of *Listeria innocua* that was suspended in either PBS or skim milk to determine the potential effect of organic matter. A linear mixed model with a randomized complete block design was performed to determine the best sanitation protocol and the effect of organic matter at $P \leq 0.05$ using SAS software (SAS 9.4). With PBS, results showed 2.08 to 4.60 log cfu/in^2 reduction on the different types of aprons, with the PAA-based sanitizer being the most effective. Different kinds of gloves showed a similar average reduction (2.15 – 4.16 log cfu/in^2). In comparison, boots showed a 1.00 – 1.70

log cfu/in^2 reduction. With skim milk as the carrier of contamination, the sanitizers achieved less than 1.00 log cfu/in^2 reduction on the aprons, showing no significant differences in effectiveness among them. Similarly, reduction levels of 0.5 to 0.8 and 0.7–1.0 log cfu/in^2 were observed on the different gloves and boots, respectively. Overall, the different sanitizers' antimicrobial activity was diminished in the presence of organic matter. This highlights the negative impact of organic matter in sanitizer effectiveness and the need to include cleaning steps to achieve the desired reduction in the bacterial population. Additionally, an extensive cleaning protocol that included cleaners, mechanical action, followed by chlorine sanitizer was evaluated. This cleaning regimen achieved a ≥3-log cfu/in^2 reductions on the different types of PPE. This study highlights the importance of detergent choice and use of scrubbing as essential steps to reduce and control *Listeria* from PPE

P199 Evaluation of the transmission of *Listeria innocua* from personal protective equipment (PPE) to the plant environment and food products. K. A. Nieto*[1], B. Martínez[1], J. Stratton[1,2], and A. Bianchini[1,2], [1]*Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE,* [2]*Food Processing Center, University of Nebraska-Lincoln, Lincoln, NE.*

Listeria monocytogenes is a foodborne pathogen that can grow and survive in the dairy processing environment. Employees and their personal protective equipment (PPE) are potential vectors of this organism during production. The objective of this study was to evaluate *Listeria innocua* transference from PPE to food products (cheeses), and dairy processing plant surfaces (cutting boards, stainless steel, and dairy brick floors). A total of 5 combinations of PPE and surfaces of interest were selected. PPE was inoculated using either Phosphate Buffered Solution (PBS) or skim milk as the carrier for *L. innocua*. A repeated-measures linear mixed model with a randomized complete block design was used to determine the significance of *Listeria innocua* transmission from each PPE to the surface of interest while considering organic matter effect ($P < 0.05$). Transmission mediated by gloves and aprons were tested using a texture analyzer to apply a constant force (2.942 N) and time (5 s) mimicking one contact transfer. Additionally, boots inoculated with *L. innocua* were used to assess bacterial transmission to floors. In general, contamination carried by organic matter led to a higher transfer of *Listeria innocua* from PPE to tested surfaces than when PBS was used ($P < 0.05$). With PBS, consecutive touches from gloves to food products led to a decline in transfer; however, *Listeria* populations were never eliminated. With skim milk, no transfer decline was observed in the different combinations of PPE and surfaces. When different combinations of PPE-surfaces were evaluated and skim milk was the carrier of contamination, on average, gloves transferred 5.33 log cfu/in^2 *L. innocua* to queso fresco, followed by 4.28 log cfu/in^2 to Cheddar cheese. A 4.01, 2.66, and 2.61 logs cfu/in^2 average transmission was observed from gloves to cutting board, aprons to stainless steel, and boots to dairy tiles. In general, bacterial transference from PPE to food contact surfaces and food products was higher than those observed between PPE and non-food contact surfaces, emphasizing the risk associated with the potential cross-contamination of the final product.

P200 Developing an affordable hyperspectral imaging system for rapid identification of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in dairy products. P. Unger*, A. Sekhon, and M. Michael, *Washington State University, Pullman, WA.*

With the increased concerns about food safety and defense, hyperspectral imaging (HSI) can serve as a potential novel technology for rapid and reliable identification method for pathogenic bacteria. The development of an affordable HSI system using a compound microscope and HSI camera offers the possibility for other researchers to explore this technology. The objective of this study was to develop and evaluate the efficacy of an affordable HSI system to identify single and mixed strains of foodborne pathogens in dairy products. This study was designed as a randomized complete block design with 3 replications. Three strains of *Listeria monocytogenes* and *Escherichia coli* O157:H7 were evaluated either as single or mixed strains with the HSI system in growth media and selected dairy products (whole milk, cottage and Cheddar cheeses). Freshly prepared single or mixed strains of respective pathogens or inoculated dairy products were streaked on selective media (PALCAM and or Sorbitol MacConkey agar) for isolation. An isolated colony was selected and mixed with 1-mL of HPLC water, vortexed for 1-min, and spread over a microscope slide. Images were captured at 2,000X magnification on the built HSI system at wavelengths of 400 nm to 1,100 nm with 5 nm band intervals. For each image, 3-cells were selected as regions of interest (ROIs) to obtain hyperspectral signatures of respective bacteria. Cells were classified by their hyperspectral signatures as either *L. monocytogenes* or *E. coli* O157:H7 using K-nearest neighbor (KNN) and cross-validation technique in R software. With the implementation of KNN ($k = 3$), classification accuracies of 58.97% and 61.53% were obtained for *E. coli* O157:H7 and *L. monocytogenes*, respectively. This preliminary work on the affordable HSI system shows great promise for the rapid identification of foodborne pathogens in a variety of dairy food matrices.

Key Words: identification, hyperspectral imaging, pathogens

P201 Need for environmental monitoring programs in small dairy plants based on initial screening of *Listeria* spp. and *monocytogenes*. T. T. Lott*, R. D. Ralyea, S. E. Roof, A. S. Harrand, A. D. Zuber Gianforte, K. Ospina, A. Trmcic, N. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Food safety incidences linked to dairy products represent a significant risk for the dairy industry. For example, in 2017 Vulto Creamery, a small dairy facility in Walton, New York, was identified as the likely source of an outbreak of listeriosis affecting 8 people in 4 states. As *Listeria monocytogenes* is the bacterium most likely to cause severe foodborne disease outbreaks linked to dairy products, the goal of this project is to develop and implement improved practices to control *L. monocytogenes* in 3 fluid milk, 3 ice cream, and 3 cheese processing facilities in New York State. To evaluate the potential *Listeria* presence in the plant environment, an initial screening was performed at each plant. The screen involved collecting 40 – 100 sponge samples at each facility with approximately 40%, 50%, and 10% of samples collected in environmental zones 2, 3, and 4, respectively. Samples were enriched in buffered *Listeria* enrichment broth (BLEB) at 30°C and plated on differential and selective media [*L. monocytogenes* plating medium (LMPM) and Modified Oxford Agar (MOX)]. Plates were then evaluated for presumptive *Listeria* spp. and *L. mono* growth. All 7 plants had samples presumptively positive for *Listeria* spp. or both *Listeria* spp. and *L. monocytogenes*; *Listeria* spp. positivity rates ranged from 4 to 50%, while *L. monocytogenes* positivity rates ranged from 0 to 8%. The initial results highlight the importance of this project as it is essential to reduce the potential for negative publicity linked to dairy, which could void the positive impact of other efforts to promote dairy consumption.

Key Words: *Listeria*, dairy foods

P202 Fluid milk spoilage associated *Paenibacillus* spp. sporulation methods assessment and novel method development.

S. J. Reichler*, N. H. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Spore-forming bacterial genera, including *Paenibacillus* spp., survive high-temperature, short-time fluid milk pasteurization. Their endospores germinate and proliferate as vegetative cells over refrigerated shelf life, causing spoilage. Though *Paenibacillus* spp. are one of the most common gram-positive bacterial contaminants of pasteurized fluid milk, present in up to 95% of containers without gram-negative contamination, little is known about their biological requirements and optimal conditions for sporulation. This presents a challenge, as accurate experimental modeling of *Paenibacillus* spp. fluid milk contamination requires endospores rather than vegetative cells for inoculation. We compiled and tested previously reported techniques for laboratory production of *Paenibacillus* endospores using isolates of 3 well-characterized spp. commonly found in fluid milk: *P. amylolyticus*, *P. odorifer*, and *P. peoriae*. We measured sporulation success qualitatively by phase-contrast microscopy and quantitatively by spore pasteurization and plating. Surface plating on AK Agar #2 and MYPGP agar produced viable endospores of *P. amylolyticus* and *P. peoriae* but failed for *P. odorifer.* We found identical results for Schaeffer's Glucose broth. A chance observation of *P. odorifer*-inoculated refrigerated fluid milk revealed the presence of endospores, suggesting milk as an alternative medium for sporulation of this challenging sp. We experimentally optimized the yield of *P. odorifer* endospores in fluid milk using a Taguchi design to minimize the number of necessary experimental replicates. Briefly, incubation temperature, butterfat, citrate content, and milk dilution were assessed at 3 levels each in combinations determined by an orthogonal array for 3 *P. odorifer* isolates, with percentage spore yields measured by plating on nonselective media before and after spore pasteurization. The resulting set of optimized sporulation conditions for *P. odorifer* (32°C incubation of undiluted skim milk containing 0.8% wt/vol sodium citrate) allowed for >75% sporulation for some isolates. Using this method, *P. odorifer* endospores can be produced for further experiments, necessary for advancing our knowledge of this important species.

Key Words: *Paenibacillus*, spore, inoculation

P203 A gentle fluid milk bacterial cell isolation and purification procedure allowing for further applications, including light microscopy.

S. J. Reichler*, N. H. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Attempts to centrifugally purify bacterial cells from milk for further applications, such basic microscopy, are stymied by the presence of butterfat and protein. Spore-forming bacteria preferentially separate into the butterfat upon centrifugation and are lost when this layer is discarded. Unacceptable quantities of casein partition into the centrifugal pellet in many spoiled samples. Both residual butterfat and precipitated casein confound the results of differential staining techniques and interfere with phase-contrast microscopy of the resuspended cells. We developed a brief and relatively simple process for centrifugal separation of bacterial cells from butterfat and protein in fluid milk, while maintaining the physical integrity of both vegetative cells and endospores. The process begins by treating the milk with a sodium citrate solution to solubilize precipitated casein. The milk is then vigorously shaken to force bacterial cells associated with the butterfat into the aqueous phase. The butterfat is physically removed from the sample after centrifugation, and any remaining is rinsed away using a mild detergent. We verified that this method produces cell suspensions pure enough for accurate phase-contrast microscopy of unstained wet mounts and brightfield microscopy of Gram-stained smears. We confirmed the viability of these suspensions by plating onto Brain Heart Infusion agar. This technique has several potential applications, including isolation of bacterial endospores grown in fluid milk medium for further experimentation. Gram staining of purified cell suspensions may also serve as a rapid preliminary diagnostic technique for fluid milk bacterial spoilage events, with several advantages over existing methods. Unlike the direct microscopic bacterial count technique, no harsh solvents are necessary, concentration of the cells allows for improved microscopic visualization, and differential staining allows for some level of insight into the nature of the spoilage event. Unlike automated bacterial enumeration instruments, such as the BactoScan, no costly and specialized equipment is required.

Key Words: spore, isolation, stain

P204 Survival and thermal resistance of *Salmonella* in dry and hydrated milk powders during storage period of 6 months.

A. S. Sekhon*, A. Singh, P. Unger, Y. Yang, M. Babb, and M. Michael, *Washington State University, Pullman, WA.*

Foodborne pathogens such as *Salmonella* can endure dry environments of milk powders for extended periods due to increased adaptability at low water activity (a_w) and proliferate when powders are hydrated. This study compared the survivability and thermal resistance of a 5-serovar *Salmonella* cocktail in dry and hydrated nonfat dry milk (NFDM) and whole milk powder (WMP) stored for 180 d at ambient temperature (~25°C). This study was designed as 2 factorial (storage day and milk powder type) randomized complete block design with 3 replications as blocks. Milk powders were spray inoculated with 5-serovar *Salmonella* cocktail and dried back to original pre-inoculation a_w. The D-values of *Salmonella* in inoculated NFDM and WMP were determined periodically (every 30 d, starting from d 1). Milk powders were also individually hydrated on each analysis day to determine D- and z-values of *Salmonella* in hydrated milk powders. The D-values were determined using thermal-death-time disks and hot-water baths at 80, 85 and 90°C for milk powders, and 59, 62 and 65°C for hydrated milk powders. The D- and z-values of *Salmonella* at specific temperatures within dry or hydrated milk powders during the storage period were compared at $P \leq 0.05$ using 2-way ANOVA and Tukey's test. The D-values of *Salmonella* in WMP on d 1 were 18.9, 9.9 and 4.4 min at 80, 85 and 90°C, respectively, which increased to 29.4, 13.6 and 6.5 min at 80, 85 and 90°C, respectively, on d 180. Whereas D-values of *Salmonella*in NFDM on d 1 were 17.9, 9.2 and 4.4 min at 80, 85 and 90°C, respectively, and stayed similar during the storage. The D-values of *Salmonella* in hydrated milk powders remained similar throughout the storage. The overall z-value of *Salmonella* in NFDM and WMP was 16.3°C, whereas in hydrated NFDM and WMP, the overall z-value was 6.4°C.

Key Words: D- and z-values, low water activity, *Salmonella*

P205 Effect of extended storage on the survivability and thermal resistance of *Listeria monocytogenes* in dry milk powders.

Y. Yang*, A. Sekhon, A. Singh, P. Unger, M. Babb, and M. Michael, *Wasghinton State University, Pullman, WA.*

Listeria monocytogenes (LM) has unique ability to survive in the low water activity (a_w) foods such as milk powders for prolonged time periods and can multiply quickly when hydrated. LM can also result in severe diseases like meningitis and even premature death of unborn children. The objective of this study was to determine survivability and thermal resistance (D- and z-values) of LM in nonfat dry milk (NFDM) and whole milk powder (WMP) during storage of 180 d. This study was designed as 2 factorial (storage and powder type) randomized complete block design with 3 replications. Milk powders were inoculated with 3-strain cocktail of LM and dried back to original a_w levels, and D- and z-values were determined every 30th day, starting on day one. Five grams of respective samples were transferred into thermal-death-time (TDT) disks, sealed, and placed in water baths set at 75, 80, and 85°C for both powders. The samples were heat treated for intervals of 10, 5, and 2.5 min at 75, 80, and 85°C, respectively and transferred immediately to an ice water bath. The samples were enumerated using injury-recovery media, and D- and z-values were calculated. Two-way ANOVA and Tukey's test at $P = 0.05$ were used for statistical analysis. D-values of LM in NFDM for day 1 were 13.1, 6.0, and 4.0 min at 75, 80, and 85°C, respectively. Whereas D-values of LM in WMP for day 1 were 12.0, 6.3, and 3.3 min at 75, 80, and 85°C, respectively. The main effect (storage day) was significant for $D_{75°C}$ and $D_{80°C}$ values of LM and no significant effect was observed for milk powder type, and milk powder type × storage day. Overall, the $D_{75°C}$ and $D_{80°C}$ values of LM increased in milk powders. However, at 85°C, the D-value of LM were similar for milk powders. In addition, the z-values of LM in milk powders were also found to be similar (18.85°C on d 1) during the storage period. The LM population decreased by 2.1and 2.2 log cfu/g in NFDM and WMP, respectively, after 180 d of storage. D- and z-values from this study will provide basic information about the effect of storage time and milk powder type on heat resistance of LM in the milk powders.

Key Words: D-values, z-values, survivability

P206 Determination of thermal inactivation parameters of *Listeria monocytogenes* in dry and hydrated milk powders.

Y. Yang, A. Sekhon, A. Singh, P. Unger, M. Babb, and M. Michael*, *Washington State University, Pullman, WA.*

Although not commonly studied, *Listeria monocytogenes* (LM) can survive and pose food safety health risks in low water activity (a_w) foods such as milk powders. Thermal processing technologies are one of the commonly used methods used to inactivate foodborne pathogens in the dairy industry. Determination of thermal inactivation parameters is crucial in developing an effective pasteurization protocol. This study aimed to determine the thermal resistance (D- and z-values) of LM in dry and hydrated nonfat dry milk (NFDM) and whole milk powder (WMP). This study was designed as a randomized complete block design with 3 replications as blocks. Respective milk powders were inoculated with a 3-strain LM cocktail and dried to their original a_w levels. The dry powders were then subjected to heat treatment using thermal-death-time disks in a hot water bath at 75, 80, and 85°C. The hydrated (13% total solids) NFDM and WMP were also subjected to heat treatment at 54, 57, and 60°C at predetermined time intervals within the range of 0 to 40 min. The surviving microbial population of LM in the dry and hydrated milk powders was enumerated using injury-recovery media: Brain Heart Infusion (BHI) agar, overlaid with PALCAM agar. The data were analyzed using one-way ANOVA, and Tukey's test was used to determine significant differences among the mean values at $P \leq 0.05$ using Minitab19. The D-values of LM were 13.1, 6.0, and 4.0 min in dry NFDM, and 12.0, 6.3, and 3.3 min in WMP at 75, 80, and 85°C respectively. The D-values in hydrated NFDM were 9.7, 3.2, and 1.0 min and that of the hydrated WMP were 8.6, 3.4, and 1.0 min in hydrated WMP at 54, 57, and 60°C, respectively. The average z-value of LM determined in NFDM was 19.6°C and 17.8°C in WMP. In the hydrated milk powders, the z-value of LM in NFDM and WMP were 6.1 and 6.3°C, respectively. Statistically, there were no significant differences between the D- and z-values of Listeria monocytogenes within the dry and hydrated milk powders. D and z-values from this research can be used to develop a thermal kill step protocol on *Listeria monocytogenes* in milk powders.

P207 Comparison of the decimal log-reductions of *Salmonella* Senftenberg with other *Salmonella* serovars in nonfat milk and powder.

A. S. Sekhon, A. Singh*, and M. Michael, *Washington State University, Pullman, WA.*

Foodborne pathogens such as *Salmonella* can survive in dry environments of nonfat dry milk (NFDM) for prolonged time periods and multiply when hydrated. It is well established that *Salmonella* Senftenberg is the most heat tolerant *Salmonella* serovar in high moisture foods; however, the scientific literature in low moisture foods, especially NFDM, is scarce. Therefore, this study was performed to determine whether *Salmonella* Senftenberg is the most heat tolerant *Salmonella* serovar in NFDM among the 5 common foodborne illness-causing *Salmonella* serovars studied. This study was designed as completely randomized with 3 replications. Five serovars of *Salmonella* used in this study were Enteritidis, Montevideo, Newport, Senftenberg, and Typhimurium. The NFDM was inoculated with individual serovars of *Salmonella* and dried back to original pre-inoculation water activity levels. The inoculated NFDM was hydrated at 13% (wt/vol) total solids just before the heat treatments. The inoculated powders (5g) or hydrated powders (5mL) for individual *Salmonella* serovars were transferred into thermal-death-time disks, sealed, and placed in hot-water baths set at 80 and 90°C for NFDM, and 59 and 65°C for hydrated NFDM. The samples were held for 0 to 60 min at respective temperatures and transferred to ice-water baths at pre-determined time intervals. The samples were enumerated using injury-recovery medium, and the average log reductions for the individual serovars were calculated. The average log reductions of individual serovars at specific temperatures within dry or hydrated NDM were compared using one-way ANOVA and Tukey's test at $\alpha = 0.05$. As expected, *Salmonella* Senftenberg was found to be most heat resistant in hydrated NFDM at 59 and 65°C; however, *Salmonella* Montevideo and *Salmonella* Typhimurium were found to be most heat resistant in NFDM at 80 and 90°C, respectively. The heat resistance of the *Salmonella* serovars is found to be different in NFDM and hydrated NFDM. The results confirm that *Salmonella* Senftenberg is not the most heat resistant serovar in NFDM, and should not be used as the sole serovar for thermal inactivation studies of low moisture dairy powders, but should be used in a *Salmonella* cocktail of several heat resistant serovars.

Key Words: *Salmonella*, nonfat dry milk (NFDM) powder

P208 Infrared spectroscopy as a screening tool for grass-fed status of Canadian dairy herds.

M. Bahadi*[1,2], D. Warner[2], R. Lacroix[2], and D. E. Santschi[2], [1]*Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

Dairy farmers in Canada who produce grass-fed milk need to prove their adherence to the stipulations of grass-fed standard by determining the ratio of linoleic acid (LA) to α-linolenic acid (ALA) throughout the year. Determination of this ratio by gas chromatography (GC) is time consuming and expensive. The objective of this pilot project was to evaluate the potential of Fourier transform infrared (FTIR) spectroscopy in screening for LA:ALA ratio as an affordable alternative to the GC analysis. Monthly milk samples were collected from bulk tanks of 12 grass-fed farms and silos of 6 milk processing facilities in Québec in 2019 and 2020. The effective total number of samples was 174. GC was used to determine LA:ALA ratio and 75 milk fatty acids including omega 6 (n-6), omega 3 (n-3), and odd and branched-chain fatty acids

(OBCFA). Principal component analysis was applied to the GC results. Scores projected on principal component 1 (PC1) revealed a tendency of separation between grass-fed and silo samples. PC1 correlation loadings revealed an inverse relationship between n-6 on one hand, and n-3 and OBCFA on the other. These results confirmed that grass-fed milk can be differentiated from conventional one by the fatty acids profile. A partial least squares (PLS) regression model was built using milk spectra collected in 2019. The calibration set consisted of spectra of 11 grass-fed and 5 silo samples (average LA:ALA ± SD of 2.64 ± 1.302; range of 0.79–4.77). The model was cross-validated using a leave-one-out approach and the external validation set consisted of 76 grass-fed samples. The root mean square error of calibration, cross-validation (RMSECV), and prediction (RMSEP) were 0.46, 0.56 and 0.51, respectively. The RMSEP was acceptable according to the Canadian grass-fed standard and the close values of the 3 measurements of error indicated that the model was stable. The RMSECV was significantly different ($P = 0.003$) from the RMSECVs of 100 permutations of the LA:ALA PLS model. We concluded that FTIR spectroscopy has the potential to be used as a screening tool for LA:ALA ratio to monitor the grass-fed status of dairy herds.

Key Words: grass-fed milk, FTIR spectroscopy, chemometrics

P209 Feasibility of mid-infrared and visible/near-infrared spectroscopy to authenticate organic bulk milk.

C. L. Manuelian, V. Vigolo, A. Costa*, and M. De Marchi, *University of Padova, Legnaro, Padova, Italy.*

It is important to have fast, inexpensive and nondestructive methods, such as infrared spectroscopy, to verify the declarations on the labeling of those products in which certain characteristics represent a premium in the sale price. Thus, we aimed to evaluate the feasibility of mid-infrared (MIR; 5,012–925 cm^{-1}) and Vis/near-infrared (Vis/NIR; 400–2,500 nm) spectroscopy to discriminate organic from conventional cow milk. Bulk milk samples (n = 225) from 24 farms (Organic = 12; Conventional = 12) located in the same area, mainly raising Italian Friesian cows, and similar management conditions, except for spending a period of time in the pasture when organic, were collected from September 2019 to August 2020. Chemical composition of the lactation total mixed ration was similar between groups. The MIR and Vis/NIR spectrum of each sample were collected, and a principal components analysis (PCA) and a partial least square discriminant analysis (PLS-DA) were done with R software. For the PLS-DA, records were split into a train set (75% records) and a test set (25% records), and only wavelengths with VIP >1 were retained. Results from PCA showed that PC1 and PC2 explained 64.3% and 93.2% of the variance with MIR and Vis/NIR spectra, respectively. However, the PCA plot showed the overlapping of both populations. The PLS-DA showed several MIR regions contributing to the model ($1 \leq VIP \leq 3.5$). Vis region contributed more to the Vis/NIR model ($1 \leq VIP \leq 3.0$), but few VIP peaks (≤ 1.4) in the NIR region were observed. Despite only milk protein content differed ($P = 0.04$) between groups, those MIR and NIR regions are linked to lactose, fat and protein content. Results from the PLS-DA revealed an accuracy of the model of 70.2% and 71.7%, for MIR and Vis/NIR, respectively, in the test set. In conclusion, both infrared-regions performed similarly, and the moderate accuracy of the PLS-DA could be related to the similarity of the selected farms between both categories (e.g., breed and total mixed ration composition). It would be interesting to incorporate other parameters such as the fat or protein profile of the milk to improve the models.

Key Words: infrared spectroscopy, organic production, PCA

P210 Influence of incorporation of whey protein isolates on the physicochemical, rheological, and microstructural properties of nonfat yogurt.

M. A. Hashim*[1,2], L. A. Nadotchii[1], A. Prockora[1], and M. Muradova[1], [1]*ITMO University, Saint Petersburg, Russia,* [2]*Agricultural Research Center, Giza, Egypt.*

Consumers give a significant awareness for healthy, naturally functional food products with clean labels and less added ingredients and external hydrocolloids. Nowadays, the demand for low and nonfat products has increased because of the problems related to obesity, diabetes and cardiovascular diseases. So, significant attention has been given to the production of low and nonfat dairy products. This study's objective was to explore the potential of whey protein isolate (WPI) to improve the quality of nonfat yogurt made by reconstituted skim milk and in the presence of full-fat and nonfat yogurts as controls. Yogurt mixes were formulated using a skim milk powder as a milk base enriched with WPI up to 6% protein content. The replacement percentage of skim milk powder by WPI in the yogurt mixes was between 3 and 9%. Nonfat and full-fat set-style yogurts (6.0 ± 0.1% protein, 15 ± 1.0% solids) were made from skim milk and whole milk, respectively. The texture, microstructure, the rheological properties (storage modulus, loss modulus, apparent viscosity) and sensory properties of the yogurt samples were analyzed in comparison with full-fat (3.0% fat, wt/wt) and nonfat (0% fat, wt/wt) yogurts. The rheological parameters were measured using a rheometer (RN 4.1, RHEOTEST Medingen GmbH, Germany). The texture was analyzed by domestic texture analyzer "Structurometre ST2," and the maximum force required to penetrate the gel was taken as a measure of the relative gel strength. The microstructure was assessed by scanning electron microscopy. The yogurt samples were evaluated by a trained panelist familiar with sensory evaluation techniques. The average and standard error were carried out for 3 replicates using an SPSS computer program. The incorporation of WPI improved the water-holding capacity of the nonfat yogurt and the rheological properties. The nonfat yogurt incorporated with 7% WPI had comparable sensory and textural characteristics to the full-fat yogurt. The firmness of yogurt samples significantly decreased by increasing the addition of WPI. The SEM micrographs showed that the addition of WPI improved the microstructure of nonfat yogurt samples by adding WPI. So, WPI can be used as a fat replacer to develop nonfat yogurt with desired features. WPI might be a natural and economical ingredient for producing nonfat fermented dairy food products.

Key Words: yogurt, whey protein isolate, rheological properties

P211 Synergistic and antagonistic ingredient interactions as a sugar reduction strategy.

A. Riak*, R. Roberts, J. Hayes, G. Ziegler, and H. Hopfer, *The Pennsylvania State University, University Park, PA.*

Approaches to combating childhood obesity include food product reformulation to decrease added sugar while maintaining product acceptability. This research is testing for perceived synergistic and antagonistic ingredient interactions between sucrose, vanillin, and cocoa powder in chocolate milk, which is the most popular flavor in the National School Lunch Program. The objectives of this study were to (i) evaluate response-surface intensities of sucrose, vanillin, and cocoa powder mixtures while assessing optimization in chocolate milk (ii) determine the presence of synergistic, additive, and antagonistic ingredient interactions using the isobole method, and (iii) measure formulation acceptability. One hundred thirty-six adults evaluated 10 of the 22 samples in a counterbalanced, incomplete block design for the response-surface and isobole findings. Final regression models were then used to create tri-variable contour plots with the Ternary package in R.

The evaluation of the contour plots highlighted the large contributions of sucrose-vanillin and sucrose-cocoa interactions to sweetness, bitterness, and chocolate flavor intensities on 100-point intensity scales. Of the 5 samples evaluated with the isobole method for both sweetness and bitterness, samples with 2.33% sucrose, 0.033% vanillin, and 0.67% cocoa, as well as samples with 4.67% sucrose, 0.017% vanillin, and 0.83% cocoa (%wt/wt) both had interaction index values below 0.9 and above 1.1 that illustrated sweetness synergy and bitterness antagonism, respectively. For both the adult (n = 142) and child (n = 61) acceptability tests utilizing 9-point hedonic scales, a mixed-effects ANOVA model ($P < 0.05$) was used to determine statistically significant differences in overall acceptability scores of the evaluated samples. Results from this indicated that samples with sucrose concentrations of 4.67% and 9.00% were liked significantly more than samples with 2.33% sucrose. These results could assist chocolate milk retailers in formulating products that decrease added sugar content while maintaining consumer acceptability.

Key Words: sensory, synergy, cross-modal interactions

P212 Understanding casein-whey protein interactions in acid gels of fibrillated model milk protein concentrate using Förster resonance energy transfer (FRET) microscopy. G. Rathod*, D. Boyle, and J. Amamcharla, *Kansas State Univerisity, Manhattan, KS.*

Förster resonance energy transfer (FRET) is a widely used technique to study interactions between 2 molecules and it is based on the non-radiative (dipole-dipole) energy transfer from a donor fluorescent dye to an acceptor fluorescent dye only when they are in close proximity (<10 nm). The objective of this study was to use the FRET technique to understand the interactions between caseins and whey proteins during acid gelation. So, 2 lots of micellar casein concentrate (MCC) and milk whey protein isolate (mWPI) were obtained from commercial sources. Proteins in MCC were labeled with the donor dye ATTO-488 NHS ester and proteins in mWPI were labeled with the acceptor dye ATTO 532-NHS ester. The labeled MCC and mWPI dispersions were mixed accordingly to prepare control model milk protein concentrate (C-MPC) and fibrillated model MPC (F-MPC). F-MPC was prepared by mixing MCC (8% protein) with fibrillated mWPI (2% protein) in equal proportions, resulting in a 5% protein dispersion. Similarly, C-MPC was obtained by mixing MCC with mWPI. The resulted C-MPC and F-MPC contained 5% protein with a casein-to-whey proteins ratio similar to milk. The protein dispersions were heated to 90°C for 10 min, cooled to 30°C, and acidified to pH 4.6 with glucono-δ-lactone to form acid gels. Acid gels were viewed using a confocal laser scanning microscope and the images were analyzed using ImageJ software and FRET analyzer plugin where bleed through analysis gave a Pearson r-value of more than 0.95 indicating that FRET interactions are highly significant ($P < 0.05$) in both MPCs during gelation. From the FRET index images, F-MPC showed 61% white area, indicating strong molecular interactions within the Förster radius. On the other hand, C-MPC showed only 4.3% white area. The results show very strong interactions between caseins and fibrils while moderate and uniform interactions in C-MPC gels. The gel strength and other rheological properties of acid gels are strongly influenced by casein and whey protein interactions. FRET analysis can be a useful tool to understand protein interactions at a molecular level.

Key Words: Förster resonance energy transfer (FRET), casein-whey proteins interactions, fibrillated model MPC

P213 Switchable solvents: A novel extraction method for milk phospholipids. K. Rathnakumar*[1] and S. I. M. Monteagudo[2], [1]*South Dakota State University, Brookings, SD,* [2]*New Mexico State University, New Mexico, NM.*

Phospholipids (PLs) found in dairy products are of great interest due to their health and nutritional benefits associated with their consumption. Dairy by-products represent a potential source of PLs, which make them an attractive feedstock for value-added opportunities of milk phospholipids. Currently, the extraction of PLs from streams of by-products result in overall low efficiencies, and it involves subsequent solvent separation and lipid recovery. The research work focuses on the extraction of milk phospholipids from by-product streams with a tertiary amine (N, N-dimethyl cyclohexylamine, CyNMe2) as a switchable hydrophilicity solvent. This solvent can be reversibly switched between a hydrophobic and hydrophilic form by simply bubbling or removing CO_2. Different dairy by-products streams, including buttermilk (BM), β-serum (BS), concentrated buttermilk (CBM), and raw cream (RC) was used to evaluate the feasibility of CyNMe2. The extraction efficiency of CyNMe2 ranged from 0.33 to 99%, depending on the type of by-product. Remarkably, CyNMe2 extracted up to 99% of the PLs directly from buttermilk (BM). The extraction of PLs from BS was further studied using ultrasound before CyNMe2 extraction. Overall, higher acoustic intensity before CyNMe2 extraction recovered 69.07 ± 0.11% of PLs, 10 times higher than the samples without ultrasound pretreatment. The recovered fraction of PLs mainly comprised phosphatidylinositol (32%), phosphatidylethanolamine (30%), and sphingomyelin (37%). The effect of the extraction temperature(25, 40, and 60°C), time (3, 10, and 18h) and solvent ratio (3/1,10/1, and 18/1 mL) was also studied. The highest yield obtained was 29.29 ± 0.06% of PLs at 60°C, at minimized solvent ratio and time (3/1 mL and 3 h). All experiments were carried out in 3 replicates and statistically analyzed using Tukey's test. Insights into the extraction mechanism of the CyNMe2 was studied through various analytical measurements, such as analysis of protein profile, particle size, zeta-potential, and microstructure such as confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM). CyNMe2 seems to disrupt the protein-membrane through ion pair formation, releasing the PLs into the aqueous medium. Therefore, CyNMe2 shows to be an effective way to concentrate PLs from by-products.

Key Words: phospholipids, dairy by-products, CyNMe2

P214 The effect of different heat treatment conditions on functional properties of polymerized liquid whey proteins. J. Wang and T. Fang*, *Jilin University, Changchun, Jilin, China.*

Fresh sweet whey was pasteurized (60°C for 30min) and pre-filtered using a screen mesh. The treated whey was subjected to microfiltration (MF, 0.1um, at 45°C for 1h). MF whey was treated by ultrafiltration using a 10 kDa cut-off membrane to 25-fold. The UF treated whey was electrodialyzed (ED) to remove 90% of salt and the final protein content of the liquid whey protein (LWP) was ~8.0%. Polymerized liquid whey protein (PLWP) was prepared by heating. Influence of heating temperature, heating time, and pH on turbidity, solubility, zeta-potential, free sulfhydryl group, surface hydrophobicity, emulsifying properties, and degree of polymerization of PLWP was studied. There were significant differences ($P < 0.05$) in surface hydrophobicity, zeta-potential, free sulfhydryl group on the surface, turbidity and emulsifying properties between LWP and PLWP. The absolute value of zeta-potential of PLWP increased in range of pH 7.0–9.0, and higher than LWP (>20MV). The free sulfhydryl group of PLWP was significantly higher ($P < 0.05$) than LWP (4.15μmol/g). The SDS-PAGE confirms the formation of polymers with large molecular weight by heating. The a-lactalbumin (a-LA) and β-lactoglobulin (β-LG) of PLWP were determined by HPLC and the degree of polymerization was calculated. The polymerization degree of LWP increased up to 85.89% with increasing heating temperature and time. The denaturation degree of a-LA with PLWP were more than 70% at 70°C, while that of β-LG increased with pH (7.0–9.0), but there was no significant difference ($P > 0.05$) in the denaturation degree at pH 7.0–7.5. The results of rheological properties of PLWP showed that the flow curves of all the PLWP with different heating conditions were suitable for the Sisko model, and all flow indices of PLWP were less than 1. The viscosity of PLWP was significantly higher ($P < 0.05$) than LWP. The apparent viscosity of PLWP increased with the temperature from 70 to 90°C at pH 7.0. All experimental analyses were carried out in triplicates. The significant differences of data between samples and control were calculated using Version SPSS11.5. The significance level was set at $P < 0.05$. One-way ANOVA was employed to find the significant differences. The PLWP (75°C for 20min at pH7.0) can be used as a thickening agent in the preparation of yogurt and it may be used as a natural and functional ingredient for formulation of milk based fermented foods.

Key Words: whey proteins

P215 Production of milk fat globule membrane-enriched ingredient derived from acid whey as potential healthful ingredient. M. Chrusciel*, J. Ortega-Anaya, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Milk fat globule membrane (MFGM) containing milk phospholipids (MPL) have recently been shown to have potential health benefits such as neuronal health and development, as well as gut function, skin health, and cancer-fighting properties. Acid whey, the by-product from the manufacturing of acid-based cheeses and yogurts, is generally regarded as containing little nutrition value due to its low pH and high-water and mineral content. However, this by-product is a valuable source of MFGM and other components when processed. In this study, acid whey was treated by membrane filtration to produce a retentate rich in MFGM, and finally spray dried to create a shelf stable powder ingredient with potential use in foods with health benefits. Fresh acid whey was ultrafiltered though a 50 kDa lab-scale membrane system in triplicate, and further treated with tri-sodium citrate and diluted ammonium hydroxide (for pH adjustment at low temperature) combined with ultrafiltration/diafiltration to remove whey proteins. The final fraction was spray dried to produce an ingredient with a total average of 4.5% of MPL measured by high-performance liquid chromatography (HPLC). Moreover, the analysis of individual classes of MPL showed a composition of 4.2 µg/mg of PI, 2.8 µg/mg of PS, 10.2 µg/mg PE, 13.1 µg/mg of PC and 15.0 µg/mg of SM. In addition, we were able to significantly reduce the content of caseins and whey proteins to a maximum of 20% of total protein in dry basis, identified by SDS-PAGE-fluorescence analysis. Migration to a pilot-plant scale is currently being conducted to calculate yields for potential industrial application. The ability to transform acid whey into a nutrient rich, functional powder ingredient in an economical and efficient way will have industrial and commercial applications as a high-value, healthful product.

Key Words: milk fat globule membrane, acid whey, membrane filtration

P216 Biofilm formation in the milk sampling devices as a result of prolonged use in a dairy processing facility. R. Kalita*[1,2] and S. Anand[1,2], [1]*Midwest Dairy Foods Research Center, Minneapolis, MN,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

During prolonged processing of milk, adhesion of bacteria cause biofilm formation in the dairy equipment surfaces. This leads to cross-contamination and shedding of bacteria in processed milk affecting the safety and quality of the milk products. The objective of this study was to determine the extent of biofilm formation in the milk sampling devices used in the dairy industry. To represent a higher incidence of *Bacillus licheniformis,* raw whole milk (at 4°C) was spiked with vegetative cells of *B. licheniformis* (4 log cfu/mL) and pasteurized for 12 h, uninterruptedly at 72°C/16 s. Commercial port-septum type of sampling devices was fitted in a pilot-scale pasteurization unit at the points of raw milk-in and pasteurized milk-out. Raw and pasteurized milk samples were drawn aseptically at different intervals of processing to examine the bacterial levels. After 12 h run, attached bacterial cells in the different parts of the sampling ports were swabbed using quick swabs and aerobic plate count was performed using tryptic soy agar with incubation at 32°C for 48 h. Selected isolates from the sampling devices were identified using MALDI-TOF. The experiment was conducted in duplicates and data were compared using ANOVA. The sampling device on the raw side found to experience higher bacterial attachment and biofilm growth (2.12 ± 0.12 log cfu/cm^2) compared with pasteurized side (1.14 ± 0.03 log cfu/cm^2), indicating a higher attachment potential in the raw side sampling device. A significant difference ($P < 0.05$) was observed between the biofilm counts in the sampling devices from raw and pasteurized sides. MALDI-TOF revealed *B. licheniformis* to be the predominant species in the sampling port biofilms on the pasteurized side. However, *B. licheniformis, Staphylococcus* sp., *E. coli*, and *Strep. uberis* were identified in the biofilms of the sampling port on the raw side. The results demonstrated that the prolonged usage may prompt biofilm formation in the sampling devices. This suggests that replacing the septum-based sampling device after a certain interval or processing of milk within a shorter period could help to prevent biofilm formation.

Key Words: biofilm, sampling device

P217 Effect of common dairy clean-in-place protocols on the surface roughness of stainless steel surfaces. T. Almalki*[1,2] and S. Anand[1,2], [1]*Midwest Dairy Food Research Center, Minneapolis, MN,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Thermoduric spore formers survive heat treatments and lead to the formation of contact surface biofilms. These biofilms are difficult to clean and cause cross-contamination of milk and dairy products during milk processing. When bacteria aggregate on the surfaces, they form resilient biofilms over time that need to be removed and washed by applying harsh CIP chemicals. These typical cleaning and disinfection protocols in dairy plants are vital in ensuring good quality products. It is hypothesized that repeated use of CIP chemicals would alter the surface roughness of native, welded, and polished SS 316 surfaces and support biofilm formation differently. The present study is focused on evaluating the role of repeated CIP process on surface properties and understanding the relationship between surface roughness and biofilm formation. Plate heat exchanger CIP protocol was followed to clean the SS 316 coupons (2X2 cm^2) for 10 consecutive cycles after forming biofilms using *Geobacillus stearothermophilus.* The selected organism was spiked into sterile skim milk samples at 6.0 log cfu/mL. After every CIP cycle, the biofilm counts were taken using standard microbiological techniques. Scanning electron micrographs were also taken to visually observe the developed biofilms. Laser scanning microscope images were taken to observe surface roughness changes pre-and post-CIP treatment. The replicate data from 3 trials for the chosen organism were analyzed and means were compared using Keyence VK Analyzer software. The surfaces roughness measurements (Ra) of pre-CIP treatment of native, welded, and polished surfaces were 0.6µm, 2.06 µm, and 1.01 µm respectively, and the measurements of post-CIP first cycle were 2.3 µm, 6.7 µm, and 2.9 µm after the first cycle. whereas the post tenth-cycle measurements were 3.8 µm, 19.2 and 4.8 µm respectively. These results demonstrate obvious changes in surface roughness after exposure to CIP chemical solutions, highest being on weldments. These observations agree with our hypothesis that repeated exposure to CIP cleaning cycles alters surface roughness and may cause cross-contamination in dairy products processing due to increased biofilm formation.

Key Words: surface, roughness, biofilm.

P218 Effect of plate-and-frame filtration temperature on the functionality of milk protein concentrate.

A. Mishra*, A. R. Hammam, V. Sunkesula, and L. E. Metzger, *South Dakota State University, Brookings, SD.*

A high level of solids in feed material saves cost of energy in spray drying. In our related research, we determined that plate-and-frame filtration (PF) system could be efficient in concentrating milk protein concentrate (MPC) when processed at elevated temperature. In this study, the functionality of concentrated MPC produced using PF system was compared with the feed; an MPC80 obtained from the ultrafiltration of skim milk. Feed containing 20% total solids and about 80% total protein based on solids was concentrated in PF system fitted with flat sheet membranes having 3.3m^2 surface area with a 10 kDa molecular weight cut-off for 3 replications. Three different PF treatments such as PF at 22°C (PF22), PF at 50°C for medium solids (PF50MS), and PF at 50°C for high solids (PF50HS) were performed. For PF22 and PF50HS treatments, filtration was continued until the transmembrane pressure difference become 900 kPa whereas for PF50MS treatment, retentate was drawn when the level of solids achieved nearly 30%. Feed and PF retentates were spray dried to get MPC powder and their functional properties were evaluated. Reconstituted MPC showed a pseudoplastic behavior and best fitted with the Herschel-Bulkley model where consistency index was decreased, and flow behavior index was increased for the high-temperature PF treatments. Rennet coagulation time was longer for the high-temperature PF treatments and required $CaCl_2$ ~0.25% for timely coagulation. Wetting time was increased whereas dissolution capacity and heat coagulation time were decreased for the powders from high temperature PF when compared with PF22 and feed. High-temperature PF did not impact emulsion formation but improved the emulsion stability. Foaming capacity was increased with increasing protein in the powder however, their stability was similar for all treatments. The level of whey protein reduced slightly at high-temperature PF on which the amount of denatured protein was also higher. This study determined that temperature optimization is important for concentrating MPC in the PF system to maintain the functional properties.

Key Words: milk protein concentrate, plate-and-frame filtration, functional properties

P219 Fermentation of whey permeate using *Brettanomyces claussenii*: Creating opportunities to develop value-added products.

V. K. Rivera Flores*, T. A. DeMarsh, and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Whey permeate has various functional benefits that have driven its use as an additive in many foods. However, no current application explores potential valorization that would enable better utilization of this once considered by-product. Through an innovative value-added approach, the lactose present in whey permeate can be hydrolyzed into galactose and glucose, which can then be strategically converted to ethanol through fermentation. If galactose remained unmodified, it could confer low-glycemic attributes and potential health benefits, to thus create low-alcoholic beverages from whey permeate with added functionality. Previous research from our group has shown that such approach is feasible. Under anaerobic conditions, *Brettanomyces claussenii* can selectively ferment glucose into ethanol from hydrolyzed lactose, leaving the galactose intact. This creates an opportunity to explore whey permeate fermentation to produce value-added products. Hence, the aim of this research is to optimize whey permeate fermentation by *B. claussenii* to maximize ethanol and galactose production, using surface response methodology. For this purpose, 5 fermentation factors were studied for their impact on ethanol and galactose yield: temperature (20–40°C), substrate concentration (5–15% TS), enzyme/substrate ratio (0–40 IU/ g lactose), inoculation level (6–8 log cfu/mL), and time (6–30 d). Anaerobic fermentations were carried out in 200 mL of substrate, and a quadratic model was built for each response to allow for optimization. Results showed that all factors were significant in the synthesis of both products. The predictive models for ethanol and galactose achieved coefficients of determination (r^2) of 92% and 95%, respectively. With optimized concentrations of 4.6% vol/vol for ethanol, and 70.8 g/L for galactose. These results demonstrate that it is possible to adjust multiple fermentation parameters to maximize ethanol and galactose production from whey permeate. Moreover, they open possibilities for the design of biomanufacturing processes to develop functional beverages that would appeal to health-conscious consumers.

Key Words: whey permeate valorization, galactose, ethanol

P220 Model fermentation of dairy effluents by *Brettanomyces claussenii* with lactose cleavage to create a value-added product.

K. Jencarelli*, M. R. Lawton, and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Disposal of dairy by-products such as acid whey is a burden on manufacturers due to high biological oxygen demand. In an effort to upcycle dairy effluents, residual sugars can be recovered. While approximately 70% of adults are lactose intolerant, lactose can be cleaved into its monomers, glucose and galactose, and sold as valuable and digestible simple sugars for other processes. Glucose can also be fermented by *Brettanomyces claussenii* into acetic acid, a key ingredient in consumer goods such as kombucha and vinegar, while retaining galactose for sugar collection. The objective of this study was to evaluate sugar utilization and time for acetic acid production in an aerobic fermentation by *B. claussenii* as an effort to upcycle dairy effluents. A 30-d aerobic fermentation was run in synthetic media (YNB, pH 4.4, 4% lactose) to emulate acid whey. Flasks inoculated with *B. claussenii* at a concentration of 3×10^6 cfu/mL were treated with lactase to cleave lactose into its monomers. The flasks were incubated at 30°C (200 RPM) and 65% relative humidity to control for evaporation. Samples were taken every other day, plated for cell enumeration and analyzed with HPLC for lactose, glucose, galactose, ethanol and acetic acid. ANOVA and Tukey's test were run to determine significant differences in sugars, acetic acid, and ethanol across fermentation time. By d 2, the lactase cleaved all of the lactose, resulting in an approximate 2% glucose and 2% galactose sugar source. Glucose was used quickly by the yeast, with complete utilization by d 2. Contrary to previous research, once glucose was depleted, *B. claussenii* utilized galactose, finishing the sugar source and completing the fermentation by d 6. Significant acetic acid production began on d 4 and reached an average concentration of 8.54 g/L by d 6 and maintained these levels for the 30-d period. Future research will be conducted to

further investigate galactose utilization by *B. claussenii* and optimize production of acetic acid and galactose from various milk substrates via a factor screen and response surface methodology.

Key Words: fermentation, lactose, acetic acid

P221 Optimizing lactose fermentations with *Brettanomyces claussenii* for production of value-added goods from dairy by-products.

M. R. Lawton*, K. G. Jencarelli, and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Functional beverages such as kombucha are popular among consumers for their health benefits. Production involves the use of alternative microorganisms such as acetic acid bacteria and non-*Saccharomyces* yeasts to produce health-promoting compounds. One such yeast, *Brettanomyces claussenii*, has the ability to aerobically ferment lactose and produce acetic acid. This microorganism opens opportunities to produce acetic acid containing beverages from dairy by-products where lactose is the main sugar, such as acid whey. The objective of this study is to optimize acetic acid production from lactose using *B. claussenii*. Several strains of *B. claussenii* were screened for their ability to ferment lactose to acetic acid. Yeast Nitrogen Base (YNB) with 4% lactose at pH 4.0 was used as synthetic medium. Flasks of YNB, inoculated with each strain to a concentration of 3×10^6 cfu/mL, were incubated at 30°C (200 rpm) for 14 d. Uninoculated YNB was used as a negative control. Samples taken at day 0, 7, and 14 were analyzed for acetic acid and lactose via HPLC. The experiment was repeated 3 times and samples were analyzed in duplicate. Analysis of variance and Tukey's test were used to determine significant differences in lactose and acetic acid content between strains on day 14. Strains Y-1414 and OYL-201 achieved highest acetic acid production, 7.31 ± 0.36 and 7.38 ± 0.22 g/L respectively. The lab strain (OYL-201) was used to further investigate the time necessary for acetic acid production. Fermentations were set up as previously stated in YNB (4% lactose) buffered at pH 4.4 to mimic acid whey conditions. Samples for the same analyses were taken every 2 d for 30 d. Levels of acetic acid were compared at each time point with ANOVA and Tukey's test. The results indicate significant acetic acid production began by day 4 (5.27 ± 1.31 g/L), achieved the maximum and plateaued by day 6 (9.28 ± 0.778 g/L). This information will be used in further experiments to optimize acetic acid production through a screening of significant fermentation factors using fractional factorial and response surface methods.

Key Words: acid whey, acetic acid, fermentation

Extension Education: Posters

P222 Exploring intragroup variation as feeding management tool. J. P. A. Rezende*, V. S. V. Dutra, V. A. Oliveira, P. Machado, and M. A. C. Danes, *University of Lavras, Lavras, MG, Brazil.*

The importance of individual cow data to better characterize feeding groups when formulating diets is well known. We hypothesized that the variation within group can also be used in feeding management. Individual milk yield and composition, days in milk (DIM), body weight (BW), group dry matter intake (DMI) and diet particle size distribution, using a 3-screen particle separator in 5 points along the bunker were collected every 2 weeks for 6 mo from the high group of a 60 lactating cows dairy farm. Average values and coefficient of variation (CV) were calculated for all variables and an initial exploratory correlation analysis was run using PROC CORR of SAS 9.3. The group evaluated had on average 18 multiparous cows, with 152 DIM and 660 kg BW, producing 27 kg/d of milk, with 3.5% fat, 3.0% crude protein and 13.7 mg/dL milk urea nitrogen (MUN). The average diet proportion retained in top, middle and bottom screen were 8.2%, 41.7% and 50.1%, respectively. Group DMI was negatively correlated with CVs of BW (r = −0.65; P = 0.05) and DIM (r = −0.60; P = 0.05), demonstrating the negative effect of group heterogeneity on intake. Another interesting observation was the correlation between the CV of milk fat content and the CV of the top (r = 0.80; $P < 0.01$) and bottom (r = 0.74; $P < 0.01$) screens, unveiling a potential use of individual milk composition as feeding management tools, such as TMR audit. However, it is important to notice that these correlations were achieved after the removal of all observation with somatic cells count (SCC) greater than one million. When the complete data set was used, these correlations were no significant. The reason for that is not clear, since SCC and the CV of SCC did not significant correlate with any of the variables. The MUN negatively correlated with the CV of the bottom screen (r = −0.73; P = 0.01) and the CV of MUN negatively correlated with the CV of the top screen (r = −0.66; P = 0.03), demonstrating that the mixing quality can also affect MUN. This initial evaluation revealed that intragroup variation, calculated from individual milk composition, should be further explored as feeding management tool.

Key Words: precision nutrition, milk composition, TMR auditing

P223 Dairy producers' perspective: Health and economic implications of the COVID-19 pandemic. N. Silva-del-Rio*[1], J. Wenz[2], F. C. Ferreira[1], M. Chahine[3], J. Dalton[4], M. de Haro Marti[5], M. Rovai[6], and M. B. Abreu1,7, [1]*University of California–Davis, Tulare, CA,* [2]*Washington State University, College of Veterinary, Pullman, WA, 3University of Idaho, Twin Falls Research and Extension Center, Twin Falls, ID,* [4]*University of Idaho, Caldwell Research and Extension Center, Caldwell, ID,* [5]*University of Idaho Extension Gooding County, Gooding, Gooding, ID,* [6]*South Dakota State University, Dairy and Food Science Department, Brookings, SD,* [7]*Universidade Federal de Viçosa, Department of Animal Science, Viçosa, MG, Brazil.*

The aim of this study was to describe dairy producers' perspectives on the health and economic implications of COVID-19 at the onset of the pandemic. Dairy producers were reached by an anonymous online survey circulated nation-wide via university and allied industry media outlets (Apr-20) and by mail [CA, ID, SD (Jun-Jul-2020), WA (Sep-20)]. Responses (n = 309) were received online (28%) and by mail (CA, 35%; ID, 16%; SD, 11%; WA, 9%) from herds with <100 (n = 45), 100 - 499 (n = 93), 500 - 1999 (n = 91) and ≥2000 (n = 70) cows. Respondents reported they had COVID-19 cases [suspected (4%); confirmed (8%)] at their premises. Regarding safety, overall producers were concerned about the health of their families (83% < 1,000 cows; 72% ≥ 1,000 cows) and their employees (56% < 1,000 cows; 88% ≥ 1,000 cows). COVID-19 information was obtained through television (38%), family and friends (26%), and social media (21%). Producers perceived that their employees were somewhat or very concerned (71%) with the pandemic; training was intended (4%) or provided (75%) in English (26%), Spanish (19%), or both (55%). The focus of training was: how to remain healthy at work (90%), at home (60%), what to do if a worker gets sick (77%), and to provide sick leave information (53%). Producers did not always offer training, and the reasons were: not necessary (38%), insufficient information available (17%), uncertainty about best approach (17%), or had no time (6%). Control measures implemented were: frequent hand-washing and sanitizer use (78%), social distancing (59%), prevention of employee gatherings (57%), limiting on-farm visitors (54%), and providing face masks (45%). The economic impact of COVID-19 concerned most dairy producers (82%); the greatest fears were being forced to reduce production, limit of the availability of goods, and lack of services. Dairy producers (76%) replied they had adequate information to develop COVID-19 emergency plans, and they had (30%) or were working (24%) on a plan. Results from this survey highlight the health and economic concerns of dairy producers during the COVID-19 pandemic and the mitigation efforts adopted.

Key Words: COVID-19, dairy producer, safety

P224 Udder dissections give dairy employees a better understanding of milk production. M. Rovai*[1], M. E. de Haro Martí[2], and M. Chahine[3], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*University of Idaho Extension, Gooding, ID,* [3]*Animal, Veterinary and Food Sciences Department, University of Idaho, Twin Falls, ID.*

Milk provides the major source of income on dairy farms and high-quality milk is the goal at every stage of the dairy operation. Employees are constantly asked to perform milking procedures correctly and consistently to achieve and maintain maximum milk quantity and quality. However, before performing the regular milking tasks, one needs to understand the function and structure of the mammary gland. It is known that hands-on activities enhance students' interest and retention. The objective of the project was to assess knowledge gains following a 3-h udder dissection workshop. The training consisted of a PowerPoint presentation followed by an interactive hands-on session. The workshop explained: 1) the importance of understanding the main mammary gland physiology functions related to milk quality; 2) the importance of each step of the milking procedures; and 3) key recommendations about mastitis prevention related to the anatomical udder structure. A total of 18 dairy employees from 20 dairies representing 120,000 cows in Idaho participated in the training session. Participants were administered an exam consisting of 9 written questions before and immediately following the training. Exam scores were compared in SAS 9.4 (SAS Institute Inc., Cary, NC) using a paired t-test. Results indicate that the training had an effect ($P < 0.0001$) on dairy workers' knowledge as indicated by the increase in average exam score from 2.13 ± 0.42 to 5.50 ± 0.38. Feedback indicated participants highly valued the hands-on experience and preferred it to traditional classroom settings. Giving dairy employees the chance to see a real udder inside and out will likely give them a different insight when they go back and milk the cows. The chance to get a different view of the udder also gives a better understanding of how mastitis can affect the animal. The knowledge originating from an udder dissection along with the classroom session may lead to a better understanding of the milking process and better milk quality by understanding the key anatomical features of the mammary gland.

Key Words: dairy farms, udder dissection, dairy workers

P225 Assessing dairy employees' mental health status in South Dakota: Workplace. L. Guifarro*[1], L. Stallones[2], J. Rosecrance[3], and M. Rovai[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Psychology Department, Colorado State University, Fort Collins, CO,* [3]*Environmental & Radiological Health Sciences Department, Colorado State University, Fort Collins, CO.*

The dairy industry's dynamics are changing, trending toward a larger number of cows on a single farm with more hired employees. While the majority of the US dairy farm workforce is Latino, little is known about the causes of distress in this group. One of the primary challenges Latino dairy workers face is finding a balance between building a better future and their ability to integrate within their new culture. The quality of milk production is directly related to the well-being of dairy farm employees, and stress can have a negative impact on both employees and the farm. Behavior and health assessments can help to identify anxiety and stress caused by various sources. This study aimed to gather information on the main causes of behavioral stress in dairy farm employees along the I-29 corridor (South Dakota) and evaluate how it affects their work performance and job satisfaction. Six focus groups (FG) of 1 h each in Spanish were conducted with workers (n = 50; 88% male) from México, Guatemala, and other Latino countries (54%, 30%, and 16%, respectively). Transcriptions of the FG were analyzed and coded line by line for each quotation by using ATLAS.TI software (Scientific Software Development GmbH). Over 38 factors contributing to overall stress and job satisfaction at the workplace were identified and grouped as: 1. Work schedule; 2. Cattle handling; 3. Equipment failures; 4. Lack of teamwork; 5. Pressure at the workplace; 6. Interruptions at work; 7. Perception of the workload; 8. Extended shifts; 9. Weather seasons; and 10. Lack of communication skills in English. This project revealed areas of stress for the dairy employees that we were not aware of and needs further consideration. The FG results provided valuable insight that can be used to create better management strategies in the future to help dairy workers to cope with stress and learn how to mitigate stress at work in a healthy manner. Based on the information gathered, a tri-fold brochure was developed with basic knowledge covering stress causes and ways to reduce it. Supported by HICAHS Community-Initiated Grant Program (Colorado State University).

Key Words: dairy workers, stress factors, focus group

P226 Use of energy drinks, alcohol, and tobacco by dairy farm employees. L. Guifarro* and M. Rovai, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Working on dairy farms may include long work shifts, long workweeks, exposure to extreme weather, high physical demands, and interruption of circadian rhythms. These factors may drive employees to find ways to keep themselves alert and productive. Because of that, energy drinks, tobacco, and alcohol use commonly co-occur in dairy farm employees. Energy drinks are nonalcoholic beverages containing stimulant compounds such as caffeine, which is marketed to reduce fatigue and improve physical/mental performance. Although frequent use of these beverages has been linked to negative health consequences, farmworkers may not be aware of that. This study aimed to determine the use of energy drinks, alcohol, and tobacco by South Dakota dairy farm employees. A personal interview was done with 70 employees from 4 farms. Participants were 76% male (women: 28 ± 1.7 and men: 34 ± 1.6 years old). The majority of workers were Mexican (46%) and Guatemalan (44%). Descriptive analysis was done using PROC FREQ of SAS v9.4. Thirty-nine percent (85% males) consumed energy drinks regularly (43% Monster and Red Bull beverages), whereas 40% (79% males) did not drink energy drinks at all. Only 21% consumed them in the past. The use of energy drinks was increased during wintertime and claimed to increase body temperature. Forty-nine percent (42% males) consumed alcohol after work on a daily basis and at social gatherings, whereas 40% have never consumed it. Nineteen percent currently smoked (only males), and 20% were former smokers (79% males). No sleep aid substances were used, and 6% of the male participants used pain killer drugs sporadically due to headaches, colds, or lack of sleep. Coffee and water were not frequently consumed. Energy drinks use at work and alcohol at home may impact employee's well-being and increase their risk for future health issues. Measuring the energy drinks consumption and alcohol use is the first step toward understanding its use and risks. A socio-culturally responsive workshop curriculum for farmworkers is needed. This approach is powerful in making meaningful connections between their cultures, languages, current living situation, and life experiences. Study supported by HICAHS (Colorado State University).

Key Words: dairy farms, energy drinks, employee health

P227 Assessing dairy employees' mental health status in South Dakota: Family and community. L. Guifarro*[1], L. Stallones[2], J. Rosecrance[3], and M. Rovai[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Psychology Department, Colorado State University, Fort Collins, CO,* [3]*Environmental & Radiological Health Sciences Department, Colorado State University, Fort Collins, CO.*

Large-scale dairy farming in SD has become of great importance. Most of the dairy workers are Latinos searching for better life opportunities and personal growth. The new life choice, the lack of a close family circle and friends, and the hardship of adaptation to a new community may be associated with distress and low performance at the workplace. The study aim was to gather information on the stress factors related to family and community in dairy workers and assess how it affects their home and neighborhood conditions. Six focus groups (FG) of 1 h each in Spanish were conducted with workers (n = 50; 88% male) from México, Guatemala, and other Latino countries (54%, 30%, and 16%, respectively). Open-ended questions related to stress-causing factors in their current home, home countries, and community were used. Additionally, participants shared different practices used to manage stress. Sessions were video and audio-recorded. Verbatim transcriptions were analyzed and coded for each quotation using ATLAS.TI software (Scientific Software Development GmbH). Around 35 and 31 factors were identified as stress related to family and community, respectively. Factors were grouped as (A) Family: 1. Lack of communication with family back home; 2. Inability to attend to family concerns in their home country; 3. Family estrangement due to longer distances; and 4. Less time shared with family due to job schedule; and (B) Community: 1. Language barrier; 2. Long-distance to grocery shopping; 3. Cultural differences; 4. Weather variations; 5. High-cost healthcare services; and 6. Lack of social support. The results showed that workers are more likely to be distressed due to their current life situation and working circumstances (e.g., culture differences, lack of family support, English barrier). Over time, stress may contribute to health problems like depression or anxiety. Therefore, the importance of employees' well-being is essential in achieving consistent and successful production performance levels. Based on the results, a tri-fold brochure was designed with basic knowledge covering stress causes and ways to reduce it based on their sharing ways to reduce stress. Instructional workshops for workers designed to promote behavioral stress awareness and strategies to better manage and cope with their specific stressors are needed. Supported by HICAHS Community-Initiated Grant Program (Colorado State University).

Key Words: stress factors, dairy workers, focus group

P228 Idaho DHIA supervisor job satisfaction and interest in testing more cows. J. C. Dalton*, *University of Idaho, Animal, Veterinary and Food Sciences Department, Caldwell Research and Extension Center, Caldwell, ID.*

Idaho lactating cow inventory was 635,000 on January 1, 2020, up 21,000 animals from January 1, 2019. Twenty-three independent Idaho DHIA supervisors tested, on average, 289,693 and 300,943 cows per month in 2019 and 2020, respectively. To determine Idaho DHIA supervisor job satisfaction and interest in testing more cows, a survey was conducted in 2 consecutive years. Supervisors (2019: n = 14; 2020: n = 11) in attendance at the 2019 and 2020 Annual Idaho DHIA Supervisor Conference were asked questions regarding length of time working as a supervisor, job satisfaction, number of cows tested per week, number of hours worked per week, and interest in testing more cows. For the years 2019 and 2020, respectively, 65% and 55% of supervisors reported being on the job more than 10 years. When asked how satisfied they were with their job as a DHIA supervisor, the majority responded that they were very satisfied (65% and 82% for 2019 and 2020, respectively). Results showed that 79% and 73% of supervisors (for 2019 and 2020, respectively) tested more than 2000 cows per week, while 69% and 60% of supervisors (for 2019 and 2020, respectively) reported working greater than 40 h per week. When asked if they were interested in testing more cows, 86% and 82% of supervisors responded yes, for 2019 and 2020, respectively. In 2020 63% of supervisors responded they felt respected by dairy personnel, managers, owners, and veterinarians. Survey results provide evidence the majority of Idaho DHIA supervisors are very satisfied with their job and are interested in testing more cows. Idaho, in 2006, was ranked the fourth-largest milk producing state in the United States, with an inventory of 500,000 lactating dairy cows. In 2020, Idaho was the third-largest milk producing state in the United States and had 635,000 cows. With continued dairy industry growth and only 23 DHIA supervisors in Idaho, it is apparent that opportunities exist for current and future supervisors interested in testing cows.

Key Words: dairy, DHIA supervisor, survey

Forages and Pastures: Posters

P229 Effect of growing degree-days and dry matter at harvest of whole-plant corn silage on total-tract neutral detergent fiber digestibility. E. Giugge*[1], J. L. Monge[2], and F. Bargo[3], [1]*Grupo Chiavassa, Carlos Pellegrini, Santa Fe, Argentina,* [2]*Universidad Nacional Villa María, Villa María, Córdoba, Argentina,* [3]*Universidad de Buenos Aires, Capital Federal, Argentina.*

In whole-plant corn silage (WPS), fiber content and fiber digestibility depend on hybrids, cycle length, and growing and soil conditions during the growing season. The variable to define silage harvesting time is the dry matter at harvest (DMatH), also if DMatH is combined with the growing degree-days (GDD) we could define fiber content and digestibility. The aim of this study was to analyze the effect of DMatH and GDD in fiber content and fiber digestibility of the WPS. We registered at a commercial dairy farm in Argentina (Chiavassa Dairy Farm; −32°02′60″ S, −61°47′59″ W) the GDD since planting to harvest time and DMatH during silage harvest and sampled 144 silo-bags (61 flint and 82 semident hybrid types) for 3 years (2017, 2018 and 2019). Before filling each silo-bag and during the silage harvest, each wagon was weighed before loading the material into the bag. Every 2 h, silage DMatH was determined with 200 g whole-plant corn chopped sample dried for 2 h in forced air oven at 135°C. GDD was calculated using the daily average temperature (registered by wireless Vantage Pro 2 weather station in the farm) minus basal temperature 10°C. After 90 d, one sample was collected manually and composed from 3 sampled points from the side of each silo-bag. The samples were analyzed by NIRS (Rock River Lab Inc.) for aNDF, ADF, lLignin (Lig), total-tract neutral detergent fiber digestibility (TTNDFD) and acid detergent indigestible crude protein (ADICP). We ran partial correlation (InfoStat, 2017) keeping year and hybrid type as fixed variables. TTNDFD was negatively correlated ($P < 0.05$) with GDD ($r = −0.56$), DMatH ($r = −0.31$), Lig ($r = −0.482$) and ADICP ($r = −0.46$). TTNDFD was positively correlated ($P < 0.05$) with aFDN ($r = 0.19$). ADICP (as a result of the conservation processes) was positively correlated with Lig ($r = 0.35$). We concluded for these ranges of GDD (1,357–1,666°C) and DMatH (34–52%) that TTNDFD decreases when GDD and DMatH increase. Also the negative relationship of ADCIP with TTNDFD suggests that is affected during the silage process.

Key Words: total-tract neutral detergent fiber digestibility (TTNDFD), corn silage, fiber

P230 Type of harvester impacts on corn silage quality in Brazilian dairy farms. G. F. M. Leão*, E. R. Gaida, C. D. Neufeldt, L. L. Damasceno, D. E. Moreira, P. H. M. Santana, A. A. Meierjurgen, C. J. Risden, H. P. Janssen, and E. M. Ribas, *Negócios Leite, Castrolanda Cooperativa Agroindustrial, Castro, PR, Brazil.*

Quality of corn silage depends on a range of factors, including the type of harvester. The goal of this study was to investigate the influence of the harvester on nutritional parameters of corn silage under dairy farm conditions. Two hundred and 53 samples were collected from 193 dairy farms located in southern of Brazil. Samples were divided in 2 treatments depending of the type of harvester used in the farm during the ensiling process: Pull-type (PT) or Self-propelled (SP). Sequentially, samples were submitted for nutritional quality analysis and data were analyzed using the MIXED procedure of SAS. Silage dry matter after the conservation process was higher (34.20 vs. 32.30%; SEM = 0.45; $P = 0.01$) and pH was lower (3.94 vs. 3.89; SEM = 0.02; $P = 0.01$) for SP silages. In addition, SP silages showed higher values of in vitro digestibility of DM (73.15 vs. 72.45%; SEM = 0.17; $P = 0.03$) and lower levels of acid detergent fiber (23.26 vs. 22.57%; SEM = 0.24; $P = 0.05$), lignin (2.21 vs. 2.08; SEM = 0.02; $P = 0.01$) and ash (3.71 vs. 3.49%; SEM = 0.05; $P = 0.01$). Values of crude protein, fat, starch, neutral detergent fiber (NDF), starch digestibility, NDF digestibility and total digestible nutrients were not affected by the treatments ($P > 0.05$). It is important to highlight that due to the high number of samples and the great variability between farms, some results are of minor biological significance. Overall, the results suggest a better conservation and nutritional quality of SP silages in dairy farm conditions.

Key Words: dairy cattle, forage conservation, pH.

P231 Effect of kernel breakage on the fermentation profile, N fractions, and in vitro starch digestibility of whole-plant corn silage and ensiled corn grain. B. A. Saylor*[1], E. C. Diepersloot[1], C. Heinzen Jr.[1], C. L. McCary[2], and L. F. Ferraretto[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The objective of this experiment was to analyze the effect of kernel breakage on the fermentation profile, N fractions, and in vitro starch digestibility (ivSD) of whole-plant corn silage (WPCS) and ensiled corn grain. Whole corn plants were harvested, and ears were separated from the forage portion and shelled. Corn kernels were either left intact or broken manually using a hammer. The remaining forage portion of the plants was chopped. Samples of the intact and broken kernels were ensiled for 0 or 30 d in quadruplicate vacuum pouches. Remaining intact and broken kernels were each reconstituted with the chopped forage portion to simulate “whole-plant” corn forage. Reconstituted whole-plant corn forage samples were also ensiled for 0 or 30 d in vacuum pouches. Data for the reconstituted WPCS, kernels separated from WPCS, and the ensiled corn grain were analyzed separately. Data were analyzed as a completely randomized design using PROC GLIMMIX of SAS. The model included kernel form, ensiling, and their interaction as fixed effects. Concentrations of N fractions and organic acids were unaffected ($P > 0.05$) by kernel form (intact or broken) in WPCS. Soluble CP and ammonia-N concentrations were affected by ensiling ($P < 0.001$), with elevated concentrations of both N fractions in WPCS stored for 30 d. In kernels separated from WPCS, kernel form had no effect ($P = 0.88$) on zein protein concentrations. However, an interaction between kernel form and ensiling was observed ($P < 0.001$) for ivSD. Starch digestibility increased with ensiling, from 23.7 to 35.7% of starch, only in kernels that were broken. In ensiled corn grain, an interaction between kernel form and ensiling was observed ($P < 0.001$) for concentrations of soluble CP and ammonia-N. These N fractions increased with ensiling to a greater extent when kernels were broken. This study gives insight into the importance of kernel breakage to improve starch digestibility in corn silage through means other than a reduction in particle size.

Key Words: corn silage, fermentation profile, ruminal starch digestibility

P232 Effects of tillage system and cutting height at harvest on dry matter yield, chemical composition, and digestibility of forage maize. O. I. Santana*[1], A. Peña-Ramos[1], J. I. Sanchez-Duarte[2], and

A. Reyes-Gonzalez[2], [1]*INIFAP Campo Experimental Pabellon, Pabellon de Arteaga, AGS, Mexico,* [2]*INIFAP Campo Experimental Laguna, Matamoros, COAH, Mexico.*

The aim was to assess the effects of 3 tillage systems [no-tillage (NT), minimal tillage (MT; only plowing), and conventional tillage (CT; subsoiling and plowing)] and 2 cutting heights at harvest (15 vs. 40 cm) on dry matter (DM) yield, chemical composition, and digestibility of forage maize. A randomized complete block design with 4 replicates and 3 × 2 factorial arrangement was conducted in a field with subplots where each tillage system was carried out for 10 yr. Experimental unit was 4 rows of 0.76 m width and 5.0 m long. A white-dent hybrid (Supremo, Aspros) was planted at 90,000 seeds/ha. Maize was harvested when kernel had one-half milkline by cutting all plants on the 2 central rows at either 15 or 40 cm stubble height. Swaths were weighed and 5 whole-plants were chopped and a sample was dried to determine DM content. Dried samples were ground for chemical analysis and for in situ digestibility of DM (DDM) and neutral detergent fiber (DNDF) at 36 h using 2 rumen-cannulated cows. Data were analyzed in R studio using the same statistical model for all variables. Forage DM yield was not affected by treatments (mean ± SD; 26.7 ± 2.8 t/ha), but DM content was greater ($P = 0.05$) in NT (33.4%) compared with MT (31.3%) and CT (31.0%). Content of NDF was greater ($P < 0.01$) in forage cut at 15 than 40 cm (50.9 vs. 47.6%, respectively). There were differences ($P = 0.04$) among tillage systems for content of nonfiber carbohydrates (36.0, 32.4 and 34.1% for NT, MT and CT, respectively), which also differed ($P < 0.01$) between cutting heights (32.5 vs. 35.8% for 15 and 40 cm, respectively). There were interactions between tillage system and cutting height for DDM and DNDF (both $P < 0.01$). Former was greater in NT and MT cut at 40 than 15 cm (74.7 vs. 66.0% and 70.1 vs. 68.6%, respectively) but similar in CT (67.1 ± 1.4%). In contrast, DNDF was similar in MT (38.9 ± 0.8%) but lower in NT and CT cut at 40 than 15 cm (37.6 vs. 33.4% and 36.1 vs. 34.3%, respectively). In conclusion, independently of tillage system used, harvesting at higher cut improved nutritional value of forage without reducing DM yield.

Key Words: crop system, digestibility, fiber.

P233 Influence of the inclusion of passion fruit waste in forage maize silage.

I. Espinoza*[1], M. Romero[1], A. Barrera[1], A. Sanchez[1], M. Medina[1], G. Muñoz[1], E. Torres[1], Y. Torres[1], M. Barros[2], and L. Montenegro[1], [1]*Universidad Tecnica Estatal de Quevedo, Quevedo, Los Rios, Ecuador,* [2]*Universidad Tecnica de Ambato, Ambato, Tungurahua, Ecuador.*

The aim of this research was to evaluate the effect of the inclusion of passion fruit (*Passiflora edulis* sims) residue in forage maize silage harvested after 60 d growth. The silage treatments on a % fresh basis were: T1 = 100% maize silage; T2 = 75% maize silage + 25% P. edulis waste; T3 = 50% maize silage 50% + 50% P. edulis waste; T4 = 25% maize silage + 75% P. edulis waste; T5 = 100% P. edulis waste, with 5 replications per treatment. The silages were made in 3 kg capacity polyvinyl chloride mini silos, with silage treatments manually packed, and kept at room temperature (28°C to 30°C) for 30 d fermentation. Crude protein (CP), dry matter (DM), organic matter (OM), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and fat were evaluated. Chemical composition data were analyzed with the GLM procedure of SAS, with treatment as a fixed effect, and the least squares means compared using the Tukey test. The treatments T1, T2 and T3 showed higher ($P < 0.05$) DM concentration (21.98, 22.62, and 22.70% respectively). The OM was higher ($P < 0.05$) in T1, T4, T5 (91.14, 90.37, and 90.83% respectively). The CP was higher ($P < 0.05$) in T3, T4 and T5 (8.63, 8.71, and 8.41% respectively). The NDF, ADF and fat were higher for T5 (68.49, 55.02, and 14.15% respectively) and lower in T3 (58.37; 41.81 and 8.13% respectively) than other treatments ($P < 0.05$). Under the conditions of this study, it can be concluded that the incorporation of waste from passion fruit in forage maize silage increases the protein and fat content, and decreases the fiber content.

Key Words: maize silage, passion fruit, chemical composition

P234 Effects of microbial inoculation and variety on fermentation profile of whole-plant sorghum silage under short-term fermentation.

M. R. Pupo*[1], E. C. Diepersloot[1], L. G. Ghizzi[2,3], J. O. Gusmão[2,4], C. Heinzen Jr.[1], C. L. McCary[2], M. O. Wallau[5], and L. F. Ferraretto[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*Department of Animal Nutrition and Animal Production, University of São Paulo, Pirassununga, São Paulo, Brazil,* [4]*Department of Animal Sciences, University of Lavras, Lavras, MG, Brazil,* [5]*Agronomy Department, University of Florida, Gainesville, FL.*

Our objective was to assess the effects of sorghum variety, microbial inoculation, and storage length on fermentation profile, nutrient composition and NDF ruminal disappearance of whole-plant sorghum silage. Samples from 3 varieties [forage sorghum, sorghum-sudangrass, and sweet sorghum] were collected at harvest, immediately inoculated [CON (50 mL distilled water) or LBLD (*Lactobacillus plantarum*, *L. buchneri*, and *L. diolivorans*; 300,000 cfu/g of wet forage)] and ensiled in experimental silos for 14, 28, or 56 d. Data were analyzed as a completely randomized design with a 3 × 2 × 3 factorial arrangement of treatments using the PROC GLIMMIX of SAS with fixed effects of variety, inoculation, storage length, and their 2- and 3-way interactions. After 56 d, pH was greatest ($P = 0.02$) in LBLD sweet sorghum and CON forage sorghum, while no differences ($P > 0.05$) were observed in sudangrass, with all pH values below 4.00. The LBLD treatments had greater ($P = 0.001$) acetic acid concentration than CON in forage sorghum. Regardless of variety, CON silage had no detectable 1,2-propanediol (1,2-PD) concentration. On the other hand, LBLD sweet sorghum and forage sorghum had greater ($P = 0.001$) levels of 1,2-PD at 56 d, with no differences ($P > 0.10$) observed for LBLD sudangrass. Neither CON nor LBLD in sudangrass or LBLD forage sorghum had detectable propionic acid. Furthermore, the concentration of propionic acid in CON sweet sorghum was undetectable at 14 d, while LBLD sweet sorghum was greater ($P = 0.001$) at 14 d and CON forage sorghum ($P = 0.001$) at 56 d. For sudangrass and sweet sorghum, LBLD decreased ($P = 0.01$) yeast counts compared with CON. The ruminal disappearance of NDF for forage sorghum was greater ($P = 0.01$) than sudangrass, while sweet sorghum was similar to other treatments ($P > 0.10$). Results indicate *L. buchneri* and *L. diolivorans* are actively involved in a heterofermentative co-fermentation resulting in the production of acetic acid and 1,2-PD. Additionally, the effects of these strains were pronounced in sweet and forage sorghum varieties before 30 d of ensiling.

Key Words: heterofermentative inoculant, sorghum silage, 1,2-propanediol

P235 Effects of hybrid, season and trait on nutrient composition, dry matter yield, and predicted milk production of whole-plant sorghum grown in Florida.

M. R. Pupo*[1], C. Heinzen Jr.[1], M. O. Wallau[2], and L. F. Ferraretto[1], [1]*Department of Animal and Dairy*

Sciences, University of Wisconsin, Madison, WI, [2]Agronomy Department, University of Florida, Gainesville, FL.

Sorghum is becoming an important forage source for dairy cows in regions susceptible to adverse growing conditions for corn production. This study aimed to retrospectively assess the effects of season [summer vs. Spring], hybrid (forage sorghum vs. Sorghum-sudangrass) and trait [brown midrib (BMR) vs. non-BMR] on nutrient composition, DM yield and predicted milk yield of whole-plant sorghum grown in Florida. Twenty-four field trials were established from 2008 to 2019 and harvested at a targeted DM of 32%. Data were analyzed as a completely randomized design with a 2 × 2 × 2 factorial arrangement of treatments with the fixed effects of hybrid, trait, season, and their interaction, and year as a random effect. Spring sorghum had ($P = 0.01$) greater predicted milk yield per Mg (1,360 vs. 1,307 kg/Mg) and ha (22,731 vs. 14,710 kg/ha) than summer. This was related to greater ($P = 0.001$) DM yield (20.7 vs.14.1 Mg/ha) and starch concentration (14.4 vs. 10.4% of DM) but reduced NDF concentration ($P = 0.02$; 56.0 vs. 57.3% of DM) in the spring than summer. Season did not affect NDF digestibility ($P = 0.63$; 47.3% of NDF, on average). Despite a tendency ($P = 0.06$) for lower DM yield (16.9 vs. 17.9 Mg/ha), forage sorghum had ($P = 0.001$) greater predicted milk yield per forage (1,424 vs. 1,244 kg/Mg) and area (19,688 vs. 17,953 kg/ha) than sudangrass. Forage sorghum had ($P = 0.001$) 5.6%-units lower NDF concentration, but 2.3, 4.2, and 6.2%-units greater NDF digestibility (% of NDF), TDN and starch concentrations, respectively. Greater ($P = 0.01$) DM yield (19.1 vs.15.7 Mg/ha) and predicted milk unit of forage (19,767 vs. 17,874 kg/ha) was observed for non-BMR than BMR. In contrast, BMR sorghum had greater ($P = 0.001$) NDF digestibility (49.8 vs. 44.8% of NDF) and corresponding greater predicted milk yield per Mg of forage (1,385 vs. 1,283 kg/Mg) than non-BMR. These results indicate summer season might negatively affect yield and nutritive value of sorghum crop for silage. Sorghum trait and hybrid could be important alternatives to minimize discrepancies between seasons.

Key Words: forage quality, brown midrib, fiber disappearance

P236 Effect of down corn plants on fermentation profile and nutrient composition in whole-plant corn forage and silage. E. C. Diepersloot*[1], J. P. Goeser[2], G. R. Dahlke[3], D. Meyer[2], and L. F. Ferraretto[1], [1]*University of Wisconsin–Madison, Madsion, WI,* [2]*Rock River Laboratory Inc., Watertown, WI,* [3]*Iowa Beef Center, Ames, IA.*

This study aimed to evaluate the effect of down corn on fermentation profile and nutrient composition of whole-plant corn forage and silage. For case study 1 (June 2019; Lee, FL), corn was blown down by a severe thunderstorm and harvested at silage maturity similarly for both down (3 fields) and standing corn (STN; 4 fields) and ensiled in Ag-Bag silos for similar periods of time before samples were collected (down = 6 silos; STN = 8 silos) and analyzed for fermentation profile and nutrient composition. For case study 2 (summer 2020; Ames, IA), corn was blown down by a derecho. Corn plants down and STN were both cut from 6 fields each to a similar stubble height, processed and forage samples were analyzed for nutrient composition. Data from each case study were analyzed separately using Proc Glimmix of SAS with the fixed effect of treatment. In study 1, STN had a lower pH ($P = 0.001$; 3.86 vs 4.02, respectively) and greater lactic acid concentration ($P = 0.001$; 4.6 vs 3.5% DM, respectively) compared with silage from down corn. Additionally, down silage had greater NDF ($P = 0.03$; 41.4 vs 36.7% DM, respectively), ADF ($P = 0.01$; 25.0 vs 20.8% DM, respectively) but lower starch ($P = 0.04$; 32.1 vs 36.6% DM, respectively) concentration. Thus, STN silage had greater predicted TDN ($P = 0.001$; 72.0 vs 67.2% DM), NE_L ($P = 0.001$; 0.71 vs 0.65 Mcal/kg, respectively) and milk yield ($P = 0.001$; 3,340 vs 2,975 kg/Mg, respectively) based on the Milk2006 predictions. Conversely, these metrics were not affected ($P > 0.05$) in study 2, highlighting the potential for quality silage to be made from down corn. In this experiment the STN corn was affected by high winds while vegetative, thus corn likely recovered to some extent. However, the results from these studies demonstrate the potential for down or lodged corn silage to have an equivalent to lesser silage quality and nutritive value. The contrasting observations between study 1 and 2 could be due to down corn silage being in the field 2 weeks longer than STN corn in study 1, and a potential interaction between maturity and plant damage.

Key Words: lodging, predicted energy content, silage quality

P237 Effects of the fungicide application on corn silage quality in dairy farms. G. F. M. Leão*, R. G. Oliveira, F. P. Basco, L. Goltz, S. T. Guerra, J. S. Ribas, H. P. Janssen, and E. M. Ribas, *Negócios Leite, Castrolanda Cooperativa Agroindustrial, Castro, PR, Brazil.*

Quality of corn silage depends on a range of factors, including management practices related to the culture. The goal of this study was to investigate the influence of the fungicide application on nutritional parameters of corn silage in dairy farm conditions. Two hundred and forty-one corn silage samples were collected from 193 dairy farms located in southern of Brazil. Samples were divided in 2 treatments depending of the fungicide application during corn management in the field: with fungicide (FUNG) or without (CONT). Sequentially, samples were submitted for nutritional quality analysis and data were analyzed using the MIXED procedure of SAS. FUNG silages presented higher values of in vitro digestibility (73.27 vs. 72.48%; SEM = 0.18; $P = 0.01$), crude protein (8.23 vs. 7.95%; SEM = 0.08; $P = 0.02$), starch digestibility (76.14 vs. 74.28%; SEM = 0.50; $P = 0.01$) and neutral detergent fiber (NDF) digestibility (49.41 vs. 48.58%; SEM = 0.30; $P = 0.04$). The FUNG silages showed lower levels of NDF (40.82 vs. 41.91%; SEM = 0.30; $P = 0.01$), acid detergent fiber (22.53 vs. 23.15%; SEM = 0.20; $P = 0.03$) and lignin (2.08 vs. 2.16; SEM = 0.02; $P = 0.04$). Values of dry matter content, pH, fat, ash, starch and total digestible nutrients were not affected by the treatments ($P > 0.05$). It is important to highlight that due to the high number of samples and the great variability between farms, some results are of minor biological significance. Overall, the results suggest a better nutritional quality of FUNG silages in dairy farm, claiming the relevance of fungicide application during the management of corn culture.

Key Words: dairy cattle, NDF digestibility, starch digestibility.

P238 Projected impact of future climate conditions on the agronomic performance of 4 binary alfalfa-grass mixtures in Quebec dairy farms. C. Payant*[1], G. Jego[2], V. Ouellet[1], P. Grenier[3], G. F. Tremblay[2], G. Bélanger[2], and É. Charbonneau[1], [1]*Université Laval, Quebec, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Quebec, QC, Canada,* [3]*Ouranos, Montreal, QC, Canada.*

Timothy is widely grown on dairy farms in association with alfalfa in Northern regions but may be more impacted by future climate conditions (FCC) than other mixtures. Our objective was to assess the agronomic performance of alfalfa with timothy, tall fescue, meadow fescue, or meadow bromegrass and corn, soybean, barley, and spring wheat on 2 virtual farms representative of Eastern Quebec (EQ) and Southwest Quebec (SWQ), Canada, under reference and FCC. The

Integrated Farm System Model (IFSM) was used to simulate yield and nutritive value of all crops grown under reference (REF: 1971–2000), near (NF: 2020–2049) and distant (DF: 2050–2079) future climates using 6 climate scenarios of different intensity with 2 representative concentration pathways (RCP 4.5 and RCP 8.5) and 4 climate models. In these scenarios growing degree-days (GDD base 5°C) are projected to increase by 161 to 412 GDD in near future and by 487 to 930 in distant future. The IFSM has been previously calibrated and validated to predict yield and nutritive value of most crops used in Quebec. For forages that were not previously calibrated, data from literature, and field results were used for additional calibration of the model. Compared with REF, yields of the 4 alfalfa-grass mixtures are expected to increase with FCC. Yield of the alfalfa-tall fescue mixture is predicted to increase the most (REF: 6.8 ± 0.8, NF: 9.0 ± 1.5, DF: 10.2 ± 1.2, and REF: 9.4 ± 1.0, NF: 12.0 ± 1.55, DF: 12.3 ± 1.4 t/ha, in EQ and SWQ) and that of the alfalfa-timothy mixture the least (REF: 7.0 ± 0.9, NF: 8.7 ± 1.2, DF: 9.6 ± 1.2, and REF: 9.2 ± 1.0, NF: 11.1 ± 1.5, DF: 11.1 ± 1.3 t/ha in EQ and SWQ). Proportion of alfalfa should increase in the mixture with FCC, increasing the crude protein and decreasing the neutral detergent fiber concentrations of the 4 mixtures. In both areas, corn and soybean yields are projected to increase, while those of wheat and barley are projected to remain stable or to slightly decrease. Our results suggest that tall fescue and meadow fescue in mixture with alfalfa could benefit more from climate change than timothy.

Key Words: alfalfa-grass mixture, whole-farm model, climate change

P239 Effects of planting density on nutritive value and in situ dry matter degradability of alfalfa cultivars adapted to the southeastern United States. S. S. Lee*[1,3], E. F. Rios[2], A. Anju[2], and D. Vyas[3], [1]*Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Gyeongsangnam-do, South Korea,* [2]*Agronomy Department, IFAS, University of Florida, Gainesville, FL,* [3]*Department of Animal Sciences, IFAS, University of Florida, Gainesville, FL.*

We aimed to evaluate the effects of different planting densities on nutritive value and in situ dry matter (DM) degradability of alfalfa *Medicago sativa* L. adapted to southeastern United States. Three alfalfa cultivars (FL77, FL99 and B805) and 2 breeding lines from the University of Florida Forage Breeding program (AP15, AP17) were seeded in 4 replicated plots at a seeding rate of 20 and 27 kg·ha^{-1}. Plots were planted at Plant Science Research and Education Center in Citra, Florida. Plots were harvested on June 24 and July 23 2020, and harvested forage was dried, ground (1 mm), and analyzed for nutritive value using near-infrared spectroscopy. Dry matter yield (DMY, kg·ha^{-1}) was analyzed for both harvests as a factorial for seeding rate and cultivars considering harvest as repeated measures. In situ ruminal DM disappearance was measured for AP15 and B805 after ruminal incubation for 0, 3, 6, 9, 12, 24, 48, 72, and 96 h in triplicates using 2 nonlactating Holstein dairy cows. Data were analyzed as 5 × 2 factorial using GLIMMIX procedure of SAS using planting densities (2 levels) and cultivars (5 levels) as fixed factors while harvest was used as random factor in the model. Mean separation was performed by a Tukey test and differences were declared significant at $P \leq 0.05$. No interaction effect was observed between planting density and cultivars on the nutritive value and DMY of alfalfa. AP15 (1908 kg·ha^{-1}) and AP17 (2,131 kg·ha^{-1}) produced the highest DMY among all cultivars. B805 produced the lowest DMY (1,362 kg·ha^{-1}). Cultivar B805 had lowest lignin concentration, greater net energy of lactation, and greater milk per ton of forage. Neutral detergent fiber degradability (NDFD) was greater for B805, FL77, and FL99 when seeded at 20 kg·ha^{-1}; however, when cultivars were seeded at 27 kg·ha^{-1}, NDFD was greater for B805 compared with other cultivars. Both the rate and extent of DM degradation was greater for B805 compared with AP15. In conclusion, B805 had greater nutritive value, while AP15 and AP17 had greater DMY compared with other cultivars.

Key Words: alfalfa, hybrid, in situ degradability

P240 Effects of seeding intensity and cutting on the nutritional quality of grass-legume mixtures. K. V. Almeida*[1], L. H. P. Silva[1,2], J. P. Sacramento[1], D. C. Reyes[1], R. G. Smith[1], N. Warren[1], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH,* [2]*Western Kentucky University, Bowling Green, KY.*

Objectives were to compare the nutritive value of 4 binary legume-grass (LG) mixtures, and 2 complex LG mixtures under different seeding strategies over 2 years following a 3-cutting schedule. We hypothesized that different LG mixtures would result in varying supplies of N and NSC, thus better balancing the RDP:energy ratio in forages. A randomized complete block design with a 6 × 3 factorial arrangement of treatments and 4 replicates for each treatment was used. Legumes selected were alfalfa (ALF), red clover (RC), birdsfoot trefoil (BFT), and white clover (WC) in binary mixtures with orchardgrass at 70:30 LG seeding rate, a high legume mixture (HI) with 70% of all 4 legumes and 30% orchardgrass, and 30:70 LG seeding rate, a low legume mixture (LO) with 30% of all 4 legumes and 70% orchardgrass. After plots were harvested, a representative sample of 200 g was collected for later analyses. Data were analyzed using GLIMMIX procedure of SAS. The statistical model included treatment and cutting as fixed effects with block and year as random factors. Main effects of treatments are shown in Table 1. Crude protein increased in HI compared with ALF and BFT, with HI and LO showing reduced NDF concentration relative to ALF and BFT. Starch was greater in LO vs. WC but it did not differ compared with the other treatments. The RC, HI, and LO treatments resulted in greatest ESC and NSC (ESC plus starch) and ALF the lowest. In vitro NDF digestibility was lower for HI and LO compared with the binary LG mixtures. In general, second and third cuttings improved nutritive value via greater CP, IVNDFD, NSC:N, and NE_L.

Table 1. Nutritional composition (% of DM unless otherwise noted) of binary and complex legume-grass mixtures

	Treatment							*P*-value
Item	ALF	RC	BFT	WC	HI	LO	SEM	Treat
CP	13.3^{c}	15.0^{a}	13.5^{bc}	14.3^{ab}	14.8^{a}	14.3^{ab}	0.51	<0.01
aNDFom	50.0^{a}	46.3^{b}	50.6^{a}	48.6^{ab}	45.4^{b}	45.8^{b}	1.28	<0.01
Starch	2.77^{ab}	2.85^{ab}	2.65^{ab}	2.14^{b}	3.12^{ab}	3.18^{a}	0.46	0.05
ESC	7.14^{b}	8.72^{a}	7.67^{ab}	8.23^{ab}	8.90^{a}	8.84^{a}	0.45	<0.01
NSC	9.92^{b}	11.6^{ab}	10.3^{ab}	10.4^{ab}	12.0^{a}	12.0^{a}	0.63	<0.01
NSC:N ratio	4.99	4.94	5.08	4.87	5.22	5.45	0.33	0.47
IVNDFD	59.7^{b}	55.8^{bc}	59.9^{b}	64.6^{a}	53.4^{c}	56.0^{bc}	1.44	<0.01
NE_L, Mcal/kg	1.40^{b}	1.49^{a}	1.40^{b}	1.47^{ab}	1.47^{ab}	1.48^{a}	0.02	<0.01

Key Words: forage, legume, sugar

P241 Effect of grazing fall-stockpiled tall fescue, meadow fescue, or orchard grass on heifer growth and greenhouse gas production. K. G. Wells*[1], M. A. Wattiaux[1], D. M. Pizarro[1], J. S. Cavadini[2], and M. S. Akins[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Marshfield*

Agricultural Research Station, University of Wisconsin–Madison, Stratford, WI.

By extending the grazing season through the fall by stockpiling pastures, dairy heifers could be raised with lower costs and in a more environmentally conscious way. Our study examined average daily gain (ADG) and the production of CH_4 and CO_2 by dairy heifers grazing stockpiled pastures during fall in Wisconsin. The study was designed as a randomized complete block using 9 pastures established in triplicate with 1 of 3 grass species mixed with red clover (tall fescue (TF), meadow fescue (MF), or orchard grass (OG)). Heifers (n = 36) with body weight ranging from 267 to 333 kg, were blocked by weight (low, medium, high) and randomly assigned to 1 of the 3 grass specie replicates with 4 heifers per pasture. Heifers grazed for 48 d from October 7 to November 24, 2020 and were allocated new pasture areas daily based on pre-grazing forage availability to allow for a dry matter intake (DMI) of 2.5% of body weight. Water was provided free-choice and mineral offered at 0.11 kg/d per heifer. Pasture consumption by heifers (DMI) was determined based on the area grazed and weekly pre- and postgrazing forage availability. Individual heifer ADG was based on weights collected over 3 consecutive days pre- and post–study. A GreenFeed pasture system (C-Lock Inc., Rapid City, SD) was used to measure CH_4 and CO_2 production (g/d per heifer). Pasture was considered the experimental unit and data were analyzed with R studio using a mixed model ANOVA to determine the fixed effect of grass specie and the random effect of heifer within species. There was no difference in DMI among treatments (9.2, 10.0, and 10.6 kg/d per heifer for MF, OG, and TF, respectively, P = 0.49), but there was a tendency in ADG (0.98, 1.07, and 0.95 kg/d for MF, OG, and TF, respectively, P = 0.09). Average CH_4 production was 206, 214, and 197 g/d per heifer for MF, OG, and TF, respectively *(P* = 0.41). Average CO_2 production was 6,861, 6973, and 7,082 g/d per heifer for MF, OG, and TF, respectively (P = 0.37). Overall, grazing stockpiled pastures resulted in optimal heifer growth with no effect of grass species on greenhouse gas production by heifers.

Key Words: dairy heifer, grazing, stockpile forage

P242 Effect of defoliation frequency around flowering time on the productivity and nutritive value of tall fescue. J. C. Marrero[1,2], F. A. Lattanzi[1], C. Cajarville[2], F. Díaz*[3], and J. M. Arroyo[1,4], [1]*Instituto Nacional de Investigación Agropecuaria, Colonia, Uruguay,* [2]*Instituto de Producción Animal de Veterinaria, Facultad de Veterinaria, Universidad de la Republica, Libertad, San José, Uruguay,* [3]*Dellait Dairy Research Center, Brookings, SD,* [4]*4Institute of Animal Science, University of Bonn, Bonn, Germany.*

Increasing the defoliation regimen of pastures allows to improve its nutritive value for ruminants but it can be detrimental for total biomass production. An experiment was carried out to study the effect of the defoliation regimen around flowering time on productivity, chemical composition and nutritional value of tall fescue. The experiments were carried out on 2-year old tall fescue (*Festuca arundinacea* cv 'INIA Fortuna') pastures, at the experimental station "La Estanzuela" (INIA, Uruguay). Three defoliation regimens –cut at 1.5 leaf-stage, at 2.5 leaf-stage, or 1.5 leaf-stage before flowering and at 2.5 leaf-stage afterward– were studied in 2 paddocks. Pasture A was not grazed during the previous winter whereas pasture B was. At each paddock, defoliation treatments were arranged in a completely randomized block design with 4 repetitions (blocks). DM, OM, NDF, CP and in vitro digestible NDF (NDFD; Ankom Technology Corp., Macedon, NY) production (kg/ha/d) were determined for each treatment and paddock for the spring season (09/01/2018 to 11/26/2018). The data were analyzed independently for each paddock using a General Linear Model ANOVA. In paddock A there was no effect of defoliation regimen (Table 1). On the contrary, in paddock B cut at 1.5 leaf-stage resulted in lower DM production. Increase in quality, higher CP and lower NDF content, were not enough to offset the lower productivity of higher defoliation frequency. These results suggest that winter management of pasture strongly affects its productive and nutritional value responses to defoliation regimen during spring.

Table 1. Effect of the defoliation regime on nutrient production (kg/ha per day) of tall fescue

	Treatment (leaf stage)				
Item	1.5	1.5–2.5	2.5	SEM	*P*-value
Location A					
DM	57.3	61.1	58.7	2.15	0.486
OM	51.6	54.7	52.4	1.92	0.521
CP	13.0	14.2	12.0	0.87	0.108
NDF	29.9	31.5	31.5	1.37	0.660
NDFD	21.4	22.2	21.0	0.90	0.634
Location B					
DM	37.7[c]	43.7[b]	57.6[a]	1.35	<0.001
OM	33.7[c]	39.0[b]	51.4[a]	1.17	<0.001
CP	8.82[b]	9.91[b]	11.3[a]	0.280	0.002
NDF	18.9[b]	22.2[b]	30.5[a]	0.77	<0.001
NDFD	13.4[b]	14.8[b]	20.7[a]	0.46	<0.001

[a–c]Means within a row with different superscripts are significantly different (P < 0.05).

Key Words: tall fescue, defoliation regime, nutritive value

P243 Physiochemical alterations during germination of hydroponically sprouted cereal grains. S. Jenkins*[1] and F. Diaz[2], [1]*HydroGreen Incorporated, CubicFarm Systems Corporation, Sioux Falls, SD,* [2]*Dellait Dairy Research Center, Brookings, SD.*

The increasing negative influence of abiotic factors on crop production coupled with the fragility of the global animal feed supply chain accentuates the importance of investigating hydroponically sprouted cereal grains for implementation in the dairy industry. Physical and chemical properties of hydroponically sprouted barley (*Hordeum vulgare*) and hard red winter wheat (*Triticum aestivum)* were investigated over a period of 7 d in an automated HydroGreen vertical farming system. Within the system, crops were seeded at a 14.6 kg $m^{2–1}$ density with environmental controls maintaining 22°C, 70% relative humidity, and an 18-h photoperiod schedule; irrigation was supplied via overhead spray nozzles throughout development. Replicated (n = 2) samples collected at 0, 24, 48, 72, 96, 120, 144, and 168 h after initial seeding revealed parallel physiochemical transformational trends for both barley and wheat. After 144 h, initial starch concentration decreased 40 percentage points (SE = 1.2 pp, P < 0.01) on average for both crops, while sugars (ethanol soluble carbohydrates) concentration increased 25.9 pp (SE = 1.1 pp, P < 0.01) from the original value. The increase in the concentration of sugars at 144 h was accompanied by significant gains in overall concentrations of ADF (+6.4 pp; SE = 0.3 pp, P < 0.01) and NDF (+10.0 pp; SE = 0.6 pp, P < 0.01). Relative moisture content of the hydroponically sprouted product increased 63% on average. Dry matter yields closely paralleled those of the reported enzymatic activity, with the highest enzymatic and dry matter yields occurring at 144 h after seeding. Compared with the original starting grain, the hydroponic sprouting process in the HydroGreen system resulted in similar energy yields, with other dramatic shifts in overall nutrient composition facili-

tated by enzyme hydrolysis. Observed results highlight the potential of hydroponically sprouted cereal grains in dairy diets and provide direction for future implementation within this feedstuff category. Further work is needed to quantify the sprouted grain's enzymatic activity and elucidate its impacts on overall diet nutrient digestibility.

Key Words: hydroponic, forage, enzymes

Growth and Development: Posters

P244 Sodium butyrate and monensin supplementation to postweaned heifer diets: Effects on growth performance, nutrient digestibility, and health.

T. C. Stahl*[1], E. Hatungimana[1], K. D. Klanderman[2], S. C. Moreland[2], and P. S. Erickson[1], [1]*University of New Hampshire, Durham, NH,* [2]*Adisseo USA, Inc., Alpharetta, GA.*

Objectives of this study were to compare sodium butyrate (SB) to monensin (MON) on growth, digestibility, and health of postweaned heifers. Forty Holstein heifers (age = 84.2 ± 1.2 d; average BW = 99.78 ± 10.77 kg) were fed diets ad libitum. Heifers were blocked by birth date and assigned to 1 of 4 treatments in a randomized complete block. The control group (CON) was fed 100 g of SBM carrier, while treatments were supplemented with carrier + 0.75 g SB/kg of BW, 1 mg MON/kg of BW, or 1 mg MON/kg of BW + 0.75 g SB/kg of BW (MSB). Amounts of orts and feed offered to each heifer were measured daily. Initial BW, skeletal measurements and blood samples were taken d1 before the receiving treatment, and every wk thereafter for 12 wk. Fecal grab samples were taken weekly from each heifer for coccidia counts. Two total-tract digestibility phases: 21–27 d (wk 3) and 63–69 d (wk 9) were used. Digestibility was measured by acid detergent insoluble ash. Single degree freedom contrasts for CON vs. ADD (control vs. additive), SB vs MON, and SB+M vs MSB (single additives vs. MSB) were determined. Compared with CON, ADD tended to increase average BW ($P = 0.10$), final BW ($P = 0.09$), and heart girth ($P = 0.10$). Compared with CON, ADD increased DMI (CON = 4.0, SB = 4.5, MON = 4.2, MSB = 4.5 kg/d; $P = 0.03$). Compared with CON, ADD decreased coccidian oocysts (CON = 1,248.9, SB = 697.9, MON = 762.5, MSB = 781.8 coccidia/kg of feces; $P = 0.03$). Compared with MON and SB, MSB tended to increase heart girth ($P = 0.07$). Compared with MSB, MON and SB tended to have greater plasma glucose ($P = 0.09$). Compared with SB, ADG/DMI was improved in MON supplemented heifers (CON = 0.27, SB = 0.25, MON = 0.28, MSB = 0.27; $P = 0.04$). Besides heart girth, no other differences were observed for skeletal measures ($P \geq 0.24$). Week 3 total-tract digestibility (DM, NDF, ADF, Hemicellulose, OM, and fat) were similar ($P \geq 0.16$). Week 9 total-tract digestibility (DM, CP, ADF, Hemicellulose, Starch, OM, and fat) were similar ($P \geq 0.12$). Overall, additive supplementation offers positive results in growth performance, and reduced coccidia counts

Key Words: sodium butyrate, monensin, coccidia

P245 Growth, feed efficiency, and health of female Holstein calves cooled during the preweaning period.

A. B. Montevecchio*[1], W. Frota[1], V. R. Merenda[1], J. G. Martin III[2], and R. C. Chebel[1], [1]*Department of Large Animal Clinical Sciences & Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Dairy Design Engineers, Gainesville, FL.*

Our objectives were to determine the effects of cooling female Holstein calves through forced air ventilation on growth, feed efficiency, and health. At birth (0d), calves were assigned randomly to: SH (n = 125) shade with no cooling, SHF (n = 101) shade with cooling through ceiling fans. Hutches were placed in a barn and SH and SHF treatments were applied in 3 alternating sections of the barn. Calves received milk replacer twice a day (0 to 13d = 3.8L, 14 to 18d = 5.6L, 19 to 49d = 7.6L, 50 to 57d = 3.8L, 58 to 64d = 0.9L) and starter ad libitum starting at 14d. All calves were weighed (BW) and had the wither (WH) height measured at birth, 71 d (weaning), and 146d. A subset of calves (SH = 54, SHF = 43) was weighed and had WH measured at 12, 19, 26, 34, 47, 54, and 61d. Intakes of milk solids and starter were evaluated daily. Health was scored in the AM, including rectal temperature (RT), every 3.5 ± 1.1d from 2 to 68d. Hutches (SH = 18, SHF = 17) were evaluated for air speed and temperature at 1000 and 1600 h and calves in these hutches had RT and respiratory frequency (RF) measured at the same time. Data were analyzed by ANOVA. The SHF treatment resulted in greater ($P < 0.01$) air velocity (AM: SH = 0.41 ± 0.05, SHF = 1.22 ± 0.05m/sec; PM: SH = 0.42 ± 0.05, SHF = 1.22 ± 0.06m/sec) and reduced ($P \leq 0.10$) air temperature in the (AM: SH = 30.5 ± 0.1, SHF = 30.2 ± 0.1°C; PM: SH = 32.7 ± 0.1, SHF = 32.5 ± 0.1°C). Calves in the SHF treatment had lower RT in the AM (38.8 ± 0.0 vs 38.7 ± 0.0°C), but RT did not ($P = 0.43$) differ in the PM. The percentage of calves with at least one RT ≥ 39.3°C tended ($P = 0.08$) to be greater for SH treatment (66.4 ± 0.1 vs 49.5 ± 0.1%). No differences in RF were observed. From 12 to 146d, BW was not ($P > 0.10$) different between treatments. Although an interaction between treatment and age affected ($P = 0.01$) WH, the WH at 71d was not ($P = 0.19$) different between treatments. Average daily gain (633.4 ± 12.5 g/d) and feed efficiency (0.60 ± 0.04 ADG/DMI) from 12 to 71d were not ($P \geq 0.71$) affected by treatment. Health scores and mortality up to 71d were not ($P > 0.20$) different between treatments. We did not detect an effect of cooling on calf growth but cooling reduced RT and the risk of hyperthermia.

Key Words: cooling, Holstein, performance

P246 Growth performance and apparent total-tract nutrient digestibility of limit-fed diets containing wet brewer's grains to Holstein heifers.

E. Hatungimana*, T. C. Stahl, and P. S. Erickson, *University of New Hampshire, Durham NH.*

The objective of this study was to evaluate the growth performance and apparent total-tract nutrient digestibility of Holstein heifers limit-fed diets containing different amounts of wet brewer's grains (WBG). A 12-wk randomized complete block study was conducted using 30 yearling Holstein heifers (378 ± 27 d of age, and BW of 357.8 ± 27.6 kg (mean ± SD)). Treatments were 0%, 10% and 20% of WBG on a DM basis and diets were formulated to be limit-fed for DMI at 2.35% of BW and provided 15% CP and 2.27 Mcal ME / kg of DM. Dry matter intake was recorded daily while BW and skeletal measurements were measured every 2 wk. During wk 12, fecal samples were collected directly from the rectum over 4 consecutive days and composited by heifer to determine apparent total-tract nutrient digestibility using acid detergent insoluble ash (ADIA) as a marker. Data were analyzed using the MIXED procedure of SAS. Dry matter intakes, BW, ADG were not different among treatments ($P = 0.2$, $P = 0.4$, $P = 0.6$ respectively). Dry matter intakes ranged from 8.6 to 9.0 kg/d. Average BW were 404.4, 411.5 and 409.3 kg for heifers fed the 0, 10 and 20%WBG diet respectively. Average daily gains were 1.03, 1.04 and 0.96 kg/d for heifers fed the 0, 10 and 20%WBG diet respectively. Skeletal measurements and body condition scores were not different among treatments. Apparent total-tract digestibilities of DM, organic matter (OM), CP, fat and hemicellulose were greater in heifers fed 0% WBG (53.6, 57.9, 45.3, 66.8 and 58.2% respectively) and 20% WBG (51.2, 55.2, 50.1, 72.3, and 55.9% respectively) than heifers fed 10% WBG (44.8, 49.7, 39.5, 65.6 and 50.8%; $P = 0.04$, $P = 0.04$, $P = 0.06$, $P = 0.06$ and $P = 0.01$ respectively). Neutral detergent fiber, ADF and fat digestibilities were not different among treatments ($P = 0.2$, $P = 0.3$, $P = 0.3$ respectively).

During the digestibility phase, DMI tended to be greater ((10.0 kg/d; $P = 0.08$) for the 10% WBG treatment These results demonstrate that limit-feeding heifers with diets containing up to 20% WBG could replace soybean and corn-based concentrates in diets without adverse consequences to heifer growth performance.

Key Words: wet brewer's grains, growth, digestibility

P247 Effect of storage of wet brewer's grains with incremental levels of salt on in vivo total-tract nutrient digestibility and purine derivative excretion in dairy heifers. E. Hatungimana*, T. C. Stahl, and P. S. Erickson, *University of New Hampshire, Durham NH.*

The objective of this study was to evaluate the in vivo total-tract nutrient digestibility and purine derivative excretion in heifers fed diets containing wet brewer's grains (WBG) treated with salt. A-12 wk replicated 4 × 4 Latin square study was conducted using 8 Holstein heifers of 224.5 ± 19.4 d of age, and BW of 483.9 ± 66.3 kg (mean ± SD). Treatments were 0%, 0.8%, 1.6% and 2.4% salt added to fresh WBG and stored for 4 d before being included in diets at 20% DM basis. Similar amounts of salt were added in reverse order to diets during the feeding time to equalize the amount of salt consumed by heifers. Diets were formulated to be limit-fed at 2.15% of BW (DM) to provide 14% CP and 2.27 Mcal ME/kg of DM. Heifers were adapted to diets for 14 d followed by 7 d of collection. Dry matter intake was recorded daily during the collection week while BW was recorded weekly. Fecal and urine samples were collected during the last 4 d of the collection period and composited by heifer. Composites of feces were used to determine in vivo total-tract nutrient digestibility using acid detergent insoluble ash (ADIA) as a marker, while composites of urine samples were used to determine purine derivative excretion. Data were analyzed using the MIXED procedure of SAS. Dry matter intake resulted in a quadratic effect ($P < 0.01$), and were 5.09, 4.97, 5.13 and 5.10 kg/d for the 0, 0.8, 1.6 and 2.4% salt treatment respectively. Body weight, ADG and feed efficiency increased linearly ($P = 0.04$, $P = 0.03$ and $P = 0.03$ respectively). Body weight ranged from 261.1 to 265.2 kg. Average daily gains ranged from 0.73 to 1.05 g/d. Digestibility of DM, OM, CP decreased linearly ($P = 0.03$, $P = 0.03$ and $P = 0.04$ respectively). Urinary volume, allantoin, and uric acid excretion and total purine derivative excretion were not affected by treating WBG with salt ($P = 0.4$, $P = 0.4$, $P = 0.3$ and $P = 0.4$ respectively). Limit-feeding heifers with diets containing WBG treated with salt decreased nutrient digestibility but may enhance heifer growth performance

Key Words: wet brewer's grain, salt, digestibility

P248 Body weight and average daily gain of one-year-old Holstein heifers supplemented with colostrum replacer. M. O. Moura*[1], V. Chiogna Junior[2,1], M. C. Rodrigues[1], M. Dias[1], and E. A. Collao-Saenz[1], [1]*Universidade Federal de Jatai, Jatai, Goias, Brazil,* [2]*Milk Mais Consultoria, Rio Verde, Goias, Brazil.*

Scientific studies evaluating long-term effects of colostrum replacers (CR) supplementation to dairy calves are limited. The average daily gain (ADG) needed to reach a target body weight depends upon body weight at weaning. The objective was to evaluate the effects of a 5-d period supplementation with colostrum replacer (CR) on BW and ADG in heifers at 12 mo. Sixty Holstein calves (39 ± 1.6 kg BW) from one herd received 6 L of colostrum (4 L after birth via esophageal feeder + 2 L after 6 h) and were assigned to 1 of 2 groups. All calves presented adequate immunity (serum IgG > 10.0 g/L). Calves assigned to the control group received 6 L of milk with 18% solids (milk + 55 g/L of a milk replacer with 22% protein:19% fat) without CR inclusion. The treatment group received 60 g of supplemental CR powder dissolved in 6 L of milk with 18% solids from d 2 to 6 of life. From d 6, all calves received the same quantity of milk (18% solids) and ad libitum calf starter. After 30 d calves received 4 L twice a day until weaning with 60 d of life. From the experimental calves, 52 heifers (n = 26 in each group) were reared under the same circumstances regarding nutrition, health, and environment, weighed the same day after 12 mo and their BW adjusted for 365 d. BW 365 and ADG were analyzed using a mixed model, treatment as fixed effect with calf birth weight as a covariable; heifer within treatment was considered random effect. There was no interaction treatment × period. Calves in CR group were 3.2 kg heavier and gained 0.05 kg/d more than control at weaning (Table 1). The BW and ADG differences observed at weaning of calves in CR treatment were not sustained until 365 d. No difference for both variables was found between treatments when the heifers reached one year of age.

Table 1. Body weight in the first year and ADG of Holstein heifers

Item	Control	CR	Min	Max	*P*-value
ADG at weaning (kg/d)	0.86 ± 0.13	0.91 ± 0.15	0.68	1.04	0.14
Weaning weight (kg)	90.8 ± 8.0	94.0 ± 8.0	78.0	108.0	0.02
ADG at 365 d (kg/d)	0.92 ± 0.07	0.95 ± 0.09	0.79	1.10	0.24
BW at 365 d (kg)	364 ± 25	372 ± 32	326	441	0.53

Key Words: calf nutrition, dairy heifer, growth

P249 Evaluation of body dimensions as predictors of body weight in slick- and wild-type–haired Puerto Rican Holstein calves. H. L. Sánchez-Rodríguez*, I. Colón-Rodríguez, N. M. Cruz-González, N. K. Pérez-Rosario, A. D. Ramos-Gerena, D. Y. Vega-Martínez, J. D. López-Colón, and K. Domenech-Pérez, *University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico.*

In Puerto Rico, commercial weighing tapes are frequently used to predict dairy cattle body weight (BW) when weighing scales are not available. However, such tapes were developed with Pennsylvania's Holstein cows; while cattle's growth may be limited in warmer countries. Thus, the present study evaluated BW relationship with thoracic perimeter (TP), withers height (WH), and barrel (BA) in 10 slick- (SLICK) and 9 wild-type–haired (WT) Holstein female calves at the University of Puerto Rico's herd. Data were collected weekly from birth to 8 weeks of age (preweaning period; Pre-WP), and then monthly, during 10 consecutive samplings (postweaning period; Post-WP). During Pre-WP, calves were individually housed; where milk (6 L/d/calf), as well as water and starter were provided. After weaning, heifers (allocated as one group) received starter (2 kg/d/heifer) and ad libitum hay and water. Data were analyzed by Proc REG (SAS). During Pre-WP, there were linear relationships between BW and TP in both the SLICK ($BW = 1.42\ TP - 72.75$; $R^2 = 0.84$; $P < 0.0001$) and WT calves ($BW = 1.52\ TP - 81.31$; $R^2 = 0.83$; $P < 0.0001$). Similarly, the BW-TP relationships were linear during the Post-WP in the SLICK ($BW = 3.22\ TP - 236.63$; $R^2 = 0.96$; $P < 0.0001$) and WT heifers ($BW = 2.97\ TP - 212.87$; $R^2 = 0.95$; $P < 0.0001$). At Pre-WP, BW and WH were linearly associated in the SLICK ($BW = 1.78\ WH - 92.25$; $R^2 = 0.75$; $P < 0.0001$) and WT calves ($BW = 2.19\ WH - 124.04$; $R^2 = 0.73$; $P < 0.0001$). Linear BW-WH relationships were observed during the Post-WP, with equations of $BW = 4.90\ WH - 345.96$ ($R^2 = 0.96$; $P < 0.0001$) and $BW = 4.93\ WH - 351.61$ ($R^2 = 0.95$; $P < 0.0001$) in the SLICK and WT heifers, respectively. During Pre-WP, BW and BA were linearly related in the

SLICK (BW = 0.75 BA – 19.64; $R^2 = 0.79$; $P < 0.0001$) and WT calves (BW = 0.88 BA – 33.49; $R^2 = 0.88$; $P < 0.0001$). Quadratic regression equations fitted the BW-BA data during Post-WP in the SLICK (BW = 0.03 BA^2 – 4.68 BA + 259.79; $R^2 = 0.94$; $P < 0.0001$) and WT heifers (BW = 0.02 BA^2 – 4.32 BA + 244.75; $R^2 = 0.96$; $P < 0.0001$). Therefore, TP, WH, or BA may represent feasible ways to predict BW in Puerto Rican Holstein calves and heifers.

Key Words: slick-haired, body dimensions, body weight

P250 Growth patterns in slick and wild-type–haired Puerto Rican female Holstein calves.

I. Colon-Rodríguez*, K. Domenech-Pérez, N. M. Cruz-González, N. K. Pérez-Rosario, A. D. Ramos-Gerena, D. Y. Vega-Martínez, J. D. López-Colón, and H. L. Sánchez-Rodríguez, *University of Puerto Rico at Mayagüez, Mayagüez, Puerto Rico.*

Doubling the birth body weight (BW) by 56 d of age has been established as the gold standard when raising dairy calves. However, this benchmark may differ for dairy calves raised in tropical countries. Therefore, the current study aimed to evaluate the growth patterns (i.e., BW) of 10 slick and 9 wild type-haired Puerto Rican Holstein female calves. At birth, contemporaneous female calves from the Agricultural Experiment Station herd (Lajas, Puerto Rico) were visually classified and enrolled into the slick or wild type-haired experimental groups. The BW experimental samplings (ES) were performed weekly, during the first 8 weeks of life (preweaning period) and then monthly, for 10 consecutive months (postweaning period). During the preweaning stage, whole pasteurized milk (6 L/calf/d; 26% CP and 28% fat; DM basis), starter (22% CP and 3% fat; DM basis), and water were provided. Starter (2 kg/heifer/d) and ad libitum hay and water were provided during the postweaning period. Data were analyzed by the GLIMMIX and REG procedures of SAS. When the pre- and postweaning periods were analyzed together, no interaction was observed between hair coat type and ES affecting calves' BW ($P = 0.8928$). Similarly, BW differences were undetected due to hair coat types ($P = 0.7489$). However, BW continually increased throughout ES ($P < 0.0001$). On average, both slick and wild type-haired calves doubled their birth BW between the 9 to 10 ES (57 to 81 d old, respectively). During the preweaning period BW increased linearly in the slick (BW = 3.19 ES + 31.72; $R^2 = 0.74$; $P < 0.0001$) and the wild type-haired calves (BW = 3.23 ES + 30.56; $R^2 = 0.73$; $P < 0.0001$). In the postweaning period, BW also increased linearly for both the slick (BW = 20.99 ES −138.29; $R^2 = 0.80$; $P < 0.0001$) and the wild type-haired heifers (BW = 19.38 ES – 122.06; $R^2 = 0.73$; $P < 0.0001$). No differences between hair coat groups were observed for BW during the evaluated periods. Generally, calves grew faster through the postweaning period. Under the established nutritional management both hair coat groups doubled their birth BW later than the established gold standard of 8 weeks of age.

Key Words: body weight, Holstein calves, hair coat

Lactation Biology: Posters

P251 Postpartum milk yield and immunoglobulin concentration, and factors related to colostrum quality at quarter level in dairy cows after parturition. E. C. Kessler[1], G. C. Pistol[1,2], R. M. Bruckmaier[1], and J. J. Gross*[1], [1]*Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Laboratory of Animal Biology, National Institute for Research and Development in Animal Biology and Nutrition, Ilfov, Romania.*

We analyzed milk production and IgG concentration at the individual quarter level in 29 multiparous Holstein cows during the first 5 milkings after calving. The objective was to investigate to which extent cow- and calf-related factors contribute to quarter milk production and IgG concentration after parturition. Cow- and calf-related factors (interval between calving and first milking, parity number, previous lactation yield, gestation length, dry period length, sex, birth weight of the calf) were assessed. Milking of first colostrum was carried out between 30 and 180 min postpartum. Further milkings were performed twice daily. Quarter milk yield at the first milking varied between 0.1 and 5.5 kg and between 1.4 and 5.1 kg at the fifth milking relative to parturition. Quarter IgG concentration ranged between 18.8 and 106.0 mg/mL at the first milking and between 0.8 and 46.1 mg/mL at the fifth milking. Mixed models (SAS) were used to assess repeatability with quarter and milking number as fixed effects, whereas effects of cow- and calf-related factors on milk yield and IgG concentration were included as class variables in GLM models. Milk yield and IgG concentration among quarters was not entirely repeatable during the first 5 successive milkings after parturition, i.e., the ranking of quarters changed (intraclass correlation coefficients for quarter milk yield and IgG concentration: 0.64 and 0.79, respectively). Milk production increased in all quarters ranging from 0.02 to 0.26 kg/h between the first 2 milkings up to values ranging from 0.11 to 0.45 kg/h between the fourth and fifth milking. First colostrum yield was not affected by any of the evaluated cow- and calf-related factors. Quarter colostrum IgG concentration was higher in cows with a higher previous lactation yield, whereas a lower colostrum IgG content was observed in cows with a longer gestation period and consequently heavier calves. In conclusion, milk yield and IgG concentration of individual quarters varied considerably, and their distribution among quarters within cows was moderately repeatable in consecutive milkings and changed partially over time. The decline of IgG concentration was not totally dependent of the concomitant increase in milk secretion with changes occurring at different rates in individual quarters. Results confirm the independency of the single mammary quarters at the onset of lactation.

Key Words: quarter, colostrum, immunoglobulin G

P252 Different microRNA contents between mammary epithelial cells and milk fat globules is not a random process? C. Leroux*[1], K. Pawlowski[1,3], D. Lago-Novais[1,4], C. Bevilacqua[2], L. Mobuchon[1,2], N. Crapart[2,5], Y. Faulconnier[1], S. Bes[1], C. Boby[1], G. Carvalho[4], and P. Martin[2], [1]*INRAe-UMRH, Saint Genès-Champanelle, France,* [2]*INRAe-Gabi, Jouy-en-Josas, France,* [3]*University of Life Sciences, Warsaw, Poland,* [4]*Universidade Federal da Bahia, Bahia, Brazil,* [5]*Excilone, Elancourt, France.*

MicroRNAs (miRNAs) are small noncoding RNAs that act as post-transcriptional regulators of gene expression. They are present in milk-derived extracellular vesicles as milk fat (MF) globules that are secreted in large amounts in milk. Discrepancies exist between the miRNA content of lactating mammary tissue (MT) and that found in milk fat. Our objective was to study the abundance of example miRNAs from different sources (MT, Mammary epithelial cell (MEC), MF), addressing the question of the origin of these miRNAs fate. Moreover, we wanted to evaluate the existence of a possible mechanism sorting miRNAs that will or will not be exported from the MEC to bovine MF. With these objectives, we used RT-qPCR to compare the abundance of 4 miRNA (*miR-125b-5p*, *miR-126–3p*, *miR-141–3p*, and *miR-204–5p*) in lactating cow MT, MF, and laser capture-microdissected MECs. After normalization (using *U6*-snoRNA and *let-7–5p*), statistical analyses, were performed on ΔCt values with a Mann-Whitney test and FDR correction. The significance level was predefined as $P_{adj} \leq 0.05$. Two miRNAs (*miR-125b-5p* and *miR-141–3p*) were detected in the MT as well as in MF and MECs. *MiR-204–5p* was detected, with reliability only in the MT (mean Ct of 25.7 whereas it was more than 30 in MF and MEC), suggesting that it is very likely expressed in a cell type other than MECs. *MiR-126–3p* was detected both in the MT (mean Ct: 17.7) and in MECs (mean Ct: 19.1) but not in MF (mean Ct: more than 30), suggesting a targeting mechanism for miRNAs in MECs. In conclusion, this study highlights differences in miRNA content between MECs and milk fat, possibly due to a mechanism for loading milk fat with miRNA cargos that may not be a random process but could involve a variable distribution in MECs or a sorting mechanism.

Key Words: miRNA, mammary epithelial cell, milk fat

P253 Comparison of miRNome from cow milk fat fraction and mammary gland tissue during inflammation. C. Leroux*[1], P.-A. Billa[1], K. Pawlowski[1,2], S. Bes[1], J. Pires[1], and Y. Faulconnier[1], [1]*INRAe-UMRH, Saint Genès-Champanelle, France,* [2]*University of Life Sciences, Warsaw, Poland.*

MicroRNAs (miRNA, small noncoding RNAs) regulate the expression of genes involved in many biological processes, including inflammation and mammary gland (MG) development and lactation. MicroRNAs are present in milk-derived extracellular vesicles including fat globules (FG) that are secreted in large amounts in milk. Our objectives were to study the bovine FG miRNome and to ensure that FG miRNAs are representative to MG miRNome during inflammation for the use of FG as a noninvasive source of miRNAs, which could be potential inflammation biomarkers. To achieve these objectives, we compared the miRNome of MG and FG, in an experimental model of inflammation in early-lactation cows. MG biopsies and milk FG were collected during the same milking, from 6 early-lactation multiparous Holstein cows, 24 h after intramammary injection of 50 µg of lipopolysaccharide to induce an inflammation. Total RNAs were extracted from FG and MG using TRIzol. Customized 8 × 60K miRNA microarrays were hybridized using a single-color method. Normalized with 75th percentile shift, data were analyzed using GeneSpring software. Statistical analyses were performed using paired *t*-tests, Benjamini-Hochberg correction, and significance considered at $P_{\text{adj}} \leq 0.10$. Analyses of MG and FG miRNome showed that 97% of the more than 2,500 detected sequences did not differ statistically between MG and FG. However, among the 47 differentially abundant miRNAs in MG compared with FG, 29 had a fold change ≥1.5. Bioinformatics analyses of coding genes potentially targeted by these miRNA revealed that they are involved cellular pathways such as calcium signaling, adherents, tight and gap junction and endocytosis pathways. Among miRNAs presenting highest FC, were let-7c, miR-126–3p and the miR-200 family, which were more abundant in MG than FG. These latter could target genes involved in cell adhesion, migration or vascular integrity, as in the epithelial-mesenchymal transition, therefore determining the epithelial cell phenotype. In conclusion, these results show that 24 h after an intramammary LPS challenge, FG miRNome is mostly representative of the MG miRNome. However, the observed differences may be related to the secretion of miRNA in FG and/or due to the LPS inflammation challenge inducing changes in mammary tissue. These hypotheses must be confirmed by further investigations.

Key Words: milk, miRNome, inflammation

P254 Influence of nutrients in milk on the in vitro growth of major bovine mastitis pathogens. R. Stürmlin[1], J. J. Gross[1], O. Wellnitz[1], L. A. Wagner[1], C. Monney[2], A. Oevermann[2], and R. M. Bruckmaier*[1], [1]*Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland,* [2]*Division of Neurological Sciences, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

The growth of 3 major bovine mastitis pathogens *Escherichia coli, Staphylococcus aureus*, and *Streptococcus uberis* was investigated in ultra-high-temperature (UHT) treated milk with different contents of fat, protein and carbohydrates. Additionally, the bacterial growth was studied in diluted milk with subsequent addition of individual nutrients (carbohydrates, nitrogen sources, minerals and B vitamins). Statistical analysis used mixed models (SAS) with type of medium and pathogen as fixed, and replicates as random factors. Data of growth rates are presented as $\log_{10}$cfu/h, calculated from the difference of $\log_{10}$-transformed cfu/mL between smears of time points 0h and 8h, and based on 3 independent setups. Varying contents of fat [0.1–25.1% wt/vol], protein [2.5–7.0%] and carbohydrates [4.0–5.0%] did not affect bacterial growth rates, except for increased growth ($P < 0.01$) of *S. uberis* in protein-enriched milk (0.754 ± 0.016 [growth rate ± SD]) compared with growth in whole milk (0.641 ± 0.014). The addition of lactose [4%] or minerals [0.2‰] to diluted milk [1:20 for *E. coli*, 1:10 for *S. aureus* and *S. uberis*] did not affect bacterial growth. The growth rate of *E. coli* was decreased when diluted milk was enriched by urea [3%] (0.507 ± 0.007; $P < 0.001$) or by a mixture of B vitamins [1%] (0.552 ± 0.012; $P < 0.001$) compared with growth in diluted milk without additives (0.654 ± 0.002). Growth rate of *S. aureus* was decreased by the addition of a mixture of amino acids [0.2‰] (0.384 ± 0.008; $P < 0.001$), urea [3%] (0.351 ± 0.011; $P < 0.001$) and B vitamins [1%] (0.349 ± 0.012; $P < 0.001$) to diluted milk in comparison to growth in diluted milk without additives (0.482 ± 0.004). The growth rate of *S. uberis* was increased by the addition of B vitamins [1%] (0.487 ± 0.014; $P < 0.05$) to diluted milk compared with growth in diluted milk without additives (0.234 ± 0.021). Changes of bacterial growth were only achieved by changes of the milk composition beyond the respective physiologically possible range. Our study confirmed that milk is abundantly saturated with nutrients for pathogen growth. We conclude that physiologically relevant changes of milk composition are unlikely to affect pathogen growth during mastitis.

Key Words: bovine mastitis, bacterial growth, pathogen

P255 Yes-associated protein expression of mammary epithelial cells from mammary glands subjected to increased milking frequency. G. Perez-Hernandez*, K. Tate, K. D. Hardin, C. Parsons, R. M. Akers, and B. A. Corl, *Virginia Tech, Blacksburg, VA.*

Increased milking frequency (IMF) during early lactation increases milk yield and has been associated with mammary epithelial cell (MEC) proliferation. Nevertheless, clear delineation if milk yield enhancement is driven by cell proliferation or cell differentiation with IMF is unclear. Yes-associated protein (YAP) promotes cell proliferation when dephosphorylated and translocated to the nucleus. The objective of this study was to evaluate the spatial expression of YAP, as a novel marker of cell proliferation, in mammary glands subjected to IMF at the beginning of lactation. Eight multiparous cows were milked at IMF for the first 21 d of lactation using the unilateral frequent milking method. Mammary biopsies from each rear quarter were obtained on d 21 and d 60. Immunofluorescence in formalin fixed paraffin embedded tissue was used to evaluate YAP expression. Data were analyzed using the UNIVARIATE procedure of SAS for descriptive statistics. The averages for intensity were analyzed using the GLIMMIX procedure. Cellular YAP staining intensity was reduced 47% from d 21 (940 ± 143) to d 60 (495 ± 143) when cows were milked twice per day. YAP epithelial staining intensity increased by 90% when 4X (938 ± 143) IMF was used compared with 2X (495 ± 143) at 60 DIM, but there was no effect of IMF at 21 DIM. YAP-positive nuclei proportion increased on d 60 (0.29 ± 0.02) compared with d 21 (0.22 ± 0.02) regardless of treatment. Mammary gland tissue samples from cows milked 4X had a higher proportion of positively stained nuclei (0.28 ± 0.02) than cows milked 2X (0.22 ± 0.02) regardless of day. More YAP in the nuclei of mammary cells of glands milked at 4X compared with glands milked 2X could indicate an increase in cell proliferation associated with IMF. This study provides new information on the relative amount and location of YAP in the mammary gland. Further research should investigate whether there is an increase in cell proliferation associated with increased YAP expression in mammary glands following IMF.

Key Words: milking frequency, cell proliferation, Yes-associated protein

P256 Intramammary infection prevalence and mammary secretion characteristics of primigravid dairy heifers. L. R. Larsen*[1], P. H. Baker[1], K. M. Enger[1], L. E. Moraes[2], and B. D. Enger[1], [1]*The Ohio State University, Wooster, OH,* [2]*The Ohio State University, Columbus, OH.*

Intramammary infections (IMI) in primigravid dairy heifers can impair growth and development of the mammary gland which reduces milk yield after calving. Detection of IMI in heifers often involves time-consuming culture-based approaches. The objective of this study was to determine if prepartum mammary secretion SCC and viscosity was associated with infection status. A total of 270 heifers were enrolled. Mammary secretion samples were aseptically collected from a randomly selected quarter of each heifer at 75 d prepartum (75PP), and another randomly selected quarter of each heifer was sampled 35 d prepartum (35PP). The remaining 2 quarters of each heifer were not utilized in this study. Mammary secretion samples underwent bacteriological examination to determine IMI status, quantitative SCC measurement, and were also visually scored for viscosity. Viscosity data were analyzed using PROC GLIMMIX, with the fixed effects of sampling day and IMI status, while heifer was a random effect. Prevalence of IMI was 26% and 28% at 75 and 35 d prepartum. Samples from 35PP were 1.7 times (95% CI: 1.1–2.8) more likely to be of thin viscosity than samples from 75PP. Uninfected samples were 68.2 (95% CI: 14.8–313.6) times more likely to be thick compared with samples infected with a major pathogen and 14.6 (95% CI: 8.6–24.7) times more likely to be thick compared with samples infected with a minor pathogen. SCC data were analyzed with PROC MIXED; sampling day and IMI status were fixed effects and heifer was a random effect. Log SCC were greater in samples infected with a major pathogen (6.6 ± 0.1) than those infected with a minor pathogen or uninfected (6.3 and 6.0 ± 0.04, respectively;

$P \leq 0.01$). These results indicate that mammary secretion viscosity and SCC measurement may be accurate and useful tools in identifying primigravid heifer quarters with IMI. The simplicity of a viscosity measure that may be taken cow-side might allow for rapid identification of IMI and subsequent treatment of infected quarters to diminish the negative physiological effects an IMI causes, ensuring increased milk production when lactation begins.

Key Words: mastitis, heifer mastitis, heifer SCC

P257 Effect of increased milking frequency during early lactation on bovine mammary epithelial cell differentiation.

G. Perez-Hernandez*, K. Tate, K. D. Hardin, C. Parsons, R. M. Akers, and B. A. Corl, *Virginia Tech, Blacksburg, VA.*

The cytological classification of mammary epithelial cells (MEC) and its use as an indicator of cell activity and cell differentiation allow investigation of the undetermined mechanism of how the mammary gland adapts to increased milk production. The objective of this study was to determine the effect of increased milking frequency (IMF) during early lactation on mammary epithelial cell differentiation through histological analysis. A scoring evaluation of the cytological differentiation of mammary tissue samples was completed on mammary biopsy samples from multiparous cows ($n = 8$) subjected to unilateral milking frequency for the first 21 d of lactation. The right udder half was milked 4× and the left udder half milked 2×. Tissue was formalin fixed, embedded in plastic-resin, and stained with Azure II for histochemical analysis. Mammary epithelial cell differentiation scoring was completed by reviewing collected images and categorizing them in 1 of 3 cell differentiation score levels, based on cell organelles characteristics as previously reported in the literature, in 0.1 increments, poor (1), intermediate (2), and complete (3). Scores were recorded and averaged for each animal by right or left udder half. Data were analyzed using the UNIVARIATE procedure for descriptive statistics, and differentiation score was analyzed using the GLIMMIX procedure of SAS. Milk yield was increased in the 4× udder halves (11.5 ± 1.1 kg/d) compared with the 2× udder halves (8.5 ± 1.1 kg/d) on 21 DIM. Mammary epithelial cell differentiation at 21 DIM was higher (2.32 ± 0.17) than d 60 (1.73 ± 0.17). Mammary gland samples from udders milked 4× exhibited the greatest mammary cell differentiation (2.41 ± 0.17) compared with 2× (1.64 ± 0.019), demonstrating an effect on mammary epithelial cell differentiation when IMF is used in early lactation. Results indicate that mammary cell differentiation is reduced at d 60 compared with d 21, coinciding with peak lactation, and increases after an IMF treatment at the beginning of the lactation in multiparous cows.

Key Words: cytological classification, MEC differentiation, increased milking frequency

P258 Proteomic analysis of the xanthosine-treated lactating goat mammary gland.

S. Choudhary[1], S. Kumar[2], A. K. Mohanty[2], R. K. Gandham[3], and R. K. Choudhary*[1], [1]*College of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India,* [2]*Animal Biotechnology Center, National Dairy Research Institute, Karnal, Haryana, India,* [3]*National Institute of Animal Biotechnology, Hyderabad, Telangana, India.*

Xanthosine (Xs) increases mammary epithelial cell proliferation in vitro and increases stem cells, and favorably affects milk secretion in goats. We determined the protein profile of mammary tissue during early lactation in the Xs-treated gland. Six primiparous Beetle goats were used in this study. Five days after kidding, one gland was infused (2 times for 3 d) with Xs, and the other gland served as control. Using 2-dimensional difference gel electrophoresis combined with MALDI-TOF/TOF mass spectrophotometry, we identified 144 differentially expressed proteins (DEP), of which 46 proteins were upregulated, and 98 were downregulated in the Xs-treated gland. These DEP analyses by ingenuity pathway analysis (IPA) showed 5 highly significant protein networks namely, 1) Energy production, Nucleic acid metabolism, 2) Cellular assembly and organization, 3) Connective tissue disorder, 4) Drug metabolism, and 5) Cell death and survival with a respective score of 46, 44, 44, 38 and 20. Pathway of cell death was downregulated in Xs-treated glands, indicating a favorable condition for survival and growth of mammary tissue. Functional annotation clustering of DEP using Database for Annotation, Visualization, and Integrated Discovery (DAVID) revealed 8 significant clusters (Enrichment score > 2.0). Interestingly, the first annotation cluster with an enrichment score of 3.58 was the cluster of antibiotic biosynthesis. Identified clusters of antibiotic synthesis were consistent with our RNa-seq data reported earlier. The novel role of Xs in nucleic acid metabolism, favoring cell survival and association of DEP in the antibiotic role is novel and requires further experimental validation. This work provides insights into protein interactions to further investigate the mechanisms underlying the role of Xs in lactating goats.

Key Words: goat mammary gland, xanthosine, protein profile

P259 An improved method for isolating epithelial cells from lactating dairy cow secretory tissue.

A. C. Hruby*[1], I. A. M. A. Teixeria[2], and M. D. Hanigan[1], [1]*Department of Dairy Science, Virginia Tech, Blacksburg, VA,* [2]*Department of Animal Science, UNESP, Jaboticabal, Brazil.*

Bovine mammary epithelial (BME) cell cultures are commonly used to better understand mammary metabolism and growth. BME cells have been established using a variety of methods including enzymatic digestion (Zavizion et al., 1992) and direct plating of explants (Hu et al., 2009). Enzymatic digestion is more expensive and cell yields are not high. Direct plating of explants results in migration of cells out from the explants that have attached to collagen-coated plates; however, fibroblasts also migrate out. Partial trypsinization (PT) is used to separate fibroblasts from BME cells in the culture system, however, details such as timing and enzyme concentrations are not well documented. Further, given that the explants are the source of the BME cells, we hypothesized that PT to remove fibroblasts followed by normal trypsinization to harvest epithelial cells could be performed, and if the explants remained attached, additional BME cells would migrate from the explant. Therefore, the objectives of this study were to 1) assess conditions for PT; and 2) assess explant retention as a method of improving BME cell yield. Secretory tissue was collected from the udder of a primiparous Jersey cow (16 DIM) at slaughter. Tissue was rinsed in Hanks' buffer until free of milk and manually reduced to 1 mm^3 using scalpels and scissors, plated at a density of 1 explant/cm^2 in collagen-coated, 75 cm^2 flasks without medium, and incubated at 38°C under 5% CO_2. Explants were kept wet. After ~4 h, 4 mL of basal growth medium was gently added to each flask. Medium was changed the following morning and every 24 h thereafter. After approximately 4 d, fibroblasts were observed emerging from the tissue pieces; at d 8.4 ± 0.2 mixed cultures were observed, and the PT was done by application of 0.05% trypsin-EDTA for 4 min. PT removed fibroblasts and did not remove well-attached explants or BME cell colonies. It was observed that clearing space around the explant allowed additional migration of cells from the explant. When pure BME cells were observed to surround the explant, BME cells were passaged (0.25% trypsin-EDTA) with care taken to avoid explant detachment. After BME cell removal, additional

BME cells migrated from the explants yielding another crop of BME cells for harvest within 1 wk. In summary, the use of PT starting at 8 d on attached cells from explants and continued harvest of remaining epithelial cells improves upon the original method.

Key Words: cell culture, fibroblast

P260 Prepartum feeding of X-Zelit increases blood ionized calcium and colostrum IgG concentrations. J. P. Campolina*[1], W. S. Frizzarini[2], P. L. J. Monteiro[2], A. Vang[2], and L. L. Hernandez[2], [1]*Universidade Federal de Minas Gerais, Belo Horizontes, MG, Brazil,* [2]*University of Wisconsin–Madison, Madison, WI.*

Colostrum is the first secretion of the mammary gland after parturition and the primary source of immune transfer for the newborn calf. Calcium is the primary mineral in colostrum, resulting in extensive demand on the cow. The aim of this study was to evaluate colostrum quality and blood ionized calcium concentrations before and after the first milking from dairy cows receiving 3 dietary treatments pre-partum. One hundred eight multiparous Holstein cows were randomly assigned to one of 3 dietary treatments 21 d before estimated calving date: control (CON; +190.24 mEq/kg; n = 32), negative dietary cation-anion difference (DCAD; −64.71 mEq/kg; n = 35), and a diet with sodium aluminum silicate (XZ; +277.40 mEq/kg with 500 g/day X-Zelit, Protekta Inc.; n = 38). After parturition, blood samples were collected to measure ionized calcium at parturition (iCaP) using a portable device (VetScan iSTAT System). After, cows were milked, colostrum weighed, and samples collected for further laboratory analysis. A second blood sample was collected to measure ionized calcium after colostrum removal (iCaM). Colostrum quality was analyzed using a Brix refractometer. Colostrum IgG concentrations were measured using a commercial bovine IgG ELISA kit (Bethyl Laboratories), and colostrum calcium concentrations were analyzed by atomic absorption. Data were analyzed in R Studio using the GLS function on NLME package. No differences between dietary treatments were observed for colostrum weight, Brix analysis or calcium content ($P > 0.05$). Increased IgG concentrations were observed in the XZ group (91.4 ± 3.9 mg/mL) compared with DCAD (80.7 ± 4.1 mg/mL) and CO (78.9 ± 4.2 mg/mL, $P = 0.04$). The XZ cows had 7% and 14% higher iCaP (1.084 ± 0.02) and 9% and 19% higher iCaM (1.089 ± 0.02) when compared with DCAD and CO cows ($P < 0.001$), respectively. Feeding XZ prepartum improved colostrum immunological composition and helped maintain normalcalcemia when compared with DCAD and CO dietary treatments.

Key Words: dairy, dry period, hypocalcemia

Physiology and Endocrinology: Posters

P261 Branched-chain amino acids and branched-chain ketoacids alter lipid metabolism and triacylglycerol content in primary liver cells enriched with hepatocytes.

K. Gallagher*, J. Laguna, and Z. Zhou, *Michigan State University, East Lansing, MI.*

Plasma branched-chain amino acids (BCAAs) are negatively associated with liver triacylglycerol (TAG) level in dairy cows in early lactation. While seminal study demonstrated that BCAAs have the lowest rate of removal by liver in dairy cows under various nutritional and physiological conditions, their transamination products, branched-chain α-keto acids (BCKAs), are mostly catabolized in the liver. Our objectives were to quantify intracellular TAG content and expression of genes related to lipid metabolism primary liver cells enriched with hepatocytes (PLEH) exposed to BCAAs or BCKAs. PLEH were isolated from 3 nonpregnant mid-lactation multiparous Holstein cows previously. A customized medium (CM) was created to mimic the profile of circulating AAs, glucose, insulin, choline, and albumin levels observed for dairy cows on d 4 postpartum. Treatments were CM, FA (CM + 1mM FA cocktail mimicking circulating FAs), 1.3BCAA (FA + 33% circulating BCAA concentrations), 2BCAA (FA + 100% of circulating BCAA concentrations), 1/3BCKA (FA + 33% of BCKA of corresponding circulating BCAA concentration), and BCKA (FA + 100% of BCKA of corresponding circulating BCAA concentration). After 72 h, neutral lipids were stained with a fluorescent dye to quantify intracellular TAG content using flow cytometer. Expression of genes controlling lipid metabolism was also quantified in PLEH after 48 h. Treatment effects were determined using PROC MIXED in SAS. As expected, intracellular TAG content in FA was higher ($P < 0.01$) compared with CM. BCAA (1.3BCAA and 2BCAA) and BCKA (1.3BCKA and 2BCKA) treatments reduced ($P < 0.01$) intracellular TAG level in PLEH. Compared with FA, increasing BCKA (1/3BCKA ($P = 0.08$) and BCKA ($P = 0.02$)) led to lower *SLC27A5* (hepatic long chain FA transporter) expression in PLEH. Expression of β-oxidation genes were also greater ($P < 0.05$) in 1.3BCAA (*HADH* and *ECHS1*) and 2BCAA (*HADH* and *ACOX1*) compared with FA. In response to BCKA, expression of β-oxidation genes (*ACOX1* and *ECHS1)* was greater ($P < 0.05$) compared with FA. Additionally, 1.3BCAA, 2BCAA and 1/3BCKA also led to higher ($P < 0.05$) expression of *UCP2* and lower ($P < 0.05$) expression of *KEAP1*, suggesting enhanced antioxidant defense compared with FA. Overall, results indicate that BCAAs and BCKAs altered lipid metabolism and reduced intracellular TAG level in PLEH.

Key Words: branched-chain amino acids, branched-chain ketoacids, lipid metabolism

P262 Different feeding conditions and their relationship with milk and blood urea nitrogen contents in Brown Swiss and Holstein dairy cows.

E. C. Kessler, R. M. Bruckmaier, and J. J. Gross*, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Milk urea nitrogen (MUN) content is closely related to plasma urea nitrogen (PUN) concentration, and reflects the balance of dietary crude protein and energy supply for rumen fermentation. The present study is based on 2 experiments and investigated if the higher MUN content in Brown Swiss (BSW) compared with Holstein (HOL) cows is related to milk production and feeding conditions. In addition, the relationships between PUN and MUN assessed either by an enzymatic method or by infrared spectroscopy were analyzed. For the first experiment, milk and blood samples (1,112 samples each) were collected in parallel biweekly from d 5 until d 150 of lactation from 72 BSW and 69 HOL cows housed at 4 different farms with diverse feeding regimens. The second experiment consisted of test-day records of 3 consecutive official milk recordings from 86 BSW and 200 HOL cows kept on the same farm under identical feeding and management conditions. Statistical analysis (SAS, v9.4) used mixed models with parity, breed, and parity × breed as fixed and cow as random effects. Concentrations of MUN in the first experiment determined either enzymatically (MUN_{ENZ}) or by FTIR (MUN_{IR}) were regressed on the PUN concentrations of the concomitantly obtained blood samples (Bland-Altmann analysis and Pearson correlations). Both MUN_{IR} and MUN_{ENZ} were highly correlated with PUN (Pearson correlation coefficients r = 0.93 and 0.89, respectively; $P < 0.0001$). Concentrations of MUN and PUN were higher in BSW compared with HOL independently of lactational stage, parity and feeding regimen ($P < 0.0001$). Protein and fat content were higher in BSW than in HOL. Primiparous cows had lower energy-corrected milk yield than multiparous cows in both breeds (27.9 vs. 31.7 kg/d, $P < 0.001$ for BSW; 29.8 vs. 35.4 kg/d, $P < 0.0001$ for HOL). Multiparous BSW had a similar milk production than primiparous HOL (31.7 vs. 29.8 kg/d, $P = 0.13$), whereas multiparous HOL had a greater ECM yield than multiparous BSW (35.4 vs. 31.7 kg/d, $P < 0.0001$). In conclusion, BSW cows have higher MUN than HOL under different as well as identical feeding conditions. The higher MUN in BSW compared with HOL cows is not related to milk production, and therefore rather genetically determined. However, the impact of potential differences in protein ingestion warrants further research.

Key Words: milk urea nitrogen, Holstein, Brown Swiss

P263 Responsiveness of bovine patatin-like phospholipase domain-containing protein 3 and transcription factors to fatty acids.

S. Erb* and H. White, *University of Wisconsin–Madison, Madison, WI.*

Bovine hepatic patatin-like phospholipase domain-containing protein 3 (PNPLA3) abundance is associated with increased liver triglyceride (TG) content and knockdown in vitro increased hepatocyte TG content. In vitro, PNPLA3 protein, but not sterol regulatory element-binding protein 1c (SREBP1c) mRNA or protein, was responsive to fatty acids (FA). In other species, PNPLA3 is regulated by FA-mediated transcription factors (TF). The objective of this study was to quantify bovine *PNPLA3*, FA-mediated gene, and TF mRNA responsiveness to FA that mimic in vivo physiological states during liver TG accumulation (ACCUM) and recovery (RECOV). Hepatocytes isolated from bull calves (n = 4) were cultured in monolayer for 24h then treated with mixtures of FA (C14:0, C16:0, C18:0, C18:1, C18:2, C22:6) combined to reflect ACCUM or RECOV (0.25, 0.5, 0.75, or 1 m*M*) or individual FA at 0.5 m*M* of RECOV ($iRECOV_{0.5}$), or 0.25 m*M* ($iFA_{0.25}$) for 24 h. Cells were harvested for RNA quantification of *FAS*, *CPT1A, CPT2, PPARGC1A*, *PPARA*, *PNPLA3*, *SIRT1, SIRT3*, and *UCP2*. Data, relative to reference genes, were transformed for normality. Response to mixture or individual FA were analyzed separately in mixed models with fixed effect of concentration and FA and random effect of calf (SAS 9.4). Linear contrasts were considered significant when $P \leq 0.10$. Expression of *PNPLA3* was not altered by FA. Expression tended to increase with RECOV vs. ACCUM for *PPARGC1A* ($P = 0.07$; 0.89 vs. 0.71 ± 0.19 arbitrary units (AU)) and *SIRT1* ($P = 0.10$; 0.88 vs. 0.77 ± 0.15 AU) but tended to decrease for *UCP2* ($P = 0.09$; 0.37 vs. 0.45 ±

0.08 AU). Increasing concentrations of RECOV tended to increase ($P = 0.08$) *FAS* and decrease ($P = 0.004$) *ChREBP*. Expression of *CPT1A*, *CPT2*, and *SIRT3* were not affected by treatments. Expression of genes were not affected by $iFA_{0.25}$ nor $iRECOV_{0.5}$ ($P \geq 0.20$). Responsiveness of genes to FA was concentration and profile dependent and was not discernable with individual FA. Previous reported increases in PNPLA3 abundance with increased concentration of RECOV are apparently due to translational regulation since mRNA expression was not altered.

Key Words: lipase, fatty liver, lipid metabolism

P264 Effect of 3 prepartum dietary strategies to manage calcium on ionized calcium concentrations of Holstein cows.

W. Frizzarini*[1], J. Campolina[2], A. Vang[1], P. M. Junior[1], and L. Hernandez[1], [1]*University of Wisconsin, Madison, WI,* [2]*Federal University of Minas Gerais, Belo Horizonte, MG, Brazil.*

Hypocalcemia is the most common metabolic disease during the periparturient period due to the high demand for calcium of the mammary gland for colostrum and milk synthesis. Our objective was to assess ionized calcium concentrations from whole blood in cows fed 3 different prepartum diets. Multiparous Holstein cows (n = 122) were randomly assigned, according to parity, to one of 3 different diets with low potassium corn silage (1.11 ± 0.03%), beginning 21 d before expected parturition date: control (CN; +190.24 mEq/kg; n = 40), control with anions (DCAD; −64.71 mEq/kg; n = 41; mean urine pH = 6.2), and control with sodium aluminum silicate (XZ; +277.40 mEq/kg with 500 g/day X-Zelit, Protekta Inc., Lucknow, Ontario, CA/Vilofoss, Fredericia, DK, n = 41). Ionized calcium (iCa, mmol/L), sodium (mmol/L) and potassium (mmol/L) were measured daily beginning 9 d before parturition (-D9) until parturition (D0), and subsequently evaluated on d 1, 2, 3, 6, 9, 12, 15, 18, and 21 postpartum. Data were analyzed using the GLIMMIX procedure of SAS. Between -D9 and -D2 (except for -D6) iCa was decreased in cows fed CN compared with DCAD and XZ diets ($P < 0.01$). On D0, cows fed DCAD and CN diets (1.01 ± 0.02 and 0.91 ± 0.02, respectively) had decreased iCa concentrations compared with XZ (1.08 ± 0.02, $P < 0.01$), and this difference was maintained on D1 (1.03 ± 0.02, 0.93 ± 0.02, and 1.12 ± 0.02, for DCAD, CN, and XZ, respectively; $P < 0.01$). On D2, cows fed XZ had higher iCa concentrations (1.16 ± 0.02, $P < 0.05$), with no differences among cows receiving DCAD and CN (1.08 ± 0.02 and 1.07 ± 0.02, respectively, $P = 0.84$). There was no difference in iCa concentration among diets on D3 ($P = 0.10$) and D6 ($P = 0.51$). The sodium concentrations were not different among dietary treatments ($P = 0.16$) but differed over time ($P < 0.01$), with the highest concentrations on D1 (144.11 ± 0.14) and lowest concentrations on D21 (136.55 ± 0.18). During the prepartum period, potassium concentrations were higher for the DCAD diet (4.44 ± 0.03) compared with CN and XZ diets (4.24 ± 0.03 and 4.12 ± 0.02, respectively). In conclusion, feeding cows with XZ diet improved calcium metabolism after parturition.

Key Words: dairy cow, transition period, calcium

P265 Effects of peripartum omega-3 fatty acid supplementation on endocannabinoid tone and inflammation in liver of dairy cows.

G. Kra[1,2], U. Moallem[1], R. Kocvarová[3], A. Nemirovski[3], J. Tam[3], GA Contreras[4], and M. Zachut*[1], [1]*Agriculture Research Organization, Volcani Center, Rishon Lezion, Israel,* [2]*Faculty of Agriculture, the Hebrew University in Jerusalem, Rehovot, Israel,* [3]*Obesity and Metabolism Laboratory, The Institute for Drug Research, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel,* [4]*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, Lansing, MI.*

Dietary supplementation of omega-3 (n-3) fatty acids may affect the endocannabinoid system (ECS) by reducing the availability of arachidonic acid (AA; C20:4n-6), and elicit anti-inflammatory effects. We aimed to examine the effects of 2 sources of n-3 FA, encapsulated flaxseed oil (FLX) or fish oil (FO) on hepatic ECS "tone," and inflammatory and metabolic markers in blood of peripartum dairy cows. Late pregnant cows were fed from 21 d prepartum a diet supplemented with encapsulated fat: i) CTL–saturated fat (n = 14); ii) FLX (n = 14); or iii) FO (n = 14). Plasma prostaglandin E metabolite (PGEM) and G-2-α metabolite (PGFM) and FA profile were examined. Liver biopsies were obtained at 10 d postpartum (n = 5 per treatment) for expression of ECS components and inflammatory genes and proteins. Endocannabinoids (eCBs) in liver and plasma at biopsy were examined by LC-MS/MS. Continuous measures were analyzed by PROC MIXED; genes, proteins, and eCBs were analyzed by GLM procedure of SAS. The n-6/n-3 ratio in plasma was lowest in FLX and highest in CTL. Milk production was similar, and feed intake during the first 21 DIM was lower in FLX than in CTL. The FLX cows had lower PGEM concentration and tended to have lower PGFM in plasma during the first week in lactation compared with CTL. In liver, the relative gene expressions of the cannabinoid receptors *CNR1* and *CNR2* as well as the proinflammatory gene *IL-6* were lower in FLX than in other groups. Protein abundances of TNF-α, IL-10 and cannabinoid receptor 1 (CB1) in liver were lower in FO than in CTL, while the abundance of NFKB was higher in FLX than in other groups. In liver, levels of eCB 2-arachidonoylglycerol (2-AG) were 1.7-fold higher in FLX than in other groups, while in plasma anandamide levels were lower in FLX and FO compared with CTL, and AA was lower in FLX than in CTL. Peripartum supplementation of n-3 FA seems to lower the ECS 'tone' in bovine liver, with some differential effects of FLX and FO on liver ECS components. We provide evidence for the involvement of ECS in bovine liver metabolism and inflammation, and dietary n-3 can attenuate ECS "tone" in liver of peripartum dairy cows.

Key Words: liver, endocannabinoid, inflammation

P266 Elevated circulating serotonin alters immune cell expression of serotonin receptor and metabolism genes in the lactating dairy cow.

M. Connelly* and L. Hernandez, *University of Wisconsin–Madison, Madison, WI.*

Serotonin's role in the periphery has been demonstrated through its action in modulating calcium metabolism, energy homeostasis, and immune function. Manipulation of the serotonergic axis has been demonstrated by administering 5-hydroxy-L-trytophan (5-HTP) and fluoxetine to alter serotonin bioavailability. Much of serotonin's action is believed to be receptor mediated, with signaling cascades and feedback also regulating serotonin transport and metabolism-related genes. Therefore, our objective was to explore alterations in expression of serotonin receptor-, metabolism-, and immune-related genes in peripheral leukocytes in response to 5-HTP infusion in the lactating dairy cow. Twelve mid-late lactation multiparous Holstein cows were randomly assigned to intravenous infusion of (1) 1 L of 1.5 mg/kg 5-HTP dissolved in saline (n = 6/treatment) or (2) 1 L of saline solution (n = 6/treatment) for 3 d in a randomized complete block design. Blood samples and Tempus Tubes were collected at 48h, 56h, and 72h relative to termination of first infusion. Peripheral leukocyte expression was analyzed via qPCR. Data were analyzed using a mixed model ANOVA with time as a repeated measure. Infusion of 5-HTP increased circulating serotonin concentrations ($P = 0.001$) and peripheral leukocyte mRNA expression of monoamine oxidase-A and serotonin receptor 7 across the experimental period (*P*

= 0.01 and $P = 0.005$, respectively). Forty-eight hours from termination of first infusion mRNA of monoamine oxidase-A, serotonin reuptake transporter, and serotonin receptor 7 were increased relative to control ($P = 0.0009$, $P = 0.03$, and $P = 0.0001$, respectively). No differences were observed in interleukin-8 in circulation or at the mRNA level ($P > 0.05$), but a decrease in tumor necrosis factor α expression occurred in 5-HTP infused cows 48h after termination of the first infusion relative to control cows ($P = 0.09$). Collectively, these data suggest infusion of 5-HTP has the ability to alter serotonin metabolism, transport and receptor dynamics and cytokine mRNA expression in peripheral leukocytes in the lactating dairy cow.

Key Words: serotonin, immune, 5-HTP

P267 Temporal profiles of plasma T3, T4, and insulin in dairy cows fed diets containing soybean meal or canola meal at 2 protein concentrations during early lactation. J. Kuehnl*[1], S. Moore[1], and K. Kalscheur[2], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Substitution of soybean meal (SBM) with canola meal (CM) increases milk production in early-lactation dairy cows. However, limited data exists regarding the effect of CM substitution on the temporal profile of plasma metabolites during early lactation. Our objective was to determine the effect of feeding a low (15.4%) or high (17.6%) crude protein (CP) diet formulated with SBM or CM as the main protein source to early-lactation dairy cows on plasma concentrations of triiodothyronine (T3), thyroxine (T4), and insulin. At calving, multiparous Holstein cows (n = 79) enrolled in a 2 × 2 factorial arrangement of treatments in a randomized complete block design. Cows were blocked by calving date. Diets were formulated to contain 55% forage and 45% concentrate mix on DM basis. Canola meal was included at 19.4% and 11.9% DM and SBM was included at 14.5% and 8.9% DM. Plasma was collected twice weekly 3 h postfeeding from the coccygeal vein of each cow during wk 1, 2, 3, 4, 6, and 8 of lactation and composited by wk for each cow. Plasma concentrations of T3, T4, and insulin were determined using ELISA. Data were analyzed using the MIXED procedure of SAS. Dietary CP concentration and protein source were not significant for any metabolite. Week of lactation was significant ($P < 0.05$) for all metabolites. The interaction of protein source and wk was significant ($P = 0.03$) for T4. Concentrations of T4 increased in CM cows during wk 3 and 4 compared with SBM cows, whereas concentrations decreased in CM cows during wk 8 compared with SBM cows. The lowest and highest concentrations of insulin occurred in wk 2 (0.20 ± 0.01 μg/L) and 8 (0.55 ± 0.03 μg/L), respectively. The lowest and highest concentrations of T3 occurred in wk 6 (1.37 ± 0.06 ng/mL) and 2 (1.57 ± 0.06 ng/mL), respectively. Overall, CP concentration did not affect any metabolite and protein source did not affect T3 nor insulin. The interaction of CP and protein source was not significant for any metabolite. The protein source and wk interaction for T4 resulted from differences measured in wk 3, 4, and 8.

Key Words: canola meal, transition period, early lactation

P268 Effect of medium-chain fatty acids added to milk replacer on growth performance in calves. A. Inukai*[1], R. Osawa[2], K. Konda[3], T. Okimura[4], T. Takeuchi[5], M. Jindo[6], N. Nakamura[7], K. Nishimura[8], Y. Ueno[9], K. Murayama[10], K. Sakamoto[11], N. Isobe[13], S. Kushibiki[12], K. Kawashima[1], T. Sugino[13], [1]*Chiba Prefectural Livestock Research Center, Chiba, Japan,* [2]*Saitama Prefectural Agricultural Technology Research Center, Saitama, Japan,* [3]*Kanagawa Prefectural Livestock Industry Technology Center, Kanagawa, Japan,* [4]*Toyama Prefectural Agricultural, Forestry and Fisheries Research Center, Toyama, Japan,* [5]*Ishikawa Prefectural Agriculture and Forestry Research Center, Ishikawa, Japan,* [6]*Yamanashi Prefectural Livestock and Dairy Technology Center, Yamanashi, Japan,* [7]*Shimane Prefectural Livestock Technology Center, Shimane, Japan,* [8]*Miyazaki Livestock Research Institute, Miyazaki, Japan,* [9]*Shinshu University, Nagano, Japan,* [10]*ZENRAKUREN, Fukushima, Japan,* [11]*YP Tech Co., Tokyo, Japan,* [12]*Institute of Livestock and Grassland Science, NARO, Tsukuba, Japan,* [13]*The Research Center for Animal Science, Graduate School of Integrated Sciences for Life, Hiroshima University, Hiroshima, Japan.*

Medium-chain fatty acids (MCFA) are rapidly metabolized in the body and can be used as an energy source for calves. MCFA can stimulate ghrelin and GH secretion in dairy cows. This study investigated the effect of adding MCFA to milk replacer (MR) on growth performance in calves. Forty-one Holstein heifer calves [Body weight (BW) at brith, 41.0 kg] were randomly assigned to one of 2 experimental MR [28% CP, 18% fat either containing 3.2% caprylic acid and 2.8% capric acid (CONT; n = 20) or containing 6.7% caprylic acid and 6.4% capric acid (MCFA; n = 21)] from 2 to 42d of age. After initial colostrum feeding, all calves were fed MR twice daily by nipple bottle. MRs were increased from 600 to 1,200 g/d over a 10 d period to adapt calves to intensive liquid feeding. Calves were fed MR at 1,200 g/d from 1.5 to 4 wk of age, were reduced to 800 g/d at 5 wk of age, and weaned at 6 wk of age. All calves fed calf starter, chopped timothy hay and water ad libitum. From 1 to 13 weeks after birth, BW was measured weekly. Feed intake was recorded daily, and the total-tract digestion test was performed at 13wk of age. Plasmaconcentrations of GH, IGF-1, progesterone and IL-6 were investigated every 2 wk to 41 wk, rumen lipopolysaccharide (LPS) activity was investigated at 8 and 13 wk of age. Data were analyzed by one-way ANOVA using Fit Model procedure of JMP 15 Pro. Calf starter and hay intakes for all calves rapidly increased at weaning (day effect: $P < 0.001$). But BW, ADG, dry matter digestibility, nitrogen retention at 13 wk and VFA concentration in rumen were not affected by MCFA. Plasma concentrations of GH and IGF-1 were higher for MCFA than for CONT group (treatment effect: $P < 0.05$). Rumen LPS activity tended to have a lower MCFA than CONT. And, plasma IL-6 level was low value for MCFA than that of CONT group. The percentage of cows showing first ovulation before 41 wk of age, calculated by plasma progesterone level, were higher for MCFA than for CONT group (treatment effect: $P < 0.05$). These results indicate that MCFA inclusion in MR stimulated GH and IGF-1 secretions before weaning, and may have positive effects on early first ovulation in calves.

Key Words: medium-chain fatty acids, milk replacer, calf

P269 Inflammatory and immunological responses of Holstein dairy cows around dry-off and calving. T. N. Marins*[1], J. Gao[1,2], J. O. S. Calix[1], Z. Qi[2], J. K. Bernard[1], and S. Tao[1], [1]*Department of Animal and Dairy Science, University of Georgia, Tifton, GA,* [2]*Department of Animal Nutrition and Feed Science, College of Animal Sciences and Technology, Huazhong Agricultural University, Wuhan, China.*

To evaluate stress, immune and inflammatory responses of multiparous dairy cows, 2 groups of cows were enrolled: around dry-off (n = 6, SCC <200,000 cells/mL before dry-off) and around calving (n = 7, SCC averaged 128,791 cells/mL after calving). Blood was collected on −8, 3, 7, and 15 d relative to dry-off (RTD), or on −7, 3, 7, and 21 d relative to calving (RTC) to analyze circulating immune cells, prolactin (PRL), cortisol and inflammatory products, and isolate peripheral blood mononuclear cells (PBMC). In vitro, PBMC were cultured with PRL, hydrocortisone, LPS, and concanavalin A (ConA), or in different combinations for 68 h to assess proliferation using MTT assay. Data were analyzed using PROC MIXED of SAS. Plasma cortisol concentration was not affected ($P > 0.10$) by time around dry-off or calving. Plasma PRL concentration was unchanged ($P > 0.10$) around calving, but declined ($P = 0.01$) after dry-off. After dry-off, serum tumor necrosis factor-α and interleukin 10 concentrations increased ($P \leq 0.07$) until d 15, but haptoglobin and fibrinogen concentrations peaked at 7 d RTD ($P = 0.02$). No differences were observed for circulating cytokines and fibrinogen from gestation to lactation, but plasma haptoglobin concentration was greater ($P = 0.07$) at 7 d RTC. Concentrations of monocytes and neutrophils reached nadir ($P \leq 0.06$) at 3 d RTD. Around calving, circulating monocytes peaked ($P = 0.01$) at 3 d RTC, but neutrophils remained unchanged ($P > 0.10$). Regardless of time, addition of hydrocortisone on the cell culture reduced ($P < 0.01$) PBMC proliferation to LPS, but PRL had no effect ($P > 0.10$). No time effect ($P > 0.10$) was observed for proliferation of PBMC collected from cows around dry-off, but PBMC collected at 21 d RTC had higher ($P \leq 0.05$) proliferative responses to LPS or ConA than other time points around calving. In conclusion, results confirmed lower PBMC proliferation around calving, and suggested that cows transitioning from late lactation to dry period maintained unchanged cell-mediated immune function. The period immediately after dry-off is characterized with upregulated systemic inflammation.

Key Words: milking cessation, parturition, inflammation

P270 Feeding rumen-protected lysine affects hepatic transcription of genes related to inflammation and oxidative stress in Holstein cows during the transition period.

L. K. Fehlberg*[1], A. R. Guadagnin[1], B. L. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*Ajinomoto Co. Inc., Tokyo, Japan.*

During the periparturient period, a negative protein balance due to decreased intake and increased demand may result in compromised immune and liver function. This experiment was conducted to determine the effects of feeding rumen-protected lysine (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) from 27 ± 5 d prepartum (0.54%DM of TMR) to 28 d postpartum (0.39%DM of TMR) on the hepatic transcription of inflammatory and oxidative stress-related genes. Seventy-five multiparous Holstein cows, blocked by parity, previous 305-d mature-equivalent milk production, expected calving date, and body condition score during the far-off dry period were assigned to 1 of 4 dietary treatments in a randomized, complete block design with a 2 × 2 factorial arrangement. Treatments consisted of TMR top-dressed prepartum with RPL (PRE-L) or without (PRE-C) and with RPL prepartum and postpartum (PRE-L POST-L), with RPL prepartum and without RPL postpartum (PRE-L POST-C), without RPL prepartum and with RPL postpartum (PRE-C POST-L), and without RPL prepartum and postpartum (PRE-C POST-C). Liver samples were collected via biopsy on −14 ± 0.8 and 14 ± 0.1 d relative to calving and analyzed for mRNA gene expression. Statistical analysis was performed using MIXED procedure of SAS. There was decreased ($P = 0.04$) expression of nuclear factor kappa B1 (*NFKB1*) postpartum for cows in PRE-L compared with PRE-C. There was also a tendency for greater ($P = 0.10$) expression of interleukin 1 β (*IL1B)* for cows in POST-L compared with POST-C. Additionally, expression of serum amyloid A3 (*SAA3*) was less ($P = 0.02$) for cows in PRE-L POST-L, PRE-L POST-C, and PRE-C POST-L compared with PRE-C POST-C. Postpartum expression of superoxide dismutase 1 (*SOD1*) was less ($P = 0.04$) for cows in PRE-L compared with cows in PRE-C. In conclusion, when cows consumed RPL prepartum and/or postpartum there was decreased oxidative stress (*SOD1*) and acute-phase response (*SAA3* and *NKFB1*) related genes in the liver and the tendency for greater proinflammatory *IL1B* gene expression postpartum likely indicates immune activation.

Key Words: immunity, lysine, transition period

P271 Whole-body mineral trafficking following immune activation.

J. Opgenorth*[1], E. J. Mayorga[1], M. A. Abeyta[1], S. Rodriguez-Jimenez[1], B. M. Goetz[1], A. D. Freestone[1], C. H. Stahl[2], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*University of Maryland, College Park, MD.*

Objectives were to evaluate mineral dynamics in tissues, fluids, and digesta following immune activation. Pigs (n = 12; 44 ± 2.6 kg) were randomly assigned to 1 of 2 treatments: 1) saline control (CON; 2 mL sterile saline; n = 6) or 2) LPS (LPS; 40 μg LPS/kg BW; *E. coli* O55:B5; n = 6) and euthanized 6 h later. Pigs were housed in metabolism stalls with restricted access to feed and water for 2 h before and during the challenge. Immediately following administration, urine and feces were continuously collected. Whole tissues of heart, kidneys, liver, spleen, and lumen contents of the stomach, small intestine, and large intestine were weighed and homogenized. Likewise, samples of skeletal muscle, pancreas, adipose tissue, bone marrow, urine, ascites, serum, and bile were obtained to determine Na, K, P, Mg, Cr, Zn, Fe, Cu, Se, Mn, Mo, and Co concentrations. Data were analyzed with PROC MIXED. LPS increased rectal temperature (39.2 and 40.7°C in CON and LPS, respectively; $P < 0.01$), and decreased circulating glucose (39%; $P < 0.01$). Serum Zn concentrations were reduced by LPS (39%; $P = 0.04$) but no changes in the other minerals were detected in circulation. Zn, P, Se, and Cu increased in ascites fluid (124, 31, 235, and 163%, respectively; $P \leq 0.09$). LPS increased Zn and Cu in the kidneys (28 and 75%, respectively; $P = 0.07$). LPS increased Fe and Mo in the spleen (86 and 70%, respectively; $P < 0.01$) and Fe concentrations in bone marrow (47%; $P = 0.02$). Muscle Mg increased 18% with LPS ($P = 0.04$). LPS increased liver Na, Mg, K, P, Mg, Cr, and Zn concentration ($P \leq 0.05$). Bile Cu tended to decrease (38%; $P = 0.07$) with LPS. LPS increased fecal Fe, Mn, Cr, and Co ($P \leq 0.05$) and tended to increase fecal Zn and Cu ($P \leq 0.07$*)*, at least partially explained by increased fecal output (104 vs 246 g for CON vs LPS, respectively). Digesta within the stomach of LPS pigs contained more Na (123%; $P = 0.04$). Small intestine contents from LPS pigs contained more Se (162%; $P < 0.01$) and tended to have increased P (70%; $P = 0.10$). Urine from LPS pigs tended to contain more P (29%; $P = 0.10$). In summary, systemic immune activation caused acute alterations in mineral trafficking throughout the body.

Key Words: immune activation, minerals

P272 Alterations in one-carbon metabolism due to methionine supplementation and lipopolysaccharide challenge in Holstein fetal liver explants.

A. Aboragah*, Y. Liang, D. N. Coleman, and J. J. Loor, *University of Illinois, Urbana, IL.*

The objective was to investigate effects of methionine (Met) supplementation on one-carbon metabolism in fetal liver tissue without and with a lipopolysaccharide (LPS) challenge to induce an inflammatory stress. Fetal liver samples were harvested at slaughter from 6 multiparous pregnant Holstein dairy cows averaging 136 ± 3 d in milk and 37 ± 6 kg milk/d before slaughter. Cows were free of clinical disease and were fed a typical corn silage/alfalfa hay-based diet. Whole liver suspended in warm DMEM:F12 medium containing 1% penicillin/streptomycin was transported to the laboratory upon harvest. Tissue slices (0.2 ± 0.02

g) from each fetus were incubated for 4 h at 37°C with each of the following treatments: ideal profile of amino acids (control; Con, Lys:Met 2.9:1), increased Met supplementation (incMet, Lys:Met 2.5:1), Con plus LPS (1 μg/mL; LPS), and incMet plus LPS (incMetLPS). A custom high-glucose DMEM devoid of essential amino acids was used to prepare each individual treatment. Tissue was used for ^{14}C radio-labeling assays to measure betaine-homocysteine S-methyltransferase (BHMT) and cystathionine-β-synthase (CBS) activity. Upon completion of the incubations, tissue was immediately frozen at −80°C until analysis of enzyme activity, Western blot, and metabolomics. Data were analyzed as a 2 × 2 factorial (without Met or with Met; without LPS or with LPS) using the MIXED procedure of SAS. The activity of CBS, rate-limiting enzyme in the transsulfuration pathway, was not affected ($P > 0.05$) and averaged 119 ± 24 nmol/h/mg protein across treatments. In contrast, there was an interaction ($P < 0.05$) for BHMT, which is involved in remethylation of homocysteine to Met. This effect was due to a 44% increase in activity in response to incMet (24.1 ± 3.2 nmol/h/mg protein) compared with Con (17.4 ± 3.2 nmol/h/mg protein). Preliminary observations suggested that the Met cycle and transsulfuration pathway are functional in bovine fetal liver. Remethylation via BHMT appears responsive to enhanced Met supply.

Key Words: methyl donors, nutritional programming, pregnancy

P273 Effects of amino acids and hyperinsulinemic clamp on plasma concentrations and mammary extraction of energy substrates.

V. Pszczolkowski*[1], H. Hu[1], J. Zhang[1,2], M. Connelly[1], A. Munsterman[1,3], and S. A. Apelo[1], [1]*University of Wisconsin–Madison, Madison, WI,* [2]*Northwest A & F University, Yangling, China,* [3]*Michigan State University, East Lansing, MI.*

Insulin and certain AA, including Leu and Met, interact to regulate mTORC1 activity in mammary epithelium. The objective of this study was to test whether Leu and Met stimulate mammary extraction efficiency of energy substrates, and if insulin potentiates the effect of AA. Six lactating Holstein cows (155 ± 9 DIM) were ruminally cannulated and had the right carotid artery subcutaneously transposed. Cows were fed a 20% protein-restricted diet and abomasally infused with water (8 L/d) or AA (Met 26 g/d, Leu 70 g/d) 8 h/d, for 7 d. The last day of each period, cows were intravenously infused with saline (0.9% NaCl, 110 mL/h) or subject to 8h hyperinsulinemic clamp alongside abomasal infusions. The experiment had an incompletely replicated Latin square design with a 2 × 2 factorial arrangement of treatments (abomasal and intravenous infusion). For the clamp, insulin was infused at 1 μg/kg/h. Normoglycemia was maintained by varying glucose (50% wt/vol saline) infusion rate based on coccygeal vein glucose concentration. Carotid arterial and subcutaneous abdominal (mammary) vein blood samples were collected at 0, 2, 4, and 6 h from start of infusions. Plasma was assayed for insulin, glucose, acetate, β-hydroxybutyrate (BHB), nonesterified fatty acids (NEFA), and triglycerides (TG) using commercial kits. Data were analyzed by ANOVA with repeated measures and Bonferroni adjustment. Interactions between abomasal and intravenous infusions were not significant. Arterial insulin was increased by insulin infusion ($P < 0.01$) with no effect of AA ($P = 0.94$). Arterial glucose was increased by AA ($P = 0.04$) and decreased by insulin ($P = 0.01$). Amino acids tended to increase arterial acetate at 6 h ($P = 0.06$). Insulin clamp decreased arterial BHB at 4 h and 6 h ($P < 0.05$), TG at 4 h ($P < 0.01$), and arterial NEFA overall ($P < 0.01$). Insulin tended to decrease mammary extraction efficiency of glucose ($P = 0.07$) and decreased it for acetate and BHB ($P < 0.01$). The arteriovenous difference for NEFA was always negative and was decreased by AA at 2 h ($P = 0.02$). Overall, AA and insulin did not improve mammary extraction efficiency of energy substrates.

Key Words: insulin clamp, amino acids, energy metabolism

P274 Metabolic alterations of heat-stressed and pair-fed lactating dairy cows during a hyperketonemic clamp.

S. Rodriguez-Jimenez*[1], E. J. Mayorga[1], M. A. Abeyta[1], B. M. Goetz[1], J. Opgenorth[1], A. D. Freestone[1], N. Reisinger[2], J. Faas[2], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*BIOMIN Research Center, Tulln, Austria.*

Study objectives were to investigate ketone clearance and metabolic consequences of hyperketonemia in heat-stressed and nutrient-restricted Holstein cows. Primiparous cows (604 ± 3 kg BW; 156 ± 5 DIM; n = 12) were allowed 10d to acclimate in thermoneutral conditions with ad libitum feed intake (baseline) and then assigned to 1 of 2 environmental treatments for 8d: 1) heat stress using an electric heat blanket with ad libitum feed intake (HS; Thermotex Therapy Systems Ltd., Calgary, AB, Canada; n = 6) or 2) thermoneutral conditions and pair-fed (PF; n = 6) to their HS counterparts. Overall, HS increased rectal temperature 2.2°C ($P < 0.01$) and decreased DMI (45%). Milk yield was reduced in the PF and HS cows by 20 and 36%, respectively compared with baseline. On d8, basal insulin was increased (82%; $P < 0.01$) in HS cows but NEFA (444 μEq/L), β-hydroxybutyrate (BHB; 0.78 mmol/L) and glucose (62 mg/dL) concentrations did not differ between environments ($P > 0.50$). Cows were then fasted and a 12 h hyperketonemic clamp was conducted. A BHB priming dose (700 μmol BHB/kg BW; KetoTech, Inc., Seymour, IL) was administered i.v. followed by continuous infusion (2.2 M) for 12 h. Blood samples were obtained every 10 min and immediately analyzed for BHB concentrations utilizing a hand-held meter (PortaCheck, Moorestown, NJ). Rate of BHB infusion (ROBI) was adjusted to achieve a “clamped” BHB concentration of 1.7 ± 0.17 mmol/L. Overall, HS increased ROBI (109 vs 43 g/h; $P < 0.01$) compared with the PF cows. During the clamp, glucose concentrations did not differ, but insulin concentrations were markedly decreased (67%; $P < 0.05$) in both groups. At h 1 of the clamp, NEFA concentrations were severely reduced (71%; $P < 0.01$) similarly among groups, but gradually increased with time and returned to basal levels at h 12. In summary, reduced feed intake only accounted for ~50% of the decrease in milk yield. Further, HS markedly increased BHB clearance (153%) compared with thermoneutral cows on the same plane of nutrition. HS-induced increased ketone utilization is similar to what we have reported during experimental immune activation (Rodriguez-Jimenez et al., 2020).

Key Words: heat stress, hyperketonemia

P275 Identification of prepartum plasma lipid biomarkers for postpartum metabolic risk in Holstein dairy cows.

J. E. Rico*[2,1] and J. W. McFadden[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD.*

Our objective was to characterize the bovine plasma lipidome during the periparturient period and to determine its association to circulating fatty acids (FA) and β-hydroxybutyrate (BHB), common proxies for disease risk. Fifty-nine multiparous Holstein cows in a commercial dairy herd (Dixie Creek Dairy, Hanford, CA) were enrolled 14 d prepartum (ap) and fed close-up and fresh cow diets to meet their dietary requirements. Blood samples were obtained at d −14, 3, and 10, relative to parturition. Risk thresholds for metabolic disease were defined as 570 μEq/L for FA and 10 mg/dL for BHB. Untargeted lipidomics was performed using LC/MS, and univariate and multivariate methods were used for data analysis. Plasma FA were 3.8-fold higher postpartum (pp), relative to ap ($P < 0.01$), and BHB tended to be higher pp, relative to ap (1.1-fold; $P = 0.10$). A total of 241 plasma lipids were detected. The lipidome of the transition period was distinguishable by day, and specific lipid signatures were found that predict time points relative to parturition (e.g., sphingomyelin [SM]-40:3 and sphingosine-18:0 were differentially expressed across time; variable importance project scores >1.77). While many lipid concentrations increased pp (e.g., SM-41:2, triglyceride (TAG)-16:0/18:1/18:1, and phosphatidylcholine (PC)-34:4; false-discovery rate [FDR] < 0.05), some lipid concentrations decreased pp (PC-36:1, TG-18:0/16:1/16:1; FDR <0.05), relative to ap. Similarly, while many TAG, SM, and PC were positively related to FA ($r = 0.50$–0.65; $P < 0.05$), some PC and TAG were inversely associated with FA ($r = 0.50$ to 0.60; $P < 0.05$). Associations between complex lipids and BHB were weak ($r = 0.15$ to 0.20; $P < 0.05$). Several ap lipids were predictive of increased pp FA (e.g., diacylglycerol-18:0/22:0 and PC-36:5 for d 3 FA; SM-34:1 and PC-34:0 for d 10 FA; AUC = 0.70 to 0.72), with similar or higher predictive value than ap FA (AUC = 0.68). Our results indicate that prepartum plasma lipidome metabolites are related to elevated postpartum plasma FA; however, their ability to predict postpartum disease requires evaluation.

Key Words: dairy cow, lipidomics, peripartum

P276 Role of dietary Ca and 1,25-dihydroxyvitamin D_3 on gastrointestinal absorption of Ca in ruminants.

A. Vieira-Neto*[1], P. R. Menta[2], C. DeWit[2], L. Fernandes[2], A. C. M. Silva[3], F. X. Amaro[3], M. A. Ballou[2], V. S. Machado[2], and J. E. P. Santos[3], [1]*Kansas State University, Manhattan, KS,* [2]*Texas Tech University, Lubbock, TX,* [3]*University of Florida, Gainesville, FL.*

Objectives were to determine the effects of dietary Ca content and 1,25-dihydroxyvitamin D_3 on Ca transport in the gastrointestinal tract (GIT). Holstein bull calves (n = 24) at 90 d of age were blocked by body weight and intake during a 5-d adaptation period and randomly assigned to 1 of 4 treatments. Treatments were arranged as a 2 × 2 factorial with 2 levels of dietary Ca (CaDef = 0.33%; vs. CaAde = 1.19% of diet DM) and 2 doses of 1,25-dihydroxyvitamin D_3 (0, placebo; PCBO vs. 50 µg; 125D) administered subcutaneously as a single injection on experimental d 5. Blood was sampled on d 4, 5, 6, 7 and 8. Feed, urine, and fecal samples were collected from d 2 to 7 to determine Ca digestibility. On d 8, calves were euthanized and tissues, including ruminal epithelia and duodenum, were subjected to RNA-seq analysis. Data for Ca digestibility and transcriptome is expected to be available for presentation at the conference. Calves fed CaDef had lower ($P < 0.01$) blood iCa before 125D treatment compared with CaAde (CaDef = 1.35 vs. CaAde = 1.43 m*M*). An interaction ($P < 0.01$) between dietary Ca and injectable 1,25-dihydroxyvitamin D_3 was observed for blood iCa because 125D increased blood iCa to a greater extent in CaAde than in CaDef calves (CaDef-PCBO = 1.38 vs. CaDef–125D = 1.46 vs. CaAde-PCBO = 1.41 vs. CaAde-125D = 1.64 m*M*). Calves fed CaDef had lower ($P = 0.01$) concentration of plasma total Ca before 1,25-dihydroxyvitamin D_3 treatment compared with CaAde (CaDef = 2.46 vs. CaAde = 2.59 m*M*). After 1,25-dihydroxyvitamin D_3 treatment, plasma total Ca concentration was lower ($P < 0.01$) in CaDef compared with CaAde, whereas it was greater ($P < 0.01$) for 125D compared with PCBO (CaDef-PCBO = 2.49 vs. CaDef–125D = 2.72 vs. CaAde-PCBO = 2.66 vs. CaAde-125D = 3.05 m*M*). Plasma Mg did not differ between dietary Ca treatments before 1,25-dihydroxyvitamin D_3 treatment. After 1,25-dihydroxyvitamin D_3treatment, plasma Mg concentration was lower ($P < 0.01$) for 125D compared with PCBO (125D = 0.77 vs. PCBO = 0.85 m*M*). Our preliminary data suggest that vitamin D-mediated changes in blood Ca are dependent on GIT Ca availability.

Key Words: vitamin D, calcium, digestibility

P277 Effects of peripartum supplementation with omega-3 from flaxseed or fish oil on expression of inflammatory genes in several tissues of dairy cows.

U. Moallem*[1], G. Kra[1,2], L. Lifshitz[1], N. Nemes-Navon[1,2], S. Druker[2], and M. Zachut[1], [1]*Department of ruminants Science, Volcani Center, Rishon LeZion, Israel,* [2]*Department of Animal Science, the Hebrew University of Jerusalem, Rehovot, Israel.*

The objectives were to determine the effects of various omega-3 fatty acids supplemented to peripartum dairy cows on inflammatory gene expression in several tissues. Forty-two 256-d pregnant multiparous cows were supplemented until 60 d postpartum (PP) with encapsulated fats, in treatments designated as i) SFA – saturated fat at 255 g/d/cow prepartum and 640 g/d/cow PP; ii) FLX – flaxseed oil at 300 g/d/cow prepartum and 750 g/d/cow PP; and iii) FO – fish oil at 300 g/d/cow prepartum and 700 g/d/cow PP. Blood samples were taken twice weekly from 21 d prepartum to 21 d PP from 8 cows from each group. Samples of cotyledons of placenta were taken from 4 to 5 cows from each group. Biopsies of adipose tissue (6 cows from each group) were obtained at ~7 DIM, and liver biopsies at 10 DIM (5 cows from each group). Biopsies of the uterus were taken at 40 DIM from 10 cows from each group, and all tissue samples were analyzed for expression of proinflammatory genes. Data were analyzed with GLM procedure of SAS. The average TNF-α concentrations from wk 1 prepartum to 2 wks PP tended to be lower in FO than in SFA ($P = 0.1$). The concentrations of IL-6 tended to be lower in FO compared with the SFA L ($P = 0.1$). In cotyledons, the relative mRNA expression of *IL1B, IL6* and *IL10* were higher in FO than in other groups. In adipose, the relative mRNA expression of *TNFA* was lower in FO than in SFA. In liver, the relative mRNA expression of *IL6* in liver tended to be lower in FLX than in SFA ($P = 0.06$), the expression of *SAA2* tended to be lower in FLX than in FO ($P = 0.06$), and *IL10* tended to be higher in FLX than in FO ($P = 0.1$). In uterus, the relative mRNA expression of *TNFA* was lower in FO ($P = 0.006$) and tended to be lower in FLX than in SFA ($P = 0.1$). The expression of *IL10* was higher in FLX than in SFA ($P = 0.02$) and tended to be higher in FO than in SFA ($P = 0.08$). This study demonstrates several anti-inflammatory effects of dietary n-3 FAs in peripartum dairy cows.s.

Key Words: omega-3, transition cow, immune

P278 Proteomic analysis of peripheral blood mononuclear cells from postpartum dairy cows supplemented peripartum with omega-3 fatty acids from flaxseed or fish oil.

G. Kra*[1,2], N. Nemes-Navon[1,2], J. R. Daddam[1], Y. Levin[3], U. Moallem[1], and M. Zachut[1], [1]*Agriculture Research Organization, Volcani Center, Rishon Lezion, Israel,* [2]*Faculty of Agriculture, the Hebrew University in Jerusalem, Rehovot, Israel,* [3]*The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel.*

Peripheral blood mononuclear cells (PBMC) consist of lymphocytes and monocytes, and proteomic analysis of PBMC is used in other species to monitor health disorders. Postpartum (PP) dairy cows experience subacute inflammation; therefore, our objective was to examine the effect of different sources of dietary n-3 fatty acids (FA), which are known as anti-inflammatory, on the proteome of PBMC in transition dairy cows. Forty-two 256-d pregnant multiparous cows were individually supple-

mented with encapsulated fats, as following: i) SFA – saturated fat at 255 g/d/cow prepartum and 640 g/d/cow PP, rich in C16:0 and C18:0; ii) FLX – flaxseed oil at 300 g/d/cow prepartum and 750 g/d/cow PP, rich in C18:3n-3; and iii) FO – fish oil at 300 g/d/cow prepartum and 700 g/d/cow PP, rich in C20:5n-3 and C22:6n-3. PBMC were isolated from blood from 5 cows from each group at wk 1 postpartum by centrifugation with Ficoll for proteomic analysis, and differential abundance of proteins were examined in FLX and FO relative to SFA (at $P < 0.05$ and fold change ± 1.5). Bioinformatics was performed using Ingenuity (Qiagen). The total n-3 FA content in plasma and PBMC was higher in FLX and FO than in SFA. In PBMC, a total of 3,807 proteins were quantified, from which 44 were differentially abundant between FLX and SFA. The abundance of NF-kappa B transcription factor p65 subunit (RELA), apolipoprotein H (APOH), kininogen 1 (KNG1) and serpin family D member 1 (SERPIND1) were higher, while albumin (ALB), C4b-binding protein, serum amyloid P component (APCS) and complement factor H (CFH) were lower in FLX vs. SFA. The main pathways enriched by the FLX treatment were: the acute-phase response signaling (based on ALB, APCS, APOH, C4BPA, RELA and SERPIND1), LXR/RXR and FXR/RXR activation (ALB, APOH, GC vitamin D binding protein, KNG1 and RELA), the coagulation system (KNG1 and SERPIND1) and the complement system (C4BPA and CFH). Other 42 proteins were different between FO and SFA PBMC; lipopolysaccharide-binding protein was higher and CD59 molecule complement regulatory protein was lower in FO vs. SFA. In conclusion, dietary n-3 FA from FLX or FO supplemented to peripartum cows were incorporated into PBMC and affected their proteome in a different manner.

Key Words: proteomics, PBMC, inflammation

Production, Management, and the Environment: Posters

P279 Economic viability and benchmarking associated with dairy farms in Brazil. J. De Souza Pinheiro*[1] and M. I. Marcondes[1,2], [1]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil,* [2]*Washington State University, Pullman, WA.*

Dairy operations have adopted benchmarking as a methodology to rank farms and stablish target indexes, however a connection between benchmarking and farmers in the tropics is still warranted. We aimed to stablish benchmarking based on economic outcomes in dairy farms in different regions. We collected data from 128 dairy farms from Minas Gerais, Brazil. Farms were grouped into Center, South and Triangle regions. The farms were subdivided into 3 groups within each region according to the return on assets (ROA, %), where 25% of the farms that presented the lowest ROA were classified as first quartile (1Q), 50% of farms were classified as intermediate quartile (IQ) and the 25% remaining farms were classified as fourth quartile (4Q). Data were analyzed in a randomized block design in a split-plot scheme, where the production systems were blocks, the regions are the main plots and the groups the split plots. Differences were declared when $P \leq 0.10$. All economic indexes were affected by regions and quartiles ($P > 0.10$). South region and 1Q had the greatest accrual operating cost (AOC) whereas Triangle and 4Q had the greatest net margin and profit. In conclusion, triangle region and fourth quartile presented the greater economic indexes, indicating the need for specific benchmarks by regions and for farms with different return on assets.

Key Words: cows, financial, milk production

P281 Profitability and environmental performance of Quebec dairy farms when 4 binary alfalfa-grass mixtures are compared under future climate conditions. C. Payant*[1], G. Jégo[2], V. Ouellet[1], P. Grenier[3], G. F. Tremblay[2], G. Bélanger[2], D. Pellerin[1], A. Vanasse[1], and É Charbonneau[1], [1]*Université Laval, Quebec, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Quebec, QC, Canada,* [3]*Ouranos, Montreal, QC, Canada.*

Objective of this study was to project the impact of choice in alfalfa-grass mixture on profitability and environmental performance of 2 Quebec dairy farms located in contrasting climates in near (NF: 2020–2049) and distant (DF: 2050–2079) future when modifications in crop production arising from climate change are considered. Yield and nutritive value of 4 alfalfa-grass binary mixtures were projected through a climatic reference period (REF: 1971–2000), and through NF and DF using a mechanistic model (Integrated Farm System Model): 1) alfalfa-timothy; 2) alfalfa-tall fescue; 3) alfalfa-meadow fescue; 4) alfalfa meadow bromegrass. Projected yield and nutritive attributes of REF, NF and DF were then transferred in an optimization model (Nutrient Cycling: Crops, Livestock, Environmental and Soil) which was adjusted beforehand to account for constraints associated with climate change, to simulate profitability and environmental performance. Results of simulations showed that relative to REF, net income could increase in both farms, with a highest difference of CAD 13.4 and 3.2 /100 kg of fat and protein corrected milk (FPCM) for the farms in the coolest and the warmest regions, respectively. The on-farm N and P balance should decrease in the future because of the increase in crop sold. Greenhouse gas emissions projections showed no major modification to the whole-farm total (mean of 1.9 and 1.8 CO_2eq/kg FPCM for the coolest and the warmest regions, respectively), but more greenhouse gas emissions is expected to be allocated to cash-crop (+0.4 and +0.1 CO_2eq/kg FPCM for the coolest and the warmest regions, respectively), and less to milk production (−0.3 and −0.1 CO_2eq/kg FPCM for the coolest and the warmest regions, respectively) in the future relative to REF. Forage mixtures with the highest net income were alfalfa with tall fescue or meadow bromegrass depending on the region and the climatic simulations. Projections of the net income and environmental parameters using a whole-farm model approach should be used to improve crop allocation on dairy farms.

Key Words: climate change, dairy farm, forage

P282 Low milk fat test in confinement and pasture-based systems: Forage source and content? M. N. Méndez*1,3, L. Grille[2], M. Oborsky[3], V. Rodríguez[2], L. Olazábal[4], J. P. Damián[5], and P. Chilibroste[3], [1]*Red Tecnológica Sectorial, Montevideo, Uruguay,* [2]*Facultad de Veterinaria, Paysandú, Uruguay,* [3]*Facultad de Agronomía, Paysandú, Uruguay,* [4]*Laboratorio Tecnológico del Uruguay, Montevideo, Uruguay,* [5]*Facultad de Veterinaria, Montevideo, Uruguay.*

We aimed to study the nutritional causes that led to low milk fat (MF) test on 16 cows fed a total mixed ration (TMR) ad libitum (TMRG) and 16 cows fed with TMR (PM, 50% of TMRG) + AM access to fresh pasture (PMRG) randomly assigned to the treatments after calving. TMR composition changed twice (experimental wk 12 and 19), generating 3 feeding periods. The hypothesis was that the low MF test in P1 and P2 was due to changes in neutral detergent fiber (NDF) content of the diet. All cows were milked at 4:00 a.m. and PM. Feed components and chemical composition of the TMR by period (P0, P1 and P2) are

Table 1 (Abstract P279). Economic indexes for dairy farms in different regions and groups

	Region			Quartile				*P*-value[2]	
Item[1]	Center	South	Triangle	First	Intermediate	Fourth	SEM	Region	Group
AOC, $/L	0.265^{b}	0.274^{a}	0.263^{b}	0.302^{a}	0.261^{b}	0.238^{c}	0.009	0.0603	<0.0001
Net margin, $/L	0.047^{c}	0.057^{b}	0.073^{a}	0.021^{c}	0.064^{b}	0.092^{a}	0.007	<0.0001	<0.0001
Profit, $/L	0.020^{c}	0.029^{b}	0.043^{a}	−0.016^{c}	0.037^{b}	0.071^{a}	0.004	<0.0001	<0.0001
ROA (%)	0.009^{b}	0.013^{a}	0.015^{a}	−0.003^{c}	0.012^{b}	0.029^{a}	0.002	0.0240	<0.0001
ATR (%)	0.169^{c}	0.189^{b}	0.207^{a}	0.133^{c}	0.184^{b}	0.248^{a}	0.008	0.0010	<0.0001

Means within a row with different superscripts differ ($P < 0.10$).

[1] AOC = accrual operating cost; [1]ROA = assets turnover rate with land; ATR = assets turnover rate.

[2] All interactions were nonsignificant (P > 0.12).

presented in Table 1. Milk samples were taken to determine MF at P1 and P2. A MIXED procedure was performed to analyze MF in P1 and P2, with treatment, period and its interaction as fixed effects and cows as random effect. Results were considered significant at $\alpha \leq 0.05$. In average TMRG cows consumed 29.1 ± 1.1 kg DM/day and produced 40.5 ± 2.1 L/day/cow, while PMRG consumed 14.3 ± 1.2 kg DM/day of TMR + 7.2 ± 2.9 kg DM/day of pasture, and produced 32.3 ± 1.6 L/day/cow. There were no effects of treatment nor interaction on MF percentage. MF decreased from P1 to P2 ($P \leq 0.01$): 3.6% *vs* 2.5% in TMRG and 3.7% *vs* 2.8% in PMRG, respectively. Although PMRG had a greater diet NDF content than TMRG (Table 1), due to pasture inclusion, MF was not different between treatments. These led to reject the hypothesis that feed fiber content was the main responsible for the low MF test. So, other TMR components such as carbohydrates from maize silage or fatty acids from concentrate could have altered ruminal environment and therefore MF production.

Table 1. Feed components and NDF (%) in 3 different periods

Diet	Components		Period		
			P0	P1	P2
TMR	Forage (%)	Maize silage	25	35	35
		Ryegrass silage	22	—	—
		Fescue hay	—	2	6
	Concentrate (%)		53	63	59
TMR	NDF (%)		42	37	35
P+TMR[1]	NDF (%)		50	41	38

[1]P+TMR = average from pasture (P) and TMR % NDF weighted by intake.

Key Words: confined and grazing cows, fiber, fat milk

P283 A 16S-metagenomic analysis of compost-bedded pack barns in dairy farms from Argentina.

J. L. Monge*[1], L. Palma[2,1], and C. Peralta[1], [1]*Universidad Nacional Villa María, Villa María, Córdoba, Argentina,* [2]*Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.*

In compost-bedded pack dairy barns (CBP) microbial communities play an essential role in the process. The aim of this work was to study the bacterial community structure at different CBP using 16S-metagenomics to identify nitrifying bacteria. Two CBP (MB: Martin Bono Dairy Farm; AT: Angela Teresa Dairy Farm) were sampled during the winter (Julie 2019) in Córdoba province, Argentina. MB was 30 mo and AT was 20 mo age. MB had concrete feed alleys, CBP was started over the natural ground without bedding addition, was deep-tilled 2x/d and the cow stockig density (CSD) averaged 14.5 m^2/cow (plus 2.4 m^2/cow concrete feed alley area). AT had not concrete feed alleys, CBP was started with 40 cm of peanut shells, with bedding addition (5.5 kg/cow/day). CBP was deep-tilled and rototilled 2×/d, CSD averaged 13.75 m^2/cow (including feed alley area without concrete). Two samples were collected per CBP at 30 cm deep. Each sample was dried by lyophilization. Dried-compost samples were homogenized with high-speed universal disintegrator (FW100 model). Total DNA was purified using PureLink Microbiome DNA Purification Kit and sequenced at INDEAR (Rosario, Santa Fe province). Raw Illumina reads were filtered as follows: 1) removeal of duplicate reads, 2) removeal of chimeric reads and 3) 16S biodiversity analysis using Geneious R11 (geneious.com). For both CBP taxonomic hits distribution results at domain level was 99% bacteria and 1% archaea. In MB taxonomic classification hits distribution at genus level for nitrifying bacteria was 5% *Nitrosococcus*, 0.06% *Nitrococcus*, 20% *Nitrosomonas* and 0.05% *Nitrobacter*; in AT was 0.02% *Nitrosococcus*, 0.004% *Nitrococcus*, 0.07% *Nitrosomonas* and 0.0005% Nitrobacter. Nitrifying bacteria showed greater values for MB than AT. We concluded that nitrifying bacteria hits distribution are affected by CBP design, CSD and the bedding use.

Key Words: compost-bedded packs barns, 16S-metagenomic, nitrifying bacteria

P284 Low milk fat test in confinement and pasture-based systems: Unsaturated fatty acids?

L. Grille*[1], M. N. Méndez[2,4], V. Rodríguez[1], L. Olazábal[3], M. Oborsky[2], J. P. Damián[5], and P. Chilibroste[2], [1]*Facultad de Veterinaria, Paysandú, Uruguay,* [2]*Facultad de Agronomía, Paysandú, Uruguay,* [3]*Laboratorio Tecnológico del Uruguay, Montevideo, Uruguay,* [4]*Red Tecnológica Sectorial, Montevideo, Uruguay,* [5]*Facultad de Veterinaria, Montevideo, Uruguay.*

We aimed to study the nutritional causes that led to low milk fat (MF) test on 16 cows fed a total mixed ration (TMR) ad libitum (TMRG) and 16 cows with access to fresh pasture supplemented with a partial mixed ration (PMRG) randomly assigned to the treatments after calving. TMR changed in experimental wk 12 and 19, generating 3 periods (P0, P1 and P2). The hypothesis was that low MF test was caused by an increase in TMR unsaturated fatty acids proportion which resulted in a surge in intermediate rumen fatty acids. Milk samples were taken to determine MF at wk 15 (P1) and 23 (P2) of lactation. Six cows of each treatment were selected to analyze milk fatty acid profile (FAP). At wk 10 (P0), P1 and P2, TMR samples were taken to determine FAP (Table 1). A MIXED procedure was performed to analyze MF and milk FAP as repeated measures, with treatment, period and its interaction as fixed effects and cows as random effect. In both groups, MF decreased from P1 to P2 ($P \leq 0.01$); 3.6% *vs* 2.5% in TMRG and 3.7% *vs* 2.8% in PMRG (P1 and P2, respectively). De novo fatty acid was affected by period ($P \leq 0.01$); 0.74% *vs* 0.43% in TMRG and 0.85% *vs* 0.54% in PMRG (P1 and P2, respectively). A stepwise regression analysis shows that C18:1 *trans* in milk explained 41% of the variability in MF content and the C18:2 *cis* explained an additional 19% variation. C18:2 *cis* in milk increased from P1 to P2 in both treatments ($P \leq 0.01$), while C18:1 *trans*-had no change between periods. The C18:2 *cis* TMR increase and de novo milk fatty acid decrease, supported by stepwise regression results, suggest an incomplete ruminal biohydrogenation with an increase in intermediate fatty acids, which might have inhibited MF synthesis. Even though there was no significant increase in C18:1 *trans* in milk, the isomer C18:1 *trans*-10 (main inhibitor of MF synthesis) could have increased without a rise in total C18:1 *trans.* Hence, TMR unsaturated fatty acids proportion increase could have decreased MF.

Table 1. Total mixed ration fatty acid profile in 3 different periods

Fatty acid (g/100 g fat)	Period		
	P0	P1	P2
C18:1 *cis*	28.5	27.8	29.2
C18:1 *trans*	0.07	0.03	0.05
C18:2n-6 *cis*	30.8	36.3	35.6
C18:2 *trans*	0.01	0.04	0.05
C18:3n-3	7.6	6.3	3.3
Total milk fat (g/100 g of TMR)	3.8	3.8	4.2

Key Words: dairy cow, fatty acid, TMR

P285 Properties of alternative bedding materials for compost-bedded pack barns.

R. A. Black*[1] and B. M. Karle[2], [1]*University of California Cooperative Extension, Santa Rosa, CA,* [2]*University of California Cooperative Extension, Orland, CA.*

Compost-bedded pack barns (CBPB) require frequent addition of bedding material to absorb moisture and sustain composting activity. The "gold standard" for CBPB bedding is kiln-dried wood shavings; however, this material may be expensive or difficult to obtain in adequate quantities. The objective of this study was to determine chemical and physical properties of by-products to understand their suitability as an alternative bedding source. Materials tested included a variety of local by-products. Water-holding capacity (WHC) was determined using the ASTM D7367–19 Standard on one sample per material. Samples were tested for Carbon to Nitrogen ratio (C:N) on one sample per material by The Pennsylvania State University Agricultural Analytical Services Laboratory. To test for dry matter, 3 samples of each material were weighed in aluminum trays, dried for 24 h at 60°C, and reweighed to determine moisture content. Dry matter data were analyzed using a mixed linear model. Results are reported in Table 1. Variability was high, indicating the need for selectivity and testing of material before use. Grapevine C:N was numerically similar to kiln-dried shavings, but had lesser WHC. Further processing of grapevines into shavings may improve suitability for bedding. Almond hulls had lower C:N but may act as an absorptive material in CBPB. Additional research monitoring composting efficiency and bacterial populations and determining optimal material combinations will further inform dairy producers of the suitability of by-products as alternative CBPB bedding.

Table 1. Physical and chemical properties of alternative bedding materials

Material	C:N	DM (% ± SE)	Water-holding capacity (%)
Kiln-dried shavings	135.4	89.9 ± 1.2^{ab}	327.8
Almond hulls A	85.8	86.9 ± 1.2^{b}	166.8
Almond hulls B	84.0	90.4 ± 1.2^{a}	356.1
Chipped almond trees	91.5	90.2 ± 1.2^{ab}	51.1
Chipped grapevines	130.9	92.7 ± 1.2^{a}	99.8
Chipped grapevine canes	79.0	92.9 ± 1.2^{a}	112.5
Chipped hemp stalks A	24.8	90.6 ± 1.2^{a}	187.9
Chipped hemp stalks B	35.8	93.2 ± 1.2^{a}	210.7
Rice hulls A	71.0	91.7 ± 1.2^{a}	139.9
Rice hulls B	74.9	91.1 ± 1.2^{a}	116.4
Rice straw	31.3	93.0 ± 1.2^{a}	411.7

^{ab}Different superscripts within a column indicate significance ($P < 0.05$).

Key Words: compost-bedded pack barn, dairy bedding

P287 Predicting dairy cattle heat stress using machine learning techniques.

C. A. Becker*, A. Aghalari, M. Marufuzzaman, and A. E. Stone, *Mississippi State University, Mississippi State, MS.*

The objectives of the study were to use a heat stress scoring system to evaluate the severity of heat stress on dairy cows using different heat abatement techniques. The scoring system ranged from 1 to 4, where 1 = no heat stress; 2 = mild heat stress; 3 = severe heat stress; and 4 = moribund. The analyses were performed in Python version 2.7, using scikit-learn libraries to predict the accuracy of the scoring system using 3 machine learning techniques: logistic regression, Gaussian naïve Bayes, and random forest. To predict the accuracy of the scoring system, these techniques used factors including temperature-humidity index, respiration rate, lying time, lying bouts, total steps, drooling, open-mouth breathing, panting, location in shade or sprinklers, somatic cell score, reticulorumen temperature, hygiene body condition score, milk yield, and milk fat and protein percent. Three different treatments, namely, portable shade structure, portable polyvinyl chloride pipe sprinkler system, or control with no heat abatement, were considered, where each treatment was replicated 3 times with 3 s-trimester lactating cows in each treatment pen. Results indicate that random forest outperformed the other 2 methods, with respect to both accuracy and precision, in predicting the sprinkler group's heat stress score. Both logistic regression and random forest were consistent in predicting scores for control, shade, and combined groups. The mean probability of predicting non-heat-stressed cows was highest for cows in the sprinkler group. Finally, the logistic regression method worked best for predicting heat-stressed cows in control, shade, and combined. The insights gained from these results could aid dairy producers to detect heat stress before it becomes severe, which could decrease the negative effects of heat stress, such as milk loss.

Key Words: heat stress; machine learning; shade; sprinklers

P288 Performance and welfare of Nili Ravi buffaloes subjected to different cooling strategies during subtropical summer.

M. Bah1,3, M. A. Rashid[2], K. Javed[1], T. N. Pasha[2], and M. Q. Shahid*[1], [1]*Department of Livestock Management, University of Veterinary and Animal Sciences, Lahore, Pakistan,* [2]*Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan,* [3]*School of Agriculture and Environmental Sciences, University of The Gambia, Serekunda, Republic of The Gambia.*

Water buffaloes wallow in water to combat heat stress during summer. With the decreasing reservoirs for wallowing, the farmers cool buffaloes by applying water using hand-held hose. This method uses a large quantity of groundwater which is becoming scarce. The objective of this study was to evaluate the effects of different cooling strategies on milk yield, physiological, and behavioral responses of Nili Ravi buffaloes during subtropical summer in Pakistan. Thirty Nili Ravi buffaloes were randomly assigned to 3 treatments: 1) CNT buffaloes cooled with application of water using hand-held hosepipe twice daily, 3 min each with a water flow rate of 50 L/min; 2) 2SS, buffaloes cooled with sprinklers twice daily; 3) 3SS, buffaloes cooled with sprinklers thrice daily. Each of the sprinkler sessions lasted for 1 h with a 12 min cycle (3 min water on, 9 min off). The trial was carried out from mid-July till the end of September, 2019. The average ambient afternoon temperature-humidity index was 86.9. The results indicated that the buffaloes in the 3SS group had lower respiration rate and rectal temperature than those in the CNT and the 2SS groups ($P < 0.0001$). The 3SS group had more daily milk yield ($P = 0.0188$) and milk fat % ($P = 0.001$) than the CNT and 2SS groups. The lying time and the lying bouts length were significantly longer in the 3SS than in the CNT and 2SS groups. Blood cortisol levels tended to be lower in the 3SS group than in the CNT and 2SS groups ($P = 0.051$). The volume of water uses for cooling in the CNT group was higher than the 2SS and 3SS groups. In conclusion, the 3SS cooling strategy had more milk yield, more milk fat, and better welfare than the CNT strategy using less groundwater and both performed better than the 2SS strategy.

Key Words: dairy buffaloes, heat stress, sprinkler cooling

P289 The evaluation of different spraying frequencies on cooling effects for cows in summer.

Z. Guo[1], S. Gao[1], L. Ma[1], L. Baumgard[2], and D. Bu*[1], [1]*Institute of Animal Sciences, Chinese*

Academy of Agricultural Sciences, Haidian, Beijing, China, [2]Iowa State University, Ames, IA.

The aim of the present study was to evaluate the effect of spraying frequencies on alleviating heat stress symptoms based on performances including physiological as well as antioxidant status, milk production and intestinal barrier of dairy cows. Twenty multiparous, lactating Holstein cows (DIM 175 ± 25d, daily milk production 27.5 ± 2.5 kg of milk/d) were housed in a freestall barn with HS condition (THI >72). The study included 2 consecutive periods (14d for each period) with 2 spraying treatments. In period 1, sprinklers were activated for 3min at 6-min intervals (3|6). In period 2, sprinklers were activated for 1.5min with 3-min intervals (1.5|3), while the fans operated 24h/d. Samples of urine, feces, milk, blood, rumen fluid, and TMR were collected on d 7 and 14 of each period, while rectal temperature, skin temperature, and respiratory rate of each cow were recorded 3 times (0700, 1400 and 2200 h) daily. Data analysis was analyzed using Proc Mixed model in SAS 9.4 with THI and DIM used as covariate. Compared with 3|6, spraying with 1.5|3 reduced the respiratory rate (64.2 vs 55.2 times per minute, $P < 0.01$) as well as skin (32.8 vs 33.8°C $P < 0.01$) and rectal temperature (38.4 vs 38.8°C, $P < 0.01$), meanwhile increased evening milk production (8.21vs 7.25 kg, $P < 0.05$) and DMI (22.02 vs 19.92 kg, $P < 0.01$). Blood concentration of LPS (2.19 vs 0.70 10^{-4} EU/mL, $P < 0.01$), NO (3.22 vs 1.72 μmol/L, $P < 0.1$) and D-lactate (17.05 vs 8.35 μmol/L, $P < 0.1$) of 1.5|3 cows were lower than 3|6 cows. The activity of SOD (7.03 vs 11.30 U/mL, $P < 0.01$) and CAT (5.35 vs 6.79 U/mL, $P < 0.01$) in blood were increased by 1.5|3 compared with 3|6. These data suggested that a spraying frequency of 1.5|3 alleviates the detrimental effects of HS compared with 3|6, suggesting that increased spraying frequency is a more efficient evaporative cooling strategy for HS dairy cows.

Key Words: heat stress, spraying frequency, cooling strategy

P290 Cooling ameliorates the decrease of milk protein of lactating Holstein cows under heat stress. S. T. Gao[1], Z. T. Guo[1], L. H. Baumgard[2], L. Ma[1], and D. P. Bu*[1], [1]*Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Department of Animal Science, Iowa State University, Ames, IA.*

The objective of the present study was to evaluate the relief effect of cooling on the decrease in milk protein concentration during heat stress, and elucidate the potential metabolic mechanism. Thirty lactating multiparous Holstein cows (DIM 175 ± 25 d, milk yield 27.5 ± 2.5 kg/d) were assigned to 1 of 3 treatments: heat stress (HS, n = 10), cooling (CL, n = 10), and cooling with pair-feeding (PFCL, n = 10). The daily feed intake of PFCL cows was equal to HS cows. The barns for PFCL and CL cows were equipped with sprinklers and fans, whereas the barn for HS cows were not. The local average THI during the experiment period ranged from 74 to 83. The spraying was activated automatically 2 times per day (1130 to 1330 h and 1500 to 1600 h) with 3 min on and 6 min off in the first 2 weeks, and 1.5 min on and 3 min off in the last 2 weeks, while the fans operated 24 h/d. The experiment lasted for 4 weeks in total and with 7d adaptation before the experiment. Milk, urine, feces, TMR, blood, and rumen fluid samples were collected weekly. Compared with HS, feed efficiency (1.24 and 1.49, $P < 0.05$), milk protein yield (0.82 and 0.94 kg/d, $P < 0.05$), and milk fat yield (0.98 and 1.26 kg/d, $P < 0.05$) were increased in PFCL compared with HS, while the differences between CL and HS were not significant. Compared with HS cows, PFCL and CL cows had lower respiratory rate (70.6, 59.1, and 60.3 bpm, respectively, $P < 0.05$), rectal temperature (38.95, 38.61, and 38.51 centigrade, $P < 0.01$), and shoulder skin temperature (33.95, 33.25, 33.40 centigrade, $P < 0.01$), while higher milk protein (3.41, 3.72, and 3.69%, $P < 0.05$) and milk fat (4.08, 4.97, 4.65%, $P < 0.01$) percentages. Both the blood activity of CAT (increased by 12.8 and 41.0%, $P < 0.01$) and GSH-Px (12.6 and 40.4%, $P < 0.01$) of PFCL and CL cows were higher than the HS cows. Compared with HS, cooling increased the blood content of glucose, methionine, threonine, and cysta by 10.7% and 10.3% ($P < 0.05$), 19.0% and 9.5% ($P < 0.05$), 15.8% and 12.0% ($P < 0.05$), and 9.5% and 23.8% ($P < 0.05$) in PFCL and CL respectively. The results indicate that cooling rescues milk protein synthesis from depression induced by heat stress, and the potential mechanism may due to decreased oxidative stress.

Key Words: heat stress, sprinklers and fans, milk protein

P291 Performance of Holstein × Gyr crossbred heifers supplemented with increasing crude protein levels in the supplement. M. M. D. Castro*[1], P. V. F. Correia[1], M. M. Ferreira[1], A. F. Machado[1], and M. I. Marcondes[2], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*2Washington State University, Pullman, WA.*

Studies evaluating the effects of increasing levels of crude protein (CP) in supplements and their interactions with season are scarce. Thus, our aim was to evaluate the effect of providing increasing levels of CP in the supplement on performance of Holstein (1/2) × Gyr (1/2) crossbred heifers grazing intensively managed *Brachiaria decumbens* throughout year. The heifers were randomly assigned to 4 treatments: 3 supplements (SUP) fed at 5g/kg of body weight, plus a control group (CON). The supplement CP levels were 12, 24 and 36%. The experimental period was divided into 4 seasons: rainy, dry and transition rainy-dry and dry-rainy. Feces and pasture were collected during 4 d, at the end of each period, chromium oxide, titanium dioxide, and indigestible neutral detergent fiber were used to estimate fecal excretion, supplement and pasture intake, respectively. The data were analyzed using the PROC MIXED of the SAS (Version 9.2) in a repeated-measures scheme. There was no interaction between SUP and period. SUP animals had greater dry matter intake, metabolizable protein and energy intake, and average daily gain (ADG, $P < 0.01$; Table 1) than CON. Among SUP treatments, there was a positive linear effect on metabolizable protein intake ($P < 0.01$), a quadratic effect (concave response) on ADG and a trend of quadratic effect (concave response) on pasture intake and DMI. In summary, SUP animals had greater intake and performance than CON and the supplementation with 24% of CP showed the best results.

Table 1. Intake and performance of Holstein × Gyr heifers supplemented with increasing crude protein levels

	Treatment[1]					*P*-value[2]			
Item	CON	S12	S24	S36	SEM	T	SUP	L	Q
Pasture (kg/d)	5.9	5.8	6.1	5.3	0.24	0.15	0.53	0.16	0.08
DM (kg/d)	5.9	6.9	7.3	6.4	0.26	<0.01	<0.01	0.13	0.06
ME (Mcal/d)	9.9	12.8	13.0	12.5	0.56	<0.01	<0.01	0.72	0.35
MP (g/d)	432.8	509.4	626.7	660.7	24.98	<0.01	<0.01	<0.01	0.02
ADG (kg/d)	0.39	0.46	0.49	0.42	0.02	<0.01	<0.01	0.19	0.04

[1]CON = control, S12 = 12% CP; S24 = 24% CP, S36 = 36% CP.

[2]T = treatments, SUP = supplemented vs non-supplemented; L = linear; Q = quadratic.

Key Words: *Brachiaria decumbens,* performance, supplementation

P292 Effect of a feed additive blend of chestnut and quebracho extracts, and saponins on the milk malondialdehyde concentration in dairy cows.

L. M. Luque*[1], F. M. Masía[1,2], M. B. Pedraza[1], M. Larrauri[1,2], J. M. Baeck[3], C. Cabral[3], and A. R. Castillo[4], [1]*Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina,* [2]*CONICET, Córdoba, Argentina,* [3]*Silvafeed S.A., Buenos Aires, Argentina,* [4]*University of California, Merced, CA.*

Malondialdehyde (MDA) is a marker used to assess the peroxidation status in dairy cows and as an indicator for mammary gland inflammation. The aim of this study was to evaluate the effect of feeding a blend of chestnut and quebracho extracts, and saponins on milk MDA concentration. Sixteen Holstein cows, balanced by milk yield and parity, were randomly assigned to 2 treatments in a 28-d crossover design (21 d of adaptation and 7-d sampling collection), control (CON), fed a basal diet composed of alfalfa hay (8.4%), corn silage (55.9%), soybean meal (20.7%), ground corn grain (13.4%), and a mineral vitamins premix (1.5%), and the blend of chestnut and quebracho extracts, and saponins (BLE) fed in the CON diet at 0.08% of DMI. The DMI and milk yield was measured daily throughout all the experimental periods. Data were analyzed using a linear mixed model with treatments, periods, and parity groups as fixed effect, and cows as random effect. The DMI was lower in BLE vs CON (25.28 vs 27.52 kg/d; $P < 0.0001$), no differences in milk yield were observed (BLE 37.68 and CON 37.41 milk L/d; $P = 0.1716$). The BLE treatment improved feed conversion efficiency (1.50 vs 1.38 milk L/Kg DMI; $P < 0.0001$). Milk MDA was lower in BLE compared with CON (489.45 vs 980.62 ng/mL; $P < 0.001$). The BLE improved the feed conversion efficiency and the peroxidative status of lactating dairy cows by reducing MDA milk contents.

Key Words: dairy cow, malondialdehyde, tannins

P293 Production costs, economic viability, and risks associated with compost-bedded pack, freestall, and drylot systems in tropical dairy farms.

J. De Souza Pinheiro*[1] and M. I. Marcondes[1,2], [1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [2]*Washington State University, Pullman, WA.*

The adoption of intensive production systems, such as compost-bedded pack (CB) and freestall (FS), has increased recently in tropical regions, mainly replacing drylot system (DL). Thus, our objectives were to compare production costs, economic outcomes, and risk of dairy operations in CB, FS, and DL systems. We collected data from 960 Brazilian farms over 120 consecutive months. All costs were modeled for 2 animal production categories: milking cows and nonmilking animals. We used a regression model that included linear and quadratic parameters, and we added the production system as a fixed variable for all parameters tested with this model. Further, we simulated annual technical and economic indexes per farm. In addition, we developed a risk analysis to measure the probability of negative profit of the farms based on a 14-year historical series of milk prices. All production costs were affected by the system. Feed, medicine, sundry, and labor costs per farm per year were greater in DL farms when milk yield (MY) was greater than 3,500 L/d. The variables milk yield, assets per liter, asset turnover rate, return on assets, operational profit, profit per cow and per liter of milk variables were greater in CB and FS with high MY (>3,000 L/d). Nonetheless, DL had greatest economic indexes with a lower MY (<3,000 L/d), with lower operating costs and greater economic outcomes. The risk analysis indicated that the probability of negative profit (risk) was reduced for CB and FS as MY increased, but DL had the lowest risk with low MY levels. In conclusion, we suggest DL as the most attractive system for tropical farms with a MY between 150 and 3,000 L/d once the DL had lowest risk and greatest profit in this production scale. Despite similar outcomes for CB and FS in most of the farms, the profit per cow ($/year), assets turnover rate (%), risk (%) and expected profit ($/L) analysis indicated that CB can be recommended for farms with MY greater than 3,200 L/d, whereas based on risk (%) and expected profit ($/L) FS would be the most profitable system in dairies producing more than 8,000 L/d of milk.

Key Words: dairy operations, milk yield, profitability

P294 Weight at calving relative to mature body weight rather than age at first calving affects milk production in primiparous Holstein cows.

M. R. Lauber*, M. S. Akins, P. D. Carvalho, and P. M. Fricke, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Our objective was to determine the effect of weight and age at first calving of primiparous Holstein cows on milk production (kg/d) during first lactation. Mature body weight (MBW; 686 kg) was estimated by weighing third- and fourth-lactation cows (n = 76) at 30 to 40 DIM, and weight at 30 DIM relative to MBW (%MBW) was calculated for each primiparous cow. Primiparous Holstein cows (n = 2,280) from a commercial dairy farm were grouped into quartiles (Q) by mean (±SEM) body weight (%MBW) at 30 DIM: Q1 (n = 570) 511.9 ± 0.9 kg (74.6%); Q2 (n = 570) 554.6 ± 0.4 kg (80.8%); Q3 (n = 570) 587.5 ± 0.4 kg (85.6%), and Q4 (n = 570) 641.8 ± 1.4 kg (93.5%). Age (mo) at first calving (AFC) for cows was 21 (n = 782), 22 (n = 846), or ≥23 (n = 652). Data analyzed included: milk production at 4, 8, and 12 wk and pregnancies per artificial insemination (P/AI) at first service as a nulliparous heifer. Milk production was analyzed using the GLM procedure of SAS with the fixed effects of weight quartile, AFC, and weight quartile x AFC. First service P/AI as nulliparous heifers were analyzed using the GLIMMIX procedure of SAS with the fixed effect of weight quartile. First service P/AI of nulliparous heifers decreased ($P < 0.01$) as weight quartile increased (71%, 60%, 53%, and 46%, respectively). The weight quartile × AFC interaction did not affect milk production at 4, 8, or 12 wk of lactation. Further, milk production did not differ ($P = 0.66$, $P = 0.82$, and $P = 0.84$) based on AFC at 4, 8, and 12 wk of lactation, respectively. Milk production at 4, 8, and 12 wk of lactation increased ($P < 0.01$) as weight quartile at 30 DIM relative to %MBW increased (wk 4: 30.6, 33.0, 33.8, and 35.9 kg/d; wk 8: 33.4, 35.9, 36.5, and 38.7 kg/d; wk 12: 34.2, 36.7, 37.3, and 39.5 kg/d). In conclusion, decreased P/AI at first service as nulliparous heifers was associated with increased weight relative to MBW at 30 DIM. Further, increased weight relative to MBW at 30 DIM rather than AFC was associated with increased milk production at 4, 8, and 12 wk in primiparous Holstein cows. Supported by NIFA USDA Hatch project 1019532.

Key Words: weight, milk production, age at first calving

P295 Effects of body condition score at calving, parity and calving season on performance of dairy cows and their offspring.

M. Poczynek[1], I. F. Carrari[1], J. H. Carneiro[1], G. F. M. Leão[2], and R. Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil,* [2]*Negócios Leite, Castrolanda Cooperativa Agroindustrial, Castro, Paraná, Brazil.*

The aim of this study was to evaluate the impact of body condition score (BCS) at calving, parity and calving season on the performance of dairy cows and their offspring. Data from 521 Holstein cows that calved a female calf and had their BCS evaluated at calving from a single commercial farm located at Southern Brazil were used. Cows were categorized into 5 BCS classes: class 1: < 3.0 (n = 19); class 2:

3.0 - 3.25 (n = 134); class 3: 3.5 - 3.75 (n = 160); class 4: 4.0 - 4.25 (n = 142); and class 5: > 4.25 (n = 66). Data were also categorized by calving order (primiparous and multiparous dams) and by 4 calving seasons. Variables with normal distribution were analyzed by GLM procedure of SAS, while reproductive and culling outputs were analyzed by GLIMMIX procedure of SAS. Daughters from primiparous dams were born lighter (39.1 ± 0.42 vs. 41.4 ± 0.29 kg; $P < 0.01$), but they had the same weight (121.5 ± 1.67 vs. 120.4 ± 1.58 kg; $P = 0.20$) than the daughters from multiparous cows at weaning. As expected, primiparous cows showed lower ($P < 0.01$) 305-d milk yields; 8,633 ± 363 vs. 10,761 ± 249 kg, respectively. Regarding calving season, cows that calved in the winter were the most productive ones and those that calved in the fall had lower milk yields ($P = 0.01$). Calves born in the winter were heavier at birth ($P < 0.01$), calved younger ($P = 0.04$) and produced more milk at first lactation ($P = 0.03$). The BCS class had an impact ($P < 0.01$) on calves' birth weight; daughters from class 1 cows (BCS <3.0) were lighter (38.01 ± 1.0 kg) than the calves from class 5 cows with BCS >4.25 (41.9 ± 0.57 kg). Calves from dams with BCS <3 (Class 1) had 31.8% culling rate until weaning, while calves from cows with BCS 3.0 - 3.25 (Class 2) had 9.6% culling rate ($P = 0.12$). Maternal BCS had no influence in both age at first calving and reproductive performance. The results suggest that maternal and environmental factors, such as calving season and parity, besides dams' body condition score at calving, are associated with different offspring performances.

Key Words: dairy calves, fetal programming, transition period

P296 Screening of macroalgae species for enteric methane mitigation effect in vitro.

D. E. Wasson*[1], H. Stefenoni[1], S. Welchez[1], C. Lage[1], S. Räisänen[1], A. Melgar[1], M. Fetter[1], C. Yarish[2], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*The University of Connecticut, Stamford, CT.*

Methanogenesis and mitigation of enteric methane emissions from ruminants have been the focus of ongoing research to address livestock contribution to climate change. This experiment investigated the effects of 70 species of macroalgae on methanogenesis and rumen fermentation in vitro. Species were analyzed for their effect on cumulative gas production and composition, and volatile fatty acid (VFA) concentration. Incubations were carried out for 24 h and replicated. Rumen inoculum was collected from 2 rumen-cannulated lactating Holstein cows fed a standard 52% forage (corn silage and alfalfa haylage) and 48% concentrate feeds diet. Dried and ground total mixed ration fed to the donor cows was used as substrate in the incubations at 0.01% (wt/vol) and macroalgae were included in the feed mix at 2.0% (dry matter basis). Negative control (total mixed ration alone) was also included in each incubation in triplicate. Gas production was continuously monitored with an automated gas production system and headspace samples were collected at 12 h and 24 h and analyzed for methane concentration. VFA concentrations were analyzed at 24 h. Data were analyzed by incubation set with the MIXED procedure of SAS with treatment in the model. Methane production (per unit of feed substrate) was decreased ($P \leq 0.001$) 99% by *Asparagopsis taxiformis* (AT). *Mastocarpus papillatus* and *Sargassum fluitans* increased ($P \leq 0.05$) methane production 11 and 10%, respectively. Total VFA concentration was decreased ($P \leq 0.05$) 5 to 8% by 3 species in addition to AT which reduced ($P = 0.03$) total VFA by 10%. Acetate proportion in total VFA was decreased ($P < 0.001$) 9% by AT, along with an increase ($P < 0.001$) in propionate by 14%. Propionate proportion was also decreased ($P \leq 0.05$) by *Laminaria farlowii* and *Ulva* spp. by 9%. AT increased ($P < 0.001$) butyrate and valerate molar proportions by 7 and 24%, respectively, whereas 3 macroalgae species decreased ($P \leq 0.05$) butyrate 3 to 5%. In this screening experiment, AT was the only macroalgae that produced a large reduction in enteric methane emission in vitro.

Key Words: enteric methane, macroalgae, rumen fermentation

P297 Effects of dietary betaine on body temperature indices, performance, and metabolism of dairy heifer calves during summer hot conditions.

M. Al-Qaisi*[1], M. Abuajamieh[1], M. A. Abdel-Majeed[1], M. A. Alnimer[1], R. Irshaid[1], A. Abdelqader[1], H. H. Titi[1], A. A. Al-Fataftah[1], and L. H. Baumgard[2], [1]*Department of Animal Production, The University of Jordan, Amman, Jordan,* [2]*Department of Animal Science, Iowa State University, Ames, IA.*

Study objectives were to evaluate the effects of supplementing dietary betaine on body temperature indices, productive performance, and metabolism in hyperthermic dairy heifer calves. Fourteen Holstein heifer calves (4 mo old) were individually housed and randomly assigned to 1 of 2 dietary treatments: 1) a control diet (CON; n = 7) or 2) a control diet supplemented with 21 g/d of natural betaine (BET; n = 7; Betafin S4; Danisco Animal Nutrition, UK) top-dressed once daily. The trial lasted for 28 d during which all animals were subjected to natural cyclic heat stress (HS) conditions (26.1 to 39.2°C; 73.2 to 84.0 THI). Rectal temperature (Tr) and respiration rate (RR) were measured twice daily (0700 and 1500 h), while dry matter intake (DMI) was measured once daily (0800 h). Hip height (HH), hip width (HW), and body length (BL) were measured on d 7, 14, 21, and 28 of the study. In addition, blood samples (collected from jugular vein) were analyzed for metabolites and complete blood count on d 7, 14, 21, and 28. Effects of treatment, day, and treatment × day were analyzed using PROC MIXED. Relative to CON, BET supplementation decreased Tr on d 23 of the experiment (39.6 vs. 39.3°C; $P = 0.04$). On the other hand, RR was similar between dietary treatments ($P = 0.73$). Feeding BET did not affect DMI, HH, HW, and BL compared with CON during HS conditions ($P \geq 0.35$). Furthermore, circulating glucose, albumin, and triglycerides were similar between dietary treatments ($P \geq 0.55$). Compared with CON, BET supplementation did not change circulating white blood cells, neutrophils, lymphocytes, and hematocrit during HS conditions ($P \geq 0.17$). In summary, BET supplementation had little to no effects on the metrics measured in this experiment.

Key Words: heat stress, betaine, dairy calves

P298 Dairy cattle body weight estimation using surveillance cameras.

H. Liu[1], M. Ramesh[1], J. P. Boerman*[2], and A. R. Reibman[1], [1]*School of Electrical and Computer Engineering, Purdue University, West Lafayette, IN,* [2]*Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Accurate body weight (BW) estimation of dairy cattle provides important information about animal health and welfare status. Observations of BW changes in dairy cattle may be used to assess both nutrient and health status of individual animals as well as populations of animals. To obtain BW information regularly, automated low-cost methods that do not intrude on normal farm operations are essential. Our objective was to utilize video images and video analytics to localize and estimate BW of animals walking from the milking parlor to freestall pens. We measured the BW (655 ± 77.1 kg; range 458 - 876 kg) and recorded the age and days in milk of lactating dairy cattle (n = 168) over 5 time points to use as our reference population directly after exiting the milking parlor. Video from 2 synchronized Ubiquiti surveillance cameras was automatically recorded to a removable hard drive as the cows walked. Each video was then analyzed using our video analytics system that combines a deep-learning module with domain knowledge to localize the cow and the location of key body points on the dairy cow, despite challenges such as occluding fences and a distracting background. From this, features were automatically extracted about the shape of the cow, including side-view body length, diagonal length, height, and area as well as front-view body width and area. The prediction model is trained with videos from 4 d and evaluated on clips from d 5. We repeat this process to train 5 different models and test each with unseen data. Three regression models were applied to estimate bodyweight of dairy cattle from these features and compared: linear regression, support vector regression with radial basis function kernel, and a random forest (RF) regressor. The RF performed best when applied to videos captured within the constraints of an operating dairy farm on 5 distinct days, demonstrating a root mean squared prediction error of 34 kg, representing less than 6% error. A model with only the front view performs better than the one with only the side view; combining both views and adding age as a feature achieves the best performance.

Key Words: video analytics, body weight, automation

P300 How Brown Swiss milk production and quality can be affected by heat waves.

A. Maggiolino*[1], V. Landi[1], N. Bartolomeo[2], U. Bernabucci[3], A. Rossoni[4], and P. De Palo[1], [1]*Department of Veterinary Medicine, Bari, Italy,* [2]*Medical Statistics, Department of Biomedical Science and Human Oncology, Bari, Italy,* [3]*Department of Agriculture and Forest Sciences, Viterbo, Italy,* [4]*Italian Brown Breeders Association, Bussolengo, Italy.*

The temperature-humidity index (THI) is effective in estimating effects of the environment on dairy cows' efficiency and welfare. Heat waves (HW) have been entered as concept of long duration of warm environmental climatic conditions, able to affect dairy cows. The present study aims to estimate the effect of HW on the Italian Brown Swiss (IBS) population production efficiency. The definition of HW was adapted to the study's aims considering it as a period lasting from 2 to 5 consecutive days with a THI (minimum, mean and maximum) over the known threshold for the IBS population. The aim of this study was to estimate the effects of HW of different duration (from 2 to 5 d) in IBS, considering some production traits: fat-corrected milk (FCM) yield, protein and fat yield, protein and fat concentration. Ten years data from test-day record system at national level were considered and merged with data from 76 weather stations. The data set was subdivided according to parity in 4 categories: first, second, third, and ≥fourth parity. A mixed-effects model for repeated measures was applied to evaluate the effects of HW on each production trait. Month, parity class and DIM class were considered as fixed effects, while herd and year were considered as random effects. When recorded THI exceeded the maximum THI threshold, cows yielded from 32g (2 d) to 40 g (5 d) of daily protein less ($P < 0.0001$). When THI exceeded the minimum THI threshold, cows produced from 30g (2 d) to 32 g (5 d) less ($P < 0.0001$) of daily protein yield. The FCM yield was lower in cows subjected to HW over the maximum THI, worsening in relation to the HW duration (from 554g for a duration of 2 d to 850g at 5 d lasting HW; $P < 0.0001$). It is clear how HW affect efficiency in this breed, although it is considered more tolerant than others. Moreover, although from a physiological perspective minimum and maximum THI values have a different meaning, both showed to be equally effective in estimating how HW affect milk production traits in IBS.

Key Words: heat stress, heat waves, Brown Swiss

P301 A bi-seasonal evaluation of somatic cell count, production measures, hygiene scores, and bedding cultures of Holstein cows housed in a compost-bedded pack barn.

G. Mould, M. Hollis, and J. Carter*, *Middle Tennessee State University, Murfreesboro, TN.*

Cows housed on compost-bedded pack barns are known to exhibit reduced somatic cell counts (SCC) compared with other types of housing. This study aimed to evaluate the relationship between SCC (transformed to somatic cell score (SCS)), milk yield (MY), conductivity (COND), activity behaviors (avg. activity (AA), avg. rest bout (RB), and avg. rest time (RT)), milk and bedding bacterial cultures (BC), and hygiene scores (HS) of cows. Holstein cows ($n = 18$) were monitored for 28 d during fall and spring seasons. Cows were sorted into high (H) and low (L) groups based on SCC from the most recent DHIA herd report of each season. Weekly milk samples were collected as a sterile composite from all 4 quarters and measured for SCC using the DeLeval Cell Counter. Milk samples testing > 250,000 cells/mL were cultured using a Tri-plate agar of Factor, MacConkey, and Focus media (University of Minnesota Easy Culture) for identification of *Staphylococcus* species, *Streptococcus* species, or gram-negative bacteria. MY, COND, AA, RB, and RT were measured daily using the Afimilk parlor system and averaged by week. Cows were scored weekly for udder cleanliness (1 = very clean to 4 = very dirty; Cook, 2002) to assess exposure to pathogens. Bedding samples were taken at the beginning and end of the project and analyzed for bacterial counts. Statistical analysis of SCS, MY, COND, AA, RB, and RT with main effects of season and treatment group were conducted using the MIXED procedure in SAS (v9.4). Analysis of BC and HS incidence were evaluated using the FREQ procedure in SAS (v9.4). High cows had lower MY ($P = 0.0001$), higher SCS ($P = 0.0003$) and lower AA ($P < 0.0001$) than L cows. Seasonally, cows exhibited lower MY in the fall ($P < 0.0001$) and higher SCS ($P = 0.0003$), AA ($P < 0.0001$) and RB ($P < 0.0001$). There were no significant differences in HS. Cows in the H group were more frequently cultured (52.0% vs. 10.7%). These results insinuate SCS and season contribute to cow productivity and udder health.

Key Words: SCC, hygiene, compost-bedded pack barn

P302 Assessing the response of ruminal bacterial and fungal microbiota to whole-rumen contents exchange in dairy cows.

M. S. Cox*[1,2], C. L. DeBlois[1], and G. Suen[1,2], [1]*Department of Bacteriology, University of Wisconsin–Madison, Madison, WI,* [2]*Microbiology Doctoral Training Program, University of Wisconsin–Madison, Madison, WI.*

With the advent of advanced methods for characterizing microbial communities, efforts are underway to improve milk production efficiency (MPE) by manipulating the rumen microbiome. Our previous work demonstrated that a total exchange of whole-rumen contents between pairs of lactating Holstein dairy cows of disparate MPE resulted in a reversal of MPE status for ~10 d. This was accompanied by a ~10-d persistence of the introduced bacterial community in the recipient rumen. However, this work did not include in-depth analysis of the microbial community response or interrogation of specific taxa correlating to production metrics. In this work, we sought to better understand the response of rumen communities to this exchange protocol, including consideration of the rumen fungi. Rumen samples were collected from 8 d before 56 d following the exchange and were subjected to 16S rRNA and ITS amplicon sequencing to assess bacterial and fungal community composition, respectively. Our results show that the rumen fungal community did not differ between hosts of disparate efficiency before the exchange (permutational multivariate ANOVA, $P > 0.05$), and no change in fungal community structure was observed over the time course (permutational multivariate ANOVA, $P > 0.05$). Rumen bacterial communities lost correlation network connectivity early in the exchange (Kruskall-Wallis, $P < 0.05$) and were able to recover in high-efficiency cows, but not in low-efficiency cows, suggesting lower resilience and elasticity in bacterial communities associated with lower efficiency. Spearman correlations of microbial taxa to production metrics identified one fungal operational taxonomic unit (OTU) in the genus Neocallimastix that correlated positively to energy-corrected milk production ($P = 0.025$) and MPE ($P < 0.001$). Within the highly abundant rumen fluid associated genus *Prevotella*, Prevotella_1 was found to be more abundant in high-efficiency cows and Prevotella_7 more abundant in low-efficiency cows (Kruskall-Wallis, $P < 0.05$), though phenotypic differences between these taxa are not known. Overall, our results suggest that the rumen bacterial community is a primary microbial driver of MPE, that ruminal fungal community variation may not significantly contribute to variation in MPE, and that more work is needed to better understand the functional roles of specific ruminal community members in modulating MPE.

Key Words: microbiota, rumen contents exchange, milk prodution efficiency

P303 Fatty acid composition changes with stage of lactation and subclinical mastitis.

S. C. Allen*[1], D. M. Barbano[2], B. E. Faulkner[1], D. H. Poole[1], M. A. Drake[1], J. Odle[1], and S. H. Ward[1], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

The objectives of this study were to evaluate the change in milk fatty acid (FA) composition throughout lactation and during periods of elevated somatic cell count (SCC) in Holstein (HO) and Jersey (JE) cows. Data were collected from April 2019 to March 2020. Cows were classified by stage of lactation: transition (0 to 14 d in milk (DIM), early (15 to 120 DIM), mid (121 to 140 DIM), and late lactation (>140 DIM). Milk samples were collected weekly and analyzed for FA composition (de novo, DNFA; mixed origin, MOFA; and preformed, PRFA). Cows were classified as having subclinical mastitis (SCM, SCC >200,000 cells/mL) or normal milk (NORM, SCC <200,000 cells/mL) at each milk sampling. Data were analyzed using PROC MIXED. Cow classification and DIM were considered independent variables, and all other variables were dependent. Significance was declared when $P < 0.05$. Both JE and HO cows increased milk production between the transition period and early lactation, and milk yield decreased as lactation progressed. Fat decreased after the transition period and subsequently increased throughout lactation in HO cows. Jersey cows had similar fat percent between early and mid lactation that declined throughout lactation. Fat yield followed the trend of milk yield in both breeds. Proportion of DNFA increased between the transition period and early lactation while proportion of PRFA decreased among both breeds (4.13 and 4.70% increase in g/100 g FA for HO and JE cows, respectively). Milk yield was reduced during SCM for both breeds (2.38 and 3.19 kg/d reduction for HO and JE cows, respectively), however milk fat percent was unaffected in HO cows and increased in JE cows during SCM. Milk from HO NORM cows tended to be greater in MOFA (g/100 g fat) compared with SCM cows. No other FA variables were affected by classification of HO cows. Both DNFA and MOFA (g/100 g milk) were greater in JE SCM cows compared with NORM cows and PRFA (g/100 g FA) was greater in JE NORM cows compared with SCM. Fatty Acid content may be indicative of energy status of cows, particularly in the transition period, and could be used to make targeted management decisions.

Key Words: de novo fatty acids, somatic cell count, mastitis

P304 The impact of season on daily rhythms of body temperature and milk synthesis.

K. Kamau*[1], R. Bomberger[2], K. Harvatine[2], and I. Salfer[1], [1]*University of Minnesota, St. Paul, MN,* [2]*The Pennsylvania State University, University Park, PA.*

Dairy cows, like other animals, possess both seasonal and circadian rhythms that coordinate behavior and physiology with the external environment. Evidence from dairy farms suggests that the circadian rhythm of body temperature and cow behavior differs among seasons. The objective of the experiment was to examine the impact of season on daily rhythms of body temperature and milk synthesis in dairy cows. We hypothesize that the rhythm of body temperature and the morning to evening ratio of milk yield will differ by season with the greatest amplitude occurring in the summer and lowest amplitude occurring in the winter. The experiment utilized 147 lactating Holstein cows in a randomized block design with data collected in 3 1-wk blocks at the beginning of each of the 4 seasons (n = 30 to 44 cows/season). Body temperature was collected every 10 min using indwelling vaginal temperature probes and activity. Milk yield was collected at 0500 and 1700 h, and the ratio of AM to PM milk yield and components was used as a proxy for the daily rhythm of milk synthesis. Daily rhythms of body temperature and activity were determined by fitting data to the linear form of cosine functions with a 24 h period in R ver. 4.0.2. A zero-amplitude test was used to determine the fit of daily rhythms and cosine rhythmometry was used to compare daily rhythms among seasons, and to characterize annual rhythms of milk yield and components. Responses were compared by using a mixed-effects model with the fixed effect of season and the random effect of block nested within season using the *nlme* package of R. Body temperature fit a rhythm in fall, spring, and summer but not winter. Spring had a lower amplitude rhythm than fall and summer ($P < 0.05$). The peak of the daily rhythm in summer (0025) occurred 2 and 4 h later than spring and fall, respectively ($P < 0.05$). Season tended to affect the AM:PM ratio of milk yield ($P = 0.09$). Season did not affect the AM:PM ratio of fat and protein yield or concentration ($P > 0.10$). Results suggest that season impacts daily rhythms of body temperature but has minimal effects on daily rhythms of milk yield.

Key Words: seasonality, daily rhythms, body temperature

P305 Effects of dietary butyrate supplementation and oral nonsteroidal anti-inflammatory drug administration on performance, inflammation, and metabolites in transition dairy cows.

L. E. Engelking*, D. J. Ambrose, and M. Oba, *University of Alberta, Edmonton, AB, Canada.*

The objective was to evaluate the effects of dietary butyrate supplementation and oral nonsteroidal anti-inflammatory drug (NSAID) administration on milk production, feed intake, serum inflammatory markers, and plasma metabolites in transition cows. Eighty-three Holstein cows were blocked by parity and calving date and randomly assigned to isoenergetic diets containing calcium butyrate at 1.42% of diet dry matter (DM) or control supplement (1.04% palm fat and 0.38% calcium carbonate of diet DM). The close-up diet contained 13.5% starch and 43.0% neutral detergent fiber, and the postpartum diet contained 22.4% starch and 34.6% neutral detergent fiber on a DM basis. Diets were fed from 28 d before expected calving to 24 d after calving. Cows received an oral NSAID (1 mL/15 kg bodyweight Meloxicam in carrier solution) or a placebo (1 mL/15 kg bodyweight food dye in carrier solution) at 12 to 24 h after calving. Butyrate supplementation did not affect milk yield ($P = 0.86$) or serum amyloid A ($P = 0.45$) and haptoglobin concentrations ($P = 0.67$). Similarly, NSAID administration did not affect milk yield ($P = 0.39$) or serum amyloid A ($P = 0.49$) and haptoglobin concentrations ($P = 0.85$). Cows fed butyrate tended to have lower milk crude protein yield compared with cows fed control diet (1.21 vs. 1.27 kg/d; $P = 0.06$). Cows fed butyrate also had higher β-hydroxybutyrate (8.30 vs. 6.81 mg/dL; $P = 0.03$) and tended to have higher plasma free fatty acids (524 vs. 365 μEq/L; $P = 0.06$) at 4 d before calving, but tended to have lower plasma free fatty acids (825 vs. 993 μEq/L; $P = 0.07$) on d 7 after calving compared with cows fed the control diet. A parity by treatment interaction was observed for DM intake ($P = 0.03$), where DM intake was lower for the NSAID treatment compared with the placebo (16.7 vs. 19.2 kg/d; $P = 0.02$) in multiparous cows fed the control diet. These results suggest dietary butyrate supplementation and oral NSAID administration may not have overall positive effects on production performance of transition dairy cows.

Key Words: inflammation, metabolites, transition cow

P306 Dairy producer attitudes towards calf rearing. E. R. Russell*, M. A. G. von Keyserlingk, and D. M. Weary, *The University of British Columbia, Vancouver, BC, Canada.*

Rearing replacement stock is integral to dairy farming. The calf rearing phase, from birth to weaning, has received considerable attention with much of the biological research focusing on housing and feeding, including the best practices to wean calves. Despite this body of research, there is tremendous variation in calf management practices. Little research has examined how dairy producers view calf rearing. Therefore, the aim of this study was to investigate the attitudes and perceptions of Western Canadian dairy producers toward calf rearing, with a focus on calf weaning and the characterization of weaning success. Eighteen participants were interviewed from British Columbia, Manitoba, and Alberta; participants were identified as the farm manager or owner (n = 15) and primary calf caretaker (n = 3). Interviews were semi-structured and conducted between July and September 2020. Participants were asked to describe their calf rearing and weaning practices, and what they viewed as successes and challenges in rearing and weaning calves. Interviews were recorded, transcribed and underwent a thematic analysis that resulted in a codebook and the identification of major themes. Four major themes were identified: (1) reliance on calf-based factors (e.g., health, growth and behavior); (2) personal beliefs and vested interest (e.g., ease, consistency and habit); (3) perceived environmental influences (e.g., facilities, environment and equipment); (4) characterization of weaning success. These results provide insight into how dairy producers view calf rearing and weaning; and can be used to design future applied research and extension projects to better address producer perceptions, beliefs, and concerns.

Key Words: calf rearing, weaning, producer views

P307 Adoption of precision technologies by Brazilian dairy farms: The farmer's perception. R. R. Silvi[1], L. G. R. Pereira*[2,5], C. A. V. Paiva[2], T. R. Tomich[2], V. T. Amorim[3], S. G. Coelho[3], J. P. Sacramento[4], M. M. Campos[2], F. S. Machado[2], R. E. P. Ferreira[5], and J. R. R. Dorea[5], [1]*Universidade Estadual de Santa Cruz, Ilhéus, BA, Brazil,* [2]*Brazilian Agricultural Research Corporation–Embrapa Dairy Cattle, Juiz de Fora, MG, Brazil,* [3]*Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [4]*Universidade Federal de São João Del Rei, São João Del Rei, MG, Brazil,* [5]*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI.*

The use of precision farming technologies, such as milking robots, automated calf feeders, wearable sensors, and others, has significantly increased in dairy operations over the last few years. The growing interest in farming technologies to reduce labor, maximize productivity, and increase profitability is becoming noticeable in several countries, including Brazil. Information regarding technology adoption, perception, and effectiveness in dairy farms could shed light on challenges that need to be addressed by scientific research and extension programs. The objective of this study was to characterize Brazilian dairy farms based on technology usage. Factors as willingness to invest in precision technologies, adoption of sensor systems, farmer profile, farm characteristics, and production indexes were investigated in 378 dairy farms, located in Brazil. A survey with 22 questions was developed and distributed via Google Forms, from July 2018 to July 2020. The most productive dairy farms were located in the south and southeast of the country. The most frequent technologies adopted by producers were automatic weight and milk flow systems (31.7%), smart gate (14.5%); sensor systems to detect mastitis (8.4%), cow activity (7.1%), and body temperature (7.9%). Producers indicated "available technical support" (mean; σ^2) (4.55; 0.80) as the most important criterion, followed by "return on investment" (4.48; 0.80), "user-friendly" (4.39; 0.88), "upfront investment cost" (4.36; 0.81) and "compatibility with the farm, management software" (4.2; 1,02). The most important factors precluding investment in precision dairy technologies were: the need for investment in other sectors of the farm (36%), uncertainty of return on investment (24%), and lack of integration with other farm systems and software (11%). Farmers indicated that the most useful technologies were: automatic weight and milk flow systems (mean; σ^2) (4.05; 1.66); sensor systems for mastitis detection (4.00; 1.57); automatic feeding systems (3.50; 2.05); cow activity (3.45; 1.95), and in-line milk analyzers (3.45; 1.95). Overall, the concerns related to data integration, return on investment and user-friendly technologies are similar to other dairy farms located in other countries. Increasing available technical support for sensing technology can potentially have a positive impact on technology adoption.

Key Words: sensors, smart farm, survey

P308 Effect of propylene glycol and vitamin B_{12} treatment on concurrent subclinical hyperketonemia and hypoglycemia in early postpartum dairy cows. A. Hubner*[1], I. F. Canisso[1], P. M. Peixoto[1], W. M. Coelho Jr.[1], L. Ribeiro[1], B. M. Aldridge[1], and F. S. Lima[2], [1]*Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL,* [2]*Department of Population Health and Reproduction, University of California, Davis, Davis, CA.*

Cow-side β-hydroxybutyrate (BHB) testing is a convenient way to screen early postpartum cows for subclinical hyperketonemia (HK). However, the large proportion of cows needing treatment is a roadblock for widespread implementation of this diagnosis strategy. Potential integration of concurrent metabolic cow-side testing could target treatment to cows with the largest benefits from treatment and improve cow-side diagnosis and treatment implementation in dairy farms. Glucose is a relevant metabolite altered during the early postpartum period that can be tested using a HK cow-side device. We hypothesized that cows with concurrent HK (BHB ≥1.2 mmol/L) and hypoglycemia (HG, glucose ≤2.2 mmol/L) would have less postpartum disease and greater milk production than cows with HK or HG after treatment. Cows (n = 2,651) from 5 commercial dairies were tested between 3 and 9 d in milk using a previously validated hand-held device (Precision Xtra, Abbott). Cows were categorized into 3 groups: HK only (HK = 230), HG only (HG = 171), and concurrent HK and HG (HKHG = 201). Cows in each group were randomly assigned to receive 10,000 μg Vitamin B12 on d 1 + 5 d of 300 mL oral propylene glycol or to remain untreated. Statistical analysis was performed with JMP Pro 15 (SAS Institute Inc.). There were no differences ($P > 0.05$) between treatment and control for the 3 metabolic groups for cows diagnosed with LDA, mastitis, and metritis.

Treatment reduced [FSdL1] ($P = 0.04$) the proportion of cows subsequently diagnosed with clinical ketosis in the HG group (HG-Treat, 2.6 ± 2.4%; HG-control 11.8 ± 2.3%). [FSdL2] Energy-corrected milk (ECM) for the first 3 DHIA tests was increased [FSdL3] ($P = 0.02$) by treatment in the HK group (HK-treat, 45.4 ± 0.9 kg; HK-control, 42.4 ± 1.0 kg) but not within the other 2 groups. In conclusion, treatment reduced clinical ketosis in HG cows and increased ECM in HK, but no benefits of treatment were found for cows with concurrent HK and HG.

Key Words: hyperketonemia, hypoglycemia, propylene glycol

P309 Association of metabolic status with postpartum health disorders diagnosed by farm personnel.

A. Hubner*[1], I. F. Canisso[1], P. M. Peixoto[1], W. M. Coelho Jr.[1], L. Ribeiro[1], B. M. Aldridge[1], and F. S. Lima[2], [1]*Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL,* [2]*Department of Population Health and Reproduction, University of California, Davis, Davis, CA.*

We hypothesized that cows with concurrent hyperketonemia (HK) and hypoglycemia (HG) would have greater incidence diseases diagnosed by farm personnel than cows without concurrent HK and HG. Cows (n = 2,651) had blood samples collected between 3 and 9 d postpartum (DPP) and whole-blood β-hydroxybutyrate (BHB) and glucose were measured using a cow-side device validated in dairy cows (Precision Xtra, Abbott). Hyperketonemia was defined as BHB ≥1.2 mmol/L and hypoglycemia was defined as glucose ≤2.2 mmol/L. Cows were categorized into 4 groups: no HK and HG (Norm, n = 1996), HK only (HK, n = 260), HG only (HG, n = 181), and concurrent HK and HG (HKHG, n = 214). Statistical analysis was carried out using JMP Pro 15 (SAS Institute Inc.), and the on-farm recorded health events clinical ketosis (CK), clinical metritis (CM), left displaced abomasum (DA), mastitis (MAST), culled (sold + dead), and any disease event (AD), which was a combination of all individual disease events, were evaluated. Cows in the HK group were more likely ($P = 0.02$) to be diagnosed with any disease when compared with cows in the Norm group (HK, 57.8 ± 3.6%, HKHG, 56.4 ± 3.9%, HG, 50.9 ± 4.3%, Norm 43.7 ± 1.6%), Cows in the HK and HKHG were more likely ($P < 0.03$) to be diagnosed with CK than cows in the Norm group (HK, 9.9 ± 1.5%, HKHG, 15.2 ± 1.6%, HG, 7.5 ± 1.8%, Norm 2.3 ± 0.6%), cows in the HKHG group were more likely ($P < 0.03$) to be diagnosed with LDA than cows in the HK or Norm group (HK, 4.3 ± 1.0%, HKHG, 7.8 ± 1.1%, HG, 5.0 ± 1.2%, Norm 0.7 ± 0.4%), cows in the HK group were more likely ($P < 0.05$) to be culled than cows in any of the other 3 groups (HK, 15.8 ± 2.0%, HKHG, 11.2 ± 2.2%, HG, 9.9 ± 2.3%, Norm 6.1 ± 1.0%). The incidence of CM was greater ($P < 0.02$) in HK than the other groups (HK, 27.6 ± 2.4%, HKHG, 17.7 ± 2.6%, HG, 5.6 ± 2.9%, Norm 10.8 ± 1.1%). Cows in the HKHG had a lower incidence ($P = 0.01$) of MAST than Norm cows (HK, 21.6 ± 3.0%, HKHG, 17.2 ± 3.2%, HG, 26.7 ± 3.6%, Norm 21.3 ± 1.3%). In conclusion cows with HKHG were more likely to be diagnosed with LDA compared with HK, however the HKHG cows were less likely than HK cows to be diagnosed with metritis.

Key Words: hyperketonemia, hypoglycemia, postpartum health

P310 Economics of fixed-timed artificial insemination with or without sexed semen in a high-producing, pasture-based dairy production system in Ireland.

D. P. Walsh*, P. Lonergan, A. G. Fahey, and M. Wallace, *School of Agriculture and Food Science, University College Dublin, Dublin, Ireland.*

Fixed-timed AI (FTAI) can increase the pregnancy rate per AI (P/AI) and improve the calving profile in seasonal pasture-based systems. Sexed semen has the potential to increase genetic gain of dairy herds through increased selection intensity on the dam side. However, the reduced P/AI of sexed to unsorted semen increases the costs associated with breeding and culling. FTAI can be employed as a complementary technology to sexed semen use by inducing ovulation in a large proportion of cows, and thus optimizing the P/AI. A stochastic simulation model was used to evaluate the impacts of FTAI on the distribution of economic profit including genetic gain. A 2 × 2 scenario design evaluated FTAI with either conventional unsorted (TCONV) or sexed (TSEX) semen and treatment applied to heifers only (H) or both heifers and cows (HC). The scenarios were compared with a baseline (BASE) in which heifers and cows were inseminated with unsorted semen after estrus detection. The mean (±SD) profit advantage (ΔPROFIT) over the baseline for TCONV-H, TCONV-HC, TSEX-H and TSEX-HC scenarios were €5.81/cow ± 5.76, €35.84/cow ± 19.28, €15.07/cow ± 8.29 and €63.25/cow ± 40.91, respectively. Combined application of both technologies has been shown to return a greater ΔPROFIT on average compared with that achievable from FTAI alone. However, the risk of not returning a ΔPROFIT varied across the scenarios with higher risk in TCONV-H and TSEX-HC. Specifically, TCONV-H and TSEX-HC had respectively a 16% and 8% chance of not returning a positive ΔPROFIT. The range in ΔPROFIT was most sensitive to the 21-d P/AI followed by genetic gain, calf and cull cow prices. FTAI with unsorted or sexed semen can increase profitability of pasture-based, dairy production systems but risk factors such as lower P/AI mean that its adoption is most suitable in herds with good baseline fertility.

Key Words: fixed-timed artificial insemination, sexed semen, stochastic model.

P311 Edge processing approaches for behavior classification of grazing cows.

B. R. dos Reis*[1], D. Fuka[2], Z. Easton[2], and R. White[1], [1]*Department of Animal Science, Virginia Tech, Blacksburg, VA,* [2]*Department of Biological Systems Engineering, Virginia Tech, Blacksburg, VA.*

Reliable, remote, real-time behavioral monitoring for pasture-based production has potential to improve efficiency and precision of management without increasing labor costs or requiring changes to housing systems. In confinement operations, behavioral monitoring using inertial measurement unit (IMU) sensors has expanded; however, real-time behavioral classification using IMU sensors in pasture-based settings has been limited due the lack of networking access, low-power supplies and the price associated with acquiring these devices. In order for IMU sensors to be effective in pasture settings, the computational load for behavioral classification must be moved to the network edge to minimize the energetic cost of transmitting data from the cow to the farm hub. Our objective was to explore behavioral-classification techniques suitable for edge processing to identify grazing, walking, laying, and standing behaviors from an open source, low-cost wearable IMU sensor placed on extensively grazed cows. Ten Angus crossbred cows were fitted with sensors for 24 h. Visual behavior observations were used as ground truth and were recorded each minute. Sensor data were logged to local storage at 100 Hz. Simplistic edge processing techniques based on ranges, means, and linear associations were insufficient to reliably classify behaviors (<10% precision). A random forest algorithm was capable of distinguishing among behaviors with improved accuracy and precision for grazing (83% sensitivity, 92% specificity, 87% precision, 89% accuracy), laying (87% sensitivity, 91% specificity, 87% precision, 89% accuracy), standing (65% sensitivity, 92% specificity, 68% precision, 87% accuracy), and walking (58% sensitivity, 95% specificity, 9% precision, 96% accuracy). As microprocessors continue to advance and become more capable of running machine learning algorithms, edge processing using random forests or similar approaches may help improve the usability of IMU sensors for behavioral monitoring in extensive production systems.

Key Words: grazing cow, behavior detection, sensor

P312 Evaluation of a protocol for the treatment of metritis in a northwest Texas dairy herd.

J. A. Garcia Buitrago*[1], G. R. Hagevoort[1], E. R. Ellen[2], and E. A. Taylor[3], [1]*Department of Animal and Ranch Sciences, Dairy Extension Program, New Mexico State University, Clovis, NM,* [2]*Department of Animal Science, Texas A&M University, College Station, TX,* [3]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX.*

To evaluate a protocol to treat of metritis in a dairy herd in the Texas Panhandle, reproductive records of Holstein-Friesian cows calving in the spring and fall of 2017 were analyzed. Thirty-three cows (16 cows in spring and 17 in fall) were diagnosed with metritis in the first postpartum week and the they were treated with a protocol of 2 doses of ceftiofur crystalline-free acid (3.3 mL/ 100 kg BW): one on the day of the diagnosis, and another 72 h later. Each treated cow was assigned a control cow calved and examined on the same day. After a 56-d voluntary waiting period the cows were enrolled in an Ovsynch protocol and subsequently inseminated. All the service dates, pregnancy confirmation date, and results were registered. The conception rate for each group (CR), open days (OD) and the number of services per conception (SC) were calculated. Records were classified by group (GR): treated (T), control (C); calving season (CS): spring (S), fall (F); and lactation (LT): primiparous (P), multiparous (M). Two-sample *t*-tests were performed for OD and SC by each of the effects GR, CS and LT using the Minitab statistical software. Results showed CR of 78.8% for GR-T and 87.9% for GR-C. OD general mean was 101 ± 47 d, the OD means were 89 ± 32 and 114.2 ± 57 d for C and T; 105 ± 61 and 99 ± 40 d for M and P; 81 ± 20 and 118 ± 57 d for F and S groups respectively. SC general mean was 2.1 ± 1.3; SPC means were 1.9 and 2.4 for C and T; 2.2 and 2.1 for M and P; 1.6 and 2.5 for F and S groups respectively. Despite that Group's CR was greater for the C group the statistics results pointed that there is not enough evidence to conclude that the OD and SC mean differ by a significance level of 0.05 due to the effect of any of the variables considered in this study. These results suggest that the cows treated with this metritis protocol had a reproductive performance similar to that of the control group.

Key Words: metritis, open days, services per conception

P313 Assessing best herd management practices in commercial dairy farms using on-farm records.

D. Warner*, R. Lacroix, J. Gunn, and D. E. Santschi, *Lactanet, Sainte-Anne-de-Bellevue, QC, Canada.*

A major challenge for extension services involves the large variability among dairy herds and, thus, identifying the main factors explaining herd performance. The objective of this observational study was to examine the association of herd-level management factors and energy-corrected milk (ECM) production across 71 commercial Holstein herds in New Brunswick, Canada. Enrolled farms were equipped with a parlor (n = 31), pipeline (24) or automatic (16) milking system. Herd performance records from September to December 2020 were assembled from a DHI database and farm practices were collected through a survey completed by advisors. Herds were segmented based on annual ECM production using *k*-means clustering. Herd performance and farm practices were assessed for clusters using, respectively, a linear mixed model and a generalized linear mixed model approach with milking system as random effect. Multivariate analysis of clusters was conducted using decision tree induction with the aim to help with the decision-making. Three herd clusters differing in ECM ($P < 0.05$) were identified as low (8,374 ± 593 kg ECM per year; mean ± SD), medium (10,303 ± 573 kg) and high (12,263 ± 773 kg) producing cluster. The herd genetic potential for ECM did not differ ($P > 0.05$) among clusters. Decision tree induction allowed to highlight the importance for reproduction indicators, with the high performing cluster explained by a good transition (Transition Cow Index), lower age at first calving, lower BHB, lower turnover and lower calving interval. A preliminary analysis of the survey results suggested that certain management practices were associated with clusters. Particularly, the low performing cluster had a greater probability of an annual trimming frequency of ≤ 1 ($P < 0.05$) and of smooth alley surface ($P < 0.07$). These results suggest that management practices influencing performance can be identified to highlight specific opportunities for improvements.

Key Words: farm management, dairy herd improvement, decision support

P314 Characterization of metabolic profile of Holstein dairy cows diagnosed with concurrent hyperketonemia and hypoglycemia.

A. Hubner*[1], I. F. Canisso[1], P. M. Peixoto[1], W. M. Coelho Jr.[1], L. Ribeiro[1], B. M. Aldridge[1], P. Menta[2], V. S. Machado[2], and F. S. Lima[3], [1]*Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL,* [2]*Department of Veterinary Sciences, Texas Tech University, Lubbock, TX,* [3]*Department of Population Health and Reproduction, University of California, Davis, Davis, CA.*

The objective of this study was to characterize the metabolic profile of cows diagnosed with hyperketonemia (HK, BHB ≥1.2 mmol/L) and hypoglycemia (HG, glucose ≤2.2 mmol/L). Glucose and BHB concentrations in whole blood were assessed using a Precision Xtra device (Abbott) in lactating dairy cows (n = 1,102) between 3- and 9 d postpartum. Cows were categorized into 4 groups: no HK or HG (Norm = 500); HK only (HK = 230), HG only (HG = 171), concurrent HK and HG (HKHG = 201). Serum collected the day of cow-side diagnosis was evaluated for nonesterified fatty acids (NEFA), total calcium (tCa), magnesium (Mg), triglycerides (Tr) and urea (Ur)[FSdL1] using an automated chemistry analyzer (Randox Daytona). Statistical analysis was carried out using JMP 15 Pro. Previously established thresholds were used to define elevated NEFA (NEFA ≥0.7mmol/L) and hypocalcemia (tCa ≤2.14 mmol/L). For Mg, Ur, and Tr, receiver operator characteristic (ROC) curve analysis were used to determining the optimal threshold to detect on-farm recorded disease for the first 100 d in milk (Mg ≤0.76; Ur ≤4.77; Tr ≥0.145). Using these thresholds prevalence of each metabolic aberration was evaluated according to cow-side status. Prevalence of hypocalcemia was greater ($P < 0.01$) in HK and HKHG than HG and Norm (HK, 35.2 ± 3.7%; HKHG, 35.3 ± 4.3%; HG, 13.5 ± 4.3%; Norm 14.4 ± 3.4%), [FSdL2] prevalence of elevated NEFA was greater ($P < 0.02$) in HKHG than HG and Norm (HK, 50.0 ± 4.7%; HKHG, 56.2 ± 5.4%; HG, 42.1 ± 5.5%; Norm 46.8 ± 4.3%), prevalence of hypomagnesemia was greater ($P < 0.01$) among HK than the other 3 groups (HK, 46.5 ± 4.3%; HKHG, 33.3 ± 4.9%; HG, 25.2 ± 5.0%; Norm 25.8 ± 3.9%), prevalence of decreased urea was greater ($P < 0.01$) among Norm cows than HG cows (HK, 68.7 ± 4.4%; HKHG, 66.7 ± 5.0%; HG, 56.7 ± 5.1%; Norm 69.8 ± 4.0%), and prevalence of increased triglycerides was not different ($P > 0.05$) among groups. Elevated HK was associated with metabolic abnormalities, but concurrent HK and

HG had no additional effects to metabolic disturbances and cows with hypoglycemia had similar metabolic profiles as healthy cows.

Key Words: hyperketonemia, hypoglycemia, metabolites

P315 Effects of an acidified diet prepartum on performance of Holstein cows and validation of 2 pH strips to measure urine pH.

L. K. Fehlberg*[1], A. Pineda[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*University of Guelph, Guelph, ON, Canada.*

There is a need for an inexpensive and accurate method to measure urine pH during the prepartum period to assess the extent of metabolic acidosis achieved and determine the subsequent effect on performance. The study aimed to determine the accuracy of Fisher pH sticks (pHF; Thermo Fischer Scientific) and pHion balance test strip (pHI; pHion Balance) compared with a portable pH meter (pHP; Accumet AP115, Thermo Fisher Scientific) in measuring urine pH (UpH) and the association of UpH on pre- and postpartum dry matter intake (DMI) and yields of milk and milk components. Cows consumed a total mixed ration with a DCAD of −118 mEq/kg for 4 wk prepartum and 397 mEq/kg for 4 wk postpartum. Prepartum UpH measurements for each cow were averaged and utilized to classify cows as Low (mean ± standard deviation; UpH ≤ 5.54; 5.44 ± 0.07), Med ($5.54 > \text{UpH} \leq 5.90$; 5.67 ± 0.09), or High ($\text{UpH} > 5.90$; 6.42 ± 0.36). Milk yield and DMI were recorded daily and averaged weekly. Cows were milked 2 × per d and milk samples were taken on d 7, 14, and 28. Bland-Altman plots and Lin's concordance correlation coefficient (CCC) were used to assess the agreement between pHP and pHF and pHI. Receiver operating characteristic curves were used to determine the threshold with pHF and pHI that best discriminated between UpH >6.0 and ≤6.0 compared with pHP and area under the curve (AUC) assessed the accuracy. At UpH threshold of 5.75 for pHF and pHI, the sensitivity, specificity, and AUC were 89.5 and 87.4, 99.1 and 97.0, and 0.94 and 0.92, respectively. The CCC was 0.93 for pHF and pHI, indicating near-perfect agreement with pHP. The UpH did not affect pre- nor postpartum DMI ($P = 0.14$). Tendencies for UpH × wk interaction for milk yield and total solids content showed decreased milk yield for Low and Med UpH cows and greater total solids content for Low UpH cows during wk 1 postpartum ($P < 0.10$). In conclusion, the pHI and pHF are accurate methods for measuring UpH. Additionally, when fed an acidified diet cows' UpH was not associated with pre- nor pospartum DMI, but when UpH was Low or Med, milk yield decreased at wk 1 postpartum.

Key Words: DCAD, pH, urine

P316 Increased calving rate reduced pre- and postpartum dry matter intake and milk yield in Holstein dairy cows.

G. M. Schuenemann*[1], B. T. Menichetti[1], and J. Fontana[2], [1]*Department of Veterinary Preventive Medicine, The Ohio State University, Columbus, OH,* [2]*Departamento de Zootecnia y Granja, Universidad Nacional de Cuyo, Mendoza, Argentina.*

The objective was to assess the association of calving rate (CR) with stocking density (SD) of pre- and postpartum pens, dry matter intake (DMI) 7 d before calving (dpp), postpartum DMI for the first 12 d in milk (DIM), and milk yield at 30 DIM (MILK) in Holstein dairy cows. A total of 3,561 animals (1,262 heifers and 2,299 multiparous) were assessed during 2 yr from a large dairy herd in Argentina. Transition heifers and cows were housed in deep bedded compost barns. Prepartum heifers and cows were comingled and exposed to an acidogenic diet for 25 ± 3 dpp. Postpartum cows were comingled for the first 25 ± 3 DIM. Linear feed bunk space was maintained at 80 ± 9 cm per cow. The mean SD was computed as the number of cows within 7 dpp or first 12 DIM. The mean DMI was computed by subtracting the total offered minus residual feed per day and then divided by the number of cows within pens once calving date was known. PROC CORR or MIXED procedures of SAS were used to assess the association of CR with SD, DMI, and MILK. Pen was the experimental unit for DMI. Correlations were adjusted by parity, season, and year. The mean CR was 23 births per week (rage: 6 to 45). The mean pre- and postpartum SD was 65 (range: 6 to 116) and 86 cows (range: 45 to 133), respectively. The mean DMI was 14 (range: 8 to 21) and 19.9 (range: 15 to 26) kg/d for pre- and postpartum cows, respectively. Mean MILK was 35.9 kg/d for primiparous and 51.8 kg/d for multiparous cows. Parity, season, and year were associated ($P < 0.05$) with CR, pre- and postpartum DMI, and MILK. Calving rate was associated with pre- ($r = 0.30$) and postpartum SD ($r = 0.38$; $P < 0.001$). Prepartum SD had a negative association with DMI within 7 dpp ($r = -0.45$; $P < 0.001$). Prepartum DMI had a positive association with postpartum DMI within the first 12 DIM ($r = 0.30$; $P < 0.001$), which was associated with milk yield ($r = 0.25$; $P < 0.001$). The combination of CR, DMI prepartum, and postpartum SD explained at least 3.2 k/d and 2.4 kg/d of the observed variation in postpartum DMI and MILK. These findings provide evidence that flow of cows determined by CR affected pre- and postpartum SD, which in turn reduced postpartum DMI and MILK.

Key Words: calving rate, dry matter intake, dairy cattle

Reproduction: Posters

P317 Association of cyclicity and estrus alerts using activity monitors in dairy cows. T. A. Burnett*, A. M. L. Madureira, A. B. Montevecchio, and J. Bauer, *The University of British Columbia, Vancouver, BC, Canada.*

The aim of this study was to determine the association between estrus alerts using an automated activity monitor (AAM) and ovarian cyclicity of lactating dairy cows. Cows (n = 62) were equipped with a leg-mounted AAM to detect the occurrence of estrus alerts. Cows were assessed using ultrasonography at the time of AAM alert and 7 d post-alert. True alerts were those with a dominant follicle > 15 mm and the absence of a large corpus luteum (CL; > 25 mm). Ovulation was verified if a new CL was recorded 7 d post-alert. Alerts were grouped as TOT (total alerts, both false and positive), TR (true positive alerts) and TRO (true positive alerts resulting in ovulation). Cyclicity was determined through weekly milk samples, collected from calving until 60 DIM, analyzed for progesterone (P4) using an ELISA kit. Cows were considered cycling when milk P4 was > 5 ng/mL for the first time. Milk production was measured as 305-d mature-equivalent yields. Pearson correlations and interclass correlation coefficient (ICC) were used to correlate the timing of cyclicity and estrus alerts, while differences between groups were tested using multivariable linear regression using SAS. The time of cyclicity was weakly correlated with the time of first TR alerts (ICC = 0.22; r = 0.25, $P < 0.05$) and first TRO alerts (ICC = 0.29; r = 0.35, $P < 0.01$), but not with first TOT alerts (ICC = 0.08; r = 0.09; $P = 0.5$). As time to cyclicity increased, the number of TRO alerts decreased ($P < 0.05$); no association was found on the number of TOT or TR alerts. However, the number of ovulations based on P4 were correlated with the number of TOT alerts (r = 0.37; $P < 0.01$), TR alerts (r = 0.46; $P < 0.001$) and TRO alerts (r = 0.52; $P < 0.001$). Time to cyclicity was not associated with time to first TOT alert, but positively associated with time to first TR ($P = 0.05$) and TRO alert ($P = 0.01$). Timing and number of alerts were not associated with milk yield, but primiparous cows had TR and TRO alerts earlier in lactation and had more events than multiparous cows. In conclusion, true alerts, especially those resulting in ovulation, are associated with cyclicity, both in the time from calving and number of events. However, total alerts (both true and false) were not correlated with first ovulation, although the number of TOT alerts over the first 60 DIM were still moderately correlated with total ovulations. Thus, the reduction of false alerts could potentially increase the precision of AAM to detect cyclicity early postpartum.

Key Words: activity monitor, cyclicity

P318 Trace-mineral supplementation reduces early pregnancy losses on an in vitro embryo production program. R. V. Sala*[1], E. Peralta[2], L. Carrenho-Sala[1], M. Fosado[2], D. C. Pereira[3], and V. A. Absalón-Medina[1,4], [1]*STgenetics, South Charleston, OH,* [2]*STgenetics, Kewaskum, WI,* [3]*STgenetics, De Forest, WI,* [4]*The Ohio State University, Columbus, OH.*

The main objective of this study was to investigate the effects of injectable trace minerals on the probability of pregnancy and pregnancy losses on an in vitro embryo production (IVP) program. Additional objectives were to measure collateral effects from luteal tissue area, among others. A total of 885 heifers were randomly allocated in 2 groups: control vs. treatment with 10 mL of injectable trace minerals (Multimin) at the beginning of the synchronization protocol consisting of a modified 5-d CIDR Synch i.e., D-8 CIDR insertion, D-3 CIDR removal plus $PGF_{2\alpha}$ and D0 GnRH where heifers were assumed in estrus viz., d 0 of estrous cycle. Moreover, heifers were evaluated by ultrasonography on D5 to determine presence and size of the CL. On D7 ± 1-d, heifers received a fresh IVP embryo and pregnancy diagnosis was performed on D32 and D60, respectively. Data on P32, P60 and pregnancy losses was fitted into a nominal logistic model using JMP version 15 of SAS. CL-tissue area in mm^2, BCS (1–5 scale), recipient age in months, times bred, IETS-based embryo evaluation and days post-estrus at embryo transfer (ET) were also included in the model as covariates. Results showed that means between control vs. treatment were not different in terms of luteal tissue area (25.3 ± 0.55 mm^2 vs. 25.7 ± 0.54 mm^2), BCS (3.04 ± 0.02 vs. 3.06 ± 0.02), age (16.2 ± 0.15 mo vs. 16.3 ± 0.20 mo) nor times bred (0.6 ± 0.05 vs. 0.6 ± 0.05). Likewise, no significant differences were found in terms of probability of pregnancy at P32 and at P60 (48.7% vs. 47.7% and 40.8% vs. 41.9%; respectively). However, there was a significant interaction between embryo stage, days post-estrus at ET and treatment that resulted in less pregnancy losses on heifers receiving trace minerals (main effect: $P = 0.03$). Post hoc analysis indicated losses were different for stage 7 embryos transferred into recipients at d 7 post-estrus compared with other subgroups. To further examine the relationship of nominal data corresponding to stage 7 embryos transferred into d 7 post-estrus uteri, a contingency analysis was performed. Results showed pregnancy losses by the presumptive d 60 of gestation were indeed lower for the trace-mineral treated group (9.5% vs. 22.2%; Fisher's Exact Test $P = 0.04$). We, therefore, conclude that an injection of trace minerals at the beginning of an estrus synchronization protocol reduced pregnancy losses when compared with controls.

Key Words: minerals, early pregnancy loss, IVP embryos

P319 The relative relationship between type 1/type 2 immunity and fertility in Holstein cows. W. Sandberg[1], A. Ahamdzadeh*[1], P. Rezamand[1], and Q. Huo[2], [1]*University of Idaho, Moscow, ID,* [2]*University of Central Florida, Orlando, FL.*

Maternal immunity shifts from a type 1/type 2 balance to a type 2-biased immunity, and lack of such a shift may contribute to unsuccessful pregnancy in animals. There may be a shift in type 1/type 2 balance as animals enter the estrus phase of the estrous cycle and before AI, which could be a predictor of their fertility. The objective of the study was to examine the relationship between relative balance between type 1 and type 2 and fertility in lactating Holstein cows subjected to timed AI for the second time or greater. After the first AI, and upon non-pregnancy diagnosis (d 0 of the experiment), lactating cows eligible for a second or greater AI (n = 221) were enrolled in a 5-d CIDR-Cosynch protocol (mean DIM = 177). On d 8, cows received GnRH and AI, and blood samples were collected form all cows to be examined for type1/type 2 balance and progesterone (P4). D2Dx nanoparticle immunity assay, a rapid blood test, was used to detect type 1/type 2 immune balance by measuring the relative quantity ratio of immunoglobulin G_1 and G_2 subclasses (IgG_1/IgG_2). Pregnancy status was confirmed by ultrasonography between 40 to 45 d after AI. Data were analyzed by general linear model and logistic regression. Mean relative quantity of IgG_1/IgG_2 was not different between non-pregnant and pregnant cows. In addition, no relationship between probability of pregnancy to AI and IgG_1/IgG_2 relative quantity were detected under these experimental conditions. There was no correlation between serum P4 concentrations and IgG_1/IgG_2 ratio. Although IgG_1/IgG_2 could not predict the probability of pregnancy to second and greater AI, the relation between type 1/type 2 balance and fertility in dairy cows requires further investigation.

Key Words: type1-type 2 immunity, fertility, lactating cow

P320 Luteal regression, follicle growth, and time to ovulation in dairy heifers treated with different formulations of prostaglandin $F_{2\alpha}$

S. G. Umana Sedo*[1], C. C. Figueiredo[1], T. D. G. Rojas[1], G. A. Duarte[1], M. B. U. Ugarte Marin[1], C. A. Crawford[1], K. G. Pohler[3], R. C. Chebel[1], T. R. Bilby[2], and R. S. Bisinotto[1], [1]*University of Florida, Gainesville, FL,* [2]*Merck Animal Health, Madison, WI,* [3]*Texas A&M University, College Station, TX.*

Objectives were to evaluate the effect of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) treatments on CL regression, follicle growth, and time to ovulation. Holstein heifers were randomly allocated to receive 1 dose of cloprostenol (CLOx1; n = 48), 1 dose of dinoprost (DINx1; n = 47), 2 doses of cloprostenol 24 h apart (CLOx2; n = 45), or 2 doses of dinoprost 24 h apart (DINx2; n = 46) starting 7 d after a synchronized estrus. Ovaries were scanned every 12 h until estrus and every 6 h thereafter until ovulation. Blood samples were collected before treatment and every 6 h until ovulation for measurement of progesterone concentrations. Luteolysis was defined as progesterone concentration <0.5 ng/mL. Binary data were analyzed using logistic regression and continuous data using ANOVA. Proportion of heifers that underwent luteolysis (CLOx1 = 82.2 ± 5.7, CLOx2 = 100.0 ± 0.0, DINx1 = 58.1 ± 7.5, DINx2 = 95.5 ± 3.1%) and that ovulated (CLOx1 = 86.7 ± 5.1, CLOx2 = 100.0 ± 0.0, DINx1 = 55.8 ± 7.6, DINx2 = 93.2 ± 3.8%) were smaller for DINx1. Time to luteolysis was affected by type of $PGF_{2\alpha}$ (CLO = 23.4 ± 1.5 vs. DIN = 30.0 ± 1.7 h) and dose (x1 = 29.1 ± 1.8 vs. x2 = 24.2 ± 1.5 h), yet, an interaction between $PGF_{2\alpha}$ type and dose was not observed (CLOx1 = 24.3 ± 2.2, CLOx2 = 22.5 ± 2.1, DINx1 = 34.0 ± 2.7, DINx2 = 25.9 ± 2.0 h). Time to ovulation was not affected by treatment (CLOx1 = 69.4 ± 1.3, CLOx2 = 71.4 ± 1.3, DINx1 = 71.9 ± 1.7, DINx2 = 71.3 ± 1.3 h). Follicle diameter in the last 60 h before ovulation did not differ between CLOx1 and CLOx2 (16.2 ± 0.2 vs. 16.3 ± 0.1 mm) but it was greater for DINx1 compared with DINx2 (16.7 ± 0.2 vs. 16.3 ± 0.1 mm). Luteal area did not differ between CLOx1 and CLOx2 (193.3 ± 5.2 vs. 190.7 ± 5.3 mm^2) whereas DINx1 had larger area than DINx2 (221.5 ± 5.1 vs. 196.4 ± 5.3 mm^2). Progesterone concentrations in the first 72 h after treatment did not differ between CLOx1 and CLOx2 (0.88 ± 0.06 vs. 0.73 ± 0.07 ng/mL) but it was greater for DINx1 compared with DINx2 (1.16 ± 0.06 vs. 0.85 ± 0.06 ng/mL), which was largely explained by heifers that did not undergo luteolysis. A similar pattern was observed for CL blood flow. Treatment did not affect time to ovulation, but CL regression and ovulatory response were impaired in heifers receiving a single dose of dinoprost.

Key Words: luteolysis, ovulation, prostaglandin

P321 Association of estrous expression detected by an automated activity monitoring system within 40 days in milk and reproductive performance of lactating Holstein cows.

S. Borchardt*[1], C. Tippenhauer[1], J.-L. Plenio[2], A. Bartel[2], A. Madureira[3], R. Cerri[3], and W. Heuwieser[1], [1]*Clinic of Animal Reproduction, Free University Berlin, Berlin, Germany,* [2]*Institute for Veterinary Epidemiology and Biostatistics, Free University Berlin, Berlin, Germany,* [3]*Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC, Canada.*

The objective of this observational study was to evaluate the association of estrous expression within 40 d in milk using a neck-mounted automated activity monitor (AAM; Heatime Pro; SCR Engineers Ltd., Netanya, Israel) with estrous characteristics of the first postpartum AI and reproductive performance in lactating Holstein cows. A total of 2,077 cows from 5 commercial dairy farms were included in the statistical analyses. Cows were classified according to the number of estrus events from d 7 until d 40 postpartum into 3 categories: 1) no estrus event (ANESTRUS); 2) one estrus event (ESTRUS1) and 3) 2 or more estrus events (ESTRUS2+). Generalized linear mixed models were used to analyze continuous or categorical data. Shared frailty models were used for time to event data. Overall, 52.7% (1,095/2,077) of cows had no estrus event detected by an AAM system from d 7 until d 40 postpartum. Herd-level prevalence of ANESTRUS ranged from 37.5% to 58.4%. Estrous expression from d 7 until d 40 postpartum affected estrous duration ($P = 0.001$) at first postpartum AI. Cows in ANESTRUS had the shortest duration (13.2 ± 0.33 h) compared with cows in ESTRUS1 (13.8 h ± 0.36) and ESTRUS2+ (14.8 h ± 0.41). Cows in ESTRUS2+ had a longer estrous duration at first postpartum AI compared with cows in ESTRUS1. For ANESTRUS, ESTRUS1, and ESTRUS2+ cows pregnancy per AI was 29.4%, 30.9%, and 37.8%, respectively. Estrous expression from d 7 until d 40 postpartum affected time to first AI and time to pregnancy. Compared with ANESTRUS cows, cows in ESTRUS1 (Hazard risk (HR) = 1.74; 95% CI 1.55 - 1.94; $P = 0.001$) and ESTRUS2+ (HR = 1.77; 95% CI 1.52 - 2.05; $P = 0.001$) had an increased hazard of being inseminated within 100 DIM. Median DIM to first AI were 70, 59 and 58 for cows in ANESTRUS, ESTRUS1, and ESTRUS2+, respectively. Compared with ANESTRUS cows, cows in ESTRUS1 (HR = 1.28; 95% CI 1.13 - 1.46; $P = 0.001$) and ESTRUS2+ (HR = 1.33; 95% CI 1.12 - 1.57; $P = 0.001$) had an increased hazard of becoming pregnant within 200 DIM. Median DIM to pregnancy were 127, 112, and 103 for ANESTRUS cows, ESTRUS1 and ESTRUS2+, respectively. In conclusion, cows with no estrous expression from 7 to 40 DIM had reduced estrous expression at first AI and inferior reproductive performance compared with cows that displayed estrous activity.

Key Words: estrous expression, automated activity monitor, reproductive performance

P322 Effects of feeding rumen-protected lysine prepartum on placental immunometabolic gene expression of Holstein cows.

A. R. Guadagnin*[1], L. K. Fehlberg[1], B. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois, Department of Animal Sciences, Urbana, IL,* [2]*Ajinomoto Co. Inc., Tokyo, Japan.*

The maternal supply of lysine during late gestation results in a greater intake and improved health of their calves during the first 6 weeks of life. We hypothesize that these effects are possible due to changes in placental metabolism, which allow for improved passage of nutrients to the fetus and immunity. Therefore, we aimed to determine the effects of feeding rumen-protected lysine (RPL, AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL) prepartum (0.54% DM of TMR) on mRNA gene expression profiles of placental samples of Holstein cows. Sixty-six (n = 66) multiparous Holstein cows were randomly assigned to 1 of 2 dietary treatments, consisting of TMR top-dressed with RPL (PRE-L) or without (control, CON), fed from 27 ± 5 d prepartum until calving. After natural delivery (6.87 ± 3.32 h), 3 placentomes from each placenta were collected. A subset of each of the placentomes was combined and flash-frozen in liquid nitrogen, to evaluate the expression of genes related to protein metabolism and inflammation. After normalization with the geometric mean of the internal control genes, the triplicate averages of the qPCR data were $\log_2$ transformed to obtain a normal distribution of residuals. Data were analyzed using the MIXED procedure in SAS. The estimates were then $\log_2$ back-transformed to express the mRNA abundance. For ease of interpretation, the response in mRNA abundance in placental tissue of cows in PRE-L versus cows in CON is represented using fold-change values [(mRNA abundance at PRE-L – mRNA abundance at CON)/mRNA abundance at CON]. The mRNA expression of methionine adenosyltransferase 2A (*MAT2A*) was 7.9 times greater ($P < 0.01$) in the placenta of cows in PRE-L than in CON. There was no difference on the mRNA expression ($P = 0.80$) of Y+L amino acid transporter 1 (*SLC7A7*), interleukin 10 (*IL10*), interleukin-1β (*IL1B*), lysine-specific histone demethylase 1A (*KDM1A*), nor euchromatic histone-lysine N-methyl transferase 2 (*EHMT2*). In conclusion, it is likely that prepartum supply of RPL allows for a greater utilization of methionine, exemplified by the greater expression of *MAT2A* in the placenta of cows that were fed RPL prepartum.

Key Words: gene expression, lysine, placenta

P323 Associations between anogenital distance and measures of fertility in lactating North American Holstein cows: A validation study.

J. E. Carrelli*[1], M. Gobikrushanth[1,2], M. Corpron[3], W. Sandberg[3], A. Ahmadzadeh[3], M. Oba[1], and D. J. Ambrose[4,1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, SK, Canada,* [3]*Department of Animal, Veterinary and Food Sciences, University of Idaho, Moscow, ID,* [4]*Livestock and Crops Research Branch, Alberta Agriculture and Forestry, Edmonton, AB, Canada.*

First reports on nulliparous Holstein heifers and first- and second-parity Holstein cows have demonstrated inverse relationships between anogenital distance (AGD) and measures of fertility in these respective populations. The objective of this study was to further validate the inverse relationship between AGD and measures of fertility in lactating cows, using a large population of North American Holstein cows. Cows (n = 5,173) from 19 herds (18 Canadian + 1 American) were subjected to AGD measurement (the distance from the center of the anus to the base of the clitoris) and reproductive performance data were obtained from herd records. Data were analyzed using UNIVARIATE, MIXED, CORR, REG, GLIMMIX, LOGISTIC, and LIFETEST procedures of SAS, where AGD and parity were considered independent variables in all statistical models. AGD was normally distributed with an overall mean (±SD) of 132.5 ± 11.8 mm (min, max: 94, 177). As the optimum AGD cut-point predictive of pregnancy was not obtainable, cows were categorized into short- (≤mean) and long-AGD (>mean) groups based on mean AGD for each parity group [first- (129 mm), second- (133 mm), and third+-parity (136 mm)]. AGD had a weak positive association with both parity (r = 0.21) and 305-d mature-equivalent milk yield (r = 0.04). Cows with short AGD had greater pregnancy to first artificial insemination (P/1stAI) than cows with long AGD (35.7 ± 2.1 vs. 31.4 ± 2.0%; $P < 0.01$); however, there was no difference in P/ second AI (37.4 ± 2.3 vs. 34.9 ± 2.2%) and third (41.8 ± 2.4 vs. 43.4 ± 2.4%) between AGD categories. Cows with short AGD tended to require fewer services per conception (2.3 ± 0.1 vs 2.4 ± 0.1; $P = 0.06$) and had fewer days open (136.9 ± 4.5 vs 140.9 ± 4.3 d; $P = 0.05$) compared with their long AGD counterparts. However, cumulative pregnancy risks up to 150 and 250 d in milk did not differ between AGD categories. Results indicate an inverse relationship between AGD and some measures of fertility in lactating cows, and is positively, but weakly associated with both parity and milk yield, strengthening the potential for AGD to be implemented as a reproductive phenotype in future breeding programs.

Key Words: dairy cattle fertility, reproductive phenotype, genetic selection

P324 Effects of dietary butyrate supplementation and oral nonsteroidal anti-inflammatory drug administration to transition cows on indicators of uterine inflammation and days to first ovulation.

L. E. Engelking*, M. Oba, and D. J. Ambrose, *University of Alberta, Edmonton, AB, Canada.*

Sixty-five Holstein cows were blocked by calving date and parity and randomly assigned to an isoenergetic diet containing calcium butyrate (BUT; 1.42% of diet dry matter (DM)) or a control (CON; 1.04% palm fat and 0.38% calcium carbonate of diet DM) from −28 to +24 d in milk (DIM). The close-up diet contained 14% starch and 43% neutral detergent fiber, and the fresh diet contained 22% starch and 35% neutral detergent fiber on a DM basis. Cows received an oral nonsteroidal anti-inflammatory drug (NSAID; 1 mL/15 kg BW; Meloxicam in carrier solution) or a placebo (1 mL/15 kg BW food dye in carrier solution) at 12 to 24 h after calving resulting in treatment groups: BUT-NSAID (n = 15), BUT-Placebo (n = 14), CON- NSAID (n = 18), and CON-Placebo (n = 18). Weekly ovarian ultrasonography was from 14 DIM until first ovulation or up to 56 DIM. Vaginal mucus was collected using a Metricheck at 28 DIM and scored from 0 to 3 (0 = clear, 3 ≥ 50% purulent or sanguineous). Endometrial cytology was performed at 28 DIM to assess uterine inflammation based on polymorphonuclear (PMN) leukocytes. Proportionate data were analyzed by Proc FREQ and the mean interval to first ovulation was determined by GLIMMIX in SAS. A smaller proportion of cows fed the butyrate diet ovulated by 14 DIM compared with cows fed the control diet (29 vs. 71%; n = 14; $P = 0.05$). The mean days to first ovulation tended to be longer in NSAID- than in placebo-treated cows (29 vs. 24 d; $P = 0.09$). Six cows did not ovulate by 56 DIM. Butyrate and NSAID treatment did not affect the mean PMN count or the proportion of low (≤18%) PMN cows; however, the proportion of cows with high (>18%) PMN tended to be greater in NSAID- than in placebo-treated cows (64 vs. 36%; n = 22; $P = 0.10$). Results may be attributed to too early NSAID administration, possibly interfering with natural postcalving inflammatory mechanisms that assist in uterine involution and return to estrus. Dietary butyrate and oral NSAID did not reduce uterine inflammation or days to first ovulation.

Key Words: reproduction, uterine inflammation, days to first ovulation

P325 Effect of a high dose of gonadotropin-releasing hormone (GnRH) at the first GnRH of the Resynch-25 on ovulatory response and fertility in lactating Holstein cows.

I. M. R. Leão*[1], M. S. El Azzi[1,2], E. Anta-Galván[1], T. Valdés-Arciniega[1], and J. P. N. Martins[1], [1]*Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin–Madison, Madison, WI,* [2]*Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, MG, Brazil.*

Our objective was to determine the effect of high vs. low dose of GnRH on ovulatory response, serum progesterone (P4) concentrations and pregnancy per AI (P/AI) in lactating Holstein cows receiving a Resynch-25 program for second+ AI. Cows (n = 2,375; primiparous: 789; multiparous:1,583) 25d after previous timed AI (TAI) were randomly assigned to receive either 100 (L) or 200 (H) μg of GnRH (gonadorelin hydrochloride; Factrel, Zoetis) for the d25 GnRH of the Resynch-25 program. On d32 after TAI, cows were diagnosed for pregnancy and checked for CL presence with ultrasound. Pregnant cows (n = 1,198) did not receive any further treatment. Non-pregnant cows without a CL on d32 (n = 126; L: 68; H: 58) were enrolled in a Ovsynch+CIDR protocol. Non-pregnant cows with a CL (n = 1,049) continued the resynch program to be rebred (d32: $PGF_{2α}$ – d33: $PGF_{2α}$ – d34(p.m.): GnRH – d35: TAI). Ovaries were scanned with ultrasound in a subset of cows (n = 549) on d 25 and 27 to determine ovulatory response (pregnant, n = 268; non-pregnant, n = 281). Blood samples (n = 285) were collected on d 25 and 32 to measure serum P4 in non-pregnant cows. A total of n = 953 cows were analyzed for treatment differences in P/AI. Binary variables were analyzed by logistic regression with PROC GLIMMIX of SAS. Most cows had a follicle ≥ 10 mm in diameter on d25 (95.8%). The proportion of cows with P4 ≥ 1.00 ng/mL on d25 was low and did not differ ($P = 0.30$) between L (48.4%) and H (42.2%). Treatment did not affect ovulatory response for all cows ($P = 0.35$; L: 27.8% vs. H: 31.5%) and for non-pregnant cows ($P = 0.42$; L: 34.7% vs. H: 39.4%). The proportion of cows with P4 ≥ 1.00 ng/mL on d32 was high and did not differ between treatments ($P = 0.45$; L: 87.9% vs. H: 90.7%). P/AI did not differ ($P = 0.72$) between L (46.4%) and H (47.6%). Ovulation after d25 GnRH tended to increase ($P = 0.06$) P/AI for cows diagnosed non-pregnant for previous TAI (ovulation: 59.8% vs. no ovulation: 46.6%). In summary, a higher dose of GnRH on d25 of the Resynch-25 was not effective in increasing ovulatory response, proportion of cows with CL on d32 and P/AI of second+ AI, based on preliminary data.

Key Words: Resynch, ovulation, GnRH

P326 Effect of gonadotropin-releasing hormone (GnRH) administered at the time of AI for dairy cows detected in estrus by an activity monitoring system or by conventional estrus detection.

A. Hubner*[1], I. F. Canisso[1], P. M. Peixoto[1], A. J. Conley[2], and F. S. Lima[2], [1]*Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL,* [2]*Department of Population Health and Reproduction, University of California, Davis, Davis, CA.*

We hypothesized that a GnRH treatment at the time of concurrent estrus detection and AI (AIDE) would increase ovulation rate, progesterone post-AI, and pregnancies per AI (P/AI). Holstein cows were randomly assigned to receive an injection of GnRH (GnRH) or remain untreated (Control) at the time of AIDE on farms using activity monitors (AM, n = 409) or conventional estrus detection methods (ED, n = 398). Ovarian structures, plasma progesterone (P4) and estradiol (E2) were assessed in a subset 107 cows from a conventional ED farm at the time of AI (For E2 and P4) and 7 d later (For P4). Cows in AM had activity level assessed and categorized as high (≥100 increase) vs. low (<100 increase). Statistical analysis was performed using logistic regression and general linear models with models including effects of treatment, ED methods, parity, season, farms, receiving or not PGF$_{2\alpha}$, service number and their interactions. There were no effects ($P = 0.98$) of GnRH on P/AI (GnRH = 35.1 ± 4.1%; Control = 35.7 ± 4.2%) or pregnancy loss (GnRH = 6.9 ± 3.0%; Control = 9.0 ± 3.1%). There was no interaction ($P = 0.88$) between treatment and method of ED nor between treatment and other variables included in the models. There was no difference ($P = 0.56$) in ovulation rate, P4 at AI, and E2 on d 0 between GnRH and Control. For quartile of E2, the highest quartile (Q4) had lower P/AI ($P < 0.02$) than cows in quartiles 1 to 3 (Q1 = 65.4 ± 9.1%, Q2 = 50.0 ± 9.1%, Q3 = 42.3 ± 9.1%, Q4 = 18.5 ± 8.8). For AM cows there was a tendency ($P = 0.06$) for higher P/AI in cows that did not received PGF$_{2\alpha}$ before ED compared with those that did receive PGF$_{2\alpha}$ (No PGF$_{2\alpha}$ = 43.0 ± 3.1%, PGF$_{2\alpha}$ = 30.6 ± 9.9%). For AM there was an interaction between ($P = 0.04$) treatment and activity level with the GnRH having a greater impact on cows with high activity level (Low-GnRH = 25.9 ± 3.4% Low-Control = 22.9 ± 3.4%, High-GnRH = 48.4 ± 3.3%, High-Control = 41.8 ± 3.3%). The current study does not support the use of GnRH to improve P/AI in heat-detected cows, but potential GnRH benefits in AIDE with low and high activity and E2 levels need further investigation.

Key Words: estrus detection, activity monitors, GnRH

P327 Pregnancy loss is preceded by elevated pregnancy-specific protein B (PSPB) in recipients of somatic cell nuclear-transfer embryos but preceded by reduced PSPB in recipients of IVF embryos.

G. Madureira*[1,5], G. Grillo[1,2], V. Gomez-Leon[1,3], J. P. Andrade[1], M. Fosado[4], R. Sala[4], R. Sartori[5], M. Mello[2], and M. Wiltbank[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI,* [2]*Departamento de Reproducao e Avaliacao Animal, Universidade Federal Rural do Rio de Janeiro, Seropedica, RJ, Brazil,* [3]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS,* [4]*Sexing Technology, Kewaskum, WI,* [5]*Department of Animal Science, University of Sao Paulo, Piracicaba, SP, Brazil.*

This study compared circulating hormones in recipients of an embryo from somatic cell nuclear-transfer (SCNT) or in vitro fertilization (IVF). Further, the effect of increasing circulating progesterone (P4) was tested by production of accessory corpora lutea (CL). A total of 123 nulliparous Holstein were randomized to receive an IVF or SCNT embryo and further randomized to receive or not human chorionic gonadotropin (hCG; 3,300 IU on days 5 and 12 [day 0 = GnRH treatment at end of synchronization]). Thus, 4 groups were created: IVF+no hCG (n = 30), IVF+hCG (n = 29), SCNT+no hCG (n = 28), and SCNT+hCG (n = 36). Ultrasound evaluations and blood samplings for P4 and pregnancy-specific protein B (PSPB) were performed on days 5, 12, 19, 21, 28, 33, 42, 47, 54 and 63. Statistical analysis were performed using PROC GLIMMIX and MIXED of SAS. Treatment with hCG caused ovulation (day 5: 96.9%; day 12: 83.1%) and increased circulating P4 (day 19: 20.8 ± 1.3 vs. 10.1 ± 1.1 ng/mL). Treatment with hCG tended to increase pregnancies per ET (P/ET) in recipients of SCNT embryos at days 28 (41.7 vs. 21.4%; $P = 0.07$) and 42 (22.2 vs. 7.1%; $P = 0.09$). At days 28 and 33, P/ET was similar for IVF and SCNT embryos, but on day 42 the P/ET was greater for IVF than SCNT embryos due to greater ($P = 0.05$) pregnancy losses in SCNT than IVF from days 33–42 (44.4 vs. 15.0%). Despite recipients of SCNT and IVF embryos having similar circulating PSPB on Day 28, recipients of SCNT embryos had greater PSPB at all later times. Interestingly, recipients of IVF embryos that maintained the pregnancy had greater PSPB on days 28 ($P = 0.006$) and 33 ($P = 0.003$) than IVF recipients that later had pregnancy loss. In contrast, recipients of SCNT embryos that later lost the pregnancy had greater ($P = 0.01$) PSPB on Day 33 than SCNT recipients that maintained the pregnancy. Thus, although reduced PSPB may predict pregnancy loss in recipients of IVF embryos, greater circulating PSPB on Day 33 was associated with later pregnancy loss in recipients of SCNT embryos, perhaps indicating excessive numbers or activity of trophoblast binucleate cells preceding pregnancy loss of SCNT embryos.

Key Words: clone embryo, human chorionic gonadotropin

P328 Impacts of transition health on reproductive performance based on detection of estrus in dairy cows.

T. C. Bruinjé*[1], E. I. Morrison[1], D. Renaud[1], E. S. Ribeiro[2], and S. J. LeBlanc[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Our objective was to investigate associations between transition health and pregnancy outcomes in a reproductive program primarily based on detection of estrus for first AI. In 1,093 Holstein cows enrolled from 2 dairies, serum calcium (Ca), haptoglobin (Hp), and nonesterified fatty acids (NEFA) were assessed at 2 and 6 (±1) d in milk, and blood β-hydroxybutyrate (BHB) measured at 4, 8, 11, and 15 (±1) d. Cows were examined for metritis at the same times, and for purulent vaginal discharge (PVD) and endometritis (ENDO; ≥ 6% PMN) at wk 5. BCS and lameness were assessed from wk −3 to 9. Additional disease data were obtained from farm records. First AI occurred based on estrus detection by activity monitors starting at 50 d. Cows not detected in estrus received timed AI (Ovsynch) around 74 d (herd A) or received PGF2a at 75 d and Ovsynch 14 d later (herd B). Pregnancy was diagnosed at 33 d after AI, and subsequent AI occurred at detected estrus or resynchronization. Metabolites were categorized based on ROC curve cutpoints associated with ENDO and PVD. Pregnancy risk at first AI (P/AI) and hazard of pregnancy by 200 d were analyzed in multivariable logistic and hazard regression models including first AI method, parity, BCS, metabolites, and disease variables, with herd as random. 78% of cows received first AI at detected estrus, P/AI was 39%, and 81% were pregnant by 200 d. Prevalence of Ca ≤ 2.1 mmol/L was 53%, Hp ≥ 1.1 g/L 42%, NEFA ≥ 0.8mmol/L 30%, ENDO 31%, and PVD 20%. Risk factors for P/AI were parity (multiparous 32% vs. primiparous 42%; $P < 0.01$), prepartum BCS ≤ 3.00 (31 vs. 39%; $P = 0.03$), high Hp at both d 2 and 6 (25 vs. > 38% if low Hp at either d 2 or 6; $P < 0.01$), and both ENDO and PVD (25 vs. > 37% if neither ENDO nor PVD; $P = 0.01$). Hazard of pregnancy was lower in cows with high Hp at d 6 (HR = 0.8; 95% CI 0.7–0.9; $P = 0.01$), PVD (HR = 0.8; 95% CI 0.6–0.9; $P = 0.01$), or BCS at wk 9 ≤ 2.75 (HR = 0.6; 95% CI 0.6–0.9; $P < 0.01$). Results suggest that early postpartum systemic inflammation, chronic reproductive tract disease, and low body condition are risk factors for reduced reproductive performance primarily based on detection of estrus.

Key Words: fertility, reproduction, transition health

P329 Impact of systemic and uterine inflammation on luteal function in postpartum dairy cows.

T. C. Bruinjé*[1], E. I. Morrison[1], D. Renaud[1], E. S. Ribeiro[2], and S. J. LeBlanc[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

The objective of this prospective cohort study was to evaluate the association of systemic and uterine inflammation on luteal function in postpartum Holstein cows. A total of 394 cows from 2 commercial herds were initially enrolled 3 wk before parturition. Serum haptoglobin (Hp), total calcium (Ca), and nonesterified fatty acids (NEFA) were measured at 2 and 6 (±1) d in milk, and blood β-hydroxybutyrate (BHB) and metritis assessed at 4, 8, 11, and 15 (±1) d. Serum progesterone (P4) were measured at 21 and again at 35 ± 3 d, when cows were examined for endometritis (≥6% PMN in cytology samples) and purulent vaginal discharge (PVD). Cows diagnosed with both Hp ≥ 0.8g/L at 2 or 6 d and endometritis, regardless of other diseases, were enrolled in the cohort study and classified as high risk for luteal dysfunction (n = 76). Cows were classified as low risk (n = 69) if they had Hp < 0.8 g/L, no record of retained placenta, metritis, endometritis, PVD, or lameness, and BCS ≥ 3 at 35 d. Cows in the cohorts had P4 measured every 3–4 d from 35 to 70 ± 3 d, and luteal phases were characterized as periods of P4 ≥ 1ng/mL. Data were analyzed using linear or hazard regression models including risk group, parity, interaction, and herd as random. BCS at wk −3 and 9 and Ca were similar between groups, and high-risk cows had greater NEFA (0.8 vs. 0.6 ± 0.1 mmol/L; $P < 0.01$) and BHB (1.2 vs. 0.9 ± 0.1 mmol/L; $P < 0.01$) than low risk cows. Hazard of cyclicity (first P4 sample ≥ 1ng/mL) by 50 d was lower in high-risk cows (HR = 0.7; 95% CI 0.5–0.9; $P = 0.01$), so day of onset of cyclicity was included as a covariate in subsequent models. High-risk cows had a longer first detected luteal phase (25 vs. 16 ± 3 d in length; $P < 0.01$), tended to have lower peak P4 (10 vs. 9 ± 0.3 ng/mL; $P = 0.10$), had fewer luteal phases by 70 d (1.6 vs. 2.2 ± 0.1; $P = 0.05$), and lower hazard of detection of estrus by 75 d (HR = 0.5; 95% CI 0.3–0.8; $P < 0.01$). Findings suggest that cows with early postpartum systemic inflammation and subsequent uterine inflammation have altered luteal function before and during the breeding period, which could underlie reduced fertility.

Key Words: fertility, ovarian function, transition health

P330 Repeatability of anogenital distance measurements at different ages and physiological states in dairy cattle.

I. Rajesh*[1], M. Gobikrushanth[1,2], J. E. Carrelli[1], M. Oba[1], and D. J. Ambrose[1,3], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*University of Saskatchewan, Saskatoon, SK, Canada,* [3]*Alberta Agriculture & Forestry, Edmonton, AB, Canada.*

Anogenital distance (AGD), measured from the anus to the clitoris, is inversely associated with fertility and moderately heritable in dairy cattle. For AGD to be considered useful in genetic selection, its repeatability (consistency in measurements at different time points) must be determined. Therefore, the objective was to determine the associations and repeatability of AGD a) from birth to breeding age (15 mo) in heifers (n = 48); and among phases of b) the estrous cycle (n = 20); c) gestation (n = 78); and d) lactation (n = 30) in cows. AGD was measured using digital calipers. The phases (proestrus, estrus, metestrus, and diestrus) of estrous cycle were established based on plasma progesterone concentrations and ovarian ultrasonography performed every 48 h. The differences in means and the association of AGD at different phases were determined by ANOVA and Pearson correlation coefficient, respectively. The AGD was modeled against time (phases) and parity, with cows being repeated subjects. Mean (±SEM) AGD differed by age, with a linear increase from birth to 15 mo (68.1 ± 0.8, 73.6 ± 0.8, 87.7 ± 0.8, 95.5 ± 0.9, 105.0 ± 1.1, 111.7 ± 1.1 mm at 0, 2, 6, 9, 12 and 15 mo, respectively; $P < 0.01$). AGD at birth and at 15 mo were not associated ($r = 0.27$; $P = 0.21$) but the AGD at 6 mo and at 15 mo were moderately associated ($r = 0.41$; $P = 0.05$). In contrast, AGD did not differ by the phases of the estrous cycle (pooled mean, 131.1 ± 2.1 mm) and lactation (pooled mean, 127.6 ± 1.4 mm), and were highly correlated among phases of estrous cycle ($r \geq 0.98$; $P < 0.01$) and lactation ($r \geq 0.95$; $P < 0.01$). The AGD measurements at 30, 90 and 180 d of gestation were strongly associated ($r \geq 0.97$; $P < 0.01$), but AGD at 270 d (mean, 142.8 ± 1.2 mm; $P < 0.01$) differed from AGD of all earlier phases of gestation (mean, 127.0 ± 1.1 mm), yet remained correlated ($r \geq 0.62$). We conclude that AGD measured in heifers at 6 mo of age is a reliable reflection of AGD at breeding age and that AGD is highly repeatable in dairy cows and could be reliably measured at any physiological state except during late gestation.

Key Words: reproductive phenotype, genetic selection, fertility

Ruminant Nutrition: Calves and Heifers: Posters

P331 Consumption of milk containing antibiotic residues increases the occurrence of fecal *Escherichia coli* antimicrobial resistance in dairy calves. H. C. Diniz Neto[1], S. G. Coelho[1], S. F. Vieira[1], J. P. Campolina*[1], and M. M. Campos[2], [1]*Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [2]*Embrapa Dairy cattle, Juiz de Fora, MG, Brazil.*

The objective of the study was to evaluate the effects of the use of waste milk containing antibiotic residue, on the resistance of fecal *Escherichia coli* in dairy calves. Thirty calves were divided into 2 groups: whole milk without antibiotic residue (WHM; n = 15) and waste milk with antibiotic residue (WAM; n = 15). Calves received 6 L of their treatment milk for 60 d of life, divided into 2 meals. At d 60, stool samples were obtained directly from the rectal ampoule. The samples were frozen for further culture and antibiogram. *E. coli* were isolated from the samples, cultured, and then it was performed a disk-diffusion antibiograma test to assess bacterial sensitivity to amoxycillin (AMO), ampicillin (AMP), ceftiofur (CEF), cefoperazone (CEFO), florfenicol (FLO), enrofloxacin (ENR), and streptomycin (STR). The samples that showed complete absence of inhibition halo were considered resistant, and samples with the presence of inhibition halo were considered sensitive. Data were analyzed by comparison between groups with the Chi-squared test using RStudio. Significance was declared at $P \leq 0.05$. Only a small portion of the WHM animals had bacteria resistant to AMO (n = 1/15), AMP (n = 2/15), and FLO (n = 1/15). Otherwise, WAM animals presented a resistant *E. coli* for all tested bases, except for STR, which was the least used base in the herd. When comparing groups, the WAM group showed superior results of resistance for AMO, AMP, CEF, CEFO, and ENR ($P < 0.05$). There were no differences between groups for FLO and STR ($P > 0.05$). The presence of antibiotics in the liquid diet of the calves exerted selective pressure, favoring the emergence of resistant bacteria in the intestinal microbiota. The waste milk used did not contain FLO, and since STR had little use for treatment protocols in the herd, this justifies the absence/lower resistance observed for these bases. The consumption of waste milk containing antibiotics residues influences the resistance of *E. coli* and reduces antibiotic-susceptible strains available for dairy calves.

Key Words: disk-diffusion test, microbiota, waste milk

P332 Effects of feeding whole milk, waste milk, and pasteurized waste milk on the development of the gastrointestinal tract of dairy bull calves. H. C. Diniz Neto[1], S. G. Coelho[1], S. F. Vieira[1], J. P. Campolina*[1], and M. M. Campos[2], [1]*Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [2]*Embrapa Dairy cattle, Juiz de Fora, MG, Brazil.*

The aim of this study was to evaluate the effects of feeding whole milk (WHM), waste milk (WAM), and pasteurized waste milk (PWM) on the development, of the gastrointestinal tract of dairy bull calves. Forty-five crossbred (Holstein-Gyr) bull calves were randomly distributed in 3 treatments: WHM (n = 15); WAM (n = 15) and PWM (n = 15). For the PWM group a fast pasteurization, ranging temperatures from 72 to 74°C for 16 s was used to reduce the bacterial count. All calves received 6 L of their treatment milk for 60 d of life, divided into 2 meals. At d 60 ± 1, the animals were euthanized and tissue samples were obtained from the dorsal (DRS) and ventral sac of the rumen (VRS), omasum (OM), abomasum (AB), duodenum (DU), and ileum (IL) for histological evaluation. Papillary height (PH) and papillary area (PA) were measured in DRS, VRS, OM, DU, and IL. Crypt depth (CD), gastric gland depth (GD) and cell proliferation (CLP) were measured in the AB. Mitotic index (MI) was determined in DRS, VRS, and OM. Data were analyzed using the linear mixed model (lme package), including calf as the random term and treatment and genetic composition as fixed variables using RStudio. Significance was declared at $P \leq 0.05$. There were no differences between groups for PH, PA, and MI for DRS, VRS, and OMA ($P > 0.05$); CD, GD, and CLP for AB ($P > 0.05$); PH, and PA for IL, and DUO ($P > 0.05$). Consumption of antibiotic residues present in milk is associated with a reduction in bacterial richness and diversity in the entire gastrointestinal tract of calves, which in a way could impair the organ development. However, microbiota richness and diversity were not measured in our study. The use of waste milk or pasteurized waste does not affect the development of the gastrointestinal tract of dairy calves.

Key Words: antibiotics, euthanasia, histology

P333 Oregano extract fed to preweaned dairy calves alters rumen bacteria microbiota. V. Fischer*[1], L. A. Ritt[1], C. Orso[1], A. K. Silveira[1], J. Frazzon[1], and D. P. Vargas[2], [1]*Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil,* [2]*University of Santa Cruz do Sul, Santa Cruz, RS, Brazil.*

This study aimed to compare the rumen bacterial population of preweaned dairy calves supplemented or not with oregano extract. Ten Holstein calves were fed 6 L/d of milk replacer, and water, concentrate and hay ad libitum. The calves were randomly assigned to 2 treatments: CON (without additive) and OE (60 mg of oregano extract/kg of BW). Weaning occurred on d 53 of the experimental period and on d 54, all calves were euthanized and their total rumen content was sampled. The V4 region of the bacterial 16S rRNA gene was amplified using F515 and R806 primers to characterize the rumen bacterial composition. Diversity was calculated using Shannon's diversity index. Data about relative frequency and diversity were analyzed by ANOVA (GLM procedure of SAS), including the fixed effect of treatment according to the completely randomized design. Taxonomic analysis identified 43 phyla; *Firmicutes*, *Bacteroidetes* and *Proteobacteria* were the most abundant. Among *Firmicutes*, 4 genera presented differences in relative abundance: *Ruminococcus* (4.19% *v.* 1.96%) and *Clostridium cluster IV* (1.95% *v.* 0.40%), were more abundant ($P \leq 0.05$) in CON, while *Succinivibrio* (1.68% *v.* 6.61%) and *Roseburia* (2.30% *v.* 5.23%) increased ($P < 0.05$) in OE. The *Bacteroidetes* phylum (56.84% *v.* 41.02%) was more abundant ($P \leq 0.05$) in CON, due to the higher abundance ($P \leq 0.05$) of *Prevotella* (53.24% *v.* 38.99%). The genera *Barnesiella* (1.00% *v.* 0.25%) and *Dysgonomonas* (2.18 *v.* 0.77%) were more abundant ($P \leq 0.05$) in CON, while *Bacteroides* (0.81% *v.* 1.52%) were more abundant ($P < 0.05$) in OE. The *Proteobacteria* phylum (3.08% *v.* 16.45%), mostly represented by the unclassified *Gammaproteobacteria* and the genus *Frischella* (0.38% *v.* 8.96%) were more abundant ($P < 0.05$) in OE compared with CON, but for *Actinobacteria* the opposite result was observed (1.33% *v.* 0.75%). The *Spirochaetes* phylum (0.58% *v.* 1.38%) was less abundant ($P < 0.05$) in CON. Rumen bacterial microbiota of OE were more diverse ($P < 0.001$) compared with CON, Shanon index = 3.165 and 3.303, respectively. Oregano extract supplemented to dairy calves reduced the abundance of gram-positive genera as *Roseburia, Clostridium cluster IV* and *Ruminococcus* while increased the abundance of gram-negative genera as *Frischella*, *Succinivibrio*, and *Bacteroides*, less suscetible to essential oils of oregano due to their outer membrane.

Oregano extract supplementation may be used to modulate rumen bacterial microbiota of preweaned calves.

Key Words: dairy calf, oregano, rumen bacteria

P334 Replacement of corn grain by reconstituted corn grain silage in the concentrate for dairy calves: Effects on intake and performance pre- and postweaning. S. C. Dondé, A. F. Toledo, A. P. Silva, M. D. Silva, J. P. G. Bernardes, R. Y. L. Ricci, E. A. Fioruci, G. F. Virgínio Jr, and C. M. M. Bittar*, *Department of Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil.*

Starter intake is the main stimulus for rumen development of dairy calves. Different starter formulations may lead to changes in the intake increases during preweaning affecting postweaning performance. The objective of this study was to evaluate the replacement of corn grain by reconstituted corn grain silage in the concentrate of dairy calves. Thirty-six Holstein newborn calves were used in a randomized block design, considering sex, birth date, and weight as blocking criteria. Calves were distributed into 2 treatments: 1) control: starter concentrate based on ground grain corn (22% crude protein; 16% neutral detergent fiber); and 2) grain silage: the same starter concentrate but with the replacement of ground grain corn with reconstituted grain corn silage. Animals were fed 6 L/d of whole milk until 56-d and the starter concentrate was available from day one until 10-wk. Calves were fed grass hay ad libitum after weaning. The ground corn was reconstituted to 35% humidity and ensiled in buckets that were opened after 60 d of fermentation. During the preweaning phase, dry matter intake (DMI) and average daily gain (ADG) increased with age ($P < 0.0001$), but were not affected by grain processing ($P > 0.05$). However, the grain silage starter improved preweaning feed efficiency (FE; $P < 0.02$). Postweaning DMI was lower for calves fed the grain silage starter ($P < 0.04$), with no differences for ADG or FE ($P > 0.05$). Total DMI increased and FE decreased with age postweaning ($P < 0.0001$). Replacement of corn grain by reconstituted corn grain silage in the concentrate may benefit calves while the rumen is under development, but after weaning it will decrease DMI. An evaluation of a longer feeding time should be done in further studies.

Table 1. Effect of supplementation of ground grain corn or grain silage on animal performance

	Diet			*P*-value		
Item	Control	Grain silage	SEM	Diet	Age	Diet × Age
Preweaning (1–56 d)						
Total DMI,[1] g/d	1,042.5	1,003.4	23.24	0.22	<0.0001	0.18
Starter DMI, g/d	303.7	256.1	27.43	0.22	<0.0001	0.16
ADG, g	691.4	708.4	21.71	0.52	<0.0001	0.53
FE	0.657	0.712	0.0174	0.02	0.015	0.47
Birth BW, kg	35.0	34.7	1.32	0.68	—	—
Weaning BW, kg	72.2	73.9	2.04	0.33	—	—
Postweaning (57–70 d)						
Total DMI,[2] g	1,638.2	1,432.1	68.09	0.04	<0.0001	0.35
ADG, g	803.3	703.3	47.72	0.15	0.387	0.56
FE	0.532	0.498	0.0395	0.54	<0.0001	0.24
Final BW, kg	79.15	79.50	1.504	0.856	—	—

[1] Milk solids and starter intake.

[2] Starter and hay intake.

Key Words: starter intake, solid diet, weight gain

P335 Replacement of corn grain by reconstituted corn grain silage in the concentrate of dairy calves: Effects on rumen fermentation and development. S. C. Dondé, A. F. Toledo, A. P. Silva, M. G. Coelho, A. M. Cezar, R. Y. L. Ricci, N. Jeronymo, R. C. Silva, and C. M. M. Bittar*, *Dept. Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil.*

One of the main objectives of the preweaning dairy calves' management is to stimulate solid diet intake to guarantee rumen development and successful weaning. The objective of this study was to understand if corn grain silage, which has a higher starch digestibility, would affect fermentation and improve rumen development. Thirty-six Holstein newborn calves were used in a randomized block design, considering sex, birth date, and weight as blocking criteria. Calves were distributed into 2 treatments: 1) control: starter concentrate based on ground grain corn (22% crude protein; 16% neutral detergent fiber); and 2) grain silage: the same starter concentrate but with the replacement of ground grain corn with reconstituted grain corn silage. Animals were fed 6 L/d of whole milk until 56 d and the starter concentrate was available from day one until 10 wk. Calves were fed grass hay ad libitum after weaning. The ground corn was reconstituted to 35% humidity and ensiled in buckets that were opened after 60 d of fermentation. Rumen fluids were collected at wk 8 (preweaning) and 10 (postweaning), by the use of an oro-esophageal tube, 2 h after concentrate feeding. Ten calves were randomly selected to be slaughtered at 10 wk to evaluate the weight of forestomach compartments. Data were analyzed using the MIXED procedure of SAS, with solid diet as a fixed effect, and the means were compared using the Tukey test. The rumen fermentation profile and the forestomach compartment weights were not affected by the corn grain processing. Reconstituted corn grain silage inclusion in starter did not improve fermentation profile that could benefit rumen development of dairy calves.

Table 1. Effect of supplementation of ground grain corn or grain silage on SCFA concentrations

	Solid diet			
Item	Control	Grain silage	SEM	*P*-value
Preweaning				
Total SCFA, mM	111.0	105.6	9.01	0.68
Acetate, mM/100 mM	45.1	45.0	2.64	1.00
Propionate, mM/100 mM	39.5	41.3	2.87	0.67
Butyrate, mM/100 mM	9.8	8.9	0.88	0.46
pH	5.6	5.5	0.09	0.62
Postweaning				
Total SCFA, mM	114.7	106.2	8.93	0.35
Acetate, mM/100 mM	48.7	46.9	0.87	0.09
Propionate, mM/100 mM	38.4	39.7	1.30	0.43
Butyrate, mM/100 mM	8.8	8.1	0.78	0.56
pH	5.9	5.7	0.13	0.22
Forestomach weight, kg				
Total	1.98	2.02	0.090	0.50
Reticulum-rumen	1.26	1.29	0.093	0.71
Omasum	0.332	0.319	0.014	0.91
Abomasum	0.403	0.397	0.018	0.83

Key Words: forestomach, short chain fatty acids, solid diet

P336 Validation of an automatic system for monitoring dairy calf weight and starter intake during the preweaning phase. M. E. Reis[1], R. C. Silva[1], R. S. Budoya[1], R. Y. L. Ricci[1], A. P. Silva[1], A. F. Toledo[1], A. M. Cezar[1], N. N. Morais Jr[2], G. G. S. Salvati[1], 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]*Federal Institute of Espírito Santo, Colatina, ES, Brazil.*

Monitoring starter intake and body weight is essential for adequate dairy calves' management and decision-making for nutrition adjustments. The study aimed to validate an automatic system for monitoring starter intake and body weight of dairy calves in individual housing systems during the preweaning phase (Systech Feeder System, Piracicaba, Sao Paulo, Brazil). Fourteen Holstein calves were housed in individual hutches from 14 to 56 d of age. Calves were bucket-fed 6 L/d of whole milk and had free access to water and calf starter. The starter device has a 10 kg capacity, but it was adjusted daily to have 2 kg. Every morning the whole device was taken off from the hutch for orts weight and feed intake calculation. After that, the device was then completed to have 2 kg of starter. The body weight was recorded twice a week on a conventional scale. The Systech Feeder system has an ultrasonic sensor that indicates the presence of the calf inside the hutch and then the body weight is recorded by equipment through weight sensors. The feeder is supported by a device with 2 weight sensors. The weight of the feeder is recorded every time that the calf is outside of the hutch, and the difference between the last and the first record of the day generated the daily intake data. The data are shown on a mobile app. A comparison was made between the data collected by the automatic system and the external scales through simple linear regression using SAS. Linear regression of starter intake data shows a high coefficient of determination ($Y = 1.04x + 9.72$; $R^2 = 0.92$; RMSE = 94.65; $P < 0.01$), suggesting that the system is efficient in registering the actual starter intake of the calves. However, body weight regression has shown a moderate to coefficient of determination ($y = 0.9528x + 4.8621$; $R^2 = 0.71$; RMSE = 4.054; $P < 0.01$), suggesting that there is an opportunity for the weighing system to be improved. Nevertheless, the data accuracy was satisfactory for commercial dairy farms, being a useful tool for farmers to evaluate the performance of preweaning dairy calves.

Key Words: feeder, technology, performance

P337 Heart girth measurements to estimate body weight of growing and mature Holstein-Friesian cattle. C. E. Bailey* and J. M. Moorby, *Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, Ceredigion, UK.*

Excellent nutritional management of replacement dairy heifers is critical to their development and for farms to meet animal development and business finance targets. Small dairy farms in particular may not have the space or resources for large animal weigh scales, and may have to rely on alternative methods of assessing heifer body weight (BW) to monitor growth and to determine nutrient requirements. Weigh tapes are a cheap, widely available alternative to weigh scales for monitoring growth targets. Our objective was to determine the relationship between heart girth (HG, cm) and BW (kg) in growing Holstein-Friesian heifers and to assess the accuracy of a commercially available weight tape. One hundred heifers started the experiment from about 7 wk old and 8,948 individual animal BW and HG measurements were recorded using a weigh crush and tape measure respectively. Animals were fed a range of diets, and measurements continued to the end of second lactation for animals that reached that. Mean data were calculated for every 2 wk of age resulting in 233 paired comparisons of BW (range 51–942 kg) and HG measurements (range 88–232 cm). A quadratic equation was fitted to investigate the relationship between HG and BW: $BW = 0.032 \times HG^2 - 3.89 \times HG + 154.4$; $R^2 = 0.999$. The weigh tape (ANImeter, Göbel, Germany) was used to predict BW from known HG, which were compared against known BW measurements. Predicted values were within 20% of the true BW >99% of the time, with a Lin's concordance correlation coefficient (L_c) of 0.999. BW were also estimated using the equations of Heinrichs et al. (1992) ($BW = 102.71 - 2.876 \times HG + 0.02655 \times HG^2$), and Sastry et al., (1982) ($W = (L \times G^2) / 300$, where W = BW in lbs, L = animal back length from withers to tail head in inches, and G = HG in inches). These resulted in lower L_c values (0.987 and 0.811 respectively) with our data. It is concluded that the correlation between HG and BW is sufficiently accurate to allow the use of BW estimated from HG measurements for monitoring heifer growth targets in the absence of weigh scales, and that commercially available tapes are appropriate for this.

Key Words: dairy heifer, nutrition, weight

P338 Effects of feeding rumen-protected lysine to Holstein cows prepartum on their calves' serum metabolites and immune function. A. R. Guadagnin*[1], L. K. Fehlberg[1], B. Thomas[1], Y. Sugimoto[2], I. Shinzato[2], and F. C. Cardoso[1], [1]*University of Illinois, Department of Animal Sciences, Urbana, IL,* [2]*Ajinomoto Co. Inc., Tokyo, Japan.*

We aimed to determine the effects of feeding rumen-protected lysine (RPL; AjiPro-L Generation 3, Ajinomoto Heartland Inc., Chicago, IL; 0.54%DM of TMR as top dress) to prepartum Holstein cows on their calves' serum metabolites and immune function. Seventy-eight Holstein calves; [male (M), n = 41; and female (F), n = 37], were assigned into 2 dietary treatments based on dams prepartum feeding with RPL (PRE-L) or without (CON). Calves were removed from their dams at calving and were given 3.8L of colostrum replacer (Land O'Lakes Bovine IgG Colostrum Replacer, Land O'Lakes, Inc. Arden Hills, Minnesota, USA). All calves were given milk replacer [Advanced Excelerate, Milk Specialties, Carpentersville, IL; 28.5% crude protein (CP), 15% fat] twice daily until 42 d of age. Grain starter (19.9% CP, 13.5% neutral detergent fiber, Ampli-Calf Starter, Purina, St. Louis, MO) was given from d 1 of age until d 56 at ad libitum access. Blood samples were collected on d 0, 2, 7, 14, and 28. Statistical analysis was performed using the MIXED procedure of SAS, modeling the fixed effects of treatment and sex, the repeated effect of time, and their interactions. Male calves in CON (CON-M) had greater ($P = 0.05$) serum concentration of phosphorus (P) (7.32 ± 0.13 mg/dL) than male calves in PRE-L (PRE-L-M; 6.96 ± 0.13 mg/dL), female calves in CON (CON-F; 7.07 ± 0.13 mg/dL), and female calves in PRE-L (PRE-L-F; 6.96 ± 0.13 mg/dL). Calves in CON-M tended to have greater ($P = 0.10$) calcium concentration (11.05 ± 0.13mg/dL) than calves in PRE-L-M (10.71 ± 0.13mg/dL), CON-F (10.72 ± 0.13mg/dL), and PRE-L-F (10.71 ± 0.13mg/dL). Calves in CON-F tended to have lesser ($P = 0.06$) serum total protein (4.59 ± 0.08g/dL) than calves in PRE-L-F (4.83 ± 0.08 g/dL), in CON-M (5.02 ± 0.08g/dL), and in PRE-L-M (4.81 ± 0.08g/dL). Calves in PRE-L tended to have greater ($P = 0.08$) percentage of phagocytic neutrophils (39.6 ± 1.59%) than calves in CON (35.9 ± 1.59%). Feeding RPL to prepartum cows alters their calves' serum metabolites, but this effect is dependent on sex, as illustrated by serum concentration of P being lesser for calves in PRE-L-M and in PRE-L-F than in CON-M, although similar in CON-F. Additionally, feeding RPL to prepartum cows tended to increase the phagocytosis of neutrophils on their offspring.

Key Words: lysine, phagocytosis, phosphorus

P339 Effects of *Saccharomyces cerevisiae* supplementation on growth performance of preweaned dairy calves: A systematic review and meta-analysis.

R. B. Lopes*[1], C. B. Winder[2], C. Bernal-Córdoba[1], and N. Silva-del-Río[1], [1]*University of California–Davis, Davis, CA,* [2]*University of Guelph, Guelph, ON, Canada.*

The objective was to quantify the effects of preweaned dairy calf supplementation with *S. cerevisiae* on feed efficiency (FE), average daily gain (ADG), milk intake and grain intake through a systematic review and meta-analysis. Manuscripts were retrieved from 5 electronic databases (Dissertations and Theses Database, Biosis, CAB Abstracts, Medline, and Scopus; Feb-Mar 2020). Eligible studies were controlled trials available in English, Portuguese, and Spanish, that evaluated the effect of probiotic supplementation on dairy calves' performance and health. The meta package of R statistical software was used to carry out a random-effects meta-analysis weighted by inverse variance method. Standardized mean difference (SMD) estimated with Hedges's *g* was used to quantify FE, ADG, milk intake and grain intake. Heterogeneity between studies was examined using the I^2 statistic. From 3,080 initially identified records, 9 manuscripts reporting 9 trials adding up 610 calves were analyzed. Studies evaluated FE (n = 6), ADG (n = 8), milk intake (n = 4), and grain intake (n = 6). At enrollment calves were 1 to 28 d old, supplementation lasted 21 to 90 d and the dose ranged from $1.5x10^6$ to $5.0x10^{11}$ cfu/d. The supplementation of *S. cerevisiae* during the preweaning period did not improve FE (SMD = −0.08; 95% CI = −1.14 to 0.97; $P = 0.84$) and considerable heterogeneity was present ($I^2 = 65.5\%$). Supplemented calves did not have a higher ADG vs. nonsupplemented calves (SMD = 0.80; 95% CI = −0.29 to 1.90; $P = 0.12$). However, substantial heterogeneity was also observed ($I^2 = 85.8\%$), indicating inconsistency of effect among studies. No effect was observed in milk intake (SMD = 0.11; 95% CI = −0.56 to 0.77; $P = 0.64$); or grain intake (SMD = 0.15; 95% CI = −0.23 to 0.52; $P = 0.36$). Low heterogeneity was detected between trials for grain intake ($I^2 = 27.0\%$) and milk intake ($I^2 = 3.1\%$). Our results suggest that supplementing *S. cerevisiae* to dairy calves has no additive effects on calf growth; however, due to the high heterogeneity detected for FE and ADG analyses, more research is needed to decisively evaluate an overall effect.

Key Words: live yeast, probiotic

P340 Performance of calves fed calf starter diets formulated with varying starch sources due to differing corn ingredient inclusion rates from birth to 8 weeks of age.

E. Dufour*[1], D. Ziegler[2], H. Chester-Jones[2], B. Ziegler[1], and A. Golombeski[1], [1]*Hubbard Feeds, Mankato, MN,* [2]*University of Minnesota Southern Research and Outreach Center, Waseca, MN.*

One hundred seven (2 to 5 d old) Holstein heifer calves (38.7 ± 0.60 kg) from 2 commercial dairies were randomly assigned to 1 of 4 calf starter (CS) treatments formulated with different corn sources and inclusion rates to evaluate performance and health through the first 56 d of life. All calves were fed a nonmedicated 20% crude protein (CP): 20% fat milk replacer (all milk protein) fed at 0.28 kg in 2 L of water 2x daily from d 1 to 35 and 1x daily from d 36 to weaning at d 42. Calves were fed a medicated (Decoquinate at 45.4 g/ton) 18% CP texturized CS free choice. Treatments were as follows: 1) CS formulated with steam-flaked corn (SFC) at low inclusion (SFL); 2) CS formulated with SFC at high inclusion (SFH); 3) CS formulated with SFC and extruded corn (EX) at a 1:0.75 ratio, respectively (EXL); and 4) CS formulated with SFC and EX at a 1:2.5 ratio, respectively (EXH). Data were analyzed using the PROC mixed procedure of SAS and repeated-measures analyses applied where appropriate. There were no differences ($P > 0.10$) in overall ADG averaging 0.69, 0.66, 0.67, and 0.66 kg/d for SFL, SFH, EXL, and EXH, respectively. Calves fed SFL and EXH had improved overall hip height gain ($P = 0.05$) compared with EXL, averaging 11.6, 11.3, and 10.4 cm, respectively, with SFH being intermediate averaging 10.9 cm. Postweaning starter intake (DM basis) tended to be greater ($P = 0.10$) for SFL calves, averaging 31.5 kg, whereas SFH, EXL and EXH averaged 29.0, 28.5 and 28.3 kg, respectively. There were no differences in overall daily DM intake, although SFL calves had numerically greater intake, averaging 1.26 kg/d compared with 1.18 for both SFH and EXL calves, and 1.16 kg/d for EXH. There were no differences in gain:feed, averaging 0.57, 0.58, 0.58, and 0.59 kg for SFL, SFH, EXL, and EXH, respectively. Under conditions of this study, there was no benefit to feeding calves a CS formulated with EX compared with conventional starters. Calves fed a conventional starter with a lower inclusion of SFC had improved structural growth and improved postweaning dry matter intake.

Key Words: calf performance, strach source, calf starter

P341 Performance of postweaned heifer calves limit-fed grain mixes formulated with inorganic or organic trace minerals and additives with free-choice hay.

E. Dufour*[1], D. Ziegler[2], H. Chester-Jones[2], B. Ziegler[1], and A. Golombeski[1], [1]*Hubbard Feeds, Mankato, MN,* [2]*University of Minnesota Souther Research and Outreach Center, Waseca, MN.*

Ninety-six (57 to 60 d old) Holstein heifers (84.3 ± 1.59 kg) were assigned to 1 of 4 grain mix treatments, formulated with inorganic trace minerals (IN) or organic trace minerals with additives (BP), in 4 replicate pens (6 heifers/pen) to evaluate feed intake and performance from 9 to 21 weeks of age. Preweaning, heifers were fed a conventional (LF) or accelerated (HF) feeding program supplemented with IN or BP through the first 8 weeks of life. Heifers were housed in 3.66 × 7.62 m pens in a naturally ventilated bedded-pack barn and limit-fed 16% CP whole corn and pellet mixes (GM) at 2.72 kg/d with free-choice hay (RFV = 122.7).

Treatments were as follows: 1) GM supplemented with IN to LF heifers (LFIN); 2) GM supplemented with BP to LF heifers (LFBP); 3) GM supplemented with IN to HF heifers (HFIN); and 4) GM supplemented with BP to HF heifers (HFBP). Data were analyzed using the PROC mixed procedure of SAS and repeated-measures analyses applied where appropriate. Overall average daily gain was similar across treatments, averaging 0.92, 0.95, 0.96, and 0.95 kg/d for LFIN, LFBP, HFIN, and HFBP, respectively. Hip height gain tended to be higher ($P \leq 0.10$) for LFIN and HFBP compared with HFIN at 8.84, 8.76, and 7.95 cm, respectively, with LFBP being intermediate averaging 8.15 cm. Overall gain:feed was similar across treatments, averaging 0.26 kg for LFIN and 0.27 kg for LFBP, HFIN, and HFBP. Body condition score at 21 weeks of age was similar across treatments, although numerically higher for HFBP heifers, averaging 3.11, compared with 2.99, 3.04, and 3.06 for LFIN, LFBP, and HFIN heifers, respectively. Total DM intake was similar across treatments, averaging 3.70 kg/d. Under conditions of this study, heifers fed a GM supplemented with organic trace mineral with additives did not improve overall performance compared with heifers fed a GM supplemented with inorganic trace minerals.

Key Words: grain mix, dairy heifer, performance

P342 Performance of calves pre- and postweaning fed pelleted calf starters formulated with select additives and/or differing energy sources.

D. Ziegler*[1], E. Dufour[2], B. Ziegler[2], and A. Golombeski[2], [1]*University of Minnesota, Waseca, MN,* [2]*Hubbard Feeds, Mankato, MN.*

One hundred eight (2 to 5 d old) Holstein heifer calves (38.4 ± 0.59 kg) from 2 commercial dairies were randomly assigned to 1 of 4 pelleted calf starter (PS) treatments formulated with additives and/or additional energy sources (ENG) to evaluate performance and health through the first 84 d of life. All calves were fed a nonmedicated 20% crude protein (CP): 20% Fat milk replacer (all milk protein) fed at 0.28 kg in 2 L of water 2x daily from d 1 to 35 and 1x daily from d 36 to weaning at d 42. Calves were fed medicated (Decoquinate at 45.4 g/ton) 20.0% CP PS free choice. Treatments were as follows: 1) PS formulated without additives or ENG (PSC); 2) PS formulated with an additive blend containing yeast products, DFM's and plant extracts (AB) without ENG (PSAB); 3) PS formulated with a combination of AB and ENG from roasted beans (PSRB) and 4) PS formulated with a combination of AB and ENG from dextrose (PSS). Data were analyzed using the PROC mixed procedure of SAS and repeated-measures analyses applied where appropriate. There was an increase in average daily gain d 1 to 56 ($P \leq 0.05$) for calves fed PSC compared with PSRB and PSS with PSAB being intermediate averaging 0.76, 0.68, 0.70, and 0.73 kg/d, respectively. Total PS and dry matter intake were greater ($P \leq 0.05$) for calves fed PSC compared with PSRB with PSAB and PSS being intermediate averaging 57.6, 78.6; 49.7, 70.7; 53.3, 74.4; and 53.8, 74.9 kg, respectively. There were no differences ($P > 0.10$) in gain:feed averaging 0.56 kg. Hip height gain d 57 to 85 was greater ($P \leq 0.05$) for PSC compared with PSRB with PSAB and PSS being intermediate averaging 8.03, 6.83, 7.42 and 7.32 cm respectively. There were no differences in health costs across treatments ($P > 0.10$). Overall daily fecal scores (1 to 4, 1 = normal, 4 = watery) d 1 to 56 were improved ($P \leq 0.05$) for PSAB compared with PSC, PSRB and PSS. Under conditions of this study, there were limited benefits to feeding calves a PS formulated with the AB or energy sourced from roasted soybeans or dextrose over a conventionally formulated PS.

Key Words: calf performance, pelleted starter, energy source

P343 Performance and health of calves pre- and postweaning fed milk replacers formulated with alternative fat sources.

D. Ziegler*[1], H. Chester-Jones[1], C. Soderholm[2], B. Hansen[2], and O. Drehmel[2], [1]*University of Minnesota, Waseca, MN,* [2]*Milk Specialties Global, Eden Prairie, MN.*

One hundred six (2 to 5 d old) individually fed Holstein heifer calves (38.9 ± 0.53 kg) from 2 commercial dairies were randomly assigned to 1 of 4 milk replacer (MR) treatments formulated with alternative fat sources to evaluate pre- (d 1 to 42) and postweaning (d 43 to 56) calf performance and health. The study was conducted between April and August, 2020. All calves were fed a medicated (Decoquinate at 45.4 g/ton) texturized calf starter (CS); 18% crude protein (CP) as fed. Water and CS were offered free choice from (d 1 to 56). All MR were formulated for 24% CP (all milk protein) and 22.0% fat fed at 0.28 kg in 2 L of water 2× daily from d 1 to d 14, 0.34 kg in 2.38 L of water 2× daily from d 15 to d 35 and 1× daily from d 36 to weaning at d 42; supplemented daily with neomycin sulfate and oxytetracycline at 22 mg/kg BW/day (d 1 to 14). Treatments were as follows: 1) MR, formulated with lard, (L); 2) MR formulated with tallow (T); 3) MR formulated with a lard/tallow blend (LT); and 4) MR formulated with all vegetable fat, (VF). Data were analyzed using the PROC MIXED procedure of SAS and repeated-measures analyses applied where appropriate. There was no difference in average daily gain ($P > 0.10$) d 1 to 56 averaging 0.68, 0.70, 0.66 and 0.72 kg/d respectively for L, T, LT and VF. Hip height gain d 1 to 56 did not differ ($P > 0.10$) across treatments averaging 12.17 cm. Total CS and DM intake did not differ ($P > 0.10$) averaging 41.5, 65.2; 43.7, 67.4; 40.4, 64.5 and 47.0, 70.6 kg respectively for L, T, LT and VF. No differences in gain:feed ($P > 0.10$) d 1 to 56 were observed averaging 0.62 kg across treatments. Average fecal scores (1 to 4, 1 = normal, 4 = watery) d 1 to 42 and overall d 1 to 56 did not differ across treatments averaging 1.32 and 1.28 respectively. There was a trend for increased health cost d 1 to 42 ($P \leq 0.10$) for T compared with VF and L with LT being intermediate averaging $0.99, 0.31, 0.41 and 0.70 respectively. These results show that both animal and vegetable fat sources offer acceptable calf health and performance benefits.

Key Words: calf performance, milk replacer, fat source

P344 Fecal bacteriome in dairy calves fed milk replacer supplemented with beta-glucans.

G. F. Virgínio Jr[1], M. E. Reis[1], A. P. Silva[1], A. F. Toledo[1], L. Greco[2], H. Montenegro[1], L. L. Coutinho[1], 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]*Kemin Animal Nutrition & Health Division South America, Valinhos, SP, Brazil.*

β-glucan has been reported to be associated with improved immunity and performance of animals. The study aimed to investigate how addition of β-glucans can affect fecal bacterial community. Holstein calves (n = 14) were individually housed in tropical shelters and blocked according to sex, date and weight at birth and randomly assigned to 1 of the treatments: (1) Control: milk replacer (14% solids, 24% CP, 18.5% Fat); (2) Beta-glucans: milk replacer supplemented with β-glucans (Aleta, 2 g/d). All calves were bucket-fed 6 L/d of milk replacer and received water and starter concentrate ad libitum starting on d 2. To evaluate the bacteriome, fecal samples were collected at wk 1, 2, 4, and 8. The bacterial community was determined by sequencing V3 and V4 region amplicons of 16S rRNA gene that was amplified by PCR, sequenced by the Illumina MiSeq platform, and analyzed using the DADA2 pipeline. No differences for diversity (Shannon and Simpson), richness (Chao1) and evenness (Pielou) was observed for treatments, but all indexes increased with age ($P < 0.001$). According to a permanova analysis

β-diversity was similar between treatments, but there were dissimilarities in the structure of the bacterial community in the preweaning period ($P = 0.01$). Sixteen bacterial phyla have been identified and Firmicutes, Bacteroidota, and Actinobacteriota were the most abundant (52.62, 25.26, and 12.58%, respectively). *Collinsella* (Actinobacteriota), *Prevotella* (Bacteroidota), and *Lactobacillus* (Firmicutes) were the most abundant genera (9.84, 9.54, and 8.82%, respectively). β-Glucan supplementation did not affect the fecal bacteriome in dairy calves.

Table 1. Fecal bacterial diversity, richness and evenness of calves supplemented or not with beta-glucans

	Treatment			P-value[1]		
Indices	Control	β-glucans	SEM	T	W	T × W
Shannon	2.43	2.44	0.11	0.925	<0.001	0.171
Simpson	0.82	0.84	0.02	0.336	<0.001	0.184
Chao1	52.51	52.15	3.30	0.930	<0.001	0.548
Pielou	0.62	0.62	0.02	0.901	<0.001	0.177

[1] T = treatment effect; W = week effect; T × W = treatment by week interaction effect.

Key Words: bacterial community, calf nutrition, microbiome

P345 Performance of calves fed milk replacer and calf starter diets formulated with inorganic or organic trace minerals and additives from birth to 8 weeks of age. E. Dufour*[1], D. Ziegler[2], H. Chester-Jones[2], B. Ziegler[1], and A. Golombeski[1], [1]*Hubbard Feeds, Mankato, MN,* [2]*University of Minnesota Southern Research and Outreach Center, Waseca, MN.*

One hundred nine (2 to 5 d old) Holstein heifer calves (40.5 ± 0.66 kg) from 2 commercial dairies were randomly assigned to 1 of 2 calf starter (CS) and 1 of 2 milk replacer (MR) treatments formulated with different trace minerals (TM) and additives to evaluate performance and health during the 56-d study. Treatments were as follows: 1) CS and MR formulated with inorganic TM, where calves were fed nonmedicated 20% crude protein (CP): 20% fat MR at 0.28 kg in 2 L of water 2× daily from d 1 to 35 and 1× daily from d 36 to weaning at d 42 (LFIN); 2) CS and MR formulated as per LFIN and fed nonmedicated 24% CP: 20% fat MR at 0.28 kg in 2 L of water 2× daily from d 1 to 7, 0.42 kg in 3 L of water 2× daily from d 8 to 42 and 1x daily from d 43 to weaning at d 49 (HFIN); 3) CS and MR formulated with organic TM and an additive containing yeast products, direct-fed microbials and other additives and fed as per LFIN (LFBP); and 4) CS and MR formulated as per LFBP and fed as per HFIN (HFBP). All calves were fed a medicated (Decoquinate at 45.4 g/ton) 18% CP texturized CS free choice through d 56. Calves fed HFBP had greater overall ADG ($P \leq 0.05$) at 0.79 kg/d compared with 0.71, 0.71, and 0.70 for LFIN, HFIN and LFBP, respectively, and higher overall hip height gain ($P \leq 0.05$) at 12.9 cm, compared with 11.3, 11.3, and 11.5 cm for LFIN, HFIN and LFBP, respectively. Total CS intake was greater ($P \leq 0.05$) for LFIN and LFBP compared with HFIN averaging 52.1, 53.1, and 35.3 kg, respectively, with HFBP being intermediate averaging 41.7 kg. Gain:feed was greater for HFBP calves ($P \leq 0.05$), averaging 0.64 kg compared with 0.55, 0.54, and 0.61 kg for LFIN, LFBP, and HFIN calves, respectively. Under conditions of this study, feeding a CS and MR supplemented with organic TM and additives improved calf performance for calves on a high MR feeding program. There were no measurable differences for calves on a low MR feeding program supplemented with inorganic or organic TM and additives.

Key Words: calf performance, trace mineral, calf starter

P346 Plasma glucose peak after feeding and fructosamine concentration as an indirect indicator of ruminal development in dairy calves. A. M. Cezar, A. P. Silva, C. R. Tomaluski, M. G. Coelho, S. C. Dondé, N. Jeronymo, M. B. Carvalho, A. F. Toledo, G. F. Virginio Jr, and C. M. M. Bittar*, *Dept. Animal Sciences, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP, Brazil.*

The glycemic status together with β-hydroxybutyrate (BHB) levels preweaning are evaluated as indicators of the transition from a pre to a functional ruminant condition. While BHB increases, glucose and fructosamine should decrease as rumen develops. However, glucose is affected by feeding time and liquid diet fed, while fructosamine is not. The objective of this study was to evaluate differences in plasma glucose peak in calves fed either whole milk (WM) or milk replacer (MR), to standardize sampling time to evaluate glycemia; and also to analyze plasma fructosamine concentrations as an indirect indicator of rumen development, replacing glucose. Sixteen Holstein calves, in a randomized complete block design, were individually housed with free access to water and starter (24% crude protein (CP); 46% nonfiber carbohydrate), and fed with 6 L of WM or MR (22.5% CP, 16.2% fat), split in 2 meals, until weaning at 56 d of age. Blood samples were collected weekly at 0 h, 1 h, 2 h, and 8 h after the morning feeding. Feed intake and fecal score were registered daily and body weights were recorded weekly. Data were analyzed as repeated measures by SAS, with treatments, time and week of sampling, and interactions as fixed effects. Feeding MR resulted in lower ADG ($P < 0.01$) and final BW (62.3 vs 72.8 kg; $P < 0.02$), which is likely explained by higher fecal score (2.13 vs 1.85; $P < 0.03$). Starter intake was not affected by liquid diet ($P > 0.05$), however, it increased with age ($P < 0.001$). Plasma glucose and fructosamine decreased with age ($P < 0.01$), while plasma BHB increased ($P < 0.01$). Plasma glucose peak was observed at 1h and 4h after feeding of WM or MR, respectively. Thus, these would be the best sampling times to assess the glycemic status according to different liquid diets. Because of its changes due to sampling time and age, plasma fructosamine did not improve the indirect evaluation of rumen development.

Table 1. Performance and plasma metabolites concentration

	Treatment			P-value[1]				
Item	WM	MR	SEM	T	W	H	T × W	T × H
Starter intake, g/d	292.0	257.0	0.42	0.57	<0.01	—	0.81	—
ADG, g/d	705.0	512.0	27.0	0.01	<0.01	—	0.24	—
Glucose, mg/dL	106.5	104.7	2.08	0.36	<0.01	<0.01	<0.01	0.01
BHB, mmol/L	0.110	0.062	0.005	0.01	<0.01	<0.01	<0.01	<0.01
Fructosamine, μmol/L	301.6	316.6	5.13	0.06	<0.01	<0.01	0.18	0.19

[1] T = treatment effect; W = week effect; H = hour effect; T × W = treatment by week interaction effect; T × H = treatment by hour interaction effect.

Key Words: milk replacer, β-hydroxybutyrate, glycemia

P347 Benefits of straw as a forage for dairy calves before and after weaning. G. Antúnez*[1], A. Bach[2,3], C. Cajarville[4], M. Vidal[2], F. Fàbregas[2], M. Ahangarani[5], and M. Terré[2], [1]*Bovine Production- IPAV, Facultad de Veterinaria, Universidad de la República, San José, Uruguay,* [2]*Department of Ruminant Production, IRTA, Caldes de Montbui, Spain,* [3]*Marlex, Sant Cugat de Vallès, Spain,* [4]*Animal Nutrition-IPAV, Facultad de Veterinaria, Universidad de la República,*

San José, Uruguay, [5]*College of Agriculture, Zabol University, Zabol, Irán.*

Forty-five Holstein male calves (3 ± 1.5 d age and 44 ± 5.7 kg of BW) individually penned with sawdust were randomly assigned in one of this forage feeding strategies: barley straw before and after weaning (SS), barley straw before and alfalfa hay after weaning (SA), or alfalfa hay before and after weaning (AA). All calves received the same milk replacer feeding program: 3L at 15% DM concentration in nipple-bottles twice a day once a day during the week before weaning at 57 d and concentrate feed ad libitum. Forage was supplied in a separated bucket at the rate of 7.5% (preweaning) and 15% (postweaning) of the previous day concentrate feed intake. Feed intake and BW were recorded daily and weekly, respectively. Rumen samples were obtained via a stomach tube at 53, 66, and 87 d of study and were composite in 3 samples of 5 animals each for further rumen microbiota analysis. A rumen epithelium sample was taken by endoscopy at 90 d to assess gene expression of selected markers (occluding, claudin-4, NHE-1, NHE-3, MCT-1, MCT-4, IL-6, TGFß). Data were analyzed with a mixed model that included the fixed effects of forage, week of study, and their interaction, and calf was considered as a random effect. The type of forage fed did not affect concentrate feed (461 ± 45.3 g/d), forage (27 ± 3.8 g/d), or total DM intake before weaning (1,239 ± 48.6 g/d). But SA and AA calves consumed less concentrate feed (3,504 vs. 2,970 ± 146.0 g/d; $P = 0.02$) and SA calves grew at lower rate after weaning than SS calves (1.08 and 1.26 kg/d, respectively). Expression of MCT-1 in the rumen epithelium was greater ($P < 0.05$) in SS than in AA and SA treatments (1.56 and 1.85, respectively). Rumen microbiota did not differ among treatments, but the relative abundance of *Acidaminococcus* and *Selenomas* genera increased ($P < 0.05$), and the relative abundance of *Alloprevotella*, *Bifidobaterium*, *Olsenella* and *Succiclasticum* genera decreased ($P < 0.05$) with calf age. In conclusion, feeding barley straw before and after weaning was more effective than feeding alfalfa hay in promoting concentrate feed intake after weaning and fostering an increase in the expression of MCT-1 in the rumen epithelium.

Key Words: calf, forage, rumen epithelium

P348 Effects of butyrate and medium-chain fatty acids supplementation on growth performance and metabolic response in dairy calves.

K. Murayama*[1,2], K. Sakamoto[3], K. Inouchi[1], and T. Sugino[2], [1]*Dairy Technology Research Institute, The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Nishishirakawa, Fukushima, Japan,* [2]*The Research Center for Animal Science, Graduate School of Integrated Science for Life, Hiroshima University, Higashi-Hiroshima, Japan,* [3]*YPTECH Co., Ltd, Chiyoda-ku, Tokyo, Japan.*

The aim of this study was to evaluate the effects of tributyrin (TB) and triglycerides containing medium-chain fatty acids (MCT) supplementation in milk replacer (MR) on growth performance and plasma metabolite and hormone concentrations in dairy calves. Sixty-three Holstein heifer calves [body weight (BW) at 8 d of age, 42.5 ± 0.39 kg; LSM ± SE] were randomly assigned to one of 4 experimental MR [28% CP, 18% fat either containing 3.2% caprylic acid and 2.8% capric acid (CONT; n = 15) or containing 6.7% caprylic acid and 6.4% capric acid (MCT; n = 16)] with/without 0.6% TB on DM basis (CONT+TB; n = 16, MCT+TB; n = 16) from 8 to 63 d of age. MRs were offered at 600 g/d (powder basis) from 8 to 14 d, up to 1,300 g/d from 15 to 21 d, 1,400 g/d from 22 to 49 d, down to 700 g/d from 50 to 56 d, 600 g/d from 57 to 63 d of age, then weaned on 64 d of age. All calves fed calf starter, chopped hay and water ad libitum. Data were analyzed by 2-way ANOVA using Fit Model procedure of JMP 15 Pro (SAS Institute Inc., Cary, NC). MCT and TB supplementation in MR did not affect dry matter intake. However, calves fed TB had greater BW at weaning transition period ($P = 0.05$; 87.9 ± 1.01 vs. 90.7 ± 0.97 kg) and after weaning ($P = 0.04$; 112.1 ± 1.50 vs. 116.5 ± 1.47 kg) compared with that of non-TB calves, respectively. And, calves fed MCT had greater feed efficiency before weaning ($P = 0.03$; 0.71 ± 0.010 vs. 0.74 ± 0.098 kg/kg) compared with that of non-MCT calves. Plasma metabolite and hormone concentrations were not affected by MCT and TB. These results suggest that MCT and TB supplementation in MR may elevate feed efficiency before weaning and elevate growth performance during weaning transition and after weaning.

Key Words: dairy calf, medium-chain fatty acid, butyrate

P349 Effects of limited forage supplementation on the intake, rumen fermentation, growth, and health condition in preweaning dairy calves.

J. Zhang*, Y. Cao, and J. Yao, *College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

Gastrointestinal development and health are the key goals in calves' rearing. Previous studies found that high-quality forage supplementation has the potential to increase rumen pH, relieve diarrhea, and/or improve growth performance. However, due to the limited gastrointestinal volume of preweaning calves, increased forage intake may decrease the intake of starter. This study aimed to evaluate the effects of limited forage supplementation on the growth, rumen fermentation, and health condition in preweaning dairy calves. Fifty-four healthy Holstein female calves were blocked by date of birth and assigned to 1 of 3 treatments that began at 30 d of age: only milk replacer and calf starter feeding group (CON), CON supplemented with 75 g/d (dry matter) alfalfa hay group (AH), and CON supplemented with 75 g/d (dry matter) oat hay group (OH). Feed and milk intakes were measured on 4 continuous days every week. Growth performance and rumen fluid and blood samples were measured or collected every 2 weeks. Health condition was checked daily. The data were analyzed using the mixed procedure of SAS 9.2 with treatment, time, and their interaction as the fixed effect. During the experimental period, the AH had greater forage intake but lower starter intake than the OH ($P < 0.01$). Milk, solid, and total dry matter intake were similar among treatments. The AH and OH had greater ($P = 0.04$) rumen pH and proportion of acetate ($P = 0.01$) but lower total volatile fatty acid concentration ($P < 0.01$) and proportions of propionate ($P < 0.01$) and valerate ($P < 0.01$) than that in the CON. The OH had a lower ruminal ammonia concentration ($P = 0.02$) than that in the CON. Treatments had no significant effects on body growth parameters, average daily gain, and feed efficiency. The AH tended to have greater blood β-hydroxybutyrate concentration ($P = 0.06$) than that in the CON. The AH and OH had lower diarrhea frequency than the CON on the third week of the experiment and the first week postweaning. In summary, even though body growth parameters were similar among treatments, limited forage supplementation in preweaning calves improved the rumen pH and health condition and influenced the fermentation parameters.

Key Words: preweaning calf, limited forage supplementation, health

P350 Impact of pork plasma on growth and health of Holstein calves: A randomized clinical trial.

D. Wood*[1], R. Blome[1], L. Ribeiro[1], A. Keunen[2], B. Keunen[2], J. Crenshaw[3], J. Campbell[3], and D. Renaud[4], [1]*Animix, Juneau, WI,* [2]*Mapleview Agri, Palmerston, ON, Canada,* [3]*APC, Ankeny, IA,* [4]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to compare the health and performance of calves fed different rates of porcine plasma in a milk replacer (CMR). Male Holstein calves (n = 320) averaging 47.8 kg body weight (BW) sourced from farms, auctions, and drovers arrived at the research facility in 4 batches of 80 head each and were housed in individual pens for the first 56 d of the experiment. Upon arrival calves were randomly assigned into one of 3 groups: (1) MR composed of whey-based proteins; (2) Inclusion of 5% spray-dried porcine plasma (15% of CP); or (3) Inclusion of 10% spray-dried porcine plasma (30% of CP). Calves were fed 2x daily a 26% CP, 20% fat CMR with amounts offered wk 1 being 0.65 kg/d, wk 2, 0.78 kg/d, wk 3, 0.91 kg/d, wk 4 and 5, 1.04 kg/d, wk 6, 0.78 kg/d, wk 7, 0.52 kg/d, and wk 8, 0.33 kg/d. Calves were also offered a texturized calf starter d 0 to 56 and then transitioned to a corn and pellet ration with refusals weighed back weekly. Calves were individually weighed upon arrival and weekly through wk 8 and upon completion of the study at d 78. Fecal consistency scores (0 = normal; 1 = semi-formed/pasty, 2 = loose, 3 = watery feces), respiratory scores (UC Davis system), and medical treatments were recorded twice daily. All statistical analyses were conducted in Stata 16 with linear regression models and Cox proportional hazard models used. Eleven calves (3.4%) died during the trial and calves spent 6.1%, 7.6%, and 8.4% of the first 28 d with a fecal score >2 in the whey, 5%, and 10% plasma groups, respectively. No differences in mortality or incidence of diarrhea were noted between groups. Despite a high incidence of respiratory disease (65% of calves treated), no differences were found between groups. On average, calves gained 42.2 kg BW pre-wean (d 0 to 56). No differences in growth were noted except on d 78 when BW was greater for calves in the control group compared with those fed a CMR with 5% plasma. Feed conversion was calculated and no differences were noted between groups. In this study, calves fed CMR composed of either 5% or 10% spray-dried porcine plasma performed comparably to a whey-protein-based milk replacer.

Key Words: plasma protein, milk replacer, dairy calves

P351 Postweaning performance growth of dairy calves is affected by preweaning feeding management.

J. Santillan[1,2], F. Frank[3], J. M. Cantet[4,5], R. A. Palladino[5], and F. Bargo*[5], [1]*Tinamu Dairy Farm, General Levalle, Córdoba, Argentina,* [2]*Graduate School UIB (INTA–FCA UNMdP), Balcarce, Buenos Aires, Argentina,* [3]*Veterinary private practice, Argentina,* [4]*Conicet, Argentina,* [5]*FIL–UBA, Buenos Aires, Argentina.*

We evaluated the effect of milk quantity (MQ: 4 vs. 8 L/d) and preweaning phase length (PWPL: 60 vs. 80 d) on growth performance of 120 female Holstein calves from a commercial dairy farm (34° 06′ 36” S, 64° 07′ 00” W). Calves were managed individually, received ad libitum water and starter (19.6% CP, 34.9% starch) and were randomly assigned to 4 treatments: 4 L/d milk 60 d (4L60d), 4 L/d milk 80 d (4L80d), 8 L/d milk 60 d (8L60d), and 8 L/d milk 80 d (8L80d). Starter and total DMI was measured daily. Body weight (BW) was recorded weekly preweaning and 2 times postweaning: at 100 d of age and at breeding age (when heifers reached 370 kg BW). Data were analyzed as repeated measures using the PROC MIXED procedure of SAS. The model included the fixed effects of treatments and the random effect of calves. Starter and total DMI preweaning were higher with longer PWPL but increments were higher with lower MQ (MQ × PWPL, $P < 0.05$): +0.295 vs. +0.058 kg/d and +0.322 and +0.107 kg/d for 4 vs. 8 L/d, respectively. A trend ($P = 0.09$) for interaction between MQ and PWPL was found for average daily gain (ADG) preweaning: calves fed with 4 L/d tended to have higher ADG when PWPL was increased. Postweaning, there was no interaction between MQ and PWPL for ADG. Calves with longer PWPL had 33% higher ADG (+0.254 kg/d; $P < 0.05$) from weaning to 100 d age; however, ADG from weaning to breeding was not affected by treatments. Our results show that early postweaning growth performance of dairy calves increases with a longer preweaning phase.

Table 1.

	Treatment					Effect, $P <$		
Item	4L60d	4L80d	8L60d	8L80d	SEM	MQ	PWPL	MQ × PWPL
Starter intake, kg DM/d	0.673^{b}	0.968^{a}	0.483^{c}	0.541^{c}	0.025	<0.01	<0.01	<0.01
Total intake, kg DM/d	1.082^{c}	1.404^{a}	1.283^{b}	1.390^{a}	0.026	<0.01	<0.01	<0.01
ADG								
0 d to weaning, kg/d	0.571^{a}	0.645^{ab}	0.675^{b}	0.667^{b}	0.024	0.01	0.17	0.09
weaning to 100 d, kg/d	0.745^{a}	0.959^{b}	0.797^{a}	1.090^{b}	0.054	0.08	<0.01	0.45
weaning to breeding, kg/d	0.892	0.935	0.911	0.925	0.018	0.80	0.11	0.41

$^{a–d}$Means with different superscripts differ ($P < 0.05$).

Key Words: growth performance, milk quantity, preweaning phase length

P352 Growth performance and development of the mammary gland of Holstein calves in northern Mexico fed preweaning milk with 15% solids and treated postweaning with growth promoters.

E. Carrillo-Moreno*[1], M. Mellado[1], E. Carrillo[2], E. Perez-Rebolloso[3], 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 feed intake, growth performance, blood metabolites and mammary gland parenchyma (PAR; ultrasound measurements) development in preweaning (0–60 d of age) Holstein female calves fed whole milk with milk replacer for 15% total solids (15% TS) or only milk (11% TS). Also, after weaning, this study investigated the effects of zilpaterol hydrochloride (ZH) and an estrogenic implant on growth performance, blood metabolites and PAR development in postweaning calves (60–90 d) previously fed milk with 15% TS. In the first phase, 78 female Holstein calves were randomly divided into 2 feeding programs (n = 39/group), 15% TS or 11% TS (5 to 8 L/day); treatments included starter ad libitum. After weaning, from 60 to 90 d, calves were randomly assigned to 4 treatments: estradiol implant (EI; 25.5 mg estradiol); zilpaterol hydrochloride (ZH; 8.3 mg/kg of DM); ZH^{+}EI or not treatments. In phase 1, calves fed 15% TS had greater ($P < 0.01$) average body weight at weaning than calves fed 11% TS (82.0 ± 5.8 vs. 74.1 ± 5.7 kg). Average daily gain was greater ($P < 0.01$) for calves fed 15% TS compared with calves given 11% TS (718 vs. 576 g/d). Calf fed 15% TS starter intake no differences ($P > 0.10$) than calves fed 11% TS. PAR area for calves fed 15% TS was 76% greater ($P < 0.01$) than calves fed 11% TS (8.7 ± 3.6 vs. 4.9 ± 1.8 cm^{2}). No differences ($P > 0.10$) in average daily gain (986 ± 79 g/d across treatments) were observed in calves receiving ZH, EI or ZH-EI with previous 15% TS or 11% TS diets. However, PAR area of calves administered ZH^{+}EI and previously fed 15% TS was 2.2 times ($P < 0.01$) greater than calves with no ZH and EI and fed 11% TS in the preweaning period. There was a postweaning treatment × preweaning

diets interaction for PAR variables. In conclusion, this study demonstrated the benefits of a feeding program supplying milk with 15% TS for enhancing overall animal growth and PAR development. Further use of ZH and EI during the postweaning period did not improve growth traits but markedly improved mammary PAR growth.

Key Words: growth, mammary gland, development.

P353 Effects of different feed types on the growth performance, rumen fermentation, and microbial community in preweaning dairy calves.

J. Zhang*[1,2], J. Shang[1], Y. Wang[1], Z. Cao[1], and S. Li[1], [1]*College of Animal Science and Technology, China Agricultural University, Beijing, China,* [2]*College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

This study aimed to evaluate the effects of corn silage and total mixed ration provided to calves after birth through weaning at 9 weeks of age. Forty-five healthy Holstein female calves were blocked by date of birth and assigned to 1 of 3 treatments that began at 2 d of age: 100% calf starter group (CON) with 20.3% crude protein (CP), 34.1% starch, and 4.5% ether extraction (EE), a mix of 75% calf starter and 15% corn silage dry matter group (CSS) with 18.8% CP, 34.5% starch, and 4.3% EE, and 100% early-lactation cows total mixed ration group (TMR) with 16.6% CP, 26.0% starch, and 5.5% EE. All dietary treatments were provided in addition to the same pasteurized normal milk feeding. Feed and milk intakes were measured daily. Body weight, withers height, body length, heart girth, and blood samples were measured or collected every 2 weeks. Rumen fluid was collected using an oral stomach tube on d 40 and 60 to measure the fermentation parameters. The bacterial community was determined based on the V3–V4 region of 16S rRNA gene amplicon sequencing of rumen fluid on d 60. Feeding cost was also measured. The intakes, growth, and fermentation data were analyzed using the mixed procedure of SAS 9.2 including treatment, week, and the interaction between them as fixed effects, and calf within treatment as a random effect. The microbial community was analyzed with the linear discriminant analysis (LDA) effect size (LEfSe). During the experimental period, feed intake and growth performance in the CON, CSS, and TMR had similar values. As expected, the week effect was significant, but treatments did no differ. Treatments had no significant effect on blood glucose, total protein, IgG, and insulin concentrations, except for that blood albumin concentration was tended to be greater ($P = 0.07$) in the CSS. Different feeds had similar ruminal fermentation parameters, except that CSS had lower ($P < 0.01$) pH than the TMR on d 40. LEfSe analysis showed that 52 taxa significantly changed among treatments. Among that, 14 genera presented in more than half of the samples were seen as the main significant genera among treatments. As one of the top 20 genera, *Acetitomaculum* was significantly enriched (LDA = 4.47) in the CON. Compared with the CON, the TMR had a lower ($P = 0.01$) total feeding cost. In summary, TMR feeding can be used in preweaning calves and decrease feeding costs without having a negative effect.

Key Words: preweaning calf, corn silage, total mixed ration

Ruminant Nutrition: Carbohydrates and Lipids: Posters

P354 Relative plasma bioavailability of omega-3 fatty acids derived from rumen-protected fish oil in lactating Holstein dairy cows.

J. E. Rico[1,2], V. Sáinz de la Maza-Escolà*[1,3], P. Deme[4], N. J. Haughey[4], R. Gervais[5], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Maryland, College Park, MD,* [3]*University of Bologna, Bologna, Italy,* [4]*Johns Hopkins University, Baltimore, MD,* [5]*Université Laval, Québec, Canada.*

Fish oil is a rich source of very-long-chain omega-3 (VLCn-3) fatty acids (FA). Feeding VLCn-3 FA may have beneficial effects on health, fertility, and milk production in the dairy cow. Rumen-protected (RP) technologies aim to prevent the ruminal biohydrogenation of VLCn-3 FA. The objective of this study was to determine the bioavailability of 2 RP fish oil products. Six mid-lactation, multiparous, pregnant Holstein dairy cows (155 ± 19 d in milk; 3.0 ± 0.5 body condition score; 3.2 ± 1.1 parity; 644 ± 23 kg body weight) were randomly assigned to treatments in a replicated 3 × 3 Latin Square design study. Treatments were provided as a single esophageal bolus in a gelatin capsule and included (1) fish oil microencapsulated with palm oil triglycerides, (2) fish oil encapsulated with modified starch, or (3) an empty gelatin capsule (i.e., control). The provision of either fish oil product provided a 10 g equivalent of VLCn-3 FA including C20:5n-3 and C22:6n-3. Cows were fed a conventional total mixed ration to meet or exceed nutrient requirements, and were milked thrice daily. Blood was sampled at 0, 2, 4, 6, 8, 10, 12, 16, 18, 20, 24, 36 h, relative to bolus administration. A 7-d washout period was utilized between each bolus delivery. Extracted plasma was analyzed for FA and phospholipid concentrations using chromatography and mass spectrometry. Data were analyzed under a mixed model with the random effect of cow and the fixed effects of treatment, hour and period. Plasma concentrations of C22:3n-3 ($P = 0.05$) and C22:5n-3 ($P < 0.01$) were increased by both fish oil supplements, relative to control. Both RP fish oil treatments increased plasma concentrations of lysophophatidylcholine-20:5, −22:5 and −22:6, and increased phosphatidylcholine (PC)-38:5 and −38:6, by h 10 ($P < 0.01$). Plasma PC-40:5 and −40:6 concentrations were greater in starch encapsulated fish oil by h 10, relative to triglyceride encapsulated fish oil ($P < 0.05$). We conclude that triglyceride or starch encapsulated fish oil increases the plasma concentrations of VLCn-3 FA and phospholipids containing these acyl chains in lactating cows.

Key Words: bioavailability, fish oil, omega-3

P355 Effects of abomasal infusions of fatty acids and one-carbon donors on milk fatty acids in lactating cows.

J. E. Rico[1], A. Javaid*[1], W. A. Myers[1], R. Gervais[2], and J. W. McFadden[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Département des Sciences Animales, Université Laval, Québec City, QC, Canada.*

Our objective was to define the effect of 16- and 22-carbon fatty acids (FA), choline chloride, and serine on milk fatty acids. In a 5 × 5 Latin square design, five rumen-cannulated Holstein cows (214 ± 4.9 DIM) were enrolled in a study with each experimental period lasting 6 d. Abomasal infusates consisted of (i) palmitic acid (PA; 98% 16:0 of total fat), (ii) PA + choline chloride (PA+C; 50 g/d choline chloride), (iii) PA + L-serine (PA+S; 170 g/d L-serine), (iv) behenic acid (BA; 92% 22:0 of total fat), and (v) docosahexaenoic acid algal oil (DHA, 47.5% 22:6 of total fat). Emulsions were formulated to provide 301 g/d of total FA and were balanced to provide a minimum of 40 and 19 g/d of 16:0 and glycerol, respectively. Cows were fed a corn silage-based diet. Milk samples were composited from samples obtained on d 5 and 6 of each experimental period and used for FA profiling. Data were analyzed using a mixed model (effects of treatment, cow and period). Milk 16:0 content was highest in PA, PA+C, and PA+S, relative to BA and DHA (e.g., 37 vs. 27% in PA and DHA, respectively; $P < 0.05$), while 16:0 yield was comparable across these treatments. Yield of 16:0 was higher in PA, relative to DHA (i.e.., 454 vs. 235 g/d; $P < 0.05$). Milk 22:0 content and yield were ~10-fold higher in BA, relative to all other treatments ($P < 0.05$). Similarly, infusing DHA resulted in higher content and yield of 22:6 in milk fat relative to all other treatments (41- and 38-fold higher, respectively; $P < 0.05$). As a consequence, the content of FA > 16-carbon (i.e., preformed) was higher in milk fat from cows infused BA and DHA, relative to PA ($P < 0.05$). The content of saturated FA was highest in PA, PA+C, and PA+S, relative to BA and DHA, whereas the content of PUFA was highest with DHA treatment ($P < 0.05$). De novo FA content in milk did not differ between PA, PA+C, and PA+S (16% of milk fat), but was higher in BA, and highest in DHA (19 and 21%, respectively; $P < 0.05$). No differences in de novo FA yield were observed with treatment. We conclude that the abomasal infusion of FA resulted in enrichment of infused 16-carbon and 22-carbon FA in milk fat.

Key Words: cow, fatty acid, milk fat

P356 Effects of choline, lipopolysaccharide, and docosahexaenoic acid on the lipidome of bovine precision-cut liver slices.

J. E. Rico*[2,1], V. Sáinz de la Maza-Escolà[3], W. A. Myers[1], B. N. Tate[1], C. Chang[1], and J. W. McFadden[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Department of Animal and Avian Sciences, University of Maryland, College Park, MD,* [3]*Dipartimento di Medicina Veterinaria, University of Bologna, Bologna, Italy.*

Choline and fatty acids (FA) are involved in phospholipid synthesis and may influence hepatic triglyceride (TG) deposition and secretion in dairy cows. Lipopolysaccharide (LPS) can induce inflammation and may increase the propensity for fatty liver in nonruminants. Liver was biopsied from pregnant Holstein dry cows (parity = 2.4 ± 0.5) and precision-cut liver slices (~200 μm) were incubated in serum-free M199 medium on titanium inserts under 95% oxygen/5% carbon dioxide within a roller culture incubator. Slices were incubated for 8h with either unsupplemented medium (no choline, LPS, or FA), 2 mM choline chloride (CC), 100ng O55:B5 LPS/mL (LPS), CC with LPS (CC+LPS), or CC with LPS and 200 μM docosahexaenoic acid (22:6; CC+LPS+DHA). Untargeted lipidomics was performed using liquid chromatography and mass spectrometry. For each experiment, 3 cows with 3 slices per cow were utilized (9 total reps/experiment). Protein-normalized, generalized log-transformed and auto-scaled data were analyzed using ANOVA and Fisher's LSD comparisons. Partial least squares discriminant analysis (PLS-DA) was performed. Analysis of variance identified differences for 32 metabolites including TG (n = 32), phosphatidylcholines (PC; n = 6), and Lysophosphatidylcholine (LPC; n = 2). The PLS-DA model distinguished slices treated with DHA, but no major lipid signatures distinguished CC from LPS. Changes in TG and PC were observed in response to treatment (variable importance of projection scores >3.0). Several TG and PC were distinctively enriched in slices treated with DHA (e.g., TG 18:0/22:4/22:6, TG 16:0/20:5/22:6, and PC 40:7, and PC 42:10, respectively; false discover rate [FDR] < 0.001). Treatment with DHA resulted in increased concentrations of TG enriched in 22:6, with no observable differences between all other treatments (e.g., TG 14:0/22:6/22:6, 14:0/22:6/22:6; $P < 0.05$). Similarly, highly unsaturated PC were increased only with DHA supplementation (e.g., PC 40:7, PC 40:8, and PC 42:10; $P < 0.05$). The robust response of the hepatic lipidome to the ex vivo supplementation with DHA appears to be independent of LPS or choline availability.

Key Words: cow, lipidome, liver slice

P357 Effect of calcium salts varying in oleic acid content on nutrient digestibility and oleic acid disappearance in continuous culture fermenters.

J. de Souza*[1], B. Wenner[2], and N. St-Pierre[1], [1]*Perdue Agribusiness, Salisbury, MD,* [2]*The Ohio State University, Columbus, OH.*

Calcium (Ca) salts of fatty acids (FA) are the most common rumen-inert FA supplements used in the dairy industry to minimize the negative impact of unsaturated FA on ruminal fermentation. The ionic bonds between Ca and other metals with FA are influenced by FA source. Our objective was to evaluate whether the content of oleic acid in a Ca-salt impacts nutrient digestibility and disappearance of oleic acid in continuous culture fermenters. In the present study, dual-flow continuous culture fermenters (n = 8) were used in replicated 4 × 2 truncated Latin squares, lasting 11 d each with 4 d of sample collection. Treatments were: control diet without supplemental FA (CON; 3.2% total FA); moderate oleic salts (MOL; diet supplemented with a Ca-salt with a moderate concentration of oleic acid – 45 g oleic acid/100 g FA); high oleic salts (HOL; diet supplemented with a Ca-salt with a high concentration of oleic acid – 75 g oleic acid/100 g FA); oleic as FA (FOL; diet supplemented with free oleic acid – 90 g oleic acid/100 g FA). MOL, HOL, and FOL were balanced to deliver the same amount of oleic acid (1.6% of diet DM) and total FA (5.7% of diet DM) using a 99% C16:0 supplement. The control diet (92 g DM/d) was a 50:50 alfalfa:concentrate pellet mixture that provided 14.8 g CP, 33 g NDF, 23 g ADF, and 14 g starch fed twice daily. Buffer dilution and solids passage rate were maintained at 7.0%/h and 5.0%/h, respectively. Data were analyzed using a mixed model including the fixed effect of treatment and fermenter, the random effect of the period, and a repeated statement for sampling day. We did not observe treatment differences for digestibility of NDF ($P = 0.44$; CON: 52.2, MOL: 54.0, HOL: 50.5, and FOL: 53.3%), ADF ($P = 0.78$; CON: 52.6, MOL: 54.2, HOL: 51.2, and FOL: 52.6%), and starch ($P = 0.63$; CON: 97.4, MOL: 97.8, HOL: 97.6, and FOL: 97.1%). The disappearance of oleic acid was greater for CON and FOL compared with MOL and HOL ($P < 0.01$; CON: 74.8, MOL: 42.9, HOL: 50.1, and FOL: 75.9%). Our results indicate that the concentration of oleic acid in the Ca-salt did not impact nutrient digestibility and disappearance of oleic acid; however, Ca-salts will have lower oleic acid disappearance than FA and the control diet.

Key Words: oleic acid, continuous culture fermenters, Ca-salts

P358 Productivity and nutrient digestibility of cows fed malt extract replacing ground corn.

M. Bugoni*, A. T. Nunes, R. G. Chesini, P. C. Vittorazzi Junior, N. T. S. Grigoletto, C. Takiya, G. Gomes da Silva, and F. P. Rennó, *Department of Animal Nutrition and Production, University of São Paulo, Pirassununga, SP, Brazil.*

Malt extract (ME) have been used in animal nutrition because of their rapidly fermentable energy content and micronutrients. The objective of this study was to evaluate the effects of ME on feed intake, milk yield and composition, productive efficiency, and total-tract apparent digestibility. Twenty-eight Holstein cows (35.3 ± 5.88 kg/d milk yield and 148 ± 78 DIM) were blocked according to milk yield, DMI, and BW and enrolled to a crossover design experiment. The treatment sequences were composed by 1) Control (CON), diet without malt extracts; and 2) Malt extract (ME; Liotécnica Tecnologia em Alimentos, Embu das Artes, Brazil), at 7.62% diet DM or replacing 46% of ground corn. Experimental periods lasted 21 d, of which the first 14 d were allowed to adaptation and the last 7 were assigned for sampling. Cows were milked twice per d and milk samples were collected for 3 d and analyzed fresh for fat, protein, and lactose. Apparent digestibility calculation was based on iNDF as an internal marker. Fecal samples were collected on d 15, 16, and 17 of each period on 9-h intervals. Ground samples of feeds, orts, and feces were placed in non-woven bags and incubated for 288 h in 2 cannulated cows. Then, samples were analyzed for NDF after removal. Data were analyzed using the MIXED procedure of SAS modeling the fixed effects of treatment, period, and their interaction besides the random effect of animal. Malt extract did not affect intake of DM and nutrients. No effects were detected on digestibility of DM and NDF. Yet, the ME group had greater digestibility of EE ($P = 0.002$) and CP ($P = 0.05$) compared with CON. Malt extract increased yields of milk ($P = 0.003$; 35.64 vs 34.43 kg/d for ME and CON, respectively), fat-corrected milk (FCM; $P = 0.001$), fat ($P = 0.004$), protein ($P = 0.026$), and lactose ($P = 0.019$). Feeding ME decreased milk urea nitrogen ($P = 0.038$). In terms of FCM, ME improved ($P = 0.015$) feed efficiency. Malt extract replacing ground corn improved yield of milk and components, feed efficiency, and nutrient digestibility.

Key Words: digestibility, starch, milk yield

P359 Effect of production level of dairy cows on the fatty acid profile of milk fat: A meta-analysis.

J. M. dos Santos Neto* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We performed a meta-analysis to evaluate the effects of production level on milk fatty acid (FA) profile of dairy cows. The data set included 405 individual observations from 182 Holstein cows from 12 studies at Michigan State University. Milk production levels were categorized as: Low (L), from 25.1 to 39.9 kg/d (33.9 ± 3.72 kg/d [mean ± SD], n = 135); Medium (M), from 40.1 to 49.8 kg/d (45.0 ± 3.03 kg/d, n = 135); and High (H), from 50.1 to 66.2 kg/d (56.5 ± 4.43 kg/d, n = 135). Diets (% DM) contained 30.6 ± 2.95 NDF, 26.4 ± 3.77 starch, and 3.27 ± 0.62 FA. Diets did not include supplemental FA. Statistical analyses were performed using the PROC MIXED of SAS, including the fixed effect of production level, and the random effects of study, period within study, and cow within study. Results are presented in the following sequence: L, M, and H and are reported as g/100 g FA in milk fat. As expected, increasing production level decreased milk fat content (4.17, 3.75, 3.55%; $P < 0.01$) and increased milk fat yield (1.48, 1.74, 1.98 kg/d; $P < 0.01$). Production level did not affect <16-carbon FA ($P = 0.60$) or >16-carbon FA ($P = 0.92$) but H reduced 16-carbon FA compared with L and M (33.1, 32.8, 32.0 g/100g; $P = 0.05$). H increased C4:0 (3.22, 3.22, 3.36 g/100g; $P = 0.02$) and tended to increase C6:0 (2.23, 2.18, 2.26 g/100g; $P = 0.07$) and decrease C16:0 (31.5, 31.4, 30.7 g/100g; $P = 0.10$). Production level had no effect on C8:0, C10:0, C12:0, C14:0, and C18:0 (all $P > 0.20$). H increased *cis*-9 C18:1 compared with M and had no effect compared with L (17.3, 16.9, 17.6 g/100g; $P = 0.04$). H and M increased C18:2 compared with L (2.52, 2.64, 2.68 g/100g; $P < 0.01$). H increased C18:3 compared with L and M; and M increased C18:3 compared with L (0.30, 0.32, 0.34 g/100g; $P < 0.01$). Further analyses should consider the effect of individual cow variation vs. stage of lactation on changes in milk FA profile. In conclusion, increasing production level decreased milk fat content, increased milk fat yield, and altered milk FA profile by decreasing C16:0 and increasing C4:0, C6:0, and 18-carbon unsaturated FA. These changes are likely associated with mammary gland plasticity to maintain milk fluidity.

Key Words: fatty acid profile, meta-analysis, milk fat

P360 Interaction of dietary unsaturated fatty acid level and acetate supply on milk fat production in Holstein cows.

A. N. Staffin*, R. Bomberger, C. Matamoros, S. L. Bennett, and K. J. Harvatine, *Penn State University, University Park, PA.*

Milk fat is a major source of dairy farm income and methods to increase milk fat yield are of strong interest to producers and nutritionists. Acetate supplementation has been reported to increase milk fat in lactating cows, but it is expected that dietary factors that impact ruminal acetate synthesis will impact response to supplementation. Unsaturated fatty acids modify the rumen microbiome and may decrease fiber digestion and acetate synthesis. The objective of this experiment was to test the interaction between acetate supplementation and dietary unsaturated fatty acid concentration. The hypothesis was that acetate would increase milk fat yield more when supplemented in diets containing high levels of unsaturated fatty acids. Twelve multiparous Holstein cows were arranged in a replicated 4x4 Latin square with a 2x2 factorial design with 21 d periods. Treatments were a no supplement control (CON), 600 g/d of acetate as sodium acetate (AC), soybean oil at 1.5% DM (UFA), and a combination of UFA and AC. Data were analyzed with a mixed model that included the random effect of cow and period and fixed effect of acetate and unsaturated fatty acid concentration in JMP Pro 15. Both AC and UFA increased milk production (1.2 and 1.7 kg/d, respectively, $P < 0.001$), although there was no interaction of AC and UFA. There was also no interaction of AC and UFA for milk fat concentration and yield, but AC increased milk fat yield 150 g/d ($P < 0.001$) and milk fat percent 0.24 units ($P = 0.01$). Milk fat concentration and yield were unaffected by UFA. Milk protein percent was slightly decreased by AC (3.02 to 2.99%, $P = 0.04$), but UFA did not affect milk protein percent and neither treatment affected milk protein yield. In conclusion, both AC and UFA increased milk production and AC increased milk fat yield and percent, regardless of dietary level of unsaturated fatty acids.

Key Words: lactation, nutrition, lipogenesis

P361 Effect of C16:0 supplementation at different lactation stages on production responses of dairy cows: A meta-analysis.

J. M. dos Santos Neto* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We performed a meta-analysis to evaluate the effects C16:0 supplementation across different stages of lactation on production responses of multiparous dairy cows. The data set was assembled with 419 individual observations from 88 Holstein cows from 4 studies. Treatments were: (1) non-fatty acid (FA) supplemented control diets (CON, n = 207); and (2) diets supplemented with a C16:0-enriched supplement containing ~80% C16:0 (PA, n = 212). Lactation stages were categorized as: (1) from 1 to 24 DIM (Fresh, n = 150), (2) from 25 to 67 DIM (Peak, n = 147), and from 145 to 215 DIM (Mid, n = 122). C16:0-enriched supplements were added at 1.5% of diet DM replacing soyhulls in CON. CON diets (% DM) contained (mean ± SD) 31.1 ± 0.69 NDF, 26.0 ± 1.50 starch, and 3.24 ± 0.32 FA. PA diets (% DM) contained 30.2 ± 0.53 NDF, 25.9 ± 1.49 starch, and 4.74 ± 0.31 FA. Statistical analyses were performed using the PROC MIXED of SAS, including the fixed effects of treatment, lactation and their interactions, and the random effects of study, period within study, and cow within study. Overall, compared with CON, PA increased the yields of milk (1.20 kg/d), milk fat (0.19 kg/d), milk protein (0.07 kg/d), ECM (2.80 kg/d), and de novo (25.0 g/d), mixed (100 g/d), and preformed milk FA (65.0 g/d; all $P < 0.05$). No interactions between treatment and lactation stage were observed for the yields of milk ($P = 0.59$), milk fat ($P = 0.96$), ECM ($P = 0.22$), or preformed milk FA ($P = 0.21$). Some interactions between treatment and lactation stage were observed; PA increased DMI in Mid (1.04 kg/d; $P = 0.10$), increased milk protein yield in Fresh (0.14 kg/d; $P < 0.01$), and de novo milk FA yield in Peak (58 g/d; $P < 0.01$) but did not alter these variables at the other lactation stages. PA increased mixed milk FA yields to different extents across lactation stages (Fresh 132 g/d, Mid 126 g/d, Peak 43 g/d; $P = 0.03$). In conclusion, C16:0 supplementation consistently increased the yields of milk, milk fat, and ECM, and interacted with lactation stage by increasing DMI in Mid, milk protein yield in Fresh, de novo milk FA yield in Peak, and mixed milk FA yield in all lactation stages.

Key Words: lactation stage, meta-analysis, palmitic acid

P362 Effect of feeding palmitic acid on milk fatty acid profile of dairy cows: A meta-regression.

J. de Souza*[1], J. M. dos Santos Neto[2], and A. L. Lock[2], [1]*Perdue AgriBusiness, Salisbury, MA,* [2]*Michigan State University, East Lansing, MI.*

We determined how the intake of palmitic acid (C16:0) alters the content of individual milk fatty acids (FA). Our analysis used individual observations (n = 1,202) from 212 Holstein cows from 14 studies. Diets (% DM) contained (mean ± SD) 30.6 ± 3.66 NDF, 27.1 ± 2.16 starch, and 4.0 ± 0.97 total FA. Intake of C16:0 averaged 371 g/d and ranged from 60 to 923 g/d. Mixed model regressions were developed taking into account study, period within study, and cow within study as random factors. Equations report the relationship between C16:0 intake (g/d) and the content of FA in milk fat (g/100 g FA). Increasing 16:0 intake linearly increased 16-carbon milk FA [$32.4 \pm 0.63 + 0.011 \pm 0.0003 \times$ 16:0 intake; $P < 0.01$], and linearly decreased < 16-carbon FA [$25.6 \pm 0.85 - 0.0037 \pm 0.0003 \times$ 16:0 intake; $P < 0.01$], and > 16-carbon FA [$42.1 \pm 1.0657 - 0.0063 \pm 0.0005 \times$ 16:0 intake; $P < 0.01$]. Thus, for every 100 g of 16:0 intake, 16-carbon FA increased by 1.13 g/100 g while < 16-carbon and > 16-carbon milk FA decreased by 0.371 and 0.657 g/100 g, respectively. For individual milk FA, increasing C16:0 intake linearly increased milk C4:0 [$2.96 \pm 0.09 + 0.0002 \pm 0.00004 \times$ 16:0 intake; $P < 0.01$] and C16:0 [$30.4 \pm 0.61 + 0.011 \pm 0.0003 \times$ 16:0 intake; $P < 0.01$]. In contrast, increasing C16:0 intake linearly decreased milk C6:0 [$2.02 \pm 0.05 - 0.0001 \pm 0.00003 \times$ 16:0 intake; $P < 0.01$], C8:0 [$1.20 \pm 0.04 - 0.0002 \pm 0.00002 \times$ 16:0 intake; $P < 0.01$], C10:0 [$3.07 \pm 0.13 - 0.0008 \pm 0.00006 \times$ 16:0 intake; $P < 0.001$], C12:0 [$3.61 \pm 0.17 - 0.001 \pm 0.00008 \times$ 16:0 intake; $P < 0.01$], C14:0 [$11.6 \pm 0.36 - 0.013 \pm 0.0004 \times$ 16:0 intake; $P < 0.01$], C18:0 [$10.1 \pm 0.41 - 0.002 \pm 0.0002 \times$ 16:0 intake; $P < 0.01$], and *cis*-9 C18:1 [$19.1 \pm 0.82 - 0.001 \pm 0.0003 \times$ 16:0 intake; $P < 0.01$]. Thus, for every 100 g of 16:0 intake, C4:0 and C16:0 increased by 0.021 and 1.10 g/100 g, and decreased C6:0, C8:0, C10:0, C12:0, C14:0, C18:0, and *cis*-9 C18:1 by 0.01, 0.02, 0.08, 0.11, 0.23, 0.22, and 0.11 g/100 g, respectively. In conclusion, increasing dietary intake of C16:0 increases the content of C16:0 and C4:0 and decreases the content of 6- to 18-carbon saturated FA in milk fat. These changes are likely associated with mammary gland plasticity maintaining milk fat fluidity.

Key Words: meta-analysis, milk fat, palmitic acid

P363 Effect of prilled saturated fats or calcium salts of fatty acids on feed intake and milk production: A meta-analysis.

F. Díaz*, J. Sánchez-Duarte, and N. Garcia, *Dellait Dairy Research Center, Brookings, SD.*

Energy density of supplemental fat may improve performance of high-producing dairy cows. The objective of this meta-analysis was to evaluate the effects of supplemented sources of prilled saturated fats (PSF; Energy Booster 100) or calcium salts of fatty acids (CaFA; Megalac; EnerG-II) on dry matter intake (DMI), milk yield and milk composition in dairy cows. Data from 8 published papers (2005–2019) were used to contrast diets containing PSF versus CaFA diets. Statistical analysis was performed using fixed or random effect models using the Metafor package for R. The degree of heterogeneity was measured with I^2 statistic, and publication bias was determined with funnel plots and Egger's regression test. Sources of heterogeneity of response were analyzed through a mixed-effect meta-regression using the level of dietary forage, concentrate, PSF, CaFA, CP, and NDF as moderator variables. Publication bias was detected for ECM, and milk fat concentration and yield. Meta-regression indicated no effects of moderator variables on study effect size ($P = 0.05$). The highest degree of heterogeneity was observed for milk yield (68.6%) and ECM (89.2%), indicating that milk responses to supplemental fats were different. Compared with CaFA supplemented diets, cows fed PSF diets increased DMI (1.15 k/d; $P = 0.002$) and tended to increase milk yield (0.66 kg/d; $P = 0.09$) and ECM (1.47 kg/d; $P = 0.08$). Milk component concentration and yield were not affected by supplemental fat source ($P = 0.05$). In conclusion, these results show that lactating dairy cows fed prilled saturated fats increased DMI and tended to produce more milk than cows receiving calcium salts of fatty acids.

Key Words: fat supplementation, milk yield, meta-analysis

P364 Effect of palmitic acid supplementation on lactating dairy cow performance.

F. Díaz*, J. Sánchez-Duarte, A. Garcia, and N. Garcia, *Dellait Dairy Research Center, Brookings, SD.*

The demand for butterfat in the United States together with higher milk fat prices have increased the use of fat supplements enriched with palmitic acid (C16:0). Recent studies have shown that feeding palmitic acid to lactating cows increases milk fat production. The objective of this study was to conduct a meta-regression to evaluate the effects of dietary C16:0 intake on lactating dairy cow performance. The study included 11 trials conducted between 2007 and 2019, with 33 treatment means in which C16:0 enriched fatty acid supplements were fed to dairy cows. Palmitic acid supplements were added to the diets at 0 – 6.0% DM so cows consumed up to 1,248 g of C16:0 daily. A mixed model meta-regression was conducted using the random effect of study weighing by the inverse of the standard error of the means squared. Linear and quadratic effects of dietary C16:0 on dry matter intake (DMI), energy-corrected milk (ECM) yield, and milk component concentration and yield were evaluated. The best fit model was chosen based on the lowest Akaike information criterion (AIC) and Root mean squared error (RMSE). Residual vs. fitted values and Q-Q plots were used to identify the heteroscedasticity and normality of the final models, respectively. Results (Table 1) indicated that increasing dietary C16:0 intake linearly increased ECM ($P = 0.005$), and milk fat concentration ($P = 0.005$) and yield ($P \leq 0.001$). However, increasing dietary C16:0 intake did not affect DMI and milk protein yield. Interestingly, milk protein concentration decreased as dietary C16:0 intake increased ($P = 0.01$). In conclusion, these results demonstrate the potential of C16:0 enriched fat supplements to improve milk fat concentration and yield in lactating dairy cows.

Table 1. Coefficients and respective standard error (SE) for predictors of the final models in dairy cows fed C16:0-enriched fat supplements

Item	n	Linear coefficient	SE	*3*-value	RMSE	AIC
DMI, kg/d	33	0.00012	0.0008	0.88	0.13	151.6
ECM, kg/d	33	0.00439	0.0014	0.005	0.06	204.9
Milk fat, %	33	0.00033	0.0001	0.005	0.12	31.2
Milk fat yield, kg/d	33	0.00024	0.00004	0.001	0.04	−11.2
Milk protein, %	33	−0.00007	0.00003	0.01	0.07	−41.2
Milk protein yield, kg/d	33	0.00005	0.00005	0.31	0.08	−3.6

Key Words: fatty acids, dairy cow, meta-analysis

P365 Effect of dietary lipid supplementation on milk production and composition by dairy ruminants.

W. B. Gallardo*, L. E. Oliveira, and I. A. M. A. Teixeira, *UNESP-Universidade Estadual Paulista, Jaboticabal, SP, Brazil.*

Our objective was to investigate how dietary lipid source affects the milk production and composition by dairy ruminants using a meta-analytical approach. We used a database composed of 88 peer-reviewed studies with dairy cows (n = 3,490), goats (n = 92), buffaloes (n = 50), and sheep (n = 665), comprising 220 treatments; the magnitude of the effect was evaluated using raw mean differences between lipid sources and the control treatment. The means were weighted by inverse variance and for heterogeneity analysis, lipid sources were divided into 10 subgroups (algae, branched-chain fatty acids (BCFA), blends, calcium salts of fatty acids (CSFA), CLA, essential oils, fish oil, oilseeds, palmitic acid, and vegetable oils). Milk production (kg/d) increased ($P < 0.01$) when BCFA (1.01 [0.26, 1.77]), CSFA (0.85 [0.38, 1.32]), essential oils (0.74 [0.35, 1.13]), palmitic acid (1.60 [0.77, 2.42]), and vegetables oils (0.30 [0.02, 0.58]) were supplemented. Milk fat and protein (g/kg) decreased ($P < 0.01$), respectively, when algae (−4.07 [−6.47, −1.66] and −0.29 [−0.51, −0.07]), blends (−4.28 [−6.01, −2.55] and −1.60 [−2.39, −0.80]), and vegetable oils (−1.64 [−2.72, −0.57] and −0.36 [−0.72, −0.01]) were supplemented. However, milk fat and protein increased ($P < 0.01$) with BCFA (3.31 [2.39, 4.24] and 0.92 [0.17, 1.67]) supplementation. In addition, milk fat decreased ($P < 0.05$) with CLA (−11.61 [−18.26, −4.96]) and fish oil (−5.00 [−9.10, −0.91]) supplementation and milk fat increased ($P < 0.05$) with palmitic acid supplementation (2.37 [0.74, 3.99]). Lipid source also altered the milk fatty acid profile (g/100g FA). Saturated fatty acids (SFA) concentration decreased ($P < 0.05$) with algae (−2.34 [−3.54, −1.15]), blends (−6.38 [−8.89, −3.86]), oilseeds (−6.11 [−8.29, −3.92]) and vegetable oils (−7.43 [−9.04, −5.81]) supplementation. The concentrations of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) increased ($P < 0.01$) with algae (1.14 [0.22, 2.06] and 0.56 [0.30, 0.83]), blends (5.84 [4.30, 7.38] and 2.05 [1.11, 2.29]), oilseeds (4.45 [2.88, 6.02] and 1.11 [0.60, 1.63]), and vegetable oils (5.71 [4.21, 7.21] and 0.68 [0.57, 0.78]) supplementation. The supplementation of dietary CSFA did not affect MUFA, but it affected PUFA (0.72 [0.07, 1.38]). In conclusion, unsaturated fatty acids supplementation increased milk production, altered fatty acid profile of milk, and decreased milk fat.

Key Words: fatty acid, milk fat, dairy ruminants

P366 Effects of medium-chain fatty acid supplementation on performance and rumen fermentation of lactating dairy cows.

M. Burdick* and M. Oba, *University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to evaluate the effects of medium-chain fatty acid (MCFA) supplementation on dry matter intake, milk and component yield, digestibility, and rumen fermentation. Thirty (n = 8 primiparous, n = 22 multiparous) Holstein cows in mid-lactation (637 ± 68.5 body weight, 98.5 ± 27.4 d in milk; mean ± SD) were used in a crossover design with 25 d diet adaptation and 3 d sample collection periods. The MCFA supplement (containing 8.0% C8:0, 5.25% C10:0, 11.75% C12:0 on DM basis) was fed at 0.25% of diet dry matter (TRT), replacing dry ground corn in control (CON). Statistical analysis was conducted using the Fit Model procedure of JMP. No differences were observed in dry matter intake ($P = 0.63$), apparent total-tract nutrient digestibility ($P > 0.20$) and body weight change ($P = 0.49$) between TRT and CON. However, there was a tendency toward a negative correlation between pre-trial milk yield and animal response to TRT, where high-producing cows increased BW to a lesser extent ($P = 0.06$; $r^2 = 0.121$). In addition, although milk yield did not differ between treatment groups ($P = 0.31$), a parity by treatment interaction was observed ($P = 0.06$), where lactose yield tended to be lower for TRT compared with CON in primiparous cows (1.45 v. 1.51 kg/d; $P < 0.01$) but it was not affected by TRT in multiparous cows. Fat and protein yield did not differ between treatments, but higher producing cows tended to decrease protein yield to a greater extent when MCFA was supplemented ($P = 0.04$; $r^2 = 0.142$). Minimum rumen pH tended to be higher in TRT compared with CON (5.66 v. 5.54; $P = 0.08$). Daily range of rumen pH was reduced in TRT compared with CON (1.17 vs. 1.40; $P = 0.02$), however, duration of acidosis (pH < 5.8, min/d) did not differ between treatment groups ($P = 0.12$). Total rumen volatile fatty acid concentration and its profile did not differ between treatment groups. These results suggest that supplementation of MCFA at 0.25% of diet dry matter may not affect overall animal performance or diet digestibility but may stabilize rumen fermentation by decreasing the fluctuation of rumen pH.

Key Words: medium-chain fatty acid, rumen health

P367 Effect of a coated omega-3 source on the performance and fertility of dairy cows in a commercial farm.

L. Royo*, M. Puyalto, and J. J. Mallo, *Norel SA, Madrid, Spain.*

With the aim of evaluating the effect of a coated omega-3 source on the performance and fertility of dairy cows, 150 Holstein cows were enrolled in a commercial trial. Animals were divided in 2 groups (n = 75), one treatment per pen due to limited facilities. Both pens received a basal diet (CP 17.4%; NDF 31.5%; starch 22%) twice daily. Control group (CT) received 400 g/d of calcium soap (0% omega-3) and omega-3 group (O3) received 440 g/d of coated omega-3 source (HIFLAX, 13.8% omega-3). Diets were isocaloric and cows were fed for 4 mo. Daily milk yield and composition (milk fat and protein content) were measure individually. Once per month individual samples of milk were taken to measure omega-3 fatty acids (FA); milk yield and composition data were summarized monthly. For fertility evaluation, days open and the number of artificial inseminations (AI) until pregnancy were recorded (n = 27). Pregnancies were evaluated 1 mo after AIs. First group of cows inseminated had been in treatment at least for 10 d. A mixed-effect model was used to analyze the data, using animal as experimental unit. Treatment, month, parity and days in milk were used as fixed effects and cows as random effect for productive parameters and treatments and days in milk were used as fixed effects and cows as random effect for the reproductive parameter's evaluation. Milk yield was no different ($P = 0.13$) between treatments, but O3 cow produce numerically more milk than CT ones. Milk fat (%) and protein (%) were no different ($P = 0.50$ and 0.88 respectively) between treatments. The content of omega-3 in milk tended ($P = 0.07$) to be higher in O3 cows, comparing with CT ones. The number of inseminations was not different ($P = 0.28$) between treatments. Days open were significantly ($P = 0.05$) reduced in O3 cows (184 d), comparing with CT ones (144 d). It was concluded that the supplementation of dairy cows with a coated source of omega-3 FA do not increase milk yield, but is a valid strategy to increase the omega-3 FA in milk and to improve the reproductive performance of dairy cows.

Key Words: omega-3, days open, fertility

P368 Effects of branched-chain amino acids on rumen branched-chain fatty acids profile during in vitro ruminal fermentation.

L. Sun[1], L. Liu[2], T. Brenna[3], Z. Wu[1], L. Ma[1], J. Xu[4], and D. Bu*[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Hunan Agricultural University, Hunan, China,* [3]*Dell Pediatric Research Institute and the Depts of Nutrition and of Chemistry, University of Texas at Austin, Austin, TX,* [4]*Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China.*

Our objective was to investigate the effects of addition of branched-chain amino acids on rumen branched-chain fatty acids (BCFA) during in vitro ruminal fermentation. The in vitro ruminal batch culture technology was used to exam the effects of 1, 2, 4, and 6 mM leucine, isoleucine or valine on ruminal fermentation parameters and BCFA profile with 24 h of incubation. Each experimental treatment had 5 replicates and experiments were repeated in triplicate. The solid and liquid phase were collected from each fermentation bottle at 24 h and used for analysis of fermentation parameters and fatty acids. Data were analyzed using the PROC MIXED procedure of SAS. Treatment effects were detected using linear, quadratic orthogonal polynomial contrasts. The addition of leucine increased gas production ($P = 0.02$) and dry matter degradation ($P = 0.02$). The volatile fatty acid (VFA) including acetate ($P = 0.02$), propionate ($P = 0.03$), isobutyrate ($P = 0.02$) and butyrate ($P = 0.07$) increased from 0 to 4 mM then decreased at 6 mM leucine. Compared with Control, at 4 mM leucine, BCFA including *iso*-14:0 ($P = 0.01$), *iso*-15:0 ($P < 0.01$), *anteiso*-15:0 ($P = 0.01$) were maximal, and decreased at 6 mM. Similarly, the addition of isoleucine increased *anteiso*-15:0 ($P < 0.01$) and *iso*-16:0 ($P < 0.01$), and valine increased *iso*-14:0 ($P = 0.01$), *iso*-15:0 ($P < 0.01$) and *anteiso*-15:0 ($P < 0.01$) at 1 to 4 mM, which had a decreasing trend at 6 mM. In conclusion, branched-chain amino acids increased rumen VFA and BCFA in a dose-dependent manner to a maximal level at ≤4 mM.

Key Words: branched-chain fatty acid, branched-chain amino acid, rumen fermentation

P369 In vitro evaluation of a novel technology used to protect polyunsaturated fatty acids from ruminal biohydrogenation.

J. P. Russi*[2], P. Dieter[1], and A. Relling[1], [1]*The Ohio State University, Wooster, OH,* [2]*One Idea LLC, Merced, CA.*

The objective of this experiment was to test a method to produce rumen-protected polyunsaturated fatty acids (PUFA). Four different products (treatments) were A) 24% fish oil, 52% lecithin, 4% sugar, and 20% water; B) 24% fish oil, 48% lecithin, 8% sugar, and 20% water; C) 32% fish oil, 44% lecithin, 4% sugar, and 20% water; and D) 32% fish oil, 40% lecithin, 8% sugar, and 20% water; prepared following the procedure under the pending US patent # 2020/0197346-A1. Treated samples and unheated controls (similar mix without a cooking step) of each treatment were incubated in vitro for 0, 4, 8, and 12 h (all in triplicates). For the incubation 0.5 g of alfalfa hay and corn grain (50:50 vol:vol), 5 mL of rumen content diluted in 15 mL of media, and 30 mg of samples were added in 50 mL test tubes at 37°C. At the end of each incubation time, tubes were flash frozen in liquid nitrogen to stop all biohydrogenation. Once all the samples were collected, fatty acids were extracted, methylated, and run on a gas chromatography. First, data were analyzed as fatty acids (FA) difference between each treated (time 0 only) and not treated samples (fixed effect of treatment, PROC MIXED SAS 9.4). Second, incubation data were analyzed as treatment, time, and their interaction (PROC MIXED SAS 9.4). Means were separated at each time using the PDIFF option of SAS. Biohydrogenation protection (%) of the sum of eicosapentaenoic acid (EPA) and eicosapentaenoic acid (DHA; FA exclusive from fish oil) was calculated using the sum of the FA concentration of the time 0 as a 100% of the FA. There were no differences ($P \geq 0.25$) in the relative amount of EPA and DHA on the prepared and not prepared samples. There was a treatment and a time effect ($P \leq 0.04$; Table 1). Products A, C, and D showed the lowest biohydrogenation of total EPA and DHA after 4h of incubation. However, after 12 h of incubation no differnece on biohydrogenation were detected. Therefore, the biohydrogenation rate depends on the method on how the product is made and the time that it stays in the rumen environment.

Table 1. Percentage of the sum of EPA and DHA after different time of in vitro incubation

Fermentation time, h	Treatment (Trt)				SEM	*P*-value		
	A	B	C	D		Time	Trt	T × Trt
4	71.8^{a}	50.9^{b}	68.3^{ab}	67.3^{ab}	6.56	<0.01	0.04	0.24
8	38.8^{ab}	31.1^{ab}	54.2^{a}	51.5^{a}				
12	34.1	41.7	38.4	50.1				

^{a,b}Means within a row with different superscripts differ at a $P < 0.05$.

Key Words: by-pass lipids, PUFA, DHA and EPA

P370 Amla (*Phyllanthus emblica*) fresh fruit as new feed source: II. Effect on milk antioxidant capacity, milk metabolites, and fatty acid profiles in lactating dairy cows. M. T. Meselu[1], L. Zhao[1], L. Sun[1], L. Ma[1], T. Callaway[3], J. Xu[2,5], and D. Bu*[1,4], [1]*Institute of Animal Science, Beijing, China,* [2]*Kunming Institute of Botany, Kunming, China,* [3]*University of Georgia, Athens,* [4]*Joint Laboratory on Integrated Crop-Tree-Livestock Systems of the Chinese Academy of Agricultural Sciences (CAAS), Ethiopian Institute of Agricultural Research (EIAR) and World Agroforestry Center (ICRAF), Beijing, China,* [5]*World Agroforestry Centre East and Central Asia, Kunming, China.*

Amla fruit, or indian gooseberry *(Phyllanthus emblica)* is a common deciduous fruit crop in Asia. The effects of feeding 3 levels of fresh amla fruit on milk antioxidant capacity and fatty acid profiles were examined in lactating dairy cows. Eight ruminally cannulated mid-lactation dairy cows were assigned to 2 treatment groups in a repeated crossover design. After a 2-wk adaptation period, during the first 6-wk experimental period (period 1), the first group of 4 cows received TMR feed without fresh amla fruit (control group). The remaining 4 cows sequentially received TMR supplemented with each of 3 levels of fresh amla fruit (e.g., 200, 400, then 600 g/d) (treatment group) at 14-d intervals (i.e., 3 subperiods). In period 2, control and treatment groups were exchanged For each subperiod, the first 10 d were adjusted to diet adaptation and the last 4 d for sampling milk and feed. Data were analyzed by using PROC MIXED of SAS. Amla fruit feeding at 400g/d increased milk ferric reducing–antioxidant power (FRAP) (+27.4%, $P = 0.02$), and ascorbic acid content in milk (+66.67%, $P = 0.04$). Phenolic content of milk was increased ($P = 0.04$) at both 400g/d (+14.3%) and 600g/d (+16.7%) supplementation compared with controls. The milk FA profile was characterized by a reduction in C12:0 ($P < 0.001$), and C14:0 ($P = 0.02$) at 200g/d by (−13.3 and −10.0%) and 400g/d by (−42.2 and −19.5%), respectively. In addition, C16:0 milk FA was reduced ($P = 0.04$) by −11.3% (200g/d) and −9.1% (400g/d) while increments in the proportions of C12:0, C14:0 and C16:0 was observed at 600g/d. Both 200 and 400g/d amla supplementation reduced de novo FA ($P < 0.001$) and mixed FA ($P = 0.04$). However, Preformed FA was increased ($P = 0.02$) at 200g/d (+18.3%), and 400g/d (+17.4%). In addition, MUFA ($P = 0.04$), UFA ($P < 0.01$) and OBCFA ($P = 0.04$) were increased at 200 and 400g/d and reduced at 600g/d amla supplementation. Our findings suggest fresh amla fruit supplementation has the potential to modulate the FA profile of milk fat in a dose-dependent manner. According to the findings, amla fruit can be incorporated into dairy cow rations at levels ranging from 200 to 400 g/d fresh, or 0.23 to 0.47% DM, to improve milk antioxidant capacity and fatty acid profiles.

Key Words: amla, antioxidant capacity, milk fatty acid

P371 Abomasal infusions of oleic acid and an exogenous emulsifier alter production responses of lactating dairy cows. J. M. dos Santos Neto*, C. M. Prom, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of abomasal infusions of oleic acid and an exogenous emulsifier on production responses of lactating dairy cows. Eight rumen-cannulated multiparous cows (110 ± 20 DIM) were assigned to a 2x2 factorial arrangement of treatments in 4 × 4 Latin squares with 18 d periods consisting of 7 d of washout and 11 d of infusion with sampling on the last 4 d. Treatments were abomasal infusions at 6 h intervals of water carrier only (CON), 60 g/d oleic acid (OA), 30 g/d polysorbate-C18:1 (Tween-80; T80), or both OA and T80 (BOTH). OA treatments were dissolved in ethanol and T80 treatments in water. Cows were fed the same diet which contained (% DM) 29% NDF, 17% CP, 28% starch, and 3.5% fatty acids (FA; 1.8% DM from a FA supplement containing 34% C16:0 and 47% C18:0). The statistical model included the random effect of cow within square and the fixed effects of OA, T80, period, and their interactions. Significance was declared at $P \leq 0.05$ for main effects and $P \leq 0.10$ for interactions. Results are presented in the following order: CON, OA, T80, and BOTH. There was no effect of treatment on DMI ($P > 0.65$). Overall, OA did not affect yield of milk or milk components (all $P > 0.14$), whereas T80 increased the yields of milk and milk lactose (both $P < 0.05$), but also did not affect the yields of milk fat and protein (both $P > 0.13$) compared with CON. Interactions between OA and T80 were observed for the yields of milk fat (1.77, 1.85, 1.83, and 1.72 kg/d; $P < 0.01$), 3.5% FCM (48.0, 50.0, 50.0, and 48.3 kg/d; $P < 0.01$), and ECM (48.1, 50.0, 50.0, and 48.6 kg/d; $P < 0.01$) with OA and T80 increasing the yields of milk fat, 3.5% FCM, and ECM compared with CON and BOTH. Interactions between OA and T80 were also observed for milk fat content (3.98, 4.00, 3.90, and 3.71%; $P = 0.10$), feed efficiency (ECM/DMI, 1.53, 1.62, 1.60, and 1.57; $P < 0.01$), and body weight (728, 729, 734, and 726 kg; $P = 0.06$). In conclusion, infusion of oleic acid or an exogenous emulsifier increased the yields of milk fat, 3.5% FCM, and ECM when infused alone. However, when infused together they had no effect on production responses.

Key Words: fatty acid digestibility, milk fat

Ruminant Nutrition: General: Posters

P372 Effect of adding different levels of fibrolytic enzyme solution on degradability and kinetics of whole-crop faba bean silage. J. Yang*[1], D. A. Christensen[1], H. (B.) Lardner[1], V. H. Guevara-Oquendo[1], B. Refat[1], O. AlZahal[2], and P. Yu[1], [1]*Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada,* [2]*AB Vista, Ruminant R&D, Marlborough, United Kingdom*

The objective of this study was to use in situ and in vitro methods to determine an optimal dose level of fibrolytic enzyme solution (derived from *Trichoderma reesei*; mixture of xylanase and cellulase; AB Vista, UK) when applied on whole-crop fava bean silage (*Vicia faba* L.). Whole-crop fava bean silage was dried at 55°C for 48 h and ground through 1-mm screens using a Wiley mill. Seven doses of the enzyme: 0, 0.25, 0.50, 0.75, 1.00, 1.25 and 1.50 mL of enzyme/ kg DM of silage were applied directly onto the substrate for both in situ and in vitro methods for 0, 6, 24 and 48 h incubation time. Four cannulated lactating dairy cows at Rayner Dairy Research and Teaching Facility (RDRTF, University of Saskatchewan, Canada) were used for in situ animal study. Two Ankom Daisy^{II} incubators were used for in vitro study. A Randomized Complete Block Design (RCBD) was used as an experimental design. Statistical analyses were performed using PROC MIXED of SAS 9.4 with significance declared at $P < 0.05$. Orthogonal polynomial contrast of SAS was used to test linear, quadratic and cubic response to increased dose level of enzyme solution. The in situ results showed that there was a cubic ($P < 0.05$) effect of enzyme dose levels on DM degradability and a quadratic ($P < 0.01$) effect on NDF degradability of whole-crop fava bean silage. There was no significant impact of the enzyme dose level on DM degradation rate (K_d), but significant linear impact on fiber degradation rate of NDF (0.98 to 2.78%/h). Similar results were showed in in vitro study, there were a quadratic ($P < 0.01$) effect on in vitro DM degradability and a cubic ($P < 0.01$) effect on in vitro NDF degradability of whole-crop fava bean silage. This study demonstrated that the new generation of fibrolytic enzyme solution has a potentially positive effect on DM and NDF degradability and kinetics of whole-crop fava bean silage. Further study is needed to examine the effect of the new generation of fibrolytic enzyme solution on lactational performance, feeding behavior, and nutrient digestibility in dairy cows fed with whole fava bean silage-based-diet.

Key Words: whole-crop faba bean silage, fibrolytic enzyme, degradation

P373 Feeding a novel probiotic and nitrate: Effects on milk yield and nutrient utilization in dairy cows. D. C. Reyes*[1], E. A. Latham[2], R. C. Anderson[3], J. P. Sacramento[1], L. H. Silva[1,4], Y. Geng[1,5], and A. F. Brito[1], [1]*Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH,* [2]*Bezoar Laboratories LLC, Bryan, TX,* [3]*Food and Safety Research Unit, USDA-ARS, College Station, TX,* [4]*Department of Agriculture and Food Science, Western Kentucky University, Bowling Green, KY,* [5]*Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China.*

We examined the effects of the denitrifying probiotic strain *Paenibacillus fortis* and nitrate on milk yield, N utilization, and apparent total-tract digestibility in dairy cows. Sixteen multiparous Holstein cows averaging 106 ± 49 DIM and 710 ± 16 kg of BW were used in a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments. Treatments were: (1) control (CON), (2) nitrate (NIT, 2% DM of calcium ammonium nitrate; 75% nitrate), (3) *P. fortis* (PROB; 2×10^9 cfu), and (4) NIT+PROB. Experimental periods lasted 18 d, with data and sample collection starting on d 12 followed by a 9-d washout period. Diets were fed as TMR, with the experimental and washout diets consisting of (DM basis) 48.5% corn silage, 11.5% mixed, mostly grass haylage, and 40% concentrate. Diets averaged 16.5% CP and 32% NDF (DM basis). *P. fortis* was top-dressed while nitrate was included in the TMR. The main effects of probiotic and nitrate, their interaction was tested using ANOVA. Dietary treatments had no effects on DMI and yields of milk, 4% FCM, and ECM. However, NIT diets decreased yields of milk protein (1.11 vs. 1.16 kg/d; $P < 0.01$) and lactose (1.70 vs. 1.76 kg/d; $P < 0.05$). Similarly, feeding NIT increased MUN (13.5 vs. 12.0 mg/dL; $P < 0.01$), but no change was observed for milk N efficiency (mean = 27.6%). An interaction was observed for PUN ($P = 0.04$) where NIT+PROB elevated PUN (15 mg/dL) relative to NIT (13.8 mg/dL) and PROB (13.1 mg/dL) but close to CON (15.2 mg/dL). A trend ($P = 0.07$) for an interaction was detected for urea N excretion as a proportion of total urinary N. Diets did not affect urinary creatinine (mean = 6.2 m*M*), urinary excretion of urea N (mean = 203.5 g/d), total purine derivatives (mean = 518.3 mmol/d), and DM digestibility (mean = 62.3%) Contrarily, NIT diets increased CP digestibility (65.1 vs. 61%; $P < 0.01$) and decreased NDF digestibility (42.7 vs. 47.9%; $P < 0.01$), implying depression of ruminal fibrolytic microorganisms. In summary, feeding *P. fortis* together with nitrate increased PUN concentration, suggesting rapid metabolism of nitrate by *P. fortis* and consequent ruminal NH_3 production.

Key Words: probiotic, nitrate, dairy cow

P374 Feeding a novel probiotic and nitrate: Effects on CH_4 emissions and rumen fermentation in dairy cows. D. C. Reyes*[1], E. A. Latham[2], R. C. Anderson[3], J. P. Sacramento[1], L. H. Silva[1,4], Y. Geng1,5, and A. F. Brito[1], [1]*Department of Agriculture, Nutrition, and Food Systems, University of New Hampshire, Durham, NH,* [2]*Bezoar Laboratories, LLC, Bryan, TX,* [3]*Food and Safety Research Unit, USDA-ARS, College Station, TX,* [4]*Department of Agriculture and Food Science, Western Kentucky University, Bowling Green, KY,* [5]*Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, China.*

Dietary nitrate has been investigated as a dietary strategy to mitigate CH_4 emissions in ruminants. However, there are concerns about nitrate toxicity. *Paenibacillus fortis* is a spore-former probiotic strain with known denitrifying capacity rapidly metabolizing nitrite to NH_3. Thus, the objective of this experiment was to investigate the effects of *P. fortis* and nitrate on CH_4 emissions and ruminal fermentation in lactating dairy cows. Sixteen multiparous Holstein cows (4 ruminally cannulated and 12 noncannulated) averaging 106 ± 49 DIM and 710 ± 16 kg of BW were used in a replicated 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments. Treatments were: (1) control (CON), (2) nitrate (NIT; 1.5% DM of nitrate), (3) *P. fortis* (PROB; 2×10^9 cfu), and (4) NIT+PROB. Experimental periods lasted 18 d, with data and sample collection starting on d 12 followed by a 9-d washout period. Diets were fed as TMR, with the experimental and washout diets consisting of (DM basis) 48.5% corn silage, 11.5% mixed, mostly grass haylage, and 40% concentrate. Diets averaged 16.5% CP and 32% NDF (DM basis). *P. fortis* was top-dressed while nitrate was included in the TMR. Gaseous emissions were measured from the noncannulated cows using a GreenFeed unit. Rumen fluid was collected from noncannulated cows. The main effects of probiotic and nitrate, and their interaction was tested using ANOVA. Feeding NIT decreased CH_4 emissions (437 vs. 480 g/d; $P = 0.02$) and CH_4 yield (18.2 vs. 20.0 g/kg of DMI; $P = 0.01$) and tended to decrease CH_4 intensity (10.8 vs. 12.3 g/kg of ECM; $P = 0.08$). Cows fed NIT diets also showed increased H_2 emissions (3.90 vs. 1.74 g/d; $P < 0.01$). In contrast, CO_2 emissions (mean = 15.1 kg/d) were not affected by treatments, as well as total VFA concentration of acetate and propionate. However, NIT diets increased the molar proportion of butyrate (9.89 vs. 9.10 mol/100 mol; $P < 0.01$) and ruminal pH (6.0 vs. 5.8; $P = 0.01$). In summary, feeding NIT decreased CH_4 production, CH_4 yield, and CH_4 intensity by an average of 9%. However, feeding *P. fortis* did not affect CH_4 emissions or rumen fermentation parameters.

Key Words: probiotic, nitrate, methane

P375 Impact of dietary carbohydrate profile on the dairy cow rumen meta-proteome. B. Mulakala*[1], K. Smith[2], M. Snider[1], A. Ayers[1], M. Honan[1], and S. Greenwood[1], [1]*The University of Vermont, Burlington, VT,* [2]*William H. Miner Agricultural Research Institute, Chazy, NY.*

The diet carbohydrate profile, particularly the starch and fiber content, is a critical factor influencing the microbe profile and volatile fatty acid production, yet little information is known about how microbial activity is affected by diet. This study characterized the rumen metaproteome in cows fed different physically effective undigested neutral detergent fiber (peuNDF240) and rumen fermentable starch (RFS) content levels. Eight cannulated Holstein cows were assigned to the study as part of a 4 × 4 Latin square design with a 2 × 2 factorial treatment design. Cows were paired randomly at the beginning of each 28-d period, and each cow pair was randomly assigned to one of 4 treatments: 1) low peuNDF240, low RFS, 2) high peuNDF240, low RFS, 3) low peuNDF240, high RFS, and 4) high peuNDF240, high RFS. Rumen fluid samples were collected from each cow on the last 2 d of each period at 3-time points (0600, 1000, and 1400 h). Samples were pooled within the time point within the cow across the day. The microbial protein fraction was isolated and analyzed using LC-MS/MS techniques. Data were analyzed for the impact of diet on microbial protein abundances using PROC MIXED of SAS. A total of 138 proteins were characterized across 22 microbial species. In total, 28 proteins ($P < 0.05$) were affected by treatments across 12 microbial species. Of these, 21 proteins were affected by RFS content, with 15 proteins having a higher abundance with higher dietary RFS. Many of these proteins are involved in energetics, glucose metabolism, and protein synthesis, for example, pyruvate-ferredoxin oxidoreductase (*P. ruminicola*), succinate dehydrogenase (*P. ruminicola*), phosphoglycerate kinase (*P. ruminicola* and *R. albus*), and 30S ribosomal protein (*C. aminophilum*). Only 2 proteins were affected by dietary peuNDF240 (phosphate dikinase and sn-glycerol-3-phosphate ABC transporter ATP-binding protein UgpC from *R. bromii*) or the interaction of peuNDF240 and RFS (5 proteins). These results suggest that the RFS content of the diet may be a greater determinant of rumen microbial protein abundances than dietary peuNDF240, and that higher RFS increases the abundance of many metabolism-related microbial proteins. This research highlights the need for further characterization of the rumen metaproteome in relation to diet and contributes to identifying dietary mechanisms that most greatly impact the core microbial metabolic pathways and ruminant productivity.

Key Words: starch, rumen metaproteome, NDF

P376 Impacts of dietary fiber content on rumination time and milk fatty acid composition. S. C. Allen*[1], D. M. Barbano[2], D. H. Poole[1], W. C. Porter[1], M. A. Drake[1], J. Odle[1], and S. H. Ward[1], [1]*North Carolina State University, Raleigh, NC,* [2]*Cornell University, Ithaca, NY.*

The objectives of this study were to evaluate the relationship between rumination time (RT) and milk FA profile and the impact of reducing dietary fiber content on that relationship. Ninety cows were fitted with rumination monitoring collars (AfiAct, AfiMilk Ltd., Israel). Thirty-four cows were used in a completely randomized block designed feeding study, where cows were blocked by parity and stratified by DIM and milk yield. Cows were assigned to either a low fiber (LF) or high fiber (HF) diet. RT of all cows with collars was monitored hourly. Weekly milk samples were analyzed for milk and FA composition: de novo (DNFA), mixed origin (MOFA), and preformed (PRFA). Weekly blood samples were analyzed for fatty acids. The relationship between RT and milk FA variables was analyzed using PROC REG and PROC CORR. All other data were analyzed using PROC MIXED. Diet and week were independent variables, and hour was independent for RT data. Significance was declared when $P < 0.05$. Dry matter intake was lower in cows fed LF (23.7 kg/d) compared with HF (24.4 kg/d). Milk yield was not different between diets ($P > 0.15$), but fat (1.06 v. 1.19 kg/d) and protein (0.87 v. 0.93 kg/d) yields were reduced in cows consuming LF diets compared with HF, respectively. DNFA, MOFA, and PRFA (g/100 g FA) was increased in cows consuming HF diets. Cows consuming HF diets had greater serum FA concentrations (162.2 v 122.9 uEq/L). Overall, RT was reduced in cows consuming LF diets (6.08 h/d) compared with HF diets (7.21 h/d). RT dropped during milking and feeding hours, but then increased until the next milking period. RT dropped during wk 2 of the study among HF cows and wk 1 through 3 among LF cows. Rumination appeared to recover after wk 3 for cows consuming LF diets, but never increased to match HF fed cows. Relationships between all FA components and RT were weak. Transitioning from a HF to LF ration negatively impacted RT, which likely affected milk fat yields, though little changes in FA were noted. Time away from feed, including time spent in the parlor or holding pen, also reduced RT and could contribute to lowered milk component production.

Key Words: rumination, de novo fatty acids, fiber

P377 Dynamic of particles in the reticulorumen of growing dairy goats. Y. C. T. Taguti*[1], M. Gindri[2], and I. A. M. de Almeida Teixeira[1], [1]*UNESP, Jaboticabal, SP, Brazil,* [2]*UMR Modélisation Systémique Appliquée aux Ruminants, Paris, France.*

The dynamics of particles in the reticulorumen (RR) is important for the understanding of the trade-offs among intake, gut capacity, and digesta passage rate (k_p). The objective was to propose pools of particles in RR of growing dairy goats which are linked by processes of comminution and k_p. For this, to avoid the effect of diet searching on RR particles (RRp, % DM), expected in a diverse database with castrated males (n = 38), intact males (n = 36), and females (n = 33) goats (15 to 45kg BW) fed with feeding levels (100, 75, and 50% of ad libitum), we expressed the RRp of a certain size as a proportion of intake of particles (DMIp, % DM) of the same size (RRp/DMIp ratio). The particles (% DM) were methodologically split in particles <0.15mm, 0.15–0.35 mm, 0.35–0.60 mm, 0.60–1.18 mm, 1.18–2.36 mm, 2.36–4.75 mm, >4.7 mm. A matrix with Pearson correlations between the RRp/DMIp ratio and the DMIp was computed. The RRp/DMIp ratio >1.18 mm was negatively related to DMIp >2.36 mm and positively related to DMIp <2.36mm. This indicates the pool of RRp >1.18 mm is slightly or not affected by the DMIp >2.36 mm and it is a transiting pool from intake to the pool of RRp <1.18 mm. The RRp/DMIp ratio 0.60–1.18 mm was not related to the DMIp <1.18 and >2.36 mm and positively related to the DMIp

1.18–2.36 mm. This suggests pool of RRp 0.60–1.18 mm is a transitory pool between large (>1.18 mm) and small particles (<0.60 mm). The RRp/DMIp ratio <0.60 mm was negatively related to DMIp <2.36 mm and positively correlated with DMIp >2.36 mm. This indicates the pool of RRp <0.60 mm is slightly or not affected by the DMIp <2.36 mm and increased by the DMIp >2.36 mm. This suggests the pool of RRp < 0.60 mm is a pool of RRp ready to scape depending on other factors, as density and opening of reticulo-omasal orifice. Thus, our findings suggest growing dairy goats have 2 pools of RRp dependent on the comminution process (pool of RRp >1.18 mm and 0.60–1.18 mm) and one dependent on scape of particles (pool of RRp <0.60 mm). Our findings will add important information for the understanding of particle dynamics in the RR and therefore on diet formulation. We thank the CNPq (135010/2020–9) and FAPESP (2008/57302–0).

Key Words: small ruminants, digest flow, fiber digestibility

P378 Ionized magnesium concentration in fresh whole blood and stored blood serum samples of grazing cows.

M. O. Moura*, D. Regalin, K. Ogliari, E. A. Q. de Lima, S. P. C. Teles, and E. A. Collao-Saenz, *Universidade Federal de Jatai, Jatai, GO, Brazil.*

Plasma ionized calcium (iCa) and magnesium (iMg) are the readily fractions available to cells. iCa and iMg measurements are more accurate and preferred to reflect the physiological status of these minerals. The aim of the present study was to evaluate if the iMg concentration in whole blood is stable in blood serum after freezing for 15 or 30 d. Whole blood of 14 lactating crossbred grazing cows (Holstein × Zebu) was collected after the first milking in the morning by puncture of the coccygeal artery. A first sample of 2 mL was taken with a heparinized syringe with lithium (Sarstedt Monovette, Brazil). Another 8 mL sample was collected in a vacuum system in sterile 10 mL tubes without anticoagulant. All samples were analyzed by blood gas analysis (Primevet Plus, USA). The fresh blood sample was analyzed within 1 h after collection, while the second sample was centrifuged and the blood serum transferred to Eppendorf divided into 2 1 mL aliquots and frozen at −20°C. After 15 and 30 d samples were thawed and analyzed. The statistical significance values were calculated using Student's *t*-test. During the freezing process the serum pH and lactate normally raise. The relationship of iCa and pH or lactate is well described and increased values commonly lead to underestimation of iCa concentration. Blood pH was higher than reference pH value for cattle (7.4) and increased after the storage period (Table 1). The observed iMg concentration was under the considered normal level of Mg in cows' plasma (0.75–1.00 mmol/L) and indicate incidence of seasonal grass tetany. Compared with fresh blood, iMg was stable in frozen serum for up to 30 d and can be used for blood gas evaluation with minimal changes in its concentration.

Table 1. Average concentration of ionic magnesium, ionic calcium, lactate and blood pH in fresh blood and frozen serum of grazing cows

				P-value	
Item	Fresh blood	Stored 15 d	Stored 30 d	Group	F × S
iMg, mmol/L	0.61 ± 0.02	0.63 ± 0.02	0.63 ± 0.02	0.86	0.65
iCa, mmol/L	1.20 ± 0.01	1.10 ± 0.01	1.05 ± 0.01	<0.01	<0.01
Lactate, mmol/L	0.70 ± 0.13	2.68 ± 0.31	2.48 ± 0.26	<0.01	<0.01
pH	7.53 ± 0.02	7.75 ± 0.03	7.80 ± 0.03	<0.01	<0.01

Key Words: blood gas analysis, hypomagnesemia, ionized calcium

P379 Effect of exogenous glucoamylase on in situ and in vitro dry matter and starch degradability of cereal grains.

L. Mu*[1], K. Arriola[1], K. Almeida[1], I. Fernandez[1], C. N. de Guzman[1], G. Hao[1], H. Sultana[1], F. Amaro[1], A. Oyebade[1], C. Heinzen[1], J. Gusmao[1], W. Li[2], S. Yu[3], and D. Vyas[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Danisco Animal Nutrition, International Flavors and Fragrances Inc., Wilmington, DE,* [3]*Danisco Animal Nutrition, International Flavors and Fragrances Inc., Aarhus, Denmark.*

We aimed to evaluate the effect of exogenous glucoamylase on ruminal in situ and in vitro dry matter degradability (DMD), in vitro starch degradability (IVSD), volatile fatty acids (VFA), and methane production using cereal grains as substrates. Six substrates (4 mm; 0.50 g per F57 bag) including sorghum (micronized and whole), barley (whole and steam-flaked), and corn (dry-rolled and steam-flaked); were incubated with exogenous glucoamylase (from *Trichoderma reesei*; 0.25 mg/g substrate DM) and buffered rumen fluid for 7 h in 6 replicates per run in 3 independent runs. In vitro DMD, IVSD, VFA, and methane production was measured after 7 h of incubation. In situ DM degradability was measured after 0, 1, 3, 7, and 12 h of ruminal incubation in 2 cannulated lactating dairy cows. Data were analyzed using the GLIMMIX procedure of SAS. Treatments and sampling time (where applicable) were used as fixed effects while run was considered random factor. In vitro DMD was increased with exogenous glucoamylase by 10, 32, 19, and 59% with whole sorghum ($P = 0.03$), whole barley ($P = 0.04$), steam-flaked barley ($P = 0.05$), and steam-flaked corn ($P = 0.04$), respectively. In situ DMD was increased with exogenous glucoamylase by 37, 4, 4, 32, 23, 73% for micronized sorghum, whole sorghum, whole barley, steam-flaked barley, dry-rolled corn, and steam-flaked corn, respectively ($P < 0.05$) after 12 h incubation. Glucoamylase supplementation increased total VFA production ($P = 0.02$) and the molar proportion of propionate ($P = 0.04$), while decreased the molar proportion of acetate ($P < 0.01$) and acetate-to-propionate ratio ($P = 0.02$) with steam-flaked corn. Lactate concentration increased with glucoamylase in micronized sorghum ($P = 0.03$) while the molar proportion of acetate decreased ($P = 0.01$). No effects were observed on methane production of any substrate with amylase supplementation ($P > 0.10$). In conclusion, glucoamylase supplementation can potentially improve DM and starch degradability when cereal grains are used as substrates in dairy cattle diets.

Key Words: dairy, glucoamylase, in vitro

P380 Effect of polyunsaturated fatty acids on corpus luteum function in dairy cows.

J. D. Iorio*[1], R. A. Palladino[3,4], N. A. Farnetano[4], L. E. Quintero Rodriguez[4], J. M. Anchordoquy[4], J. P. Anchordoquy[4], M. F. Olmeda[3], J. Simoni[1], M. G. Aguirre[1], F. Bargo[3], and M. Giuliodori[2], [1]*University of Buenos Aires, School of Agriculture, Buenos Aires City, BA, Argentina,* [2]*National University of La Plata, Faculty of Veterinary Sciences, La Plata, BA, Argentina,* [3]*University of Lomas de Zamora, Faculty of Agricultural Sciences, Lomas de Zamora, BA, Argentina,* [4]*National Scientific and Technical Research Council, Argentina.*

It is known that early pregnancy maintenance is highly dependent on progesterone (P4) synthesis, that, in turn, is associated with the perfusion of the corpus luteum (CL).The aim of this study was to evaluate the effect of supplementing flaxseed and cottonseed on the function of the CL in terms of luteal area (LA), luteal blood flow area (LBFA) and serum P4 concentration. Multiparous Holstein cows (n = 20) in early lactation were used in a completely randomized block design. Blocking criteria were DIM, number of lactations, BW, BCS and current milk production. Two isoenergetic and isonitrogenated diets were formulated for each group to meet the nutrient requirement of NRC (2001). Diets included corn silage and alfalfa pasture and the difference was given by the supplementation offered: flaxseed (1.2 kg DM/d, 273 g/d of n-3 polyunsaturated fatty acid (n-3 PUFA), FLAX) and cottonseed (3 kg DM/d, COTT). Regarding reproductive management, ovulations were synchronized by using an Ovsynch protocol preceded by a G6G presynchronization (PGF: d 0; GnRH: d 2, 8; PGF: d 15; GnRH d 17). Cows were not inseminated. Color Doppler ultrasound with a linear transductor (ESAOTE MyLab OneVET) was used to measure LA and LBFA on d 7 after ovulation (D7) (color mode: F:5 MHz, G:28%; PRF:1.0 MHz). Three different Doppler images of each clip were used to calculate LA (mm^2) and LBFA (mm^2). Images were processed with the computer software Image J (version 1.52p; National Institutes of Health; Maryland, USA) to quantify the pixels off-line. In addition, P4 concentration was measured at D7. We found that LBFA was 2.2 times higher in FLAX than in COTT group (23.60 ± 15.07 mm^2 vs. 13.25 ± 10.74 mm^2, $P = 0.1204$) respectively. Conversely, we did not find any difference in LA (295.97 ± 116.87 mm^2 vs. 298. 94 ± 53.34 mm^2, $P = 0.9487$) for FLAX and COTT, respectively) or in P4 concentration (3.43 ± 2.39 vs. 3.58 ± 2.02 ng/dl; $P = 0.8880$) for FLAX vs. COTT, respectively. We concluded that flaxseed supplementation increases LBFA probably through the stimulation of inflammatory mediator synthesis and/or luteal angiogenesis. This may be due to higher inclusion of n-3 PUFA in FLAX diet.

Key Words: PUFAs, luteal blood flow area, luteal area

P381 Pyruvate carboxylase knockdown alters lactate oxidation in Madin-Darby bovine kidney cells.

L. M. Beckett*, J. Laguna, S. Hilger, and S. S. Donkin, *Purdue University, West Lafayette, IN.*

Pyruvate carboxylase (PC) is a key enzyme at a critical control point between the tricarboxylic acid cycle and gluconeogenesis in the liver of dairy cattle, and catalyzes the conversion of pyruvate to oxaloacetate. Determination for the role of PC in energy metabolism in bovine is hampered by the lack of a metabolic model that precisely controls PC expression. The objective of this study was to develop a model to precisely reduce PC expression and to determine the effect on carbon flux. We hypothesized that a targeted reduction in PC expression would reduce lactate oxidation to CO_2, but propionate oxidation to CO_2 would not be affected. Bovine MDBK cells were cultured and transduced with lentiviral human PC short hairpin RNA (shRNA). Cells containing the PC shRNA knockdown (PCshRNA) or scrambled RNA (SRM) were selected using puromycin resistance. Based on Western blotting, PC abundance was reduced by approximately 50%. Wild type MDBK (wtMDBK) cells, SRM cells, and PCshRNA (n = 3) were grown to 90% confluency in 35 mm dishes. Cells were incubated with either 2.0 mM U-[^{14}C] lactate or 2.0 mM 2-[^{14}C] propionate for 3 h. Incubations were terminated, $^{14}CO_2$ was trapped, and samples analyzed by liquid scintillation counting. Rates of metabolism (nmol of substrate converted to $CO_2 \cdot plate^{-1} \cdot 3\ h^{-1}$) were analyzed using the PROC Mixed procedure in SAS and Tukey means separation test with significance at $P < 0.05$. The rate of lactate oxidation to CO_2 was significantly reduced for PCshRNA in comparison to wtMDBK control (8.2 vs 12.4, respectively, $P = 0.007$). There were no differences between SRM and PCshRNA cells ($P = 0.70$). There were no significant effects of PC knockdown on propionate metabolism to CO_2. The data indicates that PC knockdown specifically reduces metabolism of lactate to CO_2. Knockdown of PC in MDBK cells appears to be a useful model in characterizing the impact of precise changes in PC gene expression on lactate metabolism in bovine. This bovine model appears to have utility in understanding shifts in cellular metabolism that occur when PC expression is uniquely impaired.

Key Words: knockdown, pyruvate carboxylase, bovine

P382 Mitochondrial function during early and late lactation, of Holstein cows under 3 different productive systems.

G. Cañibe*[1], M. Garcia-Roche[1], A. Casal[2], A. Jasinsky[2], and M. Carriquiry[1], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay,* [2]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Estación Dr. Mario A. Cassinoni, Universidad de la República, Paysandú, Uruguay.*

The aim of the study was to assess the effect of 3 different productive systems on hepatic mitochondrial function in early and mid-lactation. Multiparous Holstein cows (n = 30), 698 ± 60 kg of body weight, 3.2 ± 0.3 body condition score (BCS) were assigned in a randomized block design to a total mixed ration (TMR) offered in a freestall facility (ad libitum; 29.4 kgDM/d, 40:60 forage to concentrate ratio), or to intensive grazing based systems (mixed pastures, forage allowance of 19.3 kgDM/d supplemented with 14.7 kgDM/d of TMR) in a freestall facility (PFS) or an open-sky paddock with access to water and shade (POS). Liver biopsies were collected and cryopreserved at 34 ± 13 and 171 ± 19 d postpartum (DPP). Mitochondrial function was assessed measuring oxygen consumption rates sustained by complex-I (glutamate/malate; C-I) and complex-II (succinate; C-II) substrates. Data were analyzed as repeated measures with a mixed model that included DPP, treatment and their interaction as fixed effects and block as a random effect. Energy-corrected milk yield was greater ($P < 0.05$) for TMR than PFS cows and intermediate for POS cows (38, 33 and 35 ± 1 kg/d for TMR, PFS and POS, respectively), while BCS increased ($P < 0.01$) from early to mid-lactation for TMR cows and decreased for PFS and POS cows. Oligomycin sensitive respiration –ATP synthesis-linked respiration–for C-I was affected by the treatment and DPP interaction as this increase was observed for TMR and PFS but not for POS cows (0.59 vs. 2.5 ± 0.4; 0.67 vs. 1.8 ± 0.3 and 1.1 vs. 1.5 ± 0.4 $pmolO_2 \cdot min^{-1} \cdot mg^{-1}$ for TMR, PFS and POS respectively, $P < 0.01$). In addition, for C-II, it increased from early to late lactation (2.22 ± 0.42 vs. 3.77 ± 0.55 $pmolO_2 \cdot min^{-1} \cdot mg^{-1}$, $P < 0.05$). In contrast, C-I and C-II non-mitochondrial respiration decreased from early to mid-lactation (3.94 ± 0.31 vs. 0.54 ± 0.11, 4.86 ± 0.39 vs. 0.75 ± 0.15 $pmolO_2 \cdot min^{-1} \cdot mg^{-1}$, for C-I and C-II respectively, $P < 0.05$). Our results confirm ATP synthesis is decreased while non-mitochondrial respiration is increased during early lactation and that plane of nutrition may impact mitochondrial function especially when C-I substrates are used.

Key Words: grazing vs tmr, mitochondrial function

P383 Predicting dry matter intake in dairy cows from ear tag-based estimates of chewing activity.

L. M. Campos*[1], V. L. Daley[2], A. G. Morales[1], J. M. Prestegaard[1], and M. D. Hanigan[1], [1]*Dairy Science Department, Virginia Tech, Blacksburg, VA,* [2]*Purina Animal Nutrition Center - Land O'Lakes, Gray Summit, MO.*

Diet formulation is reliant on accurate predictions of dry matter intake (DMI). However, DMI is usually estimated by group despite large animal to animal variation which often results in over- or under- feeding. Tailored individual DIM predictions would allow development of rations that optimize nutrient utilization and animal performance, and minimize nutrient excretion and financial losses. Determining individual animal DMI may be possible using pen DMI supplemented with behavior and

activity sensor data. The aim of this study was to derive a model to predict individual DMI for lactating and dry dairy cows based on data from ear tag sensors. Two independent studies were utilized to build a database with 20 animals. The observed intake data were collected via a Calan Gate system, with animals fed ad libitum 1x/d. Behavior activities were collected using the CowManager (Agis Automatisering, Harmelen, Netherlands) ear tag sensor system. Baseline data were collected for 7d before the experimental period. In Study 1 (S1), late-lactation, Holstein cows (n = 12; 800 ± 96 kg of BW; 30.5 ± 8.0 kg MY/d; 360 ± 106 DIM) were randomly assigned to a unique treatment (BASAL: 16.6% CP) in a repeated measurement design. In Study 2 (S2), non-pregnant, dry cows (n = 8; 358 ± 28.3 kg of BW) were randomly assigned to one of 3 treatments with varying CP levels (LOW: 13.2%, BASAL:16.6%, and HIGH: 19.1% CP), in a Youden square design. Trial length for S1 and S2 were 20 and 85d, respectively. Dietary treatments (DT) were formulated according to NRC (2001) requirements to meet animal needs utilizing corn silage, triticale silage, alfalfa hay and varying concentrate mixes. Statistical analysis was performed in SAS, and a mixed linear model with repeated measurements over time was considered. The selected model included the DMI kg/d as a dependent variable, ruminating (R), eating (E), physiological status (PS), and DT as fixed effects, and cow as a random effect. As expected, dry cows had lower DMI than lactating cows (16.94 vs. 21.68 kg/day; $P < 0.01$). DT ($P < 0.01$) and R time ($P = 0.03$) were influenced by DMI (kg/d). A trend for E time was observed ($P = 0.06$). Overall, our results demonstrated the ear tag sensors are important contributors to DMI model development, being R time the main variable to predict the DMI of dairy cows. Potential regressors not included in the current analysis and model evaluation will be further investigated.

Key Words: decision-making, ear tag, modeling

P384 Effect of prepartum dietary cation-anion difference strategy and level of dietary calcium (Ca) on postpartum Ca status and performance of multiparous Holstein cows.

G. Graef*[1], A. Kerwin[1], L. Ferro[1], S. Ordaz-Puga[1], C. Ryan[1], T. Westhoff[1], D. Barbano[1], K. Glosson[2], K. Zanzalari[2], J. Chapman[2], D. McLean[2], and T. Overton[1], [1]*Cornell University, Ithaca, NY,* [2]*Phibro Animal Health Corp., Teaneck, NJ.*

Periparturient hypocalcemia can be mitigated by reducing prepartum dietary DCAD; however, neither the extent of DCAD adjustment nor the level of dietary Ca fed with negative DCAD have been evaluated fully. Holstein cows (n = 98) entering second lactation or greater were enrolled in a covariate period at 32 d before expected calving. At 26 d before expected calving, cows were assigned to 1 of 4 treatments in a completely randomized design with a 2 × 2 factorial arrangement. Main effects were DCAD (PARTIAL: −2.6 mEq/100 g DM or FULL: −10.3 mEq/100 g DM) and dietary Ca (HIGH: 1.50% or LOW: 0.70% DM). All cows were fed the same postpartum diet. Urine pH (UpH) was recorded 3X weekly prepartum. Blood ionized Ca (iCa) was measured 1X weekly prepartum and at 0.5, 1, 1.5, 2, and 3 d in milk (DIM). Dry matter intakes (DMI) were calculated daily. Milk yields (MY) were measured daily to 63 DIM. Statistical analysis was conducted in SAS v. 9.4 with PROC MIXED and repeated-measures analysis. Interactions of main effects were generally not significant. Cows fed FULL had lower UpH than cows fed PARTIAL (5.64 vs. 6.71 ± 0.10; $P < 0.001$); dietary Ca did not affect UpH ($P = 0.30$). Cows fed FULL had lower prepartum DMI (13.1 vs. 14.1 ± 0.3 kg/d; $P = 0.04$); dietary Ca did not affect prepartum DMI ($P = 0.21$). Postpartum DMI from wk 1 to 9 tended to be increased by HIGH Ca (21.8 vs. 20.9 ± 0.5 kg/d; $P < 0.07$); DCAD did not affect postpartum DMI ($P = 0.70$). Circulating iCa during d 0 to 3 tended to be increased for cows fed FULL prepartum (0.98 vs. 0.94 ± 0.02 mM; $P < 0.07$). Cows fed HIGH Ca had lower iCa during d 0–3 (0.97 vs. 1.01 ± 0.02 mM; $P = 0.02$); however, cows fed HIGH Ca prepartum produced more milk in wk 1 to 3 postpartum (40.2 vs. 38.7 ± 0.9 kg/d; $P < 0.02$). In wk 1 to 9, a trend ($P = 0.10$) for an interaction of DCAD, dietary Ca, and week existed such that cows fed the PARTIAL DCAD and LOW Ca had the lowest MY and cows fed the FULL DCAD and HIGH Ca had the highest MY. Overall, results support feeding HIGH Ca in conjunction with FULL DCAD during the prepartum period.

Key Words: calcium, dietary cation-anion difference, transition cow

P385 Effect of reduced dietary levels of proteinated trace minerals on peripheral neutrophil activity in transition cows.

G. Gomes da Silva*[1], N. T. S. Grigoletto[1], T. A. Del Valle[2], A. T. Nunes[1], R. G. Chesini[1], P. C. Vittorazzi Junior[1], K. R. Santos[3], C. F. Batista[3], A. M. M. P. Della Libera[3], C. S. Takiya[1], J. Pettigrew[4], A. F. Koontz[5], L. F. Costa e Silva[5], and F. P. Rennó[1], [1]*Department of Animal Nutrition and Production, University of São Paulo, Pirassununga, SP, Brazil,* [2]*Unipampa, Itaqui, RS, Brazil,* [3]*Veterinary Clinical Immunology Research Group, Department of Internal Medicine, University of São Paulo, São Paulo, SP, Brazil,* [4]*James Pettigrew Consulting, Nicholasville, KY,* [5]*Alltech Inc., Nicholasville, KY.*

The objective of this study was to evaluate the effects of proteinated sources of Cu, Zn, Mn and Co and selenium yeast compared with inorganic sources of these minerals in diets of transition cows on peripheral neutrophils function. Twenty-four Holstein cows were distributed into 12 blocks, according to BCS, parity, and milk yield from the previous lactation, and assigned to the following treatments: 1) control (CON), typical dietary levels of inorganic Zn, Cu, Mn and Co, and Se as selenite or 2) proteinated minerals (PM), using 50% of the levels used in CON for Zn, Cu, Co, and Mn, and Se at the same level as in CON (Bioplex Co, Cu, Mn, Zn, and Sel-Plex; Alltech Inc., Nicholasville. KY). Micromineral contents in CON diets (pre- and postpartum) were formulated according to the NRC (2001) recommendations. Treatments were provided from 4 wks before the expected calving date (ECD) through 8 wks of lactation. Blood samples were collected on d −28 and −7 before the ECD, and on d 0, 7, 14, 21, and 56 postpartum. Immune function was evaluated by measuring neutrophil proportion (% leukocytes), phagocytic capacity, oxidative burst, and neutrophil viability. Measurements were performed by flow cytometry using a combination of fluorophores conjugated with antibodies and labeled bacteria. Data were analyzed using the MIXED procedure of SAS modeling the fixed effects of treatment, time, and their interaction, as well as the random effect of block. Treatments did not alter ($P > 0.10$) neutrophils proportion during the pre- and postpartum periods. Mineral sources did not influence ($P > 0.10$) neutrophil activity either in the basal state or after exposure to *Staphylococcus aureus* and *Escherichia coli*, as indicated by median fluorescence intensity of phagocytic capacity and oxidative burst. Proteinated minerals tended to increase ($P = 0.073$) the proportion of viable neutrophils during the pre-partum period. Dietary levels of trace minerals (Zn, Cu, Co, and Mn) can be reduced when proteinated sources are supplied without impairing the immune function of peripheral neutrophils.

Key Words: innate immunity, leukocyte, mineral complex

P386 Influence of methodology on estimated neutral detergent fiber from continuous culture fermenter effluent.

H. L. Maggard*, K. M. Mitchell, and B. A. Wenner, *Department of Animal Sciences, The Ohio State University, Columbus, OH.*

Dual-flow continuous culture (DFCC) fermenters fed pelleted diets generate effluent of fine particulate size by nature of the ground dry matter fed. However, the effect of reduced particulate size combined with high ash content on estimated NDF residue has been poorly characterized. Our objective was to evaluate the variation in estimated NDF from 3 commonly used methods for determining NDF digestibility: Ankom Fiber Analyzer (Ankom Technology, Macedon, NY) using F57 or F58 bags, and reflux using Berzelius beakers and filter paper. Our hypothesis was that the fine particulate effluent would clog bag pores and increase estimated NDF compared with reflux methods less reliant on solution efflux. To make this comparison, dried effluent samples (n = 58) from 4 previously published DFCC trials were assayed for NDF by F57 or F58 bags, or by reflux units using Whatman 541 in duplicate (REF). In a second experiment, samples from an additional trial (n = 29) were assayed for NDF using F57, F58, or REF, in duplicate, and compared with a commercial laboratory analysis using reflux with Whatman 934-AH filters. All samples were analyzed using the same batch of neutral detergent solution and α-amylase. For both experiments, statistical analysis included the fixed effect of method, the random effects of experimental diet and trial (for experiment 1), and ash content as a covariate. In experiment 1, F57 and F58 returned lower ($P < 0.001$) estimates for NDF (19.8% and 18.9%, respectively) compared with REF (24.8%); when directly contrasted, F57 and F58 were not different ($P = 0.14$) from each other. In experiment 2, F57 and F58 again returned lower ($P < 0.001$) estimates for NDF (20.7% and 19.2%, respectively) compared with REF (23.1%). However, contrasted F57 versus F58 indicated a tendency for F57 estimated NDF to be greater than F58 ($P = 0.06$). When compared with values from reflux with 934-AH filters, all 3 methods increased ($P < 0.001$) estimated NDF. Differences between reference and F57, F58, and REF were +3.03, +1.56, and +5.52 percentage units, respectively, and F57 tended to be greater than F58 ($P = 0.08$). These data indicate the influence of NDF method on estimates of NDF from DFCC samples; more work is needed to verify the cause of difference and the most accurate method for use in DFCC experiments.

Key Words: continuous culture, fiber digestibility, NDF method

P387 Effect of dietary metabolizable protein level on digestion and ruminal fermentation of Holstein and Ayrshire dairy cows.

F. Hassanat*[1], C. Benchaar[1], K. A. Beauchemin[2], D. R. Ouellet[1], H. Lapierre[1], and C. Côrtes[3], [1]*Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Lethrbridge Research and Development Centre, Lethbridge, AB, Canada,* [3]*Agrinova, Alma, QC, Canada.*

The objective of this study was to determine the effect of dietary metabolizable protein (MP) level on ruminal fermentation and apparent total-tract digestibility in Holstein and Ayrshire dairy cow breeds. Six Holstein (DIM = 91 ± 16; milk yield = 43.1 ± 5.1 kg; BW = 701 ± 66 kg) and Six Ayrshire (DIM = 93 ± 15; milk yield = 34.6 ± 3.0 kg; BW = 551 ± 48 kg) multiparous lactating cows were used in a replicated 3 × 3 Latin square (35-d period; 14-d adaptation) with a 2 × 3 factorial arrangement of treatments. Cows were fed (ad libitum) a TMR formulated (NRC, 2001) to provide 85%, 100% or 115% of MP requirements. Nutrients digestibility and rumen fermentation characteristics were determined over 7 and 2 consecutive days, respectively. Main effects of breed, MP level and interaction (breed × MP) were determined using the MIXED Procedure of SAS. Significance was declared at $P \leq 0.05$. Apparent total-tract dry matter digestibility was lower in Holstein versus Ayrshire cows (68.3 vs. 70.3%) and was not affected by dietary MP level. Ruminal pH and total volatile fatty acid (VFA) concentration averaged 6.27 and 134 m*M* and were not affected by breed or MP level. Molar proportions of acetate, propionate, and butyrate were not affected by breed or MP level. Ruminal NH_3 concentration and molar proportion of branched-chain VFA (isobutyrate and iso-valerate) averaged 8.07 m*M* and 2.86 mol/100 mol, respectively and were not affected by breed or MP level. Results from this study show that for both breeds, MP level has no effect on ruminal fermentation characteristics (pH, VFA concentration and molar proportions, NH_3 concentration) and DM digestibility. Ruminal fermentation characteristics did not differ between Holstein and Ayrshire dairy cows, but total-tract digestibility of DM was higher for Ayrshire compared with Holstein.

Key Words: Holstein, Ayrshire, digestion

P388 Annual rhythms of milk and milk fat and protein in dairy cattle in Brazil.

T. S. Silveira[1,2] and R. Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil,* [2]*Associação Brasileira de Criadores de Bovinos da Raça Holandesa, Curitiba, Paraná, Brazil.*

The annual rhythms of milk production and composition should be considered by the dairy industry when making management decisions, directly impacting the milk price received by the farmers. The objective of this study was to use cosine analysis to quantify the annual rhythms of milk and milk component production using both bulk tank milk samples and DHIA herd data. Milk fat and protein contents from weekly bulk tank milk samples of 2,241 herds from 2013 to 2019 were obtained from 2 large dairy coops in Southern Brazil. A cosine function with a 12-mo period by random regression was estimated with the MIXED procedure from SAS (v.9.4). The model included the random effect of year and the fixed effects of bulk tank, the linear form of a cosine function and the interaction of bulk tank and the linear form of cosine function. A zero-amplitude test was used to compare the full model containing the linear form of the cosine function to a reduced linear model. There was an interaction ($P < 0.01$) between bulk tank milk samples and milk fat and protein contents. The acrophase (time at peak) of the milk fat content ranged from June 06 to June 16, whereas the milk protein content peaked between May 31 and June 10. The amplitude (peak to mean) of the annual rhythm ranged from 0.08 to 0.13 percentage points for milk fat and 0.06 to 0.10 percentage points for milk protein. The annual rhythm of milk yield and milk fat and protein yields and contents were analyzed in 1,683,668 test-days from 241 herds enrolled in an official DHIA carried out by the Holstein Association of Paraná State (APCBRH). Milk, fat and protein yields, and fat and protein contents followed yearly annual rhythms. On average, milk yield and fat and protein yields peaked in September (spring season in the Southern hemisphere), milk fat content was highest in July, and milk protein content peaked in June. In conclusion, there is an annual rhythm of milk yield and its composition in Brazilian dairy herds that fits the cosine function.

Key Words: annual rhythms, milk composition, milk synthesis

P389 Effect of replacing a portion of inorganic chloride trace minerals with AvailaDairy on peripartum performance.

A. L. Kerwin*[1], G. M. Graef[1], C. M. Ryan[1], L. Ferro[1], S. O. Puga[1], T. A. Westhoff[2], D. M. Barbano[3], D. H. Kleinschmit[4], and T. R. Overton[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [3]*Department of Food Science, Cornell University, Ithaca, NY,* [4]*Zinpro Corporation, Eden Prairie, MN.*

The objective was to determine the effect of replacing a portion of inorganic chloride trace minerals with AA complexes of trace minerals on peripartum performance. Holstein cows were assigned randomly to treatment 1 wk after dry-off (~60 d before expected parturition) and remained on treatment through 22 wk of lactation. Cows received Zn (75 ppm), Mn (65 ppm), and Cu (10 ppm) as hydroxychlorides, and Co (1 ppm) as carbonate (ITM; n = 37) or a partial replacement (%) of ITM with AA complexes of Zn (53%), Mn (31%), and Cu (35%) and Co glucoheptonate (100%; AATM; Availa-Dairy, Zinpro Corp., Eden Prairie, MN; n = 32). Intakes through wk 8 and milk production were recorded daily. Milk composition and body weights (BW) were collected weekly. Colostrum was analyzed for composition and calf serum was analyzed for IgG. Calves were followed through 9 wk of life for calf growth. Data were analyzed in SAS with mixed-effects models and repeated-measures analysis when applicable. Prepartum dry matter intake as a percent of BW was lower in cows fed AATM (1.74 vs. 1.82 ± 0.02%; $P = 0.01$) but was not different postpartum (2.98 vs. 2.99 ± 0.04; $P = 0.95$). Cows fed AATM produced more milk from wk 1 to 8 (46.8 vs. 44.9 ± 0.7 kg/d; $P = 0.05$) and overall (wk 1 to 22; 46.5 vs. 45.1 ± 0.5 kg/d; $P = 0.05$). Energy-corrected milk did not differ between AATM and ITM fed cows (wk 1 to 8: 52.5 vs. 50.9 ± 0.9, $P = 0.20$; wk 1 to 22: 52.7 vs. 51.7 ± 0.8, $P = 0.37$), respectively. No difference was detected between AATM and ITM fed cows for milk true protein (wk 1 to 8: 1.34 vs. 1.30 ± 0.02 kg/d, $P = 0.20$; wk 1 to 22: 1.36 vs. 1.33 ± 0.02 kg/d, $P = 0.26$) and fat (wk 1 to 8: 2.08 vs. 2.02 ± 0.05 kg/d, $P = 0.44$; wk 1 to 22: 2.07 vs. 2.05 ± 0.04 kg/d, $P = 0.69$), respectively. Colostrum weight ($P = 0.42$), IgG ($P = 0.82$), Brix ($P = 0.87$) and calf serum IgG ($P = 0.85$) did not differ between treatments. Calf birth weight did not differ ($P = 0.85$) but calves from dams fed AATM (n = 12) had greater average daily gain (ADG) than ITM calves (n = 10; 0.85 vs. 0.69 ± 0.05 kg/d; $P = 0.03$). Performance benefits of feeding AATM during the dry period and lactation included increased milk yield and calf ADG.

Key Words: trace mineral, transition cow

P390 Effect of replacing a portion of inorganic chloride trace minerals with AvailaDairy on health and reproductive outcomes.

A. L. Kerwin*[1], G. M. Graef[1], C. M. Ryan[1], L. Ferro[1], S. O. Puga[1], T. A. Westhoff[2], D. H. Kleinschmit[3], and T. R. Overton[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [3]*Zinpro Corporation, Eden Prairie, MN.*

The objective was to determine the effect of replacing a portion of inorganic chloride trace minerals with AA complexes of trace minerals on health and reproductive outcomes. Holstein cows entering 2^+ lactations were assigned randomly to treatment at dry-off and remained on treatment through 154 d in milk (DIM). Cows received Zn (75 ppm), Mn (65 ppm), and Cu (10 ppm) as hydroxychlorides, and Co (1 ppm) as carbonate (ITM; n = 37) or a partial replacement of ITM with AA complexes of Zn, Mn, and Cu and Co glucoheptonate (AATM; Availa-Dairy, Zinpro Corp., Eden Prairie, MN; n = 32) targeting 0.06% of dry matter intake. Serum samples were analyzed for albumin (Alb), cholesterol (Chol), bilirubin (Bili), aspartate aminotransferase (AST), haptoglobin (Hp), β-hydroxybutyrate (BHB), and Ca. A metabolite health index (MHI) was calculated based on Bili, Chol, and Alb concentrations. A liver functionality index (LFI) was calculated to standardize changes in Alb, Chol, and Bili from 4 to 29 DIM. Greater MHI and LFI indicate better health status. Mixed-effects models were used with repeated-measures analysis, when applicable (SAS v. 9.4). Survival analysis for conception by 154 DIM was analyzed by Kaplan-Meier and Cox proportional hazards models. Disease incidence was tested with Fisher's exact test. Cows fed AATM tended to have less metritis (0% vs. 11%; $P = 0.12$) and ketosis (25% vs. 43%; $P = 0.13$) incidence. There was a treatment by time interaction for AST ($P = 0.004$) and BHB ($P = 0.07$); cows fed ITM had higher AST concentrations at 28 DIM ($P = 0.02$) and larger increases in BHB through 29 DIM. Cows fed AATM had a greater MHI at 4 DIM (0.38 vs. −0.17 ± 0.24; $P = 0.10$). There were no treatment differences for haptoglobin ($P = 0.94$), Ca ($P = 0.99$), LFI ($P = 0.23$), odds of conception ($P = 0.83$), or survival curves ($P = 0.83$); however, cows fed AATM tended to have fewer services per conception (1.5 vs. 1.9 ± 0.2; $P = 0.11$). Caution should be used when interpreting health data due to a small sample size. Overall, cows fed AATM during the dry period and early lactation had evidence of better health and improved conception to first service.

Key Words: transition cow, trace mineral, metabolite

P391 Effect of forage type on lactation performance and feed efficiency with 2 breeds of dairy cattle.

H. C. Wilson* and K. F. Kalscheur, *US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

The objective of this research was to evaluate the effect of forage type on lactation performance, milk composition, and feed efficiency of cows from 2 different breeds of cattle. Sixty cows, 30 Holstein (146 ± 29 d in milk, 1,480 ± 178 kg of BW) and 30 Jersey (136 ± 45 d in milk, 1,021 ± 96 kg of BW) were used in a 2 × 2 Latin square design with 42-d periods. Cows were fed diets formulated to contain either 80% corn silage (CS) and 20% alfalfa silage (AS) or 20% CS and 80% AS in total mixed rations with 59% forage and 41% concentrate feeds. Data were analyzed using MIXED procedures of SAS. There were no interactions for forage type by breed for any measurement ($P \geq 0.32$). Cows fed the CS-based diet had greater DMI compared with cows fed the AS-based diet (20.8 vs 20.0 kg; $P = 0.04$). There were no differences in milk yield and protein percentage or yield for cows fed either forage type. There was a tendency for cows fed AS to have greater milk fat percentage compared with cows fed CS (4.15 vs 4.02%; $P = 0.06$). There were no differences for total solids (TS, %) nor energy-corrected milk (ECM) for cows fed CS or AS. Cows fed AS had greater feed efficiency (defined as ECM/DMI) compared with cows fed CS (2.01 vs 1.91; $P < 0.01$). Holsteins had greater DMI and milk yield compared with Jersey cows (23.1 vs 17.7 kg/d and 41.4 vs 28.0 kg/d, respectively; $P < 0.01$). Holsteins had lower milk fat and protein compared with Jerseys (3.60 vs 4.48% and 3.00 vs 3.35%, respectively; $P < 0.01$). In contrast, Holsteins had slightly greater milk lactose compared with Jersey cows (4.78 vs 4.68%; $P < 0.01$). Milk TS was lower for Holstein cows compared with Jersey cows (12.5 vs 13.6%; $P < 0.01$). Holstein cows had greater ECM compared with Jersey cows (41.9 vs 32.5 kg/d; $P < 0.01$), but there were no differences in feed efficiency between breeds. There were no differences of MUN for either forage type or breed. Alfalfa silage can be fed as the main forage source in dairy diets for both Holstein and Jersey cows to achieve similar milk yields but increased feed efficiency and greater milk fat (%) compared with diets with primarily corn silage.

Key Words: alfalfa silage, Holstein, Jersey

P392 Alternate method for feeding behavior monitoring of dairy cows in a Calan Broadbent Feeding System using a 3-axis data logging accelerometer.

C. Matamoros*, R. Bomberger, and K. Harvatine, *The Pennsylvania State University, University Park, PA.*

Feeding behavior analysis provides insight into the physiological control of hunger and satiety signals that regulate dry matter intake. The objective of the study was to develop an alternate method to observe feeding behavior in a Calan Broadbent Feeding System using a 3-axis accelerometer. The study focused on using 3-axis accelerometers as these sensors have been validated for other uses in dairy research such as the determination of lying behavior in cows and calves. The sensors were deployed on the last 7 d of each experimental period of an experiment with 24 multiparous and 24 primiparous cows loosely housed in a barn equipped with a Calan Broadbent Feeding System (data not shown). The tilt angle on the Z-axis was calculated and used to determine when the door was closed or open as a proxy for feeding activity. This approach has been previously validated to measure feeding behavior with change-of-state sensors in similar housing conditions. By design, the sensors read ~0° when the door was closed and > 60° when fully open, allowing an ample range of measurement to differentiate the state of the door. The between-feeding intervals were used to further characterize feeding behavior. The minimum intermeal interval, a criterion utilized to characterize meals, was calculated by determining the intersection of the normal distributions fitted to the log-transformed between-feeding intervals. The minimum intermeal interval of the system was 31.3 min considering the best fitting model, a mixture of 3 Gaussian distribution (−2LogLikelihood = 20,729.3, AICc = 20,745.3, and BIC = 20,799.5), which agrees with previous reports in similar housing conditions. Using the minimum intermeal interval of the system, meals were characterized and meal length averaged 37.3 min/meal and frequency averaged 7.3 meals/d, which agrees with previous reports in similar housing conditions. In conclusion, the system used readily available materials and minimal labor, made no permanent modification to the feeding system, and appears to adequately estimate meal characteristics of loose housed cows.

Key Words: meal length, meal frequency, eating time

P393 Evaluation of bioavailability of different sources of rumen-protected choline in lactating dairy cows.

P. Piantoni*, M. Messman, R. Rodrigues, C. Chapman, and G. Schroeder, *Cargill Animal Nutrition and Health Innovation Center, Elk River, MN.*

Rumen-protected choline (RPC) is a methyl donor source used in transition cows to reduce the incidence of fatty liver. Bioavailability of choline in commercial products is variable and depends on their rumen protection and intestinal digestibility. Eight multiparous Holstein cows (239 ± 43 DIM; 40.2 ± 6.9 kg/d milk yield; mean ± SD) were used in a 4 × 4 Latin square design experiment with 14-d periods to evaluate choline bioavailability of 3 commercial RPC sources based on milk choline metabolites. Treatments were a control diet with no supplemental RPC (CTR), and CTR top-dressed with 60 g/d of CholineX (ChoX; NuTech Biosciences Inc.), ReaShure (ReaS; Balchem Corporation), or Valido Choline RP (ValC; Cargill Inc.). Products were not pre-conditioned and supplied 11.3, 14.4, and 11.6 g/d of choline ion, respectively. Milk samples were collected at each milking on d 13 of each period and composited based on milk yield. Choline metabolites were determined to calculate net portal flux (NPF) of choline as an indicator of bioavailability (Choline NPF = $-2.26 + 0.111[\text{Betaine}]_{\text{milk}} + 0.032[\text{Phosphocholine}]_{\text{milk}}$; de Veth et al., 2016). Data were analyzed in JMP with a model containing the fixed effect of treatment and period, and the random effects of square and cow. Treatments did not affect DMI, milk yield, feed efficiency, or contents and yields of milk fat and protein. ValC increased yield of betaine in milk compared with ReaS and CTR (0.457 vs. 0.328 and 0.310 g/d, respectively; $P < 0.05$). Yield of betaine in milk for ChoX was intermediate (0.407 g/d) and not different from the other treatments. ValC increased betaine concentration in milk compared with ReaS and CTR (111 vs. 82.6 and 74.3 μM, respectively; $P < 0.05$), but not compared with ChoX (107 μM). Concentrations and yields of choline and phosphocholine in milk were not affected by treatment. ValC increased choline NPF compared with ReaS and CTR (13.3 vs. 9.74 and 8.48 g/d, respectively; $P < 0.05$), but not compared with ChoX (12.5 g/d). ValC increased the transfer of supplemental choline compared with ReaS (41.7 vs. 8.73%; $P < 0.05$). ChoX choline transference was intermediate (35.5%) and not different from ValC and ReaS. Results from this study suggest bioavailability of RPC products vary greatly and that choline metabolites in milk can be used to determine their relative bioavailability.

Key Words: choline metabolites, bioavailability, net portal flux

P394 Partitioning variation in mineral concentrations of dry cow total mixed rations on commercial dairy farms.

B. A. Saylor*[1], E. Lynch[2], D. Sawyer[2], M. A. Wattiaux[1], J. P. Goeser[2], and L. F. Ferraretto[1], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*Rock River Laboratory Inc., Watertown, WI.*

Our objective was to quantify selected sources of variation in mineral concentrations of dry cow total mixed ration (TMR) samples from commercial dairy farms. Duplicate TMR samples collected by dairy consultants from 14 farms in the US were sent to a commercial laboratory where technicians divided each sample into 2 subsamples using a mixing and quartering technique. Each subsample was then microwave oven-dried and ground to pass through a 1.0 mm screen. These samples were then divided into 3 subsamples, each of which (n = 167) were analyzed for macro- and micro-minerals using inductively coupled plasma-optical emission spectrometry. Total variance was partitioned into that associated with sampling at the farm, with the first subsampling at the laboratory, and with the second laboratory subsampling and subsequent analysis. Data were analyzed using PROC GLIMMIX in SAS. The model included farm as a fixed effect and farm sampling, first subsampling (within farm sampling), and second subsampling (within first subsampling) as random effects. Effect of farm was significant for all minerals ($P < 0.001$). Covariance estimates were used to compare variance attributed to on-farm sampling versus subsampling at the laboratory. Covariance estimates were compared on a relative basis for each mineral. More than 25% of the variance in macromineral concentrations could be attributed to farm sampling, compared with 1.7% attributed to the first laboratory subsampling. More than 17% of the variance in micromineral concentrations could be attributed to farm sampling, compared with 1.7% attributed to the first subsampling. Across all farms, variation in mineral concentrations was greatest for Cu (CV = 44.6%) and lowest for K (CV = 13.7%). Concentrations of Ca averaged 1.2 ± 0.2% (mean ± SD) of DM, ranging from 0.6 to 1.9% of DM (CV = 19.3%). Concentrations of K averaged 1.1 ± 0.2% of DM, ranging from 0.7 to 1.4% of DM. Coefficients of variation for dietary mineral concentrations within a farm ranged from 4.1% to 12.2%. Results indicate that the majority of the variation in the TMR can be attributed to sampling at the farm level.

Key Words: dry cow, TMR, mineral

P395 Effects of high-protein corn distillers grains with and without yeast or manipulation of dietary cation-anion difference on production and nutrient digestibility of lactating dairy cows.

M. Rodriguez*[1], H. Zynda[1], J. Copelin[1], L. Rebelo[1], W. Weiss[1], M.

Wilken[2], and C. Lee[1], [1]*The Ohio State University, Wooster, OH,* [2]*ICM Inc., Colwich, KS.*

Less production, especially milk fat, often occurs when corn distillers' grains are fed to lactating cows. This study was designed to evaluate high-protein corn distillers' grain (HPDG; 42% CP, 9% EE) and whether yeast supplementation or increasing dietary cation-anion difference (DCAD) affects responses to HPDG. In a randomized block design, 40 cows were blocked and randomly assigned to: CON, a soybean meal-based diet; DG, CON with HPDG at 20% (Prairie Horizon Agri-Energy, Phillipsburg, KS; DM basis) mainly replacing soybean meal; DG-Y, DG with yeast (yeast not extracted post-distillation; Redfield Energy, Redfield, SD) at 20%; and DG-DCAD, DG supplemented with sodium bicarbonate and potassium carbonate. The DCAD of CON, DG, DG-Y, and DG-DCAD were 185, 62, 67, and 187 mEq/kg DM, respectively. The experiment was conducted for 2-wk diet adaptation followed by 3-wk data collection. All data were analyzed using the MIXED procedure of SAS (random effect of block, fixed effects of diets, wk repeated, and interaction). Milk yield tended to be lower (42.4 vs. 39.9 kg/d; $P = 0.06$) for DG, DG-Y, and DG-DCAD vs. CON with no difference in DMI among treatments. Milk protein yield tended to be lower (1.33 vs. 1.24 kg/d; $P = 0.07$) for DG, DG-Y, and DG-DCAD vs. CON. Milk fat yield decreased (1.26 vs. 1.55 kg/d; $P = 0.03$) for DG and DG-Y vs. CON, but DG-DCAD (1.43 kg/d) had no difference when compared with CON. Similarly, energy-corrected milk values were lower (38.0 vs. 43.3 kg/d; $P = 0.02$) for DG and DG-Y vs. CON, but there was no difference between DG-DCAD and CON. Digestibility of DM, and NDF were lower ($P < 0.01$) for DG, DG-Y, and DG-DCAD compared with CON. These data suggest that negative milk fat effects of HPDG is partly attributed to excess anion load, and PUFA supply from HPDG was not the sole factor as traditionally thought. Decreased milk yield by HPDG occurred due to decreases in diet digestibility regardless of yeast inclusion and DCAD manipulation and, therefore, could be caused by PUFA.

Key Words: high-protein distillers grains, yeast, dietary cation-anion difference

P396 Prokaryotic community structure in dual flow continuous cultures varying in forage, corn oil and branched-chain volatile fatty acids concentrations.

K.E. Mitchell*[1], B. A. Wenner[1], C. Lee[2], T. Park[3], M. T. Socha[4], and J. L. Firkins[1], [1]*Department of Animal Sciencesr, The Ohio State University, Columbus, OH,* [2]*Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH,* [3]*Department of Animal Science and Technology, Chung-Ang University, Anseong, Republic of Korea,* [4]*Zinpro Corporation, Eden Prairie, MN.*

Branched-chain amino acids are deaminated to branched-chain volatile fatty acids (BCVFA), which stimulate cellulolytic bacteria. Our objective was to assess how prokaryotic community structure changes when supplementing BCVFA in different dietary conditions. BCVFA were hypothesized to increase relative sequence abundance (RSA) of cellulolytic bacteria in high (HF) and low (LF) forage diets. Supplemental corn oil (CO) should decrease RSA of cellulolytic bacteria, especially with LF, but BCVFA were hypothesized to lessen the bacteriostatic effects of CO and maintain cellulolytic fitness. The study was an incomplete block design with 8 dual flow cultures used in 4 periods with treatments ($n = 4$) arranged as a 2 × 2 × 2 factorial. The factors were: HF or LF (67 or 33% forage), without or with supplemental CO (3% DM), and without or with 2.15 mmol/d each of isovalerate, isobutyrate, and 2-methylbutyrate. The diets were isonitrogenous and pH was managed to minimize diet differences. The statistical model included fixed effects of forage, CO, BCVFA, and their interactions; period and fermenter were random effects. There was a 3-way interaction ($P = 0.02$) for pH, but no treatment interactions with hour ($P > 0.19$). HF's pH ranged from 6.79 to 6.11; and LF's, from 6.77 to 6.05. LF decreased ($P < 0.04$) diversity for all indices except Simpson's ($P = 0.50$). LF decreased ($P < 0.01$) the RSA of cellulolytic bacteria including *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Butyrivibrio*. Supplementing CO decreased ($P < 0.10$) *Butyrivibrio* and *Treponema* genera; CO decreased *Ruminococcus* with LF (forage × CO, $P = 0.09$). RSA of *R. flavefaciens* increased when BCVFA were combined with LF but decreased when combined with HF (forage × BCVFA, $P = 0.09$). Supplemental BCVFA increased ($P < 0.02$) the efficiency of microbial protein synthesis by 1.54 g/kg OM truly degraded. Decreasing forage shifted RSA as expected, but CO had very few effects, probably because pH was controlled. Providing supplemental BCVFA did not increase the RSA of cellulolytic bacteria but apparently supported primary fibrolytics and other secondary bacteria proportionately in a balanced consortium.

Key Words: branched-chain volatile fatty acids (BCVFA), cellulolytic

P397 Feeding legume-based forages: Effects on N utilization in dairy cows.

J. P. Sacramento*[2,1], L. H. P. Silva[3], D. C. Reyes[1], Y. Geng[4,1], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH,* [2]*Federal University of São João del Rei, São João del Rei, MG, Brazil,* [3]*Western Kentucky University, Bowling Green, KY,* [4]*Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, PR, China.*

Previous research showed that red clover (RC) improved N utilization in dairy cows, but data on RC mixed with other cool-season legumes is lacking. Sixteen multiparous and 4 primiparous organic-certified Jersey cows averaging 116 ± 52 DIM and 502.1 ± 52.2 kg of BW were used to investigate the effects of partially replacing RC with a legume mix (LM) on N utilization. Cows were blocked in pairs by DIM or parity and, within pair, randomly assigned to treatments in a crossover design. Each experimental period lasted 24 d, with 14 d for diet adaptation and 10 d for sample collection. Two fields were planted with RC- or alfalfa-grass mix consisting (% of total) of 79:14:7 legume:meadow fescue:timothy seeding rate. Forages were harvested as baleage, with second and third cuttings used in the present study. Diets were formulated to yield a 60:40 forage:concentrate ratio and fed as TMR twice daily. Based on the botanical composition of each field, the forage portion of the treatments contained (% of diet DM): (1) 41% RC, 5% white clover, 9% weeds, and 5% grasses (RC diet), and (2) 21% RC, 10% white clover, 12% alfalfa, 11% weeds, and 5% grasses (LM diet). Diets averaged 17.7 vs. 17.1% CP for RC and LM, respectively. Spot urine samples were collected 5 times over 2 consecutive days. Cows fed the RC diet had greater N intake (605 vs. 591 g/d, $P = 0.01$) than cows fed the LM diet. However, no significant difference was observed for MUN (mean = 12 mg/dL) and PUN (mean = 16.3 mg/dL) concentrations, and milk N efficiency (mean = 20%). Likewise, urinary N excretion did not differ between diets when expressed in g/d (mean = 224 g/d) or proportion of N intake (mean = 37.5%). Urinary excretion of urea N, expressed in g/d (mean = 162 g/d) or as a proportion of urinary N (mean = 72.5%) or N intake (mean = 27%), was not affected by diets. Uric acid excretion in urine was greater in RC than LM diet (80.4 vs. 73.5 mmol/d, $P = 0.01$), but no differences were observed for that of allantoin (mean = 376 mmol/d) and total purine derivatives (mean = 453 mmol/d). In summary, partially replacing RC with a LM did not affect N utilization in lactating dairy cows.

Key Words: dairy cow, legume, nitrogen efficiency

P398 Energy of lactating dairy cattle fed increasing inclusion of new high-protein processed corn product.

A. L. Carroll*[1], D. L. Morris[1], P. J. Kononoff[1], and M. L. Jolly-Breithaupt[2], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*Flint Hills Resources, Wichita, KS.*

Advancing technologies of the corn dry-milling ethanol production process includes the mechanical separation of fiber containing particles from a portion of plant and yeast based nitrogenous particles. The resulting high-protein processed corn product (HPCoP) contains approximately 53% CP, 37% NDF, 6.5% total fatty acids. The objective of this experiment was to examine the effects of replacing non-enzymatically browned soybean meal with the HPCoP on DMI, energy utilization, and production of lactating Jersey cows. Twelve multiparous Jersey cows (95 ± 7.3 DIM) were utilized in a triplicated 4x4 Latin square design consisting of 4, 28 d periods. Cows were blocked by milk yield and randomly assigned to 1 of 4 treatment diets that contained HPCoP (DM basis) at (1) 0% HPCoP (CON); (2) 2.6% HPCoP (LoCoP); (3) 5.4% HPCoP (MdCoP); and (4) 8.0% HPCoP (HiCoP). Increasing the concentration of HPCoP tended ($P = 0.07$) to result in a quadratic effect on DMI (19.2, 19.9, 20.7, and 19.0 ± 0.62 kg for CON, LoCoP, MdCoP, and HiCoP). An increasing trend ($P = 0.08$) was observed for milk yield (27.8, 28.6, 29.8, and 29.0 ± 1.08 kg). While no difference ($P > 0.14$) was observed in the concentration of milk protein across treatments (3.40 ± 0.098%) the concentration of fat increased ($P < 0.01$) with the inclusion of HPCoP (5.05, 5.18, 5.15, 5.47 ± 0.29). No differences ($P > 0.12$) were observed in the digestibility of DM, NDF, CP, TFA, and energy averaging 66.6 ± 0.63%, 48.7 ± 2.14%, 66.1 ± 0.79%, 74.1 ± 2.56%, 66.3 ± 0.72% across treatments. The concentration of GE linearly increased ($P < 0.01$) with increasing concentrations of HPCoP (4.25, 4.26, 4.28, and 4.31 ± 0.02 Mcal/kg), but no difference ($P > 0.25$) was observed in DE and ME across treatments averaging 2.83 ± 0.035 and 2.52 ± 0.039 Mcal/kg, respectively. An increasing trend ($P = 0.09$) was observed in concentration of NE_L (1.61, 1.71, 1.73, 1.70 ± 0.054 Mcal/kg) with the ratio of NE_L:ME increasing linearly ($P = 0.03$) across treatments (0.648, 0.671, 0.682, 0.674 ± 0.0162). Results of this study suggests that the inclusion of the HPCoP can replace common feeds high in protein and support normal milk production.

Key Words: energy, corn product

P399 Feeding legume-based forages: Effects on milk yield, nutrient digestibility, and methane emissions in dairy cows.

J. P. Sacramento*[2,1], L. H. P. Silva[3], D. C. Reyes[1], Y. Geng[4,1], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH,* [2]*Federal University of São João del Rei, São João del Rei, MG, Brazil,* [3]*Western Kentucky University, Bowling Green, KY,* [4]*Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing, PR, China.*

Sixteen multiparous and 4 primiparous organic-certified Jersey cows averaging 116 ± 52 DIM and 502.1 ± 52.2 kg of BW were used to investigate the effects of partially replacing red clover (RC) with a legume mix (LM) on DMI, milk yield and composition, apparent total-tract digestibility of nutrients, and CH_4 emissions. Cows were blocked in pairs by DIM or parity and, within pair, randomly assigned to treatments in a crossover design. Each experimental period lasted 24 d, with 14 d for diet adaptation and 10 d for sample collection. Two fields were planted with RC- or alfalfa-grass mix consisting (% of total) of 79:14:7 legume:meadow fescue:timothy seeding rate. Forages were harvested as baleage, with second and third cuttings used in the study. Diets were fed twice daily as TMR, with a 60:40 forage:concentrate ratio. Based on the botanical composition of each field, the forage portion of the treatments contained (% of diet DM): (1) 41% RC, 5% white clover, 9% weeds, and 5% grasses (RC diet), and (2) 21% RC, 10% white clover, 12% alfalfa, 11% weeds, and 5% grasses (LM diet). Diets averaged 17.7 vs. 17.1% CP and 31.7 vs. 31.3% aNDFom for RC and LM, respectively. Fecal grab samples were collected over 3 consecutive d and analyzed for iNDF. Data were analyzed using PROC MIXED of SAS with significance declared at $P < 0.05$. Cows fed RC had greater DMI (21 vs. 20.4 kg/d; $P = 0.01$) than those fed LM, but no significant differences were observed for yields of milk (mean = 21.1 kg/d), 4% FCM (mean = 25.5 kg/d), and ECM (mean = 27.6 kg/d). Similarly, diets did not affect concentrations and yields of milk fat and protein, and MUN. In contrast, digestibilities of DM, OM, NDF, and ADF increased ($P < 0.05$) with feeding RC than LM (68.4, 70.4, 47.9, and 51.8% vs. 64, 66, 39.7, and 48.6%, respectively). Digestibility of CP did not differ between diets. While cows fed RC had lower CH_4 yield (18.8 vs. 19.6, respectively; $P = 0.03$), no significant differences were observed for CH_4 production (mean = 393 g/d) and CH_4 intensity (mean = 14.6 g/kg of ECM). In summary, despite improved DMI and digestibility and decreased CH_4 yield, cows fed RC did not produce more milk possibly because additional energy was not partitioned into milk synthesis.

Key Words: dairy cow, methane

P400 Effects of supplemental calcium gluconate on mid-lactation Holstein cow productivity.

S. T. Quanz*[1], L. K. Mamedova[1,2], K. E. Griswold[3], S. K. Kvidera[3], R. S. Fry[3], and B. J. Bradford[1,2], [1]*Kansas State University, Manhattan, KS,* [2]*Michigan State University, East Lansing, MI,* [3]*Micronutrients, Indianapolis, IN.*

The aim of this study was to assess the effects of supplementing encapsulated calcium gluconate (CaG) on milk component production and dry matter intake (DMI) of mid-lactation cows. Thirty multiparous Holstein cows (94 – 197 d in milk) were blocked by parity, pre-trial milk yield, body weight and condition score, then randomly assigned to treatment within block. Control cows were fed a basal diet top-dressed with palm fat at 0.04% of projected DMI, whereas treatment cows were fed the basal diet top-dressed with CaG embedded in a palm fat matrix (Selko Cremalto; Trouw Nutrition USA) at 0.07% of projected DMI. Cows underwent a 2-week basal diet covariate period followed by a 12-week treatment period. Mycotoxicosis contributed to cows being removed for metabolic problems (3 Control, 1 CaG); other cows were removed for lameness (2 Control) and mastitis (1 CaG). Data from removed cows (n = 7) were retained in the analysis until disease diagnosis. Statistical models included fixed effects of covariate period data, treatment, week, and treatment × week, with random effect of block. Covariate × treatment effects were included when significant. Supplementing CaG had no effect on DMI or yields of milk, protein, or fat ($P > 0.10$). CaG tended ($P = 0.056$) to increase milk fat content and decrease concentrations of protein ($P = 0.08$) and lactose ($P = 0.07$). In addition, CaG increased ($P < 0.05$) content and yield of de novo and mixed fatty acids in milk, with no effects on yield of preformed fatty acids in milk. A covariate × treatment interaction occurred ($P < 0.05$) for de novo and preformed fatty acid concentrations, showing that cows with greater concentrations of de novo fatty acids in the covariate period had greater increases in proportion of de novo fatty acids in response to CaG. Treatment did not affect ($P > 0.10$) energy-corrected milk (ECM) yield or feed efficiency (ECM/DMI). In conclusion, supplementation of CaG increased de novo milk fatty acid synthesis but otherwise had few impacts on productivity of mid-lactation cows. Further research is warranted to understand mechanisms linking CaG supplementation and milk fat synthesis.

Key Words: prebiotic, milk fat, feed additive

P401 Effect of dietary metabolizable protein level on enteric methane production and milk production of Holstein and Ayrshire dairy cows.

C. Benchaar[1], F. Hassanat*[1], K. A. Beauchemin[2], H. Lapierre[1], D. R. Ouellet[1], and C. Côrtes[3], [1]*Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada,* [2]*Agriculture and Agri-Food Canada, Lethrbridge Research and Development Centre, Lethbridge, AB, Canada,* [3]*Agrinova, Alma, QC, Canada.*

The objective of this study was to determine the effect of dietary metabolizable protein (MP) level on enteric methane production and milk production of Holstein and Ayrshire dairy cow breeds. Nine Holstein (DIM = 81 ± 20; milk yield = 42.3 ± 4.2 kg; BW = 701 ± 71 kg) and 9 Ayrshire (DIM = 91 ± 13; milk yield = 35.0 ± 3.4 kg; BW = 561 ± 58 kg) lactating cows were used in a replicated 3 × 3 Latin square (35-d period; 14-d adaptation) with a 2 × 3 factorial arrangement of treatments. Cows were fed (ad libitum) a TMR formulated (NRC, 2001) to provide 85%, 100% or 115% of MP requirements. Enteric CH_4 (i.e., respiration chambers) and animal performance (intake and milk production) were determined over 5 and 7 d, respectively. Main effects of breed, MP level and interaction (breed × MP) were determined using the MIXED Procedure of SAS. Linear and quadratic contrasts were used to determine effects of MP level on variable responses. Interaction breed × MP level was not significant for dry matter intake (DMI) or milk production. Intake of DM tended to increase linearly ($P = 0.10$) with increasing MP level and was higher ($P < 0.01$) for Holstein versus Ayrshire cows (25.7 vs, 20.5 kg/d). Milk yield increased linearly ($P < 0.01$) with MP level and was higher ($P < 0.01$) for Holstein versus Ayrshire cows (43.3. vs. 32.8 kg/d). Holstein cows produced more ($P < 0.01$) milk fat and milk protein (1.65, 1.32 kg/d, respectively) than did Ayrshire cows (1.33, 1.11 kg/d, respectively). Yields of milk fat and milk protein increased linearly and quadratically ($P \leq 0.01$), respectively, with increasing MP level. Feed efficiency increased linearly with increasing MP level ($P < 0.01$), but was not affected by breed. Methane emission was higher for Holstein versus Ayrshire cows (480 vs. 386 g/d, $P = 0.07$) and increased linearly ($P = 0.02$) with MP level. Increasing MP level in the diet of Holstein cows increased linearly CH_4 yield (17.5, 18.9, 19.6 g/kg DMI, $P = 0.02$) whereas no effect was observed in Ayrshire cows (averaging 19.0 g/kg DMI; breed × MP level interaction, $P \leq 0.07$). Results from this study show that milk production of Holstein and Ayrshire dairy cows responded similarly to increasing dietary MP level, even though DMI and production levels were very different between the 2 breeds. However, response of CH_4 yield to increasing dietary MP level differed between the 2 breeds.

Key Words: Holstein, Ayrshire, enteric methane

P402 Relationship between enteric methane and feeding behavior, digestibility, and nitrogen partitioning in Holstein and Jersey cows.

O. I. Santana*[1,3], M. E. Uddin[2,3], and M. A. Wattiaux[3], [1]*INIFAP Campo Experimental Pabellon, pabellón de Arteaga, AGS, Mexico,* [2]*University of California–Davis, Davis, CA,* [3]*University of Wisconsin–Madison, Madison, WI.*

Our aim was to explore the relationships between enteric methane (CH_4) production (g/d), yield (g/kg of dry matter intake, DMI), and intensity (g/kg of fat- and protein-corrected milk) with feeding behavior, digestibility, and N partitioning variables measured on mid-lactation Holstein (n = 12) and Jersey (n = 12) cows. Diets included 17% crude protein and 19 or 24% forage neutral detergent fiber (FNDF) mainly from alfalfa silage or corn silage (DM basis). Soyhull was used as a non-FNDF source to maintain dietary NDF relatively constant across diets. All 24 cows were used in Latin squares to measure CH_4 using GreenFeed equipment, rumination time (RUM) using collars, and eating time (EAT) recorded visually. Additionally, 4 Holsteins and 4 Jerseys underwent total fecal and urine collection. Pearson correlations were calculated in R Studio. Breed did not influence any relationship. CH_4 production was correlated negatively with RUM time (min/d; r = −0.31; $P < 0.05$), but positively with fecal N (g/d; r = 0.69; $P < 0.01$), urinary N (g/d; r = 0.66; $P < 0.01$), and milk N (g/d; r = 0.62; $P < 0.01$). CH_4 yield was correlated positively with EAT (min/kg DMI; r = 0.39; $P < 0.01$), RUM (min/kg DMI; r = 0.29; $P < 0.01$) and chewing (RUM+EAT) time (min/kg DMI; r = 0.35; $P < 0.01$), but negatively with cellulose digestibility (r = −0.59; $P < 0.01$). Finally, CH_4intensity was not correlated with any measured variables. There was a strong correlation between CH_4 yield and intensity (r = 0.67; $P < 0.01$) but a weak correlation between production and yield (r = 0.26; $P < 0.05$) or production and intensity (r = 0.34; $P < 0.05$). The mode of expression of CH_4influenced how CH_4 was correlated with all measured variables. On a daily basis, CH_4 production was associated negatively with RUM but positively with milk N, fecal N, and urine N. In contrast, CH_4 yield was associated negatively with cellulose digestion but positively with feeding behavior variables expressed per unit of DMI. In this study, losses of C and N were associated with one another.

Key Words: dairy, efficiency, rumination.

P403 Effect of a commercial transition cow pack on colostrum IgG concentration and productive performance of multiparous Holstein cows.

L. G. Plazas[1], L. E. Muñoz[2], J. Ruiz[1], E. Bernal*[2], J. A. Velásquez[1], and M. G. Zenobi[3], [1]*Agrícola Santa María, Bogotá, Bogotá, Colombia,* [2]*Spin Colombia SAS, Bogotá, Bogotá, Colombia,* [3]*Balchem Corp., New Hampton, NY.*

The periparturient period is a metabolically demanding time for dairy cows because of the increased nutrient requirements for milk yield. Our objective was to determine whether increasing the supply of key nutrients during the transition period affected the IgG concentration of colostrum and milk yield of cows, not only during early lactation but also its carryover effect. Three weeks before expected calving, animals were blocked by lactation number and calving date and then randomly assigned to either control group (CTRL; n = 12) or Spin Pack Transition treatment (SPT; n = 12). From 3 weeks before calving through 3 weeks after calving, cows in the SPT group were individually supplemented daily with a top-dressed SPT blend at 100 g (12.9 g of choline ion and 7 g of DL-methionine in rumen-protected form, organic Co, Zn, Mn, Cu, Cr, live yeast and prebiotic) whereas CTRL received 100 g of cornmeal. Cows were fed common, prefresh and a lactation diet (16% and 21% CP, respectively), composed of grazed kikuyu pasture (*P. clandestinum*) and concentrate. Colostrum samples collected within the first 8 h were analyzed for IgG concentrations using a digital refractometer. For each cow, milk production data were fitted to Wood's lactation curve model. The minimum number of milk records per cow were 15 and the maximum were 19, with an average of 15.6. From this model, weekly milk yield was estimated for each treatment up to 15 weeks and these data were analyzed with PROC MIXED in SAS with time as a repeated measure and previous 305 ME as a covariate. This trial was conducted in a commercial dairy farm. Significance was declared as $P \leq 0.10$ and tendency at $P < 0.15$. Cows from the SPT group tended to produce more milk (29.2 vs 25.0 ± 1.8 kg/d; $P = 0.11$). There was a treatment × time effect on milk yield; cows from the SPT group had greater production from wk 7 to wk 15 postpartum ($P < 0.11$). There was no difference in colostrum IgG concentration (Brix units 19.2 vs 23.0 for CTRL and SPT; respectively; $P = 0.19$). Caution is advised due to a limited number of animals when interpreting the data. Overall, feeding a blend of key nutrients during the transition period had an immediate effect on milk yield, but also affected milk production through 15 weeks postpartum. This is further evidence of how the provision of key nutrients during the transition period impacts further lactation performance of cows.

Key Words: milk production, transition, choline

P404 Testing palatability of hydrolyzed feather meal and solutions to increase preference for it in lactating Jersey cows.

K. Buse*, J. Hill, and P. J. Kononoff, *University of Nebraska-Lincoln, Lincoln, NE.*

Hydrolyzed feather meal (HFM) is a readily available, high-protein by-product that is often fed to dairy cattle; however, practical concerns of its palatability exist. The objectives of this study were to conduct 2 experiments to evaluate the preference of HFM compared with other common feed commodities and to determine if the addition of flavor enhancers can improve palatability. Two taste preference experiments using 8 lactating Jersey cows (196 ± 7 DIM, 25.0 ± 5.35 kg/d milk yield, 21.8 ± 2.69 kg/d DMI) were conducted to test these objectives. In experiment 1, HFM was compared with ground corn (COR), dried corn distiller's grains with solubles (DDGS), and an equal blend of all 3 (BLND). Cows were offered 1.13 kg of each in randomized arrangement within the feed bunk for 1 h or until the feed was fully consumed or consumed in the greatest amount. Feeds were offered for 9 d. All cows were offered the 4 treatments for the first 4 d. The most preferred feed for each cow was then removed, and the remaining 3 feeds were offered for 3 d. The process was repeated for the last 2 d. Feed preference was ranked from 1 to 4 with 1 being the most preferred and 4 the least; this was in the order they were removed. Experiment 2 evaluated HFM alone or with one of 3 flavor enhancers: oregano (ORG; High Quality Organics, Reno, NV), artificial licorice flavor (LIC; Luctarom 5296Z, Lucta USA, LLC, Mahwah, NJ), and artificial melon flavor mixed with a sensory feed additive (MBO; Luctarom 3618Z and Bitter Off, Lucta USA, LLC, Mahwah, NJ). The same process of ranking preference was repeated except that 0.05 kg of HFM and an additive was mixed with 0.41 kg corn silage was offered. In experiment 1, the preference ranking of DDGS was highest (1.25 ± 0.71) followed by COR, BLND, and HFM (2.00 ± 0.53, 2.64 ± 0.52, and 3.38 ± 0.74). In experiment 2, ORG was observed to be the most preferred (1.71 ± 0.95) followed by LIC (2.29 ± 1.11), MBO (2.71 ± 1.11), and HFM (2.57 ± 0.79). These results suggest that although feed preference for HFM may not be as high as other common feeds, palatability may be improved by the addition of flavoring agents.

Key Words: hydrolyzed feather meal, palatability, feed preference

P405 Effects of magnesium source and buffer inclusion on fermentation profile in a dual-flow continuous culture system.

B. C. Agustinho*[1,2], A. Ravelo[1], J. R. Vinyard[1], R. R. Lobo[1], E. Sarmikasoglou[1], J. Arce-Cordero[1], H. F. Monteiro[1], E. R. Q. Vieira[3], S. Bennett[4], C. Stoffel[5], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal, Veterinary & Food Sciences, University of Idaho, Moscow, ID,* [3]*Departamento de Zootecnia, Universidade Federal do Tocantins, Araguaína, TO, Brazil,* [4]*Penn State College of Agricultural Sciences, University Park, PA,* [5]*Papillon Agricultural Company, Easton, MD.*

The objective of the study was to evaluate the effects of replacing magnesium oxide (MgO) by calcium-magnesium carbonate (MIN-AD, Papillon Agricultural Company, Easton, MD), with or without the addition of sodium bicarbonate on ruminal pH, volatile fatty acids (VFA), and soluble Mg. Eight fermenters of a dual-flow continuous culture system were distributed in a replicated 4 × 4 Latin square design in a 2 × 2 factorial arrangement (Magnesium source: MgO vs. MIN-AD; buffer: presence or absence). The experiment consisted of 4 periods of 10 d (7 d of adaptation and 3 d of sampling). The diets were: MgO (0.21% of MgO, DM basis); MgO + buffer (0.21% of MgO + 0.5% of sodium bicarbonate, DM basis); MIN-AD (1% of MIN-AD, DM basis); and MIN-AD + buffer (1% of MIN-AD + 0.5% of sodium bicarbonate, DM basis). The diets were formulated to contain 16% CP, 1.69 Mcal/kg of NEL, 0.67% of Ca, and 0.28% of Mg. Samples were collected from the effluent containers on d 9 and 10 to determine VFA, and soluble Mg, while the pH was measured into the vessels at 0, 1, 2, 4, 6, and 8 h post morning feeding. Data were analyzed using the GLIMMIX procedure of SAS with significance declared at $P \leq 0.05$. Buffer inclusion increased pH and molar proportion of isobutyrate, while the utilization of MIN-AD decreased molar proportion of isobutyrate. No effects were observed ($P > 0.05$) for total VFA concentration, the molar proportion

Table 1 (Abstract P405).

	Treatment					Contrasts (*P*-value)		
Item	MgO	MgO + buffer	MIN-AD	MIN-AD + buffer	SEM	Mg source	Buffer	Interaction
Total VFA, mM	90.6	88.9	88.6	89.9	1.44	0.58	0.85	0.14
VFA, % of total VFA								
Acetate	47.9	49.2	48.8	48.5	0.95	0.92	0.37	0.17
Propionate	31.3	27.5	30.4	30.5	2.61	0.41	0.16	0.13
Butyrate	13.8	16.2	13.8	13.9	1.53	0.15	0.14	0.17
Valerate	3.55	3.05	3.50	3.48	0.29	0.29	0.14	0.18
Isobutyrate	0.604	0.704	0.554	0.599	0.06	0.03	0.04	0.41
Isovalerate	1.98	2.35	2.16	2.16	0.21	0.95	0.14	0.14
Mg, ppm	229	266	223	242	32.0	0.54	0.25	0.69
pH	5.72	5.86	5.74	5.77	0.09	0.38	0.05	0.20

of the other VFA, and Mg solubility. In conclusion, the main variables in the fermentative profile were not affected by the replacement of MgO with MIN-AD.

Key Words: in vitro, magnesium, mineral

P406 Effects of different levels of vitamin A supply on production and blood parameters of transition cows.

M. Rodriguez*[1], W. Weiss[1], B. Enger[1], K. Lee[2], and C. Lee[1], [1]*The Ohio State University, Wooster, OH,* [2]*The Ohio State University, Columbus, OH.*

Negative energy balance after calving promotes body fat mobilization and excessive mobilization can increase risk of hyperketonemia. Evidence exists that vitamin A (VA) is closely associated with adipose fat metabolism in nonruminant species. This study investigated whether low or high VA supply to transition cows affected production and fat mobilization from blood indicators. Sixty-three prefresh Holstein cows in a complete randomized block design were assigned to: CON, a transition diet with supplemental VA (75 kIU/d) to meet requirement (NRC 2001); LO, no supplemental VA; HI, receiving supplemental VA (190 kIU/d) 2.5 times greater than the requirement. Periods were prepartum (14 d before calving), postpartum (30 d after calving) and carryover (31 to 58 DIM; all cows were fed a common diet). Cows were fed a diet with no supplemental VA in the far-off period. Milk yield (MY) and DMI were recorded daily and blood plasma was collected to measure cholesterol, fatty acids (FA), and β-hydroxybutyrate (BHB). Whole blood was collected at 5 DIM to determine blood differential counts. Data were analyzed using PROC MIXED of SAS (block was random effect; diets, repeated week and their interaction were fixed effects). Treatment did not affect DMI during the pre- (ave. 10.6kg/d) and postpartum (18.8 kg/d) periods. There was no difference in MY in postpartum (ave. 37.1 kg/d) and carryover period (43.6 kg/d) among treatments. Milk fat content increased linearly (4.49 to 5.12%; $P = 0.02$) as VA increased (i.e., from LO to HI) but yield did not differ. Cholesterol, FA and BHB were not affected pre- and postpartum by treatments (average 21% incidence of hyperketonemia based on postpartum BHB ≥ 1,200 umol/L). Increasing VA supply linearly decreased ($P = 0.01$) segmented neutrophil (%) and linearly increased ($P = 0.01$) lymphocyte (%). In conclusion, providing VA lower or higher than the current requirement to transition cows did not affect milk production and likely had no impact on body fat mobilization and hyperketonemia. However, we found excessive VA supply may negatively affect resistance or susceptibility to disease.

Key Words: vitamin A, transition cow, hyperketonemia

P407 Relationship between attenuated total reflection-Fourier transform infrared spectroscopy protein molecular structure features in cool-climate adapted faba bean seeds and biodegradation and nutritional properties in dairy cows.

G. Deng*[1,2], M. E. Rodríguez-Espinosa[1], X. Feng[1,2], H. Zhang[2], and P. Yu[1], [1]*Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada,* [2]*College of Life Science and Engineering, Foshan University, Foshan, China.*

The objective of this study was to study an interaction between protein molecular structure from biodegradation residues and nutritional properties of fava bean seed with low and normal tannin levels. Protein molecular structures including amide I, II, α-helix and β-sheet in rumen biodegradation residues were determined by attenuated total reflection-Fourier transform infrared (ATR-Ft/IR) molecular spectroscopy. The nutritional properties included chemical and nutrient profiles, in situ rumen degradation kinetics, rumen protein degradation, and intestinal protein digestion. RCBD was used as an experimental design. Total fava bean genotype samples were 24 with 8 genotypes of fava beans with 3 university research fields. Statistical analyses were performed MIXED procedure of SAS 9.4 with significance declared at $P < 0.05$. The results showed that the spectral intensity of fava bean varieties with a normal tannin level in rumen biodegradation residues was greater ($P < 0.05$) than that with a low tannin level. The spectral intensity of amide I, II areas and peak heights, α-helix and β-sheet peak heights in the genotypes in biodegradation residuals of fava bean seeds had a unique pattern with increasing first and then decreasing ($P < 0.05$) with the increasing of rumen incubation time. The molecular structures of protein (α-helix, ratio of α-helix to β-sheet height and amide I to II area, $R^2 > 0.6$) were associated ($P < 0.05$) with in situ degradation kinetics - soluble and potential degradable fractions and rumen undegraded protein. The molecular spectral parameters in the Ft/IR fingerprint region did not form cluster among different genotypes in residual fava bean seeds in 12 and 24h incubation, which indicate they had similar protein molecular structures after incubation. In conclusion, there was an interaction between protein molecular structure from biodegradation residues and nutritional properties of fava bean seeds with normal and low level of tannin. The genotype had an impact to the protein molecular structure and protein utilization were predictable from protein spectral molecular structures after rumen biodegradation with ATR-Ft/IRS spectroscopy.

Key Words: attenuated total reflection-Fourier transform infrared spectroscopy (ATR-Ft/IR) molecular structure, degradation and digestion, low and high tannin faba bean

P408 Effects of probiotics supplementation on ruminal and plasma metabolome of early-lactation dairy cows: Targeted metabolomics approach.

A. Oyebade*[1], A. Adesogan[1], D. Vyas[1], and I. Ogunade[2], [1]*University of Florida, Gainesvile, FL,* [2]*West Virginia University, Morgantown, WV.*

This study applied a targeted quantitative metabolomics approach to evaluate the effect of 2 probiotic supplements on rumen and plasma metabolome in lactating Holstein dairy cows. Study duration comprised a 14-d covariate and 84-d treatment period. A total of 45 dairy cows were assigned to 15 blocks based on covariate energy-corrected milk yield, and randomly assigned, within each block, to 1 of 3 treatments: (1) Corn silage-based diet without probiotics (Control; CON), (2) Basal diet top-dressed with mixture of *Lactobacillus animalis* and *Propionibacterium freudenreichii* at 3×10^9 cfu/d (PRO-A), and (3) Basal diet top-dressed with a mixture of *L. animalis*, *P. freudenreichii, Bacillus subtilis*, and *B. licheniformis* at 11.8×10^9 cfu/d (PRO-B). Rumen fluid and blood samples were taken every 21 d during treatment period. Composite plasma and rumen fluid samples were analyzed using direct injection with a reverse-phase LC-MS/MS. Data analysis including metabolite set enrichment analysis (MSEA) was done using Metaboanalyst 4.0. Partial least squares discriminant analysis (PLS-DA) was used to compare CON with PRO-A and PRO-B. Based on PLS-DA model, metabolites with variable importance in projection score ≥1.5 were considered powerful group discriminators. Compared with CON, dietary supplementation of either PRO-A or PRO-B increased ruminal concentrations of lactate, several carnitines, acylcarnitines, and amino acids; however, plasma lysine concentration was reduced by supplemental PRO-A. Compared with CON, results of MSEA showed that PRO-A enriched metabolism of selenocompound, taurine and hypotaurine in the rumen while metabolisms of biotin, tryptophan, ubiquinone and arginine were enriched in plasma. Compared with CON, PRO-B supplementation upregulated ($P \leq 0.10$) pathways related to metabolisms of starch, sucrose, pyruvate and tryptophan in the rumen. This study demonstrated that dietary supplementation of either PRO-A or PRO-B altered the concentrations of several plasma and ruminal metabolites involved in essential metabolic pathways in dairy cows.

Key Words: direct-fed microbes, plasma metabolite, rumen metabolite

P409 Integrative hepatic metabolomics and proteomics reveal insights into the metabolic changes in dairy cows during the transition period. J. Zhang*[1,2], N. Gaowa[1], S. Li[1], Z. Cao[1], H. Yang[1], and Y. Wang[1], [1]*College of Animal Science and Technology, China Agricultural University, Beijing, P. R. China,* [2]*College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, P. R. China.*

The transition period from late pregnancy to early lactation is a key time in the lifecycle of dairy cows due to the markedly metabolic challenges, which are related to the health, welfare, and production efficiency as well as sustainable profitability in dairy farming. The liver is the central pivot of metabolism in cattle. However, the physiological molecular adaptation in the liver of dairy cows during the transition period has not been clearly elucidated. The objective of this study was to investigate the hepatic metabolic changes in transition cows by using integrative metabolomics and proteomics methods. Liver tissue biopsies collected from 12 healthy multiparous Holstein dairy cows on d 21 before and after calving were analyzed using gas chromatography quadrupole-time-of-flight mass spectrometry-based metabolomics and data-independent acquisition (DIA) based quantitative proteomics methods. Significantly differently produced metabolites between treatments were recognized using variable significance in projection (VIP) scores ≥1.0 and $P < 0.05$. A fold change (FC) of 1.5 and $P < 0.05$ was adjusted as the threshold for identifying differently synthesized proteins. A total of 46 metabolites (VIP ≥1.0 and $P < 0.05$) and 205 proteins (FC <0.5 or FC >1.5 and $P < 0.05$) were identified as significantly differentially expressed in the liver during the transition period. When comparing 5 selected proteins between DIA and Western blot platforms, a significant positive correlation (r >0.6 and $P < 0.05$) was observed, confirming the high relevance of the data. Integrative functional analysis of differentially expressed metabolites and proteins confirmed the upregulated amino acid degradation, ribosome proteins, fatty acid oxidation, peroxisome proliferator-activated receptor signaling pathway, TCA cycle, and gluconeogenesis in postpartum dairy cows. However, the oxidative stress defense-related proteins such as CYP2C18, CYP2D14, GSTM1, GSTM2, GSTM3, and GSTM4 were downregulated in postpartum dairy cows, which may be the reason for increased oxidative stress and subsequent increased susceptibility to production diseases and other health problems. These results may provide a better understanding of the biology of dairy cows during their transition periods, which can be helpful in further decreasing health problems and increase the profitability of dairy farming.

Key Words: transition period, metabolomics, proteomics

P410 Diversity of active ureolytic bacteria in the rumen of dairy cows: Comparison of complementary DNA and genomic DNA. L. Sijia[1,2], Z. Nan[1,2], Z. Shengguo*[1,2], and W. Jiaqi[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, P. R. China.*

Urea is used as a nonprotein source of nitrogen in feed for ruminants. Ureolytic bacteria produce urease that hydrolyzes dietary or recycled urea to ammonia, which is an important source of nitrogen (N) for microbial growth and reproduction in the rumen. The diversity of ruminal ureolytic bacteria benefits N utilization efficiency in ruminants. The urease gene (*ureC*) has been used as a marker to characterize the diversity of ruminal ureolytic bacteria at the genomic DNA (gDNA) level. However, there is no information at the RNA level to reflect the active status of bacteria. The purpose of this study was to reveal the diversity of active ureolytic bacteria in the rumen by comparing *ureC* amplicons between gDNA and complementary DNA (cDNA). Rumen fluid was collected from 4 Holstein dairy cows with rumen fistulas at 0, 2, and 6 h after morning feeding. Total microbial gDNA and RNA were isolated, and the RNA was reverse-transcribed into cDNA. The *ureC* gene amplicons of gDNA and cDNA were produced and sequenced by MiSeq. The results revealed distinct ureolytic bacterial community profiles based on gDNA and cDNA. The sampling time had no significant effect on the α and β diversity indices of the *ureC* gene. The Shannon diversity of the *ureC* gene for cDNA was greater than that for gDNA ($P < 0.05$). There were significant difference in the β diversity of *ureC* gene between gDNA and cDNA ($P < 0.01$), which indicates a shift in the community of active ureolytic bacteria. Approximately 43% of *ureC* sequences from cDNA could not be confidently classified at the phylum level. The active ureolytic bacteria were mainly from *Helicobacter*, *Herbaspirillum*, *Clostridium*, *Paenibacillus*, *Synechococcus*, and *Sphingobacterium sp*. Changes in the operational taxonomic units revealed that the top abundant *ureC* genes were mostly consistent between gDNA and cDNA, and most differences occurred in the *ureC* genes with lower abundances. These results are expected to expand current knowledge of the active *ureC* genes and ureolytic bacteria in the rumen.

Key Words: rumen, ureolytic bacteria, *ureC*

P411 Novel anionic supplement in prepartum dairy cow rations for healthy parturition and early lactation. J. R. Knapp[1], T. Takagi[2], R. S. Reed[2], and P. G. Summer*[2], [1]*Fox Hollow Consulting LLC, Lewisburg, OH,* [2]*Ajinomoto Health & Nutrition North America, Itasca, IL.*

Pre-calving diets for dairy cows are often formulated to induce metabolic acidosis to increase calcium mobilization before parturition and reduce the incidence of subclinical and clinical hypocalcemia postpartum. PF-21 (PF) is a novel feed additive that provides concentrated anions and glutamate, which is known to bypass the rumen and is associated with gut health. The study objective was to evaluate PF-21 in transition cows and compare its effects to SoyChlor (SC, Landus). Multiparous Holstein cows ($n = 48$) were fed a diet containing PF or SC, balanced to achieve a DCAD of −150 mEq/kg for 21 to 30 d prepartum. Cows were blocked by parity and expected calving date and randomly assigned to treatment. Cows were grouped in pens of 8 cows with 3 pens per treatment. Following calving, cows were fed a common diet and grouped together. Pre-partum urine pH, postpartum milk yield and composition, and health events were measured and recorded. Data were analyzed using a mixed model with repeated measures for urine pH, milk yield and component data, with treatment and pen as discrete fixed effects, time pre- or postpartum as a continuous fixed effect, and cow nested within pen x treatment as a random effect. A X^2 analysis was used to evaluate differences in incidence of health events between treatments. As expected, time postcalving significantly affected milk yield and composition. There was no treatment × time interaction, indicating that cows fed both treatments responded similarly in early lactation. Milk yield and milkfat % were numerically, but not statistically, greater for cows fed PF vs SC. Energy-corrected milk yields averaged 59.1 and 57.2 kg/d for cows fed PF and SC, respectively, for 30 d postcalving. Cows fed PF had significantly less ketosis (0 vs. 6) and numerically less hypocalcemia (4 vs. 7) than cows fed SC. PF was as effective as SC in decreasing urine pH during the dry period without compromising dry matter intake. Overall, PF was effective in achieving the metabolic acidosis necessary to increase calcium mobilization prepartum, and cows fed PF transitioned and performed well in early lactation.

Key Words: calcium mobilization, negative DCAD, glutamate

P412 Evaluation of heats of combustion of fiber contained in feed and fecal samples. J. D. Stypinski*[1], P. J. Kononoff[1], W. P. Weiss[1], and K. K. Buse[1], [1]*University of Nebraska- Lincoln, Lincoln, NE,* [2]*The Ohio State University, Columbus, OH.*

In the 7th eEdition of the *Nutrient Requirements of Dairy Cattle* (NRC, 2001), digestible energy (DE) is estimated using a summative equation. In this equation, the masses of nutritive entities are multiplied by their expected heats of combustion to yield energy values. In the case of fiber, digestible NDF is first estimated using a lignin-based equation and then that proportion is multiplied by the expected heat of combustion of starch (4.2 kcal/g). Variation in this method to estimate DE has been reported and a large proportion of this variation is associated with measures of digestibility. However, in addition to cellulose, the isolated NDF residue may contain other constituents such as lignin, ash, hemicellulose and nitrogen containing compounds and in turn, the heat of combustion may be different than starch. This study aimed to determine the heat of combustion of NDF and to consider if it is a contributing factor to the variance in DE. To do so, NDF residues were isolated from 9 feed and 8 fecal samples and then combusted. The feed samples used in this study were as follows; corn silage (n = 2), grass hay (n = 2), alfalfa hay (n = 2), wheat straw (n = 1), cottonseed hulls (n = 1), and DDGS (n = 1). The mean NDF content within forage class varied widely. The 8 fecal samples originated from individual animals enrolled in a nutrition study. Differences between the heats of combustion of feed and fecal samples were tested using a *t*-test. The mean heat of combustion of NDF residues from feed was observed to be 4.13 ± 0.21 kcal/g and was not significantly different ($P = 0.796$) from the heat of combustion of NDF residues from feces which was observed to be 4.09 ± 0.37 kcal/g. The lack of a statistical difference between feed and fecal NDF residues was surprising but indicates that NDF residue contains similar concentrations of energy even when it is not digested by the animal. Furthermore, the heat of combustion observed was less than assumed and could lead to an overestimation of energy by nutrition models. Further research should be conducted to further investigate factors that affect heat of combustion of NDF residues.

Key Words: NDF, energy, bomb calorimetry

P413 Effect of supplementing dairy cows with *Bacillus subtilis* PB6 and/or chromium propionate on health, culling, reproductive performance and metabolic parameters. A. Lago*[1], M. Morales[2], T. Harris[2], C. Moore[2], G. Camacho[1], K. Patel[1], R. Lopes[3], and N. Silva-del-Rio[3], [1]*DairyExperts Inc., Tulare, CA,* [2]*Kemin Industries Inc., Des Moines, IA,* [3]*UC Davis VMTRC, Tulare, CA.*

The objective was to evaluate the effect of supplementing *Bacillus subtilis* PB6 and/or chromium propionate on health, culling, reproductive performance and metabolic parameters. A total of 680 Holstein cows from a California Central Valley dairy were individually orally dosed with a syringe once a day from prepartum to 110 DIM with a) *Bacillus subtilis* PB6 (BS), 0.5 g of CLOSTAT 500, Kemin, Des Moines, IA, b) chromium propionate (CP), 2 g of KemTRACE Chromium also from Kemin, c) *Bacillus subtilis* PB6 and chromium propionate (BSCP), or d) placebo (PL). Multivariable mixed models were used for the analysis of all variables with the exception of Cox regression for culling. Significance was established at $P < 0.05$ and tendency at $P < 0.10$. There were no statistical differences in health events or the proportion of cows conceiving at first breeding among the different treatments. However, there was a reduction in the risk for culling. Using PL as reference, the hazard ratio for a cow to be culled was 0.48, 0.74 and 0.58 for BS, CP, and BSCP, respectively. In terms of statistical significance, culling was lower for BS ($P = 0.01$) and tended to be lower for BSCP. In addition, linear SCC score was 2.22, 2.03, 2.28 and 2.44 cells/mL, respectively, with statistically significant difference for CP vs. PL ($P = 0.01$). Prepartum FA was not significantly different among treatments. The proportion of cows with postpartum serum FA equal or greater to 0.72 mEq/L was 18.4, 14.0, 5.1 and 23.5%, respectively, and blood BHBA equal or greater to 1.2 mmol/L was 10.2, 15.0, 14.2 and 22.9%, respectively. Significant differences in proportions were determined when comparing high FA in cows assigned to BSCP vs. PL ($P = 0.01$) or BS ($P = 0.02$) cows, and when comparing high BHBA in cows assigned to BS vs. PL ($P = 0.03$). In conclusion, although there was no effect of the interventions on health events and reproductive performance, different treatments influenced meaningful metabolic parameters, SCC, and the risk of culling.

Key Words: *Bacillus*, chromium, direct-fed microbial

P414 Effects of feeding low-tannin whole-crop faba bean silage on lactational performance in high-producing dairy cows. V. H. Guevara-Oquendo*, D. A. Christensen, J. J. McKinnon, B. Tar'an, and P. Yu, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada*

The objective of this dairy trial was to determine the effect of partial (50% and 75%) and complete (100%) replacement of barley and corn silages with low tannin (snowdrop variety) whole-crop fava bean silage at late pod stage (97 d old) on high production dairy cows in terms of milk yield, efficiency, digestibility of primary nutrients and rumen fermentation characteristics. A double 4 × 4 Latin square was used as an experimental design. Statistical analyses were performed for LSD data using PROC MIXED procedure of SAS 9.4 with significance declared at $P < 0.05$. The results showed that T100 (30.60% whole-crop fava bean silage) produced higher ($P < 0.05$) fat-corrected milk (3.5% FCM) and higher ($P < 0.05$) energy-corrected milk (ECM) than control diet T0 (18.37% corn silage + 12.23% barley silage) (+4.35 and +3.48 kg/cow/d respectively), but produce similar FCM and ECM than T50 (9.18% corn silage + 6.12% barley silage + 15.30% whole-crop fava bean silage) and T75 (4.59% corn silage + 3.06% barley silage + 22.95% whole-crop fava bean silage). Additionally, the fat yield was higher ($P < 0.05$) when whole-crop fava bean silage was included in the diet (2.11 vs. 1.89 kg/cow/d). Efficiency (DMI/FCM) was higher ($P < 0.05$) when animals consumed T75 than T0 (2.21 vs. 1.91), while the digestibility of starch was similar ($P > 0.10$) among T50, T75 and T100 but were lower ($P < 0.05$) than in T0 (92.65% vs. 96.13%). Ammonia, volatile fatty acids and pH were similar ($P > 0.10$) among all the treatment. In conclusion, the inclusion of whole-crop fava bean silage at late pod stage improve fat-corrected milk, energy-corrected milk, milk fat yield and efficiency without negatively affecting the intake of dry matter. Consequently, this study showed that whole-crop fava bean silage is a highly nutritive alternative feed to improve the performance of dairy cows in western Canada.

Key Words: whole-crop faba bean silage, low-tannin variety, lactational performance of dairy cows

P415 Effects of reducing dietary cation-anion difference on milk production and milk fatty acid composition of lactating dairy cows.

H. M. Zynda*[1], J. E. Copelin[1], W. P. Weiss[1], F. Sun[2], and C. Lee[1], [1]*The Ohio State University, Wooster, OH,* [2]*Origination O2D, Woodbury, MN.*

Reducing the dietary cation-anion difference (DCAD; Na + K − Cl − S) ≤ 100 mEq/kg DM has not been extensively studied in lactating cows. While the exact mechanism is unknown, lactating cows fed a low DCAD diet may reduce DMI, milk yield, and milk fat. This study examined the effects of reduced DCAD on production and milk fatty acid profile. In a randomized complete block design, 27 cows were blocked by parity and DIM and randomly assigned to: CON, a typical lactating diet (192 mEq/kg DM); MID, the same as CON with reduced DCAD (101 mEq/kg DM); and LOW, a diet with 1.2 mEq/kg DM. An anionic product (MegAnion) was substituted for urea and soybean meal in CON to decrease DCAD for MID and LOW (isonitrogenous). The experiment lasted 7 wk (1-wk covariate followed by 6-wk data collection). All data were analyzed using the MIXED procedure of SAS (block as random effect; diets, repeated wk, and interaction as fixed effects). Urine pH was decreased quadratically with decreasing DCAD (8.26, 8.05, 6.38 for CON, MID, and LOW, respectively; $P < 0.01$). Milk yield and DMI were not different among treatments. Energy-corrected milk tended to decrease linearly ($P < 0.10$; 35.1 to 32.7 kg/d) as DCAD decreased. This is due to numerical decreases in milk yield and milk fat content, resulting in a tendency of decreased milk fat yield (1.00 to 0.86 kg/d, $P = 0.08$) as DCAD decreased. Milk protein yield was not different among treatments. Changes in milk fatty acid profiles were minimal. *Trans*-10 and *trans*-11 C18:1 were not affected (average 6.7 and 0.74%, respectively). Although *trans*-10, *cis*-12 C18:2 increased linearly ($P < 0.01$) as DCAD decreased, the concentration was low (average 0.03%). However, the concentration of linoleic acid and total PUFA in milk increased ($P < 0.05$) as DCAD decreased. Therefore, a DCAD of 1.2 mEq/kg DM may be too low for lactating cows because of reduced lactation performance, but more work may be done in the 50 mEq/kg DM range.

Key Words: dietary cation-anion difference, milk fat, milk fatty acids

P416 Effect of a blend of essential oils on dairy cows' milk performance, efficiency, and methane emissions.

G. Forgeard*[1], A. Lemesle[1], Q. Bulcke[2], PH Pomport[2], and Y. Python[2], [1]*Techna France Nutrition, Couëron, France,* [2]*Experimental Farm AgroParisTech, Thiverval-Grignon, France.*

Essentials oils and other natural active ingredients are commonly used to modulate rumen fermentation and improve protein and energy efficiency. This trial was carried out to evaluate in vivo the effect of a blend of essentials oils (Elensis, Techna) on dairy cow performance and efficiency. The experimental design was 36 cows split in 2 groups of 18 cows grouped regarding parity, days in milk, milk production, milk solids and weight. A 2 weeks pre-experimental period was used as a covariable for the statistical analysis and then the performance was measured for a 10-week period. The control diet was based on corn silage, alfalfa silage, corn cob silage, rapeseed meal, beet pulp, concentrate and minerals. The protein content of the diet was 15.8% with 27% starch and sugar. Treatment group received in addition Elensis. Individual milk production was measured daily and milk solids measured twice every week. The statistical analysis was a mixed model with SAS software. Milk yield was higher for the treatment group than control (36.4 kg / cow/d versus 35.6 kg – $P = 0.014$) and energy-corrected milk (ECM) was also significantly higher for the treatment group (+ 1.5 kg /cow/d – $P < 0.001$). Milk solids content was 5% greater for the treatment thanks to a higher level of protein (3.36 g/l versus 3.31 g/l for control – $P = 0.003$) and higher level of fat (3.91 g/l versus 3.76 g/l for control – $P = 0.004$). At the same time in another experimental design, 6 cows were split in 2 groups to measure methane emissions in 2 × 2 Latin square protocol. Each period was 4 weeks and methane was measured with the SF6 technic for 5 d during the last week. Individual intake and milk production were also measured daily. Methane emissions were lower for treatment group compare with control (18.1 g CH_4 / kg of dry matter intake versus 19.3 – $P = 0.01$ and 11.7 g CH_4 / kg of ECM versus 12.3 – $P = 0.07$). Those results were in line with the performances expected in term of milk and milk solids in dairy cow herds at the same time than reducing global methane emissions.

Key Words: dairy cow, milk performances, methane emission

P417 Effect of supplementing dairy cows with *Bacillus subtilis* PB6 and/or chromium propionate on milk yield and components.

A. Lago*[1], M. Morales[2], T. Harris[2], C. Moore[2], G. Camacho[1], K. Patel[1], R. Lopes[3], and N. Silva-del-Rio[3], [1]*DairyExperts Inc., Tulare, CA,* [2]*Kemin Industries Inc., Des Moines, IA,* [3]*UC Davis VMTRC, Tulare, CA.*

The objective was to evaluate the effect of supplementing *Bacillus subtilis* PB6 and/or chromium propionate on milk yield and components. A total of 680 Holstein cows from a California Central Valley dairy were individually orally dosed with a syringe once a day from prepartum to 110 DIM with a) *Bacillus subtilis* PB6 (BS), 0.5 g of CLOSTAT 500, Kemin, Des Moines, IA, b) chromium propionate (CP), 2 g of KemTRACE Chromium also from Kemin, c) *Bacillus subtilis* PB6 and chromium propionate (BSCP), or d) placebo (PL). Milk yield and components were measured twice a month by the local DHIA. Multivariable mixed models were used for analysis of all variables with cow within treatment as repeated measures. Significance was established at $P < 0.05$. Compared with PL no statistically significant main effect and/or interactions were found in milk yield or composition for any of the treatments and only numerical differences were observed. Cows assigned to BSCP had the highest milk production (milk yield and energy-corrected milk) and cows assigned to PL had the lowest energy-corrected milk. Also, cows assigned to CP had the highest milk fat. Cows assigned to BS produced an average of 47.82 kg of milk (47.86 kg when energy corrected), with a 3.65% fat and 2.82% protein. Cows assigned to CP produced an average of 47.41 kg of milk (47.82 kg when energy corrected), with a 3.71% fat and 2.83% protein. Cows assigned to BSCP produced an average of 48.59 kg of milk (48.55 kg when energy corrected), with a 3.65% fat and 2.82% protein. Finally, cows assigned to PL produced an average of 47.41 kg of milk (47.36 kg when energy corrected), with a 3.65% fat and 2.81% protein. However, the study sample size provided less than 20% power (in excess of 95% confidence) to detect the 1.18 kg difference in milk yield between BSCP and PL which was the largest numerical difference observed in this study. In conclusion, no statistically significant main effect and/or interactions were observed in milk yield or composition for BS, CP or BSCP compared with PL in a commercial dairy herd.

Key Words: *Bacillus*, chromium, DFM

P418 Feeding Sweet Bran, a branded modified wet corn gluten feed, to lactating dairy cows: A meta-regression. K. C. Krogstad*[1], R. J. Tempelman[1], C. Abney-Schulte[2], and B. J. Bradford[1], [1]*Department of Animal Science, Michigan State University, East Lansing, MI,* [2]*Cargill Corn Milling, Blair, NE.*

Sweet Bran (SB; Cargill, Blair, NE) is a modified wet corn gluten feed that has been a replacement for both concentrates and forages in diets fed to dairy cows. Our objective was to evaluate effects of SB inclusion rate and other dietary factors on lactating dairy cow performance. An experiment was included for analysis if it was properly replicated, investigated 2 or more SB concentrations, reported dietary nutrient and ingredient concentrations, and reported the mean and SE for milk, fat, and protein production. Twelve publications and 50 treatment means were used in the analysis. A meta-regression approach was used to evaluate SB inclusion and other dietary factors. SB inclusion (range: 0 to 61% of diet DM), diet nutrient concentrations (DM, CP, NDF, fNDF), BW, DIM, quadratic terms, each interaction with SB, and random study effect were included in the model. The lmer and step functions of the lmerTest package in R (v 4.0.2) were used for backward stepwise regression. Covariates with the greatest *P*-value were eliminated until all remaining covariates had a *P*-value ≤0.05. SB inclusion was retained in all models and main effects were retained if included in an interaction or quadratic term. Residuals were evaluated graphically and with the Shapiro-Wilk test. Cook's D identified any observations which had excessive influence on each model. Variance inflation factor was calculated to check for multicollinearity within each model. Dietary SB and NDF interacted to affect DMI (*P* = 0.006); feeding SB increased DMI but the advantage decreased as dietary NDF increased. Milk fat % was affected by the interaction of SB and fNDF (*P* = 0.018) such that increasing fNDF had positive effects on milk fat % in diets with greater amounts of SB. Milk fat and protein yields were maximized at 26% and 11% SB inclusion, respectively. Also, SB and fNDF interacted to affect milk protein yield (*P* = 0.044) such that increasing fNDF had greater negative effects when diets included greater SB concentrations. Feeding SB can be successful when included up to 30% of the diet DM but other nutrients must be considered.

Key Words: corn gluten feed, milk production, meta-analysis

P419 Evaluation of the effectiveness of the air fryer to determine dry matter in forages and diets for dairy cattle. J. Granados-Niño[1], J. Sánchez-Duarte[1,2], O. I. Santana[2,3], J. Espino-Martínez[1], M. López-Calderón[1], A. Garcia[4], and F. Diaz*[4], [1]*FAZ-UJED, Durango, México,* [2]*INIFAP, Coahuila, México,* [3]*INIFAP, Aguascalientes, México,* [4]*Dellait Dairy Research Center, Brookings, SD.*

Air fryers have been used to measure the dry matter (DM) content of livestock diets, but there are many other important activities in the farm where DM content needs to be analyzed. The aim of this study was to compare the effectiveness of the air fryer with the forced air and the microwave ovens to estimate the DM content of forages and diets on the farm. Dry matter content in samples (n = 10) of each corn at harvest (CH), corn silage (CS), triticale haylage (TH), alfalfa haylage, heifer diet before insemination, and diet for high-producing dairy cows (CD) were analyzed using ANOVA followed by the Tukey's test for multiple comparisons of means. Each sample was individually dried for 30 min at 121°C with the air fryer, for 72 h at 65°C with the forced air oven, and sequentially (3.5 min, 1.5 min, 45 s, 30 s, and 20 s) with the microwave at maximum power. The repeatability of DM estimation was evaluated in samples of CH, CS, TH, and CD within each drying method. In addition, a simple linear regression analysis was used to compare the air fryer efficiency by using the forced air oven as the standard drying method. Results of this study indicated no statistical differences between the air fryer, forced air oven, and microwave to estimate the DM content of forages and diets for dairy cattle. All methods yielded highly repeatable DM estimations with less than 5% coefficient of variation for all samples and tested method. Results in Table 1 indicate a significant ($P \leq 0.05$) and strong linear relationship ($R^2 \geq 0.91$) for all forages and diets between the air fryer and the forced air oven. Therefore, the air fryer is a good on-farm alternative for an accurate and fast estimation of the DM content in forages and dairy cattle diets.

Key Words: forages, silages, dairy diet

Table 1 (Abstract P419).

Sample (n = 10)	Intercept	SE	*P*-value	Coefficient	SE	*P*-value	R^2
Corn at harvest	4.78104	2.45579	0.08742	0.87872	0.09016	≤0.0001	0.91
Corn silage	−11.88750	4.08953	0.01969	1.35957	0.12892	≤0.0001	0.92
Triticale haylage	−2.14017	3.81493	0.59016	1.01272	0.09531	≤0.0001	0.93
Alfalfa haylage	0.58513	2.26393	0.80258	0.94374	0.05804	≤0.0001	0.97
Heifer diet	0.47459	1.33051	0.73054	0.96092	0.02667	≤0.0001	0.99
Lactating cow diet	1.00499	2.76707	0.72587	0.95148	0.05569	≤0.0001	0.97

P420 Effect of zinc source on fecal *Treponema* spp. in ad libitum-fed or feed-restricted lactating dairy cows. B. A. Wenner*[1], T. Park[2], K. Mitchell[1], S. K. Kvidera[3], K. E. Griswold[3], E. A. Horst[4], and L. H. Baumgard[4], [1]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [2]*Department of Animal Science and Technology, Chung-Ang University, Anseong, Gyeonggi-do, Korea,* [3]*Micronutrients USA LLC, Indianapolis, IN,* [4]*Department of Animal Science, Iowa State University, Ames, IA.*

Previous research has demonstrated the potential effect of dietary trace mineral source on both ruminal and fecal microbiota. However, the effect of Zn hydroxychloride on the fecal microbiome has not previously been considered. We hypothesized Zn hydroxychloride would decrease *Treponema* spp. fecal excretion relative to cows fed zinc sulfate. To test this hypothesis, lactating Holstein cows (n = 24; 685 ± 9 kg BW; 159 ± 8 DIM; parity 3 ± 0.2) were randomly assigned to 1 of 2 dietary treatments, either control (CON; 75 ppm Zn from $ZnSO_4$ or 2) or Zn hydroxychloride (HYD; 75 ppm IntelliBond Z; Micronutrients USA LLC., Indianapolis, IN). Fecal samples were collected on d −1 before dietary treatments, on d 27 of feeding dietary treatments (period 1), and again on d 34 after cows within mineral treatment were factorialized to either ad libitum (AL, unrestricted feed) or restricted (FR, 40% of ad libitum) for 5 d (period 2). Fecal sample DNA was extracted by repeated bead beating, purified, and sequenced to establish taxonomy from the SILVA database using a universal primer for the 16s rRNA gene through MiSeq. For period 1, HYD decreased the % relative abundance of *Treponema* 2 by 3-fold (14.7 vs 4.9, $P = 0.05$) but there were no other differences among genera-level OTUs except a slight increase in uncultured *Barnesiellaceae* by HYD ($P = 0.03$). In period 2, there was no interaction of HYD × FR nor any main effect of HYD on *Treponema* abundance ($P > 0.10$). However, Bray-Curtis analysis of microbial communities revealed a trend for large-scale shifts in the fecal microbiome with FR ($P < 0.10$) which can be summarized by significant increases in relative abundance of many members of *Ruminococcaceae* ($P < 0.05$) with corresponding decreases within *Prevotellaceae* ($P \leq 0.05$). Poor sequencing resolution at the species level limits our ability to infer management or gut health implications of HYD supplementation yet the inclusion of pathogenic species among *Treponema* spp. indicates a potential implication of HYD feeding on environmental conditions of the dairy cow.

Key Words: zinc, *Treponema*, fecal microbiome

P421 Zearalenone hydrolyase ZenA (ZEN*zyme*) degrades zearalenone in rumen of dairy cows. J. Faas*[1], M. Killinger[1], A. Höbartner-Gußl[1], B. Doupovec[1], D. Schatzmayr[1], T. Hartinger[2], Q. Zebeli[2], and G. Vogtentanz[1], [1]*BIOMIN Research Center, Tulln, Austria,* [2]*2 Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Vienna, Austria.*

Zearalenone (ZEN) is a mycotoxin produced by numerous species of *Fusarium* and can be commonly detected in dairy feeds. Due to its similarity to estrogens, ZEN can act as an endocrine disruptor. In ruminants, the metabolism of ZEN to α-zearalenol (α-ZEL) in the rumen can lead to a potentiation of the estrogenic effect in comparison to the parent compound ZEN. Zearalenone hydrolyase ZenA (ZEN*zyme*) catalyzes the hydrolysis of ZEN forming the non-estrogenic hydrolyzed zearalenone (HZEN). The main objective of this experiment was to test ZEN*zyme* regarding its ability to modify the metabolism of ZEN in the rumen of dairy cows. Six rumen-cannulated, nonlactating, non-gestating Holstein Friesian cows received a ZEN treatment (5 mg ZEN mixed into 200 g ground wheat) for 2 d, followed by a 2-d washout-phase. After washout, animals received the same ZEN treatment but supplemented with a total of 100 Units of ZEN*zyme* (ZEN + ZEN*zyme*) for 2 d. Daily dry matter intake was measured and rumen fluid samples were taken each day from the reticulum at various time points. ZEN, α-ZEL, β-ZEL, and HZEN concentrations were measured using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) analysis. If requirements of normal distribution and homogeneity of variances were met, an independent *t*-test was performed, if not a nonparametric test was used (Mann-Whitney U-test). Dry matter intake was similar between treatments ZEN 10.5 kg DMI/day vs. ZEN + ZEN*zyme* 11.5 kg DMI/day, $P = 0.098$). The concentration of ZEN in ruminal fluid did already differ 15 min after administration of the treatment (ZEN content: ZEN 11.40 ng/mL vs. ZEN + ZEN*zyme* 0.84 ng/mL, $P < 0.001$). The enzyme-induced shift of ZEN-metabolites could also be observed after 15 min of administration (HZEN content: ZEN 0.50 ng/mL vs. ZEN + ZEN*zyme* 11.22 ng/mL, $P < 0.001$). These differences in concentrations of ZEN and HZEN between treatments could be observed at any of the following time points. We conclude that ZEN*zyme* was effective in detoxifying ZEN by rapidly transforming ZEN into HZEN in the rumen of dairy cows.

Key Words: dairy, zearalenone

P422 Responses in blood metabolites and immune status of transition dairy cows supplemented with coated choline chloride. L. Royo*[1], M. Puyalto[1], J. J. Mallo[1], G. Elocoso[2], J. Ragues[2], and A. Bach[3], [1]*Norel SA, Madrid, Spain,* [2]*Blanca from the Pyrenees, Hostalets de Tost, Spain,* [3]*Marlex Recerca i Educació, Barcelona, Spain.*

The aim of this study was to evaluate the effect of supplementation of coated choline chloride (Chol) on blood metabolites and immune status of dairy cows during transition. Sixty Holstein cows (606 ± 84 kg BW) were randomly distributed in 2 groups (n = 30) and exposed for 60 d to 2 treatments following a complete randomized design. Treatments consisted of supplementation with 60 g/d of Bychol (CLN; 12.9 g/d of choline) 3 wk before and after calving, or no supplementation in the control group (CTR). Cows were fed a TMR in the dry period (12.2% CP, 57.3% NDF, 1.30 Mcal NEl/kg; DM basis) and a fresh period TMR (15.3% CP, 32.2% NDF, 1.69 Mcal NEl/kg) twice daily. Treatments had the same basal level of methionine. Peripheral blood samples were taken from the coccygeal vessels from all cows on days −4, 3 and 10 relative to calving to determine concentrations of fatty acids (FA), β-hydroxy butyrate (BHB), triglycerides (TG), and very low-density lipoproteins (VLDL). To evaluate the immune status, lymphocyte counts and the ratio of T4 and T8 lymphocytes subsets (T4:T8) were determined by flow cytometry. Animal was the experimental unit and data were analyzed using a mixed-effects model with the fixed effects of treatment, sampling day, and their interaction, and the random effect of cow within treatment. Initial BW entered the model as a covariate. Plasma FA concentration did not differ between treatments. Plasma BHB content tended ($P = 0.07$) to be lower in CLN group (0.33 mM) than in CTR group (0.45 mM). Plasma TG and VLDL concentration was greater ($P < 0.05$) in CLN cows on day −4 compared with CTR cows, but no differences were observed thereafter. Cows in CLN group had lower ($P = 0.01$) counts of blood lymphocytes, compared with CTR cows during the entire study. Cows in CLN group tended ($P = 0.05$) to have a greater T4:T8 ratio than CTR cows on d 3 after calving. It is concluded that dietary supplementation with Chol during the transition period tends to reduce de BHB concentration in blood and tends to increase the blood T4:T8 ratio at d 3 postcalving.

Key Words: ketosis, coated choline, transition

P423 Periparturient performance of dairy cows receiving a rumen-protected blend of B vitamins and choline during the transition period: A meta-analysis of 23 feeding trials. E. Evans[1], O. AlZahal*[2], C. Gwyn[3], and H. Leclerc[3], [1]*Technical Advisory Services, Bowmanville, ON, Canada,* [2]*AlZahal Innovation & Nutrition, Kitchener, ON, Canada,* [3]*Jefo, St. Hyacinthe QC Canada.*

Recent research supports the efficacy and use of a rumen-protected blend of B vitamins and choline for transition cows (Morrison et al., 2018, J. Dairy Sci 101:9016; Kaur et al., 2019, J. Dairy Sci 102:1642). The objective of this study was to evaluate the impact of providing the same rumen-protected blend of B vitamins and choline (RP-BVB, Jefo, St. Hyacinthe, QC, Canada) to cows in commercial herds during the transition period upon indices of health, reproduction, and milk yield. Trials were conducted between 2005 and 2018. All farms participated in a monthly third-party record-keeping system. All farms included in the analyses were deemed eligible if confirmed by the site's formulating nutritionist that the product was included in the ration, there were no changes in the method of obtaining measurements between test and control periods, and there were no unusual occurrences in herd demographics. Cows included received the RP-BVB for a minimum of 14 days prepartum to 10 days postpartum. A total of 13,116 cows from 6 countries participated. Trials were analyzed using the Meta-Essentials software program (Creative Commons, 2017) to assess risk differences in health and reproduction events, as well as effect size for milk yield. Mean risk differences, 95% confidence intervals (CI) for risk differences and rate of change in actual events are provided in Table 1. The effect size for milk yield was 1.13 kg based on values available from the 16 studies ($P = 0.003$). Results support the inclusion of the RP-BVB during the transition period to support health, reproduction, and milk yield.

Table 1. Risk differences for periparturient events for transition cows receiving a rumen-protected blend of B-vitamins and choline

Event (no. of trials)	Risk difference, incidence rate	CI	Change, % of total cases	*P*-value
Culls < 30 DIM (16)	−0.05	−0.03 to −0.06	−42	<0.001
BHB >1.2 mmol (14)	−0.08	−0.02 to −0.14	−40	0.002
Metritis (8)	−0.08	+0.03 to -0.19	−27	0.079
Mastitis <30 DIM (10)	−0.04	−0.01 to −0.07	−22	0.001
Retained placenta (13)	−0.01	0.00 to −0.03	−13	0.085
Pregnant <100 DIM (12)	0.10	+0.05 to +0.15	+33	<0.001
LDA <30 DIM (10)	0.00	0 to −0.01	0	0.868
Milk fever <30 DIM (7)	−0.01	+0.01 to −0.02	−33	0.261

Key Words: B-vitamins, choline, transition cow health

P424 Evaluation of 2 sources of monensin in mid-lactation dairy cows.

P. Piantoni*, M. Messman, C. Canale, Y. Roman-Garcia, and G. Schroeder, *Cargill Animal Nutrition and Health Innovation Center, Elk River, MN.*

Monensin (MON) is a common additive supplemented to ruminants to increase feed efficiency. The objective of this experiment was to evaluate a novel source of MON on performance of mid-lactation dairy cows. Forty-three Holstein cows (25 multiparous and 18 primiparous; 175 ± 97 DIM; 45.3 ± 10 kg/d milk yield; 692 ± 68 kg BW; mean ± SD) were used in a randomized block design experiment with a 2-wk covariate and 9-wk treatment periods. Cows were randomly assigned to treatments based on milk yield. Treatments were: Control (CTR; no MON added), Rumensin 90 (RUM; 350 mg/d monensin from Elanco), and Monovet 90 (MVet; 350 mg/d monensin from Huvepharma). All cows were fed the same diet throughout the trial (27.0% NDF, 17.2% forage NDF, 26.0% starch, and 17.8% CP) and treatments were top-dressed during the treatment period. Data were analyzed using the Lme4 package in R with a model that included the random effects of block and cow, and the fixed effects of treatment, parity, and week, and their interactions. Rumen fluid was collected from 15 ruminally cannulated cows during the covariate period and on wk 5 and 9 of treatment (n = 5). Orthogonal contrasts were used to test CTR vs. MON and RUM vs. MVet. Compared with CTR, MON tended to increase milk yield (43.8 vs. 42.3 kg/d; $P = 0.08$) but did not affect DMI or feed efficiency. The MVet treatment improved feed efficiency compared with RUM (1.70 vs. 1.58; $P = 0.02$). Treatments did not affect milk fat content and yield, milk protein yield, MUN concentration, or energy-corrected milk yield. Milk protein content tended to be lower in MVet than in RUM (3.00 vs. 3.05%; $P = 0.10$). Compared with CTR, MON increased isovaleric acid concentration in rumen fluid pre-feeding (1.19 vs. 0.878 mM; $P = 0.02$). As a percent of total volatile fatty acids, MON increased isovaleric acid (1.41 vs. 1.01%; $P < 0.01$) and tended to decrease acetic acid (59.5 vs. 63.4%; $P = 0.07$) compared with CTR pre-feeding. Pre-feeding, RUM tended to increase ($P = 0.06$) isobutyric acid on wk 9 but not on wk 5 on treatment compared with CTR. Results indicate MON tended to increase milk yield and altered volatile fatty acid profile in rumen, and that MVet is a suitable source of monensin for dairy cows.

Key Words: feed efficiency, monensin, rumen fluid

P425 Evaluation of commercially available macromineral meters for evaluation of total mixed ration uniformity.

S. R. Poldervaart* and H. A. Rossow, *University of California, Davis, Davis, CA.*

Total mixed ration (TMR) uniformity plays an important role in the overall health and milk production of a dairy cow. Factors such as operator error and variation, wagon maintenance, and mixer types can all play various roles in TMR uniformity and delivery, affecting the nutrients supplied to a cow across a mixer wagon load. The objective of this study was to 1) evaluate how consistent macromineral lab analyses (Cl, K, Ca and Mg) predicted TMR uniformity and 2) evaluate how well commercial meters (Cl using the Oakton SaltTestr (Oakton Instruments, Vernon Hills, IL), K using the LAQUA Twin K meter (Horiba Scientific, Kyoto, Japan), Ca and Mg using the Hanna Instruments Ca/Mg Meter (Hanna Instruments, Smithfield, RI) predicted macromineral concentrations compared with the lab analyses. TMR samples were collected from 2 pens (close- up and high lactating cows) at 6 commercial dairies. To determine consistency of macrominerals to predict TMR uniformity, 10 subsamples of TMR were collected from each mixer wagon load at the time of feeding and sent to Analab (a division of Agri-King Inc., Fulton, IL) for analysis using inductively coupled plasma-mass spectrometry (AOAC International, 1999; method 985.01 for Ca, Mg, K; and method 915.01 for Cl) and DM (AOAC International, 1999; methods 935.29, respectively). To evaluate the ability of commercial meters to predict macromineral variability, subsamples of TMR were soaked in room temperature deionized water in 500mL beakers for (Cl for 90min, Ca for 150min, K for 180min and Mg for 180min) as predetermined from a standard curve. All data were analyzed using linear regression in R (Version 4.0.2), with interaction between dairy and pen being significant. The Cl and K LAB values had the most consistent coefficients of variation (CV) for TMR uniformity with CV of 0.59 ± 0.05 for close-up cows and a CV of 0.205 ± 0.20 for high cows. The Cl and K meters also predicted the LAB CL and K values the best with an R^2 of 0.84 and 0.79, respectively. Therefore, LAB and meter Cl and K can be used to predict TMR uniformity. The Cl and K meters can also be used to predict Cl and K concentrations in the TMR, but these equations would need to be calibrated for each dairy and pen.

Key Words: total mixed ration uniformity, macrominerals, nutrient analysis

P426 Effects of feed restriction and premortem versus postmortem sampling on liver mineral content.

S. K. Kvidera[2], M. M. McCarthy*[2], E. A. Horst[1], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA*, [2]*Micronutrients USA LLC, Indianapolis, IN.*

Liver biopsies and necropsies are often used interchangeably to assess mineral content and status. Objectives were to evaluate effects of feed restriction (FR) and pre- vs. postmortem sampling on liver mineral content in mid-lactation dairy cows. Samples from a previous study examining the effect of FR on inflammation, metabolism, and gut health from 9 mid-lactation (153 ± 43 DIM) cows enrolled in 2 experimental periods were used (Kvidera et al., 2017). Period 1 (5 d of ad libitum feed intake) served as baseline for period 2 (5 d), during which cows received 1 of 2 treatments: 1) ad libitum-fed (n = 3), 2) FR to 40% of ad libitum intake (n = 6). Liver biopsies were collected on d 2 of P1 (covariate) and d 5 of P2 (pre-mortem, PRE). At the end of P2 (18 h after the final biopsy was taken), cows were euthanized and liver samples were harvested within 15 min of euthanasia (postmortem, POST). Liver samples were sent to Michigan State University Veterinary Diagnostic Laboratory for mineral analysis. Dry matter content was only measured on postmortem samples. Liver mineral content and DM were analyzed by ANOVA and Period 1 data were used as a covariate. The effect of treatment, time, treatment by time interaction (repeated measures only) were offered as fixed explanatory variables to the model, while cow within treatment was included as a random effect. Significance levels were based on $P < 0.05$. Liver Zn was increased 36% by FR ($P = 0.03$) and liver Fe and Cu tended to be increased (29 and 17%, respectively; $P = 0.09$). Liver dry matter content was not impacted by FR ($P = 0.69$). Compared with PRE biopsy samples, liver samples collected POST had decreased Fe (50%; $P < 0.01$) and Zn (10%; $P < 0.01$), and tended to have decreased Co (50%; $P = 0.09$). There were no significant treatment by time interactions ($P = 0.11$). In summary, FR can increase liver concentrations of Zn, Fe, and Cu. Furthermore, liver Fe, Zn, and Co content can be affected by pre- vs. postmortem sampling, and further investigation is needed into how cow health, status, and sample collection protocols may affect liver mineral results.

Key Words: iron, copper, zinc

P427 Effects of feeding a fibrolytic enzyme product on milk production and reproduction in dairy cattle.

O. Martinez*[1], G. Acetoze[2], and H. Rossow[1], [1]*Department of Population Health and Reproduction, University of California, Davis, Davis, CA,* [2]*ADM Nutrition, Chicago, IL.*

Fibrolytic enzymes have been shown to enhance nutrient availability by increasing fiber digestibility of dairy cow rations, leading to an increase in milk production. The objective of this study was to determine if a TMR supplemented with a mix of exogenous fibrolytic enzymes (EFE; RumaThrive; Wilbur-Ellis, Portland, OR) influenced milk production or reproduction on a commercial dairy. A total of 3,836 Holstein cows were randomly assigned to 1 of 4 treatments: 1) primiparous control (PC: 2 pens) fed only a TMR; 2) primiparous treatment (PT: 2 pens) fed a TMR with added EFE; 3) multiparous control (MC: 3 pens) fed only a TMR; 4) multiparous treatment (MT: 3 pens) fed a TMR with added EFE. The enzyme product had a declared minimum of 44,750 units/g of xylanase and 50,000 units/g of cellulase. Supplementation of EFE began 2 weeks before parturition (close-up period) and continued 180 d into their lactation. Cows on treatments PT and MT received EFE at a rate of 2 g/cow/d during the close-up period and 4 g/cow/d during lactation. Data were collected during the first 180 d of lactation, analyzed as a completely randomized design with repeated measures for milk production using the Mixed procedure of SAS (Statistical Analysis System, v.9.4) and Cox proportional hazard regression of Stata (v. 16.1, College Station, TX) for conception rate. Pen was the unit of interest with primiparous and multiparous pens analyzed separately. There was no difference in days to exposure of EFE between treatments. Cows in PC had higher milk fat ($P \leq 0.02$) and milk protein ($P \leq 0.01$) percentages when compared with PT cows (4.25 vs. 4.13% and 3.16 vs. 3.12%, respectively). No other milk production parameters were different between treatments. Primiparous treatment cows had a 17% higher hazard of conception when compared with PC cows ($P \leq 0.02$), meaning that at any time point there are 17% more conceptions occurring in PT cows. The results of this study indicate that feeding EFE to commercial dairy cows does not increase milk production but has the potential to decrease time to conception.

Key Words: fibrolytic, enzymes, milk

P428 Effect of biotin, folic acid, and vitamin B_{12} supplementation on whole-body glucose and protein metabolism in early-lactation cows.

M. Duplessis*, H. Lapierre, and C. L. Girard, *Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Biotin (B8), folic acid (B9), and vitamin B_{12} (B12) play major roles in energy and protein metabolism. The aim of this study was to evaluate the effects of a B8 supplement, alone or combined with B9+B12 (B9B12) on glucose, propionate (C3), and protein metabolism. From −3 to 7 wk relative to the calving, 32 cows were assigned to one of 4 treatments: 1) no supplement; 2) 20 mg/d of dietary B8; 3) 2.6 g/d of dietary B9 and 10 mg of weekly intramuscular injections of B12; 4) 20 mg/d of B8 with 2.6 g/d of B9 and 10 mg/wk of B12. At 43 (SD:3) days in milk (DIM), D-[6,6-$^2H_2$]-glucose (24.0 mmol/h; jugular vein) and [1-^{13}C]-sodium C3 (22.6 mmol/h; ruminal vein) were simultaneously infused for 4 h; the following 2 d, respectively, [^{13}C] sodium bicarbonate to evaluate CO_2 production (3.0 mmol/h) and L[1-^{13}C]leucine (3.7 mmol/h) were infused into a jugular vein for 4 h. Blood samples were taken every 15 min on d 1 and every 20 min on d 2 and 3 during the last 2 h of infusions. According to the 2x2 factorial arrangement, Proc MIXED of SAS was used to analyze data, and LSmeans and SEM are presented. Milk, protein, and lactose yields did not differ among treatments during the infusion wk ($P > 0.35$). Dry matter intake tended to be lower by 1.9 kg for cows receiving B8 compared with cows who did not ($P = 0.09$). No treatment effects ($P > 0.11$) were detected on whole-body rate of appearance (WB-Ra) of glucose (811.4 ± 34.4 mmol/h) and C3 (1,354 ± 116 mmol/h), and on the proportion of glucose synthesized from C3 (84%). The B9B12 treatment increased ($P = 0.005$) plasma glucose by 12%, while tended to decrease ($P = 0.07$) lactose yield to glucose WB-Ra ratio by 8% with B8 supplementation, whereas no effect was noted when no B8 was fed ($P > 0.30$; B8xB9B12 interaction, $P \leq 0.05$). No treatment effect ($P > 0.27$) was observed on leucine WB-Ra (133.2 ± 5.2 mmol/h), oxidized (22.6 ± 2.2 mmol/h), and used for protein synthesis (110.6 ± 4.2 mmol/h). Within the first 21 DIM, when B8 was not fed, cows receiving B9B12 produced 14% more milk and total solids compared with controls, but this was not translated into further improvement in glucose and protein metabolism during the infusion wk around 43–45 DIM.

Key Words: cyanocobalamin, folate, stable isotope

P429 Effects of feed restriction, zinc source, and liver region on liver mineral content.

S. K. Kvidera[2], M. M. McCarthy*[2], E. A. Horst[1], and L. H. Baumgard[1], [1]*Iowa State University, Ames, IA,* [2]*Micronutrients USA LLC, Indianapolis, IN.*

Liver samples from necropsied animals are frequently used to assess mineral content and status. Objectives were to evaluate the effects of feed restriction (FR), Zn source, and liver region on mineral content in postmortem liver samples. Samples from a previous 2 × 2 factorial study examining the effect of FR and Zn source on inflammation, metabolism, and gut health from 23 multiparous mid-lactation (159 ± 8 DIM) cows were utilized (Horst et al., 2020). Cows were randomly assigned to 1 of 2 supplemental Zn diets at 75 ppm: $ZnSO_4$ or Zn hydroxychloride (IBZ; IntelliBond Z, Micronutrients USA). Cows were fed their respective diets 21 d before undergoing 2 experimental periods. Period 1 (5 d) served as baseline for period 2 (P2; 5 d) where cows were assigned to groups receiving either 100% of ad libitum (AL) feed intake or FR to 40% of AL intake. Treatments were: 1) $ZnSO_4$ fed AL (n = 5), 2) $ZnSO_4$ with FR (n = 6), 3) IBZ fed AL (n = 6), and 4) IBZ with FR (n = 6). Liver samples were collected postmortem from 8 regions of the liver: 1) inferior to the left triangular ligament, 2) left lobe, 3) papillary process of caudate lobe, 4) quadrate lobe, 5) inferior to the caudal vena cava, 6) right lobe, 7) superior to the right triangular ligament, and 8) inferior to the right triangular ligament. Liver mineral content and DM were analyzed by ANOVA. The effect of treatment and region were offered as fixed explanatory variables to the model, while cow within treatment was included as a random effect. Significance levels were based on $P < 0.05$. There were no effects of diet on liver Zn ($P = 0.18$). FR decreased liver Mo (13%; $P < 0.01$) and IBZ increased liver Co (9%; $P = 0.03$). There were no significant group by diet interactions, with the exception of Mn ($P = 0.03$) where FR cows fed IBZ had increased liver Mn relative to the other treatments. No meaningful diet or group by region interactions were detected. Region significantly affected dry weight as well as Se, Fe, Zn, Mn, and Co content ($P \leq 0.05$) and tended to affect liver Cu ($P = 0.06$). Across regions of the liver, the range for Se, Fe, Zn, Mn, Co, and Cu were 0.5, 72, 12, 1, 0.02, and 146 ppm, respectively. In summary, samples from different regions of the same liver can greatly vary in mineral content.

Key Words: selenium, copper, iron

P430 Assessment of the relationship between postpartum health and mid-lactation performance, behavior, and feed efficiency in Holstein dairy cows. M. J. Martin*, K. A. Weigel, and H. M. White, *University of Wisconsin–Madison, Madison, WI.*

Postpartum health disorders are associated with negative outcomes related to milk yield, reproduction, and profitability; however, the relationship between postpartum health and mid-lactation feed efficiency and behavior is not fully understood. To investigate the relationship between postpartum health and mid-lactation performance, feed efficiency, and behavior, 179 multiparous cows were monitored for health disorders (hyperketonemia (HYK), metritis, retained placenta, hypocalcemia, mastitis, and displaced abomasum) for 21 d postpartum and then enrolled onto a 45-d trial between 50 to 200 DIM. In mid-lactation, DMI, milk yield and composition, body weight, body condition score, and sensor-derived behavioral data were collected. Feed efficiency was calculated as residual feed intake (RFI). Cows were classified as having HYK (40.2%) or not (nonHYK; 59.8%) as well as classified into health groups by frequency of postpartum health events: none (HLT; 52.5%), one (DIS; 35.2%), or 2 or more (DIS+; 12.3%). Data were analyzed using linear models (R v4.0.1). Cows with HYK had similar DMI and body weight in mid-lactation compared with nonHYK, but had lower BCS ($P = 0.05$), higher yields of energy- and fat-corrected milk ($P < 0.03$), and tended to produce more milk ($P = 0.06$). However, RFI was similar ($P = 0.13$). While there was no difference in lying or active time between HYK and nonHYK, HYK cows spent less time highly active ($P = 0.02$). Older cows (≥fourth lactation) with HYK spent more time ruminating than nonHYK ($P < 0.01$). No differences in performance or feed efficiency were detected between the health groups. There was no difference in lying or active time between health groups, but HLT cows spent more time highly active compared with DIS and similar times to DIS+ ($P = 0.01$). Older cows (≥fourth lactation) in the HLT group spent less time ruminating than DIS and DIS+ ($P < 0.05$). In conclusion, there is no relationship between postpartum health and mid-lactation feed efficiency. Postpartum health may influence mid-lactation activity and rumination behaviors.

Key Words: hyperketonemia, residual feed intake

P431 Effects of rumen-protected botanicals on lactational performance, enteric methane emission, and blood metabolites of dairy cows. T. Silvestre*[1], S. Räisänen[1], D. Wasson[1], S. Cueva[1], C. Lage[1], E. Wall[2], and A. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*AVT Natural, Kerala, India.*

This experiment investigated the effects of rumen-protected botanicals (RPBT; containing a proprietary oleoresin of capsicum combined with botanical stabilizers) on lactational performance, enteric gas emissions and blood metabolites of dairy cows. Twenty Holstein cows (days in milk 77 ± 28; 12 multiparous and 8 primiparous) were used in a replicated 4 × 4 Latin square design experiment with 4, 28-d periods. Cows were randomly assigned to 1 of 4 treatments: control or 150, 300, or 600 mg/cow/d RPBT. Data were analyzed using the MIXED procedure of SAS with square and cow within square as random effects. Contrasts were used to evaluate RPBT vs. control, and linear and quadratic effects of RPBT dose. Supplementation with RPBT did not affect dry matter intake (DMI; 27.8 kg/d; SEM = 1.25), milk and energy-corrected milk (ECM; 37.2 kg/d; SEM = 1.49) yields, feed efficiency, and milk composition ($P \geq 0.11$), except milk fat concentration and MUN tended to be greater ($P \leq 0.08$) for the RPBT treatments compared with the control. Daily methane (CH_4) emission (measured using the GreenFeed system) was linearly decreased ($P = 0.04$; 389 vs. 364 g/d; SEM = 11.8) by RPBT and CH_4 yield ($P = 0.05$; 13.3 vs. 14.0 g/kg DMI; SEM = 0.53), and intensity ($P = 0.08$; 9.96 vs. 10.5 g/kg energy-corrected milk; SEM = 0.57) were lower or tended to be lower for the RPBT treatments vs. the control. Supplementation with RPBT did not affect ($P \geq 0.16$) concentrations of blood metabolites, except for a quadratic decrease ($P = 0.05$) in serum β-hydroxybutyrate (BHB) and a tendency for a linear decrease in serum haptoglobin ($P = 0.06$). Serum insulin concentration was linearly decreased ($P = 0.01$) by RPBT in primiparous but not in multiparous cows. Overall, RPBT supplementation did not affect DMI and milk production of dairy cows. Lower insulin in primiparous cows and a decrease in BHB concentration in both primi- and multiparous cows indicates a potential positive effect of RPBT on the metabolic status of the cow. The reduction in enteric CH_4 emission suggests a potential mitigation effect of RPBT.

Key Words: plant extract, enteric methane, dairy cow

Ruminant Nutrition:
Gut Physiology, Fermentation, and Digestion: Posters

P432 The effect of blade direction on feed digestibility and particulate accumulation in dual-flow continuous culture fermenter system. X. Huang*[1], P. Yoder[2], and B. Wenner[3], [1]*Cumberland Valley Analytical Services, Waynesboro, PA,* [2]*Perdue AgriBusiness LLC, Salisbury, MD,* [3]*The Ohio State University, Columbus, OH.*

Recently, a modified dual-flow continuous culture (DFCC) fermenter system was designed to improve mixing consistency at low rotation speed. The current trial was conducted to evaluate the effect of fan blades direction on feed digestibility in the updated DFCC and fiber fraction accumulation in the fermenter at termination. Clockwise (CL) blade pitch forces mixture down against the bottom of the fermenter jar while counterclockwise (CC) pitch provides lift mixture from the base of the fermenter. We hypothesized that CC pitch orientation on a clockwise rotating motor would provide greater mixing, decreasing sedimentation of fiber fractions within the fermenter jar. Eight DFCC fermenters were assigned to a crossover design with 2 treatments of fan direction (CL vs CC) for 2 periods lasting 12 d each. Fermenters were maintained anaerobically at 39°C and 50 rpm in a clockwise motor direction. Total dilution was 7%/h with solids dilution 5%/h. Fermenters were fed 36.8 g DM twice daily composed of a 25:25:50 alfalfa:orchardgrass:concentrate pellet mix. The pH of cultures varied between 5.9 and 6.4 reaching its nadir at 4 h postfeeding. Effluent samples on the last 4 d and residual matter in the jar at the termination of each period were collected into chilled vessels (4°C) and analyzed for DM, OM, NDF, and ADF contents, and VFA and ammonia concentrations. Data were analyzed using a mixed model including treatment, day, and their interaction as fixed effects and period and fermenters as random effects. Sampling day and treatment interacted for apparent organic matter (OM) digestibility ($P < 0.01$) and NDF digestibility ($P = 0.04$). The OM and NDF digestibility were greater on d 9 than d 8 or d 10 for CC than CL, but this was likely due to lower solid outflow on d 9 under CC ($P = 0.04$). The ADF digestibility was greater on d 9 than d 8 ($P = 0.04$). Total weight of NDF and ADF residuals in the jar at the end of the period were not different between treatments ($P = 0.56$ and $P = 0.42$, respectively), indicating no effect of treatment on total NDF and ADF digestion or sedimentation within the fermenter. Overall, CC shows greater day to day variation than CL. These results further demonstrate the significant daily variation of nutrient digestion in DFCC fermenters which is susceptible to variable flow rates across days.

Key Words: continuous culture, system modification, fiber digestibility

P433 Increasing doses of carbohydrases on nutrient intake and milk composition of mid-lactation cows. P. C. Vittorazzi Junior*[1], G. Gomes da Silva[1], N. T. S. Grigoletto[1], A. T. Nunes[1], R. G. Chesini[1], T. B. de Paula e Silva[1], M. S. Dias da Silva[1], C. S. Takiya[1], M. Bugoni[1], C. Cortinhas[2], T. Acedo[2], and F. Palma[1], [1]*Department of Animal Nutrition and Production, University of São Paulo, Pirassununga, SP, Brazil,* [2]*DSM Produtos Nutricionais Brazil, SA, São Paulo, SP, Brazil.*

Few studies have evaluated enzyme preparations with glucanase activity or the combination of products rich in glucanase and xylanase in diets for ruminants. The aim of this study was to evaluate the effects of increasing doses of carbohydrases on nutrient intake and milk yield and composition of dairy cows. Thirty-six Holstein cows (160 ± 88 DIM, 30.9 ± 7.2 kg/d milk yield) were enrolled in a complete randomized block design. Cows were allowed 14 d for diet acclimation before providing enzyme treatments. Measures for covariate purposes were performed during the last 3 d of acclimation period. Cows received the following treatments for 9 wks: control (CON), and enzyme treatments at 0.05 (E0.50) or 0.75 (E0.75) g/kg DM of a blend of enzyme products with xylanase and β-glucanase activities (Ronozyme Wx and Ronozyme VP, respectively; DSM Nutritional Products). Feed intake and refusals were monitored daily, and samples were collected daily and pooled into a composite sample per wk for chemical analyses. Milk samples were collected thrice weekly and analyzed fresh for components using mid-infrared method. Data were submitted to Mixed procedure of SAS as repeated-measures modeling the fixed effects of treatment, period, and their interaction, and the random effect of animal within block. No treatment effects were detected on DM and nutrient intake. Feeding enzymes increased ($P \leq 0.036$) milk fat yield (1.39 vs 1.28 kg/d for enzymes and CON, respectively) and content. Enzymes tended to increase ($P \leq 0.056$) FCM yield (36.3 vs 34.4 kg/d for enzymes and CON, respectively) and protein content in milk. Cows fed enzyme treatments had greater ($P = 0.006$) efficiency of FCM production than those in CON (1.39 vs 1.30, for enzymes and CON, respectively). No differences in milk yield of cows were detected in this experiment when comparing the enzyme treatments fed at 0.5 or 0.75 g/kg DM. No interaction effects between treatment and time were detected. Feeding carbohydrases may improve performance of mid-lactation cows, especially in terms of milk fat production and feed efficiency relative to FCM yield.

Key Words: beta-glucanase, cellulase, fibrolytic enzyme

P434 One-carbon metabolism and nutrient transporters in the gastrointestinal tract of lactating Holstein cows. Q. Jiang*, D. N. Coleman, Y. Liang, A. Aboragah, and J. J. Loor, *University of Illinois, Urbana, IL.*

We investigated amino acid transporters, one-carbon metabolism, and protein synthesis regulatory proteins across the gastrointestinal tract of multiparous lactating Holstein cows. Cows were fed a corn silage and alfalfa hay-based diet (17.4% CP, 1.74 Mcal/kg NE_L, 358 g/d Met in the MP). Tissue samples from rumen (Ru), omasum (Om), abomasum (Ab), duodenum (Du), jejunum (Je), ileum (Il), and cecum (Ce) were harvested at slaughter from 8 multiparous Holstein dairy cows. Cows were free of clinical disease, were fed a typical corn silage/alfalfa hay-based diet, and averaged 128 ± 12 d in milk and 39 ± 5 kg milk/d before slaughter. Epithelial tissue was used for ^{14}C radio-labeling assays to measure betaine-homocysteine S-methyltransferase (BHMT) and cystathionine-β-synthase (CBS) activity, whereas methionine adenosyl transferase 1 (MAT) was measured via ELISA. Data were analyzed using PROC MIXED in SAS. Activity of BHMT did not differ ($P > 0.05$) and averaged 1.30, 0.92, 0.53, 0.43, 0.52, 0.56, and 0.38 ± 0.32 nmol/h/mg protein in Ru, Om, Ab, Du, Je, Il, and Ce, respectively. In contrast, activity of MAT and CBS differed ($P \leq 0.05$) according to site. The greatest activity ($P < 0.05$) of MAT and CBS was detected in Ru (223 ± 23 and 13.3 ± 1.7 nmol/h/mg protein) and Om (84 ± 21.8 and 21.8 ± 2.24 nmol/h/mg protein) compared with other sections of the gastrointestinal tract (49.7 and 6.6 ± 1.56 nmol/h/mg protein). It was noteworthy that MAT activity in Ce (119 ± 23.1 nmol/h/mg protein) was similar to Ru and Om, but greater ($P \leq 0.05$) compared with other sections of the gastrointestinal tract. Among nutrient transporters measured, the protein abundance of the Na-coupled neutral amino acid transporter SLC38A1 was greatest ($P \leq 0.05$) in Jej and Du compared with other segments of the gastrointestinal tract. Overall, preliminary observations suggested a greater capacity of ruminal and omasal epithelium for methionine cycle and transsulfuration activity, while duodenum and jejunum appear uniquely equipped for amino acid absorption.

Key Words: methyl donor, metabolism, digestion

P435 Effects of capsaicin supplementation on productive performance of lactation cows.

P. C. Vittorazzi Junior*, G. Gomes da Silva, N. T. S. Grigoletto, A. T. Nunes, R. G. Chesini, L. V. B. de Alcantara, F. M. Santos, C. Oliveira, M. Bugoni, and F. P. Rennó, *Department of Animal Nutrition and Production, University of São Paulo, Pirassununga, SP, Brazil.*

The aim of this study was to evaluate the effects of increasing capsaicin levels (CAPCIN; Techno Feed Ltda, Vinhedo, Brazil) on dry matter intake (DMI), milk yield (MY) and composition, and milk production efficiency of dairy cows. Thirty-six Holstein cows (160 ± 88 DIM and 30.9 ± 7.2 kg/d of MY) were enrolled in a complete randomized block design. Cows were allowed 14 d for diet acclimation before providing treatments. Measures for covariate purposes were performed during the last 3 d of acclimation period. Cows received the following treatments for 9 wks: control (diet without additives), and capsaicin at 0.75 and 1.5 g/d. capsaicin was offered mixed into the concentrate. Feed intake and refusals were monitored daily, and samples were collected daily and pooled into a sample per wk for DM analyses. Milk samples were collected 3 times a week and analyzed for contents of fat, protein, and lactose using mid-infrared method. Data were analyzed using the MIXED procedure of SAS, using contrasts to assess the effect of additive (control vs. capsaicin) and doses (0.75 vs. 1.5 g/d). Fixed effects of time, treatment and interaction between them, and random effect of block were considered in the statistical model. Capsaicin increased ($P < 0.05$) DMI and DMI in terms of body weight compared with control. Cows fed capsaicin showed an increase ($P < 0.05$) in yield of FCM and protein, fat, and lactose. In addition, capsaicin tended to increase milk yield ($P = 0.081$) and milk lactose content ($P = 0.083$). There were no effects of capsaicin on milk production efficiency. Therefore, regardless of the tested dose, capsaicin may improve solids and FCM yield without affecting milk production efficiency of mid-lactation cows.

Key Words: additive, *Capsicum oleoresin*, essential oil.

P436 The effect of glycerol administration on in vivo and in vitro ruminal parameters: A meta-analysis.

B. Yanibada*[1], D. E. Rico[2], B. Médina[3], and M.-P. Létourneau-Montminy[1], [1]*Université Laval, département des sciences animales, Québec City, QC, Canada,* [2]*Centre de recherche en sciences animales de Deschambault (CRSAD), Deschambault, QC, Canada,* [3]*Probiotech International Inc., Saint-Hyacinthe, QC, Canada.*

Glycerol is sugar alcohol, which could partially substitute corn starch in cattle diets. Significant amounts of glycerol may be rapidly absorbed across the rumen epithelium and directly supply carbon for hepatic gluconeogenesis, but another fraction is fermented by rumen microflora. Given the potential degradation of glycerol in the rumen, several studies have investigated its effects on fermentation, both in vitro and in vivo. Importantly, differences between such systems may affect glycerol fermentation responses and inferences about its metabolic effects. The objective of this study was to compare, through a meta-analysis, the effects of glycerol on ruminal fermentation using in vivo and in vitro conditions. The literature search yielded 9 in vivo and 20 in vitro studies (continuous culture n = 16; batch culture n = 4) where ruminal fermentation parameters were analyzed. Multiple regression models were created using the MIXED procedure of Minitab including the random effect of experiment. Ruminal volatile fatty acids, ammonia, and pH were the Y variables. Glycerol concentration (g/kg DM) was defined as the main predictor. Models were selected based on their fitting quality (i.e., R^2 and AIC values). Glycerol supplementation decreased pH linearly in in vitro conditions ($P = 0.03$; $R^2 = 87.7\%$), while butyrate proportion increased linearly ($P < 0.001$; $R^2 = 79.8\%$) in in vivo conditions. Regardless of study type, models predicted a linear decrease in acetate proportion ($P < 0.001$; $R^2 = 88.2\%$; $P < 0.001$; $R^2 = 87.0\%$) and a linear increase in propionate proportion ($P < 0.001$; $R^2 = 91.7\%$; $P < 0.001$; $R^2 = 83.5\%$) and of their ratio ($P < 0.001$; $R^2 = 93.2\%$; $P < 0.001$; $R^2 = 90.2\%$) for in vivo and in vitro, respectively. Results were more pronounced for in vivo studies; i.e., 100 g/kg of glycerol decreased acetate by 3.9% in vitro vs. 8.9% in vivo. These data show that regardless of study type, glycerol supplementation significantly alters the rumen fermentation patterns favoring propionate synthesis, resulting in a more glucogenic fermentation profile.

Key Words: glycerol, meta-analysis, rumen

P437 Effects of OmniGen Pro on biomarkers of stress and inflammation of Holstein cattle.

Y. Jiang*, J. D. Chapman, E. Sharman, B. Humphrey, and M. Garcia, *Phibro Animal Health Corporation, Quincy, IL.*

OmniGen Pro (OGPRO, Phibro Animal Health, Teaneck, NJ) is a new product built on the OmniGen immune foundation, developed to maintain the effectiveness of OmniGen AF while improving gastrointestinal tract function and integrity. This study examined the effects of OGPRO on biomarkers of stress and inflammation of dairy heifers during a starch challenge to induce subacute ruminal acidosis (in companion abstract, starch challenge reduced ruminal pH from 6.9 to 5.9, increased ruminal lactate from 0.1 mM to 10.4 mM, and acetate-to-propionate ratio from 4.2 to 5.1). OGPRO prevented the increase in ruminal lactate (17.8 vs. 2.93 mM) and acetate-to-propionate ratio (5.90 and 4.27) compared with control. Based on BW, 32 Holstein heifers (8-mo-old) were blocked into 2 dietary treatments, Control (CON) or OGPRO (10 g/100 kg BW). A TMR representing an early-lactation diet (16% CP, 31% starch, 28% NDF, 1.62 Mcal/kg NE_L) was offered ad libitum throughout the experiment (73 d). Following 1 d of 50% feed restriction (d70), a subacute ruminal acidosis was induced with a starch challenge by replacing 50% of dietary DM with rolled barley offered ad libitum on d 71. Blood samples were collected 4 h after feeding on d 66 and 73, and 9 h after feeding on d 71. Data were analyzed with the GLIMMIX procedure of SAS. On d 66, monocyte counts were higher in heifers fed OGPRO compared with CON (0.52 vs. $0.39 \times 10^3/\mu L$, $P = 0.01$). On d 71, treatment did not affect blood parameters ($P > 0.10$). On d73, 2 d after the starch challenge, OGPRO heifers tended to have lower plasma concentrations of cortisol (30 vs. 39 ng/mL, $P = 0.06$), haptoglobin (787 vs. 481 mg/L, $P = 0.07$), and fatty acids (152 vs. 120 μmol/L, $P = 0.09$), and had higher blood lymphocytes (6.84 vs. $6.14 \times 10^3/\mu L$, $P = 0.08$), mean corpuscular hemoglobin (35 vs. 34.5 g/dL, $P = 0.003$) and platelets (537 vs. $370 \times 10^3/\mu L$, $P = 0.01$). Overall, the findings suggest OGPRO may help to maintain a healthy metabolism and immunity of dairy cattle by reducing stress (lower cortisol and fatty acids) and inflammation (lower haptoglobin) associated with an induced subacute ruminal acidosis.

Key Words: OmniGen, immunity, subacute ruminal acidosis

P438 Palmitic acid supply and pH alter ruminal prokaryotic community composition in a continuous culture system.

L. Padilla[1], J. de Souza[2], and F. Batistel*[1], [1]*Utah State University, Logan, UT,* [2]*Perdue AgriBusiness, Salisbury, MD.*

Nonrumen bacteria incorporate exogenous long-chain saturated fatty acids (FA) to change membrane fluidity under low pH conditions. We hypothesized that rumen bacteria use a similar mechanism, thereafter, providing saturated fatty acids in the diet could support bacterial metabolism. The objective of this study was to evaluate the effects of dietary palmitic acid and pH on the prokaryotic community composition. The study was conducted as a 2 × 2 factorial treatment arrangement in a replicated 4 × 4 Latin square using continuous culture fermenters (n = 8). Treatments were a control diet without supplemental FA or the control diet plus 1.5% palmitic acid associated with normal pH (ranging from 6.6 to 7.0) or low pH (ranging from 6.0 to 6.4). The control diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate mixture. The FA treatment maintained the same nutrient input into the fermenters as the control except for FA. Daily fermenter effluent was collected over 24-h postfeeding and a 30% subsample was pooled by fermenter within period. The prokaryotic community was determined by sequencing the V4 region of the 16S rRNA gene. Data were analyzed using a mixed model including the fixed effect of pH, FA, and its interaction, and the random effects of period and fermenter. The differences were declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. No interaction between FA and pH was observed for kingdom and phylum abundance ($P \geq 0.10$). Low pH decreased archaea and increased bacteria abundance compared with normal pH ($P = 0.03$). Compared with normal pH, low pH decreased the phyla *Firmicutes* (bacteria) and *Euryarchaeota* (archaea), and increased *Desulfobacterota* (bacteria) ($P \leq 0.05$). Three interactions between FA and pH were observed at the family level. *Bacillaceae* abundance increased with palmitic acid at low pH and decreased at normal pH ($P = 0.06$). *Bifidobacteriaceae* abundance decreased with palmitic acid under low pH and it was not affected under normal pH ($P = 0.05$). *UCG-010* abundance decreased with palmitic acid at low pH and increased at normal pH ($P = 0.09$). Our preliminary data indicate that palmitic acid and pH independently affect the prokaryotic community composition. Furthermore, we did observe interactions between palmitic acid and pH at family and genus levels.

Key Words: low pH, microbiota, palmitic acid

P439 Effect of different cottonseed types on fiber digestibility and rumen fermentation in a continuous culture system.

N. Bagheri* and F. Batistel, *Utah State University, Logan, UT.*

Cottonseed is a feed ingredient with high levels of fiber, protein, and fat. However, the presence of gossypol, a toxic compound in cottonseed can limit its inclusion in the diet of dairy cows. The objective of this study was to determine the effects of feeding different types of cottonseed on NDF digestibility and rumen fermentation. The study was conducted as a replicated 4 × 4 Latin square using continuous culture fermenters (n = 8). Treatments were: 1) a control diet without cottonseed, 2) the control diet with 10% of glandless cottonseed, 3) the control diet with 10% of Upland cottonseed, and 4) the control diet with 10% of Pima cottonseed. The total gossypol concentration was 0.006, 0.033, and 0.049 g and (-) gossypol isomer concentration was 0.002, 0.016, and 0.026 g for glandless, Upland, and Pima, respectively. The basal diet (40 g DM/day) was a 50:50 orchardgrass hay:concentrate and diets were fed twice daily. The cottonseeds were slighted cracked to mimic mastication. The experimental periods were 10 d long, consisting of 6 d of adaptation and 4 d of sampling. Daily fermenter effluent was collected over 24-h postfeeding and a 30% subsample was pooled by fermenter within period for analyses. Data were analyzed using a mixed model including the fixed effect of treatment, and the random effects of period and fermenter. Differences were declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. NDF digestibility was affected by treatments ($P = 0.05$); Pima cottonseed decreased NDF digestibility compared with Control, Brownfield glandless, and Upland. Ammoniacal nitrogen, redox potential, and pH were not affected by treatments ($P \geq 0.06$). Our preliminary results indicate that the type of cottonseed differently impacted NDF digestibility, but not ammoniacal nitrogen, redox potential, and pH.

Key Words: gossypol, NDF, redox potential

P440 Palmitic, stearic, or oleic acid differently alter bacterial phospholipid fatty acid profile and bacterial community composition in a continuous culture system.

A. Sears[1], J. de Souza[2], B. Wenner[3], and F. Batistel*[1], [1]*Utah State University, Logan, UT,* [2]*Perdue AgriBusiness, Salisbury, MD,* [3]*The Ohio State University, Columbus, OH.*

We previously demonstrated that palmitic, stearic, or oleic acid differently alter NDF digestibility and rumen fermentation. Our current objective was to investigate the effect of these fatty acids (FA) on microbial growth, bacterial phospholipid FA profile, and bacterial community composition. Continuous culture fermenters were used in a replicated 4 × 4 Latin square design with 7 d of adaptation and 4 d of sampling. Treatments were (1) a control diet without supplemental FA (CON); (2) CON plus 1.5% palmitic acid; (3) CON plus 1.5% stearic acid; and 4) CON plus 1.5% oleic acid. The control diet (60 g DM/d) was a 50:50 orchardgrass hay:concentrate mixture (8.7 g CP, 21 g NDF, 11 g starch, and 1.5 g FA) fed twice daily. The FA treatments maintained the same nutrient input into the fermenters as the control except for FA. The prokaryotic community was determined by sequencing the V4 region of the 16S rRNA gene. Data were analyzed using a mixed model including the fixed effect of treatment, and the random effects of period and fermenter. Differences were declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. No treatment effect was observed for microbial growth ($P = 0.10$). The bacterial phospholipid FA profile was affected by the treatments. Palmitic increased the concentration of *anteiso* C13:0, *anteiso* C15:0, and *anteiso* C17:0 compared with the other treatments ($P \leq 0.05$). Furthermore, oleic decreased the concentration of C17:0 and *cis*-9, *cis*-12 C18:2 ($P < 0.01$), while increased the concentration of *cis*-9 C18:1 ($P < 0.01$) compared with the other treatments. Analysis of the phyla abundance showed that 2 phyla were affected by treatments. The relative abundance of *Bacteroidetes* and *Fibrobacteres* increased with palmitic compared with the other treatments ($P \leq 0.04$). *Prevotellaceae* and *Fibrobacteraceae* were the most abundant families affected by treatments. Palmitic increased the abundance of *Prevotellaceae* and *Fibrobacterceae* ($P < 0.03$) compared with the other treatments. In contrast, oleic decreased the abundance of *Fibrobacterceae* ($P = 0.01$) compared with stearic and control. Our preliminary results indicate that palmitic enhanced the concentration of *anteiso* FA in the bacterial phospholipid fraction and increased the abundance of known cellulolytic bacterial families.

Key Words: *anteiso* fatty acids, bacterial membrane, microbiota

P441 Association of residual feed intake with peripartal ruminal microbiome and milk fatty acid composition in Holstein dairy cows.

H. Zhang*[1], A. A. Elolimy[2,3], H. Akbar[4], M. Zeineldin[5], Z. Yang[1], and J. J. Loor[2], [1]*College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China,* [2]*Department of Animal Sciences & Division of Nutritional Sciences, University* *of Illinois, Urbana, IL, [3]Department of Animal Production, National Research Centre, Giza, Egypt, [4]College of Veterinary Medicine, University of Illinois, Urbana, IL, [5]Department of Animal Medicine, College of Veterinary Medicine, Benha University, Urbana, IL.*

Residual feed intake (RFI) is an inheritable character of feed efficiency in dairy cows. Objective was to evaluate differences in ruminal microbiome and milk fatty acid (FA) composition between the most- and least-efficient dairy cows during early lactation. Forty-seven multiparous Holstein cows with daily ad libitum access to a total mixed ration from −30 to 30 d in milk (DIM) were used. Cows were classified into most efficient (M-EFF, i.e., with low RFI, n = 29) and least-efficient (L-EFF, i.e., with high RFI, n = 18) based on individual RFI coefficients computed as the difference between actual and predicted dry matter intake (DMI). Predicted DMI was computed through a linear regression model. Ruminal digesta and milk samples were collected from each cow at 15 and 30 DIM. Microbiome sequencing data (16S rRNA) were analyzed with the QIIME 2 platform, whereas microbiome statistical analyses and visual explorations were performed using the web-based tool MicrobiomeAnalyst. Milk FA composition was measured via GC. The statistical model used in SAS 9.4 included RFI, time and their interactions as fixed effects. PROC CORR was used to determine Pearson correlations between relative abundance of significant bacteria and milk FA. Overall daily milk yield did not differ ($P > 0.05$) due to RFI and averaged 42 ± 1.6 kg for L-EFF and 43 ± 1.3 kg for M-EFF cows. However, M-EFF cows had lower ($P < 0.0001$) (14.9 ± 0.5 kg/d) DMI than L-EFF cows (19.2 ± 0.6 kg/d). Compared with L-EFF, M-EFF cows had greater ($P < 0.05$) relative abundance of *Lachnospiraceae*, *Lachnoclostridium*, *Papillibacter*, *Desulfovibrio*, *Sphaerochaeta*, *Acetobacter* and *Histophilus*. In contrast, relative abundance of *Bifidobacterium*, *Ruminiclostridium*, *Prevotellaceae* and *Erysipelotrichaceae bacterium* were lower in M-EFF cows. Compared with L-EFF, M-EFF cows had greater ($P < 0.05$) concentrations of long-chain monounsaturated (e.g., C16:1 *cis*-9, C18:1 *cis*-9) and lower ($P < 0.05$) concentrations of saturated FA (e.g., C12:0, C14:0, C16:0). Acetate-producing bacteria and *Prevotellaceae* were negatively correlated with monounsaturated FA in milk. Butyrate-producing bacteria (*Papillibacter*) had a negative correlation with C15:0. Overall, data suggested associations among RFI, specific microbiome, and specific milk FA profiles during early lactation.

Key Words: residual feed intake, milk fatty acid, ruminal bacteria

P442 Testing additivity of starch and aNDFom fermentation in an in vitro gas production system.

N. Schlau*[1], J. R. Knapp[2], K. Taysom[1], and D. M. Taysom[1], [1]*Dairyland Laboratories, Inc., Arcadia, WI,* [2]*Fox Hollow Consulting LLC, Lewisburg, OH.*

A statistical approach to fitting curves of sugar, starch, and aNDFom fermentation as measured by in vitro gas production (IVGP) was developed. The objective was to test whether gas produced from starch and aNDFom fermentation was additive or multiplicative. In Study 1, 12 samples with a range of starch and aNDFom contents were created from 4 cornerstone samples of dry corn, high moisture ear corn, and 2 corn silage samples. Samples were fermented in an Ankom RF system and gas production determined at 2 min intervals for 72 h. A 3-pool model utilizing exponential growth curves to describe the gas production from each carbohydrate fraction was fit using SAS NLMIXED. Acceptance criteria included goodness of fit based on the Bayesian Information Criteria, solution being a global optimum, and parameter estimates significantly different than zero and biologically reasonable. Criteria were met and a single model was adequate to fit the range of starch and aNDFom fermentation outcomes observed for a subset of samples comprising each combination of the cornerstone samples, confirming that the starch and aNDFom IVGP fermentation were fully additive and no multiplicative effects were observed. In Study 2, the 2 corn silages were treated with neutral detergent to remove starch and sugars and 12 samples were generated from cornerstone samples of dry corn, high moisture ear corn, and the 2 aNDF residues of corn silage. Gas production from aNDF residues was found to be a linear, rather than an exponential growth function, and the model was revised accordingly. The 3-pool model was fit using SAS NLMIXED with the same criteria as above. Consistent differences were found between degradation rates (Kds) for starch and aNDFom determined by fitting IVGP data as compared with Kds from in vitro starch and aNDFom assays due to the ability to fit variable lags in IVGP vs. fixed lag assumptions in the starch and aNDFom assays. In summary, the gas production from starch and aNDFom fermentation measured by IVGP was determined to be fully additive, and the approach to simultaneous fitting of multiple carbohydrate pool parameters was robust.

Key Words: in vitro digestibility, starch, aNDFom

P443 Effects of a cashew nut shell extract on nutrient digestibility and rumen microbiome in transition dairy cows.

B. M. Goetz*[1], E. A. Horst[1], E. J. Mayorga[1], M. A. Abeyta[1], S. Rodriguez-Jimenez[1], C. Hikita[3], T. Watanabe[3], J. M. Lourenco[2], T. R. Callaway[2], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Department of Animal & Dairy Science, University of Georgia, Athens, GA,* [3]*Agri-Bio Business Department, Idemitsu Kosan Co., Ltd., Tokyo, Japan.*

Objectives were to evaluate the effects of cashew nut shell extract (CNSE, consisting of 59% anacardic acid and 18% cardol) on nutrient digestibility and the rumen microbiome in periparturient Holstein cows. The formulated CNSE final granule contained 50% CNSE. Multiparous cows (n = 51) were stratified by prev 305 ME and parity and assigned to 1 of 2 treatments 21 d before expected calving: 1) CON (control diet; n = 17) or 2) CNSE-5.0 (control diet and 5.0 g/d CNSE granule; n = 34). Following parturition, 17 cows from the CNSE-5.0 treatment were reallocated into a third treatment group: CNSE-2.5 (control diet and 2.5 g/d CNSE granule; n = 17) resulting in 3 treatments postpartum: 1) CON, 2) CNSE-2.5 and 3) CNSE-5.0. Treatments were mixed with ground corn and top-dressed. A subset of cows (n = 24) were orally dosed with TiO_2 for 12 d with fecal grab collections on d 11 and 12 to determine digestibility. Additionally, a subset of cannulated cows (n = 12) were utilized for rumen fluid collection. Effects of treatment, time, and treatment × time were assessed using PROC MIXED. Prepartum and postpartum DM, OM, NDF, ADF, and starch digestibility were unaffected by CNSE administration ($P \geq 0.38$). Cows fed CNSE prepartum had decreased phylum abundance of *Chloroflexi* and increased *Elusimicrobia* ($P = 0.01$). Additionally, prepartum *Plantomycetes* tended to be decreased ($P = 0.10$) in CNSE relative to CON. Prepartum CNSE decreased genus abundance of *Methanosphaera*, *SHD-231*, *Blautia*, *Oscillospira*, and *Mogibacterium* ($P \leq 0.04$). Further, prepartum CNSE tended to decrease *Methanobrevibacter* ($P = 0.08$). Postpartum CNSE decreased phylum abundance of *Armatiomonadetes* and *Elusimicrobia* ($P < 0.05$) while also tending to decrease *Cyanonacteria*, *Fibrobacteres*, and *TM7* ($P \leq 0.08$). CNSE supplementation increased postpartum genus abundance of *Desulfobulbus* ($P = 0.03$). Further, postpartum CNSE tended to decrease abundance of *Fibrobacter* and *Dehalobacterium* ($P \leq 0.07$) while tending to increase *L7A_E11* ($P = 0.08$). In summary, CNSE supplementation altered rumen microbial abundance, notably a decrease in methanogenic bacteria, but had no detectable effect on digestibility.

Key Words: periparturient, anacardic acid, methane

P444 Effect of ruminal donor and carbohydrate type on in vitro fermentation.

N. Schlau*[1], D. R. Mertens[2], and D. Taysom[1], [1]*Dairyland Laboratories, Inc, Arcadia, WI,* [2]*Mertens Innovation & Research, LLC, Belleville, WI.*

Objective was to evaluate effects of ruminal donor diet on in vitro (IV) fermentation using purified carbohydrates (CHO). Blended ruminal fluid composited from 3 donors fed a low-(LS; 15% starch and 30% aNDFom) or high-starch (HS, 30% starch and 30% aNDFom) diet was used to measure gas production (GP) of 3 CHO types: 5 sugars (fructose, glucose, galactose, sucrose, and lactose), 4 starches (corn, CSt; pea, PSt; soluble potato starch, SPosT; and insoluble potato starch, ISPoSt), and 3 fibers (pectin; solka floc, SF; and xylan, XY) from 0 to 120h using the Ankom RF system. Undigested aNDFom (uNDFom) was measured after 12 and 120h for the SF and XY, and IV starch digestibility (IVSD) was measured after 8h for all starch sources. Duplicate samples were measured using Goering and Van Soest medium over 4 IV runs, with runs 1 and 3 using inoculum from LS and runs 2 and 4 using inoculum from HS. Samples for volatile fatty acids (VFA) were taken starting at 3 h and repeated at exponential intervals through 48h. We observed differences in IV GP kinetic parameters within the sugar and fiber sources, and in discrete lag time for starches, with CSt and PSt having the shortest (2.84 and 2.90 ± 0.22 h, respectively) and SPoSt and ISPoSt having the longest lag times (4.69 and 4.72 ± 0.22 h, respectively, $P < 0.001$). Further, IV GP distinguished among CHO Types, with starches having the slowest fractional rate of GP ($P = 0.001$) and highest maximum GP ($P < 0.001$), and sugars having the shortest discrete lag ($P < 0.001$). Sugars and fibers produced more VFA per g DM than starch from 3 - 12 h ($P < 0.05$). Higher GP and lower VFA suggests that starches were less effective energy sources. There were no differences between LS and HS among starch sources, but overall IVSD was greater for HS vs. LS (47.7 vs. 39.2 ± 1.65%, respectively, $P = 0.01$). There were no statistical differences between LS vs. HS for uNDFom of purified fibers. Results demonstrate that donor diet may affect in vitro assays and measuring IV GP can effectively characterize fermentability of CHO, which may be useful for constituents such as sugars and soluble fibers whose digestibilities are not routinely measured directly.

Key Words: ruminal donor, carbohydrate, fermentation

P445 Evaluation of 2 sources of monensin on rumen fermentation and digestion in continuous culture fermenters fed a lactating dairy cow diet.

S. El Haddad[1], P. Score[1], Y. Roman-Garcia*[1], P. Piantoni[1], H. Larson[2], and G. Schroeder[1], [1]*Cargill Animal Nutrition and Health, Innovation Campus, Elk River, MN,* [2]*Kansas State University, Olathe, KS.*

Monensin (MON) is an ionophore that modifies ruminal fermentation mainly resulting in decreased acetate:propionate ratio. The objective of this study was to evaluate a novel source of MON on rumen VFA and fermentation parameters in continuous cultures. Nine fermenters (1,955 mL) were used in a randomized block design consisting of 2 periods and 3 treatments (n = 6). Each period had 7 d of adaptation and 2 sampling days. Fermenters were fed approximately 100 g DM of a lactating cow diet once a day (94% OM, 16% CP, and 26% NDF). Liquid dilution rate was maintained at 8%/h and solid passage rate at 4%/h. The pH was maintained between 5.8 - 7.0. Treatments were: Control (CON; no MON added), Rumensin 90 (RUM; 2 mg/L MON from Elanco), and Monovet 90 (MVet; 2 mg/L MON from Huvepharma). Data were analyzed using the Lme4 package in R. The model included the random effects of period and fermenter, and the fixed effect of treatment and 2 pre-planned contrast: CON vs MON and MVet vs. RUM. Adding MON had no effect ($P > 0.10$) on percent DM, OM, NDF, ADF, and CP degradability or on total VFA production (mmol/d). MON decreased ($P < 0.01$) acetate, butyrate, and isobutyrate molar proportion and increased ($P < 0.01$) propionate molar proportion. Both sources of MON decreased ($P < 0.01$) acetate:propionate from 2.4 in CON to 1.62 in Mvet and 1.60 in RUM. Adding MON increased ($P < 0.01$) daily propionate production (74 vs. 111 vs 105 mmol/d for CON, MVet and RUM respectively) and decreased butyrate ($P = 0.01$) and isobutyrate ($P = 0.03$) daily production (mmol/d). MVet increased ($P = 0.04$) valerate daily production (mmol/d) compared with RUM. MON decreased ($P < 0.01$) NH_3-N production (545 vs. 430 vs 436 mg/d for CON, MVet and RUM respectively) but there was no difference between RUM and MVet. MVet tended to increase ($P = 0.06$) microbial-N flow (g/d) compared with RUM. There was no effect of MON on microbial efficiency (g/kg true OM or DM or NDF digested). Average daily or hourly pH was not affected by MON. In conclusion, MON shifted the fermentation profile away from acetate and in favor of propionate, and decreased microbial proteolytic activity as evidenced by lower NH_3-N production. According to this study MVet is a suitable source of MON for ruminants.

Key Words: monensin, fermentation

P446 Rumen fermentation and epithelial gene expression responses to diets continuing ingredients designed to differ in ruminally degradable protein and fiber supplies.

C. B. Gleason[1], L. M. Beckett[1,2], and R. R. White*[1], [1]*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA,* [2]*Department of Animal Sciences, Purdue University, West Lafayette, IN.*

Ruminal volatile fatty acid (VFA) concentrations are associated with diet composition and animal performance; however, details of how VFA synthesis, absorption, and interconversion respond to diets is limited. Our objective was to characterize how protein and fiber sources affect dry matter intake, rumen pH, fluid dynamics, fermentation parameters, and epithelial gene expression. Four diet treatments (soybean meal or heat-treated soybean meal and beet pulp or timothy hay) were delivered to 10 ruminally cannulated wethers (Suffolk, Dorset, or Suffolk × Dorset) using a partially replicated 4 × 4 Latin square. Soybean meals delivered varying rumen crude protein (CP) degradabilities while beet pulp and timothy hay delivered different neutral detergent fiber (NDF) degradabilities. Rumen fluid from hourly samples collected for 72 h during 7, 6-h stable isotope infusions of ^{13}C-VFA was analyzed for isotope ratio and VFA concentration during each period. The VFA fluxes were estimated from these data using a dynamic, fully-interchanging 6 pool model. Epithelial samples were collected once per period after the final fluid sample. Responses were analyzed with a linear mixed-effect model with fixed effects for fiber and protein source and their interaction, and random effects for animal and period. Dry matter intake, rumen pH, fluid pool size, and fluid passage rate were not affected by treatment ($P > 0.05$). Rumen VFA concentrations except isobutyrate increased on beet pulp compared with timothy hay ($P < 0.05$) but were unaffected by CP source ($P > 0.05$). Butyrate synthesis ($P = 0.015$) and absorption ($P = 0.045$) increased with beet pulp but synthesis and absorption of other VFA were unchanged ($P > 0.05$). Both CP and NDF treatment effects were associated with numerous VFA interconversions, predominantly in the conversions from acetate, propionate, and butyrate. Rumen epithelial genes involved in ion transportation, VFA metabolism, and epithelial integrity had similar expression among diets ($P > 0.05$). Animal intake, rumen environmental parameters, and the rumen epithelium may be fairly durable to shifts in nutrient degradability and associated changes in rumen VFA.

Key Words: degradability, rumen epithelium, sheep

P447 Characterizing effects of ingredients differing in ruminally degradable protein and fiber supplies on the rumen microbiome using next-generation sequencing.

C. B. Gleason[1], R. E. Settlage[2], L. M. Beckett[3,1], and R. R. White*[1], [1]*Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA,* [2]*Virginia Bioinformatics Institute, Virginia Tech, Blacksburg, VA,* [3]*Department of Animal Sciences, Purdue University, West Lafayette, IN.*

The dietary ratio of concentrate to forage is known to alter rumen microbial profiles, but less is known about how differing nutrient degradabilities affect the microbiome. The objective of this study was to investigate rumen microbial responses to diets composed of protein and fiber sources expected to vary in nutrient degradability. The responses of interest included relative abundances of bacterial taxa as well as estimations of community richness and diversity. Ten ruminally cannulated wethers (Suffolk, Dorset, or Suffolk × Dorset) received 4 diet treatments consisting of either beet pulp or timothy hay and soybean meal (SBM) or heat-treated soybean meal (HSBM) in a partially replicated 4 × 4 Latin square experiment. The timothy hay and beet pulp represented differing rumen degradabilities of neutral detergent fiber (NDF) while the soybean meals represented differing crude protein (CP) degradabilities. Solid and liquid samples of rumen contents were collected for microbial DNA isolation and sequenced using the MiSeq v3 600-cycle kit on the MiSeq platform. Numerous rumen bacterial population shifts were observed due to change in fiber source, with increased abundance ($P < 0.05$) of fibrolytic populations associated with timothy hay diets and decreased abundance ($P < 0.05$) with beet pulp diets. Conversely, populations of the pectin-degrading genera, *Treponema* and *Lachnospira*, increased on the beet pulp treatment ($P = 0.015$ and $P = 0.0049$, respectively). Limited impact on bacterial taxa was observed between diets differing in protein source. The Paraprevotellaceae genus YRC22 was observed to increase in abundance on HSBM diets ($P = 0.023$) and the phylum Spirochaetes tended to be more abundant on SBM than HSBM diets ($P = 0.071$). Beet pulp decreased rumen bacterial diversity ($P = 0.0027$) and tended to decrease bacterial species richness ($P = 0.051$) compared with timothy hay. Our results serve to further underscore the sensitivity of rumen microbes to changes in their preferred substrates, particularly of those associated with fiber degradation.

Key Words: next-generation sequencing, nutrients, rumen microbiome

P448 Effects of rumen acidosis on fecal pH, metabolism, and inflammatory biomarkers in lactating dairy cows acclimated to a high fiber diet.

M. A. Abeyta*[1], B. M. Goetz[1], S. Rodriguez-Jimenez[1], E. J. Mayorga[1], J. Opgenorth[1], A. D. Freestone[1], J. M. Lourenco[2], T. R. Callaway[2], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

Rumen acidosis (RA) causes systemic inflammation and disrupts normal starch flow through the gastrointestinal tract potentially leading to hindgut acidosis. Therefore, objectives were to evaluate the effects of RA on metabolism, inflammation, and fecal pH in cows acclimated to a high fiber diet (50% neutral detergent fiber and 14% starch dry matter). Four rumen-cannulated cows (231 ± 126 d in milk; 26 ± 3 kg milk yield) were acclimated to a high fiber diet for 7 d before being enrolled in a study with 2 experimental periods (P). Baseline data were collected from d 1–3 of P1 for covariate analysis. On d 4 of P1, cows were restricted to 50% of their ad libitum feed intake and acidosis was induced by manual insertion of fine ground corn to the rumen (2.75% of BW). Period 2 (4 d) began following corn administration and rumen and fecal parameters were followed for 20 h relative to acidosis induction (RAI). Effects of time were assessed using PROC MIXED of SAS. Relative to P1, post hoc analysis revealed that rumen pH decreased from 2 to 16 h RAI, with the nadir occurring at 7 h RAI (5.33 vs. 6.16; $P = 0.05$). Further, fecal pH remained steady until 8 h before markedly decreasing through 20 h RAI (4.95 vs. 6.96 at P1; $P < 0.01$). Milk yield remained unaltered by acidosis while dry matter intake numerically decreased on d 1 of P2 (31% relative to P1) before returning to baseline by d 2. Blood glucose was unaffected by RA, but insulin increased from 4 to 8 h (2.9-fold; $P = 0.05$) and remained elevated through 20 h RAI. Circulating fatty acids and blood urea nitrogen progressively decreased through 20 h relative to P1 (both 48%; $P < 0.01$). In contrast, β-hydroxybutyrate tended to increase from 4 to 12 h (61%; $P = 0.08$) before returning to P1 values at 20 h RAI. Post hoc analysis revealed a tendency for serum amyloid A to increase at 20 h relative to 0–8 h RAI (2.4-fold; $P = 0.06$) and lipopolysaccharide-binding protein tended to increase from 12 to 20 RAI (26%; $P = 0.09$). In conclusion, RA markedly reduced fecal pH, modified metabolism, and initiated an inflammatory response in lactating dairy cows acclimated to a high fiber diet.

Key Words: inflammation, acidosis, fecal pH

P449 Effects of fat-embedded calcium gluconate supplementation on inflammatory biomarkers and gastrointestinal barrier function during feed restriction.

M. A. Abeyta*[1], E. A. Horst[1], E. J. Mayorga[1], B. M. Goetz[1], S. Rodriguez-Jimenez[1], M. F. Caratzu[1], S. K. Kvidera[2], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Micronutrients USA LLC., Indianapolis, IN.*

Objectives were to evaluate the effects of rumen-protected calcium gluconate (CG) supplementation on inflammatory biomarkers and gastrointestinal (GI) barrier function during feed restriction (FR). Twenty-four lactating cows were assigned 1 of 2 iso-nitrogenous and isoenergetic dietary treatments: (1) control (CON; n = 12), or (2) CG (top-dressed at 0.07% of DM/d; Cremalto; Trouw Nutrition USA, n = 12). Cows were acclimated to their respective diets for 12 wk before study initiation. The trial consisted of 2 experimental periods (P). Period 1 (2 d) served as baseline for P2 (6 d) during which all cows received 40% of their P1 ad libitum dry matter intake (DMI). In vivo GI permeability was measured on d 1 of P1, and d 2 and 5 of P2 via pulse-dose ruminal administration of the paracellular permeability marker Cr-EDTA. Area under the curve (AUC) was calculated after 24 h of repeated blood collections for each infusion. Effects of treatment, time, and treatment × time were analyzed using PROC MIXED. By design, DMI decreased 60% relative to P1 for both treatments ($P < 0.01$). As expected, FR decreased milk yield for both diets (32%; $P < 0.01$) relative to P1. No dietary effects were observed on circulating fatty acids, insulin, glucose, β-hydroxybutyrate (BHB), or blood urea nitrogen (BUN). Feed restriction increased fatty acids and decreased insulin (4.1-fold and 58%, respectively; $P < 0.01$), while circulating glucose tended to decrease from d 1–3 of FR (3%, $P = 0.06$). Plasma BHB initially decreased on d 1 (17%) and increased from d 2–5 of P2 ($P < 0.01$). Further, BUN increased (22%) on d 1 before decreasing from d 2–5 of FR ($P < 0.01$). Regardless of diet, circulating serum amyloid A, lipopolysaccharide-binding protein, and haptoglobin increased over time, peaking on d 5 of FR (2.2-fold, 71% and 5.4-fold relative to P1, respectively; $P \leq 0.11$). Feed restriction increased the 24 h Cr-EDTA AUC on d 2 and 5 and the largest increase occurred on d 2 (30%; $P < 0.01$), however, it was unaffected by CG. Overall, FR caused leaky gut, but CG did not appear to meaningfully alter GI barrier function in this model.

Key Words: leaky gut, inflammation, barrier function

P450 Effects of Omnigen Pro on rumen fermentation parameters of Holstein cattle fed an early-lactation diet. Y. Jiang*, J. D. Chapman, E. Sharman, B. Humphrey, and M. Garcia, *Phibro Animal Health Corporation, Quincy, IL.*

OmniGen Pro (OGPRO, Phibro Animal Health, Teaneck, NJ) is a new product built on the OmniGen immune foundation, developed to maintain the effectiveness of OmniGen AF while improving gastrointestinal tract function and integrity. The aim of this study was to examine the effects of OGPRO on rumen fermentation of dairy heifers during a starch challenge. Based on BW, 32 Holstein heifers (8-mo-old) were blocked into 2 treatments, Control (CON) or OGPRO (10 g/100 kg BW). A basal TMR representing an early-lactation diet (16% CP, 31% starch, 28% NDF, 1.62 Mcal/kg NE_L) was offered ad libitum. Following one day of 50% feed restriction (d70), a starch challenge was conducted by replacing 50% of dietary DM with rolled barley offered ad libitum on d71. Rumen fluid was collected via stomach tubing, 4 h after feeding on d 66 and 73, and 9 h after feeding on d 71. Data were analyzed with the GLIMMIX procedure of SAS. OGPRO did not affect ($P > 0.10$) DMI, ADG, or feed efficiency. DMI was lower on d 72 compared with the day of challenge (d 71) regardless of treatments (5.56 vs. 9.61 kg; $P < 0.01$). Ruminal pH was lower on d71 compared with d66 and 73 (5.90 vs. 6.86 and 6.62, respectively, $P < 0.01$). Average ruminal acetate-to-propionate ratio was 4.17 on d66 and increased to 5.07 on d71 (5.90 and 4.27 for CON and OGPRO, respectively). Ruminal lactate concentration was negligible on d66 (0.065 mM) and d73 (0.32 mM). On d71, lactate was lower in OGPRO than CON (17.8 vs. 2.93 mM, $P = 0.01$). OGPRO increased ($P = 0.03$) ruminal total VFA concentration compared with CON on d 66, 71 and 73 (d 66: 96.4 vs. 89.2 mM; d 71: 89.5 vs. 81.5 mM; d 73: 79 vs. 67.7mM). On d71, OGPRO reduced ruminal acetate molar proportion (64.3 vs. 68.3%, $P = 0.03$), tended to increase butyrate molar proportion (17.3 vs. 14.7%, $P = 0.06$) and numerically reduced acetate-to-propionate ratio (4.27 vs. 5.90, $P = 0.11$). Overall, the inclusion of OGPRO in high concentrate diets improved rumen fermentation under normal feeding conditions and maintained fermentation and minimized lactate production during subacute ruminal acidosis in Holstein heifers.

Key Words: OmniGen, rumen fermentation, subacute ruminal acidosis

P451 Source of copper, manganese, and zinc affect 48-h in vitro fermentation. V. Brandao*[1], M. McCarthy[1], J. Heldt[1], N. Sriperm[3], and J. Johnston[2], [1]*Micronutrients USA LLC, Indianapolis, IN,* [2]*Fermentrics Technologies Inc., Arnprior, ON, Canada,* [3]*Nutritional Statistics, Atlanta, GA.*

Two trials were conducted aiming to evaluate the effects of trace mineral source on fermentation in a 48-h in vitro system. Treatments in the first trial consisted of: no added trace minerals (CTR1), sulfate sources of Cu, Mn, and Zn (SU1) and hydroxy sources of Cu, Mn, and Zn (IB1, IntelliBond, Micronutrients, USA). Second trial treatments were: control (CTR2), Mn oxide (OX), Mn Sulfate (SU2), hydroxy Mn (IB2), and organic Mn (OP, Optimin Mn, Micronutrients, USA). Supplemental mineral treatment levels were based on providing 222 mg/d Cu, 555 mg/d Mn, and 1,035 mg/d Zn to a lactating dairy cow consuming 25 kg/d DM and a ruminal volume of 120 L. These levels were scaled down to the appropriate amount for a 1 L fermentation vessel (Trial 1:7.25 mg $CuSO_4$, 24.3 mg $ZnSO_4$, 14.7 mg $MnSO_4$ for SU1; 30.8 mg; Trial 2: 7.8 mg OX, 14.7 mg SU2, 10.5 mg IB2, 30.8 mg OP). Four grams of dairy TMR ground to 6 mm was used as the fermentation substrate inside 5 × 10 cm bags, and each bottle contained a mix of KSU buffer (80%) and rumen fluid (20%). Bottles were placed in an insulated water bath heated to 39.5°C for a 48-h incubation period. Data were analyzed as a complete randomized design, using 4 replications/treatment, and least squares means were compared using Student's t procedure. Significance levels were based on $P < 0.05$. In trial 1, SU1 significantly reduced ($P < 0.01$) apparent organic matter disappearance (aOMD) compared with CTR1 (52.9% and 56.3%, respectively), whereas IB1 was similar to CTR1 (57.4% IB and 56.3%, respectively). Treatment SU1 tended ($P = 0.11$) to have lower apparent microbial biomass production (aMBP) compared with IB1 but similar to CTR1 (201.6 SU, 220.3 IB, 211.4 mg/g CTR). In trial 2 Mn sources from SU2 and OX significantly reduced ($P < 0.001$) aOMD relative to CTR2, whereas IB2 and OP were similar to CTR (51.4 SU2, 54.6 OX, 59.9 IB2, 60.5 OP, and 60.6% CTR2). IB2 and OP had significantly higher ($P = 0.003$) aMBP than CTR2, SU2, and OX (164.8 SU2, 158.8 OX, 173.6 IB2, 173.7 OP, and 155.6 mg/g CTR2). Overall, results indicate that improved trace mineral sources (IB and OP) have less impact on fermentation by increasing aOMD and aMBP in a 48-h in vitro fermentation system relative to SU and OX.

Key Words: apparent microbial biomass, digestibility, hydroxy trace mineral

P452 MALDI-TOF MS analysis of ruminal lipid A from total mixed ration- and pasture-fed cows: A comparative study. E. Sarmikasoglou*, J. Vinyard, K. M. S. Rahman, T. Jiranantasak, R. R. Lobo, A. Tuanyok, and A. Faciola, *University of Florida, Gainesville, FL.*

Lipopolysaccharides (LPS) are characteristic components of the outer cell wall of gram-negative bacteria and composed of 3 covalently linked regions: the O-antigen, the core oligosaccharide, and the lipid A moiety, which carries most of their endotoxic activity. The objective of this study was to isolate and compare the lipid A structures of ruminal LPS derived from TMR- and pasture-fed cows, by using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). Ruminal bacteria were collected from 2 rumen-cannulated Holstein cows; one fed a total mixed ration (60:40, concentrate: forage) and the other pasture-fed. The representativeness of each sample was validated by comparing the rumen microbiome from the cows in our study to the core rumen microbiome from previous literature. The endotoxic activity of both samples were assessed by using limulus lysate assay (LAL). Lipopolysaccharides from each respective sample were extracted with a phenol-water extraction procedure and purified via ultracentrifugation. To isolate lipid A from the core and O-antigen, pure ruminal LPS samples were hydrolyzed with 1M acetic acid at 100°C for 2 h. Lipid A derived from the TMR-fed cow exhibited tetra-acylated structure, whereas lipid A derived from pasture-fed cow exhibited penta-acylated lipid A structure. Both samples were quantified by using LAL assay and exhibited low endotoxic activity, consistent with the MALDI-TOF MS observations. Results indicate that the lipid A acylation pattern differs between animals, and that the ruminal bacteria express solely under-acylated lipid A structures contrary to hexa-acylated lipid A expressed by pathogenic bacteria, such as *Escherichia coli*.

Key Words: extraction protocol, MALDI-TOF MS, ruminal lipid A

P453 Effect of cashew nutshell extract on nutrient digestibility and rumen pH under continuous culture conditions. C. Compton*[1], O. M. Peña[1], N. Siva[3], T. C. Jenkins[1], G. J. Lascano[1], C. Hikita[2], T. Watanabe[2], and M. J. Aguerre[1], [1]*Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC,* [2]*Agri-Bio Business Department, Idemitsu Kosan Co. Ltd., Tokyo, Japan,*

[3]*Department of Biology, The Pennsylvania State University, University Park, PA.*

Various feed additives are in widespread use in ruminants' diets to modulate rumen fermentation, thus improving nutrient utilization and animal performance. The objective of this study was to determine the effects of cashew nutshell extract (CNSE, 59% anacardic acid and 18% cardol) on dry matter digestibility, organic matter digestibility, neutral detergent fiber digestibility (DMD, OMD, and NDFD, respectively), and rumen fluid pH in continuous culture fermenters. We hypothesized that incorporating incremental levels of CNSE would not negatively affect nutrient digestibility under in vitro conditions. Four treatments were randomly assigned to 8 fermenters for 2 periods of 10 d, with 7 d for adaptation and 3 d for sample collection. Treatments consisted of 4 levels of granulated CNSE formulated to contain 50% CNSE, premixed with corn grain, and added to the diets in stepwise increments equivalent to 0, 2.5, 5, and 10 g/cow/d. Fermenters were fed 58 g/d of TMR (16.6% CP, 31.4% NDF, and 26.6% starch), divided between 2 feedings at 0800 and 2000 h. Each fermenter was inoculated with rumen contents collected from 2 ruminally fistulated cows and diluted 1:1 with buffer on d 0. All fermenters received the same buffer solution, continuously, thus treatment effects of CNSE had the opportunity to alter pH. Data were analyzed with the mixed procedure of SAS as a randomized complete block design using pre-planned orthogonal contracts to test for linear and quadratic effects. Increasing levels of CNSE had no effect on DMD (54.2 to 56.2% DM; $P > 0.7097$), OMD (63.3 to 65.5% OM; $P > 0.8589$) and NDFD (57.8 to 59.9% of NDF; $P > 0.8314$). Mean culture pH was similar under all dietary conditions. The lowest pH (5.71) was observed at 2.5 g/cow/d level and the highest pH (5.77) was observed at 7.5 g/cow/d level. These results suggest that incremental levels of CNSE had no impact on nutrient digestibility under ruminal in vitro conditions.

Key Words: digestibility, dairy, fermentation

P454 The Holy Grail of ruminant energetics? Reconsidering methane energy.

J. R. Knapp*[1,2], [1]*Fox Hollow Consulting LLC, Lewisburg, OH,* [2]*The Ohio State University, Columbus, OH.*

The purpose of this review is to examine the history and biological basis of how methane energy fits into energy partitioning schemes. The simplified scheme frequently taught to animal nutrition students depicts gaseous energy (GE) as a component of apparent digestible energy (DE) along with metabolizable energy and urinary energy. Consequently, methane, as the largest component of GE, is considered to be a loss of energy to the animal. Older literature in animal nutrition and energetics describes GE as part of Intake Energy and thus a co-product of true DE. This perspective is more correct biologically, as methane is produced by microbial fermentation of feed in the gastrointestinal tract, not as part of postabsorptive metabolism by the animal. As a corollary, methane energy is not an energetic loss to the animal when it is derived from carbohydrates with β-glycosidic bonds that are not digestible by mammalian enzymes and the DE, in the form of volatile fatty acids, can only be made available by fermentation. For carbohydrates with α-glycosidic bonds such as sugars and starches that can be fermented either by microbes or digested by mammalian enzymes in the small intestine, methane energy could be considered an energetic loss to the animal when the DE from fermentation is compared with the DE theoretically available from intestinal digestion. However, experiments have shown that the DE from hindgut fermentation of starch offsets the DE from intestinal digestion. Because methane energy has often incorrectly been considered to be part of DE rather than a co-product, a lot of methane mitigation research has been justified on the basis that by decreasing methane output, more energy can be made available for maintenance, growth, lactation, etc. This is a misconception, and dietary manipulations should be directed at optimizing diet digestibility and thus optimizing the role of ruminant livestock in converting inedible feedstuffs to human-edible food rather than eliminating methane energy. Also, energy partitioning diagrams and concepts need to be reviewed and revised to correctly present methane energy as a co-product of DE in teaching students in animal nutrition.

Key Words: methane, energetics

P455 The effect of inclusion of exogenous amylolytic or cellulolytic enzymes on in fermentation of lactating dairy cow diets in a dual-flow continuous culture system.

J. R. Vinyard*[1], A. Ravelo[1], E. Sarmikasoglou[1], H. F. Monteiro[1], J. A. Arce-Cordero[1], M. L. Johnson[1], B. C. Agustinho[2], A. Bahman[1], R. R. Lobo[1], L. M. Gilbertson[1], M. N. Mills[1], M. P. L. Soltis[3], L. F. Ferrarreto[4], and A. P. Faciola[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Idaho, Moscow, ID,* [3]*University of Tennessee, Knoxville, TN,* [4]*University of Wisconsin–Madison, Madison, WI.*

The objective of this study was to determine the effects of including different exogenous amylolytic or cellulolytic enzyme products in a diet for high-producing dairy cows on in vitro ruminal fermentation. Eight dual flow continuous culture fermenters were used in a replicated 4 × 4 Latin square. The treatments were control (C), a solid combination of xylanase and glucanase (T1), a liquid α-amylase product (T2), or a solid α-amylase product (T3). Treatments were included at a rate of 0.008% of diet dry matter (DM) for T1 and T2 and 0.02% for T3 and all treatments replaced SBM compared with C. All diets had similar nutrient composition (30.2% neutral detergent fiber, 16.1% crude protein, and 30% starch; % of DM) and fermenters were fed 106 g/d split into 2 feedings. At each feeding T2 was pipetted into the respective fermenter and an equivalent amount of deionized water was added to each fermenter to eliminate potential variation. Experimental periods were 10 d (7 d adaptation and 3 d sample collection). Fermenter pH was allowed to fluctuate naturally and the dilution rates of both liquid and solid were 5.5%/h. Composite samples of daily effluent were collected and analyzed for ammonia-N (NH_3-N) and lactate concentration. Samples of ruminal fluid and pH were collected from each fermenter at 0, 1, 2, 4, 6, and 8 h after feeding to determine the change in lactate concentration and pH over time, respectively. The mean, minimum, maximum and the area under the curve for pH below 6 (AUC) were calculated for each treatment. All data were analyzed using PROC GLIMMIX of SAS with time as repeated measures. There was no effect ($P > 0.10$) of treatment on the mean, minimum, maximum, or AUC of pH, or the concentrations of NH_3-N and lactate in the effluent samples. There was also no effect ($P > 0.10$) of treatment nor an interaction between treatment and time on either pH or lactate concentration in the samples collected from the fermenter. Thus, there is no effect of exogenous amylolytic or fibrolytic enzyme inclusion on any parameters of pH or concentrations of lactate and NH_3-N.

Key Words: enzyme, fermentation, in vitro

Ruminant Nutrition: Protein/Amino Acids: Posters

P456 Rumen-protected methionine and its relationship with milk fatty acid profile in high-producing dairy cows under different planes of dietary energy. J. H. Carneiro*[1], D. R. Ribeiro[1], E. J. Askel[1], I. F. Carrari[1], M. Poczynek[1], F. Lopes[2], D. P. D. Lanna[3], J. S. Osorio[4], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil,* [2]*Adisseo South America, São Paulo, São Paulo, Brazil,* [3]*Universidade de São Paulo, Piracicaba, São Paulo, Brazil,* [4]*South Dakota State University, Brookings, SD.*

The aim of this trial was to determine the effect of methionine supplementation at 2 levels of dietary metabolizable energy (ME) on milk fatty acid (FA) profile of mid-lactation cows. A total of 112 multiparous Holstein cows (49.4 ± 5.3 kg/d of milk; 146.6 ± 68.2 DIM) from a commercial herd were blocked by lactation number, milk yield, and DIM. Cows were divided into 4 isoprotein (16.6% CP) diets: control (CON; 1.62 NE_L Mcal/kg DM); high energy [HE; CON +1.2% of DM of hydrogenated fat (Prius F 100 Nat Dry, Auster)]; methionine (MET; CON + 24g of Smartamine M, Adisseo SA); and HE+MET. The experimental period was 38 d, and the first 3 d were used as a covariate with all cows fed the CON diet. TMR was provided once a day, with or without the addition of HE treatment, while MET treatments were top-dressed twice a day. Milk samples for FA profile were individually collected on d 36 of the trial and stored −20°C until further analysis. Milk samples were analyzed by gas chromatography to determine 52 FA profile (Finnigan Focus CG, Thermo Fisher Scientific^{TM}). Data were analyzed using the MIXED procedure of SAS. The greater supply of MET improved milk fat concentration (4.13 vs. 3.87%; $P < 0.01$). Both HE ($P = 0.015$) and MET ($P < 0.01$) increased daily fat yield over CON (1.99 and 2.00 kg/d, respectively). There was no difference between treatments in proportion of De novoor mixed FA, but there was a trend ($P = 0.10$) for MET treatment to increase preformed FA (34.56 vs. 33.58 g/100g fat) and monounsaturated FA (25.13 vs. 24.25g/100g fat; $P = 0.05$) while decreasing PUFA (3.05 vs 3.44g/100d fat; $P < 0.01$). C10:0, C16:0, and C18:2*cis*9*cis*12 were reduced ($P < 0.01$) with MET, while C17:0iso, C18:1*cis*11, and C18:1*cis*13 were increased ($P < 0.05$). Higher intake of MET showed a trend ($P = 0.07$) to increase the C14:1/C14:0 ratio. No interactions were observed between HE and MET on FA profile. Changes observed in mono and polyunsaturated FA, as well as in the C14:1/C14:0 ratio, give us indications that MET may have some effect during the synthesis and modification of FA in the mammary gland.

Key Words: methionine, fatty acids, milk fat

P457 Microencapsulated methionine increased plasma methionine, milk protein yield, and milk protein efficiency. L. E. King*[1], E. Grilli[2,3], H. A. Ramirez-Ramirez[1], L. H. Baumgard[1], and J. A. D. R. N. Appuhamy[1], [1]*Department of Animal Science, Iowa State University, Ames IA,* [2]*DIMEVET, University of Bologna, Bologna. Italy,* [3]*Vetagro Inc., Chicago, IL.*

Microencapsulation is a promising technology providing excellent protection against rumen degradation and high intestinal release of nutrients. Objective of this study was to determine the effects of a rumen-protected methionine (Met) by microencapsulation (MET, Timet, VETAGRO, Italy) on blood Met status, and milk protein efficiency (MPE) of high-producing dairy cows. Multiparous Holstein cows (n = 48, 127 ± 41 DIM) were used in a replicated 4 × 4 Latin square design with 28-d periods. Dietary treatments included a TMR diet (CON) deficient in metabolizable Met (1.8% of MP, NRC, 2001), and CON supplemented with MET providing 11.0 (LM), 19.3 (MM), or 27.5 g/cow/d (HM) of Met to the diet. Dietary CP was 17.3% of DM and 64% of dietary DM was based on corn and soybean. Milk yield and DMI were recorded during the last 7 d of each period. Milk composition, arterial (coccygeal vessels) and venous (abdominal vein) essential amino acid (EAA) concentrations, arteriovenous difference (AVd) of EAA, and mammary fractional extraction efficiency (MFEE) of EAA were determined on the last 2 d. Treatment effects and the orthogonal contrasts were analyzed using the MIXED procedures of SAS with square, period within square, and treatment as fixed effects and cow within square as a random effect. Increasing MET in diet linearly increased arterial plasma concentration of Met from 2.83 for CON to 3.78 µg/mL for HM ($P < 0.01$). Plasma arginine, lysine, and tryptophan (Trp) concentrations also increased linearly for increasing MET in the diet ($P \leq 0.05$). The AVd of Met did not change for MET ($P = 0.450$) but AVd of valine and Trp decreased linearly with increasing MET ($P = 0.03$). The MFEE of all EAA except branched-chain amino acids decreased linearly as MET increased ($P < 0.050$). The supplementations of MET increased linearly milk protein yield from 1.16 for CON to 1.28 kg/d for HM ($P < 0.001$). With increasing MET, milk protein efficiency (milk N/N intake) increased linearly from 0.28 for CON to 0.31 for HM. The present microencapsulated Met increased metabolizable Met supply and milk protein yield, and decreased total manure nitrogen excretions in high-producing dairy cows.

Key Words: tryptophan, mammary uptake, efficiency

P458 Effects of dried distillers grains on milk composition and nutrient digestibility of dairy cows. R. G. Chesini*, G. Gomes da Silva, P. C. Vittorazzi Junior, N. T. S. Grigoletto, M. Bugoni, M. S. Dias da Silva, T. B. de Paula e Silva, A. T. Nunes, C. S. Takiya, and F. P. Rennó, *Department of Animal Nutrition and Production, University of São Paulo, Pirassununga, SP, Brazil.*

A balanced diet on rumen degradable and undegradable protein is critical to achieve optimum nutrient utilization and productivity. The aim of this study was to evaluate different dietary protein sources on DM intake, milk yield and composition, and apparent digestibility of nutrients in dairy cows. Twenty-seven multiparous Holstein cows (200 ± 40 DIM, 599 ± 78 kg BW, and 30.0 ± 3.92 kg/d milk yield) were blocked by DIM and milk yield, and enrolled to a 3 × 3 Latin square experiment with treatment sequences composed of (1) control (CON), soybean meal as major protein source in the concentrate; (2) rumen-protected soybean meal (SOY), bypass soybean meal replacing 30% of soybean meal in CON diet; and (3) dried distillers grains (DDG), DDG replacing 30% of soybean meal in CON diet. Each experimental period consisted of 14 d for diet acclimation and 7 d for sampling. Feed intake and refusals were monitored daily and samples collected during the sampling period for wet chemistry analyses. Fecal samples were collected every 9 h during 3 consecutive days for chemical composition. The indigestible NDF content of samples was used as an internal marker to estimate fecal DM excretion and nutrient digestibility. Milk samples were collected thrice during the sampling period and analyzed fresh for components using infrared method. Data were submitted to ANOVA using the MIXED procedure of SAS and treatment differences were evaluated by Tukey's test. Treatments containing alternative sources of protein (SOY and DDG) tended to increase ($P = 0.087$) DM and organic matter intake in comparison with CON. Cows fed either SOY or DDG exhibited greater ($P < 0.05$) milk and lactose yields. Feeding SOY or DDG tended to increase yields of protein ($P = 0.052$) and FCM ($P = 0.078$). Cows fed DDG had greater ($P < 0.05$) milk protein content compared with SOY group (3.45 vs. 3.40% for DDG and SOY, respectively). No difference on apparent digestibility of DM was detected among treatments. Replacing 30% of soybean meal by alternative protein sources (SOY or DDG) improved feed intake and productivity of cows in mid-lactation.

Key Words: by-pass soybean meal, protein sources, rumen-undegradable protein.

P459 Effects of raw or roasted soybeans on amino acid bioavailability of casein and lactation in dairy cows.

E. Petzel*[1], E. Titgemeyer[2], S. Acharya[1], E. Bailey[1], and D. Brake[1], [1]*University of Missouri, Columbia, MO,* [2]*Kansas State University, Manhattan, KS.*

Soybeans can provide ruminally degradable protein, lipid and metabolizable AA to lactating dairy cows; however, innate trypsin inhibitors in soybeans can limit protein digestion in nonruminant animals and concerns related to soy-based trypsin inhibitors can limit use of soybeans in cattle diets. An objective of this experiment was to evaluate impacts of raw or roasted soybeans on AA bioavailability of postruminal protein and performance in lactating cows. Eight ruminally cannulated Holstein cows (DIM = 144 ± 9.7 d; lactation = 2.6 ± 1.1) were divided into 2 groups that received abomasal infusions of casein or a crystalline AA analog of casein and fed a common diet that contained 10% soybeans (DM basis). Within each group, cows were placed in a 4 × 4 Latin square and provided 0 or 400 g/d abomasal infusions of casein or a crystalline AA analog of casein and fed raw or roasted soybeans for 14 d. At the end of each period plasma was collected across a 4-d period and AA bioavailability was calculated as the change in each essential plasma AA from casein divided by the change in each essential plasma AA from crystalline AA when cows were fed the same type of soybean. Data were analyzed as a strip-plot design using the MIXED procedure of SAS. Soybean type did not change ($P \geq 0.18$) bioavailability of any AA. As expected, infusion of casein or its crystalline AA analog increased essential AA and milk urea nitrogen concentration. Yet, the rate of increase was 2.4-times greater to casein than crystalline AA, but DMI also tended ($P = 0.06$) to be greater in cattle infused with casein compared with crystalline AA. Additionally, DMI was greater ($P < 0.01$) for cows fed roasted (25.1 kg) compared with raw soybeans (22.9 kg), and energy-corrected milk was greater in cows fed roasted (35.3 kg) versus raw (33.1 kg) soybeans. The proportion of fat, protein, lactose and SNF in milk did not differ between soybean type or postruminal AA source, but fat, protein, lactose and SNF yield was greater ($P \leq 0.01$) when cows were fed roasted soybeans because milk yields were greater when cows were fed roasted vs raw soybeans. These data seem to indicate that soy-based trypsin inhibitors have limited impacts on postruminal AA bioavailability.

Key Words: amino acid bioavailability, cow, trypsin inhibitor

P460 Postruminal dosing of lysine, methionine, and histidine as an assessment tools for amino acid biological availability in lactating dairy cows.

V. M. R. Malacco*[1], P. Doane[2], L. Beckett[1], S. Hilger[1], C. McCabe[1], M. Savage[1], and S. Donkin[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Archer Daniels Midland Company, Decatur, IL.*

Prediction of biological availability of rumen-protected amino acids is hampered by the lack of a direct method of assessment of postruminal (PR) biological availability (BA). This study evaluated the effect of PR dose of free Lys, Met, and His on plasma amino acids (AA) in lactating dairy cows. Three rumen-cannulated Holstein dairy cows weighing 589 ± 19 kg and producing 40.4 ± 5.0 kg/d of milk were used in a 3 × 3 Latin square design with periods consisting of 1 d of infusion and sampling and 6 d washout. AA mixtures were infused into the omasum of cows through a PVC tube inserted through the rumen. Cows were dosed with treatments (TRT) of Met, Lys, His in a 1:3:1 ratio by weight to deliver either 5g Met, 15g Lys, 5g His (1×); 10g Met, 30g Lys, 10g Hist (2×), and 15g Met, 45g Lys, 15g His (3×). Blood was collected for plasma amino acid analysis before the infusion and at 30, 60, 90, 120, 240, 360, and 480 min postinfusion (PI). Data were analyzed using the MIXED procedure of SAS with the fixed effects of TRT and period and random effects of cow within TRT. Pre-infusion plasma Met, Lys, His were 28.9, 91.9, and 47.0 µM, respectively. Plasma Lys increased within 60 min PI to 218.1, 273.1, 305.4 µM, for 1×, 2×, and 3×, respectively, and remained elevated ($P < 0.05$) through 90 min for 1× TRT and through 120 min for 2× and 3× TRT. Plasma Met increased within 30 min PI (64.8 and 108.2 µM for 1× and 2×, respectively) and remained elevated ($P < 0.05$) until 90 min for 1× and through 120 min PI for 2× TRT. Met concentration increased by 60 min PI (201.4 µM) and remained elevated ($P < 0.05$) through 120 min for 3× TRT. His concentration increased within 60 min (106.1, 169.4, 218.0 µM, for 1×, 2×, and 3×, respectively) and remained elevated for 90 min PI ($P < 0.05$) for cows receiving the 1× and through 120 min for 2× and 3× TRT. Data indicate that plasma amino acids are responsive to a dose of free AA delivered when delivered in a postruminal challenge test and that delivery of 5g Met, 15g Lys, 5g His can be determined within 60 min of infusion.

Key Words: amino acid, metabolism, dairy cow

P461 Supplementing low-forage diets with rumen-protected Met and Lys results in a reduction in mastitis risk and a modest increase of milk yield for mid-lactation multiparous Holstein cows.

M. B. Abreu*[1,2], A. Valldecabres[1], A. Correa[1], N. E. Lobos[3], M. I. Marcondes2,4, C. Peterson[5], and N. Silva-del-Rio[1], [1]*University of California–Davis, Tulare, CA,* [2]*Universidade Federal de Viçosa, Viçosa, MG, Brazil,* [3]*Corteva Agriscience, Johnston, IA,* [4]*Washington State University, Pullman, WA,* [5]*Adisseo, Alpharetta, GA.*

The aim was to evaluate the effects of rumen-protected Met and Lys (RPML) supplementation on the performance of mid-lactation multiparous Holstein cows fed low-forage diets. A total of 314 cows were randomly assigned to: control [CON; 107 g/d of dry distillers grains (DDG)] or RPML [107 g/d DDG + 107 g RPML (97.9% Smartamine ML + 2.1% Smartamine M; Adisseo USA Inc.)]. CON supplied 2.2% Met and 5.9% Lys of diet metabolizable protein (MP), while RPML supplied 2.8% Met and 7.0% Lys of MP (1.2 g Met/Mcal ME, and 3.2 g Lys/Mcal ME). Cows were group-housed and fed a 28% forage diet twice daily; treatments were top-dressed individually to each cow immediately after morning TMR delivery, at 6 a.m. for 6 wk. Milk yield was recorded daily. Individual milk samples for measuring components were collected from PM milking at d 0, and from AM/PM milkings at 2, 4 and 6 wk. Farm personnel evaluated and recorded any abnormal milk secretions or mastitis cases daily. Blood samples for plasma AA determination were taken 10 h after AM feeding at 0 and 14 d (n = 24 cows/treatment). Milk yield and components were analyzed as repeated measures using the MIXED procedure of SAS. Mastitis risk was assessed by Poisson regression using the GENMOD procedure of SAS. Compared with CON, cows fed RPML had higher plasma Met (36.0 vs. 26.9 µmol/L; $P < 0.001$) and tended to have higher plasma Lys (121.1 vs. 102.5 µmol/L; $P = 0.07$). Milk yield was higher for RPML than CON cows (46.0 vs. 45.4 kg/d; $P = 0.05$). No treatment effects were observed for ECM ($P = 0.70$) and 3.5% FCM ($P = 0.72$). Yields and concentration of both milk fat and protein were not affected by RPML supplementation ($P > 0.25$ to $P > 0.92$). Mastitis was detected on RPML (4.9%) and CON (12.5%) cows, but the risk was 0.39 times lower for RPML cows (95% CI: 0.17–0.90; $P = 0.03$). Somatic cell counts ($\log_{10}$SCC) were not different ($P = 0.92$). This study suggests that supplementing RP amino acids to adjust Met and Lys supply in low-forage diets, had positive effects in mammary gland health but modest effects on milk production.

Key Words: lysine, methionine, dairy cow

P462 Effects of 2-hydroxy-4-(methylthio)butanoate supplementation on productive performance of high-producing cows.

J. C. S. Lourenço[1], I. F. Carrari[1], E. J. Askel[1], M. Palmeira[1], G. C. Aguiar[1], H. P. Janssen[2], R. Gardinal[3], and R. Almeida*[1], [1]*Universidade Federal do Paraná, Curitiba, Paraná, Brazil,* [2]*Negócios Leite, Castrolanda Cooperativa Agroindustrial, Castro, Paraná, Brazil,* [3]*Novus do Brasil, São Paulo, São Paulo, Brazil.*

The aim of this study was to evaluate the effects of methionine analog supplementation on milk yield and its composition in a commercial dairy herd at Paraná State, Southern Brazil. One hundred fifty-eight multiparous Holstein cows, producing 48.2 ± 4.4 kg/d and 113 ± 64 DIM, were blocked by parity, milk yield, and DIM, and randomly allocated to 2 groups. In a randomized block design, the treatment consisted on daily supplementation of 35 g of MFP (Novus International Inc., USA), which contains 84% of 2-hydroxy-4-(methylthio)butanoate (HMTBa). Treatments were provided individually and top-dressed once a day mixed with corn meal. An equivalent amount of corn meal was provided to the controls. The total experimental period was 45 d, with the first 3 d used as a covariate and 2 periods of 21 d of supplementation. The milk samples were collected in the last 3 d of each experimental period, completing 9 consecutive milkings per period. The data were analyzed through MIXED procedure of SAS (v.9.4) containing the fixed effects of block, treatment, time and treatment*time interaction, the covariate, and the random effect of cow withintreatment. There was no difference between the control and HMTBa treatment for milk yield (48.15 vs. 48.79 kg/d; $P = 0.29$), but HMTBa-treated cows showed higher 4% FCM (47.65 vs. 49.46 kg/d; $P = 0.01$) and ECM (47.27 vs. 49.13 kg/d; $P < 0.01$). The cows that received HMTBa supplementation showed higher milk fat content (3.92 vs. 4.06%; $P < 0.01$) and higher milk fat yield (1.890 vs. 1.986 kg/d; $P < 0.01$). There were no differences for milk protein content between controls and HMTBa-supplemented cows (3.24 vs. 3.26%; $P = 0.40$), milk protein yield (1.549 vs. 1.573 kg/d; $P = 0.18$), milk casein content (2.57 vs. 2.59%; $P = 0.16$), and MUN (17.17 vs. 16.94 mg/dL; $P = 0.26$). HMTBa-supplemented animals showed higher milk lactose content (4.74 vs. 4.78%; $P = 0.04$), milk total solids content (12.87 vs. 13.03%; $P = 0.02$), and milk total solids yield (6.186 vs. 6.378 kg/d; $P = 0.02$). Our results demonstrate that there are positive effects of HMTBa supplementation on the synthesis of milk solids in high-producing cows.

Key Words: amino acids, methionine analog, milk fat

P463 Validation that the plasma AA dose-response technique can differentiate the methionine bioavailability of 2 rumen-protected encapsulated supplements of similar coating composition.

N. L. Whitehouse*, R. E. Luddy, and J. P. Sacramento, *University of New Hampshire, Durham, NH.*

The plasma free-AA dose-response technique has been established with the proper replication to determine methionine (Met) bioavailability within ± 3 to 5% points. Our objective was to measure the relative Met bioavailability of 2 protected sources of methionine (75% Met) with similar coating chemical composition but produced using different industrial processes. Six multiparous Holstein cows fed a Lys adequate (6.22% MP), Met deficient (1.74% of MP) diet were used in a replicated 3 × 3 Latin square with 7 d experimental periods. The 3 treatments were: 1) Control (CON), 2) CON plus 24 g/d Met from SmartamineM (SMM; Adisseo), and 3) CON plus 24 g/d Met from Kessent M (KEM; Kemin Industries, Inc.). The 2 RP-Met products were fed in 3 daily aliquots at 0500, 1300, and 2100 h. 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). Data were analyzed using the PROC MIXED to generate means and REG procedures of SAS to generate the linear regression variables. Significance was declared at $P \leq 0.05$. Average milk yield was 43.1 kg/d and DMI was 25.7 kg/d. Plasma Met, Cystathionine, Tau, and total sulfur AA (TSAA) concentrations (µ*M)* were higher for cows fed SMM compared with CON or KEM with KEM being greater than CON. Cystine was also higher for SMM and KEM compared with CON. Slope analysis confirmed the relative bioavailability of Met in KEM was 86.0% + 3.0 (0.06794/0.07903) relative to SMM. The plasma free-AA dose-response technique confirmed its robustness to differentiate the bioavailability of rumen-protected methionine products.

Table 1. Plasma AA concentrations (µ*M*) for cows fed RP-Met supplements

Item	Control	KEM	SMM	SEM	*P*-value
Methionine	23.1^{c}	46.0^{b}	50.5^{a}	2.13	<0.001
Cystine	20.8^{b}	22.0^{a}	21.9^{a}	0.42	0.04
Cystathionine	1.79^{c}	2.55^{b}	2.68^{a}	0.06	<0.0001
Homocysteine	2.77	2.46	2.74	0.23	0.38
Taurine	42.0^{c}	49.4^{b}	52.2^{a}	1.20	0.003
TSAA	90.4^{c}	122.6^{b}	130.3^{a}	3.4	<0.001
Slope for TSAA, %TAA – TSAA	—	0.06794^{b}	0.07903^{a}	0.0024	<0.001

$^{a-c}$Means within rows differ at $P < 0.05$.

Key Words: bioavailability, methionine

P464 Measurement of the difference in bioavailability of 2 sources of rumen-protected lysine using the plasma free AA dose-response technique.

N. L. Whitehouse*, R. E. Luddy, and J. P. Sacramento, *University of New Hampshire, Durham, NH.*

Precise estimates of the metabolizable lysine (mLys) contributions of rumen-protected AA technologies are needed for the industry to cost-effectively formulate rations to meet the mLys and metabolizable Met (mMet) requirements of dairy cows. Our objective was to estimate the mLys of 2 RP-Lys products hypothesized to be very different in Lys bioavailability. Six multiparous Holstein cows were used in a replicated 3 × 3 Latin square with 7-d experimental periods. The 3 treatments were: 1) Control (CON), 2) CON plus 60 g/d Lys from Smartamine ML (SMML; Adisseo; 55.0% Lys-HCl), and 3) CON plus 60 g/d Lys from LysiGEM (LGEM; Kemin Industries, Inc.; 68% Lys-HCl). All treatments were formulated to be adequate in mMet. The 2 RP-Lys products were fed in 3 daily aliquots at 0500, 1300, and 2100 h after hand mixing the product in 1.5 kg of TMR and stored at 4°C for 7–8 h before feeding. Intake, milk yield and milk 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). Data were analyzed using the PROC MIXED to generate means and REG procedures of SAS to generate the linear regression variables. Significance was declared at $P \leq 0.05$. Average milk yield was 43.5 kg/d and DMI was 28.0 kg/d. Plasma Lys concentrations (µ*M)* were higher for cows fed SMML compared with CON or LGEM, which did not differ from each other. Slopes for RP-Lys supplements expressed as %TAA−Lys was greater for SMML compared with LGEM. Relative to SMML, the Lys bioavailability of LGEM was 8.6% (0.00224/0.02595). The Lys in SMML has been previously documented to be 80% bioavailable. This would indicate the mLYS concentration of SMML would be 35.2% ± 1.7 (44% Lys x 0.80) vs 3.8% ± 1.5 (54.4% Lys x 8.6 × 0.80) for LGEM.

Table 1. Plasma AA concentrations (µM) for cows fed RP-Lys supplements

Item	Control	LGEM	SMML	SEM	*P*-value
Lys	73.1^{b}	76.1^{b}	109.1^{a}	2.79	<0.001
Met	40.6	41.1	37.8	1.52	0.11
Total sulfur AA	120	120	118	3.47	0.84
Total AA	2,107	2,132	2,219	37.1	0.13
Slope for Lys, % TAA – Lys	—	0.00224	0.02595	0.002	<0.001

^{a,b}Means within rows differ at $P < 0.05$.

Key Words: bioavailability, lysine

P465 Validation of the microbial crude protein model in Cargill's MAX System for Dairy.

Y. Roman-Garcia*, B. Ozoluko, A. Jacobs, F. Giallongo, M. Jerred, and G. Schroeder, *Cargill Animal Nutrition and Health, Innovation Campus, Elk River, MN.*

Predicting the amount of microbial crude protein (MCP) produced in the rumen is one of the most complex models in formulation needing accurate estimations of pools of rumen degradable nutrients and microbial efficiency. To evaluate Cargill's MAX System for Dairy software prediction of MCP in dairy cows we recorded data from 72 published articles from the years 2000–2020 that measured flow of MCP from the rumen under a wide range of dietary conditions and reported diet composition on an ingredient basis. The database had a total of 268 treatment observations with a milk yield mean of 31.7 kg, DMI mean of 21.4 kg and MCP mean 2,098 g (range: 462 - 4,769 g). Each ingredient reported in the article and their inclusion in the diet were entered into MAX System for Dairy software. If the article reported nutrient composition of individual ingredients these were also added. The MCP estimation was obtained from the software reports screen and recorded in the database. Using lm function in R, predicted values (x) were modeled against observed values (y) reported in the article. The intercept of the observed vs predicted equation was −67.1 (SE = 150.8) with a confidence interval of −364 to 230. The slope of the equation was 0.993 (SE = 0.067) with a confidence interval of 0.860 to 1.13. The intercept of the observed vs predicted equation was not different from 0 ($P = 0.66$) and slope was not different from 1 ($P = 0.54$) which means this equation is statistically equivalent to the identity (1:1) line. The R^2 value of the observed vs. predicted equation is 0.46 with a root mean square deviation of 951 g which suggest there is still variation not explained by the MAX System for Dairy model. To try to explain this variation the same model used in MAX System for Dairy 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 0.95 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 gives evidence that the model used by MAX System for Dairy software is a good predictor of MCP.

Key Words: microbial protein, modeling, formulation software

P466 Summarization of protein digestibility and association classification of commercial laboratory data.

D. L. Morris*[1], X. Huang[2], and R. Ward[2], [1]*Perdue AgriBusiness, Salisbury, MD,* [2]*Cumberland Valley Analytical Services, Waynesboro, PA.*

Variation exists in intestinal digestibility of high-protein feedstuffs. Data from commercial labs can be used to estimate population statistics for feedstuffs and to rank individual feeds within the greater population. The objective of this work was to summarize rumen-undegradable protein (RUP) and RUP digestibility (RUPd) measured in a commercial lab. A data set was assembled where RUP and RUPd were measured between 2015 and 2020 using the multi-step protein evaluation method (Ross method; n = 7,355). Data were initially categorized according to client definitions provided during sample submission. Mean crude protein (CP) for roasted soybean was greater than expected (45.1% vs 40%) suggesting that fat extracted soybean meals were misclassified by clients as roasted soybeans. To reclassify soybean-based feedstuffs, data for crude fat, NDF, and ash were assembled (available for 554 out of 2,454 samples). Samples were classified using k-means clustering based on CP, fat, NDF, RUP, and ash with outlier removal using a 3-step method (Yoder et al., 2014 JDS 97:5645). Four clusters were found and identified as 48% CP soybean meal (mean ± standard deviation; 52.7 ± 3.4% CP, 40.6 ± 10.9% of CP as RUP, 1.7 ± 0.6% fat, n = 50), heat-treated soybean meal (50.2 ± 2.9% CP, 79.7 ± 5.6% of CP as RUP, 1.9 ± 0.7% fat, n = 170), expeller soybean meal (46.8 ± 1.7% CP, 60.2 ± 7.1% of CP as RUP, 7.8 ± 1.4% fat, n = 231), and roasted soybeans (39.5 ± 2.0% CP, 61.2 ± 13.1% of CP as RUP, 21.1 ± 2.1% fat, n = 103). For blood meal (n = 1,124) and feather meal (n = 277), RUP on average was 87.1 ± 6.5 and 80.3 ± 5.0% of CP, respectively, and RUPd on average was 75.5 ± 18.1 and 50.4 ± 11.5% of RUP, respectively. The data for blood meal RUP and RUPd were non-normally distributed with approximately 15% of samples less than 2 standard deviations from the mean. Most other populations were normally distributed. Feedstuffs can be misclassification using client submitted information, which must be addressed when such data are used to estimate population parameters. Application of statistical techniques can be used to resolve misclassification.

Key Words: protein digestibility, sample misidentification, variation

P467 Effects of rumen-protected glutamate supplementation during the periparturient period on inflammation and digestibility of dairy cows.

S. Hisadomi*[1], A. Haruno[2], T. Fujieda[2], T. Sugino[3], and M. Oba[1], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Ajinomoto Co. Inc., Tokyo, Japan,* [3]*Graduate School of Integrated Sciences for Life, Hiroshima University, Higashi-Hiroshima, Japan.*

The aim of the study was to evaluate the effects of supplementing rumen-protected glutamate (Glu) during the calving transition period in dairy cows. As Glu is a preferred metabolic fuel in the small intestine, we hypothesized that supplementing Glu would decrease gut-derived inflammation and increase apparent total-tract digestibility (ATTD).

Fifty-two multiparous Holstein cows (parity, 2.4 ± 1.3; body condition score, 3.20 ± 0.34; mean ± SD) were blocked by parity, body condition score and expected calving date, and randomly assigned to one of the experimental diets with rumen-protected monosodium Glu prototype (RP-MSG; intestinally available Glu = 8.8%) or without (CON) at 3 weeks before expected calving date. The RP-MSG was fed at 4% of dietary dry matter before calving, and at 3% of dietary dry matter for 3 weeks after calving. Close-up diets contained 17.1% and 16.5% crude protein, 2.2% and 2.3% Met/metabolizable protein (MP), 6.6% and 6.7% Lys/MP, and 13.1% and 13.3% starch, and postpartum diets contained 18.8% and 18.3% crude protein, 2.2% and 2.2% Met/MP, 6.2% and 6.3% Lys/MP, and 22.5% and 22.7% starch on a dry matter basis, respectively for RP-MSG and CON treatments. A subset of 19 cows was used to measure ATTD. Data were analyzed using the Fit Model procedure of JMP Pro 14.3 (SAS Institute Innc., Cary, NC). Cows fed Glu did not decrease concentrations of plasma lipopolysaccharide-binding protein ($P = 0.33$), serum amyloid A ($P = 0.61$) and serum haptoglobin ($P = 0.54$). Nonetheless, cows fed Glu had higher ATTD of dry matter (70.6 vs 69.1%; $P = 0.05$), crude protein (75.1 vs 72.6%; $P = 0.03$) and fat (66.0 vs 61.2%; $P = 0.05$) in the first week after calving. However, in the third week after calving, there were no differences in ATTD of dry matter ($P = 0.14$) and fat ($P = 0.16$), and that of crude protein was lower for cows fed Glu (73.9 vs 74.7%; $P = 0.01$). These results suggest that Glu supplementation may increase the digestive capacity immediately after calving, but may not reduce inflammation.

Key Words: glutamate, inflammation, digestibility

P468 Bioavailability and rumen escape of *N*-acetyl-L-methionine in dairy cows. S. E. Räisänen*[1], C. Zhou[1,2], C. F. A. Lage[1,3], H. A. Stefenoni[1], T. Silvestre[1], D. E. Wasson[1], S. F. Cueva[1], J.-S. Eun[4], J. O. Moon[4], J. S. Park[4], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of Chinese Academy of Sciences, Beijing, P. R. China,* [3]*University of California, Davis, Davis, CA,* [4]*Institute of Biotechnology, CJ CheilJedang, Suwon, Korea.*

This experiment aimed at determining the bioavailability and rumen escape of *N*-acetyl-L-methionine (NALM) in lactating dairy cows. Four rumen-cannulated lactating Holstein cows, averaging (±SD) 239 ± 79 d in milk and MY of 41.4 ± 8.7 kg/d, were used in a 4 × 4 Latin square design experiment with 4, 12-d periods. A 12-d period for baseline data collection and determination of rumen escape of NALM preceded the Latin square experiment. For determination of rumen escape, 30 g of NALM was dosed into the rumen simultaneously with Cr-EDTA (used as a rumen fluid kinetics marker) and samples of ruminal contents were collected at 0 (before dosing), 1, 2, 4, 6, 8, 10, 14, 18, and 24 h after dosing. The rate of rumen degradation (k_d) of NALM was calculated as: (rate of NALM concentration decay – rate of Cr concentration decay). Rumen escape of NALM at different passage rates (k_p; 0.08, 0.10 and 0.16/h) was calculated as $[k_p \div (k_d + k_p)] \times 100$. Bioavailability of NALM was determined using the area under the plasma Met concentration curve (AUC) technique. Two doses of L-Met and 2 doses of NALM (providing 7.5, 15, 11.2 and 14.4 g digestible Met, respectively) were pulse-dosed into the abomasum of the cows (1 dose per cow and period) and blood was collected from the jugular vein for Met concentration analysis at 0, 1, 2, 4, 6, 8, 10, 12, 14, 18 and 24 h after dosing. The estimated relative bioavailability of NALM was 50% when dosed at 14.4 g/cow/d and 24% when dose at 28.8 g/cow/d. The estimated ruminal escape of NALM was from 14 (at $k_p = 0.08$/h) to 25% (at $k_p = 0.16$/h). Further validation of bioavailability at different NALM doses is warranted. In addition, intestinal conversion of NALM to Met needs further investigation to establish a possible saturation of the enzyme aminoacylase I (converting NALM to Met and acetate) at higher NALM doses.

Key Words: bioavailability, *N*-acetyl-L-methionine, dairy cow

P469 Evaluation of bioavailability of rumen-protected methionine supplement in lactating dairy cows. F. Francia, A. Kihal, M. A. Belaid, M. Rodriguez-Prado, and S. Calsamiglia*, *Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain.*

The objective of this study was to determine the bioavailability of a rumen-protected Methionine supplement in dairy cows using the plasma free amino acid dose-response technique. Five multiparous Holstein-Friesian cows (640 kg BW; 32 kg/d of milk) fitted with rumen cannulas were housed individually in a tie-stall barn. Cows were fed a 42:58 forage:concentrate total mixed ration formulated to meet or exceed requirements (NRC, 2001). Cows were milked twice daily. In experiment 1, 40 g of Met were dosed orally at the am feeding and blood samples were collected from the jugular vein at 0, 1, 2, 3, 4, 6, 9, 12, 24, 30 and 48 h after Met supply to determine plasma Met concentrations. In experiment 2, cows were used in a 5 × 5 Latin square. One week before the start of the experiment, blood samples were taken 8 h after feeding to measure the plasma AA concentrations used as covariate. Each experimental period consisted of 4 d for adaptation, 3 d for sample collection and 3 d for washout. Treatments were control (CTR): postruminal infusion of 15 g/d (InfLow) or 30 g/d (InfHigh) of Met: and 15 g/d (RPMLow) or 30 g/d of Met-equivalent of a rumen-protected Met (RPM, Kessent, Kemin Animal Nutrition and Health, Belgium) fed orally. Oral doses were fed in 2 equal portions before a.m. and p.m. feeding. Blood samples were collected at peak concentrations determined in experiment 1 (8 h after the Met supply) during the 3 sampling days following the same protocol described previously. Bioavailability of Met was estimated using the slope ratio method using the NLMIXED procedure of SAS. Results from experiment 1 indicated that Met plasma concentrations reached the peak around 8 h after feeding, and that time was selected for the sampling in experiment 2. In experiment 2, the lower slope of the oral treatment compared with the omasal infusion supplementation reflected the lower bioavailability due to rumen degradation and/or intestinal digestibility. The estimated of the bioavailability (ratio of slopes) of the RPM was 74.6% ± 5.2.

Key Words: bioavailability, rumen-protected methionine, dairy cow

P470 Bioavailability of different rumen-protected lysine products for dairy cattle. A. Kihal*, C. Marques, M. Rodriguez-Prado, and S. Calsamiglia, *Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain.*

The objective of this study was to determine the relative bioavailability of different sources of rumen-protected Lys and its interaction with different sources of rumen-protected Met supplements in dairy cows. Six multiparous Holstein cows (32 kg/d of milk) fitted with a ruminal cannula and an infusion line into the abomasum were used in a 6 × 6 Latin square. Treatments were pairs of Lys+Met (Lys-hydrochloride (LysHCl); LysiGEM (Kemin Animal Nutrition and Health, Emena, Belgium); and Ajipro (Ajinomoto Health & Nutrition North America, Inc., United States); DLMet, Met1, Met2, Met3, Met4 and Met5). Treatments were abomasal infusion of LysHCl+DLMet, and oral supply of LysGEM+M1; LysGEM+M2, Ajipro+M3, Ajipro+M4 and AjiPro+M5. Treatment were doses at the morning feeding (40 and 120 g for Met and Lys equivalents, respectively). Oral treatments were dosed into the rumen close to the esophageal orifice. Blood samples collected from the jugular vein at 0, 1, 2, 3, 4, 6, 9, 12, 24, 30 and 48 h after treatments. There were 3 d washout between periods. Results were used to determine the basal concentration (0 h), the maximum concentration (Cmax), the time to reach the Cmax (Tmax), and the area under the curve (AUC) of Lys. Results were analyzed with the PROC MIXED of SAS and differences declared at $P < 0.05$. The Lys basal concentration was similar among treatments (58.0 μM). The average Cmax was numerically higher (140.5 vs. 97.3 μM) and the Tmax numerically lower (5.8 h vs. 11.8) in LysiGEM vs. AjiPro, but differences were not significant. The AUC was higher in LysiGEM+M1 (1,204 units) and lower in AjiPro treatments (average 276 units). The plasma Lys concentration within the same type of Lys was different depending on the Met supplemented. The AUC of LysiGEM was 1,204 or 790 units, a 35% reduction, when mixed with M1 or M2, respectively. Similarly, the AUC of Lys from AjiPro ranged from 399 to 110, a 73% reduction, when mixed with M4 or M5, respectively. Results suggest that relative Lys bioavailability was higher in LysiGEM compared with AjiPro, and that bioavailability may be affected by the type of rumen-protected methionine supplement used.

Key Words: lysine bioavailability, methionine interaction, area under the curve

P471 Early-lactation cow response to supplementation of a methionine deficient ration with Kessent M and Smartamine M.

M. Smith*[1], S. Cronin[1], J. Mateos[2], D. Martinez del Olmo[2], F. Valdez[3], and T. Gressley[1], [1]*University of Delaware, Department of Animal and Food Sciences, Newark, DE,* [2]*Kemin Animal Nutrition and Health, Herentals, Belgium,* [3]*Kemin Agrifoods, Des Moines, IA.*

Methionine (Met) is a limiting amino acid for dairy cows that when supplemented as rumen-protected Met (RPM) can improve performance. This experiment compared production and plasma Met response of dairy cows provided a Met deficient diet supplemented with 2 RPM products. Twenty-four multiparous and 6 primiparous Holstein cows, 95 ± 20 and 71 ± 3 DIM, respectively, were used in a replicated 3 × 3 Latin square design with 21-d periods. Treatments consisted of a control diet with a predicted metabolizable Met deficiency of 17 g/d (CON), or control diet plus 13 g/d of MP Met from either Kessent M (RPM-K; Kemin Industries, Des Moines, IA), or Smartamine M (RPM-S; Adisseo Inc., Antony, France). Cows were fed ad libitum once daily, and RPM was top-dressed twice daily. Intake and milk yield were recorded daily, with milk samples collected during d 13–14 and 18–21 in each period. Weekly means of intake, milk yield, and milk composition were determined and analyzed with the GLIMMIX procedure of SAS, with the random effect of cow, and fixed effect of treatment, period, week, parity, block, and interaction of treatment by week. Blood samples were collected d 21 of each period. Plasma free Met expressed as a percentage of total nonsulfur containing amino acids was analyzed with GLIMMIX. Treatment did not affect milk yield (41.6 kg/d; $P = 0.49$) or milk protein yield (mean 1.32 kg/d; $P = 0.25$), but affected milk fat percentage ($P = 0.01$) and milk protein percentage ($P = 0.02$) and tended to affect milk fat yield ($P = 0.09$). Milk fat percentage was not different for RPM-K and RPM-S (3.76 vs. 3.73%; $P = 0.32$), and both were greater than CON (3.67%; $P \leq 0.05$). For milk fat yield, there was no difference between RPM-K and RPM-S (1.48 kg/d; $P = 0.78$), but RPM-S was greater than CON (1.45 kg/d; $P = 0.04$), and RPM-K tended to be greater than CON ($P = 0.08$). Milk protein percentage was not different between RPM-K and RPM-S (3.25%; $P = 0.96$), but both were greater than CON (3.22%; $P = 0.02$). Plasma free Met was affected by treatment ($P < 0.001$) and did not differ between RPM-K and RPM-S (2.04 and 2.05%, respectively; $P = 0.87$), but both were greater than CON (1.39%; $P < 0.001$). Overall, both RPM-K and RPM-S resulted in similar increases in milk fat, milk protein, and plasma Met compared with CON.

Key Words: methionine, lactation

P472 Canola meal improves methionine status in early lactation.

S. I. Arriola Apelo*[1], S. J. Halderson[1], S. A. E. Moore[1,2], and K. F. Kalscheur[2], [1]*Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI,* [2]*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

The objective of this study was to determine if substituting canola meal (CM) for soybean meal (SBM) at low and high dietary protein level affects plasma concentration and mammary extraction efficiency (MME) of AA in lactating dairy cows during the first 6 weeks postcalving. Seventy-nine multiparous Holstein cows were blocked by calving date and randomly assigned to 1 of 4 diets at calving. Treatments consisted of 2 dietary protein levels (low, 16.2%) or (high, 18.1%) and 2 main protein sources, SBM (8.9% in low and 11.9% in high protein) or CM (14.5% in low and 19.4% high protein), in a 2 × 2 factorial arrangement of treatments. Blood samples were collected from a coccygeal vessel (representing arterial blood) and the subcutaneous abdominal vein within d 3 to 6, d 7 to 9, wk 2, wk 4, and wk 6 after calving. Plasma AA were analyzed by liquid chromatography mass spectrometry with C-13 universally labeled AA as internal standards. Extraction efficiency was calculated as (arterial-venous)/arterial. Statistical analysis was performed with the lme function from the nlme library in RStudio. The statistical model included fixed effects of block, protein level, protein source, week, and 2-way interactions when $P < 0.10$, and the random effect of cow. Plasma concentration of total and nonessential AA were not affected by treatment or sampling date. Increasing dietary protein resulted in higher ($P < 0.012$) plasma concentration of essential AA, branched-chain AA, Arg, His, Ile, and Val, and tended to increase that of Lys ($P = 0.072$). However, high dietary protein also decreased MEE of Ile ($P < 0.002$), but only for SBM. Cows fed CM tended to increase ($P < 0.054$) plasma concentration of Met and increased ($P = 0.02$) Trp compared with cows fed SBM, regardless of the protein level. Compared with SBM, CM increased MEE of Arg ($P = 0.043$) over all weeks, whereas MEE of Lys increased ($P < 0.045$) only for wk 1 and 4. Meanwhile, MEE of Met linearly increased over time regardless of treatment ($P < 0.001$). Limited response to increased dietary protein supply suggests major deficiencies of essential AA in early lactation. Canola meal supplementation improved Met status in transition cows.

Key Words: canola meal, methionine, mammary glands

P474 Supplementation of rumen-protected lysine during the close-up period improves the vaginal discharge clearance in Holstein cows.

Y. Sugimoto*[1], H. Hayakawa[2], H. Oie[2], A. Taniguchi[2], A. Goto[2], N. Kusaba[2], N. Yamagishi[3], A. Haruno[1], M. Miura[1], T. Fujieda[1], and C. Kawashima[2], [1]*Ajinomoto Co. Inc., Chuo-ku, Tokyo, Japan,* [2]*Obihiro University of Agriculuture and Veterinary Medicine, Obihiro-shi, Hokkaido, Japan,* [3]*Osaka Prefecture University, Sakai-shi, Osaka, Japan.*

This study aimed to evaluate the effects of supplementation of rumen-protected lysine (RPL, AjiPro-L, Ajinomoto Health & Nutrition North America, Inc., USA, 80 g/d) during the close-up period on postpartum recovery of reproductive functions. 52 multiparous Holstein cows were blocked by parity, BCS and expected calving date, and randomly assigned to one of the experimental diets with RPL (n = 27) or without (CON, n = 25) at 3 weeks before expected calving date. After calving, all cows received the same diet. Onset of luteal activity was determined via ultrasonography twice a week starting at 14 d in milk (DIM) until 42 DIM. Uterine involution was monitored at 21 and 35 DIM by vaginal discharge score (MC score) assessed using a Metricheck device, a percentage of polymorphonuclear cells (PMN) in endometrial cytology samples obtained using cytobrush and cervical diameter detected by ultrasonography. Calving difficulty score and birth weight of calves were recorded. Data were analyzed using Student's t-test or Mann-Whitney U-test, depending on data distribution. Comparing within the group for the MC score were analyzed using paired t-test. Comparing between RPL and CON, there were no significant differences in the days to onset of luteal activity (25.6 ± 2.8 d vs 25.3 ± 2.4 d, $P = 0.74$, the values are described in the order: RPL vs CON), the MC score (2.7 ± 0.3 vs 2.2 ± 0.2 at 21 DIM, $P = 0.24$, 1.8 ± 0.2 vs 2.4 ± 0.3 at 35 DIM, $P = 0.11$), the percentage of PMN (32.6 ± 5.6% vs 26.2 ± 6.2% at 21 DIM, $P = 0.47$, 14.0 ± 4.2% vs 8.5 ± 2.5% at 35 DIM, $P = 0.62$), the cervical diameter (38.3 ± 0.9 cm vs 38.0 ± 1.2 cm at 21 DIM, $P = 0.89$, 33.0 ± 1.0 cm vs 35.2 ± 1.3 cm at 35 DIM, $P = 0.17$), calving difficulty score (1.4 ± 0.2 vs 1.5 ± 0.2, $P = 0.60$) and birth weight of calves (44.6 ± 0.8 kg vs 45.1 ± 0.9 kg, $P = 0.66$). Comparing within the group, the MC score significantly decreases from 21 DIM to 35 DIM in RPL (2.7 ± 0.3 to 1.8 ± 0.2, $P < 0.01$) although it did not change in CON (2.2 ± 0.2 to 2.4 ± 0.3, $P = 0.70$). These results suggest that supplementation of RPL during the close-up period improved the vaginal discharge clearance in cows.

Key Words: lysine, close-up period

P475 Relative bioavailability of 3 different rumen-undegradable methionine sources in dairy cows using the area under the curve technique.

A. Kihal*, M. E. Rodriguez-Prado, and S. Calsamiglia, *Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Barcelona, Spain.*

Most diets for high-producing dairy cows are deficient in Met. Industry has developed different methods to increase the supply of bioavailable AA to dairy cows. The 2-hydroxy-4-(methylthio)butanoic acid is a Met analog that it is converted into Met after absorption. Its esterification with an isopropyl group (HMBi) has been reported to enhance its bioavailability. The objective of this study was to determine the bioavailability of 2 HMBi products produced by different processes and compare them with an encapsulated rumen-protected Methionine using the area under the curve (AUC) method. The new HMBi product (KES, KESSENT MF Liquid Kemin Animal Nutrition and Health, Belgium) was compared with an already existing HMBi product (MtS, Metasmart Adisseo SAS, Antony, France) and a pH-sensitive coated Met (SmT, Smartamine Adisseo SAS, Antony, France). Nine multiparous lactating cows (30 kg/d of milk and 227 DIM) fed a 45:55 forage to concentrate diet were randomly assigned within square to a triplicate 3 × 3 Latin square design. Each period consisted of 3-d sampling and 3-d washout. Treatments were dosed on d 1 of each period and tail blood samples were collected at 0, 1, 2, 3, 4, 6, 9, 12, 24, 30, 48 h thereafter. The daily dose was 50 g of Met equivalent of each treatment. The HMBi treatments were administered directly into cow's mouth, whereas SmT was mixed immediately before feeding with 0.5 kg of concentrate and fully consumed within 15 min. Nonlinear models were fitted to raw data and the basal concentration at time 0 h, time at peak (Tmax), concentration at peak (Cmax) and AUC of plasma Met were determined. Differences among treatments were declared at $P < 0.05$. The Met Cbasal (26.7 ± 7.67 μM) and Cmax (210 ± 22.2 μM) were similar among treatments, but the Tmax (11.3 vs 1.4 h) was delayed and the AUC was 1.8-fold larger (3,457 vs. 1,868 arbitrary units) in SmT compared with HMBi. Results of this study indicate that the 2 HMBi products have a similar plasma kinetics and bioavailability. Smartamine had a different kinetic compared with HMBi, products with delayed Tmax, and larger AUC and relative bioavailability.

Key Words: bioavailability, methionine hydroxy analog, Smartamine

P476 Reducing metabolizable protein supply without negative effects on dairy cow performance.

J.-P. Laroche*[1], R. Gervais[1], H. Lapierre[2], D. R. Ouellet[2], G. F. Tremblay[3], C. Halde[4], M.-S. Boucher[4], and É. Charbonneau[1], [1]*Département des sciences animales, Université Laval, Québec, QC, Canada,* [2]*Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [3]*Quebec Research and Development Centre, Agriculture and Agri-Food Canada, Québec, QC, Canada,* [4]*Département de phytologie, Université Laval, Québec, QC, Canada.*

Protein requirements (rqt) expressed in metabolizable protein (MP) can lead to overfeeding some essential AA (EAA), but underfeeding others. Decreasing MP supply is often associated with a decreased milk protein yield possibly due to a reduced supply of critical EAA. The aim of this study was to measure dairy cow performance responses to a reduced MP supply when His, Lys, and Met rqt are met with a variable energy supply. Lys and Met rqt were estimated, respectively, at 2.38 and 6.95 of total MP rqt, based on a reevaluation for the NRC 2001 (JDS 93: E-Suppl.1:447); His rqt were assumed similar to Met. Using two 4 × 4 Latin squares, 8 Holstein cows were assigned to 4 diets balanced for His, Lys, and Met, with 21-d periods. Each square was fed a different alfalfa cultivar: regular vs. reduced lignin concentration. Within each square, diets were providing 103% of MP and 108% of energy rqt (NRC, 2001; AMP_AE, as a control) or providing 86% of MP rqt on average with increasing energy supply: 94% (DMP_DE), 101% (DMP_AE), and 107% (DMP_EE) of rqt. AMP_AE had 16.2% of crude protein; DMP had on average 14.2%. Measurements, taken on the last 3 d of each period, were analyzed with PROC MIXED (SAS); treatment means were compared using Tukey. Alfalfa cultivars had no effect. Compared with AMP_AE, feeding DMP_EE improved N use efficiency while maintaining energy-corrected milk (ECM), as well as milk fat and true protein yields. The DMP diets decreased total N excretion (N measured in feces and urine); AE and EE diets decreased milk urea-N (MUN) content. Reducing MP supply without negative effects on dairy cow performance is possible when energy, His, Lys, and Met rqt are met. This could reduce N excretion and the environmental impact of milk production.

Table 1.

Item	AMP_AE	DMP_DE	DMP_AE	DMP_EE	SEM	P-value
DMI, kg/d	26.0^{a}	23.9^{b}	24.5^{ab}	24.7^{ab}	0.93	0.04
ECM, kg/d	33.8^{a}	29.0^{b}	30.5^{b}	34.7^{a}	2.09	<0.01
Milk fat, kg/d	1.40^{a}	1.22^{b}	1.27^{b}	1.46^{a}	0.100	<0.01
Milk true protein, kg/d	1.11^{a}	0.93^{b}	1.00^{ab}	1.10^{a}	0.059	<0.01
MUN, mg/dL	16.2^{a}	14.8^{a}	10.5^{b}	11.0^{b}	0.56	<0.01
N use efficiency, %	25.6^{b}	26.7^{b}	27.8^{b}	31.1^{a}	0.95	<0.01
Total excreta N, g/d	463^{a}	364^{b}	358^{b}	352^{b}	19.3	<0.01

[a,b]Within a row, treatments with a different superscript differ ($P \leq 0.05$).

Key Words: amino acid, balance, energy

P477 Association between protein molecular structure and metabolizable protein supply to dairy cattle before and after rumen incubation of faba bean partitions and faba bean silage.

M. Yan*, V. H. Guevara-Oquendo, D. Christensen, H. (B.) Lardner, and P. Yu, *Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada.*

The primary objectives of this study were to determine how protein spectral features of fava bean samples were changed during rumen incubation using attenuated total reflectance- Fourier transform infrared spectroscopy (ATR-FTIR) and test the relationship between protein related spectral profiles before and after microbial digestion and protein intestinal digestibility and availability. Fava bean samples were first characterized for chemical composition and then incubated in the rumen of fistulated dairy cows for determination of CP degradation kinetics and protein intestinal digestibility. Protein related molecular spectral features (amides I and II, α-helix, β-sheet, and their ratios) before and after rumen incubation were also obtained. A randomized complete block design (RCBD) was used as an experimental design. The silage research fields were used experimental units (n = 3). Statistical analyses were performed PROC MIXED of SAS 9.4 with significance declared at $P < 0.05$. Results showed that the function of microbial digestion to the change of protein related molecular structure of fava bean could be detected ($P < 0.05$) by both univariate and multivariate molecular spectral analyses. As protein related spectral parameters, peak height of β-sheet, amide I, peak area of amide II and area ratio of amide I to amide II, spectral intensity was decreased ($P < 0.05$) with increasing time of rumen incubation. In addition, the spectral features of rumen incubation residues of fava bean had a strong correlation ($P < 0.05$) with the neutral detergent insoluble crude protein (NDICP) content, while the relationship between spectral profiles and other nutritional compositions and availability was diminishing during rumen incubation. In conclusion, the protein availability and digestion characteristic were mainly determined by its spectral profiles in original samples; the change of spectral profiles may have minimal effect on facilitating protein enzymatic hydrolysis. Overall, when evaluating nutritional value of feed for dairy cattle using ATR-FTIR spectroscopy, spectral features of original samples should be first considered.

Key Words: protein molecular structure, metabolizable protein supply, faba bean partitions and faba bean silage

P478 Using blood amino acid concentrations in dairy cows to target essential amino acids for individualized supplementation.

M. Wells*[1], J. J. M. Kim[1], L. E. Wright[1], K. Nakagawa[2], I. Shinzato[2], T. Fujieda[2], and J. P. Cant[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Ajinomoto Co. Inc., Tokyo, Kanto, Japan.*

Concentrations of individual amino acids (AA) in blood can be deficient for milk protein synthesis and impact the efficiency of dietary nitrogen capture in milk. The research objective was to test a method of supplementing AA based on a cow's blood profile. Twelve Holstein cows were fed a diet containing 1.7 Mcal/kg NE and 15.4% CP. According to a replicated 3x3 Latin square design with 10-d periods, each cow received jugular infusions for 96 h of either: 0.9% saline (Sal), an AA blend of the 3 most limiting AA in the diet according to NRC (NRCT; 32.3 g/d Lys + 25.4 g/d Val + 17.4 g/d Thr), or an equimolar AA blend of the 3 most limiting AAs based on their individual blood profile (BT). Cows on BT had blood samples taken 3 d before infusions; AA concentrations were compared with a reference profile to determine the 3 most deficient AA for each cow. Analysis of variance was conducted with period, treatment and their interaction as fixed effects, and cow as a random effect. Yields of milk and protein were similar across all treatments ($P > 0.15$). Both NRCT (3.28%) and BT (3.29%) resulted in lower milk protein content than Sal (3.38%) ($P < 0.01$). Cows receiving NRCT (981 μM) and BT (949 μM) had higher plasma concentrations of total essential AAs compared with Sal (796 μM) ($P < 0.01$). NRCT increased the concentrations of Lys (88.6 μM) and Val (310.9 μM) compared with both BT (74.4 and 236.1 μM, respectively) and Sal (70.4 and 192.1 μM, respectively) ($P < 0.05$). BT (200.2 μM) increased the concentration of Leu over NRCT (155.7 μM) and Sal (139.2 μM) ($P < 0.05$). Both AA supplementation approaches resulted in a decreased milk protein content. Refinements to the blood-targeting approach to AA supplementation are required.

Key Words: cow, amino acids, protein

P479 Impact of metabolizable protein supply across the transition period.

A. V. D. Kerckhove[1], A. Delaquis[2], F. Mueller[3], C. Gerard[4], T. Steen[5], and A. Park*[6], [1]*Federated Co-operatives Limited, Saskatoon, SK, Canada,* [2]*Sollio Agriculture, , Montréal, QC, Canada,* [3]*Kalmbach Feeds Inc., Upper Sandusky, OH,* [4]*Neovia, Saint-Nolff, France,* [5]*Tennessee Farmers Cooperative, LaVergne, TN,* [6]*Cooperative Research Farms, Richmond, VA.*

Two levels of metabolizable protein (MP) were compared in the transition period to determine the effect on performance. One hundred thirteen cows at 28 d before calving were housed and assigned to treatment in a randomized design across 15 small (4 cows per pen) pens. Thirty-one cows were fed a low/low (LL) diet (1,173 g/d – dry; 2,166 g/d – milking), 28 cows were fed a high/low (HL) diet (1,367 g/d – dry; 2,166 g/d – milking), 28 cows were fed a low/high (LH) diet (1,173 g/d – dry and 2,666 g/d – milking), and 26 cows were fed a high/high (HH) diet (1,367 g/d-dry; 2,666 g/d milking) to 35 d in milk (DIM). The 2 prepartum and 2 postpartum diets were iscaloric at 1.58 and 1.77 net energy of lactation, Mcal/kg, respectively. Cows were commingled and fed a common diet with milk yield (MY) and days to first breeding being tracked to 98 DIM. Cows were milked 3 ×/day with individual MY averaged by week. Dry matter intake (DMI) / pen and individual milk composition (2 d mean) was averaged by week. Bodyweight and condition score were recorded at initiation, calving, and 98 DIM. Plasma levels of albumin, aspartate amino transferase (AST), urea nitrogen (PUN), FA, and β-hydroxybutyrate (BHBA) levels were assayed at d 0, 3, 14, and 28 DIM. Data were analyzed with PROC MIXED for repeated measures over time. Differences were noted at $P < 0.05$ and trends at $P < 0.10$. Estimate statements were utilized to pair treatments pre and postpartum. The LH and HH diets had numerically reduced intake and lower MY during the first 35 d as well as MY (1.89 kg / d, $P = 0.001$) up to d 98. The LH and HH diets lowered energy-corrected MY (1.69 kg / d – $P = 0.02$) and tended to lower 3.5% fat-corrected MY (1.42 kg / d – $P = 0.06$). The LL and HL diets elevated milk protein percentage (0.13% - $P = 0.01$) and yield (0.08 kg /d – $P = 0.005$) during the first 35 DIM. The LH and HH diets increased PUN levels ($P = 0.003$), increased BHBA levels ($P = 0.04$), and tended to increase FA levels ($P = 0.06$). The HL and HH diet lowered AST levels ($P = 0.05$). The HL and HH diets were related to weight lost ($P = 0.02$) during lactation. The HH diet had longer DIM at first breeding ($P = 0.04$). The LH and HH diets led to reduced MY relative to the LL and HL diets in this trial.

Key Words: transition, fresh cow, metabolizable protein

P480 Amino acid supplementation during weaning decreased postweaning starter intake and growth in Holstein heifer calves.

H. K. J. P. Wickramasinghe*[1], C. A. Kaya[2], M. A. Perera[3], and J. A. D. R. N. Appuhamy[1], [1]*Department of Animal Science, Iowa State*

University, Ames, IA, [2]Dicle University, Diyarbakir, Turkey, [3]W. M. Keck Metabolomics Research Laboratory, Iowa State University, Ames, IA.

We previously showed supplementation of glutamine (Gln, 2.0% of DMI) enhanced weight gain and intestinal epithelial integrity of calves during weaning. However, blood urea increased for the Gln dose, and serum branched-chain amino acids (BCAA) were lower at 35 vs 49 d of age. Forge ahead, this study explored the effects of a lower Gln dose (1% of DMI) alone or with a BCAA supplementation on average daily gain (ADG), starter intake (SI), immune status, and serum metabolites in weaned dairy calves. Thirty-three Holstein heifer calves were assigned to 3 treatments (n = 11); no AA supplement (CTL), only Gln at 1% of DMI (GLN), or GLN plus BCAA [GLNB; Leu (17 g/d), Ile (10 g/d), and Val (11 g/d)]. Calves were weaned partially by decreasing milk volume from 9.0 to 3.0 L/d immediately on d 35. Amino acids were supplemented dissolved in milk from d 32 to 42. Weekly BW and daily SI were recorded until 70 d of age. Haptoglobin (HPT) and metabolite concentrations were measured for blood (jugular) drawn on d 35 and 42. Treatment effects were analyzed using the MIXED procedure in SAS with calf as a random effect. The AA supplements did not change serum Gln and BCAA ($P > 0.187$). Regardless of treatments, the milk restriction increased serum Ile, Gln, Gly, urea, β-hydroxybutyrate, and HPT ($P < 0.049$) and decreased Pro and glucose ($P = 0.005$). The CTL and GLN neither lost nor gained weight, whereas GLNB gained 0.31 kg/d ($P = 0.003$ for the being greater than 0) during the first week of weaning (d 35 to 42). The CTL and GLN increased ADG ($P < 0.025$) to preweaning levels but ADG of GLNB remained unchanged ($P = 0.935$) in the following week. All calves achieved 1.0 kg/d SI, on average, 17 d after partial weaning and thus weaned completely on 52 d of age. Paradoxically, GLN and GLNB were related to reduced SI ($P = 0.001$) and slower growth rate ($P = 0.012$) relative to CTL postweaning (49 to 70 d). Overall, weaning was associated with a negative energy balance and immune activation. Supplementation of BCAA improved growth at the onset of weaning but exerted a lasting negative effect alone or with Gln on SI presumably as a result of an imbalance between AA or between AA and energy.

Key Words: glutamine, haptoglobin, leucine

P481 Evaluation of noninvasive urine collection device and urine specific gravity to estimate urine output in lactating dairy cows.

P. Letelier*[1], G. I. Zanton[2], and M. A. Wattiaux[1], [1]*Department of Dairy Science, University of Wisconsin–Madison, Madison, WI,* [2]*USDA-ARS; U. S. Dairy Forage Research Center, Madison, WI.*

Bladder catheterization (BC) is the gold standard method to measure urine output in dairy cows, but it is invasive and may cause infections. External collection cup (CC) has been proposed as an alternative method. Urine spot samples (SS) can be used to estimate urine output assuming 29 mg of creatinine/kg body weight (BW). We evaluated the effect of urine collection method on urine output, urinary urea-N (UUN) and creatinine concentration and the relationship between urine specific gravity (USG) and urine output. Twelve multiparous Holstein cows were used in a split-plot, Latin square design. The cows were randomly assigned to the main plot consisting in 2 diets containing either 0.7 or 1.7% NaCl (dry matter basis). In the subplots, cows were assigned to a sequence of 3 urine collection methods in a balanced 3 × 3 Latin square design with 14-d period. Urine was measured and sampled every 4 h for 3 consecutive d. After sampling, USG was measured by refractometry (4410(PAL-10S), Cole-Palmer, USA). Urine output ranged from 18.4 to 50.6 kg/d and was 5.0 kg/d greater ($P < 0.11$) when cows were fed additional NaCl without affecting UUN excretion. Urine output averaged 33.7 and 30.1 kg/d for BC and CC, respectively ($P < 0.04$). Urine output estimated by SS was 5.0 kg/d ($P < 0.01$) lower than for BC but not different from CC (−1.4 kg/d, $P = 0.70$). Creatinine concentration in urine was higher for SS (79.7 mg/dL; $P < 0.01$) than for the BC and the CC methods (mean ± SD; 66.8 ± 1.8 mg/dL). Similarly, UUN concentration was higher for SS (705.3 mg/dL; $P < 0.01$) than for the BC and the CC methods (597.9 ± 9.3 mg/dL). UUN excretion tended to be lower when estimated with SS than measured with CC (180.5 vs. 202.4 g/d, $P = 0.08$) but neither of these methods differed from BC (196.3 g/d). USG ranged between 1.025 and 1.041 and was negatively related to urine output [urine output (kg/d) = 1,775 ± 126.1 - 1,692 ± 122.3 × USG, $P < 0.01$]. The R^2 of predicted vs. observed urine output (using both BC and CC values, n = 72) was 0.41, but was 0.72 when the prediction was based on urine USG.

Key Words: urine output, urine specific gravity, urine volume

Small Ruminants: Posters

P482 Infection dynamics of *Eimeria* spp. in sheep submitted to diets with different levels of fat babassu co-product.

G. M. Martins, A. A. Rodrigues, S. S. Reis, L. X. de Sousa*, I. A. Leme da Cunha, M. N. de Sousa, T. V. Costa Nascimento, H. N. Parente, A. G. V. de Oliveira Lima, and M. O. Maia Parente, *Federal University of Maranhão, Chapadinha, Maranhão, Brazil.*

Babassu (*Attalea speciosa* spp.) is a palm tree native to the Amazon rainforest region in South America. The oil, rich in lauric fatty acid, extracted from the seed is used after refining process. After the first step of refining, the co-product known as "borra" is available and has a great potential to be used in small ruminant diet. The greater medium-chain fatty acid proportion in this co-product, especially lauric acid, could have a preventive effect against necrotic enteritis, caused by *Eimeria* protozoa. Twenty-eight crossbred Santa Inês x Dorper lambs (20.6 ± 4.1 kg BW), naturally infected, were used in a completely randomized design. Lambs fed isonitrogenous diets (16.0 ± 0.1 CP, DM basis) with increasing levels of babassu "borra" (0, 5, 10 and 15%, DM basis) and they were monitored for 49-d experimental period. The dynamics of Eimeria spp. infection was performed by coproparasitological exams in pools made according to the level of inclusion of babassu and experimental days −7, 0, 14, 28, 42 and 49 (Table 1). During the entire duration of the experiment, oocysts of *Eimeria* spp. were detected in 95.83% of the feces samples collected, with a 100% presence in the pools made for each group according to the diets. Eight species of *Eimeria* were identified: *E. ahsata*, *E. bakuensis*, *E. crandalis*, *E. faurei*, *E. intricata*, *E. ovinoidalis*, *E. pallida*, and *E. parva*. The infection dynamics were characterized by peaks of elimination of oocysts for all levels of babassu "borra" inclusion on d 28. The addition of 9.01% babassu "borra" in the diet of lambs reduced the *Eimeria* oocysts score after d 49 of the experimental period. The addition of babassu "borra" up to 9% is effective on oocysts elimination of lamb's feces.

Table 1. Oocysts per gram of feces relative to collection intervals and and levels of fat babassu co-product

	Diet					Contrast	
Days	0	5	10	15	EPM	L	Q
−7	4,907	17,157	3,543	20,983	2,440	0.421	0.783
0	5,543	5,364	1,064	1,514	767	0.193	0.909
14	29,664	3,586	1,757	3,621	2,401	0.177	0.288
28	27,979	21,979	39,107	10,214	5,050	0.662	0.537
42	11,958	3,233	2,880	3,917	886	0.050	0.083
48[1]	11,483	1,567	2,440	4,950	911	0.158	0.046

[1] $y = 124.27x^2 - 2{,}238.5x + 11{,}026$.

Key Words: lauric acid, palm tree, small ruminant

P483 Metabolizable methionine balanced diets improved Lacaune dairy ewe performance.

F. Francia[1], A. Kihal[1], M. Rodriguez-Prado[1], G. Caja[2], X. Such[2], L. Bahloul[3], and S. Calsamiglia*[1], [1]*Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain,* [2]*Grup de Recerca en Remugants, Universitat Autonoma de Barcelona, Bellaterra, Spain,* [3]*Adisseo France SAS, Malicorne, France.*

The objective of this study was to determine the effect of supplementing 3 levels of metabolizable metionine (MetDi as %PDI) on milk yield and composition in early-lactation dairy ewes. Fifty-four multiparous Lacaune ewes (81.4 ± 2.37 kg BW; 3.1 ± 0.09 body condition) were used from the beginning of lactation. Ewes were fed ad libitum a 42:58 forage:concentrate total mixed ration formulated to meet nutrient requirements (INRA, 2007). Treatments were: control, 1.8 (CTR), 2.5 (Met25) and 2.7% (Met27) of MetDig/PDI. The rumen-protected Methionine (RPM; isopropyl ester of 2-hydroxy-4-methylthio butanoic acid; HMBi, Metasmart, Adisseo SAS, FR) was administered in the milking parlor mixed with 100 g/ewe/d concentrate offered in 2 equal portions during the a.m. and p.m. milkings. Ewes were divided into 9 balanced groups of 6 animals, according to BW and previous lactation milk yield, and milked twice daily. Treatments were randomly applied to 3 groups per treatment from lambing to d 70 in lactation. Intake was measured daily during wk 3, 6, and 10 using an indigestible marker (50 g/d PEG6000). Milk yield and composition was measured in 2 consecutive days within sampling weeks. Data were analyzed using the PROC MIXED procedure of SAS. No differences ($P < 0.10$) were observed in intake (2.89 ± 0.19 kg DM/d), milk yield (2.53 ± 0.31 kg/d) and fat content (5.33 ± 0.23%). Milk protein content was 4.94, 5.18 and 5.06% ($P < 0.07$) and milk urea was 464, 426, and 476 mg/L ($P < 0.06$) for CTR, Met25 and Met27, respectively. The significant treatment x week interactions in fat content reflected a reduction in fat content as methionine intake increased only in wk 10 ($P < 0.05$); and that in protein content reflected a higher level in Met25 in wk 6 and 10 ($P < 0.05$). The increased supply of methionine improved milk protein content. Therefore, dietary MetDi recommendation in dairy cows (2.5% metabolizable protein) may be extended to dairy ewes.

Key Words: rumen-protected methionine, ewe, milk yield and composition.

Reproduction: Posters

P484 Association between conceptus length and endometrial transcriptome on day 17 of gestation in dairy cows. P. M. Peixoto*[1], J. J. Broomfield[1], E. S. Ribeiro[2], J. E. P. Santos[1], W. W. Thatcher[1], and R. S. Bisinotto[3], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.*

The objective was to characterize endometrial transcriptome on d 17 of gestation in dairy cows according to conceptus length. Nonlactating Holstein cows (n = 48) enrolled in an experiment aimed at evaluating effects of follicular wave and progesterone concentration during ovulatory follicle growth were used. Cows were euthanized 17 d after AI and the uterine horn ipsilateral to the CL was flushed with saline. Recovered conceptuses were classified as small (1.2 to 6.9 cm; n = 10), medium (10.5 to 16.0 cm; n = 12), or large (18.0 to 26.4 cm; n = 10). Samples of inter-caruncular endometrium were dissected from the caudal, intermediate, and cranial portions of the uterine horn ipsilateral to the pregnancy. Total mRNA was extracted from endometrial tissue and subjected to transcriptome analyses using the Affymetrix Gene Chip Bovine array. Data were normalized using the GCRMA method and analyzed by robust regression using the Linear Models for Microarray library within Bioconductor in R. Transcripts with $P \leq 0.05$ after adjustment for false discovery rate and fold change ≥1.5 were considered differentially expressed. Functional analyses were conducted using the Ingenuity Pathway Analysis platform. Comparisons between cows with small vs. medium (SvsM), medium vs. large (MvsL), and small vs. large (SvsL) conceptuses yielded a total of 38, 4, and 107 differentially expressed transcripts, respectively. Top canonical pathways for SvsM and SvsL included complement system, B cell development, antigen presentation, allograft rejection signaling (SvsM only), Th1 and Th2 activation pathway. IFNγ and IFNα were identified as activated upstream regulators primarily based on increased expression of *IDO1*, *ISG20*, and *CXCL11* (SvsM and SvsL). Overall network summary revealed an upregulation of lymphocyte stimulation (*C3*, *CASP4*, *CD40*, *CXCL11*) and immune response of cells (*ATXN3*, *C3*, *CD40*), whereas IL10RA was downregulated (SvsL and SvsM). Differences in endometrial transcriptome according to conceptus length on d 17 of gestation were observed and highlighted pathways linked to immune-regulation and a potential role for interferon-regulated genes.

Key Words: uterus, embryo growth, gene expression

P485 Transcriptome changes associated with elongation of bovine conceptuses. P. M. Peixoto*[1], J. J. Broomfield[1], E. S. Ribeiro[2], J. E. P. Santos[1], W. W. Thatcher[1], and R. S. Bisinotto[3], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.*

Objective was to characterize transcriptome changes associated with elongation in bovine conceptuses during preimplantation stages. Nonlactating Holstein cows (n = 48) enrolled in an experiment aimed at evaluating effects of follicular wave and progesterone concentration during ovulatory follicle growth were used. Cows were euthanized 17 d after AI and the uterine horn ipsilateral to the CL was flushed with saline solution. Recovered conceptuses were classified as small (1.2 to 6.9 cm; n = 10), medium (10.5 to 16.0 cm; n = 12), or large (18.0 to 26.4 cm; n = 10). Total mRNA was extracted and subjected to transcriptome analyses using the Affymetrix Gene Chip Bovine array. Data were normalized using the GCRMA method and analyzed by robust regression using the Linear Models for Microarray library within Bioconductor in R. Transcripts with $P \leq 0.05$ after adjustment for false discovery rate and fold change ≥1.5 were considered differentially expressed. Functional analyses were conducted using the Ingenuity Pathway Analysis platform. Comparisons between small vs. medium (SvsM), medium vs. large (MvsL), and small vs. large (SvsL) conceptuses yielded a total of 83, 184, and 731 differentially expressed transcripts, respectively. Top canonical pathways of known involvement with embryo growth that were upregulated in large conceptuses included actin cytoskeleton (SvsL), integrin signaling (SvsL and MvsL), ephrin receptor (SvsL), mesenchymal transition by growth factor (MvsL), and regulation of calpain protease (SvsL). Estradiol-17β was predicted as an upstream regulator for SvsL and MvsL. Based on expression of transcripts, estradiol-17β is indirectly associated with upregulation of TGFB, ILB1, TNF (SvsL) and IFNG, IL6, TNF (MvsL). Overall network summary revealed upregulation of invasion of cancer cells (*ADREG5*, *BMP2*, *F2R*), proliferation of embryonic cells (*AGO4*, *BMP2*, *BRAF*), cell movement (*ACTA2*, *ACTN4*, *ADAM9*); indirect downregulation of organismal death (*HAND1*, *CALCRL*, *CEBPA*), and aplasia/hypoplasia (*HAND1*, *CALCRL*, *WIPF1*). Conceptus length on d 17 was associated with biological pathways involved with cell structure, proliferation, migration, and survival.

Key Words: embryo, gene expression, microarray

P486 Effects of 1 or 2 prostaglandin $F_{2\alpha}$ injections on luteal function and pregnancy outcome in dairy cows resynchronized with a 5-d + CIDR protocol. W. J. Sandberg*[1], K. Carnahan[1], P. Villamediana[1], W. Price[2], D. Konetchy[1], and A. Ahmadzadeh[1], [1]*University of Idaho, Animal Science, Moscow, ID,* [2]*Statistical Programs, College of Agricultural & Life Sciences, University of Idaho, Moscow, ID.*

There is evidence that 2 injections of prostaglandin $F_{2\alpha}$ (PG) in a 5-d CO-Synch + CIDR timed-AI protocol (5-d CIDR) is necessary to induce complete luteolysis by the time of AI and improve pregnancy rate. The objectives of this study were to compare the effects of a single dose of high-concentration PG (HC) or 2 doses of conventional PG (2PG) on serum progesterone (P4) concentration, and pregnancy per AI (P/AI) in Holstein cows (n = 489) re-synchronized with a 5-d CIDR program at non-pregnancy diagnosis (d 0). On d 5, cows were stratified by parity (primi- and multiparous) and number of inseminations and assigned randomly to receive (at CIDR removal) either one dose of PG (HC; 25 mg; s.q.; n = 247) or 2 doses of PG (2PG; 25 mg each, i.m.; n = 242), 24 h apart. Estrual behavior was monitored from d 5 to d 8, and if cows were detected in estrus, they were bred on that day. On d 8, cows not detected in estrus were administered a second dose of GnRH and inseminated at a fixed time AI (TAI). Blood samples were collected (n = 242), on d 5 and 8 to measure serum P4 concentrations and determine luteolytic response. Pregnancy was confirmed via ultrasonography 40 to 45 d after TAI. Data were analyzed by logistic regression (GLIMMIX). No difference in P/AI was detected between treatments ($P = 0.11$), as mean P/AI was 20.0% vs. 25.7% for HC and 2PG, respectively. There was a treatment by parity effect on P4 ($P < 0.05$). Mean serum P4 concentrations at TAI were greater ($P < 0.05$) in HC compared with

2PG (n = 115), only in multiparous cows (n = 69). Regardless of parity, mean P4 at TAI was <0.5 ng/mL for both treatment groups (0.44 ± 0.06 and 0.17 ± 0.06 ng/mL for HC and 2PG, respectively). Incidence of luteolysis (defined as P4 ≥ 1.0 on d 5 and <0.7 ng/mL at TAI) was greater ($P < 0.05$) in 2PG (97%) than HC (87%) cows. Overall, 2PG was more effective in inducing luteolysis before TAI than HC, however, no difference in P/AI was detected between treatments. Thus, based the current data, one dose of HC may potentially be used as option in the 5-d CIDR program with fewer animal handlings, injections, and labor compared with 2 doses of 2PG.

Key Words: prostaglandin $F_{2\alpha}$, resynchronization, progesterone

P488 Use of a cow-side immune test to improve prediction of metritis.

J. Prim*[1], Q. Huo[2], A. Mirzaei[1], K. Galvao[1], and R. Chebel[1], [1]*University of Florida, Gainesville, FL,* [2]*University of Central Florida, Orlando, FL.*

Two branches of immune function work together to protect the animals from invading pathogens: cell-mediated immunity (type 1), and antibody-mediated immunity (type 2). Maintaining a proper balance of type1/type2 immunity is necessary to provide optimal protection to the animals against infectious diseases. The objectives were to investigate the benefits of adding information from a cow-side immune test to metritis-prediction models. Cows (n = 197) were examined by study personnel at 4, 7, and 10 DIM for the diagnosis of metritis (watery, fetid, pink/brown uterine discharge) and acute metritis (rectal temperature >39.5°C). Blood samples collected within 24 h of calving were used to determine the balance between type 1 and type 2 immune response by measuring the relative quantity of IgG_1 and IgG_2 in the blood serum (D2Dx immunity test). A high-test score indicates a stronger type 1 response, whereas a low-test score indicates a stronger type 2 response. Logistic regression was used to create multivariable models to predict the risk of metritis through a step-wise backward elimination ($P < 0.20$). The area under the curve and the max-scaled adjusted r-square were used to evaluate the precision of the models. Dairy, parity, gestation length, calf sex, dystocia, stillbirth, twining, vaginal laceration (VL), and retained fetal membranes (RFM) were initially included in the model. The final model to predict metritis included parity, twining, VL, and RFM and had AUC = 0.86 and max-rescaled r-square = 0.43. When D2Dx results were added to the model to predict metritis, the final model included parity, twining, VL, RFM, and D2Dx results and had AUC = 0.87 and max-rescaled r-square = 0.46. The only variable retained in the initial model to predict acute metritis was calf sex and the model had AUC = 0.63 and max-rescaled r-square = 0.04. Conversely, when D2Dx immunity test results were added to the model to predict acute metritis, the model included D2Dx results and had AUC = 0.67 and max-rescaled r-square = 0.10. Our preliminary results suggest that the cow-side test used herein could improve the accuracy of models to predict metritis.

Key Words: metritis, immunity, prediction

Dairy Foods Symposium: Norm Olson Recognition Symposium

S100 What I will always fondly remember about Norm Olson, my "Best of Class" mentor. M. Johnson*, *Wisconsin Center for Dairy Research, Madison, WI.*

We acknowledge the mentors we have by their research accomplishments but we need to also include a more personnel side of their story. I had the privilege of seeing Norm in action both in and outside of the research arena. In 1979 Norm received funds from the State of Wisconsin to initiate the Cheese Research Institute. We had 2 employees and I was one of them. With funding originating from the Dairy Check-off Program, the Institute was absorbed into the first of the eventual 6 national dairy research centers, the Wisconsin Center for Dairy Research and we now have a staff of over 47 not including student hourlies. While I could make cheese, or should I say follow a recipe, I had no idea about the complex chemistry it entailed. Norm saw to it very quickly to teach me those details about cheese making. Norm was also very keen on bringing the best cheese technologists and dairy chemists from around the world to do a sabbatical with him. It was through interactions with those scientists that I got further lessons in the chemistry of cheese and Norm helped me apply them to real-world cheese making. Norm would take me out on troubleshooting field trips to various cheese factories around the state and we would discuss their issues in more detail on the drive home. As always, I would think about those discussions and days later Norm would very obligingly spend hours discussing new ideas. As part of the Institute Norm initiated a Cheese Technology conference to promote our research efforts and have experts talk about relevant topics about cheese. This event, eventually was incorporated into the Wisconsin Cheese Makers Association Annual meeting (Cheese Expo) which at the time, had no platform for speakers but only presented awards from their cheese judging contest at a large banquet. A few hundred people attended either event but currently the attendees at the WCMA event are a couple thousand each year. Norms legacy lives on in the presentation of the Norm Olson Scholarship awarded at this event annually. Last year Norm was awarded the Babcock Award by WCMA which recognizes contributions to education, and partnering with industry to promote dairy innovation and excellence.

S101 The biochemistry of cheese ripening and acceleration of its maturation: Overview and the contribution of the late Dr. Norman F. Olson. P. McSweeney*, *University College Cork, Cork, Ireland.*

Rennet-coagulated cheese varieties are ripened (matured) for periods ranging from less than 2 weeks to over 2 years, during which time the flavor and texture characteristic of the variety develop. The process of flavor development in cheese during ripening is extremely complex and involves a range of microbiological and biochemical changes. The principal biochemical events that occur during ripening involve changes to the caseins, and peptides and amino acids derived therefrom (proteolysis and amino acid catabolism), changes to lipids and free fatty acids (lipolysis and fatty acid metabolism), and the metabolism of residual lactose and of lactate and citrate. The ripening of hard cheeses is an expensive process and its acceleration has been the focus of much research in recent decades. Approaches to accelerate and control cheese ripening include addition of enzymes, the use of attenuated, adjunct or genetically modified starter cultures, and the use of high hydrostatic pressures and elevated ripening temperatures. The biochemistry of cheese ripening and the acceleration and control of cheese ripening were among the many research themes of late Dr. Norman F. Olson, former director of the Wisconsin Center for Dairy Research. His work in this area included aspects such as the proteolytic specificity of cheese-related proteinases on the caseins, the effect of proteolysis on the functional properties of cheese, cheese starter systems and their enzymes, microencapsulation of constituents to accelerate ripening, factors affecting the development of calcium lactate crystals and the use of attenuated cultures to accelerate ripening; he also performed early studies on the pink defect in Cheddar. Dr. Olson's work helped establish a firm foundation for our knowledge on the biochemistry of cheese ripening and the acceleration and control of cheese flavor development; the corpus of his work has made an important contribution to dairy research.

S102 Cheese structure: The beginning of cheese quality. D. Everett*, *AgResearch and Riddet Institute, Palmerston North, New Zealand.*

Structure exists on a spectrum of scale lengths, ranging from molecular to that which is visible to the human eye, and from nanometers to the size of the finished cheese. Cheese structure is affected by composition (including pH), cultures (both starter and nonstarter), the type of coagulant, processing conditions, the biochemistry of ripening, packaging, and storage conditions (time, temperature). It has been often stated that structurally poor cheese almost invariably results in uncharacteristic and poor flavor, but that good structure does not always result in good flavor. As such, a first step in producing high-quality cheese is to get the structure and texture right. Cheese structure was one of many research themes explored and published by Dr. Norm Olson, from 1958 when he was a graduate student at the University of Wisconsin, until he retired as a Wisconsin Distinguished Professor of Food Science from the UW 4 decades later. Dr. Olson was a Fellow and a former President of ADSA, and senior editor of the *Journal of Dairy Science*. He was instrumental in setting up the Walter V. Price Cheese Research Institute at the UW in 1976, which in 1985 became the Center for Dairy Research, the first, and one of eventually 6 in the United States. Much of his work involved one of the original pasteurized process cheese melting tests, crystal formation in cheese brought about by nonstarter microbial action, casein micelle structure, the role of α_{s1}-casein as a molecular backbone, confocal imaging of cheese structure, and the most recent work on bacterial surface characterization and the adhesion energetics of cheese cultures to the surface of milk fat globules, some of which he co-published with his graduate students. This work has established a foundation for current and future research into areas such as cheese as a vehicle for microencapsulating health-conferring ingredients, the effect of redox potential on flavor development, and digestibility of cheese with release and absorption of molecular components in the intestinal tract.

S103 Rennet coagulation: A critical but complex process. J. Lucey*, *University of Wisconsin–Madison, Madison, WI.*

Rennet coagulation is the critical first step in the cheesemaking process. It took until 1956 to finally identify and isolate the specific casein fraction (κ-casein) acted on by rennet. And it was 1981 before Walstra demonstrated that rennet action reduced the size of casein micelles confirming that there were κ-casein "hairs" on the surface. Norm Olson made important contributions in the rennet coagulation area including exploring how the firmness at cutting impacted yield and retention of milk components. Selecting the optimum firmness at cutting needs to balance several factors. If the coagulum is cut too soon shattering may occur, with losses of fat and curd fines. If the cutting is excessively delayed, then tearing of the firm coagulum can occur, especially in concentrated milks. Cutting gels when they are soft results in greater syneresis, while cutting firm gels helps increase the moisture content of cheese. He recognized that coagulation sensors that could operate in the cheese vat would be helpful for cheesemakers to select the optimum cutting time, and he evaluated several different prototypes. Nowadays several commercial coagulation sensors are widely used in the industry. As the use of membrane filtration to concentrate milk was being developed, he explored how milk concentration impacted rennet coagulation and cheese yield. This continues to be an important topic today. He also contributed to the development of the Carlson model for rennet coagulation, this was one of the first models that tried to include kinetic parameters to account for both the enzymatic and aggregation reactions. This model was recently modified to successfully fit the gelation profiles for rennet gels undergoing syneresis. Looking forward, I believe various challenges or gaps remain including how to handle the coagulation of highly concentrated milk including predicting when to cut these gels, the need for robust inline sensors for the syneresis process, and it is not fully clear what is the mechanism responsible for the increase in gel stiffness with time (2 suggestions include rearrangements of particles already in the network or a percolation type model where more clusters are incorporated into the network over time).

NANP Nutrition Models Workshop

S104 Tutorial on R software. T. Hackmann*, *University of California, Davis, Davis, CA.*

R statistical software will be used throughout the NANP Nutrition Models Workshop. The objective of this tutorial is to familiarize workshop attendees with use of this software. Specifically, this tutorial will walk attendees through basic data manipulation, visualization, and other functions. Assistance will be available for attendees who have difficulty installing the software on their laptops.

Key Words: R, tutorial, modeling

S105 Techniques for estimating parameters in compartmental models. K. F. Reed*, *Cornell University, Ithaca, NY.*

Compartmental models are a form of mechanistic models in which the researcher's hypothesis about the behavior of the real-world system is described through a series of differential equations. There are many ways to arrive at numerical estimates for parameters in the differential equations that define the system of interest. These methods are distinct from empirical statistical methods for model fitting or ANOVA in that, in most cases, they are not bound by assumptions about the distribution of the parameter itself or the outcome of interest (often the solution of the system of differential equations). The common thread that remains in both empirical and compartmental model parameter selection is the objective to minimize the distance between observed and model predicted values. Selection of the method for parameter estimation in compartmental models depends on the model objective, the availability of data, and the choice of probabilistic framework. I will present an overview of 4 methods for estimating parameters in compartmental models: direct measurement, meta-analysis, least squares optimization, and Bayesian model fitting. I will discuss appropriate applications and limitations of each method before providing a detailed description of a technique for compartmental model parameter estimation using optimization in R statistical software. Important steps in this methodology are definition of the system of interest in the form of differential equations, finding numerical solutions to system outcomes, and choice of package and search algorithm to find parameter estimates that minimize solution distance from observed data.

Key Words: compartmental model, parameter estimation, optimization

S106 A case study of compartmental model parameter estimation in R statistical software. K. F. Reed*, *Cornell University, Ithaca, NY.*

I will present a compartmental model case study of rumen protein digestion for participants in R statistical software. We will work through steps to define the system as a series of differential equations, use numerical integration to find solutions to the system, and implement a method for optimizing parameters of interest for the provided observations of system outcomes. We will discuss the key factors and decisions at each step of the modeling process and assess the impact of those choices on model outcomes. I will highlight the importance of harmonizing units and time-steps between parameter definitions and data to alert participants to potential pitfalls in implementation of this technique. Finally, we will compare models of increasing complexity and discuss mathematical techniques to adapt the optimization methodology to different types of data.

Key Words: compartmental model, parameter estimation, optimization

S107 Cross-validation and bootstrapping for determining bias and error rates of models. J. A. D. R. N. Appuhamy*, *Department of Animal Science, Iowa State University, Ames, IA.*

Cross-validation (CV) and bootstrapping are resampling methods that refit a model to samples drawn from the data. CV helps in choosing a "best" model associated with the lowest prediction error rate, whereas bootstrapping allows determining the uncertainty of parameter estimates. One may be tempted to use whole data set to develop and evaluate a model simultaneously. This approach however has issues such as overfitting and thus selects models that would potentially fail on an independent data set. These limitations can be overcome successfully with CV. Traditionally, CV is applied by splitting the data into 2 sets training, test that are used for model development and evaluation, respectively. This method called Hold-out is not recommended particularly for small data sets as the error rate would depend exclusively on the split and be misleading for a different split. Data splitting methods such as K-fold and leave-one-out are recommended to overcome those limitations. K-fold CV involves dividing the data into K number of samples and holding out one as the test set to determine the error rate. In leave-one-out CV, only one observation is held out at a time as the test set. In both cases, the true error rate for models with continuous responses is generally estimated as the average of the separate error estimates. Bootstrapping is a powerful statistical tool involving resampling with replacement and commonly used to quantify standard error or the confidence interval of statistical estimates. Consequently, bootstrapping allows for determining bias, standard errors, and confidence intervals of statistical estimates. Traditionally, the uncertainty of model parameters is estimated by deriving the sampling distribution based on assumptions about distribution of the population. In contrast, bootstrapping allows estimating the uncertainty without explicitly deriving the sampling distribution that way although it is important to keep in mind that the bootstrap depends on the bootstrap principle "Sampling with replacement behaves on the original sample the way the original sample behaves on a population." This lesson will cover the principles and implementations of CV and bootstrapping for models frequently used in animal nutrition.

Key Words: confidence interval, K-fold cross validation, standard error

S108 Cross validation and bootstrapping linear models in R: An exercise. J. A. D. R. N. Appuhamy*, *Department of Animal Science, Iowa State University, Ames, IA.*

Here we demonstrate a few applications of cross-validation and bootstrapping in evaluating the predictive ability and determining uncertainty of the parameter estimates of a linear regression model using R, a freely available and widely used statistical programming language. The packages such as "design," "DAAG," "caret," and "boot" are capable of performing cross-validation of linear models in R. The "boot" package particularly provides extensive facilities for bootstrapping and thus estimating the standard error or confidence interval of a single statistic (e.g., mean), or a vector (e.g., regression coefficients). A data set including a given number of enteric methane emission (CH_4) measurements, and corresponding dry matter intake (DMI) and dietary fat content is used. A simple linear regression model to predict CH_4 is first developed including DMI and evaluated separately using Hold-up, K-fold, and leave-one-out cross validation methods. The outputs are discussed and the methods are compared related to the variability of MSPE, and computational cost. The K-fold cross validation is performed with traditional K = 10 (90% of data for training and 10% for test), and compared with lower (K = 5) and higher (K = 20) number of folds. One of the issues with K-fold cross validation is that it often has a high variability if performed multiple times on the same data. The replicated K-fold cross-validation method addresses this issue by performing the whole process several times averaging over replications. Therefore, we perform replicated K-fold cross validation and compare the MSPE with previous values. We then use our simple prediction model to demonstrate an application of nonparametric bootstrapping to estimate bias, standard error, and 95% confidence interval of the parameter estimate. Histograms and normal quantile-comparison plots for the bootstrap replications are obtained and discussed. If time permits, the bootstrapping will be repeated with a multiple regression model including both DMI and dietary fat content. The data and all the R scripts will be available in advance for download.

Key Words: confidence interval, K-fold cross validation, standard error

S109 Automated model selection: Part I (lecture). V. L. Daley*[1], L. M. Campos[2], A. C. Hruby[2], and M. D. Hanigan[2], [1]*Purina Animal Nutrition Center - Land O'Lakes, Gray Summit, MO,* [2]*Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

Automated model selection (AMS) involves searching for the best models from a set of candidate models, also known as multi-model inference. The objective of this lecture is to present the concepts of AMS and illustrate how this procedure can be used in the development of empirical models in Animal Science. First, the hypothesis, objectives, and potential variables associated with the subject of study will be discussed. Further, key concepts in development of a meta-analytic data set will be presented. Data should be assessed for biological coherence and outliers need to be identified and removed. For the AMS framework, a mixed model is fitted using all predictor variables that potentially affect the dependent variable (global model), then a set of submodels are derived from the fixed terms of the global model. The parameters are estimated using maximum likelihood methods. The AMS approach can use one or more information criterion, but the Akaike's information criterion (AIC) is often adopted. All candidate models are ranked by AIC, and the best set of candidate models are selected based on AIC and biological coherence. Variance inflation factors (VIF) can be calculated to evaluate collinearity among model predictors, and may be used for model discrimination. The final stage involves evaluation of selected model accuracy and precision (often < 10 models) based on root mean square errors (RMSE) and concordance correlation coefficients (CCC) with (adjusted) and without (unadjusted) the random model effects. Cross evaluations should be performed to estimate model reliability. AMS is useful when a large set of potential predictor variables are available, and avoids bias inherent to forward or backward elimination approaches. However, it can involve millions of model derivations with results maintained in memory. Thus parallel computing is encouraged, and the computer must have large memory chips to store the results matrix during processing and manipulation.

Key Words: multi-model inference, empirical models, review

S110 Automated model selection: Part II (exercises). V. L. Daley*[1], L. M. Campos[2], A. C. Hruby[2], and M. D. Hanigan[2], [1]*Purina Animal Nutrition Center - Land O'Lakes, Gray Summit, MO,* [2]*Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

Automated model selection (AMS) can be applied in different research areas for the selection of the best fitting models. The attendees should have basic experience in R programing, data analysis, and empirical models. The objective of this exercise is to apply the AMS approach as a tool for the selection of prediction models. A hypothetical example in Animal Science is used to help the audience better understand and apply AMS. This exercise will use RStudio, which can be freely downloaded from the internet. As an example, the investigator will develop empirical models using the "MuMIn" package and parallel computation in R. A meta-analytical data set from the National Animal Nutrition Program (NANP, https://animalnutrition.org) is available for this exercise. Data quality and range are checked to identify and remove outliers, and data are visualized utilizing "ggplot2" package. Then, all the rows with missing values for any of the predictor variables are removed to ensure that the number of observations are similar when individual terms are added and removed from the model during model selection. A global mixed model applying "lme4" package is fitted to the data using all potential predictor variables from the data set. A set of candidate models is generated using combinations of the fixed terms of the global model. Akaike's information criterion (AIC) are calculated and used to rank the models. The candidate models are collected in a data set named "all models" for future use. The models with the lowest AIC values are collected in another data set named "best models." The estimated parameters of best candidate models are automatically collected. Only candidate models with a variance inflation factor (VIF) less than 10 are kept in the "best models" data set. Evaluation of the biological coherence and ANOVA to compare the models are performed. The best candidate models are also evaluated using for root mean squared error of prediction (RMSE) and concordance correlation coefficient (CCC). The best models are automatically collected in a table developed by "sjPlot" package. During the exercise, additional instructors will be available to help participants. The attendees will be able to undertake AMS to select the best models and apply those models in modeling research or development of prediction tools for industry use.

Key Words: multi-model inference, mixed models, Akaike information criterion

S111 Molly and other dynamic models: Lecture. H. A. Rossow*, *UC Davis VMTRC, Davis, CA.*

Mathematical models are tools to examine existing theories, find gaps in knowledge and explain phenomena such as nutrient digestion and metabolism. Simulated data from a model can be used to examine model behavior, as in sensitivity analysis, and compared with experimental data to determine if the model makes biological 'sense'. The objective of this session is to explore how concepts or theories of rumen digestion, nutrient metabolism and lactation physiology are translated into mechanistic mathematical equations and combined into a whole-animal model using the Molly model as an example. Molly is a mechanistic model of a dairy cow composed of a digestive element and an animal element. The rumen digestive element converts chemical composition of the diet to volatile fatty acids, microbial growth and absorbed nutrients using physical attributes of the diet such as proportions of large and small particles and water passage. The animal element converts products from the digestive element into tissues (protein), waste products, heat production or secreted products (milk, milk fat, etc.). Two processes that have been translated into equations in the Molly model will be examined in this session: (1) Passage of digesta through the rumen, described in Baldwin et al., 1977 (Agric. Syst. 2:255–288), and (2) Milk production by the mammary gland, described in Neal and Thornley, 1983 [J. Agric. Sci. (Camb.) 101:389–400]. In the lecture, representations of these processes will be examined using conceptual diagrams and differential equations. Then a full simulation will be used to demonstrate how rumen digestion and nutrient metabolism fit into the model.

Key Words: computer simulation model, dairy cow, metabolism

S112 Molly and other dynamic models: Exercise. H. A. Rossow*, *UC Davis VMTRC, Davis, CA.*

Mechanistic models representing physiological processes can be connected to represent whole-animal systems. When the models use Michaelis-Menten type kinetic equations, examples of ways to connect physiological processes are (1) including central pools (blood pools) of nutrients available for physiological processes, (2) using differential equations which represent nutrient input and output relationships for nutrient pools and, (3) assuming a physiologic process is the same across all tissues, i.e., protein synthesis. Based on the 2 example processes discussed in the lecture: (1) Passage of digesta through the rumen, described in Baldwin et al., 1977 (Agric. Syst. 2:255–288), and (2) Milk production by the mammary gland, described in Neal and Thornley, 1983 [J. Agric. Sci. (Camb.) 101:389–400], participants will conduct a simulation exercise which uses these modeled processes in Molly to predict lactation performance. In the exercise, participants will observe effects of altering rumen digestion on production of the dairy cow to understand how metabolic processes can be represented by mathematical equations to provide a conceptual framework that improves our understanding of animal biology.

Key Words: computer simulation model, dairy cow, metabolism

ADSA Southern Branch Symposium: Managing Heat Stress in a Warmer Planet

S113 The role of inflammation in chronic heat stress and its effects on growth, efficiency, and well-being in livestock. D. Yates*, *University of Nebraska-Lincoln, Lincoln, NE.*

Heat stress slows growth and diminishes efficiency in livestock. Our recent studies show that both muscle and fat are diminished by chronic heat stress, and that reduced feed intake is only partially responsible for these deficits. Indeed, chronic heat stress (THI = 85; 30 d) in feedlot lambs reduced average daily gain and loin eye area even when thermoneutral controls were pair-fed. Moreover, 9th-12th rib sections from heat-stressed lambs were smaller due to reduced muscle and fat components. Growth was also less efficient due to impaired metabolic homeostasis. Despite normal circulating insulin, heat-stressed lambs exhibited lower glucose and blood urea nitrogen but greater triglycerides and cholesterol. Muscle from heat-stressed lambs exhibited reduced insulin-stimulated glucose oxidation despite normal glucose uptake. These deficits collectively manifested in reduced feed efficiency. Factors mediating heat stress (beyond reduced feed intake) are not particularly well studied, but our studies show that elevated inflammatory activity appears to be a key contributor. Heat-stressed lambs exhibited greater circulating monocytes and granulocytes as well as TNFα. This may explain pathologies such as hoof wall overgrowth, adrenal and left ventricular hypertrophy, and reduced blood pressure and pulse rate in these lambs. Moreover, our recent preliminary evidence indicates that supplementing inflammatory agents lessens some impacts of heat stress. Fish oil, a source of ω-3 fatty acids, moderated heat stress-induced rises in leukocytes, improved average daily gain and feed efficiency, and partially recovered the lost fat fractions in several muscles. Serial dexamethasone injection moderated heat stress-induced hyperventilation and improved several indicators of metabolic function. Although β agonists induce inflammation in unstressed animals, supplementing ractopamine during chronic heat stress moderated the rise in TNFα, perhaps by desensitizing cytokine-producing tissues. This partially mitigated higher body temperatures and respiratory rates, left ventricle hypertrophy, and the loss of fat in the loin. These findings demonstrate that inflammation during chronic heat stress is a target for intervention strategies to overcome the impacts of heat stress on livestock.

S114 Managing heat stress in a warmer planet: Impact of increasing cutaneous heat loss. R. J. Collier*, *University of Idaho, Moscow, ID.*

A combination of genetics and good management has steadily increased milk production in the dairy industry. However, this increase in milk production also increased the amount of metabolic heat produced by dairy cows. Higher metabolic heat production requires additional cooling of cows during HS. Standard heat-abatement techniques rely on water and/or fans to either cool down the environment cows are housed in or to apply water to increase the rate of evaporation that typically occurs from the skin of a cow. These systems are operating at or near capacity. Future opportunities lie in the use of passive cooling techniques that require low energy input and increasing cutaneous heat loss (CHL) from cattle. In cattle, CHL is regulated by apocrine sweat glands. However, very little is known about the regulation of these glands. Successful isolation of apocrine sweat glands was accomplished through mechanical shearing of skin from cow udders, a 2-step collagenase digestion process, and use of neutral red dye, which is selectively taken up by these glands. The isolated glands were plated and growth of sweat gland apocrine cells was observed in 3 to 5 d on both plastic and collagen, utilizing keratinocyte media supplemented with bovine pituitary extract (bPE), epidermal growth factor (EGF), and fetal bovine serum (FBS). Two cell types were identified in culture. The first cell was identified as epithelium due to its morphological paved cobblestone appearance and positive expression of epithelial specific antigen. These cells were also positive for cytokeratin (CK)4, CK6, and CK18. The second cell type was positive for calponin and alpha smooth muscle actin (ACTA2), seen in myoepithelium, and fibroblast specific protein 1 (S100A4), specific for fibroblasts. Therefore, this cell type was identified as myofibroblasts and was also positive for CK4, CK6, and CK18. The third objective was to identify receptor populations by looking at the cDNA expression through conventional PCR of receptors in bovine whole skin, isolated apocrine sweat glands, and a mixed population of cultured apocrine sweat gland cells. For the serotonin (5-HT) system, we identified isoforms 5-HT 1A, 1B, 1D, 1F, 2B, 5A, 6, and 7 in whole skin, apocrine sweat glands, and cultured cells, while 5-HT 4B was in whole skin and apocrine sweat glands. For the prolactin (PRL) system, 2 isoforms were identified: PRL receptor long (PRLr-L) and PRL receptor short (PRLr-S). We found that PRLr-S was in whole skin, apocrine sweat glands, and cultured cells, while PRLr-L was only in whole skin. For the adrenergic (ADR) receptors, α1A, α1B, α2A, β1, and β2 ADR receptors were in whole skin, apocrine sweat glands, and cultured cells. Total growth hormone receptor (tGHr) was found in whole skin, apocrine sweat glands, and cultured cells. The presence of these receptors in the apocrine glands and cultured cells indicates that the 5-HT, PRL, GH, and ADR systems are involved in regulation of CHL in cattle.

Key Words: heat stress, cutaneous heat loss, sweat glands

S115 Heat abatement options for pastured dairy cattle. A. E. Stone*, *Mississippi State University, Starkville, MS.*

Heat stress is both a production and welfare issue for the dairy industry. As temperatures climb and an increase in food production is needed, alleviating heat stress on dairy farms is more important than ever. The dairy industry has made great progress in effectively cooling cows in confinement systems with sprinklers and fans via evaporative cooling. The southeastern United States is classified as humid and subtropical with high ambient temperatures, humidity, and rainfall. In regions with a subtropical climate, the high relative humidity limits the rate of heat loss via evaporative cooling. Producers in the southeastern region implement pasture-based systems more readily because of water and land availability along with a longer growing season. Pasture-based operations have fewer heat abatement options in general, but severely lack opportunities for forced evaporative cooling systems that could benefit them as much as they have benefitted confinement operations. Trees, permanent shade structures, and portable shade structures are available heat abatement options for pasture-based dairy producers, but all these systems have limitations that prevent both effective and consistent cooling. To apply the concept of evaporative cooling on pasture, the dairy team at Mississippi State University has created the "Mississippi Mister," a portable polyvinyl chloride pipe sprinkler system, to evaluate if the positive effects of evaporative cooling can be replicated outdoors. New systems, like this one, can be compared to current options through evaluating cow behavior, physiology, and production by using precision dairy monitoring technologies. The end goal is to develop heat abatement solutions that fit the Southeastern dairy producers' needs throughout the year but that could also be employed throughout the rest of the world as needed for shorter or less continuous durations. These systems need to be easy to use, sturdy enough to sustain bad weather and clumsy cows, and return on their investment. Ideally, systems could be incorporated into all life stages, from calf to lactating cow to dry cow. The industry needs innovative solutions to solve the heat stress problem on pasture-based operations in order to improve production and welfare.

S116 Managing heat stress in grazing dairy cows.

F. R. Dunshea*[1],[2] and S. S. Chauhan[1] [1]*Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC, Australia,* [2]*Faculty of Biological Sciences, The University of Leeds, Leeds, United Kingdom.*

Grazing lactating dairy cows are at a high risk of heat stress (HS) because they are outdoors for most of the day, have a high metabolic rate, and by necessity need to exercise to be milked and consume pasture. To reduce their heat load, dairy cows will decrease feed intake, milk production and activity as well as seek out shade or other means to physically cool down when the temperature-humidity index (THI) increases. Physiological responses to high THI include increased respiration and panting rate and diversion of blood from the viscera to the periphery to dissipate heat. If the nighttime THI decreases sufficiently, the cows can dissipate heat and reduce the impacts of high ambient temperatures. However, if the THI remains high at night, the heat load will accumulate and the cow may become heat stressed. For example, in a study conducted on a robotic pasture-based dairy during summer at THI of <72, 72–82, and >82 milk yields were 25.2, 24.1, and 22.0 kg/d ($P < 0.001$), respectively. Rectal temperature (67, 85, and 109 breaths/min, $P < 0.001$) and body temperature determined by infrared imagery (38.4, 39.4, and 41.5°C, $P < 0.001$) also increased with increasing THI. Shade seeking also increased with increasing THI. Strategies to reduce the effects of HS in grazing dairy cows include physical measures such as the provision of shade and cooling mechanisms such as cooling fans and misting in the milking parlor. A number of dietary strategies such as dietary betaine, chromium and antioxidant supplementation and providing slowly fermentable starch concentrates can partially mitigate against HS. In this paper, we will discuss some of these strategies.

Key Words: heat stress, nutritional intervention, physiological response

S117 Effect of in utero hyperthermia on postnatal sweating physiology.

J. Laporta*, *Department of Animal and Dairy Sciences, University of Wisconsin–Madison, Madison, WI.*

Skin glands are classified as either sebaceous or sweat glands. Sweat glands are the primary means for cutaneous evaporative heat loss in mammals, while sebaceous glands work in tandem with the sweat-producing glands to regulate body temperature. Sweating allows the body to control its internal temperature in response to thermal stress. The primary way humans stay cool during hot weather is by sweating and losing heat through the skin surface. Cattle sweat glands are not the most effective, and thus, their main way of losing heat is through their breath (i.e., panting). Our laboratory is currently investigating how skin characteristics (i.e., hair length, skin gland morphology, and function) might be altered by fetal exposure to intrauterine hyperthermia during late gestation, and how this might lead to long-term effects on the offspring thermoregulatory capacity later in life. We recorded thermal indices (weekly from birth to weaning) from heifers that were either in utero cooled (IUCL, n = 24) or in utero heat stressed (IUHT, n = 24) during the last 56 d of gestation. Hair samples and skin biopsies (neck and rump locations) were collected from a subset of IUCL (n = 8) and IUHT heifers (n = 8) at birth (d 0) and weaning (d 63). Heifers gestated under IUHT had reduced neck stratus corneous area and reduced sebaceous gland area at birth, while in the rump, IUHT heifers had smaller but more sebaceous glands. At weaning in the neck location, IUHT heifers had smaller and fewer sweat glands, fewer sebaceous glands, shorter short hairs, and longer long hairs in their coat. In the rump, IUHT heifers had longer sweat gland depth. The rectal temperature and sweating rate, in both the rump and neck locations, was higher for IUHT heifers when measured weekly from birth to weaning. When thermoregulatory responses were recorded every four hours over a 36-hour period one week before weaning, IUHT heifers had higher rump sweating rates in the early morning hours and during the evening hours relative to IUCL. In summary, intrauterine hyperthermia modifies hair length, skin gland morphology and possibly function, leading to different postnatal thermoregulatory capacity.

Animal Health Symposium: Adipose Tissue in Transition Dairy Cows as an Integrator of Metabolic and Inflammatory Cues in Health and Disease

S118 Adipose tissue insulin resistance: Homeorhetic adaptation or risk factor for periparturient disease. G. R. Opsomer*[1], J. De Koster[2], and O. B. Pascottini[1], [1]*Fac Vet Med, Ghent University, Merelbeke, Belgium,* [2]*Zoetis, Zaventem, Belgium.*

There is still a lot of debate about the role of insulin resistance in dairy cows. Especially in overconditioned cows, insulin resistance has been said to contribute to the fat cow syndrome. Accurately assessing insulin sensitivity remains a challenging task in pregnant and lactating cows, since in these animals, large amounts of glucose are sequestred in an insulin independent way. Using the hyperinsulinemic euglycemic clamp test, we showed that fat accumulation at the end of pregnancy, negatively influences insulin sensitivity and responsiveness of the glucose metabolism, while insulin action at the level of the NEFA metabolism is preserved. Overconditioned cows have larger adipocytes, which release more NEFA, both in the basal state as well as upon stimulation with catecholamines. The basal lipolytic activity of adipose tissue fragments is higher in larger adipocytes but not different for adipocytes originating from the omental versus subcutaneous depot. Due to the higher basal and stimulated lipolytic activity of their adipocytes, overconditioned cows are prone to excessive mobilization of body fat and elevated NEFA levels. The inhibitory effect of insulin on NEFA release remains preserved in overconditioned cows. In fat tissue of overconditioned cows before calving, we found a higher abundance of proinflammatory cytokines like leptin and IL6 and CD14. This is indicative for infiltration with proinflammatorily activated macrophages. Generally, the internal fat depots (mesenteric, omental, perirenal and intrapelvic) demonstrate a more proinflammatory expression profile compared with the subcutaneous fat depots. All together, this expression profile is indicative for a proinflammatory state of the adipose depots from overconditioned cows, which might dysregulate inflammatory processes and contribute to metabolic and infectious disorders in the perparturient period. In these overconditioned cows, we detected furthermore an accumulation of fat in the pancreas which was associated with a lower insulin release by the pancreas following a glucose load.

Key Words: adipose tissue, insulin resistance, periparturient homeorhesis

S119 Adipose tissue endocrinology in the periparturient period of dairy cows. H. Sauerwein*[1], S. Haeussler[1], H. Sadri[1,2], and M. H. Ghaffari[1], [1]*University of Bonn, Bonn, NRW, Germany,* [2]*University of Tabriz, Tabriz, Iran.*

The involvement of adipose tissue (AT) in metabolism is not limited to energy storage but turned out to be much more complex. We now know that besides lipid metabolism, AT is important in glucose homeostasis and amino acid metabolism, and also has a role in inflammatory processes. With the discovery of leptin in 1994, the concept of AT being able to secrete messenger molecules, collectively termed as adipokines, and acting in an endo-, para-, and autocrine manner emerged. Moreover, based on its asset of receptors, many stimuli from other tissues reaching AT via the bloodstream can also elicit distinct responses and thus integrate AT as a control element in the regulatory circuits of the whole body's functions. The protein secretome of human differentiated adipocytes was described to comprise more than 400 different proteins. However, in dairy cows the characterization of the physiological time course of adipokines in AT during the transition from pregnancy to lactation is largely limited to the mRNA level; for the protein level, the analytical methods are limited and available assays often lack sound validation. Besides proteinaceous adipokines, small compounds such as steroids can also be secreted from AT. Due to the lipophilic nature of steroids, they are stored in AT, but during the past years, AT became also known as being able to metabolize and even to generate steroid hormones de novo. In high-yielding dairy cows, AT is substantially mobilized due to increased energy requirements related to lactation. Whether the steroidogenic system in AT is affected and may change during the common loss of body fat is largely unknown. Moreover, most research about AT in transition dairy cows is based on subcutaneous AT, whereas other depots have scarcely been investigated. This contribution aims at reviewing the changes in adipokine mRNA and, where available, protein expression with time relative to calving in high-yielding dairy cows under different conditions, for example, parity, body condition, diet, specific feed supplements, and health disturbances, will provide an insight into steroidogenic pathways in dairy cows' AT, and will address differences between fat depots where possible.

Key Words: adipose tissue, steroids, adipokines

S120 A proteomics approach to unravel adipose tissue inflammatory responses in peripartum cows. M. Zachut*, *Agriculture Research Organization, Volcani Center, Rishon Lezion, Israel.*

Proteomic analysis explores the repertoire of proteins at a given state. In recent years, proteomics of subcutaneous adipose tissue (AT) revealed numerous inflammatory proteins in AT from peripartum (PP) dairy cows, highlighting the presence of complement and acute-phase proteins in AT. Bioinformatics analyses pointed to the key role of inflammatory pathways in AT of PP cows. Proteomics of AT from cows with a high degree of metabolic stress, represented by increased AT lipolysis postpartum, showed differential abundance of complement and acute-phase proteins in AT compared with cows with a low degree of metabolic stress. In cows that had an insulin-resistant (IR) AT, the top differential function was the inflammatory response; and inflammatory signals are known to induce IR. Hence, proteomics of AT demonstrate that metabolic stress and lipolysis enrich AT with inflammatory proteins, possibly contributing to subacute inflammation in PP cows. Proteomics of AT from heat-stressed late pregnant cows revealed enrichment of the Nrf2-mediated oxidative stress response and acute-phase response. PP cows suffer from oxidative stress related to AT lipolysis, and both oxidative stress and lipolysis affect inflammation. Therefore, increased oxidative stress in AT, associated with lipolysis and/or heat stress, could increase AT inflammation, as reflected by the AT proteome. In ketotic cows, proteomic analysis showed a downregulation of key innate immune response proteins in AT, suggesting that the health status affects the proteome and AT inflammation. Systemic treatment of postpartum cows with anti-inflammatory sodium salicylate unexpectedly enriched the AT proteome with inflammatory pathways of the complement system, cytokine signaling, and acute-phase response, perhaps due to immune cell recruitment. In conclusion, biotic and abiotic stressors, the health status and treatment with anti-inflammatory agents affect the abundance of inflammatory proteins in AT. Proteomics of AT improves our understanding of AT inflammation, and adds information on novel proteins in AT of PP cows.

Key Words: adipose, proteomics, peripartum

S121 Oxidative stress in the adipose tissue: Magnifying the risk for periparturient disease.

G. A. Contreras*, *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

During the periparturient period, intense lipolysis in adipose tissues (AT) mobilizes fatty acid (FA) reserves to meet dairy cows' high energy needs. At the same time, lipolysis generates reactive oxygen and nitrogen species (RM) by different mechanisms, such as the activation of FA mitochondrial oxidation, NADPH oxidases that produce superoxide and hydrogen peroxide, and AT macrophages' myeloperoxidases that release singlet oxygen. To neutralize RM, the AT activates antioxidant defenses, including the glutathione (GSH), superoxide dismutase, and the nuclear factor erythroid 2–related factor 2 (Nrf2) systems, and mitochondrial uncoupling. However, in cows with intense and protracted lipolysis during the first month of lactation, the production of RM rapidly depletes antioxidant systems and oxidative stress (OS) develops. The effects of OS in AT are harmful and include the activation of inflammatory responses and the inhibition of insulin signaling. RM damage cellular components of AT including phospholipid membranes and peptides, promote the activation of inflammatory pathways (e.g., NFκB), and enhance the expression of chemotactic cytokines that recruit macrophages. Relevant to the AT is that RM oxidize FA released from triglycerides during lipolysis producing oxylipins. Among these, oxidized linoleic acid metabolites such as 9- and 12-hydroxyloctadecadienoic acids (HODE) are abundantly produced during OS and induce a proinflammatory polarization of macrophages. By intensifying AT inflammation, OS impairs adipocytes' responses to insulin leading to a vicious circle where OS exacerbates AT lipolysis and inflammation, which in turn further promote OS. To prevent periparturient OS in AT, it is necessary to enhance the antioxidant defenses in AT, especially in cows that are more susceptible to excessive lipolysis such as those with high body condition score and during metabolic diseases such as clinical hyperketonemia and hypocalcemia. However, our current knowledge of AT antioxidant function and its responses to antioxidant supplementation is limited and should be the focus of future research.

Key Words: lipolysis, oxidative stress, adipose tissue macrophages

S122 Adipose tissue in transition dairy cows: What is our current understanding and how can we apply this to herd management?

S. Mann*, *Cornell University, College of Veterinary Medicine, Ithaca, NY.*

The biology of the transition cow is intricately linked with changes in internal and external adipose tissue depots. Historically these were viewed primarily as a readily available source of energy, and a storage compartment for energy surplus. The adipose tissue has emerged as an integrated, metabolically dynamic organ with endocrine and paracrine activity that assumes physiological functions beyond storage and delivery of energy. It produces adipokines and harbors immunologically active cells whose composition and inflammatory profile change throughout the transition period depending on disease state, energy availability, body condition, and the degree of oxidative stress. Adipose tissue may be a potential contributor to postpartum disease and changes in insulin signaling, particularly when its biology according to our current understanding appears to be dysregulated. However, it clearly has important and beneficial physiological functions and takes part in whole-body homeorhesis in the transition period. While efforts have concentrated on describing the detrimental effects of overconditioning on the risk of ketosis and negative health outcomes postpartum, underconditioning can also be detrimental to health and production outcomes. Cow health and management strategies to minimize adipose tissue pathology have focused on cow-level strategies rather than adipocentric treatment strategies. Maximizing dry matter intake early postpartum, providing cow comfort and a feeding program that aims to minimize changes in both fat deposition prepartum, and excessive mobilization postpartum through diet formulation and targeted supplementation are key factors to consider for cow-level management. Recording of body condition through lactation and transition periods can help identify opportunities for improvement of volatile or detrimental conditioning of cows in the stage of the production cycle where they arise. Identification and use of specific strategies to directly address adipose tissue pathology or alter its composition to improve health and productivity remains a current knowledge gap.

Key Words: adipose, management, transition cow

Breeding and Genetics Symposium: Genetics of Milk

S123 Genes that alter milk composition and their marketability. H. Bovehuis*, *Animal Breeding and Genomics Group, Wageningen University, Wageningen, the Netherlands.*

Bovine milk contains around 3 to 4% protein, which consists of 6 major milk proteins: α-lactalbumin, β-lactoglobulin, α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein. Together these milk proteins make up about 90% of the total milk protein content. The remaining 10% consists of minor proteins, like bovine serum albumin, γ-casein, immunoglobulins, lactoferrin and many proteins that appear in low concentrations. The high nutritional value of milk is partly due to the properties of its proteins. The caseins provide many essential amino acids, and are also carriers of phosphate and calcium. Whey proteins are rich sources of essential amino acids like methionine and cysteine and, therefore, have a high nutritional value. Interestingly, human milk does not contain β-lactoglobulin. Milk protein composition shows both quantitative as well as qualitative variation. Quantitative variation in milk protein composition is based on variation in absolute or relative amounts of the individual milk proteins. Qualitative (genetic) variation in milk protein composition is the result of different protein variants. Recent studies demonstrated that quantitative variation in milk protein composition is strongly affected by genetic factors. Heritability estimates for the 6 major milk proteins range from 0.25 to 0.80. In addition to protein or genetic variants, variation also occurs as a result from posttranslational modification, such as phosphorylation and glycosylation. Recently it was shown that these posttranslational processes are also strongly affected by genetic factors. Dairy products like cheese, milk formula and milk powder will benefit from a product-specific milk protein composition. There is substantial genetic variation in milk protein composition, and, different protein variants as well as genomic regions that contribute to this genetic variation have been identified. The genetic variation in milk protein composition can be exploited by means of selection. This selection can be either based on phenotypic information or on genotypic information. Given that milk protein composition is strongly affected by a small number of genes with very large effects, selection for known protein variants, using polymorphisms in known genes, might be a good selection strategy.

S124 Genomic testing for milk composition: Past, present, future. L. Mastro*, R. Kephart, R. Ferretti, and R. Tait, *Neogen GeneSeek Operations, Lincoln, NE.*

Dairy producers often identify an opportunity to differentiate their milk product based on the interests of the consumer market and commercial dairy milk processors. Fortunately, the genotype of the cow has a direct effect on the attributes of the dairy products produced. Specifically, producers are interested in genomic testing to determine milk protein composition traits such as α-casein, β-casein, κ-casein, and β-lactoglobulin, each of which plays a different role in metabolism and protein synthesis in the human body. Genotype results for milk protein traits are also of interest to milk processors due to the impact on coagulation and renneting efficiencies as well as overall marketability. Research on milk protein allele frequency by dairy breed has demonstrated variation exists based on population studied. This knowledge gain has led to increased interest in milk protein genetic status of individual selection candidates. Further, selection of sires with preferable β-casein and κ-casein genotypes has increased desirable allele frequencies in recent years. Of the milk protein composition traits, the one most commonly subjected to selection is β-casein due to the perceived consumer health benefits. Consumer brands have entered the market with a variety of dairy products selected for the A2 variant family group of the β-casein protein. Within the β-casein family groups, subvariants exist; however, little is known about the resulting milk properties of some of the subvariants present. As more is learned about the quality attributes of these additional variants, selection or management strategies will likely evolve as well. Overall, milk protein composition traits will continue to be subject to genomic testing by dairy producers due to their importance to the future of milk marketing.

Key Words: milk proteins, genomic testing, dairy cattle

S125 The effects of the rumen microbiome on milk fat composition. D. Bickhart*, *USDA ARS Dairy Forage Research Center, Madison, WI.*

The fat content of milk represents a composite of many different types and lengths of fatty acids that can vary in abundance depending on several different variables. Many of these fatty acids provide human health benefits; however, unsaturated fatty acid species can promote cardiovascular diseases if not consumed in moderation. It is therefore increasingly important to not only identify the factors that promote the production of milk fat in general, but also to identify factors that influence the content of fatty acids produced by individual cows. For example, linoleic acid is a polyunsaturated omega-3 fatty acid found in dairy feed that can provide human health benefits. Through rumen microbial activity, 70–95% of the consumed linoleic acid is biohydrogenated to either healthy or less healthy intermediates depending on the concentration of specific rumen microbial populations in the rumen. The picture is further complicated by the discovery of host-genetic effects on fatty acid content in milk, with the abundance of specific species having moderate to high heritabilities (0.23–0.47). New models that incorporate both microbial and host genetic effects on milk production are therefore necessary to predict which cows are likely to produce healthier fatty acid profiles. Recent innovations in the assessment of rumen microbial contents of cattle through buccal swabs could provide useful input to these models. If used in tandem with existing host genotyping tools, rumen microbial profiles could provide a new means of improving the quality and profitability of milk through selection and benefit producers interested in selling high-quality milk products.

Key Words: milk fat, microbiome, genomic selection

S126 No abstract provided

S127 Dairy processors view on milk marketing and the rise of plant-based protein products. C. J. Hollander*, *Danone, Amsterdam, the Netherlands.*

Danone is a leading global food and beverages company with a unique health-focused portfolio of iconic global and unique local brands across the world. Our vision — Danone, One Planet. One Health — reflects a strong belief that the health of people and the health of the planet are interconnected. This vision is a sign of our commitment to embrace the food revolution, as a new generation increasingly challenges the mainstream food system, while asking for a fresh approach. With consumers demanding more quality and naturality through organic, GMO-free or from grass-fed cows. For this we rely on farming and we want to support and lead the adoption of regenerative agriculture models. As agriculture is part of the solution and not the problem. This strong relationship we have with our milk suppliers is key. Sourcing milk from family farms, with some in a long-term milk supply contract where the farmer gets paid for the milk based on the cost of production with a secured margin, is an important lever. This motivates farmer to find more efficiency improvements and that benefits the whole value chain. To support farmers further in this journey a new strong collaboration, called Farming for Generations, between 8 global agricultural sector leaders started in 2019. Over the next 3 years, farmers will be involved each step of the way, ensuring that practices are fit for purpose in terms of economic viability and environmental sustainability and suited to local customs and geographic specificities. With farmers at the heart of the project's governance, Farming for Generations aims to make a difference by proposing new solutions but also by identifying the ways they can be implemented on the ground to deliver real impact at scale. Farming for Generations will focus on improving the impact of dairy farming across 3 areas: 1. Protecting Soil, Water and Biodiversity, 2. Respecting Animal Welfare and 3. empowering generations of farmers. In this presentation some examples of where the genetics of the dairy cow plays a role in providing a healthy dairy product to consumers, sustainable margin for the farmer and ensuring the right cow in the right place.

Dairy Foods Symposium: Reducing Dairy Food Loss and Waste

S128 The global impact of food loss and waste. J. C. Buzby*, *United States Department of Agriculture, Washington, DC.*

The Food and Agriculture Organization (FAO) of the United Nations (UN) estimates that one-third of all food, globally, is lost or wasted each year. When food is lost or wasted, so too are opportunities for improved food security, economic growth, and environmental prosperity. Wholesome food that is currently wasted could help feed families in need. An estimated 820 million people are hungry at some point during the year, and the world population is expected to grow by around 2 billion people by 2050. If food is landfilled and rots, it creates methane gas, which is a powerful greenhouse gas. Reducing food waste can save or make money because food waste means that all the land, water, labor, energy, and other inputs that are used in producing, processing, transporting, preparing, storing, and disposing of the discarded food are also wasted.

S129 Controlling spoilage to reduce food loss and waste in dairy foods. N. Martin*, *Cornell University, Ithaca, NY.*

Premature spoilage of dairy products, in particular fluid milk, is a major contributor to dairy food waste. Microbial spoilage of fluid milk typically occurs by either gram-negative bacteria introduced into the product after pasteurization (i.e., postpasteurization contamination; PPC) or by psychrotolerant gram-positive spore-forming bacteria (PASB) that enter the fluid milk continuum at the farm, survive pasteurization and then subsequently grow to spoilage levels during refrigerated storage. Studies show that PPC and PASB each account for approximately 50% of fluid milk spoilage. Reducing premature fluid milk spoilage by these different groups of organisms, and ultimately reduce the food waste because of this spoilage, requires a comprehensive approach at both the farm and processing levels of the fluid milk continuum. Specifically, studies have shown that controlling the entry of spoilage PASB at the farm is achievable through milking parlor management practices and employee training. Furthermore, PPC in the fluid milk processing facility often occurs sporadically and in low levels and is consequently a challenge to detect quickly to mitigate the contamination. Reducing premature spoilage by PPC requires that the dairy industry adopt testing methods that allow for early detection. Ultimately, implementing strategies to reduce premature dairy product spoilage will reduce dairy food waste.

Key Words: premature spoilage, postpasteurization contamination, sporeformers

S130 The role of consumers in dairy food waste. C. Campbell* and G. Henningsen, *Iowa State University, Ames, IA.*

An estimated 40% of food produced in the United States is wasted, which poses a significant barrier to achieving a sustainable future. So much so, that the United Nations Sustainable Development Goal #12, to "ensure sustainable consumption and production patterns," includes a goal to "halve per capita global food waste at the retail and consumer level, and reduce food losses along the production and supply chains by 2030." Annually, consumers waste approximately 90 billion pounds of food, equating to roughly 1 pound per person per day. More specifically, consumer waste is the largest contributor to the food waste problem when compared with other steps along the supply chain, such as production, postharvest handling and storage, processing, and distribution. Furthermore, American families toss out about 25% of the food and beverages they buy. When considering the type of waste coming from households, fresh fruits and vegetables rank highest at 22%, with dairy products, at 19%, following in close second. A variety of factors contribute to why consumers waste so much food. For dairy, commonly referenced reasons are related to the misunderstanding of date labels, poor planning of purchases, spoilage before consuming products, and improper storage. This wasted food accumulates in landfills and produces methane when decomposing, resulting in severe environmental consequences related to ozone depletion and climate change. Even milk can have negative environmental consequences when disposed of down the drain. Another important environmental consideration is the role of packaging. Food packaging greatly extends the shelf life of products thereby reducing food waste. However, consideration of the implications of the packaging waste created from the consumption of dairy products should also be considered. This presentation will provide background knowledge on consumer-related food waste with a special focus on waste of dairy products. In addition, we will discuss the highest referenced reasons for this waste, potential strategies for reduction, as well as the role packaging plays in the conversation.

Key Words: consumer, dairy, food waste

S131 Novel solutions for valorization of dairy waste. J. L. Guzman*, *Capro-X, Ithaca, NY.*

Capro-X is an ag-tech spin-out from Cornell University focused on bringing greater sustainability to the dairy industry by upcycling wastes and by-products into useful green chemicals. Capro-X's "WheyAway" uses a fermentation platform employing natural, non-GMO microbes to treat and upcycle problematic wastes and by-products from dairy product processing into treated water and green chemicals. Today, the company is evaluating the WheyAway technology at the pilot stage at a Greek yogurt plant near Capro-X's headquarters in Ithaca, NY, and scaling to a demonstration system in the coming year with assistance from the National Science Foundation and Environmental Protection Agency. The green chemicals produced by the WheyAway are typically sourced from unsustainable palm – Capro-X plans to provide sustainable alternatives to the flavor/fragrance industry in the next year, and to begin evaluating their green chemicals as healthful feed additives for livestock, and ultimately biofuels. I will provide an overview of Capro-X's WheyAway fermentation process and chemicals produced; outline opportunities at various scales where I see industrial biotechnology benefiting the dairy industry the most by valorizing low-value wastes and by-products to increase the sustainability, economics, and consumer appeal for dairy production and processing; and provide insights for fellow entrepreneurs considering founding their own startups.

Growth and Development and Midwest Branch Joint Symposium: Feeding and Managing the Preruminant Calf

S132 Nutritional regulation of growth and metabolic development in calves: Colostrum and milk. A. C. Welboren[1], K. S. Hare[1], J. N. Wilms,[1,2], L. N. Leal,[2] and M. A. Steele*[1] [1]*Department of Animal Biosciences, Animal Science and Nutrition, University of Guelph, Guelph, ON, Canada,* [2]*Trouw Nutrition Research and Development, Amersfoort, the Netherlands.*

Over the past decade, there has been great progress in our understanding of neonatal and preweaning nutritional programming and their effects on dairy calf growth, metabolism, and future productivity. The quality and quantity of the first meal of colostrum has been shown to have a critical impact on the growth, health and development of calves. Although the primary focus of colostrum research has been on the passive transfer of immunoglobulins, colostrum contains a myriad of other bioactives that can influence growth, metabolism, and gut development. Pinpointing the causes of variation in these colostral bioactives and their biological roles is an exciting area of research that requires attention. Another area with great potential is gradually transitioning from colostrum to milk to confer growth and health benefits during the preweaning period. The findings from recent studies call into question our current recommendations for feeding calves during the neonatal period and highlight the potential of phase feeding programs for the dairy industry. With respect to the preweaning period, the long-term consequences of preweaning nutrition and growth rates on lifetime productivity has been well documented, yet the biological mechanisms governing these responses are not understood. Since half of dairy calves rely on milk replacer for nutrition, recent research has started to evaluate milk replacer formulations, which have evolved to be strikingly different in macro and micronutrients compared with whole milk. New studies have shown that altering the carbohydrate to fat ratio, as well as the carbohydrate and fatty acid profiles, impacts growth and metabolism during the preweaning period. Recent research focusing on total preweaning ME and MP intake, primarily from milk, has been shown to program dairy calves and impact lifetime metabolism and performance. The long-term consequences of these alterations however are unclear. In summary, there is great potential to explore the long-term consequences of neonatal and preweaning nutritional programs; yet a more intensive and rigorous effort is necessary.

S133 Low rumen pH in calves: Problem or opportunity? B. C. Agustinho[1], and A. H. Laarman*[2], [1]*Department of Animal, Veterinary, and Food Sciences, University of Idaho, Moscow ID,* [2]*Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton AB, Canada.*

Historically, feeding calf starter was seen as the primary nutritional goal of pre-weaning calf nutrition. Due to ample evidence that butyrate, produced via fermentation of calf starter, promotes rumen papillae development, maximizing calf starter intake became a primary goal of young calf development. As high intakes of rapidly fermentable calf starter create acidotic pressure in the rumen, concerns arose regarding the incidence and harm of subacute ruminal acidosis in calves. In cows, subacute ruminal acidosis is well-documented history of adversely affecting fiber digestion, rumen health, barrier function, and milk production. Consequently, occurrence and mitigation of subacute ruminal acidosis became an additional focal point in calf nutrition management. In the last ten years, the advent of continuous rumen pH recorders demonstrated that low rumen pH in calves occurred much more frequently and dramatically than previously thought, with animal-to-animal variation spanning physiological, subacute, and acute ruminal acidosis ranges. Despite these low rumen pH occurrences, calves exhibited no clinical signs nor compromised growth, suggesting rumen pH does not affect calves in the same way it does cows. Recently, more studies demonstrated that feeding calf starter, while promoting morphological development, fails to increase nutrient absorption capacity. Instead, changes in cellular physiology suggest an adaptive response to promote cellular remodeling, especially during the weaning transition. What triggers the adaptive response towards epithelial remodeling is unclear. Research on different weaning strategies may contribute to our understanding of which weaning signals may contribute to epithelial adaptation. What is needed now is to shift the rumen development paradigm from morphological changes to physiological changes so that more precise ruminal development targets can be developed.

Key Words: ruminal acidosis, rumen development, epithelial remodeling

S134 Preweaned calf rearing options for dairy producers. B. J. Heins*, M. I. Endres, and K. T. Sharpe *University of Minnesota, West Central Research and Outreach Center, Morris, MN.*

Dairy replacement feeding and management systems have undergone major evolution in the last 25 to 30 years. As herd sizes increased, individual hutches were introduced to protect calves from contaminated and overcrowded environments. Higher levels of milk feeding are recommended to promote early growth, and now some farmers are adopting extended suckling until calves are weaned. Recently, pair-raised calves have been shown to improve growth and animal health, as well as improved consumer perception of dairy calf housing. Group calf rearing offers opportunities to reduce labor and to aid in socializing calves, but performance of group-managed calves in enlarged hutches is not well documented in dairy production systems. Consumers of dairy products are interested in management practices related to raising dairy calves on cows, but research-based studies on this topic are lacking. The maintenance of health and growth of dairy calves is very important in their first few months of life. Successful group feeding of dairy calves is enhanced with aggressive suckling during infancy and early consumption of high-quality calf starter. An ongoing research study at the University of Minnesota West Central Research and Outreach Center (Morris, MN) dairy will evaluate the growth, health and, most importantly, the economic performance of dairy calves in individual housing, group housing, pair housing, and raising calves with cows on pasture in the context of calf health, behavior and welfare. Future studies will evaluate the economic impacts, as well as production effects of these calves when they come into milk. The results will help improve the long-term profitability and social sustainability of the dairy industry by improving its public image.

Key Words: calves, calf rearing, group rearing

Milk Protein and Enzymes Symposium: Opportunities to Create New Products with Mixed Dairy and Plant Proteins

S135 Sensory properties of plant and dairy foods: Opportunities? M. A. Drake*, M. D. Yates, H. R. M. Keefer, and C. M. Racette, *North Carolina State University, Raleigh, NC.*

Fluid milk consumption has steadily declined in the United States since the 1970s while sales of plant-based milk alternatives continue to increase. Similarly, positive messaging for plant proteins and other plant-based dairy alternatives (PBDAs) continue to influence consumers. The sensory and functional properties of plant proteins and PBDAs are quite distinct from dairy proteins and dairy foods. Consumers desire foods that are healthy, natural and sustainable. The sensory properties of plant and dairy foods, consumer perception of these products and possible opportunities for synergy will be discussed.

Key Words: dairy foods, plant proteins, consumers

S136 Processing dairy proteins with plant proteins: Challenges and opportunities. M. Corredig*, *Aarhus University, Aarhus N, Denmark.*

The current climate crisis requires a dietary shift to more sustainable sources of proteins. The use of blends of dairy and alternative proteins will enable a rapid transition by providing new formulations containing nontraditional ingredients and with properties close to current consumers´ expectations. Dairy proteins are well known not only because of their nutritional value, but also their processing functionality. On the other hand, the utilization of alternative proteins, i.e., plant protein ingredients, present challenges, in relation to their functionality. They often do not measure up when utilized in conventional processes. However, their behavior in the presence of non-dairy proteins is grossly unknown. For example, soy and pea concentrates and isolates have been studied in mixes with caseins and whey proteins, but these studies are just at their infancy. The emulsifying and gelation behavior of these plant proteins differ substantially from those of dairy proteins, leading to tremendous opportunities to control the dynamics of their molecular and supramolecular interactions. Heating can induce gel formation, or facilitate protein dissociation, providing aggregates that will then gel with cooling. Specific enzymatic treatments can induce precise, targeted gelation of the individual components in a mix. In multiphase systems containing oil droplets, the interactions between the proteins adsorbed at the interface and those present in the continous phase will also play an important role in the structure of the final gel. Finally, the level of refinement and processing history of the proteins will have to be well defined. A better knowledge of the processing dynamics will provide inspiration for formulations with functionalities accepted by the consumer and with a well-balanced nutritional composition.

Key Words: proteins, interfaces, gelation

S137 The power of fermentation: Modulating taste and texture in plant bases with novel culture solutions. M. Nygaard*, *Chr. Hansen, Hoersholm, Denmark.*

While animal milk is naturally rich in nutrients that support the growth of microbial cultures, plant bases are often lacking key components resulting in a higher complexity in processing and sensory outcome, compared with dairy yogurt. In this study specific lactic acid strains were selected which can help improve flavor by partially masking the vegetal off-flavors often associated with plant-based products. Furthermore, strains with a high capacity for producing extracellular polysaccharides (EPS), which forms a unique network structure when combined with protein, were selected to increase the viscosity, mouthfeel and creaminess of the dairy alternatives. Results obtained across a variety of plant bases demonstrate the ability of the new innovative starter cultures to positively contribute to the flavor and texture during fermentation, providing manufacturers with a tool to help improve the sensory quality of dairy alternatives.

Key Words: plant-based, sensory, flavor

S138 Could dairy factories pivot to plant proteins? S. Loveday*, *Smart Foods Innovation Centre of Excellence, AgResearch New Zealand Ltd., Auckland, New Zealand.*

There is increasing consumer demand for plant-based foods that are produced using protein concentrate and isolate ingredients. In the New Zealand context, milk production is linked to pasture growth, and thus highly seasonal. During the winter months, dairy factories process milk volumes well below their capacity, and some commentators have suggested that dairy factories could process plant proteins. This presentation explores the potential for dairy processors to pivot existing dairy processing infrastructure toward processing plant material for protein extraction, and discusses the opportunities and pitfalls that pivoting would present.

Animal Health and National Mastitis Council Joint Symposium: Management Strategies to Enhance Health of Dairy Cows During the Transition Period

S139 Variation in estimation methods and economic impacts of transition cow diseases. F. Ferreira*, *Veterinary Medicine Teaching and Research Center, Department of Population Health and Reproduction, School of Veterinary Medicine, University of California–Davis, Tulare, CA.*

There is a wealth of literature measuring the economic impacts of transition diseases in dairy production systems. There is no doubt about their negative economic impact, however, different methods and inputs used may lead to large variations in the final costs of the diseases. Ultimately, economic evaluations serve to compare alternative courses of action in terms of their costs and consequences. The purpose of this review was to discuss the variation in methods used in economic studies of transition cow diseases and their economic estimations. A total of 21 papers reporting the economics of transition cow diseases in the US, Canada, and the Netherlands were reviewed. Most papers estimated the total burden of disease, followed by cost-effectiveness analysis of prevention strategies. Methods used included partial budget analysis, dynamic programming, simulation models, and meta-analysis. Costs of diseases were highly variable. In 2021 US dollars, reported costs per case of mastitis varied from $88 to $542, and $91 to $298 per lactating cow per year. For retained fetal membranes and metritis, estimated costs varied from $188 to $884/case. A case of hyperketonemia and its associated costs due to greater risk of displaced abomasum, metritis, culling, and other diseases was reported to be $426 (primiparous) and $291 (multiparous). A case of digital dermatitis varied from $67 to $163, and a case of sole ulcer varied from $187 to $267, with the overall cost of lameness varying from $199 to $366/case. The variation observed in these values may be explained by differences in the methods used, inputs chosen and included in the analysis, market conditions, and assumptions regarding losses considered and incidence of the disease. This highlights the need for specific farm economic evaluations, as they vary according to herd- and cow-specific factors. Economic studies focusing on the cost-effectiveness of preventive practices are needed because there is a substitution relationship between failure costs and preventive costs (Hogeveen and Van Der Voort, 2017). Last, the reporting of transition cow diseases economics may benefit from clear guidelines to improve transparency, completeness, and facilitate the interpretation of study findings.

S140 Using automated health monitoring systems to improve health monitoring and management of transition dairy cows J. Giordano*, M. M. Perez, C. Rial, M. L. Stangaferro, and A. L. Laplacette, *Department of Animal Science, Cornell University, Ithaca, NY.*

Monitoring transition cows through sensor-generated behavioral, physiological, and performance parameters incorporated into automated health monitoring systems (AHMS) could help improve herd health through non-invasive, timely, and accurate detection of cows with health disorders (HD). Such benefits of AHMS depend upon detection of specific alterations to normal patterns of sensor-monitored parameters in cows suffering HD. In this regard, multiple recent studies demonstrated shifts in the trajectory of sensor-monitored parameters days before and after diagnosis of clinical HD. Moreover, disease-specific signature patterns for several sensor-parameters were identified consistent with the biological alterations caused by different HD. The predictive value of these sensor-parameter signature patterns may increase if sensor data is coupled with other non-sensor data associated with cow health outcomes. Thus, recent efforts focused on the exploration of different methods to integrate automatically and in real time large streams of cow, herd management, and environmental sensor and non-sensor data from multiple sources. Promising results have been observed when these multidimensional data streams were used to predict cow health status using advanced data analytics such as machine learning. From a herd management perspective, AHMS might help reduce cow manipulation and interventions in early lactation while reducing labor needs. Recent research from our group and others demonstrated that health monitoring programs based primarily on, or with the assistance of data from AHMS were comparable to traditional health monitoring programs based on compulsory clinical examination. Importantly, programs based primarily on AHMS did not negatively affect milk yield, reproductive outcomes, and herd exit while reducing the number of interventions with cows. Thus, the use of AHMS for transition cow monitoring and management is promising and likely to become more widespread as our understanding of sensor-monitored parameters improves, novel approaches for data integration and analytics are developed, and the value of these technologies for herd management is demonstrated.

Key Words: automation and technology, fresh cow, disease

S141 Optimizing transition cow success in robotically milked dairy facilities. B. Treichler*, *Select Milk Producers, Oneida, WI.*

The "transition period" refers to the time frame in a cow's life that includes the end of lactation, cessation of milking, far-off dry period, close-up period, colostrogenesis, calving and maternity, colostrum harvest, initiation of lactation, and the immediate postpartum period. Dairy scientists and veterinarians have long been focused on the transition period, because it represents a time of massive metabolic shifts and physiologic changes for the cows. No matter the style of dairying, or region of the world where a dairy is located, the cows' ability to navigate the metabolic changes and disease challenges associated with this transition period set the foundation for the future performance of the individual cows and the dairy. The unique nature of how dairies utilizing robotic milk systems (RMS) are designed, laid out, and managed, creates specific challenges and opportunities for those cows and their managers to minimize transition stress. This session will look at what high performing RMS dairies and their management teams are doing to improve their cow's ability to navigate the transition period, as well as where they face challenges that many RMS dairies struggle to overcome. We will examine specific opportunities and challenges related to the following:

- What tools exist for RMS dairies to manage the end of lactation to better prepare the cow for cessation of lactation?
- What are some considerations when thinking about procedures and strategies for "drying-off" cows in RMS facilities?
- What are the key concepts around managing close-up and maternity animals in RMS facilities?
- What factors drive decisions around colostrum harvest and the milking of fresh animals?• How should we approach training fresh cows to

the RMS, and which facilities considerations lend themselves to better milking attributes of fresh cows and heifers?

- How should RMS dairies evaluate the opportunities and risks associated with grouping strategies?
- What advantages does increased data provide RMS dairies and how can they couple data streams and dashboards with boots on the ground strategies for early disease detection, diagnosis, and management?

S142 Opportunities to improve resilience and health traits in dairy breeding programs.

H. Mulder*[1], M. Poppe[1], M. van Pelt[2], and R. Veerkamp[1], [1]*Wageningen University and Research Animal Breeding and Genomics, Wageningen, the Netherlands,* [2]*Cooperation CRV, Animal Evaluation Unit, Arnhem, the Netherlands.*

Worldwide, dairy farms are rapidly growing in number of cows. Therefore, there is an increasing demand for high-producing cows that are healthy and easy to manage. The number of breeding values for health, reproduction and longevity traits is growing and these breeding values are successfully used in breeding programs. These breeding values do not directly capture resilience. Resilience is the capacity to cope with environmental disturbances such as pathogens or heat waves. Longitudinal data such as daily milk yield records, offer opportunities to develop resilience indicators for breeding. The aims of this presentation are to show how resilience indicators can be defined based on daily milk yield records, their genetic parameters and how they can be used in breeding programs. Daily milk yield records of automatic milking systems from ~200,000 cows in the Netherlands were used. Lactation curves were fitted per cow to obtain daily deviations from the curve. The log variance (LNVAR) and the lag-one autocorrelation (RAUTO) of these deviations were used as resilience indicators. Both resilience indicators showed heritable variation and their heritabilities were 0.18–0.2 for LNVAR and 0.06–0.08 for RAUTO in lactations 1–3. Lower LNVAR was genetically associated with better udder health, better longevity, less ketosis, better fertility, higher body condition score and higher dry matter intake at the same level of milk yield. For RAUTO, most genetic correlations with health and longevity traits were favorable but closer to zero. In a validation study, low LNVAR was associated with low milk loss during heat waves or herd perturbations, while low RAUTO was associated with fast recovery and reduced perturbation length. Therefore, it is recommended using LNVAR and RAUTO in a resilience index. Breeding on such a resilience index will not only decrease disease incidence and improve recovery from diseases and environmental disturbances, but also improve longevity and fertility of future generations of cows. Big data from sensors is expected to offer further opportunities to increase resilience by breeding.

Key Words: resilience, health, longitudinal data

S143 Negatively controlled trial investigating the effect of dry cow therapy on clinical mastitis and culling.

S. Rowe*[1,2], M. Dziuba[3], B. Boyum[4], S. Godden[2], E. Royster[2], and L. Caixeta[2], [1]*University of Sydney, Camden, NSW, Australia,* [2]*University of Minnesota, St Paul, MN,* [3]*Michigan State University, East Lansing, MI,* [4]*Riverview LLP, Morris, MN.*

We hypothesized that dry cow therapy (DCT) was not necessary in well managed herds, such that the use of an internal teat sealant (ITS) alone would be sufficient to maintain cow health and welfare after dry-off. Therefore, the objective of this negatively controlled clinical trial was to determine the effect of DCT on clinical mastitis and removal from the herd during the dry period and the first 90d of the subsequent lactation. The study was conducted in a commercial dairy herd in South Dakota during June 2020 to January 2021. Dry-off sessions (n = 43) over a 2-mo period were scheduled in advance such that all cows at a given session were dried off either using an internal teat sealant alone (ITS-only, n = 1,108 cows) or a commercial intramammary DCT product containing cloxacillin 500 mg, followed by ITS (ABX+ITS, n = 1,331 cows). Dry-off procedures were conducted by farm workers, with the same staff administering treatments to both treatment groups. Outcomes of interest were clinical mastitis (CM) during 1–90 d in milk (DIM) and removal from the herd between dry-off and 90 DIM. Farm personnel were blinded to the treatment group when collecting outcome data. Hazard ratios (HR) for the effect of the treatment group on these outcomes were estimated using Cox proportional hazards, adjusting for the clustered treatment allocation strategy. Parity, previous clinical mastitis history and dry period length were offered to the models as covariates. Risk of CM during 1–90 DIM was lower in ITS+ABX cows (5.9%), when compared with ITS-only cows (10.8%, HR = 0.53, 95%CI: 0.37–0.76). Risk of removal from the herd during the dry period was slightly lower in ITS+ABX cows (1.1 vs 2.7%, HR = 0.44, 95%CI: 0.23–0.82). Risk of removal from the herd during 1–90 DIM was similar in ITS+ABX (11.1%) and ITS-only cows (11.5%, HR = 1.06, 95%CI: 0.85–1.32). The beneficial effects of DCT were consistently observed across strata of parity, previous clinical mastitis history and dry period length. These findings demonstrate that the cessation of DCT can be detrimental to cow health and welfare.

Key Words: dry cow therapy, mastitis, antimicrobial use

S144 Feeding prepartum cows for better health during transition into lactation.

A. Vieira-Neto*[1] and J. E. P. Santos[2], [1]*Kansas State University, Manhattan, KS,* [2]*University of Florida, Gainesville, FL.*

Nutritional strategies to improve metabolism and health during the transition period have been extensively researched. Acidogenic diets have been fed for over 50 years with the aim to induce a state of metabolic acidosis that improves Ca homeostasis. A meta-analysis reported that reducing the DCAD of diets fed prepartum not only increased postpartum Ca concentrations, but also reduced the incidence of diseases which likely explains the greater dry matter intake and milk yield observed for those cows and, therefore, suggesting that the benefits of feeding acidogenic diets go beyond Ca homeostasis. Concerns have been raised about the advantage of feeding acidogenic diet to nulliparous cows, as they are less likely to develop hypocalcemia. Two recent experiments reported neither benefits nor detrimental effects when feeding acidogenic diets to nulliparous cows. In addition, extended exposure to acidogenic diets during the prepartum, 42 d, compared with 21 d, reduced milk yield and reproductive performance. Although 3 d of feeding acidogenic diets was sufficient to elicit a greater tissue responsiveness to parathyroid hormone and improve Ca homeostasis, the recommendation to feed such diets to prepartum cows remains at 21 d because of the large gestation length variability. Feeding diets that induce a severe metabolic acidosis is not recommended because it can interfere with energy metabolism, thus the suggested target DCAD ranges between −50 and −100 mEq/kg. Dairy cows are prone to hepatic steatosis and supplementation of diets with rumen-protected choline has been shown to reduce hepatic triacylglycerol concentrations in feed-restricted cows, presumably by enhancing hepatic lipoprotein secretion. Supplementation with choline during the transition period has been shown to increase yield of energy-corrected milk irrespective of reduction in fatty liver. Furthermore, recent data demonstrated that benefits to supplemental choline during the transition period is observed regardless of body condition score of cows. In summary, nutritional strategies to improve mineral and energy metabolism are well documented and effective to mitigate economical losses during the transition period in dairy cows.

Key Words: hypocalcemia, choline, transition period

S145 Feeding the fresh group for better health and lactation performance. T. Overton*, *Department of Animal Science, Cornell University, Ithaca, NY.*

Specific diets have been fed to fresh cows on dairy farms for many years, although in general these diets have been based largely upon the high lactating diet with a few modifications (e.g., slightly greater fiber content, slightly lower starch content, and inclusion of specific nutrients or additives demonstrated to improve health and performance). Furthermore, until recently there were very few controlled experiments focused specifically on nutrition of the early postpartum dairy cow; therefore, success or failure of specific postpartum nutritional strategies was largely determined anecdotally. Research conducted over the past few years has focused largely on carbohydrate nutrition [primarily starch and neutral detergent fiber (NDF)], protein and amino acid nutrition, specific fatty acids with roles in immune function and inflammation, and specific nutrients or additives. The level and fermentability of dietary starch fed during the immediate postpartum period has been a controversial topic, although most research supports feeding moderately higher levels of starch. One provision to this recommendation may be to ensure that sufficient physically effective NDF accompanies the higher levels of starch to decrease risk for subclinical ruminal acidosis during the postpartum period. Recent research supports prior experiments demonstrating proof of concept that metabolizable protein supplied substantially above that provided by diets typically fed to lactating cows improves cow performance in early lactation, particularly when the additional rumen-undegradable protein supplied is balanced for amino acids. Several studies support the concept that specific long-chain fatty acids may attenuate immune function and inflammation during the postpartum period. Finally, research supports inclusion of certain nutrients (e.g., choline, methionine, chromium, biotin, improved trace mineral sources) and feed additives (e.g., monensin, yeast and yeast culture) in diets for fresh cows; although the vast majority of studies conducted with these nutrients and additives included them in diets fed during both the prepartum and postpartum periods.

Key Words: transition cow, carbohydrate, protein

S146 Treatment and culling decisions for mastitis in the first 30 days postpartum. P. Ruegg*, *College of Veterinary Medicine, Michigan State University, East Lansing MI.*

Despite impressive improvements in bulk tank somatic cell counts, mastitis continues to be the most significant cause of morbidity, mortality and premature culling of dairy cows and its occurrence results in decreased profitability and frequent use of antimicrobials. Mastitis occurs when bacterial challenge exceeds both the anatomical defenses of the teat and the immunological defenses of the mammary gland and an active infection is established. Due to anatomical changes and immune suppression, both defense mechanisms are least effective in the immediate postpartum period. Thus, prevention and management of mastitis that occurs in early lactation are critical for maintaining udder health and herd profitability. Occurrence of mastitis in the first month postpartum is often a result of exposures that occurred during the preceding dry or transition periods. Subclinical mastitis in early lactation can be a result of cows maintaining chronic infections caused by contagious pathogens that persisted across the dry period. These cows are at high risk for new clinical cases, have low expectations for spontaneous or treatment cures and often serve as a reservoir for transmission of infections to other cows. In contrast, many cases of clinical mastitis that occur during this period are a result of new infections caused by environmental pathogens. Appropriate management of these cases is critical because cases that occur during this period often recur and can have long lasting economic impacts that include reduced productivity and fertility and increased risks for early removal. While prevention is the most important aspect of controlling mastitis, after infection has been established, interventions such as culling, treatment using antibiotics or nonantimicrobial intervention strategies must be considered. Decisions about use of antibiotics or retention of affected cows must be based on knowledge of etiology and incorporate cow-specific factors that are predictive of case outcomes. In this presentation we will review the strategies for managing mastitis during this critical period with emphasis on practical recommendations for when antimicrobial therapy is indicated

Key Words: mastitis

Breeding and Genetics Symposium: Crossbreeding

S147 Capitalizing on breed differences and heterosis. L. B. Hansen*, *University of Minnesota, St. Paul, MN.*

Mankind is blessed with high-quality and differentiated "breeds" (populations) of dairy cattle that have meaningful population size and effective genetic improvement programs for use in the temperate regions of the world. The "breeds" are Holstein (including Friesian and Red Holstein), Ayrshire (including the sizeable Nordic Red population), Jersey, Normande, Guernsey, Fleckvieh, Montbéliarde, and Brown Swiss. These breeds are divided into 2 subgroups – those originating in Great Britain and northern mainland Europe (Holstein, Ayrshire, Jersey, Normande, and Guernsey) and those originating in the Alpine region of Europe (Fleckvieh, Montbéliarde, and Brown Swiss). Crossing of breeds within the 2 subgroups provides heterosis of about 3% for production traits, but crossing of breeds across the 2 subgroups provides heterosis of about 10% for production traits. Estimates of heterosis for functional traits such as fertility, health, and survival usually are greater than 10% for crosses among all the breeds. Dairy cattle differ from beef cattle and other meat-animal species because the major output from dairy cattle is the efficient production of milk from a female rather than the production of meat by offspring from a terminal cross. Consequently, a 2-breed or 3-breed mating strategy is routinely implemented for crossbreeding in dairy cattle, and a 3-breed rotation has gained popularity and is routinely recommended to achieve a high level of mean heterosis across generations. Breeds for crossbreeding must be chosen based on complementarity and on suitability for environmental conditions of specific production systems. Research from a large-scale 10-year field study documented the 3-breed rotation of Holstein, Viking Red, and Montbéliarde resulted in lactating cows with statistically significant advantages for longer herdlife, enhanced fertility, reduced health treatment cost, and lessened stillbirth rates compared with their pure Holstein herdmates. From the same study, production of milk solids (kg) was similar for the crossbreds and profit per day in the herd was significantly higher (+10.5%) for the crossbreds compared with their pure Holstein herdmates.

Key Words: breeds, crossbreeding, heterosis

S148 Genomic evaluations for crossbreed dairy cattle. B. Harris*, E. Reynolds, C. Couldrey, M. Nilforooshan, A. Winkelman, and R. Sherlock, *Livestock Improvement Corp., Hamilton, Waikato, New Zealand.*

Genetic evaluation of dairy cattle, both pedigree-based and genomic, is generally done within breed. New Zealand (NZ) is the exception in this regard where across-breed genetic evaluation was implemented in 1996 and genomic evaluation using a multi-breed reference population began in 2007. Across-breed evaluation was implemented because a substantial proportion of the dairy cattle population is crossbred; our Holstein-Friesian sires are an admixed population of North American Holstein-Friesian and NZ Friesian, 10% to 15% of our progeny-tested sires are Friesian-Jersey crossbreds and more than 50% of cows have both Friesian and Jersey ancestry. Across-breed evaluations allow the animals to be ranked and compared within and across bred. This paper traces the evolution of NZ's genomic evaluation models. Evaluation currently incorporates data from the informative 35K from the Illumina 50K SNP panel. Analysis of the population structure via principal components analysis of the SNP data on 160K animals indicated the presence of breed differences, that is, the SNP frequencies differ across breed. When not modeled correctly, breed stratification may result in inflation in the genomic prediction. The SNP data will also be used to calculate the levels of genomic inbreeding and dominance. These factors will be fitted in the single-step marker model to assess their impact on the genomic evaluations. Other research in the pipeline includes identifying the most informative SNP from imputed whole-genome sequence data instead of the 50K SNP by undertaking an across-breed genome-wide association study using imputed whole-genome sequence data (16m variants) on 160k animals. Also in the pipeline, we are exploring the use of breed specific genome maps (NZ Jersey and NZ Holstein Friesian) to improve sequence mapping and imputation and identify areas specific to each breed. Multiple-trait marker models are being developed. Currently, the lactation information for production traits are evaluated separately. We aim to have models that include all lactations for each of the milk production traits. Other examples are multitrait models to evaluate fertility and body condition score.

Key Words: dairy cattle, genomics, crossbreeding

S149 Genomic evaluation with multibreed and crossbred data. I. Misztal*, *University of Georgia, Athens, GA.*

Joint genomic evaluation of multiple breeds and crossbreds potentially offers multiple benefits. Purebreds can benefit from the crossbred information and crossbreds can be evaluated based on information from their breeds of origin. Also, purebreds can benefit from the information on the other purebreds if they share quantitative trait nucleotides (QTN) with similar substitution effects. Based on studies across species, the joint evaluation requires more research to be viable. The simplest multibreed evaluation includes all animals as a single population. When multiple breeds share SNP effects, the accuracy of evaluation decreases compared with single trait models, more with larger data. In simulations, the accuracy for one breed based on another breed were low even if the breeds were simulated with identical QTNs and substitution effects. When breeds are unequal size, the predictions are accurate for the largest breed(s) but are worse compared with single breed analyses for smaller breeds. When GEBV of crossbreds are predicted as proportion of purebred evaluations, the accuracies are limited; the accuracies for the crossbreds appear reasonable with a crossbred reference population. Use of the crossbred information has a small impact on the accuracy of the purebred population. The accuracies are similar whether purebreds and crossbreds are analyzed jointly or as a different trait per breed combination. The accuracies slightly improve with SNP selection especially with preselected SNP based on sequence data, however, the benefits are trait dependent. The SNP preselection seems to be more effective with smaller data using simpler models, but less or none when using large data and single-step models. The accuracies also seem to increase slightly with phased genomic data for the F1 crossbreds. Without accounting for breed type, realized accuracies for complex populations may be strongly biased upwards, with genomic predictions predicting breed types and not the individuals within the breed type. The genomic selection works mostly by estimation of clusters of chromosome segments, and the clusters are different for each breed and type of crossbred. High accuracy of multibreed predictions may be realized with identification of many QTNs explaining a large part fraction of the additive variance, if QTN substitution effects are similar across breed types.

Key Words: genomic evaluation, crossbred, multibreed

S150 Economics of using beef semen in dairy herds.

V. E. Cabrera*, *University of Wisconsin, Madison, WI.*

Profitability of beef semen in dairy is principally influenced by (1) dairy and beef market calf prices, (2) reproductive performance, and (3) semen combination strategies. Due to the complex interaction among these factors and their inherent changing conditions, the quest for an optimal strategy is best served by the application of an integrated model and a decision support tool adaptable to ever changing farm and market conditions. As such, we have developed a decision support tool to calculate the income from calves over semen cost (ICOSC) in response to user-defined beef semen crossbreeding strategies. The model follows a Markov-chain approach in which animal (heifers and cows) status (age, months after calving, lactation, pregnancy, calving) is simulated on a monthly basis. Replacement balance is calculated as the difference between demand and supply of springers in function of selected semen utilization protocols, which could include beef, sexed, and/or conventional. A case study was performed in a 1,000-cow virtual dairy farm set at medium reproductive performance (i.e., 55% conception rate for first breeding heifers and 20% 21-d pregnancy rate for cows when using conventional semen) and 35% turnover rate. Five strategies of beef semen utilization on adult cows (0 to 100% in 25% intervals) were combined with 6 strategies of sexed semen use: none (NS), first breeding heifers (1H), 2 first breedings heifers (2H), 2H + 20% top genetic cows (TOP), 2H + first breeding primiparous (1C), and 1C + first breeding second-lactation cows (2C). All animals not bred to either sexed or beef were bred to conventional semen. Having a price of beef calves 4 times greater than the price of dairy calves and the price of sexed semen 2.3 times greater than the conventional or beef semen determined that the optimal breeding protocol that concurrently maximized ICOSC (+US$2,001) and still produced enough replacements was 100% beef semen after imposing 2C sexed semen protocol. This strategy was consistently the best option under several feasible market conditions, but not when reproductive performance changed drastically.

Key Words: crossbred, economic decision-making, simulation

S151 Selecting Angus sires for the growing beef on dairy market.

S. P. Miller*[1], J. A. Archer[2], F. Hely[2], C. Quinton[2], K. Retallick[1], D. Moser[1], and P. Amer[2], [1]*Angus Genetics, Saint Joseph, MO,* [2]*AbacusBio Limited, Dunedin, New Zealand.*

Widespread use of sexed semen among other factors has resulted in a dramatic increase in cows being mated to beef bulls on American dairy farms. To aid dairy farmers in their choice of bulls and to enhance the end beef product being produced the American Angus Association has developed specific selection indexes. Indexes developed identify the best Angus bulls, a predominant breed in this market, to use on Holstein and Jersey cows. Through interviews and site visits with key participants in the dairy beef supply chain, production and economic parameters were sourced to inform the modification of the standard American Angus terminal sire index $B. $Angus-on-Holstein Value ($AxH) and $Angus-on-Jersey Value ($AxJ) were developed and although highly correlated to each other (0.96), were considered to rank bulls different enough, especially at the top end, that both were needed. Generally, the dairy indexes identify bulls with the best $B but avoid 3 particular traits that are problematic in the beef on dairy supply chain, with nonlinear emphasis. Calving ease had relatively lower emphasis with a similar penalty in both the $AxH and $AxJ indexes, where muscling was heavily weighted with greatest emphasis in $AxJ and a penalty for excessive yearling height EPD was implemented in $AxH in an effort to reduce excessively long carcasses in that cross. The moderate correlation between $Beef with the $AxH (0.72) and $AxJ (0.78) indexes illustrates the major re-ranking that will exist with these new indexes compared with the standard terminal index for straight beef matings and hence their need in the marketplace. These new indexes provide dairy farmers and players in the supply chain through to slaughter a tool to select Angus bulls to produce calves that are better suited to the requirements of this rapidly growing sector.

Key Words: selection index, cattle, Holstein

Dairy Foods Symposium: Nourish to Flourish—The Role of Product, Process, and Package in Driving Milk Consumption Among Children

S152 Connecting dairy, school meals, and student consumption. K. Wilson*, *Urban School Food Alliance, Madison, WI.*

In schools across America, school breakfast, lunch, and after school snack/supper programs are teaching students what a balanced meal looks like. These meals are balanced in nutrients as well as portion sizes. With over 31 million children participating in school lunch alone, these programs have the potential to influence life -long eating habits. As we continue to fight childhood obesity and other food related health disparities, it is critical that we find ways to encourage children to consume dairy products, particularly fluid milk, that are rich in many essential nutrients. With an increasing number of alternative beverage choices and marketing aimed at children, we face a significant challenge keeping milk on the tray, particularly in secondary schools. This session will explore some of the deterrents of milk consumption in the school environment as well as innovative ideas being used to increase consumption and what is needed to assist districts with this issue.

S153 Optimizing acceptability of sugar- and sodium-reduced chocolate milk via cross-modal interactions. A. Riak[1,2] and H. Hopfer*[1,2], [1]*Department of Food Science, The Pennsylvania State University, University Park, PA,* [2]*Sensory Evaluation Center, The Pennsylvania State University, University Park, PA.*

Sugar reduction is a key goal for dairy producers. To accommodate increasing consumer desires to reduce sugar consumption, as well as a need to promote the healthfulness of dairy products, it is in the best interest of dairy producers to optimize products with reduced sugar content. This is particularly important in flavored milks, where traditionally high levels of sugar have caused a great deal of concern. The main strategies for reducing added sugar in fluid milk are sugar substitutes, lactose hydrolyzation, and gradual reduction. All these sugar reduction strategies are promising; however, they are not sufficient to allow for a large reduction in the amount of added sugar. Thus, further strategies and other approaches need to be explored. Cross-modal enhancement of perceived sweetness by vanillin was recently demonstrated in skim milk, and vanillin may also be able to decrease perceived bitterness. This project aims to optimize the synergistic interactions between sucrose, cocoa, and vanillin in a low-fat milk system, leading to a chocolate milk that maintains acceptability and satisfies current and future NSLP requirements with regards to added sugar and sodium content. Synergistic combinations of sucrose, vanillin, and cocoa in a skim milk chocolate matrix are determined via a dose-response experiment with 100 consumers, able to model higher-order interaction effects, followed by acceptability testing with school children (8–14 yr) of optimized chocolate milks. The obtained dose-response relationships could be used by producers to predict acceptability and sensory attributes, as a function of ingredient levels, thus, linking formulation to consumer perception and liking. They could be further combined with other sugar reduction strategies to possibly achieve even further added sugar reduction.

Key Words: chocolate milk, sensory perception, sugar reduction

S154 Predicting the impact of the school milk distribution chain and processing factors on the quality of school milk using Monte Carlo simulations. T. T. Lott*, N. H. Martin, A. N. Stelick, S. I. Murphy, A. Trmcic, and M. Wiedmann, *Cornell University, Ithaca, NY.*

The decline in fluid milk consumption is most severe among school aged children. Current school milk quality can influence children's future decisions regarding fluid milk consumption. Historic data on fluid milk quality has shown higher total bacterial counts and lower sensory scores at d 14 postprocessing in single serve (i.e., school milk) as compared with gallon and half-gallon containers. Most of the high total bacterial counts are due to postpasteurization contamination with gram-negative bacteria. The goal of this project was to understand the factors that influence school milk quality (i) during processing and (ii) throughout the distribution chain and (iii) to implement strategies to improve overall quality. This was achieved through (1) conducting in-plant observations, surveys, and shelf-life testing which included total bacterial counts, total gram-negative counts, and sensory scores on d 1, 7, and 14; and (2) use of continuous temperature monitoring across the distribution chain using data loggers placed in individual school milk cartons. Additionally, schools were given surveys regarding milk handling with a focus on factors that may influence product quality. Milk types tested for shelf life included white skim, white 1%, chocolate skim, and chocolate 1%. Preliminary data from temperature tracking showed that the greatest risk of temperature abuse occurs upon delivery of milk to the school. Survey and temperature data revealed that the greatest variability of temperature is between individual school coolers. Postpasteurization contamination was found in all milk flavors and fat levels with highest incidence in chocolate milk. In-plant surveys revealed that the carton-forming mandrels were consistently identified by plant personnel as filler parts that are the most difficult to clean and consistently failed ATP swab checks. Temperature data and processing factors that affect quality will be used to create a Monte Carlo simulation model that will help producers make data driven decisions to improve school milk quality.

Key Words: school milk, Monte Carlo simulation, postpasteurization contamination

Dairy Foods Symposium: Making Lactose the Carb of Choice

S155 Lactose: Designed to deliver. T. Huppertz*[1,2], [1]*FrieslandCampina, Amersfoort, the Netherlands,* [2]*Wageningen University and Research, Wageningen, the Netherlands.*

Despite global milk production yielding approximately 40 billion kg of lactose each, lactose only contributes a small proportion of total energy from carbohydrate. It does, however, play various important roles in the human diet and development. The β-1–4-glycosidic link between galactose and glucose makes lactose more resistant than other carbohydrates to oral fermentation, and therefore the least cariogenic of the main saccharides. As the main nutritional source of galactose, it provides a versatile carbohydrate source which can be directly available for energy, but also can be stored as glycogen and enhance fat oxidation. Compared with other carbohydrates, lactose has been linked to lower dopamine release and less carbohydrate craving effects. Combined with known effects on gut health and micronutrient uptake in the intestines, this highlights an array of important features of lactose in human nutrition. These are further improved by the dairy product matrices in which lactose is currently consumed; for example milk, yogurt and fermented milk products, all of which show many benefits to human health. Thus, while similar to many other disaccharides in atomic composition and molar mass, the specific structure of lactose makes it a unique and versatile carbohydrate, which is truly designed to deliver.

S156 Mining value-added, naturally derived sweeteners from dairy co-product streams. Z. Wang* and J. M. Goddard, *Cornell University, Ithaca, NY.*

The valorization of whey protein into a high value-added ingredient underscores the market potential for co-products of cheese production. After purification of whey protein concentrate, whey permeate remains with a high lactose content and cannot be directly released into wastewater. In recent years, consumers and producers alike have increasingly demanded a reduction of added sugars in processed foods and beverages. Rare sugars are monosaccharides with near-equivalent sweetness intensity as sucrose, but a fraction of the caloric density and minimal adverse health impacts. Present in very small amounts in nature, an opportunity remains to produce rare sugars from 'waste' sugars such as lactose. Tagatose and allulose, are of particular interest as they have been granted Generally Recognized as Safe (GRAS) status by the US Food and Drug Administration. While enzymatic conversion of lactose to rare sugars is possible, challenges remain in designing a system that preserves enzyme activity and stability over multiple cycles of reuse. The long-range goal of this project is to immobilize a series of enzymes onto a cellulosic support to convert the lactose in the whey permeate into a rare sugar syrup. Lactose is first hydrolyzed into glucose and galactose using β-galactosidase (lactase). Glucose will be isomerized into D-allulose using xylose isomerase and D-psicose 3-epimerase. Galactose will be isomerized into D-tagatose using L-arabinose isomerase. We have successfully produced a recombinant lactase with a cellulose binding module, which permits site-directed immobilization of the recombinant enzyme onto a cellulosic support under 2 h at 18°C. The immobilized lactase presented enhanced thermostability at 50°C compared with free lactase, an important consideration for higher temperature bioprocessing. The immobilized lactase retained over 70% of its initial activity whereas the free lactase retained less than 10% of its initial activity at an incubation temperature of 50°C over 50 h. Outcomes of this research will lead to new applications in the reuse of dairy co-products, promoting economic and environmental sustainability of global dairy processing.

Key Words: dairy, enzymes, rare sugars

S157 Polylactose: An emerging ingredient for human health. T. Schoenfuss*, *University of Minnesota, St. Paul, MN.*

The polymerization of sugars via a condensation reaction to oligo and polysaccharides using heat and acid has been developed using a continuous process to produce polylactose, oligosacharides from lactose. Polylactose has a degree of polymerization that ranges from 3 to 11. We conducted an animal study with polylactose and 2 well-established prebiotic dietary fibers, polydextrose and fructooligosaccharides. Polydextrose, fructooligosaccharides and polylactose were fed as part of a high fat diet to induce mild obesity, an impaired blood glucose control, and a fatty liver. The findings demonstrated that polylactose is highly fermentable, as measured by the drop in cecal pH (the cecum being the first part of the large intestine in rats) and an increase in cecum weight. Polylactose was found to be more fermentable than both polydextrose and fructooligosaccharides. The greater fermentation of polylactose suggests it had a more profound effect on the colonic microflora. In terms of health benefits to the host, polylactose significantly reduced body fat, as measured by a lighter epididymal fat pad, when compared to the high fat control diet. In addition, plasma leptin concentrations showed a trend towards a lower concentration when compared to the high fat diet control. The microbiome of the rats was compared by Unweighted Unifrac and Jaccard Dissimilarity PCA. The microbiome of treated rats fell into 3 distinct groups: high- and low-fat controls in one, the other 2 prebiotics in a second group, and polylactose alone in a third group. The populations of *Bifidobacteria* and *Lactobacillus* was increased in the polylactose treatment which demonstrates this fiber had positive effects on the rat microbiome in this study.

Key Words: lactose, polymerization, prebiotic

S158 Lactose-derived oligosaccharides: Sources, technology, bioactivity, and health benefits. D. Barile*, *University of California–Davis, Davis, CA.*

Milk oligosaccharides (OS) possess a multitude of bioactivities, including the abilities to act as prebiotics and prevent serious diseases in infants. They are indigestible, complex sugars that have demonstrated roles in infants' immune system development by promoting the growth of beneficial bacteria and limiting pathogen adhesion in the gut. Infant formulas contain little to no OS due to the limited availability of human milk and difficulty of synthetically producing the many complex OS structures. Milk OS are highly concentrated in human milk but they are much less abundant in bovine milk, and their presence in the dairy marketplace is scarce. With improved production and isolation strategies, these compounds could be recovered from dairy processing streams for use as ingredients in infant formula and adult therapeutics. Currently, little is known about variations in OS production among dairy cattle and the factors that impact milk OS abundances, such as breed and diet. Therefore, it is important to develop methods for measuring milk OS abundance in a large sampling of dairy cattle by implementing novel techniques for high-throughput milk OS profiling. Examining the influence of genetics and diets on bioactive compound formation will provide insights into ways that both milk OS abundance and the value of liquid milk production can be increased. Because bovine milk oligosaccharides are not identical to human milk oligosaccharides and generally present smaller structures, enzymatic modification of milk OS is considered an intriguing alternative to replicate the many benefits afforded by oligosaccharides that are heavily sialylated and fucosylated. This line of research could lead to the development of personalized dairy products with enhanced bioactive content for specific human health needs.

Key Words: oligosaccharides, bioactive compounds, dairy streams

EAAP Exchange Symposium: Limits in Production Growth on Level of Cow, Farm, and Industry

S159 Limits of milk production in dairy cows: Perspectives from physiology. J. J. Gross* and R. M. Bruckmaier, *Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Milk production of dairy cows continues to increase through animal breeding, which is required to ensure a sufficient income for the farmer. However, production systems vary tremendously between countries in terms of environment, feeding and husbandry, intensity, and genetics of cows. Milk production does not appear to be limited by the productive capacity of the mammary gland. Limits are much more determined by the provision of energy and nutrients by the animal which challenges feed uptake as well as the activity of intermediary metabolism. Increasing the energy density in the diet by energy-rich ingredients such as fat and starch has limitations as the breakdown of dietary fiber in rumen ecosystem decreases under acidotic conditions and excessive lipid supply. Rumen acidosis and constraints in rumen fermentation process can be avoided by feeding rumen-protected or by-pass starch and fat. However, rumen microbes may not be limited in their supply with crude protein, essential amino acids and energy. On the other hand, duodenal absorption capacity for starch and fat is limited. By-pass components in dairy cow rations (e.g., soybean meal as protein source) are controversially discussed in terms of their ecologic footprint. Limited feeding of concentrates (e.g., due to economic considerations or exclusive herbage-based production systems) gives the impression of dairying close to nature, but lowers milk production in high-producing animals and further affects animal health. Low-genetic merit cows could be an alternative for these production systems. A reduced metabolic load during peak lactation can be achieved by cows with a more moderate start into lactation at simultaneously high lactational persistency. This may reduce the propensity for various production diseases. Management tools such as extended lactation through short dry periods or late service can support the genetic adaptations. Furthermore, functional feed additives improving the metabolic status result in an elevated feed intake and save resources for milk production and the immune system. Despite efforts in management, physiological limitations remain unsolved in high-yielding dairy cows.

Key Words: milk production, limits, physiology

S160 Opportunities to improve the productivity of dairy cattle from conception through calving. M. E. Van Amburgh* and R. A. Molano, *Cornell University, Ithaca, NY.*

The productivity of dairy cattle is initially determined by genetics, and further shaped by the environment and the interaction between both factors. From conception forward, environmental signals have been identified that play a key role in the phenotypic expression of the innate capacity of the animal. During gestation, factors such as nutrient status of the dam and heat stress impact maternal blood flow, nutrient transfer, conceptus development, organ and muscle development of the fetus, and ultimately growth and BW at maturity. After birth, other environmental signals can continue to alter the calf's productive capacity. There are significant data characterizing the role of non-nutrient, non-immunoglobulin components in colostrum on immunity, gastrointestinal development, nutrient absorption and anabolic status of the calf. Longitudinal studies demonstrate greater growth and anabolic status in calves receiving colostrum up to 4 d of life. Further, the heifer's nutrient intake above maintenance stimulates aspects of development resulting in improved productivity as a lactating cow. In a large study, the preweaning growth rate was significant and positively correlated with milk production during the first and subsequent lactations. Preweaning growth accounted for 22% of the variation in first-lactation milk yield, which was approximately 4 times the variation explained by genetic merit. These investigators also observed the same relationship between the rate of gain from birth to first breeding and first-lactation milk yield and between average daily gain from weaning to breeding and the cumulative milk production through 3 lactations. Additional work has associated this enhanced nutrient availability with increased mammary gland development, and improved body weight and composition at calving, as part of mechanisms facilitating improved performance. Likewise, several studies demonstrate these effects are interdependent and could be cumulative. Thus, new and emerging information on epigenetics, coupled with the attainment of basic growth benchmarks, represents a valuable approach to improve productivity in dairy cattle.

Key Words: heifers, epigenetics, productivity

S161 Long-term productivity changes in the dairy industry and projected impacts on global food production. R. White*, *Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA.*

Genetic selection of animals with improved feed efficiency, coupled with precise and accurate feeding strategies designed to meet the needs of those animals, shows promise as one of the most effective means of continuing to enhance sustainability of dairy production globally. The objective of this review is to summarize the relationships between genetic selection intensity, feed efficiency, and environmental impact of dairy production systems. A secondary objective is to extrapolate how chances in efficiency affect global dairy environmental impacts based on current heterogeneity in efficiencies experienced worldwide. Genetic selection for improved feed efficiency, coupled with improved feeding practices was simulated using baseline inputs reflecting average production practices and efficiency in the United States. The cost of implementing dietary changes to reduce environmental impact dramatically reduces (75%) when selection is used to affect a 15% improvement in feed efficiency. Globally the coefficient of variation in the production of milk per animal is nearly 100%. Over the period from 1960 to 2020, mean milk production per animal has increased by 30.8 kg per animal per year. The difference between the 95th and 5th percentile of countries in terms of milk production per animal has increased by 191 kg per animal per year. This expanding efficiency range has occurred because the most efficient countries are continuing to improve efficiency. Minimum efficiencies have not changed over time. Efficiency progress achieved by the most efficient countries has been extremely linear over the past 3 decades, showing minimal evidence of a saturation or a plateau efficiency. Countries in the 25th to 50th percentile of efficiency (i.e., those with low efficiency) contribute 40% of greenhouse gas emissions. The proportion of emissions associated with this group of countries increased by nearly 1% per year between 1960 and 2020. Efficiency improvements in countries with below average efficiency will contribute notably to enhancing environmental impacts of global dairy production.

Key Words: efficiency, genetic improvement, nutrition

S162 About the essential role of dairy in future food production within the planetary boundary. M. Scholten*, *Wageningen University & Research, Wageningen, the Netherlands.*

Dairy is generally considered a healthy, nutritious food source. However, dairy production itself is today seen as less healthy for the planet, due to land and water use as well as greenhouse gas emissions. The call for a reduction of the dairy production enforced by banning dairy consumption increases. But that is a too simplistic conception about the future food system. Future food security in a world with increasing consumption will be all about resource security. Circularity in using resources for fertilizing crops and feeding livestock is a key to sustainability. This means that agricultural biomass that is not directly edible as food, such as grass, herbs and crop residuals, can best be valorized as feed in livestock producing food. And chemically produced fertilizers can best be replaced by fertilizers converted from residual agricultural biomass such as crop residuals and livestock manure. And that is where cows come into the circular food system. Ruminants with a great capacity to digest feedstocks that otherwise remain unused, while producing a high-quality manure. This results in an optimized resource use in an integrated, ruminant inclusive food production. The global challenge of resource security is as important as food security, climate change and biodiversity losses. Ruminants are essential for resource security in future food systems providing sustainable nutrition security within planetary boundaries [United Nations Sustainable development goals (UN-SDG) 13, 14, and 15] in connection to health boundaries (UN-SDG 2 and 3), to address responsible consumption and production (UN-SDG 12) in a future society based on a circular biobased economy. Dairy livestock inclusive mixed food systems have the best environmental performance. The essentials of dairy production customized to this transition of our food system will be addressed: selection of circular feed, valorization of manure and selection of breeds that are king in circularity. This will be illustrated for various dairy farm systems worldwide: from intensive feedlot systems to extensive pasture-based systems, as well as for typical small holder systems in low- and middle-income countries.

Reproduction Symposium: Do We Need Estrus?

S163 Activity monitors: Is it possible to design a selective reproductive program according to the cow's needs? R. Chebel*[1,2], [1]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The advancement of automated technologies (AT) for the detection of estrus has created new opportunities for the management of lactating dairy cows. In addition, the association of such technologies with genomic information has bridged a gap in our understanding of the associations between reproductive traits and estrus phenotype. In a recent study, it was possible to identify according to estrus-characteristics, as early as 42 DIM, cows that have shorter interval from calving to pregnancy. In these studies, greater heat index (determined changes in activity and rumination) in estrus occurring before 42 DIM was associated with greater hazard of pregnancy and reduced interval from calving to pregnancy. This creates an opportunity to decide, before the end of the voluntary waiting period, if a specific cow should be submitted to an ovulation synchronization protocol or insemination in estrus for first postpartum service. Several experiments have compared reproductive management using AT versus strict fixed-time AI for first service. By design, the rate of first insemination is faster for cows subjected to insemination at detected estrus with AT but by a set DIM, all cows are inseminated as is expected when fixed-time AI protocols are implemented. In an experiment in FL, the use of AT for first and subsequent services resulted in greater rate of pregnancy because it reduced the interval to first and second services and increased pregnancy per service, demonstrating that AT may prove beneficial in circumstances where visual detection of estrus lacks accuracy. Recently, we demonstrated that daughter pregnancy rate is positively associated with estrus phenotypes, which may aid in the identification of cows that could be managed mainly through AT. Ovulation synchronization protocols have been and will continue to be formidable tools for the reproductive management of dairy cows. In light of the fact that even cows subjected to such protocols have greater risk of pregnancy when they express estrus, the quest for estrus-expression shall benefit dairy herds independent of their reproductive management strategies.

Key Words: estrus, automated technology, reproduction

S164 Do we need estrus? Influences of estrous intensity on fertility in dairy cattle. R. L. A. Cerri*[1], T. A. Burnett[2,1], A. M. L. Madureira[1], A. L. Moore[1], J. Denis-Robichaud[1], J. L. M. Vasconcelos[3], W. Heuwieser[4], and S. Borchardt[4], [1]*University of British Columbia, Vancouver, BC, Canada,* [2]*University of Guelph, Guelph, ON, Canada,* [3]*Sao Paulo State University, Botucatu, Brazil,* [4]*Free University Berlin, Berlin, Germany.*

Recent studies have demonstrated how estrus events and intensity are associated with ovulation, ovarian and uterine function, and fertility. Data sets that included spontaneous and induced estrus have shown that greater relative increase and longer duration of estrus, captured by different activity monitors, significantly improve pregnancy per AI (P/AI; around 15% units across different studies) and decrease pregnancy losses (13.9 vs. 21.7%). Intensity and duration of estrus were weakly associated with follicle diameter and concentration of estradiol at estrus, but the odds of ovulation failure was reduced by 3-fold in cows displaying estrus with high relative increase in physical activity. Data from 2 studies in beef cows showed that estrus behavior near AI also modified transcripts related to the immune system, adhesion molecules and prostaglandin synthesis in the endometrium, as well as apoptosis, progesterone synthesis and prostaglandin receptor in the corpus luteum. Most recently, studies from the USA and Canada demonstrated that reproductive programs using activity monitors and emphasizing detection of estrus can be successful and comparable to intensive timed AI protocol-based programs. One last study from our laboratory also concluded that the inclusion of GnRH at AI greatly improved P/AI from cows displaying low intensity estrus, demonstrating the potential use of activity monitors as a tool in targeted protocols. Quantitative information from estrus events could be used to improve estrous detection quality and develop decision-making strategies at the farm level. Further studies in this field should aim to (1) better understand ovarian, embryo and endometrium mechanisms associated with either the expression or intensity of estrus, and (2) refine the collection of phenotypes related to estrus (i.e., relative increase, absolute increase, baseline levels, duration, and repeatability within cow) to improve estrous detection and possibly genetic selection.

Key Words: activity monitors, dairy cow, estrous expression

S165 The implications of spontaneous versus synchronized ovulations on the reproductive performance of high-producing dairy cows. P. M. Fricke* and M. C. Wiltbank, *University of Wisconsin, Madison, WI.*

In species classified as induced ovulators, the LH surge is induced by the act of coitus thereby precisely timing insemination relative to ovulation. Lactating dairy cows are spontaneous ovulators. Thus, the LH surge is induced by a cascade of endocrine events beginning with an increase in follicular estradiol in the absence of luteal progesterone. A GnRH surge from the hypothalamus is then followed by an LH surge from the anterior pituitary, followed by ovulation. Increased estradiol in the absence of progesterone causes behavioral estrus which is manifested by increased physical activity and standing to be mounted. Detection of behavioral estrus either visually, through the use of estrus detection aids, or via automated activity monitoring systems is widely used to determine timing of AI. Cow-related factors that limit service rates include the association of high milk production and duration of estrus, ovulation failure after estrus, ovulation in the absence of behavioral estrus, and anovular conditions which affect 20% to 30% of dairy cows at the end of the voluntary waiting period. One factor that limits fertility to spontaneous estrus is the high variation among cows in the timing of ovulation relative to increased activity resulting in imprecise timing of AI relative to ovulation. Another factor is the high rate of hepatic metabolism of estradiol and progesterone associated with high feed intake in high-producing cows. Under this endocrine milieu, development of preovulatory follicles from deviation until ovulation occurs in a prolonged low-progesterone environment marked by high frequency LH pulses that overstimulate the oocyte and compromise fertility similar to a persistent follicle. Development of the Ovsynch protocol turned dairy cows into induced ovulators, which allowed for increased service rates and precise timing of AI relative to ovulation. Subsequent modifications of the Ovsynch protocol, including presynchronization strategies and complete induction of luteal regression, lead to fertility programs that yield greater fertility than that of a detected estrus in high-producing dairy cows.

Key Words: estrus, timed AI, dairy cow

S166 Artificial insemination after detected estrus underpins efficient seasonal-calving pasture-based systems. S. T. Butler* and S. G. Moore, *Teagasc, Fermoy, Co. Cork, Ireland.*

The overarching objective of seasonal-calving, pasture-based dairy production systems is efficient conversion of grazed grass to milk, and is reliant on compact calving in late winter/early spring to synchronize herd feed demand with seasonal variation in pasture growth. Breeding commences on a fixed calendar date in late spring, and excellent submission rates (≥90% of all cows eligible for breeding submitted to AI within 21 d) and pregnancy per AI (≥60%) are required to achieve a rapid rate of herd pregnancy establishment. If 90% of all non-pregnant cows eligible for breeding are submitted in each of 4 consecutive 3-wk blocks, and 60% P/AI is achieved, it is theoretically possible to have 54%, 79%, 90%, and 96% of the lactating herd pregnant by wk 3, 6, 9, and 12 after the farm mating start date, respectively. These metrics are achievable without extensive usage of synchronization. Is fixed-time AI (FTAI) a cost that would ultimately improve profitability on seasonal-calving, pasture-based dairy farms? Most herd owners see little need for whole herd synchronization if most cows will express overt estrus during the first 3 weeks of breeding, allowing target submission rates to be achieved. Although FTAI may facilitate breeding a large proportion of the herd on a single day, this could ultimately cause problems at the start of the following calving season (inadequate calving facilities; unpredictable grass supply and grazing access). Protocols that include pre-synchronization [e.g., Presynch-Ovsynch (35 d), Double-Ovsynch (28 d)] are not used in seasonal-calving systems due to the excessive time required for implementation. Instead, Ovsynch (10 d) with a progesterone insert for the first 7 or 8 d is used on a targeted subset of cows that will derive most benefit (anestrous cows, cows that calved in the latter half of the calving period), or to mitigate risk of reduced P/AI as a result of using sexed semen. Genetic selection for fertility traits is increasing intensity of estrous behavior and improving P/AI, allowing most cows to be bred following detected estrus.

Key Words: seasonal calving, estrous detection, reproduction

Animal Behavior and Well-Being Symposium: Translating Animal Welfare Science—Animal Experiences, Dairy Production Implications, and Societal Viewpoints

S167 Decision-making as an indicator of affective state: A cost-benefit framework. S. J. J. Adcock*, *University of Wisconsin, Madison, WI.*

Decision-making paradigms for assessing cattle affect, such as cognitive bias tasks, are based on the theory that affective states function to organize behavioral responses to incoming sensory cues. In animal welfare studies, an individual's previous aversive experience is thought to lead to a negative affective state which functions to reduce risk-taking. Thus, when faced with an ambiguous cue that may signal the arrival of a reward or threat, an animal in a negative state would be more likely to have a risk-averse response than one in a more positive state. However, in behavioral ecology, negative states are typically associated with risk-proneness, not risk aversion. These conflicting predictions may be reconciled by considering the optimal threshold for a decision outcome as an inverse function of the expected payoff in terms of evolutionary fitness. For example, when faced with an ambiguous cue that may signal food or danger, the optimal decision (approach or avoidance) is the one that maximizes fitness. Internal or external events, and their resulting affective states, may alter the fitness payoffs associated with the 2 decision outcomes. An aversive experience such as disbudding may increase the cost of approaching the ambiguous cue as injured animals are more vulnerable to predation, and so should favor avoidance. However, disbudding may also increase the benefits of feeding via maintaining energy reserves for healing or via the soothing/analgesic effects of sucking milk, and so should favor a low threshold for approach. Thus, whether disbudded calves are more or less likely to approach the ambiguous cue compared with uninjured calves depends on the relative change of costs and benefits associated with this behavioral response. Under a cost-benefit framework, affective states bias decisions in whichever direction maximizes fitness, such that a negative state could favor a risk-averse or risk-prone outcome depending on the behavioral conflict in question. Dissecting the fitness payoffs associated with a decision outcome should help to structure hypotheses, explain seemingly counterintuitive results, and reduce inferential error in studies of cattle affect.

Key Words: welfare, emotion, behavior

S168 How do we humanely feed a hungry world? Supply chain challenges and solutions and what it means to dairy farmers. J. B. Walker*, *Danone, Broomfield, CO.*

Projected population growth has brought additional concerns about the trade-offs we may face with regard to animal welfare in grappling with our mission to "feed the world." This seems a fair question given that it was advances in agriculture and its intensification, in addition to advances in public health, that fueled the exponential population growth seen over the last 200 years. While the population is expected to grow at an astonishing rate, the production of food is expected to experience even greater increases due to increases in wealth and economic development. Relevant in this growth is the fact that production will be driven by consumptive preferences, which will likely shift toward animal protein and milk in addition to fruits and vegetables. These facts have fueled anxiety for some seeking to improve animal welfare. The concept of animal welfare focused on protecting animals from suffering is seen by many in the field as insufficient if not obsolete. The idea that animals in agriculture should be provided for in such a way that animals' lives are good and not merely void of suffering is becoming the focus of some supply chain leaders. Still some in agriculture have posited that attempts to provide for positive welfare will essentially tie our hands in an attempt to feed the world. Is promoting positive animal welfare a competing interest of the global challenge to feed the world in the year 2050? To address this question, we must first answer the following: What do we mean by positive animal welfare? Does increasing efficiency and productivity pose a threat to animal welfare? Should providing for the positive welfare of farm animals be a choice or is it a moral imperative? The reality is, these questions have been asked and answered. It is now in the hands of the supply chain to design and deliver the path forward. Success will depend on understanding both the value and risk of certification, the role audits play in animal welfare programs, challenges in driving meaningful improvement in animal welfare and that rhetoric without results sets the stage for failure.

Key Words: animal welfare, audits

S169 Global challenges and implications for animal agriculture and opportunities for animal welfare. J. Marchant-Forde*, *USDA-ARS, Livestock Behavior Research Unit, West Lafayette, IN.*

Animal agriculture is under seemingly opposing pressures from society. On one side is the increasing population and an increasing global demand for food and especially animal products. On the other are societal concerns about sustainability, animal agriculture's contribution to global warming and climate change, food safety and the ethics of animal production from a welfare perspective. As animal welfare scientists, we often sit comfortably within our own bubble, looking at animal welfare issues within a given production system or a specific management procedure. It is increasingly important that we scale up; that we seek to recognize and explain how our research fits into the bigger picture and how it helps address the grand challenges facing agriculture in general and animal agriculture specifically, and that we engage in multidisciplinary studies that are addressing these grand challenges directly. As animal agriculture evolves, animal welfare must be part of the conversation. In some countries, there is pressure to intensify animal production, for example increasing feedlots and zero-grazing ; in others, the pressure is to extensify, to move to year-round grazing or develop silvopastoral systems. Climate change is increasing heat stress and severe weather events and changing the geographical distribution of vectors and the severity of outbreaks of livestock diseases. Increasing demand for animal protein is driving an increase in live export, especially to developing countries where, after long sea journeys, animals may be processed through slaughterhouses with questionable standards of operation. All of these are exposing more livestock to situations that can impact their welfare and, in all scenarios, animal welfare scientists have a role, or rather, a duty to engage and work to mitigate negative impact and develop production systems that are environmentally, economically and socially sustainable.

Reproduction Symposium: Prebreeding Predictors of Fertility

S173 Use of multiple biological, management, and performance data for the design of targeted reproductive management strategies for dairy cows. J. Giordano*, *Department of Animal Science, Cornell University, Ithaca, NY.*

As the reproductive efficiency of dairy cattle continues to improve in response to better management and use of technology, novel reproductive management approaches will be required to improve herd performance, profitability, and sustainability. A potential approach currently being explored is "targeted reproductive management." This approach is based on the possibility of identifying cows with different reproductive and performance potential using multiple traditional and novel sources of biological, management, and performance data. Once identified, subgroups of cows with unique biological and performance features are targeted with management strategies specifically designed to optimize cow performance, herd profitability, or alternative outcomes of interest. Major steps in the development of targeted reproductive management programs are (1) identification of sources of variation in cow performance; (2) creation of management strategies to optimize outcomes of interest for subgroups of cows; (3) development, evaluation, and deployment of tools for on-farm implementation. Areas currently explored for targeted management include use of genomic predictions and data from on-farm sensors that monitor behavioral, physiological, and performance parameters. Recent research demonstrated that cohorts of cows with different reproductive performance and response to programs that favored AI at detected estrus or timed AI were identified using genomic predictions. Similarly, cows that became pregnant to first service had signature patterns of sensor parameters before AI. Thus, sensor data might be used to predict the likelihood of pregnancy. Once the most valuable predictive sources of variation are identified and their impact quantified, novel analytic methods (e.g., machine learning) for prediction will likely be required. These tools must identify groups of cows for targeted management in real time and with no human input. Despite some encouraging research evidence supporting the development of targeted reproductive management strategies, extensive work is required before implementation.

Key Words: targeted management, predictions, fertility

S174 Genomic markers of fertility in Holstein heifers. J. C. Dalton*[1], J. N. Kiser[2], E. M. Keuter[2], C. M. Seabury[3], M. Neupane[2], J. G. N. Moraes[4], G. W. Burns[6], T. E. Spencer[5], and H. L. Neibergs[2], [1]*University of Idaho, Animal, Veterinary and Food Sciences Department, Caldwell Research and Extension Center, Caldwell, ID,* [2]*Washington State University, Department of Animal Sciences, Pullman, WA,* [3]*Texas A&M University, Department of Veterinary Pathobiology, College of Veterinary Medicine, College Station, TX,* [4]*Oklahoma State University, Department of Animal and Food Sciences, Stillwater, OK,* [5]*University of Missouri, Division of Animal Sciences, Columbia, MO,* [6]*Michigan State University, Department of Obstetrics, Gynecology and Reproductive Biology, Grand Rapids, MI.*

Over the past 70 years, selection for increased milk production in dairy cattle has been very successful; however, fertility remains a challenge as the average Holstein heifer conception rate (HCR) to first artificial insemination (AI) in the United States is estimated at 55 to 60%. Taken together with the cost to raise heifers, which has been higher than the value of heifers at calving in recent years, it is important to investigate opportunities to increase efficiencies in heifer selection that will provide replacements of increased value. A current area of opportunity is to use genomic technology to improve heifer fertility. Unfortunately, few loci associated with HCR have been identified and validated. Consequently, objectives were 1) to identify loci associated with HCR to first AI (HCR1), and 2) to validate loci previously associated with fertility (Kiser et al., 2019 *BMC Genomics* 20:576). Blood samples were collected from Holstein heifers (n = 3,359) housed in pre-breeding pens at a commercial heifer raising facility. Records were obtained after AI to conventional semen at observed estrus, with pregnancy determined at d 35 via palpation of uterine contents. Deoxyribonucleic acid, previously extracted from blood samples, was genotyped using the Illumina Bovine HD BeadChip. Genome-wide association analyses (GWAA) were performed with additive, dominant, and recessive models using the Efficient Mixed Model Association eXpedited (EMMAX) method with a genomic relationship matrix. The GWAA compared heifers pregnant to first AI (n = 497) to heifers pregnant on or after fourth AI (n = 405), which also included heifers that never conceived. Genotyped heifers included similar proportions of the tails of the phenotypic distribution represented by highly fertile and subfertile or infertile heifers. The GWAA identified 228 loci associated with HCR1. Loci previously associated with fertility were in linkage disequilibrium with 5 HCR1 loci and were considered the same locus for validation. Loci associated with HCR1 have been identified and validated and may be used to improve HCR through genomic selection. Project supported by Agriculture and Food Research Initiative Competitive Grant 2013-68004-20365 and 2018-67015-27577 from USDA NIFA.

Key Words: fertility, genomics, heifers

S175 Using leukocyte transcriptome analysis for predicting fertility potential in heifers. F. Biase*, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Artificial insemination is a preferred breeding method for heifers as it advances the genetic background of the herd. Reproductive efficiency in heifers is key for the success of beef and dairy production systems. In beef operations, heifers that do not become pregnant at the end of the breeding season represent an irreparable economic loss. Likewise, dairy heifers may yield optimum productivity and profit when calving between 22 and 24 mo depending on the rearing costs, with considerable economic losses with delayed age at first calving. Based on recent farm data, over 10% of heifers do not become pregnant and are culled from the herd, posing economic loses to the farmers and reducing the sustainability of productions systems. We have been working on the development of means to assist the decision-making process of reproductive management of heifers. Using liquid biopsy, we have profiled the transcriptome of peripheral white blood cells of beef heifers at the time of artificial insemination in 2 consecutive breeding seasons. Data from both years show that transcript levels for several protein-coding genes are statistically significant among heifers that become pregnant to artificial insemination, pregnant to natural breeding, or did not become pregnant. Next, we posed the hypothesis that data from one breeding season can be used to predict the reproductive outcome of the next breeding season. We focused on 2 independent sets of heifers bred on consecutive years (2015 and 2016) at the same farm, that became pregnant by artificial insemination or did not become pregnant. Using machine learning algorithms, we determined that the data from 2015 could predict the next year's breeding outcome with high accuracy. Taken together our work has demonstrated the need for the development of new tools for assisting the reproductive management in heifers. One promising venue for helping producers to identify heifers that have low reproductive potential is the profiling of bloodborne transcripts. We have advanced the efforts to develop the molecular profile of heifers and the results are promising.

Key Words: infertility, heifer, transcriptome

S176 Understanding endometrial function for early pregnancy success in catttle.

N. Forde*, *University of Leeds, Leeds, West Yorkshire, UK.*

In cattle the majority of pregnancy loss occurs in the first 3 weeks of pregnancy during which time the embryo is susceptible to the impact of altered metabolite concentrations in circulation associated with nutritional stress in postpartum dairy cows. If we are to enhance pregnancy success, and ensure that offspring are robust, a key area that requires attention is understanding the mechanism by which changes in maternal circulation alter ability of the endometrium to support early pregnancy and development. The role of the specific stressors on endometrial function and consequences for embryo development are less well understood. This presentation will focus on (1) how changes to hormones and nutritional stressors in maternal circulation alter the interactions between the developing embryo and the maternal environment; (2) demonstrate the development and use of an endometrium-on-a-chip these to understand these interactions; (3) understanding how novel proteins produced by the conceptus alter the endometrium to facilitate receptivity to implantation, and 4) how extracellular vesicles from embryos with different developmental potential contain different cargo which may modify the molecular communication between the developing conceptus and the endometrium. Collectively these data may shed light on why pregnancy loss occurs or indeed where we can intervene to support successful pregnancy in dairy cattle.

Key Words: endometrium, conceptus-maternal interactions, uterus

Ruminant Nutrition Symposium: Bioavailability of Amino Acids—Methods and Lessons Learned

S177 The plasma dose response technique: Its application in determining relative bioavailability of rumen protected amino acids. N. L. Whitehouse*, *University of New Hampshire, Durham, NH.*

The plasma-dose response technique (slope-ratio assay) has been used in swine amino acid nutrition to determine the effects that supplying limiting amino acids has on plasma amino acid concentrations in sows and growth in young animals. For the plasma free AA dose-response technique to be a widely accepted method for determining relative bioavailability (RBV) of RPAA supplements, a positive linear response between increasing amounts of infused or fed AA and plasma AA concentrations must be unequivocally established. This has been shown in literature for both Met and Lys. Rulquin and Kowalczyk in 2003 were the first to use the plasma free AA dose-response technique to measure bioavailability of Lys and Met in rumen protected AA (RPAA) supplements. Their work showed linear responses for both Lys and Met with a large amount of cow variation for Lys. The plasma-dose response technique used today differs for Rulquin and Kowalczyk in that it is replicated Latin square design with 7 d periods and the cows receive all treatments. The RPAA supplements are placed in TMR for 8 h before being feed to the cows and infusions are continuously infused into the abomasum vis the rumen cannula, except for when cows are being milked. The diet is balanced to meet the nutritional needs for the cow so that energy is not a limiting factor. Using this technique, 39 Lys products have been evaluated with their RBV ranging from 5 to 85% and 17 Met products with their RBV ranging 15 to 80%. Therefore, the plasma free AA dose-technique is a robust, but simple, method of providing accurate and reliable estimates of bioavailability starting with product evaluation begins with addition to ration. Linearity of response is the only required assumption; proven for the most limiting AA and minimal steps and calculations are involved, minimizing experimental error.

S178 The isotope dilution technique: Its application in determining bioavailability of amino acids. M. D. Hanigan*, *Virginia Tech, Blacksburg, VA.*

Determining the fraction of each amino acid (AA) in a feed ingredient that is absorbed is technically difficult in ruminants due to microbial remodeling of protein, digestive tract anatomy, and endogenous AA flows. Measurements of disappearance from the small intestine have rarely been undertaken and have been observed to have 25 to 50% error of determination. Milk protein responses also typically have large errors of determination and nonlinear responses that complicate derivations. Plasma AA concentration responses generally have errors of determination of 5 to 12% for ruminally protected Met products, but are 20% or more for Lys when each is supplemented in relatively large quantities. The lower error for Met is due to the slow conversion of the D-isomer to the L form and thus larger concentration responses. Isotope dilution-based measurements typically have lower errors of determination due to the high precision of mass spectrometry. The method makes use of a constant infusion of a mix of ^{13}C-labelled AA into the jugular with blood sampling over a 2- to 8-h period. Blood AA enrichment is inversely proportional to AA absorption, and the latter can be directly derived from the dilution data. With careful animal feeding management and supplementation of the AA of interest to achieve at least a 10% increase in total supply, errors of determination are generally less than 15%, and all of the essential AA can be simultaneously assessed. Doubling the proportional supplementation results in a halving of the error of determination. Because the isotope is infused into the jugular vein, true AA entry is slightly underestimated due to first-pass splanchnic use. Abomasal infusions of crystalline essential AA provides that loss, which averages just under 8%, allowing correction of the measured appearance. Essential AA bio-availabilities were derived for feather meal and blood meal with approximately 10% errors of determination when substituting only 2% of the diet. Based on these results, the method appears to be inherently more precise than other methods of assessment and is applicable to all of the essential AA.

Key Words: amino acid, bioavailability, ruminant

S179 Ruminant feed amino acid bioavailability estimates through the use of old and new classical techniques. G. I. Zanton*, *USDA-ARS, Dairy Forage Research Center, Madison, WI.*

Dairy cattle N use efficiency (NUE) is often in the range of 25 to 35% of ingested N secreted as milk protein N. Improving NUE without reducing productivity can occur through either reducing N intake, increasing metabolizable protein (MP) quality and amino acid (AA) composition, or both while maintaining or increasing milk protein N. Although the biological requirement is for metabolizable AA, most protein feeding recommendations for dairy cattle are based on MP, which is the sum of small intestinally digestible and absorbed protein from ruminal microbes, rumen undegraded feed, and endogenous sources. Bioavailability is a concept that has been used and studied for many years and is defined by Ammerman et al. (in Bioavailability of Nutrients for Animals, 1995) as “the degree to which an ingested nutrient in a particular source is absorbed in a form that can be utilized in metabolism by the animal.” As applied in the context of the MP system, feed AA bioavailability refers to digestible rumen undegradable protein (or digestible, rumen escape AA). There are various methods that have been developed over time to predict feedstuff or rumen escape AA bioavailability based on in vivo, in situ, or in vitro methodologies. Each of these methods have strengths and weaknesses in accurately and precisely predicting AA bioavailability for use in formulation models to match absorbable AA supply with demand and optimize NUE. The objective of this presentation is to discuss the concept of bioavailability as applied to the dairy cow, review some of the more classical techniques for estimating AA bioavailability, and to emphasize their strengths and weaknesses.

Key Words: bioavailability, methods, ruminant

Teaching/Undergraduate and Graduate Education Symposium: Education and Workforce Development in the Online Learning World

S180 Undergraduate student perceptions of 2 terms of remote delivery of course-work due to COVID-19 restrictions. F. Robinson*, M. Johnson, and W. Adamowicz, *University of Alberta, Edmonton, AB, Canada.*

The transition to remote teaching due to COVID-19 restrictions partway through the Winter 2020 term resulted in an abrupt change in teaching methods at the University of Alberta. Following that, during the Fall 2020 term, course materials were presented mostly online, with some in-person laboratories. Two online, anonymous surveys were conducted, one at the end of each term, to elicit responses from undergraduate students in Agricultural, Life and Environmental Sciences to learning in this online environment. Responses were received from 432 students (41.6% response rate) in the Winter 2020 term and from 347 students (20.9% response rate) in the Fall 2020 term. In both surveys, students cited that they lacked motivation and experienced difficulty in staying engaged in online classes, (Winter term: 35.1% of respondents; Fall term: 72.9%). Some students reported experiencing stress, anxiety, and, in some cases, other mental health issues (Winter term: 5.8%; Fall term: 73.2%). During both terms, students tended to favor synchronous online courses that were recorded. The recording of lectures was appreciated due to shared computer access, internet/hardware issues and varied home/life situations. In the Winter term, the abrupt switch was most challenging to students because of reported poor communication with professors, resulting in confusion in expectations (25.5%). Other significant challenges faced by students during the subsequent Fall term were exams and exam proctoring (46.7%), group project work (42.1%), and internet connectivity issues (35.2%). Students were asked to list issues they wanted instructors to be aware of. Generally, the responses included the following: be considerate and accommodating, do not increase the workload or make exams harder than usual, record lectures, be aware of students in different time zones and with varying internet connectivity, be engaging and communicate effectively. In the Fall term survey, 53.0% of students stated that they thought that instructors were doing their best to present a high-quality online teaching experience.

Key Words: online learning, COVID-19, pandemic

S181 Any concerns of COVID-19 pandemic-related impacts on post-secondary curricular and co-curricular academic programs, the education and development of students, and success of future graduates entering the workforce? J. M. Fernandez* and A. D. Goecker, *College of Agriculture, Purdue University, West Lafayette, IN.*

Students pursuing college degrees in agriculture (Ag) and related disciplines obtain hands-on, practical application type of experiences during school, improving their employability in agriculture jobs. The recent release of the "USDA NIFA Employment Opportunities for College Graduates in Food, Agriculture, Renewable Natural Resources and the Environment, United States, 2020-2025" (Fernandez et al., 2020. https://www.purdue.edu/usda/employment/) reports an average of 59,400 open positions annually for graduates (a 2.6% increase in job opportunities from previous report). Of these, 61% will be filled by graduates with Ag and related degrees, and the remainder (39%) by non-ag graduates. The proportion of positions will be: 42% business and management; 31% science and engineering; 14% education, communication and government services; and 13% in food and biomaterial production. In a study conducted by APLU ("From Academia to the Workforce: Critical Growth Areas for Students Today." Crawford, P., and W. Fink. 2020. APLU, Washington, DC. https://www.aplu.org/library/from-academia-to-the-workforce-critical-growth-areas-for-students-today/file), employers identified the top 3 skills deemed most important for success in the workplace: (1) listen effectively, (2) communicate accurately and concisely, and (3) identify and analyze problems, and the top 3 skills they perceived as showing the largest preparedness gap in new hires: (1) understand role and realistic career expectations, (2) recognize and deal constructively with conflict, and (3) accept critique and direction in the workplace. The question we must wrestle with today is how has the pandemic affected the delivery of quality educational and co-curricular programing, how has this impacted student preparedness, and what impact might this have on graduates as they enter the agricultural workforce?

Key Words: education, workforce development, COVID-19

S182 Discussion-based strategies for remote teaching and learning. M. Wattiaux*, M. Erickson, and D. Pizarro, *Department of Animal and Dairy Sciences, University of Wisconsin, Madison, WI.*

Remote learning has become a dominant teaching modality at US universities following COVID-19 restrictions. Early pandemic research highlighted the challenges of maintaining the quality of student-student and student-instructor interactions. In response, we implemented discussion-based learning in 2 upper-level (1- and 3-credit; spring 2020) and 1 introductory (3-credit; Fall 2020) animal sciences courses after converting to remote instruction. Our teaching methods included (1) employing a "flipped" classroom with pre-class readings, quizzes, and open-ended prompts followed with expectations and working agreements for in-class discussion; (2) designing lesson plans to communicate learning objectives and supporting activities; (3) using breakout rooms, Google Docs, and other technologies to structure discussion described in lesson plans. In spring, we surveyed course participants to explore their perceptions of discussion-based learning practices. Participants in the upper-level courses provided feedback in a mixed-method survey based on the Community of Inquiry Framework (n = 44, response rate = 84.6%). These upper-level course respondents quantitatively expressed perceiving high social presence, cognitive presence, and teaching presence in discussion-based sessions. Their qualitative responses revealed contrasting beliefs and expectations about remote learning affecting engagement and satisfaction. Fall introductory course students participated in a survey experiment assessing specific pre-class reading activities designed to prepare for discussion (n = 77, response rate = 96.3%). Overall, introductory course participants expressed moderate self-efficacy for engaging in academic discussions with their peers (means = 3.3, SD = 0.9, anchored scale from "1; not at all" to "5; extremely" confident) and displayed more favorable motivational profiles with open-ended reading support activities. Our results suggest that careful design and implementation of student-centered activities for before, during, and after "flipped" classes substantially influenced student perceptions of engagement and satisfaction in remote discussion-based learning.

Key Words: remote learning, discussion, teaching

S183 Experiences in engaging undergraduate students in dairy club activities online.

L. Hansen*, *Department of Animal Science, University of Minnesota, St. Paul, MN.*

Almost all courses at the University of Minnesota were taught remotely after spring break for spring semester 2020, during fall semester 2020, and during spring semester 2021. For the 2020–21 academic year, the Gopher Dairy Club moved to Zoom for its monthly meetings. The meetings were conducted amazingly well with excellent attendance and participation of the club's 90 members. Summer internship reports were presented during the Zoom monthly meetings as usual, which is very valuable for new members of the club. The business meetings and planning for allowed events continued as usual. However, many of the routine activities of the Gopher Dairy Club were canceled. Most noteworthy was the Gopher Dairy Bar (sales of milk shakes) at the Minnesota State Fair, which is the major source of income for the club. However, the club maintains adequate reserves, so it was able to continue with all activities that could take place. Club activities that were canceled included hosting the state FFA dairy cattle evaluation contest, hosting the Gopher Dairy Camp for junior high youth, hosting the annual alumni reunion during the Minnesota State Fair, traveling to World Dairy Expo, hosting a fall invitational FFA dairy judging contest, organizing an annual career fair that includes a sit-down dinner for animal-interest students called the Food Animal Networking Evening, implementing the annual 12-d senior trip to California, participating in the Midwest Regional SAD-ADSA, and hosting the club's annual recognition banquet. However, all committee structures were maintained through the pandemic, so these annual activities will be able to be reinstituted for 2021–22.

Key Words: dairy club, COVID-19, extracurricular engagement

S184 Student and volunteer experiences in virtual Dairy Challenge events.

A. Carpenter*, *Department of Animal Science, Iowa State University, Ames, IA.*

The North American Intercollegiate Dairy Challenge Inc. is responsible for a series of dairy farm evaluation contests held at the regional and national level. In 2020–2021, the regional Dairy Challenge contests were held virtually. Student debriefing notes and judge and mentor surveys are collected anonymously each year. Surveys were collected from the Midwest judges (n = 13) and mentors (n = 14) as well as the student debriefing sessions from the Midwest (n = 15), Southern (n = 13), and Western (n = 9) regions. Judges in the Midwest contest unanimously agreed that students were adequately prepared for the contest. The majority of mentor respondents agreed that students were able to engage and commit to participation (85%) and that students were successful in their efforts to evaluate a working dairy (93%). The majority of judge respondents (62%) indicated that the slower delivery of the Midwest contest was "about right," while the remaining respondents (39%) indicated that it was "too long." The majority of mentor respondents (79%) indicated that the slower delivery of the Midwest contest was "about right," while only 21% indicated that it was "too long." All teams across regions indicated that the judges' comments were beneficial. For the Midwest contest, 81% of teams indicated that they participated in the career fair, and 94% indicated that they were satisfied with sponsor interactions; however, in the Southern contest, only 54% of teams participated in the career fair, with 78% of teams indicating that they were satisfied with sponsor interactions. The Southern contest was held first, and the Midwest planning committee was informed by the Southern region's challenges and successes. For the Western contest, 78% of teams indicated that they planned to participate in the career fair, and 44% of teams indicated that they were satisfied with sponsor interactions, although debriefing information was collected for the Western region before the career fair was held. Overall, student reviews on the virtual format were mixed, with multiple teams indicating that the contests were well-run for virtual format but an in-person format was preferred.

Key Words: Dairy Challenge, COVID-19, extracurricular engagement

CSAS Milk Synthesis Symposium: Unlocking the Mechanisms of Milk Protein and Fat Synthesis

S185 Impact of post-absorptive energy source on mammary gland metabolism. K. Nichols*[1,2], [1]*Animal Nutrition Group, Wageningen University and Research, Wageningen, the Netherlands*

[2]Trouw Nutrition R&D, Amersfoort, the Netherlands

Increased motivation to optimize resource use efficiency and implement precision-feeding of dairy cattle has elevated the importance of understanding the effect of not only energy level but also energy source (lipogenic, glucogenic, or aminogenic) delivered from feed components on the efficiency of milk synthesis. Variable efficiencies of individual amino acid (AA) use by dairy cattle have been recognized, but knowledge gaps remain around the dynamics of these efficiencies with respect to dietary lipogenic and glucogenic energy, and how post-absorptive macronutrient supply can be most effectively managed in a feeding scheme for dairy cows. A series of recent studies utilized arteriovenous difference methodology and climate respiration chambers to characterize the effect of lipogenic and glucogenic energy at low and high metabolizable protein (MP) levels on mammary gland metabolite utilization and whole-body nitrogen (N) and energy partitioning. These studies determined the differences in mammary gland metabolite utilization stimulated by glucogenic and lipogenic energy, and their interactions with MP level. This work shows that the source of post-absorptive energy will affect N and energy partitioning between body storage, milk synthesis, and excretion, and that this partitioning was generally independent of MP level. Increased absorptive supply of fatty acids directed energy metabolites into milk, largely did not impact mammary AA metabolism, and increased milk N efficiency (milk N/feed N) at low MP levels. Increased absorptive supply of glucose increased the efficiency of group 2 AA (Ile, Leu, Val, Arg, Lys, Thr) sequestration by the mammary gland, and increased milk N efficiency regardless of MP level. Overall, dairy cattle display impressive flexibility to synthesize milk protein, fat, and lactose from lipogenic, glucogenic, and aminogenic substrates.

Key Words: glucogenic, lipogenic, nitrogen efficiency

S186 An update on amino acid metabolism in support of lactation: The barrel has some rotten staves. M. D. Hanigan*, *Virginia Tech, Blacksburg, VA*

Mitchell and Block (1946) based their conceptual model of amino acid (AA) limitations and order on earlier soil fertility work (Whitson and Walster, 1909). The model assumed fixed efficiencies of conversion, which can be true for a narrow range of inputs when applied to AA conversions to milk protein, but is not true for the full range of feeding conditions. Variable AA efficiency is driven by responses of splanchnic and mammary tissues to the supply and composition of absorbed nutrients. Marginal use of essential AA by the portal-drained viscera increases as absorbed flux increases, but total apparent use increases substantially if blood concentrations also increase due to catabolism from arterial blood. Marginal hepatic use from arterial and portal blood also increases as supply increases except for the branched-chain AA which are not directly catabolized by the tissue. Mammary uptake of AA is quite variable and driven by both supply and tissue needs. In general, uptake efficiency declines as supply of AA increases thus returning a greater proportion of AA to general circulation. Efficiency of removal is generally not greater than 60% for essential AA, but can vary from 10% to greater than 90% depending on supply and mammary demand. Mammary tissue demand is driven by tissue potential, but regulated by the supply of energy yielding substrates, several essential AA, and one or more hormones. Based on cell culture experiments, regulation appears to occur via the mTOR system with additive and independent effects of several essential AA, energy supply, and insulin, but in vivo confirmation of mTOR action has been elusive. Despite this limitation, experimental and simulation evidence of independent effects of essential AA on milk protein are extensive and conclusive. Observed performance responses to provision of individual AA are inconsistent with the first-limiting AA concept, and use of such a concept to predict performance results in very biased predictions. Conversely, representing the effects of energy and 7 essential AA on milk protein production results in unbiased predictions with high precision.

S187 The role of leaky gut and inflammation on milk component losses during heat stress: A mechanistic view. D. E. Rico*, *CRSAD, Deschambault, QC, Canada.*

Observational data in lactating dairy cows show reduced synthesis of milk fat, protein, and lactose in the summer months. This may be associated with changes in several factors including heat stress, forage quality, photoperiod, and even herd composition. Importantly, controlled studies show similar reductions in milk components under heat stress conditions, suggesting that heat stress may be a central factor explaining component losses observed in the summer. Of note, heat stress reduces blood flow to the intestine and thus, it increases intestinal barrier permeability (i.e., leaky gut), resulting in increased systemic inflammation and altered nutrient partitioning. Recent studies suggest that immune system activation in response to leaky gut may play a central role in explaining component losses. Indeed, a hyperactivated immune system will utilise key nutrients such as glucose and amino acids, thus reducing their availability for mammary synthesis of lactose and protein. In addition, substrates for mammary lipid synthesis, such as long-chain fatty acids (e.g., 18:0) from adipose tissue are reduced. This occurs concomitantly with increased concentrations of plasma insulin, despite the commonly observed reductions in dry matter intake during heat stress. This seminar will review the potential for nutritional interventions to mitigate the impacts of heat stress on lactation performance and gut health of dairy cows. The use of milk fatty acid profile as a tool to diagnose heat stress will also be discussed.

Key Words: heat stress, fat, protein, lactose

Dairy Foods Fall Webinar: The Future of Probiotics

W100 Dairy and probiotics pairing for intestinal health. M. Marco*, *University of California, Department of Food Science and Technology, Davis, CA.*

Yogurt and other fermented dairy products are the most common food carriers of probiotic bacteria to the human digestive tract. Despite the popularity of these foods, the importance of the milk matrix for conferring probiotic efficacy is not well understood. This lack of knowledge jeopardizes the continued use of dairy as the preferred food for probiotic delivery. Our studies are addressing this question by investigating the separate and combined effects of milk and probiotic *Lacticaseibacillus casei* on inflammation and epithelial barrier integrity in the digestive tract. Milk alone was sufficient to reduce intestinal inflammation and improve recovery in a mouse model of colitis. This effect was altered when milk and *L. casei* were combined, thereby showing how the anti-inflammatory properties of milk change when consumed with certain probiotic strains. Similarly, only the combination of milk and *L. casei* resulted in prevention of epithelial damage caused by proinflammatory cytokines in cell culture in vitro. Taken together, these findings show the positive role of dairy in the digestive tract and provide opportunities for optimizing the preparation and delivery of probiotics in foods tailored for specific health outcomes.

Key Words: probioitics, yogurt, *L. casei*

W101 The biological relevance of prophages in the probiotic gut symbiont *Limosilactobacillus reuteri* is context-dependent. J. P. van Pijkeren*, *University of Wisconsin, Madison, WI.*

Lysogens are bacteria that contain dormant phages (prophages) inserted into their genome. Many lactic acid bacteria, including probiotics, are lysogens. Prophage activation and subsequent virion production can impact individual cells and community composition. However, our understanding of lytic-lysogenic dynamics is limited to nonexistent in probiotic bacteria. Acquiring such knowledge is important to optimize biotechnological processes, but it will also reveal important insight into the ecology of probiotics. Using in-house developed genome editing tools, we established the probiotic gut symbiont *Limosilactobacillus reuteri* as a model to study its prophages. We uncovered that *L. reuteri* prophages are activated during gastrointestinal transit and that phage production is further increased in response to a fructose-rich diet. Fructose metabolism and exposure to acetic-, butyric-, propionic- but not lactic acid boosted phage production via the AckA pathway. To understand to what extent *L. reuteri* prophages impact ecology, we studied *L. reuteri* and its isogenic mutants lacking prophages in conventional and gnotobiotic mice. We found that, in conventional mice, phage production reduces bacterial fitness. However, in gnotobiotic mice, phage production provides *L. reuteri* with a competitive advantage over a sensitive host. In conclusion, our findings may be leveraged toward optimizing industrial production of probiotics and open up previously unexplored avenues to develop dietary approaches to promote phage production toward probiotic engraftment in the intestine.

Key Words: probiotics, lysogens, bacteria

W102 What is a *Lactobacillus*? The why, who, and how of the reclassification of the genus *Lactobacillus*. M. Gänzle*, *University of Alberta, Edmonton, AB, Canada.*

Why? In March 2020, the genus *Lactobacillus* comprised 260 species that displayed a level of diversity that by far exceeded the level occurring in other bacterial genera and even families. Core genome phylogenetic analysis of lactobacilli after 2015 revealed the discrepancy between the taxonomy of these genera and other bacterial taxa [1–3]. This discrepancy impeded research aimed at understanding the ecology, physiology, evolution and applications of this important group of organisms. The lack of appropriate taxonomic terminology also impeded regulatory approaches to assess the safety of lactobacilli [4] because species that are genetically distinct and metabolically diverse are assigned to the same genus. Who? In October 2018, a team of 15 scientists including groups from the University of Verona, University College Cork, University of Antwerp, the University of Alberta and Huazhong Agricultural University joined forces to re-evaluate the genetic relatedness and phylogeny of the species within the genus *Lactobacillus* and its sister taxa through a polyphasic approach that includes core genome phylogeny as well as metabolic and ecological traits of the organisms. The proposal for the reclassification of lactobacilli was published in April 2020 [5]. How? We considered average amino acid identity (AAI) and core-gene average amino acid identity (cAAI), core genome phylogeny, signature genes, and metabolic as well as ecological traits. Within the lactobacilli, 26 clusters or single-species lineages were identified that were reliably separated and share conserved phenotypes and clade-specific genes. Twenty-three of these clades were described as new genera, the description of the genus *Lactobacillus* was emended and the genus *Paralactobacillus* was revived. This communication will describe the team effort to achieve the taxonomic re-assessment of lactobacilli and outline the impact on research and the regulatory approach on lactobacilli after the online publication in April 2020. References: [1] *Appl. Environ. Microbiol.* 81:7233; [2] *Nat. Commun.* 6:8322; [3] *FEMS Microbiol. Rev.* 41:S27; [4] *EFSA J.* 16:5206; [5] *Int. J. System. Evol. Microbiol.* 70:2782.

Equids Milk Production Fall Webinar: Scientific Challenges and Perspectives

W103 Milk production by mares and jennies: From history and traditions to future perspectives. K. Potocnik*, *University of Ljubljana, Biotechnical Faculty, Department of Animal Science, Ljubljana, Slovenia.*

Equid milk (EM) is produced by mares and jennies. Traditionally, horses are bred in cold climates and donkeys in arid regions. Consequently, the production of mare's milk (MM) is linked to the regions of Central, Northern, and Eastern Europe, as well as the steppes of Asia. Jennie's milk (JM) production is present in the arid climates of Southern Europe and Asia. The characteristics of MM and JM are very similar to each other, and their nutrient content is considered to be the closest to human milk. A decade ago, archeological research found that the Botai culture used MM during the era of horse domestication. Written historical records of EM use date back to 1000 BC. Historical sources describe EM's beneficial effects on human health. Despite the lack of modern research to confirm or refute these benefits, we can conclude from the available scientific literature that EM has many bioactive nutrients that have a positive effect on human health. Recently, the importance of prebiotic effects in alleviating the symptoms of autoimmune diseases has been increasingly emphasized. Public recognition of these facts has increased market demand for EM in the past decades. In countries that have traditionally produced EM, this milk is often used as preventive care for its health benefits. On the other hand, European and North American consumers of EM tend to be patients with chronic diseases. There are many challenges in the production and processing (PP) of EM. Guidelines for EM PP are practically nonexistent. From the hygienic point of view, the PP practice of EM production in the Euroasian steppe is unacceptable to the standards of Europe and North America. Moreover, the EM production sector is unorganized. In Europe, it is based on individual breeders who do not cooperate. The prospects of the EM sector lie in integration and cooperation between all stakeholders. They need to raise public awareness about the characteristics of EM and harmonize the PP methods of EM production. Research on EM effects on human health, as well as research of the PP methodology of this valuable food, will play a crucial role in the future development of the sector.

Key Words: equid milk, alternative use, niche production

W104 Mammary gland physiology and management of dairy mares and jennies. P. De Palo*, *University of Bari A. Moro - Department of Veterinary Medicine, Valenzano, Italy.*

The constantly increasing trend of consumers' aptitude for food sustainability, food functional effects, social role of agricultural activities, traditional food, biodiversity, represents a great opportunity for the introduction on the market of milk and dairy products from equids. This opportunity explains the change of trend of head numbers bred in some areas (i.e., Europe), as well as the increasing interest toward these topics by scientific research. Even if the market accepts these new/ancient foods, milk production from donkeys and warm blood horses presents several issues due to the unavailability of deep knowledge, also due to the totally different structure and physiology of lactation in these animals compared with dairy ruminants. The lecture will focus mainly on the principal issues regarding farm management of dairy jennies and mares. After a brief overview on the main morphological and physiological aspects of mammary gland and galactopoiesis, the lecture will highlight the main challenges for improving milk yield and quality, moving from the recent knowledge to the future perspectives for scientific research and for the farm management. Provided data and information will highlight also the very large differences between horse and donkey species with regards to milk yield. The main areas that need more and more investigation for supporting in a concrete way this supply chain are linked to the milking technologies. What do we already know about milking frequency and mechanical milking technical parameters? What do we know about jennies' adaptation to milking parlor and to milking routine? Moreover, what about the management of the foals? Do they have to naturally suckle from their dams or there are some innovations that allow farmers to increase milk yield for selling, without worsening both galactopoietic patterns and animal welfare and performances? Another main issue is linked to the need of increase knowledge on the relation between milk yield, metabolism, nutritional requirements and feeding strategies in lactating jennies and heavy breed mares.

Key Words: dairy equids, management, welfare

W105 Valorization of donkey milk: Technology, functionality, and future prospects. P. Papademas*, M. Ioannou, P. Mousikos, and M. Aspri, *Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Limassol, Cyprus.*

Donkey milk is considered by many to be the best alternative to human milk, especially for cow's milk protein (CMPA) allergy infants, due to its unique chemical composition (nutrients and bioactive compounds), good palatability and clinical tolerability. As the milk's bioactive compounds (i.e., lysozyme, immunoglobulins) will include constituents with antimicrobial, antioxidant and immunomodulatory activities, donkey milk should be destined for consumption primarily by special consumer groups (i.e., the elderly, infants, immunocompromised). Therefore, donkey milk valorization technologies should be carefully designed to produce a safe for consumption milk and to (a) preserve or even enhance the functional (i.e., potential bioactivity) properties and (b) increase the shelf life of the end product as daily milk production is minimal. The fermentation of donkey milk by carefully selected/characterized indigenous microflora and/or by commercial probiotic bacteria could enhance the milk's nutritional value and prolong its shelf life. Additionally, there is a growing interest for minimal processing of foods in recent years, therefore another aim of our studies was to produce a minimally processed freeze-dried donkey milk powder where the inactivation of pathogens was achieved by a novel non-thermal turbulent flow UV process. The effect of processing (i.e., UVC milk treatment, freeze-drying, fermentation) on key milk constituents was also studied after in vitro digestion using internationally accepted protocols (INFOGEST 2.0), to reveal the fate of any biological activity after the consumption of the product. The future study of donkey milk involves the better understanding of the milk constituents' interactions and their effect of biological activities by combining carefully designed clinical studies.

Key Words: donkey milk, fermentation, minimal processing

W106 Functional role of donkey milk, well-being, and health: Which evidence in humans? M. Serafini*, *Faculty of Bioscience and Technologies for Agricultural, Food and Environment, Teramo University, Teramo, Italy.*

The use of donkey milk (DM) for human consumption is known from ancient times, however only during the last few years there has been an increasing interest of the scientific community for its nutritional and functional properties. Composition of DM is very similar to human milk (HM) in terms of gross composition and of protein profile. DM has a high palatability, stimulates intestinal absorption of calcium, providing an excellent substrate for the development of enteric flora. DM has a lower fat content than HM, showing a more favorable fatty acid (FA) composition, as it is richer in polyunsaturated FA (PUFA), higher concentration of C18:3n-3 and n-3 FA, and a lower saturated FA (SFA) content than CM, as well as a lower n-6 to n-3 FA ratio. DM has been shown to exert antioxidant and immuno-modulating properties and to release nitric oxide, a potent vasodilator, in cells of immune system. On these bases, DM represent a promising "functional" food, potentially able to modulate lipid function, human microbiota, oxidative stress and antioxidant status, cardiovascular and immune function. However, despite the promising nutritional and functional properties, the available literature reveals a striking lack of evidences in humans, with only few successful trials testing, in infants with protein-induced enterocolitis and intolerance, children with cow's milk allergy and elderly. According to the EC regulation No 1924/2006 on nutrition and health claims on foods, human-based evidence is fundamental for establishing a functional effect of a food. The evidence in human is crucial because variables like bioavailability and homeostatic mechanisms, play a key role in determining the efficacy of the tested food. In this work, emphasis will be placed on the nutritional and functional properties of DM in the food matrix and on its ability to modulate lipid, redox, immune status in vivo, reviewing the body of evidences obtained in humans. Increasing the scientific knowledge about the functional effect of DM at different stages of human life is crucial to provide practical advices for DM consumption to maintain human well-being and health.

Key Words: donkey milk, humans, functional food

Forages and Pastures Fall Webinar

W107 Connecting forage data to on-farm performance.

J. Goeser*[1,2], [1]*Rock River Laboratory Inc., Watertown, WI,* [2]*University of Wisconsin–Madison, Madison, WI,* [3]*Cows Agree Consulting LLC, McFarland, WI.*

Forage represents 15 to 35% of dairy production costs and the intersection between agronomic and dairy cattle performance. Quality can be defined in many ways, including total digestible nutrient (TDN) content as described by the 2001 NRC or CNCPS. Summing digestible nutrient fractions (nutrient content × nutrient digestibility) results in TDN. This net nutritive value is useful to relate to on-farm performance measures. Alternatively, individual TDN components can be evaluated. Fiber (NDFD) and starch digestibility have been related to dairy cattle performance in published literature. For example, a one-unit increase in NDFD has been related to 0.17 and 0.25 kg gains in intake and 4% fat-corrected milk. Recent commercial dairy survey results suggest relationships could be greater, where a one-unit increase in NDFD corresponded to 0.20 and 0.45 kg increases in intake and energy-corrected milk. In determining TDN components, feed analysis laboratories analyze samples in an ideal environment. Many routine nutritive measures do not consider particle size or feed hygiene attributes, both of which are known to affect performance. Particle affects starch digestion, with kernel or berry processing score indicating forage grain potential. Controlled research in the feed hygiene space is difficult, thus farm surveys correlating hygienic risk factors to performance may be warranted. The agronomic performance associated with NDFD or other TDN factors also warrants consideration. Greater milk yield may not equate to economic returns if agronomic yield and added acres to feed the herd offset dairy cattle performance. Future quality or feeding studies evaluating seed genetics or forage management should include replicated field measures, alongside replicated cows or pens, to account for field variance factors. In summary, forage quality data should be related to both agronomic and dairy cattle performance, to account for performance through the complete dairy farm life cycle. Performance metrics should also equate to dairy farm economics. Commercial dairy survey studies and literature review methods such as that detailed by Oba and Allen (1999) will improve our forage quality understanding relative to farm performance.

W108 Physical characteristics of forages and impact on the lactating dairy cow.

P. J. Kononoff*, *University of Nebraska-Lincoln, Lincoln, NE.*

Dairy cattle are grass and roughage eaters, and it is well established that they require coarse roughage to maintain normal rumen function, overall health and production. The physical nature of fiber consumed by the dairy cow is known to affect feed intake, eating and ruminating activities, rumen fermentation and ultimately milk production and composition. Fragility, a characteristic defined as the rate at which plant tissues are further fragmented into small particles, is also an important physical characteristic of forages. Although some methods to measure forage fragility exist, they are not routinely used. Because of the complexity of the interactions between the physical characteristics of forage and rumen fermentation, it is not surprising that observed responses on milk production are mixed when forage particle size is varied. Increased digestibility and intake likely factors that support higher milk and milk protein production in studies that test reduction in forage particle size. Because the production of milk fat is often linked to increases in rumen pH; it is often expected that increasing forage particle size will increase rumination activity, saliva production, pH, and ultimately milk fat, yet this response is not always observed. Explanations for a lack of response may vary but include decreased digestibility and nutrient availability and increased sorting behavior. Although forages represent a large proportion of the feed ingredients included in a TMR, the types of forages included may also vary. In the case of corn silage, physical characteristics may have major effects on total-tract NDF and starch digestibility, yet only NDF digestibility may be affected in legumes and grass silages. The likely increases in intake and digestibility as well as reduced sorting activity are major reasons why finer particle size is recommended for corn silage compared with legumes and grasses. The links between physical characteristics of forages and milk production are complex yet improving the understanding of the relationship between particle size and rumen fermentation should lead to improved feeding practices.

Key Words: particle size, forage, milk

W109 Linking forage quality to performance in grazing dairy herds.

K. J. Soder*, *USDA-ARS, University Park, PA.*

High-quality forages are the pillar of any dairy operation to achieve optimal levels of milk production. Forage quality in pastures can be impacted daily by factors such as forage species composition, weather patterns, and grazing management that create unique challenges in maintaining animal health and consistent productivity in grazing dairy herds. Supplementation strategies, including conserved forages, concentrates, by-products, and TMR, are often used to balance nutritional deficiencies in pastures. However, by the time the results of a pasture forage test are received, the cows have either already grazed that paddock, or the growing forages have significantly matured, causing producers and nutritionists to question the value of such forage tests. Additionally, some grazing strategies (e.g., mob grazing) and milk markets (e.g., organic, grass-fed) limit the use of some supplementation strategies, therefore improving forage quality is of utmost importance to meet the nutritional needs of dairy cows. While cool-season perennial grass-legume pastures are the mainstay in temperate environments of the US, as weather patterns change and production methods evolve, alternative forages are being included in grazing systems to improve forage quality and/or extend the grazing season. Forage options to be discussed in this symposium include establishing novel forage mixtures, interseeding forages, grazing cover crops, use of warm- and cool-season annuals, and grazing brassicas. Another critical aspect in linking forage quality to animal performance is understanding grazing behavior of dairy cows in relation to forage conditions. This review will give a summary of research on the link between pasture forage quality and performance of grazing dairy cows, including various management strategies to improve animal and pasture production for improved economic and environmental sustainability of pasture-based dairy farms. Future research needs will also be discussed, including high-energy forages, changing weather patterns, cow genetics adapted to grazing systems, and changing milk markets.

Key Words: animal performance, forage quality, grazing

W110 Enteric methane production in relation to animal performance.

A. N. Hristov*, *The Pennsylvania State University, University Park, PA.*

Enteric methane (ECH$_4$) mitigation practices must be considered within the context of their effects on animal productivity, health, and economic feasibility. Cost, undesirable side effects, and lack of production responses can limit adoption of practices with proven effectiveness. For example, decreasing ECH_4 by up to 30–40% (as already demonstrated for some feed additives) will result in sizable feed energy savings, but production responses to the additional metabolizable energy are uncertain and may be difficult to measure in commercial settings. The proportion of feed gross energy intake lost as ECH_4 energy (Y_m factor) varies largely depending on diet composition. In the GLOBAL NETWORK (GN; an international consortium of scientists) dairy database of 2,566 observations, Y_m varied from 3.6 to 9.8% (SD = 1.04) for EU data and from 2.7 to 8.4% for US data. One of the factors that affects Y_m is the amount of neutral detergent fiber (NDF) in the diet. An analysis of the GN database showed a weak but statistically significant ($P < 0.001$) relationship of dietary NDF concentration and Y_m in lactating dairy cows: Y_m linearly increased by about 0.07% for every percentage increase in NDF concentration. Numerous strategies for mitigating livestock ECH_4 emission have been developed and tested. A meta-analysis by the GN project examined 103 mitigation strategy combinations from a database of over 3,600 treatment means. Rumen manipulation strategies decreased the daily ECH_4 emissions, the Y_m factor, ECH_4 yield (g ECH_4/kg dry matter intake), and ECH_4 intensity (g ECH_4/kg milk yield) on average by up to 12%. Feed additives, such as the inhibitor 3-nitrooxypropanol, decrease ECH_4 emission by 25 to 30%. Others, such as the red macroalga *Asparagopsis taxiformis* can decrease ECH_4 emission by up to 80%, but there are uncertainties about persistency of the effect, stability of the bromoforms (the macroalga's active compounds), palatability, milk quality for human consumption, and effects on animal health. Thus, practical implementation of ECH_4 mitigation practices will depend on proven long-term effects, economic feasibility, government policies, and consumer acceptance.

Key Words: enteric methane, mitigation, ruminant animal

Lactation Biology Fall Webinar: Mammary Innate and Adaptive Immune Responses During Involution and Disease

W111 Milk, teat, and skin microbiomes and their role in mammary health and immunity.

E. Ganda*, *Penn State University, University Park, PA.*

Investigations on the total collection of microbes (microbiomes) found in milk and the mammary gland environment have challenged our previous perception that the udder is a sterile environment. Knowledge acquired on the mammary microbiome was largely made possible because of the advent of culture-independent sequencing-based technologies that allow for the characterization of previously uncultured bacteria in complex environments. Although substantial debate exists regarding the functional activity level of the organisms identified in the mammary gland environment, the existence of a milk and mammary gland microbiome has been well established in several species. Another microbial component that plays an important role in mammary health is the teat skin flora. Based on ecological principles or niche partitioning and competition, the teat skin microbiota can be conducive to improved mammary health through interference in host-pathogen interactions. By acting as a gatekeeper between the environment and the mammary gland microbiome, the teat skin can either serve as a reservoir of or a competitive niche against mammary pathogens. Despite the substantial advances made in the study of the mammary gland microbiota in the past years, several questions remain unanswered – including how the milk microbiota is originated, what are the different factors and the extent to which these factors can influence microbiota composition, and how can management practices or potential therapeutic approaches be utilized to improve mammary health through microbiome modulation. In this presentation, we will discuss the current literature on milk and mammary gland microbiota, their potential role in mammary health and immunity, and demonstrate how innovative technologies have been used to investigate the impact of different treatment and management strategies on the milk microbiota and mammary health.

Key Words: microbiome, mammary health, host-pathogen interaction

W112 Metabolic transitions and lipid mediators as modulators of mammary gland inflammation and oxidative stress.

G. A. Contreras*, A. Abuelo, and L. Sordillo, *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

The lactation cycle in dairy cows is characterized by 2 major functional transitions of the mammary gland, from late lactation to involution and from late gestation to early lactation. As with any tissue remodeling process, both transitions involve inflammation and lipolysis activity. Mammary gland inflammatory responses are essential to facilitate involution and enhance mastitis resistance that is necessary for optimal milk production. Lipolysis, carried out by phospholipases, hormone-sensitive lipase, and lipoprotein lipases, is necessary for tissue remodeling. Also, lipolysis modulates inflammation as it releases unsaturated fatty acids (UFA) that are substrates for the biosynthesis of potent lipid mediators of inflammation such as oxylipids. Successful lactational transitions are characterized by shifts in the pool of oxylipids from a proinflammatory to a resolving profile. When aberrant inflammation occurs, these shifts are truncated due to altered oxylipid biosynthesis driven by excessive accumulation of reactive oxygen species (i.e., oxidative stress) and lipolysis dysregulation. These factors modify oxylipid profiles by changing a) the type of UFA substrates released by lipolysis and b) the subsequent metabolism of UFA through various oxidizing pathways. Supplementation of antioxidants and specific fatty acids during the nonlactating and early-lactation periods can modulate lipolysis, oxylipid biosynthesis, and oxidative stress and can improve the efficiency of the inflammatory response during mammary gland transitions. For example, including lipolysis inhibitors in the diet could modify the content and profile of UFA available for oxylipid biosynthesis. Alternatively, increasing the dietary content of antioxidants may limit the activity of oxidizing pathways with known proinflammatory oxylipid products. Understanding how inflammatory processes during lactational transitions are affected by lipolysis, oxylipid biosynthesis, and oxidative stress is key to developing mastitis prevention and control programs for the dairy industry.

Key Words: oxilipids, oxidative stress, antioxidants

W113 Use of applied and genomic strategies to investigate and develop novel solutions to promote mammary gland health.

G. Pighetti*, *University of Tennessee, Knoxville, TN.*

Dairy cows are constantly exposed to mastitis pathogens. As an industry, we have developed and continually improved upon several strategies that help dairy cows resist and eliminate infections. Despite this, mastitis remains present on all dairies, remains the dominant reason cows are treated with antibiotics, and explains why approximately 1 in 4 cows are culled from the herd. Research that focuses on understanding the immunological-basis for differential cow responses, can provide valuable insight to specific mechanisms that contribute to the severity and duration of intramammary infections. Differential cow responses can be induced through environmental stressors, nutritional supplementation or limitation, transitioning from late gestation to early lactation, and immune modulators. These studies have provided valuable knowledge regarding immune pathways and system interactions that contribute to mastitis susceptibility and resistance. The advent of genomic technologies has enabled us to dig deeper and identify potential drivers of subclinical and clinical mastitis. We and others have used this technology, in combination with experimental intramammary challenge, to identify critical lynchpins that can influence the severity and duration of intramammary infection. This presentation will discuss the key lynchpins identified, their relationships to mammary immunity during the nonlactating dry period and early lactation in dairy cattle, and their potential as novel regulators of mastitis.

Key Words: mastitis, mammary immunity, genomic

W114 Environmental effects on mammary immunity and health.

G. E. Dahl*[1] and T. B. McFadden[2], [1]*University of Florida, Gainesville, FL,* [2]*University of Missouri, Columbia, MO.*

Environmental impacts on pathogen abundance and access are precursors to mastitis. Indeed, high heat and humidity and unsanitary housing and equipment are associated with greater pathogen load and exposure. While less is known about impacts of environment on the cow's ability to resist infection, several indicators suggest that it can affect pathogen responses. Mastitis incidence and bulk tank SCC vary with season, typically peaking in summer. Recent controlled studies have revealed that heat stress exposure results in changes in the microbiome of the cow and her environment, which may relate to negative impacts on milk quality and cow health. Alternatively, specific pathogen loads may vary based on housing dynamics rather than associations with physical environment. Indeed, housing related stressors such as overcrowding and social group challenge influence secretion of glucocorticoids, and affect pathogen resistance. Two key seasonal variables are photoperiod and temperature, specifically heat stress consequent to elevated temperature and humidity. Shifts in light duration regulate immune function in other species but apparently have limited impact on udder health of lactating cows. In contrast, in dry cows, short days increase PBMC number and are associated with lower SCC in the next lactation, versus long days. With heat stress, elevated body temperature directly affects expression of immune-related genes in mammary tissue. Responses depend on duration of exposure and feature acute upregulation of immune-signaling pathways followed by enrichment of other immune-related pathways after prolonged exposure. Most responses are transient and recover within one week. Functionally, heat stress impairs some aspects of acquired immunity in dry cows, including antigen responses and lymphocyte proliferation, but apparently not innate immune function. However, heat stress in late gestation reduces neutrophil phagocytosis and killing in vitro, and neutrophils in circulation are reduced in vivo as are responses to pathogen challenge in the subsequent lactation. Holistic understanding of the complex interplay of environment, pathogens and host is needed to inform advances in this area.

Key Words: heat stress, photoperiod, mastitis

W115 Applications of nutritional immunology to improve mammary health. B. J. Bradford* and T. H. Swartz, *Michigan State University, East Lansing, MI.*

Interest in nutritional immunology has grown in recent years, with much of the applied work in dairy cattle naturally focusing on prevention or resolution of mastitis. Some of the earliest work on nutritional influences on mastitis utilized both epidemiological and intervention studies to demonstrate that mastitis incidence and herd-level SCC are increased when insufficient selenium or vitamin E are fed, particularly in the peripartum period. Since that work, similar methods have been used to explore potential impacts of other nutrients on mastitis, but other than evidence for choline decreasing SCC, no strong links have been uncovered. Beyond essential nutrients, a variety of feed additives including yeast or yeast components have been used in an attempt to modulate immune responses, with some evidence of success in field studies and very limited data from challenge studies. This dietary work has occurred against the backdrop of the growing field of immunometabolism, which seeks to understand both how immune cells utilize nutrients and how disease processes alter whole-body metabolism. This has helped to shift the focus from dietary nutrients alone to also considering metabolites that may influence immune response to mastitis, with key insights into the impacts of calcium, essential amino acids, glucose, and β-hydroxybutyrate concentrations on immune cells. Although concentrations of some key nutrients may not be limiting in milk (e.g., calcium), some of these metabolites are likely to impact immune responses to mastitis. To date, most of the immunometabolism research related to mastitis has focused on innate immunity, but there is opportunity to test nutrient effects on the adaptive immune system as well, with potentially longer-term impacts on T and B cell populations. Although in vitro studies provide opportunities to understand mechanisms and develop hypotheses, we believe that a combination of large-scale clinical studies and intensive challenge studies are necessary to move the field forward. With advancing technologies to carry out both types of research, the opportunities to better understand nutritional impacts on mastitis are exciting.

Key Words: immunometabolism, innate immunity, adaptive immunity

Production, Management, and the Environment Fall Webinar: Advances in Enteric Methane Mitigation in Dairy Cattle—The Last Decade and Future Prospects

W116 Rethinking methane: Animal agriculture's path to climate neutrality. F. Mitloehner*, *University of California–Davis, Davis, CA.*

Animal agriculture is often shouldered with a large part of the blame when it comes to climate change, and that is in part due to the fact that we have not been looking at all greenhouse gases correctly. While methane – the main greenhouse gas associated with animal agriculture – is a potent climate pollutant that we can and need to reduce, it warms our atmosphere differently than other gases because of its short lifespan. Methane persists in our atmosphere for about a dozen years before it is broken down via oxidation, and it is that atmospheric removal that is often neglected when trying to characterize methane's warming impact. Furthermore, if we can reduce methane emissions to the point where more is being broken down in the atmosphere than is being emitted, we'll see animal agriculture go from being blamed for climate change to being recognized as a major climate solution. By rethinking methane, we can see that animal agriculture's path to climate neutrality is within reach as scalable solutions offer the global community tools to fight global climate change.

W117 An overview of successful strategies to mitigate enteric methane in dairy farms. A. Hristov*, *Pennsylvania State University, University Park, PA.*

Intensive research in the past decade has resulted in a better understanding of factors driving enteric methane (ECH4) emission in ruminants. Large databases have been created that allow prediction of emissions based on simplified or more complex, region-specific models. Analyses based on these databases have confirmed that dry matter intake (DMI) is the most important factor in predicting ECH4 emission from dairy cows with other factors, such as dietary neutral detergent fiber and milk yield and composition, improving prediction accuracy. Meta-analyses have also identified successful strategies for mitigation of ECH4 emission. Lipids, nitrates, tanniferous forages (pasture-based production systems), and the synthetic inhibitor 3-nitrooxypropanol (3-NOP) are among the recommended strategies for mitigating ECH4 emission. A meta-analysis of experiments conducted at the Pennsylvania State University with 3-NOP with lactating dairy cows (n = 185; duration of 31 to 105 d) showed an average reduction in daily ECH4 emission of 24% (or 101 g/cow/d; SE = 8.05). There was an exponential relationship between daily methane emission and 3-NOP intake {SE shown in parentheses; Daily methane, g/cow/d = 258.5 (42.40) + 159.8 (43.66) × exp[−0.596 (0.3381) × 3-NOP dose, g/cow/d]; adjusted $R^2 = 0.68$, $P < 0.001$}. Similarly, the relationships of 3-NOP intake and methane yield and intensity were exponential {Methane yield, g/kg dry matter intake (DMI) = 11.30 (0.865) + 5.045 (0.978) × exp[−0.887 (0.4112) × 3-NOP dose, g/cow/d]; adjusted $R^2 = 0.76$, $P < 0.001$} and {Methane intensity, g/kg energy-corrected milk = 6.541 (0.928) + 4.247 (1.0037) × exp[−0.7437 (0.3916) × 3-NOP dose, g/cow/d]; adjusted $R^2 = 0.71$, $P < 0.001$}, respectively. In these experiments, there was no effect of 3-NOP on DMI or milk production, but milk fat concentration was increased ($P = 0.05$) by 0.15%-units, compared with the control. Other mitigation strategies, such as supplementation with the red macroalga *Asparagopsis taxiformis* have also shown promising results, but much more research is needed before they can be recommended for adoption by the dairy industry.

Key Words: enteric methane, mitigation, dairy cow

W118 Modeling the enteric methane mitigation effect of feed additives and their impact on dairy farm-gate emissions. E. Kebreab*[1], J. Li[1], M. E. Uddin[1], and J. Tricarico[2], [1]*University of California, Davis, CA,* [2]*Innovation Center for US Dairy, Rosemont, IL.*

Enteric fermentation is the largest anthropogenic source of methane (CH_4) emissions in the United States. In 2019, enteric CH_4 emissions were 179 MMT CO_2 equivalents (CO_2e; 27.1% of total CH_4 emissions). Studies have shown that several feed additives are effective in reducing enteric methane emissions. According to Food and Agriculture Organization of the United Nations feed additive guideline, a life cycle assessment approach needs to be taken to conduct a cradle-to-farm gate environmental impact analysis. Emissions associated with crop production, feed additive production, enteric methane, farm management, and manure storage need to be calculated and expressed as kg CO_2e per kg of energy-corrected milk. Ideally, the effectiveness of feed additives in reducing CH_4 emissions need to be determined through a meta-analysis. This is because the effectiveness may depend on animal life stage, feed additive dose, dietary composition such as NDF, and EE and DMI. For example, a meta-analysis of 3-nitroxypropanol and nitrate showed that although their overall effectiveness was −30.6 and −13.9%, respectively, type of cattle (dairy or beef), dose, DMI and BW were significant moderators. An example of feed additive impact in California dairy farms showed that the carbon footprint for baseline, 3-nitroxypropanol and nitrate offered during lactation were 1.12, 0.993, and 1.08 kg CO_2e/kg energy-corrected milk, respectively. The average net reduction rates for 3-nitroxypropanol and nitrate were 11.7% and 3.95% at the farm-gate, respectively. In both cases, using feed additives on the whole herd slightly improved overall carbon footprint reductions compared with limiting its use during the lactation phase. Although both 3-nitroxypropanol and nitrate had effects on decreasing the total greenhouse gas emission, the former was much more effective with no known animal safety issues in reducing the carbon footprint of dairy production in California. The combination of meta-analysis and life cycle assessment is critical to understand the full potential for enteric methane reduction that feed additives offer.

Key Words: methane, feed additives

W119 Understanding the role of the rumen microbiota and metagenome in enteric methane mitigation and productivity in dairy cows. D. Pitta*[1], A. Hristov[2], N. Indugu[1], A. Melgar[2], H. Stefanoni[2], B. Vecchiarelli[1], M. Hennessy[1], and J. Bender[1], [1]*Department of Clinical Studies, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, PA,* [2]*Department of Animal Science, The Pennsylvania State University, University Park, PA.*

Ruminants are one of the largest sources of global methane (CH_4) emissions. Enteric CH_4 is exclusively produced by methanogenic archaea as an end product of microbial fermentation in the reticulorumen. Several enteric CH_4 mitigation approaches have been proposed including the development of alternative electron receptors, novel CH_4 inhibitors, and other dietary or animal management strategies. However, responses to these CH_4 mitigation strategies have been inconsistent owing to a lack of understanding of the mechanistic basis of methanogenesis and how individual methanogens contribute to total CH_4 formation. Methanogenic archaea are an ecologically diverse group of strictly anaerobic microorganisms with a distinct energy metabolism that results in the formation of CH_4 from the reduction of carbon dioxide (CO_2), methanol, or methylamines or the cleavage of acetate. In the rumen, it was assumed that methanogens that utilize molecular hydrogen (H_2) and CO_2 and/or formate are the predominant species, followed by a small proportion of methylotrophic methanogens. Recent research has revealed that methylotrophic methanogens may have a greater share in overall CH_4 production compared with CO_2-reducing methanogens than previously thought. Using advanced genomic tools coupled with metagenomics and metatranscriptomic approaches, our group demonstrated how natural variation in CH_4 yield in dairy cows (high and low methane-yield phenotypes) as well as potent inhibitors (natural and synthetic compounds) inhibited CH_4 formation and how this inhibition was accompanied by differences in inhibiting individual methanogens and consequently methanogenesis. Furthermore, our research also demonstrated shifts in specific bacteria-methanogenic archaea cohorts under inhibited methanogenesis mediated by H_2 concentrations, thus offering opportunities to further reduce CH_4 formation and also improving productivity in dairy cows.

Key Words: methanogenesis, bacteria-archaea cohort, hydrogen metabolism

W120 Is it possible to selectively breed low enteric methane-producing dairy cows and maintain health and productivity?

Y. de Haas*[1], A. E. van Breukelen[1], R. F. Veerkamp[1], G. de Jong[2], and M. N. Aldridge[1], [1]*Animal Breeding and Genomics of Wageningen University and Research, Wageningen, the Netherlands,* [2]*CRV, Arnhem, the Netherlands.*

The global livestock sector, particularly ruminants, contribute substantially to the total anthropogenic greenhouse gases. Management and dietary solutions to reduce enteric methane (CH_4) emissions are extensively researched. Animal breeding that exploits natural variation in CH_4 emissions is an additional mitigation solution that is cost-effective, permanent, and cumulative. We quantified the effect of including CH_4 production in the Dutch breeding goal using selection index theory. The current Dutch national index contains 15 traits, related to milk yield, longevity, health, fertility, conformation and feed efficiency. From the literature we obtained a heritability of 0.21 for enteric CH_4 production, and genetic correlations of 0.4 with milk lactose, protein, fat and dry matter intake. Correlations between enteric CH_4 production and other traits in the breeding goal were set to zero. When including CH_4 production in the current breeding goal with a zero economic value, CH_4 production increases each year by 1.5 g/d as a correlated response. When extrapolating this, the average daily CH_4 production of 392 g/d in 2018 will increase to 442 g/d in 2050 (+13%). However, expressing the CH_4 production as CH_4 intensity in the same period shows a reduction of 13%. By putting economic weight on CH_4 production in the breeding goal, selective breeding can reduce the CH_4 intensity even by 24% in 2050. Correlated responses of selection for lower emitting cows show that it is possible to both increase production and reduce the enteric CH_4 emission per cow, and also health traits can still be improved. This shows that breeding is a valuable contribution to the whole set of mitigation strategies that could be applied to achieve the goals for 2050 set in the Paris COP agreement in 2015.

Key Words: greenhouse gases, mitigation, breeding

W121 The Greener Cattle Initiative was developed to support research on enteric methane mitigation from ruminants.

J. Tricarico*[1] and T. Kurt[2], [1]*Innovation Center for US Dairy, Rosemont, IL,* [2]*Foundation for Food and Agriculture Research, Washington, DC.*

Enteric methane continues to be a major source of greenhouse gas emissions from milk and beef production systems that contribute to global warming. The Foundation for Food and Agriculture Research (FFAR) and the Dairy Research Institute, a non-profit organization affiliated with the Innovation Center for US Dairy, developed a collaborative program to align resources and fund projects to identify, develop and/or validate new and existing practices that mitigate enteric methane emissions from dairy cattle. This collaborative program is called the Greener Cattle Initiative. The program will develop requests for proposals and award grants on projects similar, but not limited, to the following examples. Research, development, and evaluation of additives fed at less than 1% dietary DM that inhibit enteric methane emissions, ingredients that alter ruminal metabolic pathways away from methanogenesis when fed at quantities requiring diet reformulation, technologies such as sensors, robots, and artificial intelligence systems to monitor enteric methane emissions or related physiological indicators and manage animals to mitigate emissions, selection traits and programs that allow breeding of low methane-emitting cattle, and socioeconomic analysis of enteric methane mitigation practices. Communicating the resulting research findings will help dairy farmers mitigate enteric methane emission and respond to stakeholder questions about reducing the impact of milk production on global warming. The Greener Cattle Initiative program will continue incorporating participants from the food and agriculture industry, commodity groups, and non-profits who share common objectives and contribute in-kind and matching funds to the program up to a total of 10 organizations. We anticipate up to US$5M funding will be awarded through this initiative. The Greener Cattle Initiative expected contributions to research and communication of its findings support the US dairy industry's goals of carbon neutrality by 2050 and will enable the industry to provide consumers with critical information to drive demand for milk, and dairy ingredients and foods.

Key Words: dairy, enteric methane, funding

Author Index

Numbers following names refer to abstract numbers. A number alone indicates an oral presentation; SC preceding the number indicates a student competition, a P indicates a poster, an S indicates a symposium presentation, and a W indicates a presentation in the post-conference webinar series. Student competition abstracts are listed first, followed by orals, posters, symposia presentations, and webinar presentations.

The author index is created directly and automatically from the submitted abstracts. If an author's name is entered differently on multiple abstracts, the entries in this index will reflect those discrepancies. Efforts have been made to make this index consistent; however, error from author entry contributes to inaccuracies.

Key Word Index

Numbers following names refer to abstract numbers. A number alone indicates an oral presentation; SC preceding the number indicates a student competition, a P indicates a poster, an S indicates a symposium presentation, and a W indicates a presentation in the post-conference webinar series. In this index, student competition abstracts are listed first, followed by orals, posters, symposia presentations, and webinar presentations.

The index is created directly and automatically from the submitted abstracts. Efforts have been made to make this index consistent; however, error from author entry contributes to inaccuracies.