8th International Feed Conference
Present and Future Challenges

FEED 2023

Milan, October 9-10

UNIVERSITÀ DEGLI STUDI DI MILANO

divas
DIPARTIMENTO DI MEDICINA VETERINARIA E SCIENZE ANIMALI

ABSTRACT BOOK

Chair: Prof. Luciano Pinotti
Chair of the Organizing Committee and
Chair of the Scientific Committee

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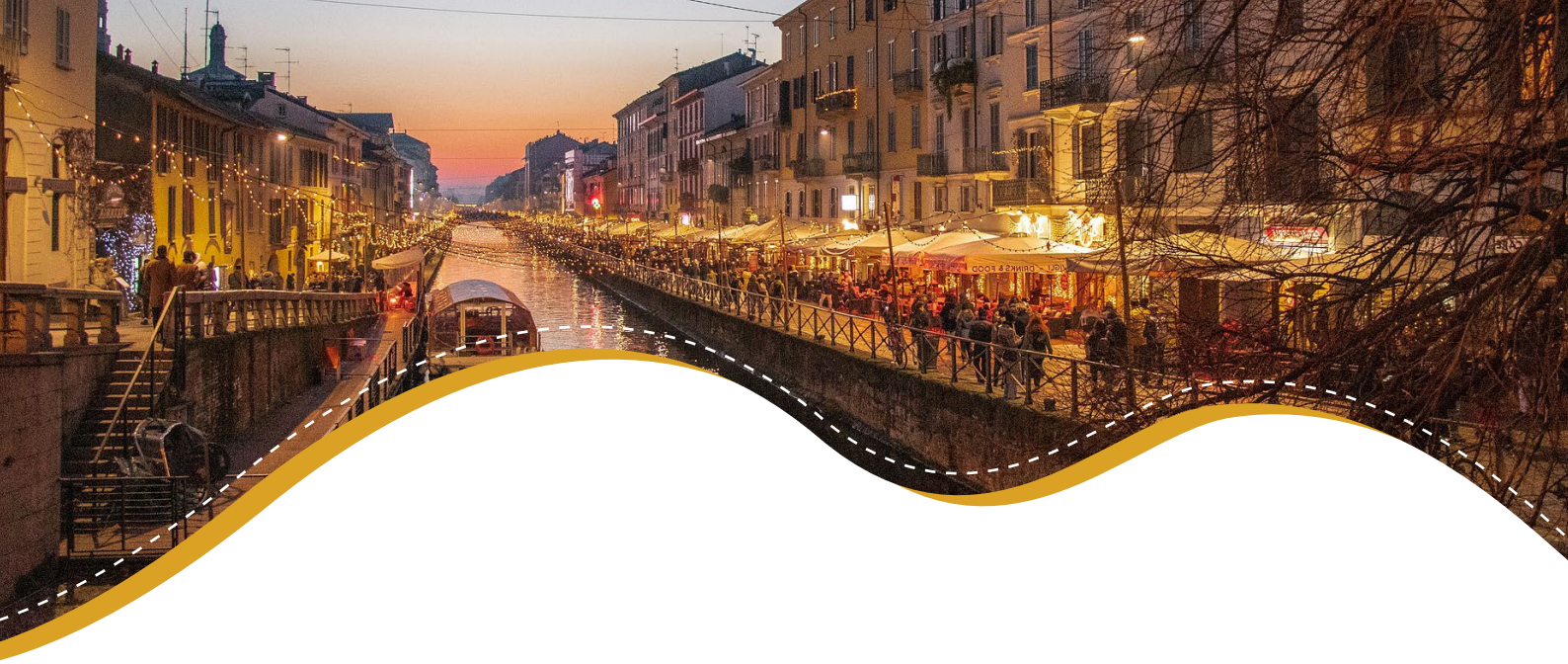

Dear Colleagues and Friends,

On behalf of the Local Organizing Committee, it is my pleasure to welcome you to the 8th International Feeding Meeting "Present and Future Challenges", at the Università degli Studi di Milano (Italy) October 9-10, 2023.

As seen in 2021, the availability of sufficient and safe feedingstuff is still a key challenge in modern agriculture. While the topic of undesirable substances in feed remains a major issue, the globalisation of the feed business has further reinforced the need for efficient tools for traceability of feed ingredients. Moreover, the constantly increasing demand for food from animal origin, along with limited resources, triggers the need for evaluating new sources of feed ingredients such as insects and efficient feed production. Additionally, the impact of climate change on feed production should be also taken into account. Keeping the feed safe and sustainable therefore requires a multidisciplinary approach, bringing together all stakeholders, including industry.

The 2023 International Feed Conference covers all the current interesting areas for animal feed, which will be presented in the following two sessions:
- Circular feed and additives
- Feed quality, safety, authentication and traceability

In today's world, sharing scientific knowledge, research findings, laboratory methods and strategies within the scientific community has become a necessity. The aim of this conference is to bring together, at a single event, scientists, researchers, laboratory personnel, policy-makers from governmental and non-governmental organizations and people from industry where they can share their knowledge, scientific experiences and experiments on subjects crucial to animal feed. With the participation of international experts, we hope that productive discussions will stimulate new creative ideas to translate new discoveries into better practices and applications.

For this eighth edition - FEED2023 – participants can attend in both online or directly at the venue.
Keynote and regular speakers will deliver their presentations in front of the audience in Milan.
Discussions will only be open to the audience in the conference venue.

Welcome to FEED2023!

Prof. Luciano Pinotti
Chair of the Organizing Committee and Chair of the Scientific Committee

Scientific Committee

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Keynote Speakers

Anton van den Brink

European Compound Feed & Premixes Association

Anton van den Brink is the Deputy Secretary General for the European Compound Feed & Premixes Association (FEFAC). He started at FEFAC in 2013 as Communication Advisor and held the position of Senior Policy & Communication Manager from 2018 to June 2022. For several years he has been actively engaged on the sustainability-related topics connected to European compound feed manufacturing, such as responsible soy sourcing and environmental footprinting (PEFCR Feed for Food-Producing Animals). In that capacity he has played a leading role in the development of the FEFAC Sustainability Charter 2030, the FEFAC Soy Sourcing Guidelines 2021 and the publications on Circular Feed (June 2022) and CoProducts (June 2019).
Anton is also Executive Director of EFFPA, the European Former Foodstuff Processors Association.
Anton graduated in Persuasive Communication at the University of Amsterdam (2012). He lives in Mechelen, Belgium, with his wife and daughter.

Abstract
In June 2022 FEFAC made the publication “Circular Feed – Upcylcing Optimised Nutrient Recovery Through Animal Nutrition”. It provides a conceptual approach to Circular Feed, an overview of examples and a vision of future possibilties for the animal nutrition sector to upcycle nutrients that currently are not covered by the regulatory framework. FEFAC points to the essential need to obtain market and consumer acceptance of the future transition of European feed manufacturing, assisting the livestock and aquaculture sectors to become part of more circular, low-carbon food production systems. FEFAC also advocates for setting up a clear hierarchy for nutrient-rich biomass, prioritising the food chain use of nutrients over non-food use, bearing in mind the examples of circular feed are increasingly absorbed by producers of biogas.

Keynote Speakers

Silvia Ampuero
Federal Department of Economic Affairs, Education and Research EAER Agroscope Competence Division, Method Development and Analytics

Silvia Ampuero Kragten, Dr ès sc., obtained a degree in chemical engineering, 1989, and a PhD from the Materials Science department, 1994, from the Swiss Federal Institute of Technology (EPFL), Lausanne. She has been working for over 20 years at Agroscope, the Swiss Centre of Excellence for Agricultural Research, as associate scientist in the Method Development and Analytics division dedicated to the animal production and nutrition sector. Her research interests are mainly focused on secondary plant metabolites and chemometrics. She has contributed to several national and European projects in the fields of tannins and boar taint analysis, including with an electronic nose based on mass spectroscopy. She is also responsible for the NIRS models used as prescreening tool by the Swiss official feed inspection authority and the pork carcass quality control tool used by Swiss commercial slaughterhouses, based on NIRS models to predict fatty acid profile in fresh adipose tissue.

Abstract
NIR portable instruments. Development of a cloud-based application with a handheld spectrometer: Pocket Feed Lab

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The undeniable advantages of benchtop NIR spectrometers as analytical tools capable of rapid, non-invasive, inexpensive and environmentally friendly analysis (no chemicals required) of a wide variety of samples prompted the development of portable spectrometers to also allow on-site analysis. As a result, a wide diversity of novel technologies are being implemented on miniaturized spectrometers, which inevitably results in con-trasting performance. Based on the literature, a brief overview of the technological building blocks of handheld spectrometers will be presented. The focus will be on the development, for commercial purposes, of a cloud-based application for the analysis of poultry feed and poultry feed ingredients with a new portable instrument based on MEMS (micro-opto-electromechanical systems). The advent of such systems, like the “Pocket Feed lab” presented here, may enable precision feeding at the feed mill and farm level, thereby improving animal nutrition, reducing feed costs, wastage of excreted nutrients, environmental pollution and ultimately GHG emis-sions, making them interesting as climate change mitigation tools in livestock production.

Keynote Speakers

Nicola Walker
Global Principal Scientist (Ruminants), dsm-firmenich

Nicola is the Principal Scientist on the dsm-firmenich methane inhibition project investigating the mode of action of the novel methane inhibitor Bovaer® (3-Nitro-oxypropanol, 3-NOP). As part of this project, she has conducted a range of trials in dairy cows, beef cattle and calves, measuring the effect of the inhibitor on methane emissions to help aid registration and commercialisation of Bovaer® as a viable solution to reduce the carbon footprint of ruminant production systems globally. An experienced animal nutritionist, she obtained a PhD in rumen microbiology from the University of Aberdeen, Scotland. Over the last 34 years Nicola has worked as a research scientist in both academia and industry in the UK, NZ, France, Canada and Switzerland, investigating a range of different eubiotic feed additives for their impact on the rumen microbial ecosystem and host animal in terms of health and production with the aim of improving sustainability of livestock production.

Abstract

From Farm to Fork: Innovative feed additives and reducing emissions from livestock

The world's population is expected to exceed 9.8 billion by 2050. With this increase in population growth comes an increasing consumer demand for the availability of healthier, balanced, and nutrient dense diets. Meat, milk, and eggs are an excellent way of fulfilling this need for a source of protein and nutrients for human consumption. However, this increasing consumer demand also places significant pressure on the planet's natural resources and the environment. Current estimations are that the livestock sector is responsible for the production of 14.5% total anthropogenic Green House Gas (GHG) emissions, with 6% originating from enteric fermentation by ruminants. Thus, the challenge currently facing the livestock sector, is to be able to provide enough food for a growing population, but at the same time implement measures and mitigation strategies to reduce the environmental impact of livestock production.

Fortunately, dietary and innovative feed additive mitigation strategies exist, or are under development, to reduce the product environmental footprint (PEF; kg GHG/kg end product) of each segment of the livestock sector. This is achieved either by tackling emissions directly using specific mitigating solutions or indirectly by increasing animal performance and feed efficiency, resulting in a reduction of the PEF.

Ruminant production has been singled out as a sector of interest, not only because ruminants are considered the main source of methane emissions from enteric fermentation and methane (CH_4) is regarded as a potent GHG with a global warming potential (GWP) 28x that of CO_2), but also because it is a relatively short lived GHG. Implementation of any methane mitigating strategy can therefore have a relatively high impact in the short-term. When targeting enteric fermentation, dietary strategies include supplying more energy by improving forage quality, addition of concentrate feeds and dietary lipids, upcycling of fibrous by-products that cannot be used by other species and improving feed processing. Further improvements can be achieved through supplementation with feed additives that either improve rumen function, increase feed digestibility or which directly target either methanogenesis or reduce excessive proteolysis and nitrogen excretion. The most extensively researched methane mitigator is Bovaer® (3-nitrooxypropanol, 3-NOP, dsm-firmenich) which is the first zootechnical feed additive authorised in a new EU category as a feed additive with a positive effect on the environment. This innovative feed additive has been demonstrated to be efficacious and safe. With more than 65 peer reviewed papers, efficacy has been demonstrated in dairy cows, beef cattle and calves under a variety of different diets and management systems, with significant reductions in methane (up to 90%) observed. Synergy with other methane mitigating feed additives and strategies have also been demonstrated. A prediction model for dairy cows has been developed to be able to account for the effect of dose rate and diet composition on the percentage reduction of methane achievable which has been validated and implemented into different carbon accounting tools.

Manure management and animal management in terms of breeding, genetics, improving reproduction, fertility and health and welfare can also be used in combination with these feed additives and dietary strategies to reduce the impact of livestock production on the environment. Similar opportunities to reduce the PEF also exist for monogastric animals. With monogastric animals, the focus would be more on the reduction of both phosphorous (P) and nitrogen (N) emissions, rather than methane. Several different mitigation strategies have already been put in place ranging from reducing crude protein levels in diets or tailoring CP levels to suit the stage of growth and this used in conjunction with eubiotic feed additives to improve health, production, and feed efficiency. The use of phytase feed additives in monogastrics has long been recognised for reducing P excretion and improving host performance. Carbohydrases have been successfully used to improve feed utilization; probiotics, prebiotics and synbiotics to improve gut health. New Omic technologies have been implemented to identify new targets within the gut microbiome or to demonstrate effectiveness of these strategies to modulate the gut microbiome, leading to new innovative feed additives being developed. Over the next few years, it is expected that further developments will occur, opening the way for completely new and innovative feed additives that are especially equipped to reduce the PEF and improve the sustainability of livestock production.

Guidelines, models and carbon accounting tools have also been developed to estimate baseline emissions within each production system and credit any mitigation steps taken. By implementing these mitigating strategies and tools, the opportunity exists to make a significant step forward in improving the future sustainability of livestock production.

Keynote Speakers

Mia Eeckhout
Department Food Technology, Safety and Health Research group cereal and bakery technology (food and feed) Ghent University

Mia Eeckhout is a senior full professor at Ghent University. She is teaching Feed Technology for M.Sc. students since 1989 and performs research in this domain for over 30 years. She also provides tailor made training in Technology of Compound Feed Manufacturing including GMP and HACCP for plant managers, nutritionists and process operators within international animal feed companies. She conducts applied scientific research in the field of compound feed production at the request of and in close collaboration with the Belgian animal feed industry. She coordinated numerous research projects in a Belgian, European and global context on innovative feed ingredients, feed technology and feed safety. From 2015 to 2020 she was chair of the public-private platform Feed Design Lab, Wanssum, the Netherlands.

Abstract
Doing it better with less: the main drive for innovation in the feed mill.

When we dive into the production process of animal feed in 2023, we see at first sight little difference from what was already being done more than 60 years ago. Animal feed production, originally the production of flour or mixing ground raw materials, grew from the milling sector and became an individual activity within the agrofood sector. In the 60's the first pellet mills were put into use. They are still the heart of the compound feed factory. But, although we are still talking about a process of dosing, grinding, mixing and granulating, this industry is also subject to innovation. Driven by the challenges of the current and coming decades, in which animal husbandry and therefore also the production of animal feed, are being questioned more than ever, innovation found his way to the feed mill. Raw material availability, energy prices, environmental impact, lack of experienced employees, focus on animal health and wellbeing, declining profitability of animal husbandry.... are daily items on the agenda of the current company management. Innovation in very diverse areas is essential to do better, to deal with the many challenges. The lecture gives a review of a series of innovations that have found their way into the animal feed sector in last decades and how they contribute to "doing it better with less" .

Oral Communications

O01

Circular Feed and additives

THE INCLUSION OF SALTY AND SUGARY FORMER FOOD PRODUCTS IN THE FEED OF GROWING-FINISHING PIGS DOES NOT IMPAIR DIET DIGESTIBILITY

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A more sustainable feeding strategy in pig production needs to be achieved in terms of reducing food waste and environmental footprint. One of the potential alternatives is reintroducing food industry leftovers, also known as ex-food or Former Food Products (FFPs), into the feed chain. The presence of manufacturing errors, packaging defects, and logistic challenges, makes these products no longer suitable for the human market. Due to the richness in lipids, starch and energy, FFPs-based diets could substitute conventional feed ingredients (e.g., wheat, barley). In this study, FFPs were divided in two main categories (sugary and salty) and they were used in in grower (G: fed from 20-60 kg body weight) and finisher (F; fed from 60-110 kg body weight) diets of pigs in order to replace conventional ingredients and investigate their effects on energy and nutrient digestibility. Thirty-six Swiss Large White castrated male pigs originating from five different litters were assigned within litter to three grower and finisher experimental diets: 1) standard diet (ST-G; ST-F), 0% FFPs; 2) 30% conventional ingredients replaced by either sugary FFPs (SU-G, SU-F) or 3) salty FFPs (SA-G, SA-F). The grower and finisher diets were formulated to be iso-energetic and iso-nitrogenous. Fecal samples from 24 selected pigs (eight pigs per treatment) were collected in the grower and finisher phase to assess the apparent total tract digestibility (ATTD) of gross energy, crude fiber (CF), and crude protein (CP). Data were analyzed with the MIXED procedure of SAS. The dietary treatment was set as fixed effect and the litter origin as random effect. Least squares means were calculated and considered statistically significant at $P < 0.05$. In the grower and finisher period, the energy ATTD of the salty diets was greater ($P < 0.05$) than that of the standard diets, with intermediate values for the sugary diets. In the finisher but not the grower period, the CF ATTD of the standard diet was greater ($P < 0.05$) than that of the salty and sugary diets. The CP ATTD did not differ among the groups. Despite the inclusion of 30% FFPs in the feed of growing-finishing pigs affected digestibility of energy, CF and CP in different ways, growth performance traits were unaffected, which indicates that these differences were not sufficiently large to have a significant phenotypic impact.

O02

Circular Feed and additives

COMBINED INCLUSION OF FORMER FOODSTUFF AND WET DISTILLER GRAIN IN DAIRY COW DIETS TO REDUCE THE ENVIRONMENTAL IMPACT OF CHEESE PRODUCTION.

L. Mammi [2], F. Ghiaccio [2], R. Colleluori [2], G. Buonaiuto [2], D. Cavallini [2], A. Federiconi [2], A. Palmonari [2], V. Massa [1], A. Giorgino [1], A. Formigoni [2]

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Former foodstuff and by products coming from bakery and grain industry represent a promising way to reduce the environmental impact of animal nutrition and reduce food losses, but the effects of these ingredients on cheese quality has not been sufficiently reported.

For this purpose, a double crossover trial was set up at the dairy farm of the University of Bologna (Italy), to evaluate the impact of these feeds on cheese production and quality. 86 Holstein cows (192 ± 108 days in milk (DIM), 1.96 ± 1.1 number of lactation and 34.2 ± 10.2 kg/d of milk yield (MY)) were divided in 2 homogeneous groups, housed in 2 comparable pens. In the trial, traditional starch and protein sources were partially replaced with sustainable “circular” feeds: a wet wheat distiller grain with solubles (WDGS) and a pelleted former foodstuff feed (FF) (Dalma mangimi, CN, Italy).

The trial was composed by 4 periods of 5 wks each (4 adaptation and 1 experimental), during which 2 diets, including only traditional (CTR) or traditional and “circular” feeds (WDGS+FF) were alternatively offered to each group. The diets, composed by dry hay and concentrates with no silages, were balanced for starch (21.6±3.4), crude protein (14.2±1.2) and NDF (37.9 ±3.1) content, % of dry matter (DM). During the experimental weeks, milk produced by each group was collected separately and used to produce 40 cheeses (20/treatment).

Diet composition, in vitro starch digestibility and fatty acids (FA) profile of experimental feed was assessed, as well as fiber digestibility (TTDpdNDF) determined by NIR on fecal samples collected twice a week on 12 cows (6/group). Bulk milk of each group was analysed for composition, somatic cell count (SCC), urea, cheesemaking properties and FA profile. Cheese was analysed for composition, FA and organoleptic characteristics after 3 months of ripening.

Land occupation (LO, m2/y), net fresh water use (NFW, lt) and global warming potential (GWP, kg Co2 eq.) deriving by the diets ingredients were calculated for kg of milk produced. DMI (22.5±0.3, kg/d) and MY (32.6± 0.3, kg/d), remained similar with the 2 diets, while TTDpdNDF was higher ($P<0.05$) with the WDGS+FF diet compared to the CTR: 82.12 vs 79.62, % of pdNDF. Milk composition differed for fat %, that resulted to be lower in the WDGS+FF (3.4 vs 3.7, %, $P<0.05$), as well as De Novo FA (17.3 vs 18.3, g/100g FA, $P=0.06$). Cheese-making properties of milk (LDG), cheese yield and its organoleptic characteristics were not affected by the diet. Cheese fat % tended to be lower (31.8 vs 32.8, %, $P=0.06$) and preformed FA tended to be higher (36.8 vs 35.0, g/100g FA, $P=0.06$). The environmental impact of the circular diet was significantly lower: for each kg of milk, it was calculated a reduction of 24% m2/y of LO, 31% lt of NFW and 25% kg Co2 eq. of GWP coming from feed. These results support the possibility to include circular feeds in cows diets also for cheese production, but proper nutritional characterization is essential.

O03

Circular Feed and additives

BIOCONVERSION OF AGRO-INDUSTRIAL RESIDUES TO VOLATILE FATTY ACIDS (VFAS): A SUSTAINABLE APPROACH FOR RUMINANT FEED SUPPLEMENTATION

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The scientific interest in volatile fatty acids (VFAs) as an energy source and chemical precursor in ruminant diets has been longstanding, as it has significant implications for animal physiology and well-being. The main objective of this study is to explore the production of volatile fatty acids (VFAs) through the anaerobic digestion of agri-industrial residues. Additionally, the study aims to develop an approach for assessing the viability of VFAs as replacements for traditional energy sources in ruminant feed, providing a sustainable alternative for supplementing ruminant diets. In this regard, the bioconversion of abundant food industry residues, apple pomace (AP) and potato protein liquor (PPL), into VFAs was explored, while adhering to regulations governing the ruminant feed supplement production. To accomplish this, a semi-continuous immersed anaerobic membrane bioreactor (iMBR) was employed for fermentation and in situ recovery of VFAs, at different organic loading rates (OLR).
The highest VFAs concentration of 28.6 g/L (0.751-2 g COD VFAs/gVS added) was achieved at an OLR of 3.7 gVS/L.day. Furthermore, the feasibility of substituting different levels of energy (10%, 20%, and 30%) of the silage and concentrate with the residue derived VFAs mixture was investigated using the Menke gas method. Changes in gas and VFA concentration and compositions, pH, and ammonium nitrogen content, were analyzed. The findings of this study reveal that substituting 20% of the concentrate's energy with VFAs solution has no adverse effects on methane production or pH levels. However, it significantly enhances the production and accumulation of VFAs in the rumen fluid-buffer media ($p<0.05$). Conversely, replacing 10% of the silage energy with VFAs leads to an increase in methane production and further enhances the accumulation of VFAs in the rumen fluid-buffer media ($p<0.05$). These results suggest a potent approach for supplementing feed energy through readily digestible VFAs mixture.
Keyword: Agro-industrial residues, Anaerobic digestion, Membrane bioreactor, Sustainability, Ruminant feed alternative

O04

Feed quality, safety and authentication

MICROPLASTICS INTERACT WITH THE RUMINAL MICROBIOME EX VIVO

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Background:
Microplastics already circulate within the feed chain. So far, little is known about the interaction of these materials within digestive systems. This study obtained first data on ruminal interactions ex vivo.
Methods:
The experiment was conducted using the Hohenheim Gas Test. The microplastic species polylactide (PLA), polyhydroxy butyric acid (PHB), high density polyethylene (HDPE), polyvinyl chloride (PVC) and polypropylene (PP) were applied in different particle size ranges (<125µm, 125-500µm), at dosages of 0, 0.5, 5, 35, 70 mg/incubation cylinder. Different microplastic variants were incubated in rumen fluid (24h, 39°C, 1rpm) with either 250 mg hay or barley measuring the cumulative microbial gas production. Cylinders were placed on ice stopping fermentation and cylinder contents were sampled without losses. During sample homogenization, aliquots were taken for further analysis. To determine dry mass degradation, the remaining part of the samples were freeze dried. Statistical analysis regarding gas production and dry mass degradation comprised multifactorial ANOVA. An untargeted metabolomics approach was done using NMR spectroscopy for analysis and Chenomx NMR suit (Chenomx Inc. v.8.6) to identify the metabolites. Principal component analysis (PCA) and partial least square discriminant analysis was done using MetaboAnalyst. For Metaproteomics, samples were analyzed using Nano-LC-ESI-MS/MS and proteins were identified using a rumen metagenomic database.
Results:
Cumulative gas production decreased irrespective of dosage while total dry matter degradation increased with dosage ($p<0.0001$). Metabolites of negative controls clustered against all microplastic variants, which furthermore showed a significant clustering according to microplastic species. For barley incubations, dimethylamine, isobutyrate and methylamine had the strongest discriminatory power between plastic species, and dimethylamine and formate for hay incubations. Microbial proteins in barley incubations indicated a change in the microbial composition towards lower Bacteroidetes and Fibrobacterota, but enhanced Firmicutes abundance by microplastic. From a functional perspective, higher activities of the protein groups `replication and repair`, and `translation`, but decreased activities of `carbohydrate metabolism and transport` and `amino acids metabolism` (HDPE, PHB, and PLA, <125 µm, $p<0.05$) were observed, especially in the presence of barley.
Conclusions:
Our data indicates that microplastics irrespective of their chemical nature and particle size distribution interact with the rumen microbiome ex vivo. While the particles appear to be partially degraded by the microbes, plastic affected the microbial phylum composition and downregulated proteins of the microbial amino acid and carbohydrate metabolism. The upregulation of microbial proteins of replication, repair and translation indicates an active response to challenging conditions due to microplastics.

O05

Circular Feed and additives

DETERMINATION OF MICROPLASTICS ADDITIVES IN FEED CONTAINING FORMER FOOD

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Former food products (FFP) reused in animal feed may represent a promising alternative to cereals, which should remain a primary source for human nutrition. In the EU, 3.5 million tons of FFP are processed into animal feed. Packaging residues (paper, cardboard, aluminum, plastics and regenerated cellulose) presence in FFP is unavoidable, therefore undesirable substances occurrence has to be carefully evaluated. Phthalates are widely used as industrial additives in the production of food contact materials (FCM), flooring materials and cosmetics. They are ubiquitous molecules and are increasingly found in environmental matrices. The aim of this research is to identify specific markers of microplastic and phthalates contamination level in feed containing FFP.
A quantitative method was developed and validated for the determination of six phthalates in feed by gas chromatography interfaced to a triple quadrupole (GC-MS\ MS, TRACE 1310 GC Thermo, TSQ 8000 EVO Thermo). Investigated compound were dibutyl phthalate (DBP), diethyl phthalate (DEP), benzyl butyl phthalate (BzBP), dimethyl phthalate (DMP), bis-diethylhexyl phthalate (DEHP) and di-n-octyl phthalate (DnOP). Fourteen feeds containing FFP were analysed. Samples were collected as part of the official control of packaging residues in feed, containing former food products. Extraction and clean-up phases were carried out by applying a modified QuEChERS method and separation was performed on a capillary column (30 m, 0.25 mm ID, 0.25 µm df, Rxi-5Sil MS Restek), in a temperature-programmed oven and an injection system with Programmed Temperature Vaporization in splitless mode (PTV injector). Raman and mid-infrared spectrophotometric methods were optimized to differentiate and recognise different types of microplastics in feed.
The quantitative determination was carried out in isotopic dilution. The linearity range was 5-500 µg/L (Determination Coefficient: R2> 0.995). Limit of quantification (LOQ) was 0.02 mg/kg for DMP, DnOP, BzBP and 0.2 for DEHP, DEP, DBP. DEHP was detected in 5 out of the 14 samples analysed and concentrations ranged from 0.210 to 0.898 mg/kg. In two samples, DEHP was associated to DMP and BzBP, 0.081 and 0.113 mg/kg respectively.
Feed containing FFP might be one of the routes of ingestion of microplastics; food is the main source of phthalate exposure in humans therefore assessment of contamination levels of phthalates in feed it is of great importance. Investigate to detect phthalates in these matrices can be a good strategy to balance sustainability, safety and circular economy. Due to their lipophilic nature, phthalates can accumulate in the tissues of animals intended for human consumption. Their occurrence is of concern due to their xenogeneic activity, reducing fertility in both men and women. Measuring and quantifying the occurrence of these pollutants in feed could provide a useful tool for a risk-based approach.

O06

Circular Feed and additives

TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF HEMP CO-PRODUCTS AFTER GREEN EXTRACTION AND EX VIVO DIGESTION SIMULATION

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The valorisation of biomass generated along the agri-food chain into co-products represent a valid approach to produce alternative and sustainable feed ingredients. Although to date, some co-products have already been included in the diets of farm animals, the characterisation of co-products from hemp and hempseeds (Cannabis sativa L.), is still at an early stage, despite the trend is growing steadily throughout Europe; a tendency that will allow for increased volumes to be allocated to the feed sector. While hemp has already been characterised in the literature, the co-products are still little investigated, especially from a functional point of view. For this reason, the aim of the present research is to study the total phenolic content (TPC, Folin-Ciocalteu) and antioxidant activity (ABTS assay)) of hemp co-products such as panel (HP), leaves/flowers mix (HLs) and hulls (HHs), after green extraction (water: EtOH, W:E) and ex vivo digestion process. For the green extractions, 150mg of samples were incubated for 1h, at room temperature, under shaking, with different concentrations of W:E (100:0, 75:25, 50:50, 25:75, 0:100). At the end of the incubation, the samples were centrifuged for 5 min at 4000 rpm and subjected to multiple extraction process (n=3). Considering ex vivo digestion, after collecting gastric and intestinal fluids from swine (days 50-110, n=24 at slaughter house), they were pooled and kept at 4oC until use. Then, 500mg of hemp co-products were subjected to gastric digestion (39oC x 2h) in gastric fluid, and further exposed to intestinal fluid (39oC x 2h). Aliquots were taken at the beginning of gastric digestion (0h), at the end of gastric digestion (2h) and at the end of the intestinal phase (4h), to assess TPC and antioxidant activity during ex vivo digestion. Data are expressed as mean ± SEM. Considering green extraction, data obtained showed a valuable TPC content and antioxidant activity, in particular with 50:50 W:E extraction, even when compared to references co-products (e.g. grape marcs). Although the HP showed lower values than the other matrices, the HLs and HHs showed higher levels, with statistically significant differences for TPC (3551.12±98.54 and 1647.11±60.53 mg Tannic Acid Equivalent (TAE)/100g respectively) than grape marcs (1123.29±34.47 mg TAE/100g) ($p<0.05$). These values were also confirmed for ABTS. In particular, HLs (6950,10±546,82 mg Trolox Equivalent (TE)/100g) showed statistically significant differences compared to HHs and grape marcs (2106,58±232,72 mg TE/100g and 1454,14±122,80 mg TE/100g respectively). Considering ex vivo digestion, HLs and HHs showed higher values than HP and grape marcs at each stage of the digestive process. These results encourage further investigations both to identify and characterise phenols and antioxidants responsible of such activities and to define the best level of inclusion of these matrices in feed to ensure high animal health and high production performance.

O07

Circular Feed and additives

ASCOPHYLLUM NODOSUM AND LITHOTHAMNIUM CALCAREUM AS FUNCTIONAL FEED ADDITIVES ALTERNATIVES TO ANTIBIOTICS IN F4+ ESCHERICHIA COLI CHALLENGED PIGLETS

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In pig farming, weaning is a critical moment during the life of young animals where there is a high incidence of multifactorial disorders, particularly from gastrointestinal origin, such as post-weaning disease, enterotoxemia and systemic pathologies such as oedema disease mainly caused by pathogenic strains of E. coli. For reducing their detrimental effect on animal health, farmers and veterinarians' resort to antibiotics for treating affected piglets. Due to the raising issue of antimicrobial resistance, novel strategies are needed for preserving the last effective antibiotic molecules. In this scenario, functional nutrition is one of available strategies to promote animal health and decrease the risk of pathology development, particularly in young animals that often are not completely immunocompetent. The goal of the following study was to assess an innovative dietary supplementation of the combination of Ascophyllum nodosum and Lithothamnium calcareum on animal health, E. coli shedding, intestinal morphology of jejunum, gene expression, and oxidative status in F4+ Escherichia coli challenged piglets. Forty-eight post-weaning pigs (28±2 days) were enrolled in two different groups (n=24/group) balanced per weight and sex: control group was fed with a commercial diet (CTRL), and seaweeds group was fed with commercial diet supplemented with 1.5% of A. nodosum and 0.5% of L. calcareum for 27 days (ALGAE). After 13 days, 50% of animals/group were challenged with a single dose of 10^8 UFC/dose of E. coli F4+ obtaining two infected groups (CTRL+ and ALGAE+, n=12/group). Zootechnical performance were registered by monitoring the body weight on a weekly basis. Faecal samples were collected from day 13 to 18 for the evaluation of E. coli shedding by plate counting. After 27 days serum samples were collected to evaluate the oxidative status and antioxidant barrier through colorimetric tests. At the end of the trial, 6 animals/group were slaughtered, and intestinal sections were sampled for histological evaluation and gene expression by RT-qPCR. Results revealed a higher body weight in ALGAE+ group compared to CTRL+ after 20 days ($p < 0.0001$). E. coli shedding showed a similar trend in ALGAE+ and CTRL+ groups during the 5 days post-challenge confirming adopted procedures for oral infection. Higher antioxidant barrier was registered in blood serum of seaweeds supplemented group compared to commercial diet pigs at 27 days of trial ($p < 0.05$). Jejunum morphology showed lower villus height and width in CTRL+ compared to ALGAE+ ($p < 0.05$). Gene expression of duodenum section showed a tendency to increase the mRNA transcription of transforming growth factor beta in CTRL+ compared to ALGAE+ after 27 days ($p < 0.09$). Obtained findings encourages further studies for development of functional algae-based formula to increase animal health, preventing intestinal disorders caused by pathogenic E. coli, thus decreasing the antibiotic treatments in weaned piglets.

O08

Circular Feed and additives

EFFICACY OF A SPORE-FORMING BACTERIA (BACILLUS COAGULANS) ON THE HEALTH OF WEANING HOLSTEIN FRIESIAN FEMALE CALVES

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Bacillus coagulans is a lactic acid-producing and spore-forming bacterium. This combination makes this bacteria a unique probiotic in animal nutrition and its beneficial effects can be attributed to several modes of action. The safety and efficacy of Bacillus coagulans DSM 32016 (Technospore®), when used as a zootechnical additive for piglets (suckling and weaned), other growing Suidae, chickens for fattening, other poultry for fattening and ornamental birds were attested by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) in 2020. The study aimed to evaluate the efficacy and effects of Bacillus coagulans DSM 32016 administration on the health of young Holstein Friesian female calves. After birth, the animals were housed in individual boxes with free access to water. A total number of 20 calves were randomly divided into 2 homogeneous groups on the basis of body weight from the seventh day of life (0 d of trial) until 63 days of age (56 d of trial). Control group was fed with a standard milk replacer (MRP) plus concentrate and treated group was fed MRP and concentrate supplemented with $1*10^9$ CFU/kg Bacillus coagulans DSM 32016. Withers height, length (from withers to the base of tail), hip width, chest width and girth were registered on day 0, 28 and 56 on trial to perform morphological evaluations. The following samples were collected: colostrum for IgG content, feces (0, 28 and 56 days on trial) for Cryptosporidium spp analysis and fecal microbiota characterization by 16S rRNA-gene sequencing. Blood samples were collected at 0, 28 and 56 days on trial for haematological, metabolic parameters (NEFA, BHB and glucose) and antioxidant capacity (OXY-Adsorbent test) assessments. The morphological evaluations revealed that on day 56 on trial the treated animals registered a higher wither height if compared to control calves (94.44 ± 4.92 cm vs 89.38 ± 1.81 cm; $p<0.05$). The value of IgG monitored in the first milking colostrum (6 to 12 hours) were above 50 mg/ml with a positive immune transfer to calves. Haematological and metabolic parameters did not show any differences between treated and control. Results revealed that the gut microbiota was dominated by Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria phyla which together made up for 95% of all microbes. As in similar studies the consumption of Bacillus coagulans was able to increase population of Lactobacillus and Bifidobacterium in the gut. On day 14 of trial a slight difference was highlighted in terms of point prevalence of Cryptosporidium spp. in favour of the treated group (40%) in comparison to control (45%). The administration of Bacillus coagulans significantly affected the antioxidant capacity. Serum antioxidant capacity was significantly ($p<0.05$) enhanced after 63d (439.77 ± 81.90 control vs 546.16 ± 116.20 µmol HClO/ml treated). The results obtained confirmed the positive effect of Bacillus coagulans administration in supporting calves at weaning.

O09

Circular Feed and additives

YEAST MIXTURE BENEFITS GUT HEALTH OF POST-WEANING PIGLETS

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Intestinal dysbiosis in the postweaning period has negative repercussions on the overall health of the animals, with subsequent impaired performance. Yeast supplementation has been proven effective in modulating gut microbial population and the reduction of weaning stress. Yeast is one of the main brewing by-products, generating 2 to 4 kg of yeast waste per 100 L of beer. The recovery and reuse of these by-products to obtain functional ingredients and feed additives contributes to the concept of circular economy in the agri-food sector.

Yeast market for animal feeding is currently dominated by S. cerevisiae, but Kluyveromyces spp. and Pichia spp. are gaining increasing attention thanks to their pool of extracellular enzymes. At the moment, few data are available on any possible cumulative effect of different yeast strains administration. Thus, the present study aimed to investigate the efficacy of a yeast mixture (YM, Levustim B0399, Vetoquinol; Kluyveromyces marxianus fragilis B0399, Pichia guilliermondii, and Saccharomyces cerevisiae, intact inactivated cells) on growth performance and gut health of weaned piglets.

Forty-eight male piglets (27±1.7 d, 7.19±0.54 kg) were divided into two homogeneous groups and enrolled in a 28-days trial. Piglets were fed a basal diet with (T) or without (C) inclusion of 0.8% YM (weeks 1-2) and 0.6% (weeks 3-4). Growth performance was determined at the beginning, on the trial's second and fourth weeks. Fecal collection was performed at 4, 14, 21, and 28d for microbiota analysis. At 28d, ileum tissue was sampled for morphological analysis and for analysing the overall mucins profile using Alcian-Blue combined Periodic acid-Shiff (PAS) staining. Growth performance and fecal score were analysed by a MIXED procedure of SAS for repeated measures, while morphological parameters were analysed using a GLM procedure. Significance was declared for $p < 0.05$. No significant differences were observed for growth performance among the experimental groups, while increased fecal consistency was outlined in T group ($p < 0.01$). At all the sampling times, Firmicutes and Bacteroidetes resulted the most abundant phyla in both experimental groups (70% and 20% of the total fecal microbiota, respectively). The administration of YM determined changes in the relative abundance of some taxa, among which Bifidobacterium ($p = 0.006$), Coprococcus 2 ($p = 0.015$), and Clostridium Sensu Stricto 1 ($p = 0.019$). In terms of morphology, villus height, and width were significantly affected by yeast supplementation ($p < 0.001$; $p = 0.014$, respectively). The Mucin profile revealed that mucus in ileum goblet cells was heterogenous, with a higher presence of PAS-positive mucins in the villi of T piglets ($p= 0.037$). Overall, the administration of a YM showed to exert beneficial effects on the gut health of post-weaning piglets, reducing potentially harmful bacteria while improving beneficial genera and intestinal morphology.

O10

Circular Feed and additives

EVALUATION OF PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF COCOA (THEOBROMA CACAO L.) BY-PRODUCTS TO EXPLORE THEIR POTENTIAL AS ANIMAL FEED ADDITIVES

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The production and distribution of food exert significant strain on natural resources and around 20% of food produced in the European Union (EU) goes to waste. To create a more sustainable and competitive Europe, the European Circular Economy Action Plan focuses on minimizing waste and obtaining superior secondary resources. Cocoa processing by-products contain compounds of interest for various industries (i.e., food, cosmetics, chemical industries): polyphenols, methylxanthines, dietary fibres and lipids. These qualities make cocoa bean shells an interesting matrix for reuse in animal feed, in line with the circular economy aim of optimizing available resources and reducing food waste.
In this study, we conducted a comprehensive characterization of cocoa bean shells using in vitro methods such as the Folin-Ciocalteu assay, DPPH Assay, and vanillin test. Three different samples, S1 (flakes), S1K (chunky flakes), and S2 (pellets), obtained from cocoa bean by-products, supplied by a Swiss former foodstuff processor, were evaluated for their total polyphenol content, overall antioxidant capacity, and flavan-3-ols. A comprehensive characterization of the cocoa bean shells was also conducted using High-Performance Liquid Chromatography coupled with Ultraviolet Detection (HPLC-UV). Target analytes were identified by comparing the retention times of chromatographic peaks with those of standards.
The findings from the in vitro methods showed that sample S2 had the highest phenolic compounds and flavan-3-ols concentration. Sample S1 and S1K exhibited comparable values in terms of antioxidant activity, total polyphenols, and flavan-3-ols, although both were lower than Sample S2. These results were consistent with the outcomes obtained by HPLC-UV analysis. Samples S1 and S1K, both in flake form, showed a similar quantitative content of analytes, while Sample S2, in pellet form, had higher concentrations. Theobromine was the most abundant analyte in all samples, followed by caffeine, epicatechin, and p-hydroxybenzoic acid. These results suggest that the physical form (pellet or flake) of cocoa bean by-products affects the concentration of analytes and antioxidant properties.
Overall, cocoa bean shells can serve as a valuable source of polyphenols and flavan-3-ols with known antioxidant activity, suitable as high-quality secondary raw materials in the feed industry. However, it is crucial to consider the theobromine and caffeine content due to their potential toxicity to animals. While Theobroma cacao L. is an authorized feed additive in the EU (Directive 70/524/EEC), it is essential to obtain proper formulation when incorporating cocoa or its by-products into animal feed, following established guidelines and complying with the maximum levels of theobromine in animal feed (Directive 2002/32/EC), for ensuring the safety and well-being of animals.

O11

Circular Feed and additives

SUPPLEMENTING BROILER DIET WITH A NEW GENERATION EMULSIFIER: EFFECTS ON PERFORMANCES, NUTRIENT ABSORPTION, AND COST OPTIMIZATION.

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The administration of emulsifiers is useful in diets for commercial hybrids of broiler chickens that require feeds with a high energy content. Lipid energy sources, such as oils, are commonly included in diets for their capacity of easily raising the caloric content of feed. On the other hand, the reduction of complex energy sources could be an ideal strategy to increase the sustainability of the feed production chain. Given that, we decided to investigate the effects of a novel emulsifier (Nutriemul P, Sevecom part of Barentz) on broiler ROSS 308 chickens performances and apparent total tract digestibility (ATTD) in a low-energy diet. Immediately after hatching, a total of 720 broiler ROSS 308 male chicks were randomly divided into 4 homogeneous experimental groups: a positive control group (PC) fed with a normal energy content in diet, a negative control group (NC), fed a basal diet formulated with a reduction of 70 kcal in terms of energy based on the modulation of soybean oil inclusion levels, and two treatment groups, T1 and T2, where the same low-energy diet was characterized by the inclusion of two different levels of emulsifier, 250 and 500 g/ton respectively. Homogeneity of the feed mixture was assessed through Micro Tracer system, including inert iron particles coated with food coloring at 0.5 % of complete feed. Performances were evaluated on day 0, 10, 21 and 42 of trial. Celite 535® was included at 0.5% of complete feed in finisher diet to enhance the acid-insoluble ash (AIA) content. Faecal samples were collected on day 21 and 42 on trial to further establish DM, OM, CP, EE, AME, ASH, and AIA content and calculate the total tract digestibility (ATTD) of the considered nutrients. Performances were evaluated through a MIXED procedure of SAS. ATTD of nutrients was analyzed by a GLM procedure of SAS. The MT evaluation revealed an excellent quality of the mixing process. At the end of the trial BW was markedly higher in T1 and T2 group comparing both with NC and PC (2845.67 ± 90.69 g and 2773.83 ± 93.56 g vs 2557.52 ± 83.68 g and 2608.97 ± 95.64 g; $p<0.01$). ADG was significantly higher from 0 d to 42 d in T1 and T2 If compared to NC and PC groups (68.37 ± 2.21 g and 66.63 ± 2.29 g vs 61.34 ± 2.05 g and 62.60 ± 2.33 g; $p<0.01$). ATTD of DM and OM was enhanced by the inclusion of emulsifier in T1 and T2 ($p<0.05$). Ash digestibility was better at the end of the trial in T1 and T2 ($p<0.01$) while CP ATTD depicted a more pronounced digestibility rate in the treatment groups at 21 d ($p< 0.05$) and 42 d ($p<0.01$). Furthermore, EE and AME ATTD were increased by both dosages of emulsifier ($p<0.05$). These results suggested the possibility to improve the performances of broiler ROSS 308 through the administration of a new generation emulsifier which increases the digestibility rate of dietary nutrients contributing to the reduction of the use of complex energy sources in favor of a more sustainable approach to animal nutrition.

O12

Circular Feed and additives

SOIL NEMATODE C. ELEGANS INFECTION MODEL FOR SCREENING OF NOVEL PLANT-BASED ANTIMICROBIALS AS FEED ADDITIVES

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Pathogen infections play an important role in human and animal health as well as in food safety. Due to bans of antibiotic growth promotors or the pharmacological use of zinc oxide in the feed industry as well as increasing concerns regarding antibiotic-resistant bacteria, natural compounds (phytochemicals) gained more and more attention. Phytochemicals comprise secondary plant metabolites such as polyphenols, flavonoids or terpenes and were already shown to provide anti-inflammatory or antioxidant properties. Here, we utilize a multi-screening approach for the detection of novel, plant-based antimicrobials for applications as feed additives (e.g., broiler, piglets). In this work, 300 plant extracts were tested for either quorum sensing or bacteriocidic effects.
First, local, water-soluble plant extracts were measured in a plate-based assay utilizing the bacterial biosensor Chromobacterium violaceum. Absorbance measurement of violacein production was performed which directly correlates with the inhibition grade. Next, positive hits were further screened in a 96-well approach with selected pathogens including Pseudomonas aeruginosa or Salmonella enterica. Finally, the most effective extracts were applied in our C. elegans infection model to monitor alterations in nematode health span. C. elegans was pre-treated with the plant extracts followed by infection with pathogens to validate their potential protective or recovering effects. Common pathogens were previously identified to not only infect humans or higher animals but also C. elegans. The soil nematode possesses an innate immune system, thus capable of responding to bacterial infections. Hence, the use of C. elegans as an infection model provides a powerful tool for understanding host-pathogen interactions and developing alternative treatments for infectious human and animal diseases.
In the Chromobacterium assay, we identified 8 plant extracts with high inhibitory effects (>90%) and additional 15 compounds with moderate inhibitory effects (>50%). Furthermore, biofilm formation as well as pyocyanin and pyoverdine production were inhibited for several extracts in the Pseudomonas screening approach (10-70%). Thus, novel compounds with potential antimicrobials traits for the feed sector were identified on a bacterial level. Previously, we identified several suitable read-out parameters based on literature including gene expression markers (e.g., MAPK and IIS pathway; irg-1, gst-4), fluorescent gene reporters and lifespan to monitor pathogen infections in C. elegans. Currently, the extracts of interest are analysed in our C. elegans infection model.
To sum up, pathogen infection depicts severe issues for the food and feed industry. Natural compounds could act as novel antimicrobials when added as feed additives. We were able to identify several extracts of interest in bacteria-based screening assays. The potential use is currently further examined in the whole-animal model organism C. elegans.

O13

Feed quality, safety and authentication

MEASURES AGAINST ANTIMICROBIAL RESISTANCE: METHODS OF ANALYSIS FOR 24 ANTIMICROBIAL ACTIVE SUBSTANCES IN FEED AT CROSS-CONTAMINATION LEVEL TO SUPPORT EU LEGISLATION ENFORCEMENT

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In animal husbandry, antibiotics are used to ensure animal welfare and to provide appropriate cure in case of bacteria induced illnesses similarly as in human medicine. Medicated feed is one of the routes for oral administration of veterinary medicinal products. In usual production schemes, there are no dedicated production lines for medicated compound feed versus non-medicated or for a compound feed supplemented with one antibiotic versus a compound feed supplemented with a different antibiotic. Consequently, there is a high probability that traces of the antibiotic present in the firstly produced compound feed remains in the production line and contaminates the next compound feed produced on that same line. This cross-contamination, besides the potential impact on the health of the animal due to the unforeseen presence of the given antibiotic in non-target feed, also contributes to the emergence of antimicrobial resistance (AMR), which is currently a real threat for both animal and human health. Combating AMR is a top priority worldwide that is also addressed at European level. In this context, the European Commission (EC) has committed to issue delegated acts for the enforcement of Regulation (EU) 2019/4 that specify the maximum acceptable cross-contamination levels in compound feed for twenty-four antibiotics listed in Annex II of Regulation (EU) 2019/4.

The Directorate General for Food and Health (DG SANTE) of the European Commission has therefore requested support from the Joint Research Centre of the European Commission for the provision of methods of analysis for the twenty-four antimicrobial active substances in feed at cross-contamination level. The availability of suitable and reliable analytical methods is indeed of key importance for facilitating the implementation and monitoring the efficiency of the Measures against Antimicrobial Resistance taken by the European Commission.

The current contribution will present the three analytical methods developed and validated at the Joint Research Centre, which enable to detect traces of these 24 antibiotics belonging to 10 therapeutic families in compound feed. The performance characteristics established showed the fitness for purpose of these three methods for the determination of the twenty-four antibiotics in five different types of compound feed (swine, bovine, ovine, poultry and rabbit compound feeds) at levels as low as achievable using common High Performance Liquid Chromatography coupled to Tandem Mass Spectrometry instruments routinely available in official control laboratories for feed analysis.

These findings will be used by DG SANTE as one criterion for the set-up of the maximum levels for cross-contamination acceptable for the twenty-four antibiotics listed in the Annex of Regulation (EU) 2019/4.

Keywords: antibacterial substances, compound feed, cross-contamination, antimicrobial resistance

O14

Feed quality, safety and authentication

DETERMINATION OF AMINOGLYCOSIDE ANTIBIOTICS IN FEED AT CROSS-CONTAMINATION LEVELS FOR ENFORCING EU LEGISLATION

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Infections by multidrug-resistant bacteria are responsible for about 33 000 annual deaths in the European Union (EU) and 700 000 worldwide. Moreover, antimicrobial resistance (AMR) is of growing concern causing an estimated annual cost of 1.5 billion EUR in the EU due to healthcare expenditures and productivity losses. Combating AMR is thus a top priority worldwide and requires concerted action by several high-level institutions and organisations such as the European Commission (EC), the European Medicines Agency (EMA), the European Food Safety Authority (EFSA), just to name a few only at European level.
Ensuring the lowest possible levels of unauthorised antibiotics are present in compound feed due to cross-contamination is certainly an effective measure to reduce the spread of AMR. Four aminoglycoside antibiotics (apramycin, paromomycin, neomycin and spectinomycin) are included in the list of priority antibiotics to be controlled in feed at cross-contamination levels (Regulation EU 2019/4). These antibiotics are challenging to analyse due to their high polarity and the ability to interact with labware and feed components. Four published standard operating procedures based on alternative principles of sample preparation (mixed mode cation exchange, weak cation exchange, reversed phase, and molecularly imprinted polymer SPE associated with various extraction protocols) were investigated for the analysis of pig feed. None of them provided satisfactory results serving the needs for a method to be applied in the frame of official control.
This poster presents the rational for abandoning the above-mentioned protocols and proposing an amended one. The different sample preparation stages (extraction, pH adjustment and SPE) were carefully assessed and key parameters have been identified and optimised. The developed method involves an efficient clean-up and analyte concentration hence, enabling the quantification of aminoglycosides in feed by LC-MS/MS at very low levels. This work is part of the overall JRC commitment and effort to provide the EC policy makers and the Member States with robust and validated methods as reliable analytical tools for monitoring the 24 antibiotics listed in Regulation EU 2019/4 at cross-contamination levels.

O15

Feed quality, safety and authentication

NEW APPROACH METHODOLOGIES FOR MECHANISTIC TOXICOLOGY-BASED FEED SAFETY ASSESSMENTS

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New Approach Methodologies (NAM) comprise novel approaches including multi-level omics, in silico and computational tools which in their totality allow for systems-level mechanistic effect assessments of the exposure to xenobiotics in vivo and in vitro. In the past, the use of omics in farmed animals has been hampered by a general lack of publicly available genomic information. An uptick in sequencing efforts and a wider application of automated genome annotation pipelines has in part remedied this challenge. To further advance the applicability of omics approaches for feed safety studies, the application of proteogenomics strategies (which include an integrative analysis of genomics, transcriptomics, and proteomics data) was suggested. Here, we show how a proteogenomics analysis workflow developed at the Bafna lab at the University of California San Diego (UCSD) can be utilized in mechanistic toxicity studies in non-model species using Atlantic salmon (Salmo salar) as an example. Based on transcript data of several salmon tissues obtained from public repositories, and in-house generated RNA-Seq data of primary salmon hepatocyte and salmon liver samples, proteogenomic databases were constructed. Searching these data against corresponding proteomic LC-MS/MS datasets led to the identification of several thousand peptides which provided direct evidence of translation for computationally predicted RefSeq gene structures. In addition, proteogenomic events were detected that corrected earlier gene annotations and identified novel (previously unannotated) genes. Protein abundance matrices derived from the proteogenomic dataset revealed distinct expression patterns of proteins involved in the defense against xenobiotics across the different salmon tissues. At the same time, across in vivo and in vitro salmon liver systems, a large degree of homology in protein expression of chemical defense proteins was observed providing molecular support for the feasibility of in vitro – in vivo extrapolation (IVIVE) in farmed fish. The proteogenomic salmon data is currently being used to mechanistically assess and describe Mode of Action (MOA) and Adverse Outcome Pathways (AOP) related to pirimiphos-methyl (PM) exposure; a xenobiotic found to be among the most dominant pesticides present in plant-based salmon feeds. Taken together, our work shows that routine application of NAMs in feed safety research is feasible and can contribute to the advancement of the mechanistic understanding of target tissue toxicity assessments and IVIVE modeling in farmed animal species. It is envisaged that the present work will contribute to the closing of regulatory data gaps aiding work initiated by the European Commission (EC) and CODEX which, in anticipation of setting maximum residue levels (ML) for pesticides in seafood including farmed fish, have started to devise protocols for sample analysis of pesticides in fish products.

O16

Feed quality, safety and authentication

CONTRANS: A TOOL TO ESTIMATE FEED-TO-FOOD CONTAMINANT TRANSFER IN FARM ANIMALS

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Predictive toxicokinetic models are mathematical tools that integrate knowledge on the transfer of contaminants from feed to food. They are based on in vivo, ex vivo and in vitro experimental studies, as well as other in silico tools, and can be used to simulate the absorption, distribution, metabolism and excretion of undesirable substances such as persistent organic pollutants [e.g. “dioxins” or per- and polyfluoroalkyl substances (PFAS)], plant toxins [e.g. quinolizidine alkaloids (QAs)] or heavy metals in farm animals. The existence of a published model does not directly benefit risk assessment or management, as it consists of abstract algorithms. To mitigate this problem, we developed ConTrans as an easy-to-use graphical tool to empower risk analysis specialists to perform feed-to-food contaminant transfer estimations based on such models.
ConTrans was designed to estimate transfer into a wide variety of substance-animal product combinations using a single interface. The user can configure exposure scenarios with feed and/or drinking water, as well as different depuration periods.
As an application example, PFAS have gained prominence due to their potential to bioaccumulate in livestock and wildlife and lead to consumer exposure via edible tissues, eggs and milk. In 2020, the EU risk assessment (EFSA) concluded that the exposure of parts of the European population to 4 PFAS is of concern. Subsequently, EU’s risk management established maximum levels (MLs) for the same 4 PFAS in foods of animal origin. ConTrans can help to understand the relationship between existing MLs in food and their consequences for allowable PFAS feed and water levels. Relatively low concentrations in feed can lead to the MLs being exceeded in food of animal origin, yet no MLs exist for feed.
ConTrans includes the first predictive tool for the transfer of 6 PFAS into hen eggs, integrating knowledge from multiple studies. As an example result, a diet of 115 g/d DM (dry matter) contaminated with 0.42 µg/kg DM perfluorooctane sulfonic acid (PFOS) for laying hens is estimated to result in an egg concentration of 1.0 µg/kg wet weight (WW), or exactly the ML for eggs, which is reached asymptotically about 30 days after the start of exposure.
ConTrans can help to understand the relationship between existing MLs in food and their consequences for allowable feed and drinking water levels. An interesting result is a cross-species comparison: for selected PFAS, the MLs for pork correspond to very low allowable feed concentrations, up to an order of magnitude lower than those allowable for feed destined for beef. Such low theoretically allowable concentrations in feed highlight the need to develop and widely implement analytical techniques with limits of detection (LODs) for individual PFAS below 0.01 µg/kg 88% DM in feed.
These results illustrate how ConTrans helps feed and food scientists make quantitative predictions of the transfer from oral exposure to food of animal origin.

O17

Feed quality, safety and authentication

QUALITY ASSURANCE AND CONTROL OF METHODS TO EXAMINE VISUALLY RECOGNIZABLE SUBSTANCES IN FEED AND FOOD

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In 2022 a guideline was published, with the aid of an international group of experts, by Wageningen Food Safety Research (WFSR) to examine, clarify, and to help people apply validation studies and the development of methods in the visual inspection of feed and food materials.
The guideline presents an elementary overview of the validation and application methods to examine visually recognizable substances in feed and food. Although quality parameters and criteria for visual inspection methods have been frequently taken from other major disciplines, such as analytical chemistry and microbiology, it is necessary to acknowledge that visual examination is a completely different discipline, with different required approaches. The basic principle of visual inspection is the examination of undesired substances or ingredients occurring as visible units of extremely large dimensions when compared to molecules. As a consequence, the statistical background and physical distribution are principally different from those of molecules, which affects the design and validation of visual inspection methods.
The guidance will present and discuss specific applications of relevant quality parameters in visual detection methods, including microscopy, and a framework of dedicated sets of quality parameters for the domain of visual monitoring methods. The latter with separate parameters for quantitative, qualitative, and estimation methods. Elements for the design of visual examination methods are presented and discussed in relation to quality parameters.

O18

Feed quality, safety and authentication

RANKING OF CHEMICAL HAZARDS FOR RISK-BASED MONITORING OF FARMED FISH, FEED AND FEED MATERIALS

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In the European Union (EU), food safety is ensured through several measures, including official monitoring of chemical substances in food and feed products to control the safety in the food and feed chain. Following the implementation of the EU regulation on official controls (EU 2017/625), monitoring programs must be risk-based, focusing on those products and hazards that pose the highest risk for human health.
In aquaculture production of farmed fish, contaminants can originate at different stages of the production of food, including feed raw materials, complete processed feed, and other sources. In Norway, monitoring programs for farmed fish, fish feed and feed materials have been performed annually since the late 1990s. Prioritization of contaminants has been based on legislation, scientific literature and other knowledge on potential risks with farming of fish. However, a systematic approach for the prioritization is now required, ensuring risk-based monitoring of the Norwegian aquaculture production chain.
Food safety comprises both a legal and a health dimension. The work was based on a method developed by van Asselt et al. [1] for risk-based monitoring plans for chemical contaminants in food products, and was further developed to also include feed and feed materials. Contaminants in the monitoring programs were evaluated considering the following factors (1) monitoring requirements with respect to legal limit values, (2) consumer health risk, (3) risk of exposure through transfer along the production chain, (4) risks to animal health or/and the environment, (5) existing data from the monitoring of aquaculture production in Norway and (6) risks arising from knowledge gaps. Existing data was retrieved from three monitoring programs, i.e. the program for farmed fish, fish feed and feed materials.
Chemical substances currently included in the programs were ranked by priority according to different risk factors, including legal action limits and indications of human and animal health risks. Following this approach, the substances were classified into low, medium or high priority for monitoring in aquaculture feed and/or farmed fish. In addition to a prioritization of compounds already included in the programs, emerging compounds identified by EFSA and the Norwegian scientific committee (VKM) were evaluated for their relevance in feed and fish. Furthermore, knowledge gaps were highlighted for the sampling regime, data for different fish species, novel feed materials, the nutrient composition and environmental effects.

O19

Feed quality, safety and authentication

ASSESSMENT OF THE ACCURACY OF NIRS TECHNOLOGY FOR DETERMINING THE PROXIMATE COMPOSITION AND AMINO ACID CONTENT OF COMMERCIAL SOYBEAN MEAL SAMPLES

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In total, 30 commercial samples of soybean meal (SBM) from the USA (n = 11), Brazil (n = 10), and Argentina (n = 9) were randomly collected by trained personnel from European feed mills. DM, CP, amino acids, EEh, and CF were analyzed by wet chemistry (WCh) and NIRS. All wet analyses were performed in the same laboratory using official methods. NIRS values were determined by 4 specialized European laboratories (A, B, C, and D) using their own calibration models. Independently of the SBM origin, the average and range of WCh values (as fed bases) were: 88.7% (85.6-91.1%) for DM, 46.2% (44.2-49.8%) for CP, 2.41% (1.40-3.30%) for EEh, 4.66% (4.10-5.20%) for CF, 2.91% (2.75-3.06%) for Lys, 0.63% (0.53-0.68%) for Met, and 0.67% (0.59-0.75%) for Cys. Bias, slope, and unexplained error were the statistics used to investigate the accuracy of WCh vs. NIRS, including comparisons among the 4 labs. The analysis was supported by a scatter plot of the ratio of NIRS to WCh (di) vs. NIRS values (ni) on a logarithmic scale, including bias confidence limits (BCL), limits of agreement, and any other trend that could be detected in the plot. DM content was properly determined by the 4 labs, with 95% of the ratios within the 0.981 to 1.031. CP values were close to reference values, with a mean ratio between 0.992 and 1.048. The BCL variation among labs implied that the under- and overestimation for the CP varied between 2% and 5%, respectively. For EEh and CF, the variability of the ratio ranged from 0.49 to 2.37 and from 0.47 to 1.29, respectively. The scatter plot trends showed that the magnitude of the detected error depended on the variable measured. For Lys, NIRS values were similar among companies, with lab “C” showing the lowest rate and an underestimation of around 7%, whereas for lab “D” the overestimation was as high as 7%. For Met, lab “C” had a slightly higher prediction error than all the others, with over- and underestimation being of 6% and 4%, respectively. The error for Cys varied by 4% and 9%, for under- and overestimation, respectively. NIRS data from labs “A” and “D” were similar to WCh data, with a SSEP of 0.02. In summary, the data showed that NIRS technology is a valid alternative to WCh to estimate the chemical constituents of commercial SBM. However, its accuracy depends on the analyzed component, the company that developed the calibration data, and factors, such as the country of origin of the SBM, that affects the chemical composition of the samples.
Keywords: amino acids, near-infrared spectroscopy technology, proximal chemical analyzes, soybean meal, wet chemistry.

O20

Feed quality, safety and authentication

SPECTRA LIBRARY-BASED PROTEOMICS FOR FEED FORENSICS IN A CIRCULAR ECONOMY

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Under the auspices of an increased push towards circular food production systems, processed animal proteins (PAPs) have again become part of the animal feed chain. To ensure safety in the feed system, detecting and identifying the tissue and species-specific origin of PAPs are essential. In parallel to the official PAP monitoring methods, including microscopy and polymerase chain reaction (PCR) assays, alternative methods such as liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics are being developed for authentication purposes. The development and advancement of LC-MS/MS-based tools are hampered by a need for sequence information for target species used in feed products. We have shown that alongside targeted LC-MS/MS assays, untargeted proteomics and spectral library matching approaches (SLM) can be implemented for protein-focused feed forensics allowing for tissue and species identification and quantification of PAPs without any genomic information.
The SLM approach is based on bottom-up proteomics, where samples are digested using trypsin, and resulting peptides are analyzed using LC-MS/MS. For proteomics data processing, tandem mass spectra are analyzed using compareMS2 for quality control and molecular phylogenetic screening. Subsequently, an SLM approach is implemented using SpectraST in the Trans-Proteomic Pipeline (TPP). To ensure the reproducibility of our methods, following the FAIR (Findable, Accessible, Interoperable, and Reusable) principle, all data and spectral libraries created in our laboratory are made available in public repositories such as the Mass Spectrometry Interactive Virtual Environment (MassIVE) platform.
Our research to date shows that SLM-based proteomics successfully differentiated PAPs at the tissue level, such as bovine milk and blood. The approach enabled the identification of the origin of insect PAPs fed on prohibited bovine material and the authentication of insect species authorized to be used as feed material. The untargeted proteomics SLM workflow implemented during our work also successfully separated different fish species in mixed samples and conventionally farmed from transgenic and organic soy. Our data indicates that untargeted proteomics bioinformatics tools allow for rapid and comprehensive efficient protein fingerprinting in feed and food samples.
Current work focuses on developing a dedicated web-based service in which we record all tissue and species-specific data, and our collaborators are made available to the feed authorities and researchers. The SLM database is currently being developed, and following proper quality testing, the database will be publicly released for feed and food forensics purposes.

O21

Feed quality, safety and authentication

INSECT MEAL IN FEED: USE OF NEAR-INFRARED SPECTROSCOPY (NIRS) TECHNIQUES TO SUPPORT THE DETECTION OF AUTHORIZED AND UNAUTHORIZED INSECT SPECIES

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Since the bovine spongiform encephalopathy (BSE) crisis, many restrictive measures have been taken with regard to animal feed. Since 2017, the European authorities have allowed seven species of insects to be used in aquaculture. More recently, in 2021, this authorisation was extended to pigs and poultry, with the inclusion of an eighth insect species. The correct characterisation and identification of insect meals and their detection are required to ensure the application of the legislation. To date, the official control methods have shown some limitations, such as the need for expertise in light microscopy (LM) and Real-Time Polymerase Chain Reaction (RT-PCR) being a targeted method that does not allow the detection of several products at the same time. Furthermore, these different methods do not provide any insight into the chemical composition of the species studied.
In this context, various studies on insect meals have been carried out as part of the EU Farmyng project and the European Union Reference Laboratory for Animal Proteins in feedingstuffs (EURL-AP). Firstly, a sample bank of well-referenced and characterized insect meals has been built. This sample bank has been used as a starting point to develop NIRS models to predict chemical composition of insect meals. The first results gave a RMSEP of 2.21, 2.97 and 1.22 % for protein, fat and chitin contents respectively.
Secondly, Near-Infrared Microscopy (NIRM), combined with chemometrics, was investigated in order to develop a screening method to detect insect particles in feed without any chemical extraction step as is the case with current official methods. Different blends have been created from ruminant feed adulterated at levels of 1 %, 5 % and 10 % w/w with either H. illucens larvae meal or T. molitor larvae meal. Based on PLS-DA analysis, several spectra of insect particles in the blend were identified. The performance of the model was very good, with an accuracy for the respective detection of H. illucens and T. molitor particles always above 96% and 98%.

O22

Feed quality, safety and authentication

DEFINING A COMMON CUT-OFF OF REAL-TIME PCR METHODS IN A NETWORK OF LABORATORIES FOR THE DETECTION OF PROCESSED ANIMAL PROTEINS

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After the bovine spongiform encephalopathy (BSE) epidemic, a European legislation entered progressively into force to eradicate BSE with, as result, the total feed ban of processed animal proteins (PAPs) with some few exceptions such as fishmeal for young ruminants. The drastic decrease of BSE cases led the European Commission to envisage a progressive lift of the feed ban. To reach this goal, the polymerase chain reaction (PCR) was added to the light microscopy (LM) as official control method. Indeed, PCR is specific to a taxon whereas LM cannot discern e.g. cattle bones from pig ones.
CRA-W worked first on the development of a sensitive bovine PCR method. Nevertheless, this method is characterised by a regular occurrence of late signals not necessarily linked to the presence of unauthorized feed material. Therefore, a protocol defining a common cut-off of the method had to be outlined in order to interpret the PCR results similarly within a network of laboratories whatever the PCR platform used.
A cut-off is generally expressed as a Ct value, which as such is not transferable. It was then analysed to what copy number this functional cut-off in Ct corresponded. Astonishingly it appeared to be below one copy. This is not necessarily impossible as the Ct for one copy maybe variable. In practice, we finally arrived to the conclusion that the defined cut-off corresponded to the upper confidence limit at 95% of the Ct for one copy of the target. A protocol was designed to calculate its value on any PCR platform. The protocol is based on a reverse regression where the logarithm of the copy number is the dependent variable and the Ct value the independent variable. To generate sufficient variability the calibrants used are replicated on a same plate but also on different plates. It was analysed how to define a robust value for the upper confidence limit of the Ct at one copy with a rather reduced set of data. The transferability of the protocol has been successfully checked by an interlaboratory study with more than 20 thermocyclers from different brands and models. Although the so-defined cut-off in terms of Ct varied between 37.70 and 43.64 cycles (which on a thermocycler is almost a difference from 1 to 64 copies), it allowed to define a robust and common way to interpret the PCR results of the bovine target whatever the thermocycler used.
Three PCR methods answering to the minimum performances requirements and targeting ruminant, pig and poultry DNA respectively were validated through interlaboratory studies. For each method, the protocol was adapted to obtain the desired sensitivity. Its use is publicly available in procedures available on the website of the EURL Animal Proteins (https://www.eurl.craw.eu). The plasmid calibrants to use for the cut-off determination of the three PCR methods are now produced by the JRC and commercially available as European Certified Reference Materials (ERMs) at https://crm.jrc.ec.europa.eu/.

O23

Feed quality, safety and authentication

THE EMERGING MYCOTOXIN ENNIATIN B CAUSES ADVERSE HEALTH EFFECTS IN ATLANTIC SALMON FARMED ON PLANT-BASED FEEDS, WHILE BEAUVERICIN DOES NOT.

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The extensive use of plant ingredients in novel aquafeeds have introduced mycotoxins to the farming of seafood. The emerging enniatin B (ENNB) and beauvericin (BEA) mycotoxins have been found in the novel aquafeeds and farmed fish. Little is known about the potential toxicity of ENNs and BEA in farmed fish and their feed-to-organ transfer. Atlantic salmon (Salmo salar) pre-smolt (75.3±8.10g) were fed four graded levels of spiked chemical pure ENNB or BEA feeds for three months, in triplicate tanks. Organismal adverse health end-point assessment included intestinal function (protein digestibility), disturbed hematology (red blood cell formation), bone formation (spinal deformity), overall energy use (feed utilization), and lipid oxidative status (vitamin E). Both dietary BEA and ENNB had a low (<~0.01 %) transfer to organs (kidney>liver>brain>muscle), with a higher transfer for ENNB compared to BEA. BEA caused a growth reduction combined with a decreased protein digestion and feed conversion rate- ENNB caused a stunted growth, unrelated to feed utilization capacity. In addition, ENNB caused anemia while BEA gave an oxidative stress response. Lower bench-mark dose regression assessment showed that high background levels of ENNB in commercial salmon feed could pose a risk for animal health, but not in the case of BEA.

Posters

P01

Circular Feed and additives

A SURVEY OF OLIGOELEMENTS AS FEED ADDITIVES FOR ANIMAL HEALTH

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BACKGROUND
The maximum contents of these nutritional additives consider physiological requirements of animals as well as average requirements in the diet, need to meet the needs of most members of animal populations and possible inefficiencies in the use of the nutrients. Feed additives may not be put on the market unless they have been authorised following a scientific evaluation carried out by EFSA demonstrating that the additive has no harmful effects on animal health and on the environment. A total of 271 samples were analysed between 2020 and 2022 to assess the compliance with the mandatory requirements set by the European legislation (Regulation 1334/2003 UE and 767/2009 UE) for the authorisation of additives belonging to the group of trace elements (cobalt, copper, iron, manganese, selenium, and zinc) in feeding stuffs and on the placing on the market and use of feed.
METHODS
Feed samples (complementary n= 142, compound n= 86; feed additives, premixtures, medicated feed, feed materials =23) were homogenized, then 1.5 g of sample was added with. 7 mL of HNO3 (70% v / v) and 1.5 mL of H2O2 (30% v/v) and subjected to mineralization in a microwave digestion lab station (ETHOS 1, Milestone S.r.l, Sorisole, BG). Quantification of Cu, Fe, Mn, Se and Zn was performed by Atomic Absorption Spectroscopy (Analytic Jena, Zeenit 700P), Co by Inductively Coupled Mass Spectrometry (Agilent 1260 Infinity II) and results expressed in mg/kg ± measurement uncertainty.
RESULTS
All feed samples were collected in the frame of Italian National Nutrition Plan.
Cobalt (n=67) was found in the range <LOQ (0.010)-19 mg/kg, mean 0.74, median 0.20
Iron (n=33) was found in the range <LOQ (20)-427 mg/kg, mean 145, median 124
Manganese (n=13) was found in the range 68-162 mg/kg, mean 101, median 96
Copper (n=55) was found in the range <LOQ (10)-36 mg/kg, mean 13, median 14
Selenium (n=51) was found in the range <LOQ (22)-68 mg/kg, mean 2.0, median 0.28
Zinc (n=51) was found in the range <LOQ (20)-10124 mg/kg, mean 361, median 93
CONCLUSIONS
No samples were found exceeding the maximum limits set by Regulation 1334/2003 for feed additives authorization. 3 out of 271 samples (1.1%) were not compliant with the labelling tolerance set out in Annex IV labelling particulars for feed materials and compound feed for food-producing animals.

P02

Circular Feed and additives

TRADITIONAL VS CIRCULAR DIETS: EFFECT OF THE PARTIAL REPLACEMENT OF CORN AND SOYBEAN MEALS WITH BAKERY FORMER FOOD PRODUCTS AND WET DISTILLERS GRAINS ON PRODUCTION PERFORMANCE, DIGESTIBILITY AND ENVIRONMENTAL SUSTAINABILITY IN BEEF HEIFERS

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The beef cattle sector is facing many sustainability challenges, such as greenhouse gases (GHG) emissions, deforestation, land and water use and pollution and human-edible resources consumption, combined with an increase in the demand of meat. Losses along the food chain represent another global dilemma with negative environmental, social, and economic consequences.
Sustainable feeding strategies are required to improve those aspects in livestock farming. The use of alternative resources such as food losses no longer suitable for human consumption instead of cereal grains and soybean can be an innovative way to valorise those losses, reduce food–feed competition, and mitigate the environmental impact of beef cattle.
The effects of the partial substitution of corn and soybean meals with bakery former food products (BFF) and liquid wheat distillers grains (WDGs), in beef cattle on environmental sustainability and production efficiency were evaluated. Newly arrived Limousine heifers (n = 408), housed in an Italian fattening farm, were divided in two groups, balanced for initial weight and body conformation: (i) Traditional corn–soybean meals diet; (ii) Circular diet with average as-fed 1.5 kg BFF and 1.5 kg WDGs as substitute for 1.6 kg corn and 0.3 kg soybean meals. The environmental impact of the production of the feeds involved in the partial substitution of corn and soybean meals with BFF and liquid WDGs was analysed considering GHG emissions (kg CO2 eq), water (H2O, L), and land use (LU, m2) as well as inclusion of human-edible feeds (HE, kg).
Individual weights were collected at enrolment, day 92 and day 145, and the relative average daily gains (ADG) were calculated. The pen feed intake (FI) was evaluated weekly, comparing the feed given and the feed refused after 24h, corrected for the dry matter of the two diets. Pen feed conversion rate (FCR) was then calculated by comparing the average FI with the ADG per pen and period. Apparent total tract digestibility (aTTD) was evaluated monthly using a portable NIR instrument by comparing the chemical composition of the fresh feed and faeces, collected from 20 heifers for each pen per group 24 hours later. Health status was monitored daily. Carcass weights and muscle and fattening scores were recorded individually at slaughterhouse. Colorimetric characteristics and pH were evaluated in 20 animals per group at 24 h post-mortem.
The Circular diet led to a reduction per kg of cold carcass weight (CCW) of 1.00 kg CO2 eq of GHG, 72.38 L of H2O, 1.20 m2 of LU, and 0.95 kg of HE ($p<0.0001$). Growth performances, carcass characteristics, and health status were not affected ($p>0.05$). The aTTDs of sugar and pectin were significantly higher ($p<0.0001$) in the Circular group.
Replacing traditional feed ingredients with BFF and WDGs reduced the environmental impact of the diet of fattening Limousine heifers and the food competition between humans and beef cattle, in accordance also with circular economy principles.

P03

Circular Feed and additives

THE IMPACT OF HARVESTING TIME AND NUTRITIONAL ASPECTS OF HEMP (CANNABIS SATIVA L.) ON ITS ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS

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Antibiotic resistance is a growing threat to both human and animal health and welfare. Among bacterial pathogens, Staphylococcus aureus is the species which currently possess the major challenges in terms of bacterial resistance. In addition, S. aureus has been identified as the so-called “ESKAPE” microorganisms which have caused significant morbidity and mortality. Thus, there is a growing need for the development of alternatives to antibiotics in the prevention and treatment of S. aureus infections. One of the new alternatives with a promising antibacterial effect is hemp (Cannabis sativa L.) and its products. Hemp contains a number of bioactive compounds, including cannabinoids, known for their anti-inflammatory, antibacterial, or antioxidant properties. The aim of this study was to determine the antibacterial activity of cannabis ethanolic extracts towards two strains of S. aureus (both methicillin-sensitive and methicillin-resistant ones) and determine the effect of plant nutrition and a week of its harvesting. Plants were grown in 4 different nutrition cycles and were harvested weekly at 1-7 weeks of its vegetation. Antibacterial activity was determined by a broth microdilution method and expressed as MIC (the lowest of tested concentrations with ε 80% bacterial growth reduction). Our results showed that all cannabis extracts tested exhibited a certain degree of growth-inhibitory effect towards both strains of S. aureus, ranging from 32 to 256 µg/mL. The highest antibacterial activity was observed at 5th-7th week of plant growth (MIC = 32 – 64 µg/mL) across all nutritional treatments tested in this study. These results suggest that cannabis extracts tested in this work can be used for further testing and a subsequent development of a new anti-staphylococcal agents.

P04

Circular Feed and additives

AMINO ACID REQUIREMENTS OF MEALWORM AND BLACK SOLDIER FLY LARVAE

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The amino acid requirements of each animal depends on the species and on its phase of growth. The diet should therefore vary according to the animals' needs. The essential amino acid requirements of most farm animals have been extensively studied and diets have been formulated that fully meet their needs. Knowing the amino acid requirements of the most commonly reared insects for feed and food purposes could further optimise this innovative breeding sector. Based on this question, the amino acid requirements of the 2 most common species of farmed insects, mealworms (MW, Tenebrio molitor) and black soldier fly larvae (BSFL, Hermetia illucens) were studied in this project. According to needs of pigs and evidence found in the literature, it was decided to focus on insect requirements of lysine, methionine, threonine, phenylalanine and tryptophan. Based on these assumptions, semi-artificial diets with a base of 25% wheat bran for MWs and chicken feed and water (30/70) for BSFLs supplemented with sugar and 14 synthetic amino acids (the 10 essential amino acids for mammals + glutamic acid, glycine, alanine and aspartic acid) were formulated. All the diets were isoenergetic and isonitrogenic. Of the five amino acids studied, one was given in different doses for each experiment. Glutamic acid, which is not essential, was used as a substitute for the amino acid examined. Finally, growth (measured by weight gained) and survival rate were measured. For lysine no additional growth was noted from a content of 0.35 g/100 g. For methionine the value was from 0.13 g/100 g. For what concerns the threonine a significant difference was noted as the amount increased and the minimum dose appeared to be 0.42 g/100 g. With tryptophan the level of inclusion did not lead to differences and at 0.06 g/100 g the maximum growth had already been reached. In conclusion, a report with the optimal formulation standards and guidelines will be drafted with a focus on the use of the results of this research in the formulation of optimal diets with attention to environmental sustainability.

P05

Circular Feed and additives

REDUCING AGGRESSIVENESS IN WEANED IBERIAN PIGLETS USING A PLANT EXTRACT BLEND

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Mixing is a common practice in swine farms. Pigs fight more after mixing in order to reestablish a new social hierarchy. Aggressive behavior between unfamiliar pigs results in animal welfare issues such as skin lesions which may affect economic returns. On the other hand, pure iberian pigs are considered semi-wild animals being more aggressive that the purely domestic breed. Moreover, they are prone to develop skin diseases such as exudative dermatitis that can be accelerated by a poorly healed lesions and subsequently superficial infections of the skin. BehavePro® is a feed supplement containing a blend of selected botanical extracts that may help to reduce the stress caused by mixing piglets and therefore, to reduce the aggression. The aim of the current trial is to evaluate the effect of the addition of BehavePro® into the prestarter feed, offered from 7 to 32 days of age, on aggressiveness post-mixing at weaning. Around 38 7 d-old pure iberian piglet litters were split into 2 groups: A) Control diet without natural feed solution, and B) Diet containing natural solution at level of 0.3%. Feed and water were offered ad libitum. On day 28 (at weaning), piglets of the control group were split into 2 different pen group size: large (a pen with 75-100 piglets) and small (a pen with 25-40 piglets) while all piglets fed with BehavePro were alloted in large pens. The experimental unit was the pen and each treatment was replicated 5 times. Skin lesions in vivo were recorded 24 hours after weaning according to Welfare Quality® Protocol (2009). The number of fresh scratches by body region was scoring with three levels per pig: 1 for up to 5, 2 for a number between 6 and 10, and 3 for more than 10. Data were analyzed statistically using proc GLM of SAS (SAS v9.3). Reducing the group size decreased the number of skin scratches of the pig (16.71 and 13.76 skin lesions per piglet for large- and small group fed control diet; respectively, $P<0.001$). The highest reduction in the number of scratches was observed in the group fed with BehavePro® in a large group (8.84 scratches), irrespectively of the body region studied. A reduced group size increased the percentage of piglets with score 1 while BehavePro® inclusion did it in the large group (28.2, 13.3, 43.8% piglets for small- and large group fed with control and large group fed with BehavePro® diet, respectively; $P = 0.002$). Contrary, larger group increased the percentage of piglets with score 2 (77.8 vs. 67.1%; $P = 0.023$) and 3 (8.9% vs. 4.7%; $P = 0.004$) while BehavePro® inclusion reduced both (56.2 and 0.0% for the score 2 and 3, respectively). Therefore, there was a higher reduction of the incidence of skin lesions with the use of BehavePro® in the large group than decreasing the group size. The use of BehavePro® at level of 0.3% into a prestarter diet offered during the lactation reduced a 47% skin lesions 24 h post-mixing at weaning probably due to calmer piglets in the BehavePro® group.

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RELATIONSHIP BETWEEN POTENTIATED ZINC OXIDE AND MONOVALENT COPPER IN A DIETARY ADMINISTRATION FOR WEANLING PIGLETS

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The aim of this study was to evaluate different dietary level of inclusion of Zn and Cu according to pharmacological dose of ZnO, European and non-European levels for 14d after weaning of piglets. The endpoints considered were growth performance, serum Zn/Cu status, faecal score, blood biomarkers of intestinal integrity and faecal microbiota. A total of 120 piglets (7,143 ± 0,924 kg) were divided into 4 experimental treatments: positive control (PC, 2500 ppm of Zinc through ZnO) and 3 treatments in which Cu and Zn supplemented through potentiated ZnO and Cu_2O according to different European and non-European levels of inclusion: EU (120 ppm of Zn; 140 ppm of Cu), non-EU+ (300 ppm of Zn; 200 ppm of Cu) and non-EU- (300 ppm of Zn; 140 ppm of Cu). Performance data were analysed using the MIXED procedure of SAS. Serum concentrations of diamine oxidase (DAO) and Zn/Cu serum concentrations were evaluated at 0d and 14d using the GLM procedure of SAS. Fecal samples (14d) were collected and the V3–V4 hypervariable regions of the bacterial 16S gene were sequenced in one MiSeq (Illumina) run. BW, ADG, ADFI, FCR and FE were not affected by the experimental treatments. Faecal consistency score during the first 14 days of trial tended to be lower in the three treatment groups while in PC the high concentration of Zn favoured a better faecal consistency. The initial serum Zn and Cu concentrations at 0d was similar for the dietary treatment groups. After 14d, Zn serum concentration was significantly higher in the PC (15.71 ± 6.25 µg/l, $p<0.05$). No differences were highlighted in serum zinc concentration between the treatment groups. Copper concentrations revealed statistically significant results ($p<0.05$) on day 14 after weaning in T1, T2 and T3 groups showing a higher concentration (17,65 ± 5,49 µmol/l, 17,73± 3,46 µmol/l 18,33 ± 4,13 µmol/l respectively) of Cu compared to PC (11,87 ± 3,60 µmol/l). These results suggested that the European level of inclusion of both potentiated Zn and monovalent Cu is enough to reach the same Cu and Zn plasma levels of non-EU treatments. Plasma DAO level, as indirect markers of intestinal permeability, was negatively ($p<0.05$) affected in non-EU- treatment. Firmicutes and Bacteroidetes were the most abundant phyla in faecal microbiota. Non-EU- contributed to a significant decrease in biodiversity in fecal microbiota as depicted with Shannon for diversity and Simpson for evenness indexes ($p<0.05$). In faecal samples collected on day14 the presence of genera linked to a higher disrupt of the gut barrier (Escherichia-Shigella) was depicted in non-EU- indicating significant modifications of the microbial community. These results suggest the need of a balanced supplementation of Cu and Zn through more bioavailable sources. The use of potentiated ZnO and Cu_2O according to European levels could represent a valid strategy to enhance weanling piglets gut health and to reduce the environmental impact.

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FUNCTIONAL CHARACTERISATION OF EUGLENA GRACILIS FOLLOWING GROWTH MEDIUM ENRICHMENT

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In recent years, microalgae, in particular Euglena gracilis, has been a candidate in the food/feed industry due to its high nutritional (high protein and lipid content) and functional properties due to its ability to produce vitamin C, E and paramylon, a high molecular weight ®-1,3 glucan with immunomodulatory properties. However, the inclusion of E. gracilis in the diets of farm animals is little investigated, especially its antioxidant activity. Moreover, microalgae are known for their variability and adaptability in their composition and nutritional properties, mainly due to their cultivation's conditions. For these reasons, the aim of the present work is to study the total phenolic content (TPC, Folin-Ciocalteu assay) and antioxidant activity (ABTS and FRAP assays) of E. gracilis grown under three different nutritional conditions. In particular, the algae growth media are characterised as follows: HEgM (yeast extract, soy peptones and casein broth); ETX (animal derived amino-acidic (AA) extract;) DOE-ETX (animal derived AA extract, MgSO4, KH2PO4, and a mixture of microelements). The samples undergo green chemical extraction and ex vivo digestion. For green extractions, 150 mg of Euglena HEgM, ETX, DOE-ETX were incubated for 1h, at room temperature, under shaking, with different concentrations of water:ethanol (W:E) (100:0, 75:25, 50:50, 25:75, 0:100). At the end of the incubation, the samples were centrifuged for 5 min at 4000 rpm and subjected to multiple extraction process (n=3). Considering ex vivo digestion, after collecting gastric and intestinal fluids from swine (50-110 d, n=24 at slaughter house), they were pooled and kept at 4oC until use. Then, 500 mg of Euglena HEgM, ETX, DOE-ETX were subjected to gastric digestion (39oC x 2h) in gastric fluid, and further exposed to intestinal fluid (39oC x 2h). Aliquots were taken at the beginning/end of gastric phase and at the end of the intestinal phase, to assess TPC and antioxidant activities. Data are expressed as mean±SEM. Considering green extraction, DOE-ETX showed a high TPC with statistically significant differences compared to ETX and HEgM, especially following the 25:75 W:E extraction, with values of 1104.4±68.2; 310.9±7.0 and 117.2±6.2 mg Tannic Acid Equivalent (TAE)/100g respectively ($p<0.05$). These values were also confirmed for ABTS (630.8±19.0; 41.8±7.0; 21.5±2.3 mg Trolox Equivalent (TE)/100g). Following ex vivo digestion, DOE-ETX showed good results for TPC (0h:501.0±12.0; 2h:584.9±6.7; 4h:465.2±37.4 mg TAE/100g). Although the functional profile was improved following medium enrichment, the production yields showed an inverse trend (0.67; 0.81; 1 g/l*day) for DOET-ETX, ETX and HEgM, respectively. These results confirmed the potential of E. gracilis as a valuable source of functional ingredients for feed application. Further investigations will be of paramount importance to optimise growth medium formulation to obtain high algae yield with improved functional characteristics.

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PROCESSED FORMER FOODSTUFF BASED ON BAKERY BY-PRODUCT INCLUDED IN COMPOUND FEED DIET DESTINED TO POULTRY CAN ENHANCE BROILER PERFORMANCE WITHOUT IMPAIRING MEAT SENSORIAL PROPERTIES

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Reduction of feed-food competition is nowadays recognised as one of the most effective solutions to increase livestock sustainability and improve circularity in animal feed. Among the proposed strategies, recent studies suggested that the use of Former foodstuff (FF) as feed ingredients could be a win-win strategy for both food and feed industries. Within the FFs, bakery by-products (BBPs) have been investigated in ruminants and swine diets as sustainable ingredients with positive results. The present work aims at filling the gap concerning their use in poultry. For this purpose, 200 one-day-old male ROSS-308 chicks were assigned to four dietary treatments balanced by average live weight (LW) (38.0 g ± 0.11 - 5 replicates, 10 birds/pen). Within each diet BBP were included as replace for corn-soybean meal on a w/w ratio obtaining 4 different feeds: control (CTR: commercial feed), L-BBP (6.25% BBP), M-BBP (12.5% BBP), and H-BBP (25% BBP). In vivo LW and Average daily feed intake (ADFI) were registered, and average daily gain (ADG) and feed conversion rate (FCR) were calculated. At day 36, birds were slaughtered, and chicken breast samples (n=5/group: for each analysis) were taken and stored at −20°°C. A discriminant analysis were then used involving consumers in two section in a binomial test. Performance data were analysed by one-way ANOVA using polynomial contrasts to test the linear and quadratic responses to increased BBP inclusion rate. Sensorial data were analysed using ANOVA and considered significant for $P<0.05$. No differences emerged in LW and ADG, while ADFI (CTR: 62.52, L-BBP: 60.59, M-BBP: 60.67, H-BBP: 57.53, P= 0.026) and, consequently, FCR (CTR: 1.57, L-BBP: 1.49, M-BBP: 1.54, H-BBP: 1.39, P=0.002) resulted positively affected by the higher dose of BBP. Discriminant analysis revealed no difference between dietary groups. For our panel, the different inclusion levels of BBP in the broiler diet did not influence the perception of the final product. Results obtained are encouraging. The decrease in feed intake and the absence in final LW can result in important advantages for the breeder in terms of both economic and environmental sustainability. On the other side, the absence of differences highlighted by the discriminant analyses are crucial for the final consumer accustomed to buying a product of constant quality. Overall, even if more data are needed and more studies should be performed to confirm, we can state that including up to 25% of BBP in poultry diets can represent an effective new sustainable nutritional protocol for broilers farming. Furthermore, the LCA data of the two diets supplied during the test have been evaluated with an important environmental saving in terms of water consumption, CO_2 emissions and reduction of land use.

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INFLUENCE OF MAIN CHEMICAL CONSTITUENTS ON IN VITRO FERMENTATION USING FROZEN RUMEN FLUID: PRELIMINARY RESULTS

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The study of the nutritional value of feed is an essential prerequisite for nutritionists and livestock operators. The assessment of nutritive value of feed is determined by chemical components, as well as their rate and extent of digestion. The description of digestive and metabolic processes can be done by using in vitro models that simulate the in vivo digestion processes with different levels of complexity. Compared to in vivo experiments, in vitro methods have the advantage not only of being less expensive and less time-consuming, but they allow one to maintain experimental conditions more precisely and reproducible than do in vivo trials. Gas production (GP) test has become a valuable tool in the evaluation and selection of feeds for ruminants, since it provides information on the fermentability, digestibility and nutritional value of feeds. These techniques require rumen microbial inoculum taken directly from fistulated animals that is a practice subjected to criticism not only from the cost demanding and difficulty of keeping such rumen-fistulated animals, but the ethical issues poses questions on the compliance of animal welfare leading to seek to other alternatives. Ruminal fluid (RF) taken from slaughtered animals as microbial inoculum is an alternative source. Another mitigating question is the feasibility usage of frozen RF for GP test. Storing microbial fluid while maintaining its microbial activity is an approach that enable the standardization of in vitro studies, giving ample time to determine useful parameters and to work on a large number of substrates. The aim of this preliminary study conducted on Ankom system is to test the frozen RF on in vitro fermentation of different substrates along with the assessment of proximate parameters and polyphenolic content. Six substrates (maize, bran, sorghum, animated oats, soft bread and hard bread), present locally in the Maltese Islands, were selected. The data for cumulative GP (as mL of gas produced per g dry matter) were processed by the three-parameters Gompertz sigmoidal function. The GP results showed the R2 higher than 0.96 for all the substrates (maize =0.98; bran = 0.98; animated =0. 98; sorghum = 1; soft bread = 0.97; hard bread = 0.96). Animated oats showed the lowest fermentation kinetics (65.48 mL/g) according to its Crude protein, Neutral Detergent Fiber and Acid Detergent Fiber content, while hard bread the highest one (192.59 mL/g), followed by maize, sorghum, soft bread and bran respectively 184.8, 180.64, 178.04, and 130.6 mL/g. The antioxidant activity may be partially related to the polyphenol content: bran and animated oats showed highest values, respectively 26 and 24 mg/100g, while soft and hard bread the lowest one respectively 9.8 and 11 mg/100g. The results highlight that the frozen ruminal fluid seems potentially valid to replace the fresh one, showing rate of fermentation for all the substrates according to their chemical composition.

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EFFECT OF DIFFERENT ENZYME PREPARATIONS ON NUTRIENT DIGESTIBILITY IN BROILER CHICKENS FED CORN-SOYBEAN BASED DIET

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A broiler chickens' study was conducted to evaluate the effect of commercially available enzymes on ileal nutrient digestibility and apparent metabolizable energy (AME). A control diet was formulated based on corn-soy with a phytase background (1000 FTU/kg) applied with full matrix for calcium, phosphorus, energy, and amino acids. The other 3 treatments were based on the control diet, and they were either supplemented with a xylanase (X) and ®-glucanase (BG) product (XBG); an enzyme cocktail containing multiple non-starch polysaccharide-degrading enzymes (cocktail); or X, amylase (A), protease (P) combination product (XAP). A total of 240 male broiler chicks (Ross 308) were allocated to the 4 treatments (12 replicates of 5 birds/cage). TiO2 was used as a marker. Excreta samples and ileal digesta were collected on d-23 and d-24, respectively, and analyzed for gross energy and nitrogen (N). Data were subjected to one-way ANOVA and means were separated by Fisher's test. The results showed that XBG improved ($P<0.05$) ileal N and energy digestibility compared to the control. No difference ($P>0.05$) was observed between birds fed XBG and XAP for N digestibility. All enzymes improved ($P<0.05$) AME compared to the control, but XBG and XAP had significantly higher AME compared to the birds fed the cocktail enzyme treatment. The result of this study confirms the efficacy of carbohydrase enzymes to improve the nutritive value of corn-soybean diets and suggests that the enzyme efficacy and degree of response may vary depending on the enzyme preparation.

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EVALUATION OF THE PREBIOTIC ACTIVITY OF ASCOPHYLLUM NODOSUM AND LITHOTHAMNIUM CALCAREUM IN CO-CULTURE WITH LIMOSILACTOBACILLUS REUTERI STRAIN

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In line with the principles of sustainable development animal nutrition is called to face different challenges. In this sense searching for new functional ingredients, that in addition to their nutritional value are capable of supplying the body with bioactive molecules is a crucial for a sustainable development of animal production. Among these, algae given their advantages and their richness in several bioactive compounds could be a valuable choice.
Given these evidences, this study aimed to evaluate, in vitro, antioxidant and antimicrobial capabilities, of Ascophyllum nodosum and Lithothamnium calcareum when they are used as prebiotic substances, to this end Limosilactobacillus reuteri strain was used as microorganism model microorganism.
First, the influence of algae inclusion on the growth curve of L. reuteri was evaluated. Afterward, functional activities of A. nodosum and L. calcareum as prebiotics on L. reuteri strain were evaluated after different hours of co-culture (24; 48; 72; 96). The antioxidant capacity was determined by the ABTS Radical Cation Decolorization Assay. The growth inhibition capacity of L. reuteri and algae, against porcine O138 E. coli was evaluated following the microdilution bacterial growth method. Furthermore, in order to assess the antimicrobial effect a Minimum Inhibitory Concentration assay (MIC) was performed.
The inclusion of algae was seen not to affect, significantly, the growth curve of L. reuteri, thus making them a potential prebiotic for the probiotic strain under consideration. Regarding the functional activities of A. nodosum and L. calcareum as prebiotics for L. reuteri strain the presence of different bioactive compounds in these algal extracts, particularly when placed in combination, correlated with the enzymes produced by L. reuteri allowed an improvement in antioxidant activity. In fact, the antioxidant capacity, after 72hrs and 96hrs of co-culture, results significantly (p-value: 0.0258 and 0.0127 respectively) higher than that of the control with values of PI% that are respectively 63.52% and 64.81% against 48.60% of the control. The same co-culture time points also succeed in observing increased growth inhibition of E. coli. In fact, it can be observed that the combination of the two algal extracts is able to inhibit significantly ($p < 0.05$) the growth of E. coli. This result was confirmed also by MIC showing lower minimum inhibitory concentrations after 72 and 96 hours of co-culture (respectively 1 mg/ml and 10 mg/ml). The results obtained from this study disclose that A. nodosum and L. calcareum, especially if used in combination can be considered valid prebiotics on Limosilactobacillus reuteri strain. Further in vitro studies will be conducted to complete the knowledge about the employment of the algal species as prebiotic, in order to subsequently evaluate their effect also in vivo.

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CAMELINA COLD PRESSED CAKE - A FUNCTIONAL INGREDIENT IN THE PET FOOD

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Pet food has long been one of the most attractive sectors in the world, and it is now beginning to grow and acquire significance in Serbia. Functional ingredients with plant origins that influence the health of animals are receiving more and more attention. Regarding fatty acids, vegetable oils are well-known as energy sources for animal nutrition. Camelina sativa, also known as wild flax, is an oil crop from the Brassicaceae family cultivated across Europe by the middle of the 20th century. Camelina can be grown on insufficient agricultural grounds and requires little nitrogen or water to thrive. It can be grown in rotation with wheat and other cereals to improve the soil's health and could be a good crop regarding climate change. According to numerous studies, camelina oil plays a crucial role in the human and animal bodies. The linolenic (omega-3) and linoleic (omega-6) fatty acids and bioactive substances like tocopherol help alleviate inflammatory processes. A product of camelina processing and oil production, a cold-pressed cake could be a valuable ingredient in animal nutrition. This study examined the nutritional value of cold-pressed camelina cakes (two varieties) and their amino acid and liposoluble vitamin contents. All chemical analyses were done according to standard ISO methods. The amino acid content determined by the Biochrom Analyzer after hydrolyzing crude proteins. Liposoluble vitamins are determined by the HPLC method. Analzying the results of chemical composition, there are no significant differences between the two varieties of camelina. Both varieties contain about 35% proteins, 5% ash, 16% crude fat, and 9% cellulose. The level of phytic acid is also significant and is between 3,37 and 3,60% which major benefit is decreasing the risk of cancer and diabetes control. Potassium and magnesium are the most significant elements in both camelina varieties (9270.6–9508.1 mg/kg and 3722.8–3544 mg/kg, respectively). The amino acid profile showed a significant level of glutamic acid (10,4 and 11,00%). Glutamic acid helps nerve cells exchange information between cells and may be involved in learning and memory. The content of arginine, one of many amino acids the body needs to function properly is significant. Like other amino acids, arginine plays a role in building protein. The content of arginine is between 9.22 and 9.36%. Camelina cake has strong antioxidant capacity thanks to the content of gama tocopherol, which is 18.9 mg/kg in one variety and 16.2 mg/kg in another. Concerning results, camelina cake has the potential for use in animal nutrition, particularly in pet food. The level of magnesium and potassium, antioxidative properties, and amino acid profile could be beneficial for animal health by improving metabolic processes and the skin and hair health.
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INCLUSION OF FERMENTED GUAR MEAL IN WEANING PIGLETS DIET AS ALTERNATIVE SOURCE OF VEGETABLE PROTEIN IN PORK INDUSTRY

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Weaning is a very crucial period in the productive life of the piglet in pork industry. Nutritionally, it requires high attention to avoid digestive disturbances and diarrheas. Inclusion of dietary specialties like vegetables protein concentrates in the prestarter and starter formulations is common in order to cope with the high protein requirement levels. In this category of feed ingredients, fermented feeds represent an interesting option. Guar meal is a co-product derived from the extraction of galactomannan polysaccharide guar gum (Cyamopsis tetragonalobulos) from the endosperm of the bean and is a good candidate to be considered as a specialty for weaning piglet diet. A trial was conceived to evaluate the use of a fermented guar meal at two different inclusion levels on performance of weaning piglets. The trial lasted 42 days. 42 piglets (age: 28 days; average body weight: 7.67 ± 0.34 kg) were housed in single box at the Experimental Zootechnical Centre of the Università degli Studi di Milano, in Lodi, Italy. Animals were fed with a prestarer diet from 0 to day 14 and a starter diet from 14 to day 42. Animals were randomly assigned to three homogenous groups for weight, sex and litter (n=14): a control group (C), without experimental supplementation; a prestarter diet supplemented with 7% of fermented guar meal (Mycoprime®, Panghea spa, Italy) and a starter diet supplemented with 5% of fermented guar meal (T1); a prestarter diet supplemented with 10% of fermented guar meal and a starter diet supplemented with 10% of fermented guar meal (T2). Individual feed intake (FI) and live body weight (LBW) was recorded at days 0, 14, 28, 42. From the results obtained, average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were calculated. Collected data were analyzed by mixed procedure of SAS. Significant level was considered for $p<0.05$ and tendency for $0.05<p<0.1$. No differences were observed between C and T1 for LBW, ADFI, ADG at days 14 (10.92±0.56 vs. 10.94±0.56; 0.39±0.04 vs. 0.36±0.04; 0.24±0.03 vs. 0.24±0.03; P>0.05), 28 (17.76±0.56 vs 17.63±0.56; 0.77±0.04 vs. 0.80±0.04; 0.49±0.03 vs. 0.48±0.03; ; P>0.05) and 42 (27.68±0.56 vs. 27.23±0.56; 1.19±0.04 vs. 1.22±0.04 ; 0.71±0.03 vs. 0.69±0.03; P>0.05). Lower performances were observed in T2 compared with C and T1 at days 28 and 42 for LBW, ADFI and ADG (d28, 16.38±0.56; 1.02±0.04; 0.39±0.03; d42, 24.22±0.56; 1.02±0.04; 0.56±0.03; P<0.05), but not at day 14 (10.95±0.56; 0.34±0.04; 0.23±0.03; P>0.05). FCR tended to be lower for C compared with T2 (1.66 vs. 1.75; $0.05<P<0.1$) and did not differ between C and T1. In conclusion, fermented guar meal was able to replace protein concentrates like soybean concentrates in prestarter and starter weaning piglets diets without reducing performances at inclusions of 7% in prestarter and 5% in starter diets while reduced performances at the highest level (10%).

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EFFECT OF DIETARY INCLUSION OF CHLORELLA ALGAE ON THE NUTRITIVE VALUE AND PHYSICAL PROPERTIES OF PELLETED FEED FOR LAYING HENS

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BACKGROUND: Protein deficiency in animal nutrition poses a significant global issue, leading to a large amount of research directed towards the search for new and sustainable protein sources. The use of alternative feed ingredients that can meet animals' requirements, and being easily cultivable and economically viable, could reduce the burden on agricultural sector and ensure sustainable animal husbandry. In recent years, algae have gained much attention as promising protein-rich and functional ingredients in animal nutrition. The inclusion of algae in poultry feed offers the greatest perspective for their commercial use in animal nutrition. The aim of the current study was to investigate the effect of incorporation of Chlorella sp. as a substitute for traditional protein sources in laying hens diet on the nutritive value and physical characteristics of the pelleted feed.
METHODS: Chlorella sp. was added in the increasing amount (0, 5 and 10%) to partially replace corn, soybean meal and sunflower meal in the laying hens' diet. The feed ingredients were milled using a hammer mill, mixed in a twin shaft paddle mixer and then pelleted on a flat die pellet press.
RESULTS: It was observed that the dietary inclusion of Chlorella caused an increase Fe content, but decrease in Na and K content in the pellets. The content of Ca in the pellets declined with 5% of Chlorella supplementation, while increased with the addition of 10% of Chlorella. The addition of Chlorella led to a significant decrease in the content of monounsaturated fatty acids but caused rise in the level of polyunsaturated fatty acids (PUFA), which was due to high share omega-3 fatty acids (particularly ⟨-linolenic acid) in Chlorella. The proportion of essential (EAA) and nonessential amino acids (NAA) in the pellets increased with higher inclusion of Chlorella. Of the EAA, leucine, lysine valine, and phenylalanine were the most abundant in the pellets, while glutamic acid, aspartic acid, proline and arginine were the major NAA in the pellets. In addition, it was observed that 5% of Chlorella did not affect bulk density of the produced pellets, while 10% of Chlorella influenced an increase in the bulk density. Pellet durability index was unaffected by the addition of Chlorella. Pellets containing 5% and 10% of Chlorella were less hard compared to the control pellets, indicating that the pellets could be more easily consumed by the animal due to their lower hardness.
CONCLUSIONS: The use of Chlorella as a protein source in lying hens' diet can improve nutritive profile and physical properties of pelleted feed. However, due to the high price of Chlorella, its maximum possible exploitation has yet to be achieved in order for production to be economically viable.

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AQUACULTURE AND FOOD SYSTEM: EARTHWORMS FROM WASTE BIOREFINERY TO EEL FEEDING AND CONSERVATION

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The last 20 years have seen a dramatic decline in the number of eels (Anguilla anguilla) reaching European river systems. The European eel is listed as “critically endangered” under the IUCN Red List of Threatened Species. One of the strategies to counteract this decline is to improve the aquacultural system, allowing a reduction in wild elvers and juvenile eel harvesting. Using natural food to grow captive eels for restocking will increase their fitness and survival. The study seeks to evaluate the feasibility of earthworms as feed components for elvers and eels, to be used for restocking and to produce a stock of juvenile eels to be released in vocated areas. Earthworms will be reared on wastes of different origins (fruit and vegetable, household/catering waste, and sewage sludge), and their safety for eel and humans as final consumers will be evaluated. Different feeding trials will be conducted, and wastes, earthworms, vermicompost, and eels will be characterized in terms of food safety. The performance will be evaluated and compared
among the different diets used. Expected results include validation of the three different waste biotransformation by earthworms (bioconversion efficiency and LCA), safety characterization of earthworms as a novel feed ingredient for eel, and derived food for human consumption (as by EU legislation); production of high-fitness juvenile eels to be used for restocking; contribution to eel
conservation by releasing batches of juvenile eel to the wild. Because of the study structure (short duration, involvement of network of earthworm farmers interested in the production of earthworms from waste biorefinery and of eel farmers and fishermen associations), the expected results of the project may contribute not only to advances of knowledge of farmed eel restocking and conservation and the realistic possibility to boost the development of a circular economy valorizing organic substrates while reducing partially their adverse environmental impacts and their management costs, immediately applicable. This also scopes in line with PNRR mission. AQUAFEEL will develop an innovative approach to create a standardized model of research-based skill competence and technology to be implemented, as shared innovation, into the traditional extensive eels farming system of national marginal areas (Po-valley) with relevant value in terms of cultural landscape interest, where the eel is one of the most important species critically endangered by a dramatic decline in the number of eels reaching this area. The study will promote scientific and technological advances by exploiting the current knowledge combined with a joined-up approach involving multi-actors as co-creators of innovations. The proposed approach addresses challenges in line with the “farm-to-fork” strategies for a transition into fair, healthy, and resilient eels’ conservation and food production systems in the marginal area scenario.

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Circular Feed and additives

THE USE OF CO-PRODUCTS OF THE AGRI-FOOD INDUSTRY IN FEEDING DAIRY RUMINANTS

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From the perspective of the circular economy, it is crucial to provide alternatives to conventional sources of energy and protein, in order to decrease the level of feed vs food competition. Camelina (Camelina Sativa) cake, and Cardoon (Cynara Cardunculus) cake are by-products of the agri-food industry with high potential in ruminants feeding. These compounds are rich in proteins, essential amino acids, fiber, and contain bioactive compounds such as unsaturated fatty acids (UFA) and polyphenols. The inclusion of Camelina and Cardoon co-products in dairy ruminant diet, in different physiological moments, was evaluated. Several trials with transitioning dairy goats, late lactation dairy goats, goat kids, heifers and dairy cows were performed. Camelina and Cardoon were administered in the form of camelina cake (CAME), cardoon cake (CD) and a mixture between cardoon and camelina cakes (CACD). The direct effects on metabolism and microbiota and the effects on the composition of the milk were evaluated. Moreover, the effects of the maternal milk from goats supplemented with this co-product on goat kids, were also evaluated. Significant level was considered for $P < 0.05$ and tendency for $0.05< P <0.1$. Camelina and Cardoon did not significantly affect intake, rumen pH levels and performance ($P > 0.05$) in transitioning dairy goats, late lactation dairy goats, heifers, and dairy cows. The intake of milk from goats supplemented with cardoon and camelina cakes did not show any differences in performance between the goat kids ($P >0.05$). The co-products did not significantly affect protein, urea, lactose, fat content and somatic cells counts ($P >0.05$) in goat's milk while were able to modify unsaturated fatty acid content at 4 days in milking (CACD = 2.15 ± 0.11 VS C = 1.7 ± 0.10; CAME = 1.66 ± 0.10; CD = 1.66 ± 10 g/100g; $P <0.05$). Moreover, milk from goats supplemented with camelina and cardoon cakes showed higher total phenolic compounds (TPC; 22.96 ± 6.76 and 32.49 ± 2.08 mg GAE/l; $P <0.05$) and antioxidant capacity (AOC; 207.9 ± 4.25 and 195.4 ± 26.6 µmol TE/mL; $P <0.05$). The administration of camelina cake in heifers influenced rumen microbiota both at 28 and 56 days of treatment, showing significant ‹-diversity values between CAME and C group. Higher expression levels for genes involved in follicular developmental competence were detected in CAME group compared with C, suggesting a positive effect of treatment diet on fertility in heifers. In dairy cows, CAME had a reduced milk fat percentage (4.27±0.14 vs. 4.72±0.14%; $P < 0.05$) compared to C but not a reduced production of fat (1.11±0.05 vs. 1.17±0.05 kg; $P >0.05$). Saturated milk fatty acids were also reduced in CAME (2.8 vs. 3.14 g/100 g; $P < 0.05$) and linolenic acid and rumen biohydrogenation intermediates were increased in CAME. Overall, these co-products could be excellent protein sources alternatives to soy also because more environmentally sustainable.

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Circular Feed and additives

EFFECT OF PHYTOTHERAPIC ADDITIVES OR RUMEN MODIFIERS ON DAIRY CATTLE COLOSTRUM YIELD AND QUALITY

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Colostrum is paramount to neonatal nutrition and passive immunity. In fact, as first source of nutrients for the calves, it is reach not only in immunoglobulins, but also in lipids, proteins, vitamins, and minerals. Therefore, good maternal nutrition during the transition period is important not only to promote cows' health and performance but also colostrum quality. Several metabolic enhancers, and rumen modifiers are often used to mitigate the effects of the negative energy balance in the transition cows, hence, to improve colostrum yield and quality. Therefore, the objective of this study was to compare the effect of a commercial rumen modifier releasing monensin and of a phytotherapic intraruminal bolus on colostrum yield and quality. 75 Holstein dairy cows, blocked by parity (2 to 4), BCS, previous lactation yield and length were allocated to either a control group (CTL), a monensin group (MON) receiving , 20 days prior to the expecting calving date, an intraruminal bolus of 2.9 g of sodic monensin gradually released over 95 days, and a phytoterapic group (PHYTO) receiving, 5 days pre-partum, an intraruminal bolus composed of 5 g of Echinacea purpurea dry extract, 10 g of vitamin E, 5 g of l-carnitine and 5 g of silymarin (Silibum marianum) in a collagen capsule and released in the rumen during the first 24 hours after the administration. At parturition, colostrum yield (first and second milking) was recorded, and its density was assessed using a refractometer. Furthermore, a sample of colostrum was collected for analyses on humidity, dry matter (DM, %), ash, fat and protein content, Alanine (Ala), arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), and valine (Val), and mineral content. The effect of the treatments was assessed by ANCOVA using the Production, Functionality and Type index (PFT) as covariate in the general linear model of Sas 9.4. No differences were observed among treatments regarding the colostrum yield and density. However, a trend for lower concentration of Isol was observed in the treatments compared to the CTL treatment ($P>0.1$). Furthermore, a trend for increased content of K and Na (<0.1), and increased levels of Si ($P<0.05$) were observed in the treatments compared to the control, but no differences between treatments were observed. In conclusion, neither of the treatments assisted in improving colostrum yield and quality therefore, further research is needed to assess the effect of the treatments on cows performance during the transition period to evaluate if a phytotherapic treatment could be indeed a valid alternative to an antibiotic-based rumen modifier.

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SUGARY AND SALTY FORMER FOOD PRODUCTS SLIGHTLY AFFECT FAECAL MICROBIOTA WHEN USED TO PARTIALLY REPLACE CEREALS IN PIGS' DIET.

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Food industry leftovers, also called former foodstuffs products (FFPs) are promising alternative ingredients to improve the circular economy in food production. This study investigated the effects of salty and sugary FFPs on gut microbiota and intestinal integrity of growing and finishing pigs. Thirty-six Swiss Large White male castrated pigs were assigned to the three grower and finisher experimental diets: 1) standard diet (ST), 2) 30% conventional ingredients replaced by sugary FFPs (SU) and 3) 30% conventional ingredients replaced by salty FFPs (SA). At the beginning of the trial (T1), at the end of the growing period (T2) and 1 day before the slaughter (T3), faeces were collected from the rectal ampulla, snap-frozen, and used for next-generation sequencing to analyse the composition and the alpha and beta diversity indexes of the microbial population and for the quantification of the volatile fatty acids (VFAs). All microbiota data analyses were run in R v4.0.3 (Boston, MA, USA). The R packages used were phyloseq v1.26.1, vegan v2.5–5, microbiome v1.12.0, and microbiomeutilities. v1.00.14. The VFA profile was determined by gas-liquid chromatography (Gaschromatograph Series II Agilent 6850, Agilent Technologies 2000, USA).
For each time point, no difference in the alpha and beta diversity indexes were found between the dietary treatments. At T3, the core microbiota of the SU pigs was composed by 12 ASVs, while in the ST and SU groups it was characterized by 9 and 8 ASVs, respectively. The Linear Discriminant analysis of effect size (LefSe) showed that at T1, no differential taxa between groups were found. However, differences ($P<0.05$) at the genus level between ST, SU and SA groups were found at T2 and T3. The diets did not influence the production of VFA in faeces and the only differences observed were related to the time point of samples collection. When used to partially replace traditional ingredients in growing-finishing pig's diet, both SU and SA FFPs led to very mild effect on the gut microbiota.

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Circular Feed and additives

A NEW FORMULATION WITH FERMENTATIVE S ACETYL GLUTATHIONE AND SILYBIN FOR DOGS WITH LIVER DISEASE

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Introduction

The liver synthesizes glutathione (the major intracellular antioxidant, and glutathione peroxidase GPx which protects against oxidative damage(1 GSH and GPx decrease in dogs and cats with various diseases, so clinicians recommend interventions to overcome these deficiencies Veterinary literature reports the use of natural feed supplements with antioxidant activities.

Aim

A randomized control trial was conducted in dogs with liver disease to test the hepatoprotective effect and GPx level variation following the administration of a supplement containing S acetyl glutathione (derived from Saccharomyces cerevisiae fermentation, S ilybin orange bioflavonoid, and vitamin B 2 B 12 and E.

Materials and methods

-We included a total of 24 adult owned dogs with a diagnosis of liver disease (cholangiohepatitis
-All dogs were treated at the beginning of the study with antibiotics (oral amoxicillin clavulanate BID 12 5 25 mg/kg), anti inflammatory drugs (oral prednisolone SID 0 5 mg/Kg), and intravenous fluid therapy
- Diet for all dogs Vetsolution monge epatic 50 Vetsolution digest 50 from at least 14 days before starting the trial
- 12 dogs were randomly assigned to the treatment group (TRT) and received the supplement at a dose of one tablet/ 15 kg BW, while the other 12 dogs were assigned to the control group (CTR) and did not receive the supplement The TRT consisted of 5 male (mean age 6.8 yr) and the CTR consisted of 6 male (mean age 6.7 yr).
- Duration of the study 35 days equally divided in six experimental times (T 0 T 5
- At each time point the following parameters were evaluated BW, complete blood analysis and erythrocyte GPx
- Blood parameters were analyzed by a regression model built as a generalized linear mixed model (with
Gaussian likelihood (R software) The model included a nonlinear variable describing the link between each time point within and between the CTR and TRT group, sex, body weight (and age The model accounts for repeated measurements (random effect) and the heterogeneity of individuals.

Results

- The product under study was well tollerated by all the animals. During the trial no adverse effects vomiting/diarrea) were observed confirming the safety of the product. No dogs were excluded during the trial.
- No significant alteration of blood counts (values within normal ranges) and no evidence of other diseases reported.
- The supplement had no or limited effects on some of the biochemical values relevant to monitor liver disease Tab 2
Most of the liver key parameters (ALT, AST, ALP, GGT and BIL) are significantly reduced from T4 on in the TRT group but not in the CTR group A significant increase in the erytrocite GPx level even from T2 in the TRT group and nearly reaching the minimum phisological limit (300 Ug/Hb) at the end of the treatment.

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Feed quality, safety and authentication

EFFECTS OF ADDING THE FUNCTIONAL ADDITIVES IN THE DIET OF LAYING HENS ON PHYSICAL, NUTRITIVE AND SENSORY QUALITY OF EGGS

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Eggs are cheap and nutritionally valuable products of animal origin. Using specially designed mixtures for the production of table eggs, it is possible to produce functional eggs with ω-3 fatty acids, pigments, vitamins as well as with good sensory profile of the eggs. Thus, this study aimed to investigate the effects of inclusion of co-extrudates based on hemp seed, flaxseed and camelina seed in combination with natural pigments in hen's diet on nutritional and sensory quality of eggs.
Two hundred and forty Lohmann Brown laying hens were divided into eight groups (two controls, C1 and C2, and six experimental treatments, T1-T6) and fed for four weeks. The laying hens were fed with corn-soybean meal based diet (C1 and C2). Flax-corn meal co-extrudate was added in the amount of 13.5% (T1) and 22.5% (T2), camelina-corn meal co-extrudate was added in the amount of 16.6% (T3) and 27.6% (T4) while hemp-corn meal co-extrudate was added in the amount of 18.4% (T5) and 30.7% (T6) in the based diet. C1 contained up to 3% fat without added pigments, while C2 contained up to 5% fat and synthetic pigments. The same amount of natural pigments (1% carrot and 0.5% paprika) was added in all experimental groups. Content of fat in T1, T3 and T5 was 3%, while in T2, T4 and T6 was 5%. No significant changes ($p>0.05$) in the physical parameters of egg quality were observed for eggs from the experimental groups when compared with eggs from control groups. All experimental groups achieved desirable yolk color demanded by consumers, from 12.67 to 13.28 RYCF value (Roche Yolk Color Fan). The content of polyunsaturated fatty acids in experimental eggs was significantly higher ($p<0.001$) than that in eggs originated from hens fed with control diets. The ω-6/ω-3 ratios in experimental groups (T1-T6) T1, T2, T3, T4, T5 and T6 respectively, were significantly lower (1.43, 1.01, 1.74, 1.73, 5.09 and 5.00, respectively, $p<0.001$) than those in treatments C1 and C2 (9.40 and 8.88, respectively). The addition of co-extruded flaxseed in treatments T1 and T2 had a negative effect ($p<0.05$) on the sensory quality of the eggs (taste of eggs) while the addition of co-extruded camelina seed and hemp seed did not influence taste or smell of eggs ($p>0.05$).
Based on the obtained results it can be concluded that with the addition of natural pigment, as well as with selected co-extrudates, it is possible to design functional eggs that will have good egg quality parameters, optimal yolk color, increased content of ω-3 fatty acids, better ω-6/ω-3 ratio, as well as good sensory profile of the eggs.
Keywords: functional eggs, flaxseed, camelina seed, hemp seed, natural pigments

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Feed quality, safety and authentication

FATAL CYANIDE POISONING IN CATTLE FROM SORGHUM FORAGES

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Sorghum vulgare is part of Graminacee family and it is very appreciated in hot and arid regions for its resistance to drought and heat. The stems and leaves are used for feed for livestock. Dhurrin is the cianogenetic glucoside (CNG) of Sorghum vulgare; the epidermal cells of leaf and roots of sorghum contain Dhurrin, which may degrade into hydrogen cyanide (HCN). CNGs are nitrogen-substituted secondary plants metabolites formed by the condensation of cyanohydrin derivates with D-glucose. Traditionally, dhurrin has received considerable attention due to its frequent occurence in agri-feed. High levels of dhurrin in plants represent health risks for grazing animals. In August 2022, 50 cows were died from poisoning by fresh sorghum plants at an Italian farm in Sommariva Bosco (Turin, Italy). In the same month other 105 cows were poisoned by young plants of sorghum in Moretta (CN), in Bra (CN), in Asti and in Cossato (BI). The samples collected from IZSPLV and analyzed by National Reference Laboratory for Plant Toxins in Food (LNR-TVN) at the Food Chemical Department in Bologna, IZSLER.This study investigates the presence of dhurrin in 67 samples of sorghum collected in Piemonte and Emilia Romagna regions. The LNR -TVN has developed an ultra-high-performance liquid chromatography-triple quadruple tandem mass spectrometry (LC-MS/MS) to identify and quantify dhurrin. Chromatographic analysis was performed using XEVO TQ-Xs Waters Acquity UPLC I Class plus, with a column UPLC BEH C8 Waters. ESI-MS analysis was operated in positive mode. For the analyte, two characteristic ions were selected for the identification and the quantification. Samples were extracted with aqueous methanol (80%) solution. The limit of quantification (LOQ) of dhurrin in agri-feed was 50 mg/kg (corresponding to 4.3mg/kg HCN). According to EFSA Journal 2019, 1 g of dhurrin releases 86.7 mg HCN potential. Dhurrin was detected in 42 samples of sorghum fresh leaves analyzed, and the occurrence ranges from 111 to 10717 mg/kg (9.6-929 mg/kg of HCN). In 25 samples the quantitative of dhurrin was under the LOQ.Sorghum is an ancient, old world cereal that was domesticated in Africa and spread throughout the world to became one of the most important feed crops known to man. The expected increase of temperature and decrease of precipitation as the result of global climate change also European farmers moved toward sorghum's cultivation. During the summer 2022 in Piemonte (Italy) adverse climate and minimal water stress conditions caused high concentrations of dhurrin in young plants of sorghum, it caused the death of cattle. From August to now, the increase of controls on sorghum forages, collected to local farmers, reduces the risk of poisoned. To improve agricultural and harvesting practices will be necessary to ensure the safety of sorghum.

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Feed quality, safety and authentication

FLUORIDE IN ITALIAN FEED: 5 YEARS OF MONITORING

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Background: The sources of fluoride contamination are both natural and anthropogenic and are related respectively to geogenic processes or industrial origin or irrigation of soils with fluoride-enriched water. Through the polluted soil and water, fluoride can enter the food-chain. The extended intake of high fluoride doses can lead to a series of illnesses involving the dental apparatus and the skeletal system, but also gastric, renal, and metabolic disorders. As stated in EFSA opinion, the adequate intake of fluoride from all sources (including non-dietary sources) is 0.05 mg/kg body weight per day for both children and adults, including pregnant and lactating women. Fluoride is a non-essential nutrient, but the setting of an adequate intake is appropriate because of the beneficial effects of dietary fluoride on prevention of dental caries. In animal feed, instead, fluoride is considered an undesirable substance. Directive 32/2002/EC of the European Parliament and Council on undesirable substances in foodstuffs set maximum limits for fluoride in feed materials, vermiculite (E 561), complementary feed, and complete feed.
Methods: Fluoride in feed is quantified by ion-sensitive electrode method after hydrochloric acid treatment. An ionometer equipped with ISE electrode for fluoride, pH measuring electrode, reference electrode, automatic dispenser and magnetic stirrer was used for the quantitative determination of fluoride in feedstuffs.
The developed method has the following performances:
• linearity (correlation coefficient, R2, ε 0,99 between 20 and 4000 mg/Kg),
• limit of quantitation (LOQ) of 20 mg/Kg for complete feed for cattle, sheep and goats during lactation and 40 mg/Kg for all others respectively
• repeatability (Relative Standard Deviation lower than 5% at 20, 40, 100, 500, 1500 and 4000 mg/kg),
• recovery between 94% and 108% for all levels
• extended measurement uncertainty: 20%
Results: During the 5-year monitoring period, the number of samples with fluoride content below the quantification limit increased proportionality to the decrease of samples with quantifiable fluoride content. Raw materials, additives, complementary feed, and complete feed were considered (70, 4, 102 and 83 samples respectively) and all has shown fluoride content < LOQ. No exceedances of maximum limits were registered.
Conclusions: Results have shown that an appropriate monitoring plan has led to awareness of the fluoride problem in the feed industry. The presence of official controls therefore efficiently ensures animal health.

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Feed quality, safety and authentication

VALIDATION OF A METHOD FOR THE DETERMINATION OF SELENOMETHIONINE IN FEED BY HPLC-ICP-MS

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Task:
Selenomethionine (SeMet) is an approved additive, but so far only the total selenium content has been analyzed for official feed control in Austria.
In order to be able to determine the amount of selenomethionine added, the method for the determination of selenomethionine by HPLC-ICPMS published by the EURL-FA had to be validated.
Method:
In the first step, selenomethionine is extracted for 16 h using enzymatic extraction with proteases and lipases. This enzymatic extraction has to be carried out 3 times, the supernatant is combined. After centrifugation and filtration, the different Selenium-compounds are separated selectively by HPLC and detected by ICPMS at m/z 78. Hydrogen is used as reaction gas, and to avoid any interferences m/z 77 is also monitored.
Conclusion:
A robust and suitable method for SeMet in feed and feed additives was established and validated in the laboratory.
For measurement by HPLC-ICPMS m/z 78, with Hydrogen as reaction gas, was chosen. But m/z 77 with Hydrogen as reaction gas is also a possible mass.
Even very high contents (150-fold) of inorganic Se didn´t interfere with SeMet.
The precision and accuracy were satisfying, the recovery rate was approx. 110 %
The limit of detection was 0.03 µg/l, the limit of quantification 0.09 µg/l.
The measurement uncertainty of the method was calculated with 22 to 28 %.
One big disadvantage of the method is, that she is very time consuming and has a long turnaround time (at least 3 days).
Literature:
EURL-FA; SeMet determination in selenized yeasts by HPLC – ICP MS – 3b817
link: https://ec.europa.eu/jrc/sites/default/files/m2-fa-3b817.pdf

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Feed quality, safety and authentication

RAPID ALERT SUPPLY NETWORK EXTRACTOR (RASNEX 2.0) TOOL TO MINE UNSTRUCTURED SUPPLY CHAIN INFORMATION FROM FOOD AND FEED CONTAMINATION NOTIFICATIONS

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National competent authorities of member countries use the Rapid Alert System for Food and Feed (RASFF) to report information on (i) any direct or indirect human health risk arising from food or feed, (ii) serious risk to animal health or the environment in relation to feed. During a contamination or outbreak, it is of utmost importance to rapidly identify involved supply chain actors and withdraw causative products from the market, since the impacts on human and/or animal health and economic damages grow with duration of the event.
A major challenge for risk managers and risk assessors is to gain and maintain an overview during contamination events, as relevant information is continuously updated. Recent feed related incidences (e.g. Salmonella, Ethoxyquin) illustrated a need for a software system capable of supporting investigations on supply chains as well as exposure assessments in crisis situations. To address these needs, the Rapid Alert Supply Network Extractor (RASNEX 2.0), was developed within a collaborative project between German Federal Institute for Risk Assessment (BfR) and the European Food Safety Agency (EFSA).
RASFF notifications provide information about contamination events in standardized forms which contain free (unstructured) text fields and attached documents (e.g. PDF files, Excels in various forms such as invoices and delivery receipts). RASNEX is an open-source tool that extracts actors involved in contamination events or outbreaks from the structured parts of the notifications.
Using modern methods from AI, namely Natural Language Processing, RASNEX 2.0 builds on the original project, by enhancing to extract company names and relevant addresses from the unstructured texts found in the notifications and their nonstandardized attached documents.
Extraction of this information enables the user to more precisely follow the event(s) along the supply chain and react in an impactful manner.
The underlying models were trained on RASFF data and are shown to perform well, identifying international companies and address in various formats and languages used in the EU. The models are integrated into a dashboard that allows users to interactively work with the extracted data, update and make corrections, before downloading it into a standardized format for further processing and analysis.
RASNEX generates a graphical mapping of actors of the supply network provided and extracted by the software (e.g. applied onto the example of an Ethoxyquin outbreak).
RASNEX is a user-friendly tool that facilitates and tremendously accelerates the extraction of relevant information from RASFF notifications on national and European levels.

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Feed quality, safety and authentication

VALIDATION OF A METHOD FOR THE DETERMINATION OF BETA-AGONISTS IN FEED BY LIQUID CHROMATOGRAPHY WITH TANDEM MASS SPECTROMETRY ACCORDING TO THE COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

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The control of the presence of unauthorized substances in feed is one of the key aspects in order to ensure the safety of the animals. And for the food producing animals it is even more important, as they can be transferred to the human chain food potentially becoming a health risk for the animals and the consumers. In the European Union (EU) this issue is reflected in the Farm to Fork Strategy, as a global strategy to ensure a fair, healthy and environmental-friendly food system.
Specifically, in the Council Directive 96/22/EC the European Union banned the presence of anabolic substances in stock farming. Among others, it includes the prohibition of the presence of beta-agonists in feed. How the member states should control these substances in food and feed across the European Union is regulated in the Regulation (EU) 2017/625.
In Spain, the control of the unauthorized and prohibited substances is yearly planed in the National Residue Plan (PNIR), where are included the matrices and substances that should be controlled and how many samples should be collected. The Laboratori Agroalimentari is one of the designed laboratories for the analysis of part of these samples.
One key aspect in the control of the unauthorized substances is that the laboratories approved for the official residue control should ensure the quality and the compatibility of their analytical results. To ensure this, the analytical methods should be validated harmoniously following the same specifications. These specifications were, until 2021, regulated by the Commission Decision 2002/657/EC. The analytical methods used in official control must be validated accordingly to this Decision. In 2021, the European Commission adopted the Regulation (EU) 2021/808 that repealed the Commission Decision 2002/657/EC, giving the laboratories 5 years to revalidate the methods for official control with the new parameters.
The Laboratory Agroalimentari analyzes beta-agonists in around 175 samples of feed every year (2020: 165 samples, 2021: 176 samples, 2022: 181 samples). This method consists in a solid/liquid extraction with a mixture of methanol/water, horizontally shake for 30 minutes, centrifugation and a Solid Phase Extraction (SPE) with OASIS MCX 30 mg and a posterior injection in a liquid chromatography with tandem triple quadruple mass spectrometry detector (LC-MS/MS).
Due to the change in the regulation the Laboratory Agroalimentari started to update and revalidate the methods that are under the scope of the PNIR program. This included the method for the analysis of beta-Agonists in feed. This validation was performed the European Union Reference Laboratories Guidance Document on Confirmation Method Validation. The validation proved that the method complies with the Regulation (EU) 2021/808 for the analysis of beta-agonists in feed and it has been accredited, with the new parameters, accredited according to UNE-EN ISO/IEC 17025.

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Feed quality, safety and authentication

ANTIBIOTIC USE AND APPROPRIATENESS OF TREATMENTS IN PIEDMONTESE SWINE FARMS FOR FATTENING

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BACKGROUND - The swine industry in Italy represents the main critical issue in animal husbandry on the use of antibiotics, as they are administered massively as group therapy in medicated feed or in drinking water for the treatment of alleged multi-factorial diseases. In addition, inappropriate and excessive use of drugs occurs to compensate for poor hygiene and welfare conditions, and the frequent and unjustified use of the Critically Important Antibiotics, now banned by Reg. EU 6/2019, or the Highly Important Antibiotics.
This paper aims to investigate the use of antibiotics in medicated feed in some Piedmontese swine farms (SITI 3 - finishing) at the time of grounding and the appropriateness of treatments in the year 2022.
METHODS - The selection, made in a targeted way among the percentages of companies at risk according to the Classyfarm system or to the regional criteria, concerned 5 farms of the district Local Health Company Torino3 (ASL TO3), 5 farms of the district ASL TO4 and 10 farms of the district ASL Cuneo1, for a total of 20 farms.
The number of animals and their weight at the time of introduction, the amount of medicated feed introduced within 7 days of grounding and the defined daily intake (DDD) were recorded for each farm.
A questionnaire on the use of the drug was then formulated to be combined with existing ministerial checklists and used during the inspection activity.
RESULTS - As a result of researches carried out in relation to the consumption of antibiotics, it has emerged that the use of medicated feed takes place for the convenience of administration and for the prevention of diseases. The DDDs calculated are directly related to the number of animals introduced into the farm, the origin, the type of pigs and the veterinarian working in the farm. Furthermore there is a correlation with the percentage of mortality detected by the integrated risk assessment system.
CONCLUSIONS - Oral group treatment with medicated feed requires specific targeted measures to prevent under-dosing or non-homogeneous mixing of the active substance in the feed. Such practices contribute dangerously to the development of resistance to antibiotics. The appropriateness of treatment should be carefully assessed by the veterinarian at the time of prescription based of the diagnosis. Finally, particular attention must be paid to the production of the medicated feed in order to achieve a homogeneous distribution of the active substances. The medicated drinking waters are also a problematic means of administration of the drug; the active molecules are often distributed unevenly with carry-over phenomena at the end of treatment.
In conclusion, it should be noted that the Competent Authority has carried out an epidemiological investigation in the selected farms in order to assess the appropriateness and aware use of the antibiotic, with the aim of reducing its use and preventing resistance.
Keywords— medicated feed, antibiotic-resistance, swine farm

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DETECTING DIFFERENT TYPES OF OUTLIER IN NON-TARGETED SPECTROSCOPIC DATA USING A USER-FRIENDLY ROUTINE

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The detection of "anomalous" samples in food and feed control is of importance to ensure consumer safety and to uncover fraudulent practices. Spectroscopic techniques, such as nuclear magnetic resonance spectroscopy (NMR) and infrared spectroscopy (IR), are increasingly used for this purpose in a non-targeted manner. However, it is essential that appropriate quality assurance measures are implemented in such an approach to obtain reliable results and to identify systematic errors, similar to classical targeted methods. Quality control samples are typically measured along with the routine samples, even in some non-targeted studies. While these samples are usually evaluated quantitatively, appropriate multivariate data evaluation methods are often overlooked. They enable, however, the utilisation of the whole spectral information for the evaluation.
Recently the multivariate analysis of quality assurance samples using various distance and density based outlier detection methods was reported. This previous study of our working group provided a promising approach to evaluate quality assurance samples in a multivariate manner similar to quality control charts that are well established in targeted analysis. In this current work presented, we adapted the approach to enable the evaluation of ^{1}H-NMR measurements with appropriate pre-processing of the raw data. The entire data analysis process has been developed as a user-friendly KNIME® workflow for routine analysis, including input of raw data, pre-processing, statistical analysis and interactive summary of the results.
The workflow is currently trained on the detection of different types of outliers in representative matrices. Samples have been measured under different conditions (operator, temperature, number of scans etc.) to simulate systematic and random errors during the sample preparation and measurement. These samples are used to test the limits of the outlier detection methods and to get an impression of the capabilities of the workflow. The outlier detection workflow is not only restricted in detecting systematic errors in measurement procedures, but might also be applied for other outlier problems, for example detecting spoiled or adulterated feed materials.

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A NEW SCREENING METHOD FOR THE SEARCH FOR NITROFURANS IN FEED AND DRINKING WATER BY LC-MS/MS

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Nitrofurans are a class of synthetic antimicrobial compounds, active against both Gram-positive and negative bacteria. Their use in food-producing animals has raised concerns due to their potentially harmful effects on human health, such as carcinogenicity, mutagenicity, allergies, and development effects on fetuses and young children. Residues of nitrofurans in animal products, such as meat, poultry, eggs, and milk, can pose a risk to consumers if ingested. Therefore, regulations and monitoring programs have banned their use in food-producing animals and established to control the presence of nitrofurans in food products. In the Italian national official control plan on animal feed (P.N.A.A.), nitrofurans are among the prohibited substances which cannot be present in feed or drinking water. The objective of this work was to develop and validate a screening method for the detection of nitrofurans (NF), such as furaltadone, nitrofurazone, nitrofurantoin, and furazolidone, in animal feed and drinking water using LC-MS/MS. The feed samples are extracted with acetone, suitably diluted, and finally subjected to analysis by liquid chromatography combined with a mass detector (LC-MS/MS). The drinking water samples are filtered, acidified with formic acid, and analyzed by LC-MS/MS. To verify specificity, 20 blank feed samples and 20 blank drinking water samples were analyzed. The samples were kept at room temperature for the period necessary for the tests. The method has a detection capability (CC®) of 0.5 mg/kg for feed and 1 µg/L for drinking water samples. The CC® was verified, according to Reg. 808/2021, by the analysis of 20 blank feed samples and 20 blank drinking water samples spiked with nitrofurans. The robustness to slight changes was evaluated by taking into consideration the only important and critical variable i.e., the acidity, in terms of concentration of formic acid in the standard reading pool and in the sample solutions, considered an indispensable factor to obtain the maximum sensitivity response and good chromatography (Gaussian peaks). From the tests carried out, it has been demonstrated that slight changes in this chemical feature do not generate significant variations, i.e., they have no effect on the performance of the method. Based on the results obtained from the validation, the method is judged suitable for the qualitative screening of nitrofurans in feed and drinking water. It is fast, easy, and implies the analysis of multiple molecules at the same time.

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DETERMINATION OF QUINOLIZIDINE ALKALOIDS (QAS) IN FEED AND FOOD WITH LC-MS/MS – RESULTS OF THE IMPLEMENTATION PART OF A METHOD VALIDATION STUDY

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Following the BfR Opinion on 'Risk assessment of the occurrence of alkaloids in lupin seeds' in 2017 and EFSA's 'Scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particu-lar in lupins and lupin-derived products' in 2019, the German Federal Institute for Risk Assessment (BfR), decided to develop analytical methods to quantify these ana-lytes. To this end, two analytical methods for the determination of QAs in sweet (0.02 to 0.5% QAs) and bitter (5 to 8% QAs) lupin seeds, feed and food with low wa-ter content as well as in foods with higher water content by means of a high-performance liquid chromatography (HPLC) tandem mass spectrometric (MS/MS) method has been successfully in-house validated.

It is essential to have a robust, reliable and harmonised method to monitor the QA content in feed and foodstuffs in order to protect animal health as well as consumer health. Therefore, a method validation study is set up as a tiered approach, divided into three parts; namely two parts of method implementation for different matrices fol-lowed by the main part of the method validation study.

The purpose of the first two parts was to assess the transferability of the method to other laboratories (Germany and Europe), to enable the participants to become fa-miliar with the method and to observe possible challenges during implementation of both methods.

The method for feed (e. g. Blue sweet lupin (BSL) seeds) and dry foods (e. g. lupin flour) has been tested in part one of the study. In the second part of the study, a method especially developed for the analysis of QAs in wet food samples (e. g. milk) was investigated. More recently, a transfer of QAs from feed to cow's milk has been proven at the BfR facilities. Furthermore, wet lupin-based dairy substitutes, such as lupin-drink as well as lupin-based spreads, have become commercially available.

At the beginning of the method validation study, the method included nine QAs. For the second part of the study, the scope of analytes included in the method protocol has been expanded to ten QAs.

Feedback of the participants, identified challenges of the two methods and results of the preliminary first and second implementation part of the method validation study will be presented.

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SALMONELLA DETECTION IN LIVESTOCK FEED: ANALYSIS OF DATA COLLECTED IN THE FRAMEWORK OF THE NATIONAL PLAN FOR FEED IN ITALY FROM 2012 TO 2020

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BACKGROUND
Salmonellosis is the most commonly reported foodborne infection in humans in Italy. It is often related to the consumption of animal-based foods, whose safety strictly depends on the sanitary status of the livestock they originate from. Livestock may be exposed to zoonotic salmonellae via feed, thus the evaluation of Salmonella prevalence, serovar distribution and the application of prompt sanitary measures in case of positive findings in this matrix is crucial to prevent animal and food contamination. The Italian multi-annual National Plan for Feed (PNAA) provides specific indications for Salmonella spp. monitoring and surveillance in feed for livestock. While monitoring is aimed at collecting data for prevalence, surveillance relies on risk-based criteria. This study aimed at providing an overview of data on Salmonella occurrence in feed samples collected in the context of the PNAA in the period 2012-2020, with a particular focus on serotyping results.
METHODS
Data resulting from the official sampling in the context of PNAA 2012-2014, 2015-2017 and 2018-2020, both for monitoring and surveillance purposes, were collected and analysed. PNAA data were transferred by the Ministry of Health through electronic files to the National Reference Laboratory for Salmonella, which made them available to epidemiologists for analysis. Data were organised for harmonisation over the years and error corrections, and the final dataset was subjected to descriptive statistical analysis.
RESULTS
Throughout the studied period (9 years), 8330 samples were collected for monitoring purposes. A total of 79 Salmonella isolates were detected and 30 different serotypes were identified. The most detected serovar was S. Senftenberg (SS) (12,76%), followed by S. Typhimurium (ST) (10,13%), S. Mbandaka (SM) (7,59%) and S. Llandoff (SL) (7,59%). The isolation of SS, SM and SL occurred primarily in oil seeds, whereas ST was mostly found in cereals and compound rations for pigs and poultry. Oil seeds and products thereof were the matrix with the highest prevalence recorded (3,72%), followed by bovine compound feeds (0,99%), feed ingredients of animal origin (0,91%), and cereals and derivatives (0,88%). As regards the surveillance activities, which are mainly focused on feed for poultry and pigs, 4475 samples were collected, from which 65 Salmonella isolates were detected. A total of 22 different serotypes were recorded, among which SS (20,00%), SM (16,92%), ST (10,77%) and S. Agona (SA) (10,77%) were the most frequently detected ones. Soybean seeds and derivatives were the materials with the highest prevalence recorded (2,06%), followed by compound feeds for pigs (1,45%) and poultry (0,73%).
CONCLUSIONS
Results show that few Salmonella serovars were more frequently detected in feed. Among those, SS, SM, SA and ST are often isolated in poultry, while ST and SA in pigs. This demonstrates that feed control is fundamental to limiting the dissemination of salmonellae in livestock.

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DEVELOPMENT OF A MICRO-NIRS- APP-CLOUD SYSTEM FOR ON-SITE DETERMINATION OF THE NUTRITIONAL COMPOSITION OF POULTRY FEED AND ITS VARIOUS INGREDIENTS

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Precision feeding can improve poultry nutrition and at the same time reduce the excretion of surplus nutrients, thereby improving the sustainability of poultry production while reducing feed costs. Near infrared spectroscopy (NIRS) is a useful tool for precision feeding as it allows to accurately analyze feed and feed ingredients and enables precise formulation of rations according to known nutritional requirements, eventually avoiding over-formulation. The purpose of the Pocket Feed Lab project is to develop a portable micro-NIRS for the on-site analysis of poultry feed and poultry feed ingredients. The micro-NIRS sensor developed by aikemy GmbH is based on MEMS (micro electro mechanical system) technology. It connects with a mobile app via Bluetooth and finally to cloud services for data analysis and storage. Results are displayed in near-real time as percentage values in the app. Furthermore, the comparison of the results with user pre-specified test targets provides an immediate pass/fail score for each nutrient. The potential of three different MEMS, covering different regions of the NIRS wavelength range, was explored for use in the final dual-MEMS design. Calibrations are being developed to predict crude protein, crude fiber, crude fat, water soluble carbohydrates, crude ash and moisture. Over 1000 samples (poultry feed and its various ingredients: corn, wheat, soybean meal/press cake and distillers' grain) were collected in Switzerland, different European countries and the USA. They were analyzed according to official methods at the accredited analytical laboratory of Agroscope. The training sample set covers the protein content recommendations suggested by different research centers and is in line with the nutrient composition of available commercial products reported by the Swiss poultry sector. In general, the statistics of the preliminary prediction models show a good potential of the micro-NIRS system in terms of coefficient of determination (R^2), root mean square error of prediction (RMSEP), and ratio of the standard deviation of the validation set to the standard error of prediction (RPD). The results suggest that this system will be a useful tool for precision feeding in poultry production. Based on the current knowledge, the application of this device could be extended to additional nutritional parameters as well as other feeds and feed ingredients.

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IMPROVEMENT OF THE SAFETY AND EFFICACY OF PROPIONIC ACID-BASED MOULD INHIBITORS FOR STORED GRAINS

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Mould growth in stored grains can lead to a reduction in nutrient quality. Furthermore, the presence of mycotoxins may result in detrimental effects on animal performance and health. Propionic acid represents a powerful mould inhibitor, widely used for the protection of grains, however, challenges persist with its pungent odour and the corrosivity of its vapours.
Esterification of propionic acid and monopropylene glycol (MPG) was hypothesized, to reduce the disadvantages of propionic acid and further improve its efficacy. Fatty acids esterified to propylene glycol are allowed as feed materials, according to EU regulations.
A dose response study was performed, to compare the antifungal capacity of extracted propylene glycol propionate esters with propionic acid on a molar basis. Propylene glycol propionate and propionic acid were applied onto barley, under challenging conditions of 20.2 % moisture, at dosage levels ranging from 0.02 mol/kg to 0.08 mol/kg. The evaluation of CO_2 production in the barley samples demonstrated that 0.06 mol/kg and 0.08 mol/kg propylene glycol propionate performed significantly better in controlling mould growth versus the same dosages of propionic acid (General Linear Models procedure, $p < 0.05$).
The influence of esterifying propionic acid with MPG (30 % yield) in terms of volatility was assessed under accelerated conditions (40 °C for 6 hours, purged with a constant air flow). The evaporation was determined by high-performance liquid chromatography (HPLC). When partially esterified, the evaporation rate of propionic acid was reduced 14 times compared to free propionic acid.
A novel mould inhibitor, Myco CURB® LC liquid was developed based on propionic acid, ammonium propionate and propylene glycol propionate. Its efficacy was validated under field conditions, in collaboration with Adesco (Ireland). Freshly harvested barley was allocated into two treatments: 1) Myco CURB LC liquid (4 kg/T) and 2) Myco CURB ES liquid (4 kg/T) as a reference. Although Myco CURB LC liquid contains a lower amount of total active ingredients, the efficacy of both products was similar. Myco CURB LC liquid and Myco CURB ES liquid were both able to decrease the mould counts from 31000 CFU/g to < 100 CFU/g within the first 2 weeks of storage. After 9 months, the mould growth was still completely under control in both barley batches.
Analyses of air samples - taken in the hall during product application and 2 weeks post-treatment in the headspace of the storage silos revealed that the levels of propionic acid were reduced by 45% and 89%; and for ammonia by 84% and 86%, respectively. All levels were well below the short-term exposure limit (STEL OEL) reference values for propionic acid (62 mg/Nm³) and ammonia (36 mg/Nm³).
In conclusion, propylene glycol propionate was shown to improve the efficacy, odour and corrosivity of vapours versus traditional mould inhibitors.

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DIGITAL MICROSCOPY : A NEW TOOL TOWARDS BETTER AUTHENTICATION AND EXPERTISE DEVELOPMENT

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Authentication and fraud detection in the feed and food still relies, aside other technics, on the conventional observation method by light microscopy. Although this method has been proven to be efficient, it is nevertheless showing shortages. For instance, one of the limitations is the working distance between the objective and the observed structure with as a consequence the impossibility of obtaining large fields of observation, as well as difficulty for micromanipulation. Over the past years digital microscopy evolved fast offering new tools and opening new perspectives in research areas as diverse as electronic component industry, forensics, biology and material science industry.
The use of digital microscopy was applied by the European Union Reference Laboratory for Animal Protein detection in feedingstuffs (EURL-AP) to widen their expertise in animal proteins authentication and detection. The technic, as a complement to light microscopy, offers many advantages in both the understanding and better recognition of animal remains (e.g insect PAPs and marine invertebrates' microstructural identification features), in bridging the physical gap between macroscopy and microscopy (e.g using magnification ranges from 20x up to 2000x on entire objects such as larvae) or generating high quality images with sharp evenly focussed areas by automated stitching, tiling and multi-focus algorithms. Finally, digital microscopy demonstrated its usefulness as a key technic for building up a virtual training platform by the realisation of virtual slides (each composed of over 1000 records) intended for on-line training of experts in animal constituent's identification.
Functions for image optimisation combined with automated measurements (e.g. counts, 2D measures or 3D reconstructions) are also offering new perspectives of the utilisation of digital microscopy as a source of high-quality imaging for the establishment of training libraries for deep learning.

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STUDY OF TRUCKLOAD CROSS-CONTAMINATION OF SALINOMYCIN AT ONE FEEDMILL USING A MICROTRACER (R)

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Background:
Coccidiostats formulated in broiler feed- can be toxic if they reach feeds where they are not intended.
Nicarbazin reaching poultry breeder feed is one example, salinomycin reaching turkey feed and monensin sodium reaching horse feeds are other examples.
Manufacturers of coccidiostats have formulated Microtracers® in their products to allow feed manufacturers to test feed before it leaves their feed mills for the tracer as evidence of the coccidiostat. This is to detect manufacturing errors where coccidiostats could cause toxicity or drug residues in poultry meat. Testing is often performed qualitatively before feed is shipped or quantitatively in testing "retain" truckload samples by the feed mill laboratory.
In one study performed in Italy, tracer counts correlated well with vitamin E assay results.
Method:
At a USA feed mill, Microtracer FS-Blue#2 (colored uniformly sized stainless steel particles, 50,000/gram) was formulated in Biocox ® salinomycin to yield. 2 grams/2,000-lbs of feed or 50 particles/454 grams. "Retain" truckload feed samples from consecutive loads were tested quantitatively for the tracer. The analysis required grinding the pelleted feed to mash, pouring it through a laboratory magnetic separator, sprinkling the tracer onto an 18cm filter paper wetted with 60% ethanol, drying the paper and counting the spots that developed.
Results:
115 samples formulated with salinomycin and 90 samples formulated without salinomycin were analyzed.
The 115 samples formulated with salinomycin yielded an average of 37 particles from analysis of 265 gram samples for a Recovery of 64% of the formulated tracer. Four samples contained 5 particles or fewer evidencing a failure to add the drug as formulated.
The 90 samples formulated without salinomycin (turkey feed) yielded an average of 2 particles per 265 grams of feed or 3.5% of the formulated tracer. Thirteen of the samples had counts of 5 particles or more or 14% of the tracer found in the feeds formulated with the drug. The highest count 16 would equate to 43% of the tracer formulated with the drug.
Conclusion:
Salinomycin formulated in broiler feeds at 60 ppm can lead to depressed rate of growth in 7 week old turkeys at 22ppm or 37% its formulated level and can be toxic to adult turkeys with a mortality of 35% when fed at 60 ppm. In this study, The turkey feed sample with the highest count of 16 had an estimated salinomycin or 26ppm enough to depress growth but not to kill the turkeys.
The EU in 2009 set a tolerance of 3% of the formulated level of coccidiostats or about l.8ppm for salinomycin in feeds not formulated with it. This would equate to 1 particle in 265 grams of feed. 44 of 90 samples had one or more tracer particles suggesting 49% of the feeds not formulated with the coccidiostat may have failed to meet EU standards.

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VIRAL DETECTION IN MASS-REARING CRICKETS (ACHETA DOMESTICUS) USED AS FEED AND FOOD.

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Viruses have different relationships with insects, as they exploit them as vectors to infect animals or plants, establish symbiosis with entomological parasitoids and set up multiple co-infections that could be difficult to diagnose. Diseases caused by insect pathogens can be detrimental to reared insects and may cause significant economic loss to producers. Mass farming systems of edible insects are relatively new, and little is known about factors determining the quality and yield of production processes. Achieving this knowledge could be essential to avoid insect diseases, particularly viral ones, and therefore guarantee the success of insect breeding. In our study, we investigated the presence of viral pathogens in insects reared in farmed conditions. We focused on a food and feed production system where a single insect species, Acheta domesticus (Orthoptera: Gryllidae), was reared. Two techniques were employed to reveal the viral presence, i) negative staining electron microscopy (nsEM), which can permit to identify any virus showing typical morphological patterns (so-called "catch-all" technique), and ii) RT-PCR with two different protocols for the detection of the Iridovirus and Cricket Paralysis Virus (CrPV). Diagnosis was carried out on specimens at different times (7, 14, 21, 28, 35 and 49 days after the hatching). The nsEM highlighted the presence of Iridovirus in alive and dead individuals and immature and breeding specimens, but not in eggs. The presence of Iridovirus in immature specimens was associated with small (20-30nm) roundish virions, likely resembling dicistrovirus (CrPV) and/or densovirus (AdDV). In specimens of the early nymphal stages (both dead and alive), iridoviruses have also been associated with picornaviruses and icosahedral particles (approximately 50-60 nm) similar at birnaviruses. By using a specific PCR method, the identification of Iridovirus only was confirmed, whereas the identity of small particles is still pending. These results confirm that at least two viruses, particularly iridovirus stating the high viral load in dead crickets, are closely related to mass rearing of A. domesticus and to mortality events. The use of diagnostic techniques (EM and PCR) could help to develop strategies for the surveillance and identification of viruses that can compromise the production process of insects used for feed and food. Detection of viruses in mass rearing insects requires, therefore, a systematic approach with consequential steps, starting by clinical evaluation followed by laboratory analysis to detect aetiological agents.

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SIZE AND SHAPE ATTRIBUTES OF PACKAGING REMNANTS IN FORMER FOOD PRODUCTS

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Former food products (FFPs) are alternative feed ingredients used in livestock diets. They are surplus rejected by human market but do not pose microbiological or hygienic risks when considered as feedstuffs. However, although the processes of transforming FFPs into animal feed often include mechanical unpacking and grinding, the final products may be still prone to packaging contamination. Common materials of packaging remnants in FFPs are aluminum, cellulose, and plastic. Therefore, it is important to investigate and to provide information regarding the size and shape attributes of these materials so as to improve the processing techniques in feed industry.
A total of 441 packaging remnants from 17 sources of FFPs were included in this study. Fourier transform infrared spectroscopy coupled with an optical microscope was used to identify the material of the packaging remnants, which resulted in a categorization of remnants consisting of 44 aluminum, 308 cellulose, and 89 plastic remnants. The categorized remnants were observed with a stereomicroscope and were subsequently measured by a digital camera and an image analysis software. Each measurement contains 21 size descriptors and 9 shape descriptors some of which were derived from calculations. The values obtained were standardized for plotting and statistical analysis.
A principal component analysis showed that one size descriptor, strongly correlated with area, explained 84% of the total variability in the size of remnants. The distribution of values for both size and shape descriptors overlapped between the three materials though aluminum remnants tended to have smaller areas compared to cellulose and plastic ones. Also, aluminum remnants showed a narrower range in descriptors such as roundness and solidity than remnants composed of the other two materials. Through the information provided by the image analysis and the measurements, it was concluded that the obtained values in terms of size and shape attributes were distributed broadly rather than following a specific pattern. Nevertheless, the measured values of aluminum remnants seemed to be more consistent compared to the ones of cellulose and plastic remnants. Namely, aluminum remnants tended to be smaller in size and have narrower distribution in shapes. This could be associated with the intrinsic property of aluminum used in food packaging and the processing protocols run in the feed plant. The most common form of aluminum in FFP packaging is aluminum foil which is very thin and malleable. After intensive mechanical processing, aluminum remnants could be folded and become more compact, which contributes to their smaller sizes and more regular shapes. Overall, this study aimed at providing information about size and shape attributes of packaging remnants in three different materials. To distinguish between aluminum, cellulose, and plastic remnants, other properties of the materials have to be considered.

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DETECTION OF MICROPLASTICS IN FECES OF PIG FED FORMER FOOD PRODUCTS

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Former food products (FFPs) have been authorized as alternative feed ingredients for livestock in Europe. However, recent scientific evidence suggests that in feed produced from FFPs, there was presence of plastic packaging remnants. Accordingly, these contaminants, especially microplastics (MPs), are known to occur in biological matrices including feces. This is of serious animal health and environmental concern as well as food safety issues for consumers. Hence, the aim of this study was to test the effectiveness of the selected method for MPs sampling, digestion, and extraction in pig feces.
A total of 36 fecal samples were collected from growing-finishing pigs fed a control diet or diets containing either 30% sugary FFPs or 30% salty FFPs (n=12 per group). The feces were directly collected from the rectum and placed in an aluminum bag. The sugary FFPs included confectionary products while the salty FFPs were made up of bakery products or pasta. Additionally, in a previously study, MP has been detected in the same FFP sources used in our current trial. In each fecal sample, three replicates of 3 g of feces were obtained from random spots. To digest organic matters in feces, 25 mL of 30% H2O2 was added to 3 g of feces in a beaker for a week at room temperature. An additional three samples were spiked with pieces of blue polypropylene to check if the digestion process can change the color and shape of the plastics. Later, 100 mL of saturated NaCl solution (density: 1.2 g/cm3) was added to the sample for density separation. After settling overnight, the samples were filtered. Then, pretreated feces were inspected under a stereomicroscope to detect possible MPs. The suspected particles found were placed in a petri dish to be analyzed by the Fourier transform infrared spectroscopy for confirmation.
The results from spiked samples showed that neither the color nor the shape of plastics was changed due to the H2O2 digestion. However, with the chosen method, it was not able to detect any MPs in the collected feces, which may be the result of several factors. In the current study, the original MP contamination level in FFPs was relatively low and the inclusion level of FFPs in the pigs' diet was 30%, which led to a dilution effect in the final diet. Considering the feed intake of pigs and the possible retention of MPs in their intestine, the amounts of MPs excreted could be less than ingested. In addition, the distribution of MPs in feces was not even, which made the detection of MPs more challenging. Therefore, more research is needed to clearly understand the fate of MPs in FFPs, whether they remain in animal's intestine or they are excreted via feces or urine as well as the proportion of retention and excretion. Furthermore, as there is currently no standard protocol for MPs extraction in animal feces, other methods can be tested to see their effectiveness and to develop an optimized protocol.

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Feed quality, safety and authentication

DETECTION OF DOWNGRADED FAST FOOD IN INSECT FEED BY MASS SPECTROMETRY-BASED PROTEOMICS

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Since 2021, insect meal has been authorised in feed intended for pigs and poultry, in addition to fish feed. Despite these regulatory changes that seem to increase the insect market, its use in feed remains limited and still more expensive than other protein sources. One of the reasons for this high price is linked to the limitation of authorised substrates for breeding. From a legislation point of view, edible insects are considered farmed animal and, therefore, must comply EU animal by-products regulation.
Following the same sustainability strategy, the EU has also adapted legislation to promote the use of another feed source, former foodstuffs (FFS). FFS retain a significant nutritional value and their use aligns with the current trend of circular economy. However, FFS containing meat or fish remain prohibited. But, is it possible to control? At the end of the insect rearing, insects are separated from feed residue and incorrect procedure may lead to the presence of residual feed materials.
The objective of this study was to develop a UHPLC mass spectrometry-based proteomics method to detect the presence of animal proteins in insect meal. Meat products from different species (beef, pork and chicken) and under various forms (raw, cooked or fast food form (beef burger, ham and nuggets)), were used to identify specific peptides markers. Different substrates adulterated or not with 10 % of each fast food, were also prepared for insect rearing. These substrates were analysed before and at the end of the rearing trial in order to evaluate the sensitivity of the method. Analyses were performed by liquid chromatography (Acquity UHPLC system, Waters) coupled with a triple quadrupole mass spectrometer (Xevo TQ-XS, Waters). Additionally, samples were characterised by PCR.
Sixteen bovine markers, seven porcine markers and fifteen poultry markers derived from various proteins such as haemoglobin, collagen, myosin, albumin, carbonic anhydrase, beta-enolase, myosin-binding protein and pyruvate kinase were selected for their specificity and ability to detect the targeted meat in its processed or unprocessed forms. Results obtained on the substrates have showed that the degradation of the proteins varied from a protein to another. Additional analyses will be performed in order to characterise this degradation.

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Feed quality, safety and authentication

USEFULNESS OF AN ARTIFICIAL INTERNAL CONTROL FOR THE DETECTION OF PCR INHIBITORS IN THE ANALYSIS OF RUMINANT DNA IN ANIMAL FEED

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Following the bovine spongiform encephalopathy crisis in the 1980s, in Europe there is a strict control for the presence of constituents of animal origin in feed materials and compound feed.
The Agrifood Laboratory, attached to the Department of Climate Action, Food and Rural Agenda of the Generalitat de Catalunya and based in Cabrils (Barcelona), participates in the official control of agri-food products safety and quality and means of production - including the control for the detection of animal constituents in feed - processing samples from inspections, litigation procedures and intended for foreign trade. The Laboratory has accreditation according to the UNE-EN ISO/IEC 17025 standard.
The European official control is based on the combination of two techniques, microscopy and Real-time PCR (Polymerase Chain Reaction), which are used depending on the constituents of animal origin and the animals to which the feed is intended.
In the case of the Real-time PCR, it is known that some feed components can inhibit it. For this reason, the European SOP (Standard Operating Procedure) for ruminant DNA detection (Detection of ruminant DNA in feed using real-time PCR) requires the evaluation of PCR inhibition, referring to ISO 24276: 2006, to avoid a false-negative result.
Our laboratory uses an internal PCR control based on the amplification of an artificial target. Its detection is done by using specific primers and a Taqman probe labelled with a different fluorophore than that used to detect ruminant DNA. This design allows the internal control to be amplified in the same reaction as the ruminant DNA target.
We have observed that the internal control is amplified at lower CTs (Threshold Cycles) than the established cut off on our platform, which is required by the SOP. Amplification occurs both in the negative PCR controls (without the presence of any DNA) and in the positive controls (presence of ruminant DNA), showing the same fluorescence intensity, which indicates that its use does not interfere in the detection of ruminant DNA. When PCR inhibitors are present, a clear increase in the number of internal control CTs has been detected. In those cases, as indicated in the SOP, we have been able to verify that the dilution of the extracted sample decreases the presence of inhibitors, allowing the PCR reaction to be carried out.
In conclusion, the designed artificial internal PCR control is useful for the detection of PCR inhibition and therefore helps in discarding false negative results for the presence of ruminant DNA.

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Feed quality, safety and authentication

SURVEY OF REGULATED TRACE ELEMENTS IN PET FOOD ON THE DANISH MARKET

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The current European Union feed legislation (2002/32/EC and later amendments) includes maximum levels (MLs) for the trace elements arsenic, cadmium, lead, and mercury in pet food, including feed for cats and dogs. In the EURL-MN (the European Union reference laboratory for metals and nitrogenous compounds in feed and food) we did a survey of the content of regulated elements in a range of feeds for cats and dogs. The measured levels were compared with current MLs.
A total of 32 samples were purchased in physical and online shops. The samples were selected based on their main protein source (vegetables, poultry, by-products, or fish) and included both canned (wet) and pelleted (dry) feeds and covered a differentiated price range (cheap – expensive). Samples were homogenised and analysed as is. Total arsenic, cadmium, lead, and mercury were determined by inductively coupled plasma mass spectrometry (ICP-MS) after microwave-assisted acid pressure digestion. The certified reference material DORM-4 was included in the analysis.
Arsenic, cadmium, and lead were detected in all samples, while mercury was only detected in samples containing fish and in one sample containing by-products. The highest levels of arsenic (up to 2,8 mg/kg) and cadmium (up to 0,35 mg/kg) were found in samples containing fish. High levels of cadmium (up to 0,29 mg/kg) were also found in samples with vegetables as the main protein source. The level of lead varied across all samples, irrespectively of main protein source. None of the pet food samples had levels exceeding the MLs.
The survey of 32 pet food on the Danish market showed that although the feeds contain regulated trace elements, none of the pet food contained levels exceeding the current MLs. This suggest that feed safety is ensured and that pet food producers comply with the European Union feed legislation.

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Feed quality, safety and authentication

DETECTION OF RUMINANT PROCESSED ANIMAL PROTEIN IN FEED BY LC-MS/MS

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BACKGROUND
The prohibition of feeding animal proteins was established to prevent TSE (transmissible spongiforme encephalopathy) and has been relaxed gradually since 2007. Since 2013, processed animal proteins (PAPs) derived from non-ruminant origin and in 2017 PAPs derived from insects were authorized for the use in aquaculture feedingstuffs. Recently, the feed ban was further lifted and non-ruminant PAPs were authorized under consideration of the intra-species recycling ban. Ruminant PAPs and blood products are still unauthorized. Next to the unauthorized materials, gelatine or dairy products coming from ruminants were permissible at any time. The official control method for the determination of animal proteins in feeding-stuff (as laid down in regulation (EG) 152/2009), is the combined application of light microscopy possibly followed by PCR or in some case only by PCR. However, the current methods are no longer sufficient because a positive PCR result does not automatically indicate that non-authorized PAPs were used.
METHODS
A LC-MS/MS based targeted screening method on peptide level to distinguish between authorized and unauthorized PAPs deriving from ruminants was developed. First selective peptides of ruminant proteins were selected based on untargeted discovery proteomics by data dependent (DDA) and data independent analysis (DIA) approaches. Afterwards, a parallel reaction monitoring (PRM) method was established and optimized. Proteins were extracted by 2 M urea (pH 9.2), alkylated, digested with trypsin and obtained peptides purified by SPE with stage-tips. The measurements were performed by a high-resolution UPLC-QToF-MS system.
RESULTS
The final PRM targeted method contains 19 unique peptides of 7 specific ruminant proteins. It enables the determination of the cell origin (blood, muscle, gelatine, dairy products) of the PAPs within the species Ruminantia. Selectivity and trueness was proofed by the measurement of spiked sample materials containing 0.1 % ruminant PAP, 2 % milk powder and blank samples. Obtained results revealed that the developed method was able to distinguish between authorized and non-authorized ruminant ingredients. Unfortunately, BSA is not a suitable marker for ruminant PAPs due to its presence in milk powder.
CONCLUSIONS
The official control method for the analysis of PAPs in the EU (light microscopy and/or PCR) can identify particles derived from terrestrial vertebrates, fish and terrestrial invertebrates while the PCR is able to identify the species, but not the cell compartment within the species. This analytical gap can be closed by the developed PRM method, which can define the origin of PAP reliably at protein level. The performed validation process revealed excellent results for selectivity and trueness for the method; thus, the developed analytical procedure is suitable as screening method to analyse the composition of feedingstuff and help to uphold feed and food security within the EU.

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Feed quality, safety and authentication

STRATEGIES FOR THE PREVENTION OF DATURA STRAMONIUM AND ITS ALKALOIDS IN SOYBEANS

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BACKGROUND
Datura stramonium(jimsonweed) is an important agricultural weed and contains toxic tropane alkaloids, namely scopolamine and atropine. Both can cause acute symptoms of poisoning in humans and animals. The origin of contamination of the harvested crop with tropane alkaloids occurs due to the presence of the species in the fields at the time of harvest. D. stramonium mainly occurs in late harvested crops, such as maize, sunflower, and soybean. Conventional cleaning of the crop is not sufficient, as the toxic plant juice contaminates the fresh crop during harvesting.
At present, in animal feed maximum levels are set in directive 2002/32/EG for 1000 mg/kg for harmful botanical impurities, while for food maximum permissible levels are in force since September 2022 as laid down in Commission Regulation (EU) 2023/915. Due to the increased occurrence of tropane alkaloids in feed and food and its toxic effect, it is necessary to keep the contamination as low as possible and to establish strategies for this in the field as well as after harvest.
METHODS
Within a national research project (StopDatura) strategies to prevent the contamination of tropane alkaloids based on the following three pillars are developed:
1. Safe crop production — development of strategies for detection and control of D. stramonium in soybean incorporating drone technology
2. Safe raw materials and products — evaluation and establishment of rapid methods for the analysis of tropane alkaloids in the harvested soybean crop
3. Awareness raising — development of a code of practice
RESULTS
A drone-based tool was developed for the detection of D. stramonium in the field. In a next step, soybean fields with different levels of infestation with D. stramonium were identified with the help of the drone. The harvest was sampled and then examined visually for the contamination with seeds and also for tropane alkaloids by means of LC-MS/MS. Furthermore, the applicability of two rapid test kits was tested.
The results show a high variation of infestation of D. stramonium in the fields, which is also reflected in the levels of tropane alkaloids in the samples.
CONCLUSIONS
In the future, D. stramonium will require more attention and control measures in order to reduce the contamination of harvested crops and to provide safe food and feed. At present, it is noted that the level of awareness and negative effects of the plant toxins are not yet sufficiently known. Therefore, improvement in the method of analysis (screening methods) and awareness-raising measures must be implemented.

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Feed quality, safety and authentication

MONITORING ON PACKAGING MATERIAL RESIDUES IN FEED – SURVEY AMONG IAG FEED MICROSCOPY MEMBERS AND OTHER EUROPEAN OFFICIAL CONTROL UNITS - IMPACT ON THE SAFETY OF FEEDS USING FORMER FOOD PRODUCTS.

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Macro- and microscopic evaluation of feed includes detection of animal proteins, botanic impurities, label control, customs and of prohibited ingredients such as packaging materials (PM; Regulation (EC) 767/2009). In addition, detection of micro-plastics (possible degradation products of some of the PM) is getting attention.
PM can harm animals or disturb their feed intake, pollute the environment and can be considered as undesired impurities in feeds. These materials do not consist of a definite molecule, group of molecule, living species or definite bodies. They can be plastic foil, hard plastic, metal pieces, paper, wood, or some combination of materials. Their features (sharp, pointed) can be as important as the material itself. This is a typical topic for microscopy detection and evaluation.
In this poster presentation, a review of the work done on detection of PM in 14 monitoring entities (institute, laboratories) is presented:
-since 2012 some institute analyze more than 20 samples each year, the incidence of non-compliant samples will be presented.
-a majority of the entities have an active monitoring, whereas others have passive surveillance (done while performing other microscopy analyses), the results are compared.
-protocols used depend on sample types and analysts, harmonization is sought.
With these results, an overview of the situation and the opportunities for a safe utilization of former food products in Europe will be discussed.

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Feed quality, safety and authentication

EUROPEAN UNION REFERENCE LABORATORY FOR FEED ADDITIVES (EURL-FA): WHAT DID WE LEARN FROM 11 YEARS' EXPERIENCE OF ORGANISING PROFICIENCY TESTING?

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Feed additives cover quite different products such as trace elements, vitamins, coccidiostats, and colourants. These products are individually authorised within the European Union and the corresponding legal acts often contain target levels for their use in compound feed. Moreover, European legislation requires proper labelling of the compound feed supplemented with the feed additive, also specifying the content of this feed additive.
One of the tasks of the EURL-FA is the annual organisation of proficiency testings (PT) to evaluate the capability of the Member States' laboratories to correctly determine the content of selected feed additives in compound feed. PTs are based on the principle that the organiser prepares blind test material containing the target feed additives at a level of interest, which is the assigned value. The participating laboratories receive the samples, analyse them and report the results back to the PT organiser. After statistical analysis, each laboratory receives a score, indicating the closeness of their result with the assigned value and a judgement, whether the difference between both values is still acceptable or not.
A major challenge encountered by the EURL-FA derives from the broad spectrum of analytical methods employed for the determination of feed additives. This aspect has a significant impact on the organisation of proficiency testings (PTs), thus requiring tailor-made decision for each PT. For instance, the test material may be prepared using pure substances, ready-to-use preparations containing the feed additive or commercially available compound feed already supplemented with the target feed additive. There are also different options to establish the assigned value of the study. These examples underscore the complexity inherent in the organization of PTs within the domain of feed additive analysis.
The purpose of the poster is to show interesting lessons that we learned when comparing the results of PTs from quite different methods and the evolution of the PT participant's results over time.

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Feed technology and novel processing techniques

FIBERS CAN IMPROVE PHYSICAL QUALITY OF THE PET FOOD KIBBLES

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Introduction

Nutritionally balanced pet food is an utmost priority of the industry. However, the physical quality of the pet food kibbles is an equally important aspect. Each ingredient included in the pet food kibble has a unique nutritional composition and contributes uniquely to the physical structure of the final product.

Materials and Methods

Raw materials used for the pet food diets were milled with an Alpine hammermill on a 1 mm maximum particle size. Mixing was done in an IsDeCa® 60L mixer. Fibers peanut-husk, fibersol, and nano-cellulose were added as fiber sources with inclusion levels of 0.7% and 1.4%. All diets were produced by a twin screw extruder (BCTG-62, Bühler) except for two different screw speeds, 475 rpm, and 650 rpm. The extrusion die size was 7 mm. Durability (PDI%) was done with New Holmen (TekPro) in triplicate. Hardness analyses were done with Kahl pellet tester (Amandus KAHL GmbH) with 30 repetitions.

Results and Discussion

The screw speed and the fiber source influence the enhanced PDI% of kibbles ($p<0,05$). The screw speed of 650 rpm during extrusion showed to have similar PDI% when compared to all other fibers, except fibersol and control diet. Fibersol in both dosages 0.7% and 1.4% in the diet and produced at 650 rpm showed to have the highest PDI%. However, the control diet upon the same screw speed showed to have the lowest PDI% ($p<0.05$). Hardness analyses revealed that the control diet produced at 650 rpm were harder (2.33 kg/mm²) than the same diet produced at 475 rpm or the diets with different fiber source ($p>0.05$). It has been shown that diets containing fibersol (0.7 and 1.4%), nano-fiber (1.4%) and produced at 475 rpm and peanut husk (0.7%) produced at both 475 and 650 rpm had higher hardness ($p>0.05$) than nano-fiber, peanut-husk, and fibersol, all with 1.4% inclusion and produced at 650 rpm. The lowest hardness was observed for pet food kibbles containing 1.4% peanut-husk produced at 475 rpm. The highest expansion and bulk density change (34.14%) during extrusion screw speed alteration from 475 rpm to 650 rpm was observed in the diet containing fibersol 1.4% and the lowest (3.72%) containing 1.4% peanut-husk.

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Feed technology and novel processing techniques

MULTI-ANALYTE METHOD FOR THE DETERMINATION OF TRACE ELEMENTS AND HEAVY METALS IN ANIMAL FEED BY ICP-MS

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This poster demonstrates the use of inductively coupled plasma-mass spectrometry (ICP-MS) to determine trace elements and heavy metals of interest in organic and inorganic feed samples.
The principal responsibility of the Animal Feedingstuffs Section in The State Laboratory is in providing a comprehensive analytical and advisory service to Government Departments and Offices thereby enabling them to implement and formulate the technical aspects of National and EU Legislation. Our main client is the Department of Agriculture, Food and the Marine (DAFM) – from whom we receive approx. 1,500 samples per year.
We carry out analysis on different feed material types: compound feedingstuffs, feedmaterials and premixtures. Feed composition is extremely variable which means matrix effects must be controlled. Feed samples contain many different elements at varying concentrations, which could lead to severe bias in the results, if interferences are not eliminated efficiently. A multi-analyte method was developed and tailored for use on two different ICP-MS systems.
The method produces precise and accurate results for digested feed samples using both the Agilent 7900 as well the NexION 350X ICP-MS. The combination of the ICP-MS and ESI prepFAST Autodilution System allows for rapid and accurate analysis of 16 elements in three different modes (to eliminate all interferences), while reducing the amount of manual interaction during sample preparation.

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Feed technology and novel processing techniques

EVALUATION OF A PORTABLE X-RAY FLUORESCENCE DEVICE FOR A SUSTAINABLE MINERAL NUTRITION IN DAIRY HERDS

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[1]*ANIMINE*

Forages are extremely variable in mineral content and can contain antagonists that reduce the availability of some minerals (i.e Cu, S, Mo and Fe). Imprecise supplementation of macro (P, Ca…) and micro-minerals (Zn, Cu, Mn…) will result in nutritional imbalances, economic losses, and environmental impacts. The mineral content of ruminant's basal diet is not analysed in routine due to the high cost of traditional analytical methods performed in laboratories. Proposing an innovative handheld analytical tool for cheap and immediate mineral analysis at the farm, will give the opportunity to perform a precise mineral supplementation. The objective of this work was to validate the analysis of selected minerals (Ca, P, K, Mg, Na, S, Zn, Cu, Mn, Fe and Mo) in 89 forages with a portable device based on X-Ray Fluorescence (XRF) technique in comparison to wet chemistry method. Twenty samples of grass, 21 of hay, 11 of haylage, 21 of maize silage and 16 samples of grass silage were first collected all over Auvergne-Rhônes-Alpes area in France, then dried and ground. Forage mineral content was directly determined by XRF, in parallel, the corresponding samples were analysed by ICP-AES. The relationship between XRF and ICP-AES methods was given by the coefficient of determination (R^2) resulting from the linear correlation established between XRF and ICP-AES results. Mean absolute error (MAE) between ICP and XRF was also calculated. As a result, mineral content measured by the reference method, was variable between the five types of forages with maize silage being the lowest concentrated in minerals; macro (g/kg DM) :P=1.92, Ca=2.16, K=11.1 and S=0.924 and micro-minerals (mg/kg DM): Cu=4.67, Fe=147.4, Zn=21.1, Mo=0.5 and Mn=30.7. Grass and grass silage contained the highest contents of antagonists, with maximum (mg/kg DM) reaching 3.7 for Mo, 12 for Cu, 676 for Fe and 3.75 g/kg DM for S. Globally, the correlation between portable XRF and ICP-AES methods was dependent on the chemical element. Magnesium and sodium, due to XRF physical limits, were poorly (R^2=0.02 for Mg) or not at all detected (Na). Copper, iron and sulphur contents were quantified by the portable XRF, R^2 were respectively 0.70, 0.78 and 0.94 and MAE were respectively 13%, 24% and 8%. Because of its very low concentration, ranging between 0.4 and 3.7 mg/kg DM, molybdenum was a challenge for the portable XRF analysis (R^2=0.49, MAE=61%). For the other minerals, R^2 ranged between 0.89 and 0.96 and MAE between 6 and 18%. Although, the portable XRF analysis showed globally promising results to quantify mineral content in forage, further analytical development is needed for molybdenum.

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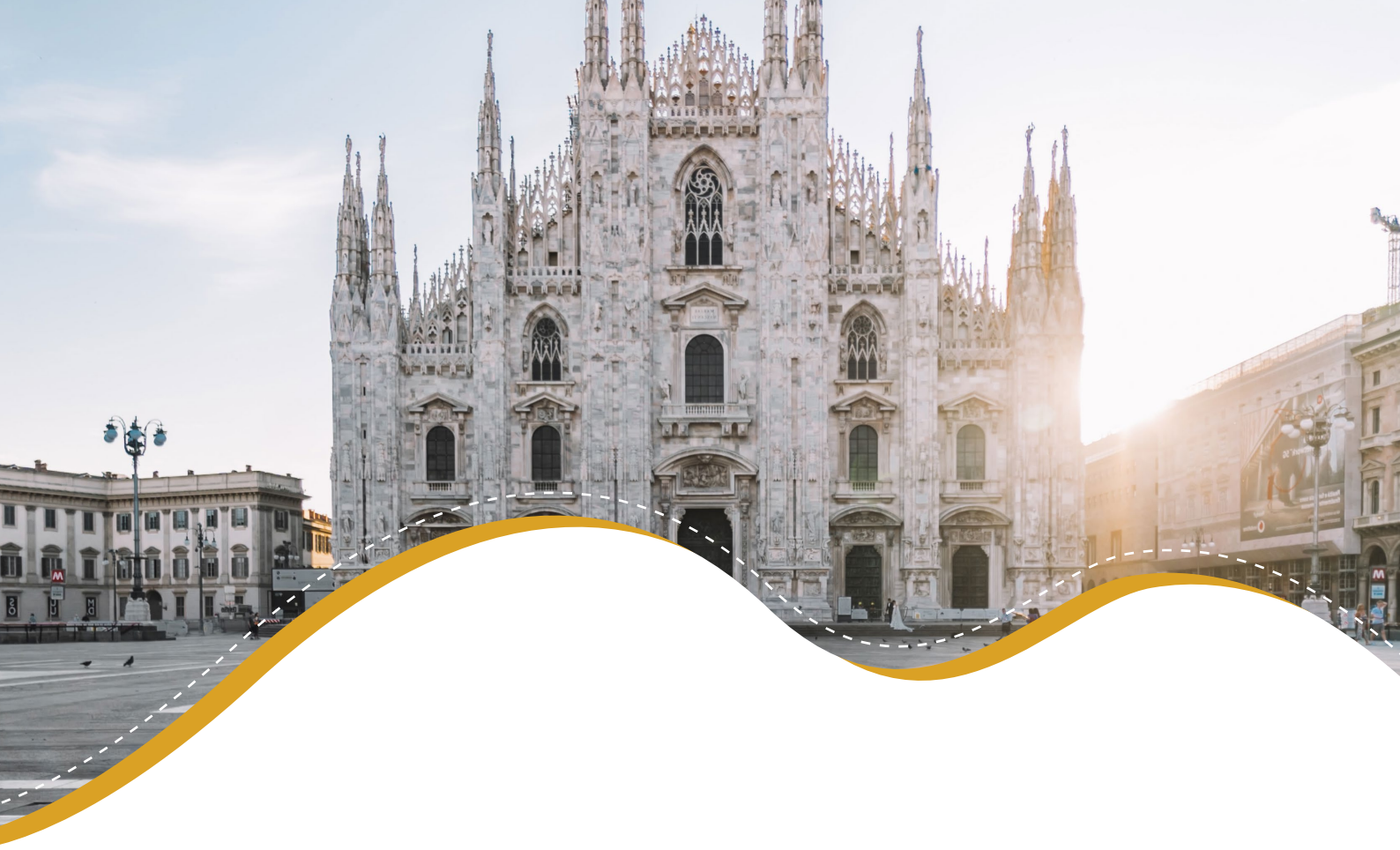

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