

Feed 2018

6th International Feed Conference:
Present and Future Challenges

25 - 26 October 2018
Bergen - Norway



Programme & Abstracts

Foreword

Welcome to Bergen and Feed 2018!

Feed 2018 is the sixth in a series of conferences held biannually, and was initiated by the European Union's leading reference laboratories and research institutions in animal feed. The focus of the feed conference is development of analytical methods and research to support risk assessment and legislation in a fast developing industry sector. The conference provides a meeting place for scientists, industry representatives, NGOs and public organizations from across Europe to debate current and future challenges for feed.

The world's growing supply of food has been successful in fueling the exceptional growth of the human population over the last century. However, the continued population growth presents challenges for future food production. Land use for crops and farm animals may soon reach its carrying capacity. Increased food production from the ocean may reduce some of the pressure on agriculture to improve food security.

Hosting the conference in Norway gives a unique opportunity to focus on aquaculture in addition to agricultural feed production. The aquaculture sector is a fast growing food-producing sector with a rapid implementation of novel feed ingredients that requires new risk assessment and development of legislation. For example, recent EU legislation on processed animal proteins from insects has been implemented first for aquacultured species. Novel feed ingredients and better use of traditional feed resources through a transition to a more circular bioeconomy are vital to achieve feed security and increased production of animal protein. However, upcycling of former food products and the use of feed ingredients not naturally encountered by a farmed animal poses both opportunities and threats in the feed and food production chain. Such hazards must be met by further developing analytical approaches and studying emerging risks in order to ensure feed and food safety. Moreover, dialogue is a key element to meet these challenges and this conference aims to bring together participants from different sectors, including the animal feed industry, and to share recent scientific advances in the feed area.

Bergen is a city with a long history and tradition in seafood trade with Europe and is often described as a "city with a small town charm and atmosphere". We hope you will enjoy your time in Bergen and this conference!

On behalf of the organizing committee

Robin Ørnstrud

Organizing Committee

Feed Safety Research Group
Institute of Marine Research
Bergen, Norway



Robin Ørnstrud (chair)

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MarcBerntssen
Kai Lie
Anne-Katrine Lundebye
Sylvain Merel

Josef Rasinger*
Veronika Sele
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Liv Søfteland
Paul Whatmore*

* absent on the picture

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Marc Berntssen	Senior scientist at the Institute of Marine Research
Simon Davies	Professor at Harper Adams University
Jacob de Jong	Programme leader feed, Wageningen University and Research centre
Ron Hoogenboom	Programme leader food contaminants, Wageningen University and Research centre
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Han Lujia	Professor at China Agricultural University
Daniela Marchis	Scientist of the National Reference Centre for Animal Feed Control, University of Torino
Mari Moren	Research director at Nofima, nutrition and feed technology
Robin Ørnsrud	Head of the feed safety group at the Institute of Marine Research
Luciano Pinotti	Associate professor at the University of Milan
Jens Sloth	Head of research group for nano-bio science at Denmark Technical University
Irmengard Strnad	Head of Institute of Animal Nutrition and Feed, Austrian Agency for Health and Food Safety
Leo van Raamsdonk	Senior scientist at Wageningen University and Research centre
Philippe Vermeulen	Senior scientist at Walloon Agricultural Research Centre
Ursula Vincent	Scientific officer at the European Commission Joint Research Centre
Christoph von Holst	Scientific officer at the European Commission Joint Research Centre, operating manager of the European Union Reference Laboratory for feed additives

Keynote Speakers

Session 1: New feed materials and feed security

Dag Aksnes



Dr. Dag Aksnes is a professor at the Department of Biological Sciences, University of Bergen, Norway. He recently co-chaired a working group of the SAPEA (Science Advice for Policy by European Academies), which produced the evidence review report “Food from the ocean”. The report addressed the question of how the oceans can help satisfying the global demand for food.

Session 2: New analytical approaches in feed traceability and safety

Carsten Fauhl-Hassek



Dr. Carsten Fauhl-Hassek is the Head of the unit Product Identity, Supply Chains and Traceability at the Federal Institute for Risk Assessment (BfR), Berlin, Germany. His research is focused on (i) the development, validation and assessment of methods for the analysis of wine, spirits and fruit juices, (ii) the use of stable isotope and fingerprinting based techniques in the determination of food and feed authenticity and (iii) the application and further development of statistical tools for data intensive analyses.

Keynote Speakers

Session 3: Feed safety - Contaminants and additives

Daniela Battaglia



Daniela Battaglia is an officer at the Animal Production and Health Division of the Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy. She organised the Joint FAO/WHO Expert Meeting on Hazards Associated with Animal Feed, in 2015. The meeting addressed chemical, microbiological and physical hazards in feed from the perspective of human and animal health. She will provide an updated overview of the current state of knowledge on hazards associated with feed.

Session 4: Feed of the future – The industry perspective

Alexander Döring



Alexander Döring graduated as an Agricultural Economist at the University of Bonn (1991). He is Secretary General of the European Feed Manufacturers' Federation (FEFAC) for over 20 years, being appointed in 1994. In his working experience as FEFAC Secretary General, he has been a regular participant to DG SANTE & DG AGRI Advisory & Civil Dialogue Groups, EFSA stakeholder platforms, FVO seminars & training workshops on Feed Hygiene, TAIEX workshops on Feed Safety management as well as meetings of international institutions such as Codex Alimentarius, FAO, OIE and IFIF.

Programme

Thursday 25th of October 2018

08:30 – 09:30 Registration and coffee
Poster set up

09:30 – 09:40 **Opening of the conference Feed 2018.**
Dr. Gro-Ingunn Hemre, research director at the Institute of Marine Research (IMR), deputy leader of the Norwegian Scientific Committee for Food and Environment

Session 1: New feed materials and feed security

09:45 – 10:15 **Keynote lecture:** Options for obtaining more food from the ocean.
Dag Aksnes

10:15 – 10:35 Heavy metal and mycotoxin excretion/accumulation in insects for feed and food (O1).
Nathan Meijer

10:35 – 10:55 Species-specific discrimination of insect protein in aquafeed using proteomic tools (O2).
Ikram Belghit

10:55 – 11:10 **Coffee break and poster viewing**

11:10 – 11:30 Effects of bakery/confectionary former food products as cereal substitute on growth performance and gut microbiota in post-weaning piglets (O3).
Luciano Pinotti

11:30 – 11:50 Mineral nutrition in Gilthead seabream (*Sparus aurata*) fed diets high in alternative ingredients (O4).
David Domínguez

Session 2: New analytical approaches in feed traceability and safety

- 11:50 – 12:20 **Keynote lecture:** Analytical challenges for ensuring feed integrity.
Carsten Fauhl-Hassek
- 12:20 – 12:40 The concept of “Technical zero” to be used in the PCR analyses for the detection of processed animal proteins in feedingstuffs (O5).
Olivier Fumière
- 12:40 – 13:00 Quantitative Immunoaffinity-Based Mass Spectrometry for the Species and Tissue Differentiation of Processed Animal Proteins and Blood Products in Feed (O6).
Oliver Poetz
- 13:00 – 14:00 **Lunch at Hotel Terminus and poster viewing**
- 14:00 – 14:20 UHPLC-MS/MS method for sensitive, specific and simultaneous detection of bovine blood meal, blood products and milk products in compound feed (O7).
Marie-Caroline Lecrenier
- 14:20 – 14:40 Candidate standard method for the determination of carotenoids in animal feedingstuffs: results of the collaborative study (O8).
Ursula Vincent
- 14:40 – 15:00 Fast on-site screening of oils and fats by spectroscopics: a one-class model approach (O9).
Yannick Weesepeel
- 15:00 – 15:20 New approaches for the authentication of feed – Study on the differentiation of the geographical origin of grain maize by spectroscopic methods (O10).
Elisabeth Achten
- 15:20 – 15:45 **Coffee break and poster viewing**

Session 3 Feed safety - Contaminants and additives

- 15:45 – 16:15 **Keynote lecture:** Hazards associated with feed; a worldwide overview.
Daniela Battaglia
- 16:15 – 16:35 Toxicity prediction for ethoxyquin and its transformation products using computational in silico tools (O11).
Josef Rasinger
- 16:35 – 16:55 Safe limits of selenomethionine and selenite supplementation to plant-based Atlantic salmon feeds (O12).
Marc Berntssen
- 16:55 – 17:00 **Information from the organizing committee on guided tour of the city of Bergen and the conference dinner**
- 17:00 – 18:00 **Coffee break and poster viewing**
- 18:30 – 19:00 **Guided tour of the city of Bergen**
- 19:00 **Meeting at Fløibanen (Vetrlidsallmenningen 21) for conference dinner**

Friday 26th of October 2018

Session 3 Feed safety - Contaminants and additives

- 09:05 – 09:25 Kinetics and effects of deoxynivalenol and ochratoxin A in dietary exposed Atlantic salmon (*Salmo salar*) (O13).
Aksel Bernhoff
- 09:25 – 09:45 Chronic Wasting Disease: a new hazard in feed (O14).
LWD van Raamsdonk
- 09:45 – 10:10 **Coffee break and poster viewing**

Session 4: Feed of the future – The industry perspective

- 10:10 – 10:40 Keynote lecture: FEFAC vision 2030 & key working priorities.
Alexander Döring
- 10:40 – 11:00 Growth, skin and gut health effects of variable essential amino acid, micromineral and vitamin supplementation in low fish meal diets on Atlantic salmon smoltification and post transfer performance: a full factorial design (O15).
Katerina Kousolaki
- 11:05 – 11:25 Natural solutions contributing to reduce antibiotic usage in animal feed (O16).
Angela Atkinson
- 11:30 – 11:50 Alternatives to water-plasticization in the fish feed extrusion process – reduced drying costs and improved physical pellet quality (O17).
Tor Andreas Samuelsen
- 11:55 – 12:15 Effect of Protease and Beta-Glucanase on Rheological and Quality Characteristics of Compacted *Candida utilis* Pellets (O18).
Dejan Miladinovic
- 12:20 – 12:40 **Poster prize**
Information about Feed2020
Closing ceremony
- 12:40 – 13:00 **Poster removal**
- 13:00 – 14:00 **Lunch at Hotel Terminus**

Abstracts

O1. Heavy metal and mycotoxin excretion/accumulation in *Hermetia illucens*

van der Fels-Klerx H.J., Meijer N.

RIKILT Wageningen University & Research, Wageningen, The Netherlands

Before insects can be used on the large scale as ingredient in European animal feed production, the safety of this novel feed ingredient should be investigated and ensured. To date, data on insect safety is collected by means of surveys and controlled experiments. Recently, several experiments investigating the potential accumulation/excretion of several chemical contaminants from the substrate by *Hermetia illucens* (black soldier fly, BSF) were performed. One study focused on heavy metals, and covered cadmium, lead, and arsenic. Another study covered the mycotoxins aflatoxin B1, deoxynivalenol (DON), ochratoxin A and zearalenone, as well as the mixture of these four mycotoxins.

Substrate that was based on compound feed formulation for broilers (used as feed for insects) was spiked with the particular contaminant at various concentrations, relative to the European Commission maximum limits or guidance levels. Next, BSF larvae were reared on the spiked substrate. After harvest, insect larvae and the residual material were chemically analysed for the presence of the contaminants and, in case of mycotoxins, also for well-known mycotoxin metabolites.

From the heavy metal study, bio-accumulation was seen for lead and cadmium in the BSF larvae. Results from the mycotoxin study showed that BSF larvae excreted or metabolized the four mycotoxins present in their substrate.

More general, from these two studies, it can be concluded that the accumulation/excretion rate of chemical contaminants by BSF depends on the particular contaminant. Hence, more of such studies are needed to investigate other potential chemical accumulation/excretion by BSF from the substrate.

Keywords: Insect, ingredient, safety, chemical contaminant

O2. Species specific discrimination of insect protein in aquafeed using proteomic tools

Belghit I¹, Lock E.J.¹, Fumiere O.², Lecrenier M.C.², Dieu M.³, Palmblad M.⁴,
Berntssen M.¹, Rasinger J.D.¹

¹Institute of Marine Research, Bergen, Norway

*²Département Qualité des Productions Agricoles, Centre Wallon de Recherches
Agronomiques (CRA-W) Gembloux, Belgium*

³University of Namur, Namur, Belgium

⁴Leids Universitair Medisch Centrum, Leiden, the Netherlands

Aquaculture is amongst the most efficient ways to produce animal protein for human consumption, and this sector is expected to continue to grow worldwide. Inclusion of novel protein sources, like insect meal, may help to mitigate the expected scarcities of feed resources. Indeed, insect ingredients hold a great potential as a source of nutrients for different fish species. In addition, insect production on organic side-streams can valorize many types of organic material by producing protein for aquafeed. However, considering as animal protein, insect should comply with legal constraints and guarantee the safe use in fish feed ingredients. Furthermore, there is a need for detection of the insect ingredients identity used in aquafeed. In the current study, we used a proteomics tools, for the detection and differentiation between eighteen different insect meal samples, from the species *Hermetia illucens*, *Tenebrio molitor*, *Alphitobius diaperinus* and *Acheta domesticus*, belonging to the Arthropoda, Coleoptera and the Orthoptera phylum. The gel-free shotgun proteomics approaches in combination with direct spectral comparison were able to differentiate specifically the insect meal samples, according to the taxonomic classification of the insect species. Thus, this biological fingerprinting methodology is a useful tool for species specific discrimination of insect's protein.

Keywords: Insect meal, Aquafeed, Shotgun proteomics

O3. Effects of bakery/confectionary former food products as cereal substitute on growth performance and gut microbiota in post-weaning piglets

Tretola M., Ottoboni M., Luciano A., Rossi L., Baldi A., Pinotti L.

*Department of Health, Animal Science and Food Safety,
Università degli Studi di Milano, Milano, Italy*

Former Foodstuffs (FFPs) are products that have lost their commercial value on the human consumption market, due to for example production errors. However, their nutritional value for animal feed purposes is not at all affected. Consequently, biscuits, bread, chocolate bars, pasta, savoury snacks and sweets, high in energy content in the form of sugar, starch, oil or fat can be considered an appealing alternative feed ingredient. Although FFPs composition, may vary to a large extent, they have been indicated as energy sources mainly. Accordingly, in this study, conventional cereal grains have been partially replaced by FFPs in post-weaning piglet's diets in order to investigate the effects of these alternative feed ingredients on growth performance and gut microbiota. The diets were iso-energetic (16 MJ/kg DM) and iso-nitrogenous (20.5% DM), and contained all essential amino acids in the recommended amounts. After an adaptation period (7d), post-weaning piglets (n=12, 28d old) were housed for 12d in individual pen and assigned to two experimental groups: CRT, receiving a standard diet and FFP, receiving a diet in which 30% of conventional cereals (wheat, barley, corn) were substituted by 30% FFPs. Both diets were in grounded forms and piglets had ad libitum access to the feed and fresh water throughout the whole trial. Individual feed intake was recorded daily, piglet's bodyweight (BW, kg) was recorded on d1, 5, 9 and 12 of the experiment. Average daily gain (ADG kg/day), average daily feed intake (ADFI kg/day) and Feed Conversion Ratio (FCR kg/kg) have been calculated. Raw data means were analyzed by IBM SPSS Statistics version 25 software (SPSS Inc.). In order to characterize gut microbiota composition, bacterial DNA has been extracted from stool samples, and the 16s rRNA gene has been sequenced by Next Generation Sequencing approach. At the end of the experiment no differences in BW, ADG, ADFI, have been observed between groups. Conversely, piglets on the FFP diet showed a better FCR ($P<0.05$). The gut microbiota did not show differences in microbial taxa composition, while further investigations are necessary to clarify the effects of FFPs on gut bacterial abundance and biodiversity.

Keywords: former food, growth, microbiota, weaning pig

04. Mineral nutrition in Gilthead seabream (*Sparus aurata*) fed diets high in alternative ingredients

Domínguez D.¹, Sehnine Z.¹, Sarmiento P.¹, Fontanillas R.², Prabhu P.A.J.³, Izquierdo M.S.¹

¹*Grupo de Investigación en Acuicultura (IU-ECOQUA), University of Las Palmas de Gran Canaria, Telde, Gran Canaria, Spain*

²*Skretting Aquaculture Research Centre AS, Stavanger, Norway*

³*Institute of Marine Research, Bergen, Norway*

Aquafeeds for marine species cultured in the Mediterranean Sea is still highly dependent on fish derived ingredients due to their high content in essential fatty acids. However, these are non-renewable and increasingly scarce ingredients, which in turn, suffer great market changes. The industry is increasing the use of alternative ingredients, namely those of vegetable origin, however the nutritional profile of these ingredients differs with those of marine origin. Furthermore, the requirements for the major aquaculture species produced in the EU for most of the essential amino acids and fatty acids have been currently established. However references for mineral requirements for Gilthead seabream are scarce. This lack of information forces feed producers to base the micronutrient formulation of their feeds on the available information of other species. A widely used tactic in previous years was to add minerals “in excess”, which increases production costs and can lead to mineral imbalance. Furthermore, if we take into consideration that the price of the feed stands for the highest production cost it is clear the importance to determine requirements that will enable the reduction of production costs.

The present study might contribute to understand the requirements of the major microminerals for seabream by means of a series of trials, reducing considerably the time and resources necessary to perform this type of trial. Several trials were independently conducted on seabream fingerlings using a plant-based diet with low levels of marine ingredients. Feeds were supplemented with one of the minerals to study, such as Se, supplied as sodium selenite; Mn as MnSO₄; or Cu as CuSO₄. Growth and productive parameters were monitored and samples for biochemical, mineral, histological, gene expression and X-ray studies were taken at the end of the trial.

During the experimental period seabream almost tripled their body weight in all trials. Results varied depending on the mineral supplemented. Some affected overall performance and growth, others had effects on tissue morphology and mineral retention, or on oxidative defence. Overall the results pointed out the importance of determining optimum dietary levels of micro-minerals in plant based diets for gilthead seabream, especially to reduce oxidative risk.

Keywords: Mineral, Aquaculture, Seabream requirements

05. The concept of “Technical zero” to be used in the PCR analyses for the detection of processed animal proteins in feedingstuffs

Fumiere O., Marien A., Ninane V., Planchon V., Lecrenier M.C.,
Veys P., Baeten V., Berben G.

*CRA-W, Walloon Agricultural Research Centre, European Union Reference Laboratory
for Animal Proteins in feedingstuffs, Gembloux, Belgium*

Two official methods are currently used for the detection of processed animal proteins (PAPs): light microscopy and PCR. In 2013, PCR being able to determine the species origin of PAPs detected by light microscopy was included in the legislation to check for absence of ruminant PAPs when re-introducing non ruminant PAPs in aquafeed. Nevertheless, PCR is unable to determine whether the origin of an amplification signal is due to DNA coming from PAPs or from any authorized feed material like dairy products. This analytical limitation is manageable for aquafeed where use of authorized ruminant material is scarce. If the lifting of the feed ban was enlarged to pig and poultry feed then the occurrence of positive PCR results wrongly assimilated to the presence of unauthorized material could drastically increase. On request of DG Sante, the EURL-AP worked on the concept of “Technical zero” applicable to PCR results. It can be assimilated to a threshold below which no action should be taken because of the high probability that the PCR signal results from the presence of authorized constituents of animal origin.

The ruminant PCR method described in the EURL-AP SOP requires the use of European reference material consisting of plasmids at certified copy numbers to calibrate PCR platforms. The so-built calibration curve also enables to determine if a “Technical zero” corresponding to a defined copy number is or not exceeded by a sample.

Based on theoretical assumptions about the distribution of copy numbers per mass unit of the ruminant target used in the PCR within the various possible PAP samples available on the European market, tables were provided to convert the copy numbers of several possible levels of the “Technical zero” in mass fractions with a probability of occurrence associated to it. These data were used by EFSA to reiterate their quantitative risk assessment, to evaluate what is the risk that would be associated to the introduction of a “Technical zero” and thereby allow the risk managers to integrate or not this concept in the legislation. The EFSA opinion relating to this is now publicly available (<http://www.efsa.europa.eu/en/efsajournal/pub/5314>).

Keywords: PAPs, PCR, Feed safety, Technical zero

06. Quantitative immunoaffinity-based mass spectrometry for the species and tissue differentiation of processed animal proteins and blood products in feed

Steinhilber A.E.¹, Schmidt F.F.¹, Planatscher H.², Niedzwiecka A.³, Zagon J.³, Braeuning A.³, Lampen A.³, Joos T.O.¹, Poetz O.^{1,2}

¹*Natural and Medical Sciences Institute, University of Tuebingen, Germany*

²*Signatope GmbH; Germany*

³*Federal Institute for Risk Assessment, Germany*

The ban of processed animal proteins (PAPs) in feed for farmed animals introduced in 2001 was one of the main EU measures to control the bovine spongiform encephalopathy crisis. To enforce the ban, light microscopy was introduced as official method for the detection of illegal PAPs in feed. Polymerase chain reaction was later adopted as second official method to improve the species-specific detection of ruminant PAPs. Nevertheless, there is still an analytical gap for PAP detection. Since the reintroduction of non-ruminant PAPs in aquaculture feeding and with the ban for an intra-species feeding, there is a need for species differentiation methods. Legal protein additives call for methods capable of differentiating also the tissue source. Furthermore, discussions about quantitative tests came up to replace the zero-tolerance-concept by thresholds. So far, no analytical method was able to parallelly detect and differentiate PAPs down to a level of 0.1% (w/w) in common feed matrices.

To address this issue, we developed and validated two multiplex mass spectrometry-based immunoassays in combination with a new sample preparation procedure termed heterogeneous phase digestion. The workflow comprises a direct tryptic digestion of PAPs in suspension, an immunoaffinity enrichment of released peptides, and an LC-MS/MS-based analysis for peptide quantification using isotope-labeled standard peptides. One multiplex MS-based immunoassay uses a group-specific antibody that recognizes homologous peptides from the 9 livestock species' alpha-2-macroglobulin: cattle, sheep/goat, pig, horse, turkey, goose, chicken and duck. Another multiplex MS-based immunoassay uses 7 peptide-specific antibodies that recognize ruminant-specific peptides from the tissue types meat, bone, cartilage, blood and milk. Analytical parameters were validated and the assay performance was demonstrated by the analysis of proficiency test samples which were initially part of a ring trial organized by the European Reference Laboratory for Animal Protein (Gembloux, Belgium). In these samples species and tissue source were clearly detected and unambiguously identified down to 0.1% w/w.

For the very first time, a mass spectrometry-based method is capable of differentiating and quantifying illegal PAPs and blood products in feed over a concentration range of 4 orders of magnitude with a detection limit of 0.05%.

Keywords: Feed fraud, processed animal protein, Immuno-LC-MS

07. UHPLC-MS/MS method for sensitive, specific and simultaneous detection of bovine blood meal, blood products and milk products in compound feed

Lecrenier M.C.¹, Dieu M.^{2,3}, Berben G.¹, Fumiere O.¹,
Marien A.¹, Veys P.¹, Gillard N.⁴, Baeten V.¹

¹CRA-W, Walloon Agricultural Research Centre, Gembloux, Belgium

²Laboratory of Cellular Biochemistry and Biology (URBC) – NARILIS, University of
Namur, Namur, Belgium

³MaSUN, Mass Spectrometry Facility, University of Namur, Namur, Belgium

⁴CER Groupe, Marloie, Belgium

Feed availability is one of the biggest challenges for the future. Solutions will be found by increasing the production efficiency and finding new sources without jeopardizing feed quality and safety.

Animal by-products are an interesting source of feed materials. These materials are rich in proteins of high nutritional value and have an economic interest since their non-use results to a logical loss of gains. However, since the mad cow disease crisis, their use has been strictly regulated. In 2013, non-ruminant processed animal proteins (PAPs) were reauthorised in aquafeed but ruminant PAPs remain forbidden. Official controls are based on a combination of light microscopy and PCR. But sometimes these methods are unable to distinguish some feed materials.

The objective of this work was to develop a sensitive method using ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) for the specific detection of bovine blood-derived products and milk powder in feed. This method has the advantage to be species and tissue specific. Peptide biomarkers identified in previous studies were used. The sample preparation and the analytical method were designed to provide a fast, simple and powerful method suitable for routine. Proteins were extracted in a buffer containing 200 mM TRIS-HCl pH 9.2, 2 M urea followed by trypsin digestion and purification with tC18 SPE (Waters). Analyses were performed by liquid chromatography (Acquity UHPLC system, Waters) coupled with a triple quadrupole mass spectrometer (Xevo TQS, Waters). Labelled peptides were used as internal standard in order to compare the results independently of the retention time variation due to matrix effect.

Various commercial aquafeed batches artificially adulterated at levels of 0.1 % to 1 % (w/w) with bovine blood meal, bovine blood products or milk powder were analysed in order to assess the influence of matrix and to evaluate sensitivity and specificity of the method.

The method was able to detect all adulterants at 0.1 % level in all matrices. This makes the method suitable for application in feed control and offers an innovative and complementary solution for the simultaneously identification of authorised and unauthorised animal by-products such as PAPs.

Keywords: PAPs, Mass spectrometry, Aquafeed, Blood, Milk

08. Candidate standard method for the determination of carotenoids in animal feedingstuffs: Results of the collaborative study

Vincent U., Serano F., von Holst C.

European Commission, Joint Research Centre, Belgium

Carotenoids are feed additives, which, when fed to animals, add colour to food of animal origin and for which legal limits have been established (Regulation (EC) No1831/2003). Improved methods of analysis of carotenoids are therefore required to carry out official controls to ensure the verification of compliance with feed and food law, labelling claims, animal health and animal welfare rules (Regulation (EU) 2017/625). Carotenoids present a variety of stereoisomers and in particular geometrical isomers (E/Z); these are inter-convertible in solution and present different physical properties. In particular their absorption coefficient and the wavelength of maximum light absorbance are different.

A multi-analyte HPLC method that enables the reliable quantification of authorised carotenoids in feed has been successfully validated in-house. This new method has, among others, the important advantage that it is applicable to all the different sources of carotenoids (chemical and natural additives), which until now required the use of product specific methods. The new method involves a straightforward sample preparation, avoids the use of chlorinated solvents, is based on reverse phase HPLC and provides reliable quantification thanks to the isosbestic concept which makes it fit for the purpose of official control of carotenoids in fish and poultry feeds at authorised levels. The method has been selected as candidate method for standardisation under the third mandate of the European Commission to the European standardisation body CEN. In this frame, the Joint Research Centre, appointed by CEN, organised a collaborative study for its validation.

The collaborative study has been carried out on 9 different feed materials containing target analytes at relevant content levels.

The obtained repeatability and reproducibility values ranged from 2% to 16% and from 7% to 30%, respectively, depending on the analyte and on the feed.

Furthermore the analytical recovery calculated from the results reported from the laboratories was ranging from 70 to 108%.

Those values of precision and trueness demonstrated the fitness for purpose of the method that was thus considered as validated and transferable to the control laboratories within the frame of official control. The method is currently undergoing the formal European standard drafting process.

Keywords: Carotenoids, Feed additives, Feed, Official control

09. Fast on-site screening of oils and fats by spectroscopics: a one-class model approach

Weesepoel Y.¹, de Jong J.¹, Khodabux D.¹, Zuidema T.¹, Alewijn M.¹, Klomp A.², Blomsma C.³

¹RIKILT – Wageningen University and Research, Wageningen, The Netherlands

²MVO – The Netherlands Oils and Fats Industry, Zoetermeer, The Netherlands

³Vernof – The association of Dutch Producers of Edible Oils and Fats, Zoetermeer, The Netherlands

Food and feed fraud cases often come as a thunderbolt in clear skies. The use of specific analysis methods is therefore not always sensible, because they are often aimed at one particular adulterant. Certainly in the case of oils and fats, there are large product flows and a high number of potential low-grade oils, fats and derived products such as fatty acids and (deo-) distillates that can be (mis)used in the production of compound feeds. Therefore, there is a need for a low-cost, rapid screening method that can determine the authenticity of an oil, fat or derived product independently of the admixed component.

This project aims to address this problem by utilizing low-cost and miniaturized spectroscopic equipment in combination with multivariate statistics in order to screen an oil or fat sample on-site within seconds. In the first phase of the project, a large number of pilot applications is explored by using a conceptual modular scanner, covering the spectrum of ultraviolet, visible and near infrared wavelengths. Samples for each pilot application were supplied by several producers, traders and resellers of the oil or fat products of interest, over a time-span of 12 months. The feasibility of the pilots was assessed by using so-called 'one-class model' multivariate statistics and compared to the traditional targeted statistical methods like partial-least squares regression. One-class statistics can be used for authentication independently of the adulterant applied and may therefore be a powerful tool for an early warning system. A disadvantage of the one-class approach is the increased risk of false positive and false negatives results. A solution may lie in a combinatorial approach of both one-class and targeted chemometrics.

This research was financially supported by TKI Agri & Food, Ministry of Economic Affairs The Netherlands under project number AF-16091.

Keywords: integrity, on-site miniaturised spectroscopics, chemometrics

O10. New approaches for the authentication of feed – Study on the differentiation of the geographical origin of grain maize by spectroscopic methods

Achten E.¹, Schütz D.², Fischer M.², Fauhl-Hassek C.¹, Horn B.¹

¹*German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Berlin, Germany*

²*University of Hamburg, Hamburg School of Food Science, Institute of Food Chemistry, Hamburg, Germany*

To ensure quality of food products and safety for the producing animals, authentication of feed is of up-coming importance and reliable analytical strategies need to be developed. Globalization and increasing complex supply chains on the one hand and the growing awareness of the consumer towards fraudulent practices in the supply chain on the other hand are major drivers. In addition to document-based traceability, analytical authentication can be performed on the product itself to confirm the stated declaration on the product label, such as the geographical origin, established composition or production process.

Classical targeted analytical methods measure single parameters in the product, for example typical feed quality parameters such as moisture, crude protein, fat and ash content. However, this approach is typically not sufficient to verify the authenticity of a product. Non-targeted methods become increasingly important to investigate even complex authenticity issues. Spectroscopic techniques in combination with multivariate data analysis can be used to classify samples according to specific features such as geographical origin or to detect deviations from a normal composition.

The presented study focusses on the differentiation of the geographical origin of grain maize. This major feed crop is well suited as demonstrator matrix as it is grown and traded worldwide. Fourier transform infrared and proton nuclear magnetic resonance spectroscopy were applied to a set of samples that includes grain maize samples from three countries on different continents. The collected data were used to build classification models to differentiate the geographical origin of grain maize. In order to improve the performance of the selected models sample preparation and data preprocessing were optimized and will be discussed. The results of this feasibility study might be used as starting point for further developments into routine checks at import points for example.

Keywords: maize authenticity, geographical origin, spectroscopy, chemometrics

O11. Toxicity prediction for ethoxyquin and its transformation products using computational in silico tools

Rasinger J.D.¹, Merel S.¹, Berntssen M.¹, Frenzel F.²,
Lampen A.², Braeuning A.², Ørnsrud R.¹

¹*Institute of Marine Research (IMR), Bergen, Norway*

²*German Federal Institute for Risk Assessment (BfR), Department of Food Safety, Berlin, Germany*

Ethoxyquin (EQ; 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) has been used as an antioxidant in feed for pets, livestock and aquaculture. Concerns regarding the safety of EQ and its transformation products (TP) led to a suspension of the authorisation of EQ as a feed additive for all animal species and categories. A re-consideration of this assessment by the European Commission (EC) is possible if supplementary data concerning the safety of use and the efficacy of this additive are brought forward and if current data gaps in its safety assessment are filled. Using traveling-wave ion mobility spectrometry (TWIMS) coupled to quadrupole time-of-flight mass spectrometry (QTOFMS) 27 EQ TP were identified in oxidation experiments; 25 of these were detected in fish feed and 24 in fish from EQ exposure experiments. Based on these data, computational analyses were performed to in silico predict toxic effects of EQ and its TP. The computer models employed included VEGA [1], T.E.S.T [2], LAZAR [3], the OECD QSAR toolbox [4] and Derek Nexus [5]. The predictions obtained from these tools were combined employing different recently published strategies to facilitate a prioritization of EQ TP according to their predicted toxicities in order to highlight the compounds of most concern, which need to be analysed further using in vitro or in vivo models of toxicity. It is expected that the present work will aid current efforts to fill data gaps in the hazard characterization of EQ and its TP.

[1] <https://www.vegahub.eu/>

[2] <https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test>

[3] <http://lazar.in-silico.de/predict>

[4] <http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm>

[5] <https://www.lhasalimited.org/>

Keywords: feed safety, (Q)SAR, data mining

O12. Safe limits of selenomethionine and selenite supplementation to plant-based Atlantic salmon feeds

Berntssen M.H.G.¹, Betancor M.², Caballero M.J.³, Hillestad M.⁴, Rasinger J.¹, Hamre K.¹, Sele V.¹, Amlund H.¹, Ørnstrud R.¹

¹*Institute of Marine Research (IMR), Bergen, Norway*

²*Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, United Kingdom*

³*Department of Morphology, Veterinary School, University of Las Palmas de Gran Canaria, Arucas, Las Palmas, Spain*

⁴*BioMar AS, Trondheim, Norway*

The use of plant-based feeds warrants the supplementation with selenium (Se) to cover the requirement for Atlantic salmon. Se is a trace element with a narrow range between requirement and toxicity. Information on safe upper limit for Atlantic salmon feed supplementation is lacking. Salmon (147 g) were fed a low natural background organic Se diet (0.45 mg Se kg⁻¹, wet weight (ww)) fortified with 5 graded levels of inorganic sodium selenite (0.45, 5.4, 11.0, 29.4, or 60.0 mg kg⁻¹ ww) or organic selenomethionine (SeMet) (0.45, 6.2, 16.2, 21, or 39 mg kg⁻¹ ww), in triplicate for 3 months. Excess Se supplementation was assessed by targeted biomarkers of Se toxicity pathways as well as general adverse effect parameters. Safe limits were set by model-fitting the effect data in a dose-response (lower bound) bench mark dose (BMDL) evaluation. Fish fed the two highest selenite levels showed mortality while fish fed SeMet had no mortality. Fish fed 5.4-11 mg selenite kg⁻¹ feed showed significantly increased liver oxidative stress, as seen from altered hepatic GSH and vitamin E levels, and liver damage as seen from increased plasma ALAT and liver histopathology such as degeneration and focal necrosis. Fish fed SeMet mainly showed liver pathology and kidney dysfunction as seen from altered plasma creatinine and total plasma proteins in fish fed ≥ 21 mg kg⁻¹. For selenite exposed fish, a safe feed limit was set at 1-2 mg kg⁻¹ ww feed (daily dose 0.01-0.02 mg kg BW⁻¹ day⁻¹), based on plasma ALAT increase, liver vitamin E depletion, and liver histopathology. For SeMet fed fish, the safe feed limit was higher than for selenite with a BMDL of 2.8 mg kg⁻¹ ww (dose 0.03 mg kg BW⁻¹ day⁻¹), based on liver histopathology and plasma creatinine. In conclusion, with regards to fish health, salmon seemed to tolerate the supplementation of selenite or SeMet to a level of total selenium of respectively 1-2 or 3 mg kg⁻¹ feed, respectively, in a high plant-based salmon feed with background levels of 0.45 mg Se kg⁻¹.

Keywords: Atlantic salmon, selenite, selenomethionine, toxicity

O13. Kinetics and effects of deoxynivalenol and ochratoxin A in dietary exposed Atlantic salmon (*Salmo salar*)

Bernhoft A.¹, Moldal T.¹, Høgåsen H.R.¹, Rosenlund G.²,
Berntssen M.H.G.³, Alexander J.⁴

¹Norwegian Veterinary Institute

²Skretting Aquaculture Research Centre AS

³Institute of Marine Research, Norway

⁴Norwegian Institute of Public Health

Post-smolt Atlantic salmon were fed with standard feed added one of five concentrations of either pure deoxynivalenol (DON; 0.5-6 mg/kg) or pure ochratoxin A (OTA; 0.2-2.4 mg/kg), or no added toxins for up to eight weeks. DON was distributed to various tissues including muscle, and the concentrations increased significantly from three to eight weeks of exposure. OTA was mainly found in liver and kidney, and the concentrations in liver decreased significantly from three to eight weeks. OTA was eliminated faster than DON from various tissues. The risk to human health from the consumption of salmon fed diets containing maximum recommended levels of these toxins was considered to be negligible.

Performance effects (feed intake and -efficiency, gain, length and condition factor), various clinical biochemical parameters, packed cell volume and vaccination response against *Aeromonas salmonicidae* were all inversely correlated with DON dose, whereas relative liver weight increased with DON dose. DON was also found to impair the epithelial barrier (decreased relative expression of markers for three tight junction proteins and increased relative expression of a marker for proliferating cell nuclear antigen) and modulate the cytokine signalling in the intestine compared when tested at highest concentration, compared with controls. In fish fed OTA, the effects were rather small.

For DON, a BMDL₂₀ of 0.3 mg/kg feed for reduced total protein in plasma, a BMDL₅ of 0.5 mg/kg feed for reduced condition factor, and a NOAEL of 1 mg/kg feed were derived. For OTA, a BMDL or NOAEL could not be derived (>2.4 mg/kg).

Keywords: deoxynivalenol, ochratoxin, Atlantic salmon, toxicokinetics, health

O14. Chronic Wasting Disease: a new hazard in feed?

Raamsdonk L.W.D. van

RIKILT Wageningen UR, Wageningen, The Netherlands

There is a severe set of European Union regulations for the use of animal by-products in feed. Several epidemics in the past, such as swine fever, foot and mouth disease and most notably Bovine Spongiform Encephalopathy (BSE), are at the root of these prohibitions. Among a range of different areas in processing and trade, the precise prohibitions, restrictions, exemptions and derogations for the use of animal by-products in feed cover 18 pages in Regulation (EU) 999/2001 Annex IV. BSE is a specific form of prion diseases, Transmissible Spongiform Encephalopathies (TSEs). Scrapie is known as the TSE version targeting sheep. A less known TSE, Chronic Wasting Disease (CWD), is known from Northern America among elk, moose, reindeer and deer species. It is valuable to survey possible hazards for feed in European cattle husbandry.

The first incidence of CWD in Europe was encountered in Norway in 2016. Since then, incidences were found in Sweden and Finland as well, summing up to more than 20 animals involved. The most frequently involved species is reindeer, while some incidences occurred in moose and deer. Strains of CWD prions can be found in faeces, urine, nasal secretions, blood, meat, saliva, milk, semen and antler velvet, which is a much larger range of vectors than found in BSE. Maternal transmission has been found. Plants edible for foraging ruminants are shown to be capable to take up possible prions in their physiologic systems, ready for congestion. The variety of transmission routes implies that CWD can be considered highly contagious, unprecedented among TSEs. The strains of CWD appear to be variable and new versions arise. Recently macaques, a type of apes considered a good model for human response in animal trials, appeared to be susceptible to CWD prions. Several *in vitro* and *in vivo* human model systems showed responses resembling sporadic Creutzfeldt-Jacob disease after infection with CWD prions. Also in Europe, the until now limited number of incidences are already proved to be caused by different strains.

Consequences for animal feed production and application could include:

- The current feed bans for animal by-products in European legislation might be modified in the view of this new hazard.
- The legal term Specified Risk Material might render useless in the view that CWD prions can be present in every animal tissue and organ.
- It is theoretically possible that new CWD strains come into existence which are infectious for cattle.
- In the desire of a circular economy, (mixed) by-products of the food production need to be used in feed production. Insects are the most relevant group of animals to be fed with these by-products. At the same time, insects are known to be capable of transferring prions.

Keywords: Transmissible Spongiform Encephalopathies (TSEs), Chronic Wasting Disease (CWD), circular economy

O15. Growth, skin and gut health effects of variable essential amino acid, micromineral and vitamin supplementation in low fish meal diets on Atlantic salmon smoltification and post transfer performance: a full factorial design

Kousoulaki K.¹, Ytteborg E.¹, Krasnov A.¹, Sweetman J.²

¹*NOFIMA*

²*Alltech Inc*

Responsible growth of fish farming requires simultaneous development of knowledge and technologies that secure fish performance, health and welfare in constantly changing conditions. Salmon farming mortality rates average 20%, and smoltification, sea water transfer, handling at vaccination and therapies, stocking densities and water quality are bottlenecks contributing to stress and limiting fish growth and survival. Along with improving farming practices it is important to balance diets that enable growth of robust fish that withstand and recover stress and disease. Essential nutrient requirements in fish have been defined mainly under optimal conditions with marine diets. Stress tolerance and skin ulcers at smolt transfer are likely to be linked to sub-optimal mineral and vitamin nutrition, whereas poor smoltification and transfer performance may be linked to the fish's poor essential amino acid and mineral status. As a small step to the general goal of developing safe sustainable salmon diets, we run a feeding trial applying a 23 full factorial design to investigate the combined effects on Atlantic salmon smoltification and post transfer performance of variable supplementation of essential amino acids (Lys, Met, Thr and Arg), microminerals (Zn, Fe and Se) and vitamins (Vit E, C and astaxanthin) in low fishmeal diets. We chose nutrient levels in our design based on benchmarking chemical analyses of commercial feeds and previous trial findings. Fish performance, nutrient digestibility, skin and intestinal health were evaluated, the latter by means of qPCR, global transcriptomics and immunohistochemistry.

The results revealed the potential for significant improvement of Atlantic salmon parr-smolt performance by simultaneous increase of the dietary levels of the essential amino acids Met, Lys, Thr and Arg. Moreover, a negative effect of high supplementation levels of vitamin C, E and astaxanthin was demonstrated, in terms of survival and growth. High Zn, Fe and Se supplementation resulted in several positive effects, e.g. enabling performance improvement from high essential amino acid and ameliorating the negative effects of high dietary vitamin supplementation. Compensatory mechanisms in terms of protein and energy metabolism, immune modulation and skin repair systems were observed in relation to the variable fish performance responses in this study.

Keywords: minerals, vitamins, aminoacids, smoltification, salmon, aquaculture

O16. Natural solutions contributing to reduce antibiotic usage in animal feed

Atkinson A., Le Roux J.F.

Nuproxa Switzerland, Etoy, Switzerland

Today, customers are highly concerned by their food quality and its mode of production. There are requesting more sustainability in agriculture and livestock farming with also giving more attention to animal welfare and to animal feed's composition. However, top priority has been given to production of safe foods, which does not require quantities of drugs, for example by banning the use of antibiotics in the feed. Production of animal without antibiotic in specific production channels is now a reality in many countries. Nevertheless, anticoccidial are sometimes tolerated, even if they are classified as antibiotics. Some of those molecules are also known for their toxicity on some animals (Horses intoxicated with monensin e.g.) and need withdrawal periods to limit their residues in the meat.

In this context, numerous researches are done to find natural alternatives to the synthetic molecules in the feed (antibiotics, anticoccidials, e.g.). Among these solutions, plants-based products are serious candidates. Phytotherapy is namely used since millenary in human nutrition in many countries and even for animals. It exists namely a science, called zoopharmacognosy, studying the usage of plant by animals in order to improve their health. That's why, the use of plants in the feed of animals is very interesting. Thanks to the diversity of actives present in the herbs, the mode of action on the animal metabolism is larger and permits to prevent some disorders. A natural solution has been found to contribute to the gut flora management. Tannins and saponins in the plants interact with the components of the microorganism membranes and are able to perturbate their development or activity. The plants enable to keep the flora well balanced and contribute to limit pathogens' development. Besides direct interactions with microorganisms, components of the plants have shown effects to enhance immunity and to reduce impact of oxidative stress for the animals. Such natural solutions are really interesting to improve safety and sustainability of the feed with being cost-effective and thus to provide better quality food to customers.

Keywords: natural, herbs, phytogetic, anticoccidial, safety, feed

O17. Alternatives to water-plasticization in the fish feed extrusion process – reduced drying costs and improved physical pellet quality

Samuelsen T.A.¹, Ahmad R.², Oterhals Å¹,

¹*NOFIMA*

²*Cargill*

Increased use of plant proteins in fish feed formulations has become essential to ensure a sustainable growth of the aquaculture industry. Plant proteins are not a uniform group of ingredients and have different nutritional and technical properties. A combination of different plant proteins as soya protein concentrate, wheat gluten and sunflower are therefore used to replace fishmeal. Large scale manufacture of fish feed is extensively based on extrusion. The process involves use of high temperature achieved by steam injection and mechanical heat dissipation to transform the feed mix into a plasticized and flowable material that can be shaped through dies, cut into pellets, dried and added oil in a coating operation. Proteins exist in an amorphous state and undergo both glass and flow transition in this process. Normally a plasticizer is added to reduce these temperatures and improve transformation. Water is the main plasticizer used; however, any low molecular weight compound may give a plasticization effect.

Fishmeal contains high levels of water-soluble compounds that act as plasticizers in the extrusion process and improve physical pellet quality. The plasticizing effect of the amino acid proline has been documented in a soy protein concentrate model system using a closed-chamber capillary rheometer. The studies demonstrate that free amino acids and low molecular weight water-soluble peptides can replace moisture as plasticizer in the extrusion process. In industrial manufacturing practice any protein hydrolysate with a high degree of hydrolyzation may be a cost effective processing aid that will act as both nutrient and plasticizer in feed formulations. Due to the need for higher moisture level during extrusion processing of plant-based diets, especially diets high in soya protein concentrate, these require more energy for drying compared to fishmeal based diets. The use of protein based plasticizers to partly replace water open up the possibility for thermomechanical transformation in the extrusion process at reduced moisture level with a potential for significant reduction of the energy consumption in the drying step. The information can be used by the feed manufacturing industry to better understand how to impact and control the extrusion process and physical feed quality.

Keywords: Amino acids, extrusion, plasticizer, water-soluble peptides

O18. Effect of protease and beta-glucanase on rheological and quality characteristics of compacted *Candida utilis* pellets

Miladinovic D., Tong A.

Biovit-NMBU

Finding sustainable resources that will assure the food security is a challenging goal. The aquacultural development through the blue revolution enables the food security. Challenges for the aquaculture development due to raw materials are scarcity of resources and exploiting the agricultural land. Finding the alternative raw materials which do not influence negatively on the environment and that do not require large land-production area is necessary. Traditionally fishmeal and soybean-meal are important protein-sources for aquacultural diets due to their optimal amino-acidic composition. Utmost proteins included in the aquatic-feed are considered unsustainable or with negative health consequences when given over long feeding period. Proteins from single cell organisms may overcome unsustainable aquacultural development and have the health benefits for farmed fish. Sustainable feed manufacturing using such novel ingredients also requires a basic knowledge of its rheological and quality characteristics during production and post-production.

This study explains the reaction between the biomass derived from *Candida utilis* and enzymes protease and endo/exo-1.3-beta-glucanase. Evaluation of interaction between the enzymes and yeast biomass on physical pellet quality was done by analyzing the rheological characteristics, pellet hardness, underwater pellet swelling rate, moisture content, water activity and surface contact angle with water and oil. Ten treatments with three different enzymatic dosages (1-fold, 10-folds and 100-folds) were performed. The protease alone showed to influence changes in pellet quality when compared to endo/exo-1.3-beta-glucanase or mixture of protease and endo/exo-1.3-beta-glucanase. Mixture of endo/exo-1.3-beta-glucanase and protease had higher influences on decreasing the viscosity of the yeast-biomass during pelleting, as an indication of possible decrease of electrical energy consumption during pelleting. Protease and mixture of protease and endo/exo-1.3-beta-glucanase increased the oil absorption rate, which is a good indicator for better oil infusion during vacuum coating. Protease increased the pellet hardness. Endo/exo-1.3-beta-glucanase significantly decreased the pellet hardness. Enzymes decreased the water activity. Protease (100-folds) decreased pellet swelling when the pellets was submerged in water as an indication that protease may influence better water stability of feed pellets. Further studies of the enzymatic influence on the yeast-biomass included in the feed diet is necessary.

Keywords: candida-utilis, enzymes, pellet-quality, rheology, sustainability, feed

P1. Zinc in salmon meal: Up-concentration effect and implication for use in animal feeds

Prabhu A., Lock E.J., Waagbo R., Ørnsrud R.

Institute of Marine Research, Bergen, Norway

Salmon meal (SM), a rendered product from salmon by-products, is a valuable animal (except ruminants in the EU), fish (except salmonids) and pet food ingredient. The rendering process yields 20-23% of SM from the by-product biomass which might lead to varied degree of up-concentration in certain metals. In this perspective, we aimed to develop a mathematical model to predict the up-concentration of Zn in SM produced from salmon by-products.

Based on observed range in whole body Zn concentration of harvest size Atlantic salmon (4 kg, 30 to 60 mg Zn/kg), fillet yield (65 or 75%), and assumption that rest of all by-products are collectively used for SM, the up-concentration of Zn in the final SM product was predicted. Concentration of Zn in whole body, fillets, and that of moisture and lipid in the by-product biomass and SM product were taken into consideration in the model. The predicted estimates were compared with analyzed data from the Norwegian feed surveillance program.

The predicted Zn concentrations in SM ranged between 349 to 1012 mg/kg depending on the zinc status and fillet yield of salmon assumed in the model. The two analyzed Zn concentration data in SM from the feed surveillance program (2012-2018) were found to be 2300 mg/kg (in 2013) and 1400 mg/kg (in 2018) mg/kg.

Zinc concentration in SM, both predicted and analyzed varied considerably. The major factors influencing high variability in model predicted values are the Zn status and fillet yield assumed. One other factor important to be understood is the proportion of the different by-products used for SM rendering and their contribution to the total Zn concentration of SM. These warrant the need to study Zn concentrations in various salmon by-product fractions and possibly devise strategies to avoid exceedingly high Zn levels in SM. The maximal allowable limits for total Zn levels in dry animal feeds range between 150-200 mg/kg in the EU, depending on animal species. If unchecked, Zn could be a decisive factor in limiting the utilization of SM in animal feed and pet food industry, through restricting the inclusion of Zn additives in formulations containing SM.

Keywords: zinc, salmon meal, modelling, feed safety

P2. Seaweeds from Northwester Mediterranean Sea (Italy) as potential animal feed: trace metals and rare earths elements occurrence

Squadrone S.¹, Brizio P.¹, Stella C.¹, Nurra N.², Mussat-Sartor R.², Battuello M.², Pessani D.², Capra C.¹, Riva A.¹, Mantia M.¹, Abete M.C.¹

¹*Istitutoooprofilattico del Piemonte, Liguria e Valle d'Aosta*

²*University of Turin*

Seaweeds have been used as animal feed since a long time and are consumed as food in several cultures. Macroalgae are known to be a source of protein, fibre, polyunsaturated fat, and minerals. However, macroalgae are able to concentrate metals from seawater, that are up to 20 times more concentrated than in terrestrial plant. The concentrations of trace elements and rare earths elements (REE) were determined in 16 dominant macroalga species belonging to Chlorophyta, Ochrophyta and Rhodophyta phylum, collected in the Ligurian Sea (Italy).

Seaweeds samples were rinsed with distilled water, freeze-dried and homogenized. The concentrations of trace elements (aluminium, antimony, arsenic, beryllium, cadmium, cobalt, chromium, copper, iron, lead, mercury, manganese, molybdenum, nickel, selenium, tin, thallium, vanadium and zinc) and rare earths elements (lanthanides, scandium and yttrium) was detected by Inductively Coupled Plasma Mass Spectrometry (ICP-MS), after microwave digestion with concentrated nitric acid and hydrogen peroxide. Quantification limit (LOQ) was 0.010 mg Kg⁻¹.

Total metals concentration was found in the order Chlorophyta (8230 mg Kg⁻¹ dry weight) > Ochrophyta (6435 mg Kg⁻¹) > Rhodophyta (4728 mg Kg⁻¹). □REE (the sum of lanthanides, Sc and Y) was found comparable in Chlorophyta (12 mg Kg⁻¹) and Ochrophyta (13 mg Kg⁻¹) but lower in Rhodophyta (8.4 mg Kg⁻¹).

The highest mean levels of As, Co, Cu, Fe, Mn, Sb, Sn and Tl were found in Chlorophyta; while the highest concentrations of Al, Be, Cd, Cr, Ni, Pb, Se and Zn in Ochrophyta. Only V has shown the highest level in Rhodophyta. In the three macroalgae phylum comparable low levels of Hg were detected. European Community (2015/186 UE Regulation) set maximum limits for As, Cd, Hg and Pb in feed materials. Between these elements, only lead could constitute a limitation especially in Ochrophyta and Chlorophyta in which we recorded Pb values close to the limits.

As we generally found the lowest metals and REE values in Rhodophyta, that moreover tend to be rich in protein (up to 50%) and with limited amounts of iodine (0.03–0.04) we can suggest privileging red seaweeds in choosing feed ingredients.

Keywords: seaweeds, trace metals, rare earths elements

P3. Evaluation of predicted glycemic index in bakery/confectionary former food products and in former food based pig diet

Ottoboni M., Tretola M., Luciano A., Fusi E., Giromini C., Pinotti L.

*Department of Health, Animal Science and Food Safety,
University of Milan, Milano, Italy.*

This study evaluated the predicted glycemic index (pGI) in FFPs, and in two pig compound feeds containing or not FFPs. For this purpose, 6 samples of FFPs and a pig compound feed were used. FFPs were based on bakery and confectionary ex-food, while the pig compound feed was formulated substituting 30% of cereals with FFPs (feedFFP30). All samples were analyzed using an in vitro based on the Englyst-assay that simulates gastric and small intestinal digestion, and that has been proposed for determine hydrolysis index (HI) and predict the glycemic index (pGI) of cereal-based foods entering pig diets. Corn meal, flaked wheat, and a conventional pig compound feed (feedCTR) were included as control feed ingredients. In the assay white bread was used as reference material. Results obtained distinguished two main group of samples, namely: Low HI sample with HI lower than 100 including corn meal, feedCTR, feedFFP30, flaked wheat and FFP4; and high HI samples including all the other FFPs tested. The same classification can be adopted for pGI. Thus combining HI and pGI results it can be suggested that most of the FFPs tested in the present study were characterized by a high glycemic index potential that seems linked to the starch/sugars HI. The inclusion of FFPs in a commercial compound feed, and its effect on HI and pGI merit further investigations.

Keywords: former food, carbohydrate digestion, glycemic index

P4. Feed to fillet transfer of undesirable substances in Atlantic salmon fed feeds containing insect meal

Amlund H., Belghit I., Biancarosa I., Lock E.J., Ørnsrud R.

Institute of Marine Research, Bergen, Norway

Insects are a promising source of protein and lipids in feed for farmed animals. In the European Union (EU), the use of insect meal and insect oil is allowed in feed for fish. Insect meal and insect oil may contain undesirable substances, such as metals and mycotoxins. The European Food Safety Authority (EFSA) highlighted the lack of data on undesirable substances in insects and products thereof in their recent risk profile [1].

Atlantic salmon (*Salmo salar* L.) (approximately 1500 g) were fed experimental diets in triplicate. Fishmeal were gradually substituted with insect meal, and the four diets had an inclusion level of 0, 33, 66 and 100% insect meal, respectively. After 16 weeks of feeding, samples of fillet were collected. The level of undesirable substances (i.e. those included in EU Directive 2002/32/EC and amendments) in the insect meal and the experimental diets were documented, and transfer of undesirable substances from feed to fillet were investigated.

Results for the study will be presented and implications for feed and food safety will be discussed.

[1] EFSA Scientific Committee (2015). Scientific Opinion on a risk profile related to production and consumption of insects as food and feed. EFSA Journal 2015, 13(10):4257.

Keywords: insects, feed safety, food safety

P5. Natural solutions as alternative to synthetic products in farm animals' diets

Atkinson A., Le Roux J.F.

Nuproxa Switzerland, Etoy, Switzerland

Today, in a context of a growing world population and ongoing accumulation of greenhouse gases, the challenge of providing food while protecting the environment is a priority.

Providing enough food of good quality is related to the management of the farm animals (feeding, welfare, e.g.). One of the aspects often blamed is the use of diverse synthetic molecules in the animal feed. Synthesis of chemical molecules can often be damaging to environment, China where most of vitamin synthesis factories have been moved during the last decades has been forced to impose one year ago strong limitations to reduce environmental damages. Moreover, chemical production of synthetic feed additives can be dangerous for workers and inhabitants living around factories. For example, in 2013, a huge explosion happened in a choline chloride plant in China and has affected nearby residences. Supported by customers demand for more sustainability and more safety, investigations are conducted to find natural alternatives to synthetic products.

Phytotherapy has been used since decades in the human life whether for nutrition or for medicinal application. Same observations are true for animals able to eat specific plants in case of health disorders (Huffman and al., 1996).

Replace synthetic products in the feed with polyherbal mixtures is possible. For example, natural option exists for the chemically synthesized choline chloride used in the feed. Choline is well known for its role of methyl donor and its benefits for lipid metabolism.

A natural polyherbal mixture is able to substitute choline chloride in the feed thanks to its content in phosphatidylcholine and to its action on PPAR α , nuclear receptors involved in regulation of many metabolisms. This natural alternative to synthetic choline provides same level of zootechnical performance while being cost-effective. Moreover, besides its effects on animal, this natural option does have the disadvantages of the synthetics such as difficult handling, deterioration of other feed components (vitamins, pigments, e.g.). Trials in field and experimental farms have been conducted to validate this substitution and to be able to provide a bioequivalence matrix for the customers.

Keywords: natural, phytogetic, plants, sustainability, choline, feed

P6. The development of a detection method for poultry in feed and in animal proteins

Raamsdonk L.W.D. van¹, Prins T.W.¹, Scholtens I.M.J.¹, Dahlmans H.², Vrijenhoek T.³, Heijden L. van der², Sebille A. van³, Raaijmakers D.³

¹RIKILT Wageningen UR, ²Nutricontrol, ³Masterlab, Trouw Nutrition

There is a range of definitions in European legislation for the circumscription of poultry. These definitions range from intended for monitoring food safety issues (narrow: chicken, geese, duck, turkey, Guinea fowl; e.g. Regulation (EC) 882/2004, Regulation (EU) 212/2013), for trade purposes (wide: narrow circumscription with the addition of quails, pigeons, pheasants, partridges and ratites; e.g. Directive 2009/158/EC, Regulation (EU) 139/2013) or for enforcing hygiene rules for avoiding zoonotic diseases ("Poultry" means farmed birds; e.g. Regulation (EC) 853/2004). This variety of definitions hampers the development of a method for monitoring.

A second aspect is the goal for monitoring. Where the monitoring of ruminant processed animal proteins (PAPs) in feed is aiming at eradication of Bovine Spongiform Encephalopathies (BSE), the prohibition of poultry PAP in poultry feed is part of the species-to-species ban. In the absence of a safety issue, the ALARA principle (as low as reasonable possible) is not necessary to apply and a cut-off close to the legally required technical limit for monitoring methods (0.1 % (w/w)) is sufficient.

The available legal framework imply that specific requirements should be applied in the design of the method. For the range of species to be detected the narrow definition was adopted, since the monitoring method is intended for application in the area of food safety. In the view that the species chicken and turkey on one side and the genera duck and geese on the other side belong to two different main groups of birds, a special design was worked out. One detection system for chicken and turkey (Galliformes; at the species level), and a second one for duck and geese (Anseriformes; at the genus level) were designed, based on an *in silico* analysis of DNA diversity in chicken, turkey, two duck species and three goose species, and specifically excluding ostrich, common wood pigeon and two gull species. These two methods were constructed to be able to run complementary in one reaction as a single poultry detection method.

With regard to specificity as demanded by the chosen definition of poultry, experiments with rendered material revealed positive signals for chicken, turkey, four duck species and two geese species, and late signals for helmeted guinea fowl and turkestan hill dove. 32 other species, among them a number of birds, gave no signal. A secondary achievement is the possibility to detect chicken/turkey and duck/geese separately.

The sensitivity of the method was adjusted by calculation of a cut-off value close to the signal of a representative sample of a mixture of DNA of chicken, turkey and duck at a total level of 0.1% (w/w) in a salmon sperm matrix. Results reveal that a sufficient adjustment can be achieved with a good reproducibility. The results show that different master mixes will show different responses, which influences the sensitivity of the method.

Keywords: species-to-species ban, poultry detection in feed

P7. Image analysis of feed ingredients by deep learning

Raamsdonk L.W.D. van¹, Daniels F.², Hedemann B.¹

¹RIKILT Wageningen UR, ²FBR Wageningen UR

Establishment of the composition of feed based on visual observations is usually achieved by experienced technicians. Recognition of visual information, however, also takes place via automatic systems, such as in passport control, fingerprint recognition, license plates on cars, or the interpretation of images from security cameras. Currently, Convolutional Neural Networks (CNN) are outperforming previous identification methods for such tasks. A Short Innovation Project was carried out in order to identify the opportunities for the automatic identification of ingredients from animal feed. A model system was designed and tested for the simultaneous identification of starch grains of three major ingredients: wheat, corn and potato. Theano was used as deep learning framework to implement and explore various types of CNN architectures. In phase 1 a library of 600 images with visual light information was constructed for training of the network, consisting of one type of starch per image. Additionally image sets were used for testing the neural network and for validation. It appeared that pre-filtering of the background enhanced the success rate of proper identification. In the final stage of phase 1 correct identification was achieved for more than 95% for each of the three types. These results were sufficient for starting the training of the model for identification of grains in mixtures. Images with mixtures of the three types of grains were produced and tested in phase 2. Every image was available for training in two versions: an unlabelled version, and a labelled version with indication of the type for every starch grain visible. The annotation was based on expert judgment, supported by a third image made with polarised light, and a check of a second expert. The neural network developed and sufficiently tested in phase 1 produced no optimal results. Three new models were developed and tested. The most promising model was optimised by using a post-processing approach and tested for its success rate. The identification was successful with rates of approx. 87% (potato and wheat) and approx. 93% (corn). The results reveal that further optimisation of the procedure of deep learning could be possible. The currently applied models are pixel based: the pixel information is used for the discrimination between the three targets and the background. This implies that important information such as size and shape of the starch grains was currently not used for identification. Modified strategies using networks such as Mask R-CNN include masks for individual instances, i.e., not only labelling each pixel but also grouping pixels together to form an object. The current achievements can be applied in those applications where a relatively simple setting exists such as the recognition of somatic cells in milk, and nucleus deviations in cells in a cell culture (image analyser). For compound feeds over 600 different ingredients are allowed according to the Feed Catalogue, which implies that complicated combinations can be present. A strategy can include several aspects, such as simplifying the composition by preparing specific subsamples and by applying dedicated algorithms.

Keywords: image analysis, feed ingredients, composition analysis, deep learning

P8. Characterization of hydrolysed proteins in feed

Raamsdonk L.W.D. van¹, Jardy N.², Weiner A.³, Bremer M.G.E.G.¹, Paprocka I.³, Bescond M.², Vonsovic S.¹, Genouel C.², Kwiatek K.³, Prins T.W.¹

¹RIKILT Wageningen UR, ²Service Commun des Laboratoires de Rennes (SCL35), France, ³NVRI, Department of Hygiene of Animal Feedingstuffs, Pulawy, Poland

European legislation provides a set of restrictions to the use of animal material in animal feed, put in force for the eradication of Transmissible Spongiform Encephalopathies (TSEs), most notably the bovine form (BSE). The prohibitions and derogations form a quite complicated structure, specifying a range of materials and destined animals for feeding. The limitations vary from a strict prohibition of any use except incineration to full application in animal feed. The definition of the legal term “animal protein” includes a series of materials with their own specified restrictions, as well as a set of excluded materials (Regulation (EU) 142/2011). These excluded materials cover milk and egg derivatives as well as some specific materials such as tricalcium phosphate and hydrolysed proteins from animal origin. This poster will emphasize on the background and strategy of the characterization of the hydrolysed proteins. Regulation (EC) 999/2001 Annex IV prohibits in Chapter 1 the use of hydrolysed proteins in general sense, making derogations for hydrolysed proteins derived from parts of non-ruminant animals, and from ruminant hides and skins, in Chapter 2. This derogation provides a broader range of application than applies for animal proteins as included in the definition. There are strict procedures for the processing of animal material for the production of hydrolysed proteins. Annex IV of Regulation (EU) 142/2011 lists alkaline and heat treatment, and adds the requirement of peptides with a size below 10,000 Dalton in case of ruminant origin of the proteins. The definition of “hydrolysis” in the Feed Catalogue (Regulation (EU) 68/2013) adds the treatment with acid or with enzymes. The complicated set of restrictions and opportunities for application includes three aspects: the procedure for hydrolysis, a peptide length requirement, and the source of the material. Procedure: hydrolysis in a strict, chemical sense means shortening of the principal chain of amino acids. This can be achieved by a treatment using an acid, an alkali or an enzyme. Consequently, this is the procedure that will finally result in shorter chains, i.e. in lengths shorter than 10,000 Dalton. Heat treatment, as applied to feather meal, is primarily meant to cut the S-bonds in the tertiary structure of a protein, without shortening the chain length. Chain length: a strategy for establishing the lengths of the peptides is only feasible for those materials that are hydrolysed in a strict sense, i.e. by alkali, acid or enzymes. Material primarily based on a chain length below 10,000 Dalton might be used without further restrictions. Source: in those cases that the chain length of the peptides exceed the limit of 10,000 Dalton, a strategy for establishing the absence of ruminant proteins needs to be applied.

Keywords: hydrolysed proteins in feed, degree of hydrolysatation, identification, 10 000 Dalton limit

P9. Occurrence of polar pesticides in feed and feed ingredients

Tienstra M., Memelink J., Zomer P., Mol H.

RIKILT – Wageningen University & Research, Wageningen, The Netherlands

Pesticides are widely used in agriculture and may leave residues in products used as feed or feed ingredients. In the EU, pesticide residues in feed are regulated under CR 2002/32 and CR 396/2005. Pesticides are a wide range of substances with a variety of physical-chemical properties. The majority of them can be efficiently analysed by multi-methods based on LC-MS/MS and GC-MS/MS. However, a number of pesticides, most notably very polar pesticides, are not amenable to multi-residue methods. Examples include: glyphosate, glufosinate and metabolites, ethephon, paraquat, diquat, difenzoquat, chlormequat, daminozide, cyromazin, trimethylsulfonium, chlorate and perchlorate. For their determination, several additional dedicated analysis methods are required. Since this substantially increases the overall analysis costs per sample, not many samples are analysed for the polar pesticides and they may go unnoticed. In 2016 it became apparent that paraquat (banned in the EU) was found in feed commodities at levels exceeding the MRL. This triggered the inclusion of polar pesticides in the Dutch National Feed monitoring program. Based on the QuPPE approach [1], three methods were developed and validated that together covered all polar pesticides mentioned above. The methods were applied for analysis of approximately 400 samples in 2017-1st half 2018. The most frequently found polar pesticide was glyphosate (34% of all samples) mostly in soybean/by-products and cereals, followed by chlormequat (13%), mepiquat (7%), paraquat (7%), diquat (6%) perchlorate (3%), daminozide (2%), trimethylsulfonium (2%), glufosinate/metabolites (2%) and cyromazine (0.2%). Of these the MRL was exceeded in a number of cases, for paraquat virtually always (mostly soybean/by-products), and to a lesser extent for diquat, mepiquat, chlormequat and glyphosate. This work shows the relevance of inclusion of polar pesticides in the monitoring and enforcement programs.

- [1] http://www.eurl-pesticides.eu/userfiles/file/EurlSRM/meth_QuPPE-PO_EurlSRM.pdf

Keywords: polar pesticides, feed, feed ingredients, LC-MS/MS

P10. Harmonization of methods of analysis for natural toxins in feed – Activities of CEN/TC 327/WG 5

Witsenburg J.¹, Bodi D.², Gray K.³, Mulder P.⁴, Stroka J.⁵, Mol H.⁴

¹*Nederlands Normalisatie-instituut (NEN), Delft, The Netherlands*

²*Bundesinstitut für Risikobewertung (BfR), Berlin, Germany*

³*LGC, Teddington, United Kingdom*

⁴*RIKILT Wageningen University & Research, Wageningen, The Netherlands*

⁵*Joint Research Centre F.5 – Food and Feed Compliance, Geel, Belgium*

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 “Animal feeding stuffs: Methods of sampling and analysis”. The work of CEN/TC 327 is financially supported by standardization requests from the European Commission.

Currently CEN/TC 327/WG 5 “Natural toxins” is drafting methods for the determination of:

- Ergot alkaloids and tropane alkaloids by LC-MS/MS (prEN 17256)
- Free gossypol by LC-MS/MS
- Intact glucosinolates in rapeseed by LC-MS/MS
- Mycotoxins (multi-method) by LC-MS/MS (prEN 17194)
- Pyrrolizidine alkaloids by LC-MS/MS
- Theobromine by LC-UV and LC-MS/MS (prEN 17270).

Furthermore, a document with criteria for methods of analysis for mycotoxins is under preparation.

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

www.nen.nl and www.cen.eu

Keywords: CEN/TC327, standardization, european, analysis, natural toxins

P11. Harmonization of methods of analysis for elements in feed and their chemical species – Activities of CEN/TC 327/WG 4

Eykelhoff J.¹, Sloth J.² Danier J.³

¹*Nederlands Normalisatie-instituut (NEN), Delft, The Netherlands*

²*National Food Institute, Technical University of Denmark (DTU), Søborg, Denmark*

³*Bavarian State Ministry of the Environment and Consumer Protection (STMUV), Munich, Germany*

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis". The work of CEN/TC 327 is financially supported by standardization requests from the European Commission.

Currently CEN/TC 327/WG 4 "Elements and their chemical species" is drafting methods for the determination of:

- Fluoride content after HAT by ISE (revision of EN 16279:2012; new work item proposal under preparation)
- Inorganic arsenic by anion-exchange HPLC-ICP-MS.

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

www.nen.nl and www.cen.eu

Keywords: CEN/TC327, standardization, european, analysis, elements, chemistry

P12. Harmonization of methods of analysis for additives and drugs in feed – Activities of CEN/TC 327/WG 3

Mosies C.¹, Petrová J.², Schönherr J.³, Strnad I.⁴, Vincent U.⁵,
von Wright A.⁶, von Holst C.⁵

¹*Nederlands Normalisatie-instituut (NEN), Delft, The Netherlands*

²*Central Institute for Supervising and Testing in Agriculture (UKZUZ), Prague, Czech Republic*

³*Saxon State Company for Environment and Agriculture (BfUL), Radebeul, Germany*

⁴*Austrian Agency for Health and Food Safety (AGES), Linz/Vienna, Austria*

⁵*Joint Research Centre F.5 – Food and Feed Compliance, Geel, Belgium*

⁶*Biological Safety Solutions, Kuopio, Finland*

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 “Animal feeding stuffs: Methods of sampling and analysis”. The work of CEN/TC 327 is financially supported by standardization requests from the European Commission.

Currently CEN/TC 327/WG 3 “Feed additives and drugs” is drafting methods for the determination of:

- Bacteria through PFGE typing
- Benzoic and sorbic acids by HPLC (prEN 17298)
- Carotenoids by RP-HPLC-UV
- Coccidiostats and antibiotics with HPLC-MS/MS (prEN 17299)
- Organic acids by IC-CD (prEN 17294)
- Various probiotics (revision of EN 1578x-series).
- Vitamin A, D and E content by SPE clean-up and HPLC
- Yeast.

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

www.nen.nl and www.cen.eu

Keywords: CEN/TC327, standardization, european, analysis, additives, drugs

P13. Harmonization of methods of analysis for organic contaminants in feed – Activities of CEN/TC 327/WG 1

Wagner M.¹, Gebbink W.², Fry H.³, Lacoste F.⁴, van Leeuwen S.², de Jong J.²

¹*Nederlands Normalisatie-instituut (NEN), Delft, The Netherlands*

²*RIKILT Wageningen University & Research, Wageningen, The Netherlands*

³*Bundesinstitut für Risikobewertung (BfR), Berlin, Germany*

⁴*ITERG, Pessac, France*

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 “Animal feeding stuffs: Methods of sampling and analysis”. The work of CEN/TC 327 is financially supported by standardization requests from the European Commission.

Currently CEN/TC 327/WG 1 “Organic contaminants” is drafting CEN standards with methods for the analysis of:

- Dioxins, dioxin-like PCBs and non-dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS (revision of EN 16215:2012)
- Melamine and cyanuric acid by LC-MS/MS (prEN 17212)
- Organochlorine pesticides and non-dioxin-like PCBs by GC/ECD (revision of EN 15742:2009)
- Organochlorine pesticides and non-dioxin-like PCBs by GC/MS (revision of EN 15741:2009)
- Pentachlorophenol by LC-MS/MS
- Saturated hydrocarbons in vegetable oil by GC-FID.

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

www.nen.nl and www.cen.eu

Keywords: CEN/TC327, standardization, european, analysis, organic contaminants

P14. Harmonization of methods of sampling and analysis of feed – Activities of CEN/TC 327

Mosies C.¹, Venemans A.¹, Sobiech-Matura K.², Ellermann N.³, van Tuinen S.⁴

¹*Nederlands Normalisatie-instituut (NEN), Delft, The Netherlands*

²*Joint Research Centre G.2 – Standards for Nuclear Safety, Security and Safeguards, Geel, Belgium*

³*Ministeriet for Fødevarer, Landbrug og Fiskeri, Ringsted, Denmark*

⁴*RIKILT Wageningen University & Research, Wageningen, The Netherlands*

It is important for the European feed sector, national governments and the European Commission that the safety and quality of animal feed, including feed materials, pre-mixtures and feed additives, is guaranteed. For a uniform judgement of conformity of products to the requirements, in particular in the framework of quality assurance and regulatory control, validated and harmonised methods are needed when purchasing, producing or selling animal feed. In the European Union and the European Economic Area, European Standards play an important role in meeting the specific European requirements. The European Committee for Standardization (CEN) is the organisation responsible for European standardization in a wide variety of sectors. One of the technical committees is CEN/TC 327 "Animal feeding stuffs: Methods of sampling and analysis". The work of CEN/TC 327 is financially supported by standardization requests from the European Commission.

Currently, CEN/TC 327/WG 2 "Composition" supports ISO/TC 34/SC 10 "Animal feeding stuffs" with the drafting of standards for the determination of the

- Content of fatty acids – Part 1: Preparation of methyl esters (revision of CEN ISO/TS 17764-1:2006)
- Content of fatty acids – Part 2: Gas chromatographic method (revision of CEN ISO/TS 17764-2:2006).

Currently, CEN/TC 327/WG 6 "Radioactivity measurements" is drafting a method for the

- Determination of the radionuclides ¹³¹I, ¹³⁴Cs and ¹³⁷Cs in feed with gamma ray spectroscopy.

One of the most important requirements for standardization is the successful validation of a method by means of a full international collaborative study. The drafting and validation of these methods is in various stages of progress. For some of the items mentioned, dedicated posters will be presented by the project leaders.

www.nen.nl and www.cen.eu

Keywords: CEN/TC327, standardization, european, analysis, composition, radioactivity

P15. Moving the lab to the farm for feed analysis and better animal feeding

Chamberland N., Vermeulen Ph., Lecler B., Decruyenaere V.,
Froidmont E., Baeten V.

Walloon Agricultural Research Centre (CRA-W), Gembloux, Belgium

In the framework of smart farming, the Walloon Agricultural Research Center (CRA-W) is currently testing a new approach using NIR spectroscopy applied to the context of dairy farming in Wallonia, Belgium.

The aim of the project is to develop and validate new analytical methods for predicting quality parameters such as dry matter, composition (Starch, Crude Protein, ADF, NDF, Ash and Fat) and digestibility of wet forages directly on farm (specifically maize silage, grass silage and fresh grass). Benefits for dairy farmers will be a reduction of wasted feed, an improvement of feeding efficiency (higher milk yield per kg of DM intake) and a better knowledge of the quality of their forage along the time enabling diet adaptations.

Fifteen dairy farms have been selected in Wallonia between May and October 2018 to collect fresh wet samples. NIR spectra were measured directly on site with three portable NIR spectrometers: the FieldForSpec 4 from ASD (350-2400 nm), the NIR4FARM from AUNIR (950-1750 nm) and the flameNIR from OceanOptics (940-1665 nm). These instruments allow acquiring NIR spectra directly on farm.

Moreover, samples were also measured with a benchtop XDS instrument from FOSS (400-2400 nm) in the Food and Feed Quality Unit of the CRA-W. Transfer of NIR database will be performed from the FOSS XDS to the NIR4FARM to assess the potential of the portable system to predict quality parameters with a transferred database.

Reference values were obtained by prediction with a FOSS XDS on dry and grounded samples from forages collected on farm using robust prediction models developed by the CRA-W since 30 years.

Validation of the built models will be performed during the 2019-2020 seasons with another set of farms.

The outcome of this project will be the development of an user-friendly tool for dairy farmers to predict on site the composition of their forages, enabling the calculation of their nutritional value and the adaptation of animal's feeding for a better sustainability.

Keywords: feed, forage, NIR, handheld instrument, quality

P16. Results from the CEN Collaborative Study on Pentachlorophenol (PCP) in Feed Materials and Compound Feed

Gebbink W.A., Elbers I., van der Weg G., van Leeuwen S.P.J., de Jong J.

RIKILT, Wageningen Research, Wageningen, Netherlands

European Commission Mandate M/523 called for the standardisation for a method for the pentachlorophenol (PCP) analysis in compound feed and feed materials [including guar gum and fatty acid distillates (FAD)]. An LC-MS/MS method was developed for the determination of PCP in compound feed, guar gum and fatty acid distillates with a limit of quantitation of 10 µg/kg. For the developed standard a collaborative trial was organised early 2018 for the validation of the standard. A total of five samples were prepared containing PCP between 9 and 22 µg/kg, determined by homogeneity testing. These five samples included two compound feed samples, one guar gum sample and two FAD samples, and blind duplicates of these five samples were sent to participating laboratories. A copy of the standard was sent along with the samples and laboratories were asked to follow this method.

A total of twenty-one laboratories registered for participation in the collaborative study, fourteen located in Europe, six in Asia and one in North-America. Sixteen of them submitted results for one or more of the five samples. The submitted results will be assessed by Mandel h and k plots for the identification of consistent irregular data, and irregular data will be discarded, if applicable. After statistical evaluation of the data according to ISO 5725, the HorRat (Horwitz ratio) will be calculated for the two feed samples, guar gum sample and two fatty acid distillate samples. This poster will present the outcome of the collaborative trial on PCP in feed materials and compound feed.

Keywords: collaborative trial, pentachlorophenol, feed, feed materials

P17. Fate of the antioxidant ethoxyquin from feed to salmon filet: application of liquid chromatography coupled to ion mobility and high resolution mass spectrometry

Merel S., Regueiro J., Berntssen M.H.G., Hannisdal R., Ørnsrud R., Negreira N.

Institute of Marine Research (IMR), Bergen, Norway

Ethoxyquin is commonly present in fish feed and acts as an antioxidant. The quick oxidation of ethoxyquin prevents the oxidation of other components responsible for the nutritional quality of the feed. Identifying the transformation products of ethoxyquin and examining their occurrence and transfer from fish feed to salmon filet is important for risk assessment purposes. Previous research relying on chemical bench-scale oxidation and the analysis of fish feed allowed identifying 37 transformation products of ethoxyquin. Therefore, this study aimed at extending the current knowledge to the identification of transformation products in fish muscle. To achieve this objective, salmonids were fed during 90 days with fortified feed containing ethoxyquin at 0.5 mg/kg, 119 mg/kg, and 1173 mg/kg. In addition, the occurrence of ethoxyquin and its transformation products was also assessed in filets from 12 commercial Norwegian farmed salmon. All salmon filets were extracted in acetonitrile and analyzed by liquid chromatography with traveling-wave ion mobility spectrometry coupled to high resolution mass spectrometry. Salmon filets from the feeding trial showed the occurrence of ethoxyquin along with 23 transformation products resulting from dimerization, oxygenation, cleavage, cleavage combined with oxygenation, cleavage combined with conjugation, and other alterations. Among them, 10 were characterized for the first time. In filets of farmed salmon intended for human consumption, ethoxyquin was detected in 75% of the samples. In addition, 24 transformation products were also detected with a frequency ranging from 8% to 100%. In all salmon filets from both the feeding trial and fish farms, transformation products resulting from dimerization were by far the most abundant. In particular, the ethoxyquin dimer 1,8-EQDM was the main transformation product. Finally, ion mobility spectrometry provided additional confidence for compound identification during screening analysis. In complex matrices or when the abundance is too low to allow the detection of characteristic fragments, collision cross section with a 2% deviation lowers potential interferences and false positives. The current study allowed a comprehensive knowledge of the fate of ethoxyquin from fish feed to salmon filet, and brought the total number of transformation products identified to 47.

Keywords: ethoxyquin, transformation products, ion mobility, QTOF

P18. Determination of ergot alkaloids and tropane alkaloids in feed materials and compound feeds by LC-MS/MS. Results of a collaborative trial

Mulder P.P.J., Pereboom-de Fauw D.P.K.H., Mol J.G.J., de Jong J.

RIKILT Wageningen University & Research, Wageningen, the Netherlands.

Ergot alkaloids are mycotoxins produced by the fungal species *Claviceps purpurea*. The fungus may infest plant species of the Poaceae family (true grasses), producing dark coloured bodies, called sclerotia or (rye) ergot. Economically important cereal grains that may be infected by *C. purpurea* are rye, wheat, triticale, barley, millet and oats. The sclerotia contain a suit of ergot alkaloids, of which twelve have been recognised as major components: ergocornine, ergocristine, α -ergocryptine, ergometrine, ergosine, ergotamine and their respective epimers.

Tropane alkaloids are plant toxins produced by several species within the family of Solanaceae (nightshades). The most relevant are *Datura* (thornapple), *Hyoscyamus* (henbane) and *Atropa* (belladonna, deadly nightshade). Seeds and other plant parts contain substantial amounts of atropine (hyoscyamine) and scopolamine, which are the most important toxic principles. Nightshades can be present as weeds in arable fields and may be co-harvested, resulting in contaminated feed products.

CEN is the organisation that is responsible for European standardization of methods. The technical committee CEN/TC 327 drafts methods for organic and inorganic contaminants, including mycotoxins and plant toxins, in feed. The European Commission has mandated the standardization of an LC-MS/MS based method for ergot and tropane alkaloids in feed grains and compound feeds.

In the proposed method alkaloids are extracted from the sample with methanol/water 60/40 containing 0.4% formic acid. After centrifugation, a portion of the supernatant is further purified by passing it through a 30 kD ultrafilter. The filtrate is analysed by LC-MS/MS. Quantification is performed by one-point standard addition to the sample.

Fourteen laboratories participated in the main collaborative trial and 13 laboratories submitted results. One dataset was discarded because the sensitivity of the instrument proved insufficient to meet minimum required performance criteria. The accepted datasets were evaluated using robust statistics.

The method has been successfully validated in the following matrices: rye, barley, wheat, complete feed for bovine, porcine and poultry. The validated range is approximately 10 to 250 $\mu\text{g}/\text{kg}$ for individual alkaloids. Determination of concentrations above 250 $\mu\text{g}/\text{kg}$ is possible by applying a higher spiking level and dilution of the sample extract, but this was not validated in the collaborative trial.

Keywords: Ergot+tropane alkaloids, feed, LC-MS/MS, collaborative trial

P19. Comprehensive analysis of 9-cis, 13-cis, and all-trans (enantiomers and mesoform) Astaxanthin in salmon through liquid chromatography with ion mobility and high resolution mass spectrometry

Merel S., Oveland E., Ørnsrud R., Hamre K.

Institute of Marine Research (IMR), Bergen, Norway

The carotenoid astaxanthin is used as nutritional supplement and antioxidant but its main application is as feed additive in aquaculture in order to provide its color to the salmon filet. However, in Europe, the regulation (EU) 2015/1415 restricts the concentration of astaxanthin to a maximum of 100 mg/kg in fish feed. Therefore the accurate quantification of this compound in different matrices is necessary. However, such task remains challenging since astaxanthin consists of several stereoisomers, including three diastereoisomers (9-cis-astaxanthin, 13-cis-astaxanthin and all-trans-astaxanthin), two enantiomers and a mesoform of all-trans-astaxanthin. The abundance ratio of these astaxanthin isomers varies between wild salmon and farmed salmon, therefore revealing the origin of food products. When usual method might allow discriminating diastereoisomers, discriminating the enantiomers and mesoform of all-trans-astaxanthin requires chiral chromatography. Therefore this study proposes an alternative analytical method based on liquid chromatography coupled to ion mobility and high resolution mass spectrometry. A reversed phase C18 column commonly used for the analysis of trace organic contaminant with a gradient consisting of water (with 10 mM ammonium acetate) and methanol proved successful to discriminate all three diastereoisomers of astaxanthin standard. The second dimension to the separation added by ion mobility spectrometry allowed further discriminating all enantiomers of all-trans-astaxanthin. However this discrimination based on ion mobility was dramatically improved when considering the ion $[M+Na]^+$ rather than the ion $[M+H]^+$. In addition, high resolution mass spectrometry and specific fragments together provides much higher confidence regarding the identification of a compound in comparison to other detection methods based on spectrophotometry. This method allowed the detection of astaxanthin below 1 pg on column and over 3 orders of magnitude. The application of this method incorporating ion mobility spectrometry proved successful for the analysis of astaxanthin in samples of farmed salmon, discriminating all stereoisomers within 15 minutes of measurement time.

Keywords: astaxanthin, ion mobility, QTOF, isomers

P20. Bio-based screening for contaminants and residues. “From whole animals to onsite biosensors”

Bovee T.F.H., Peters J., Pikkemaat M., Van Ginkel L.A., Hoogenboom L.A.P., Gerssen A.

RIKILT Wageningen University & Research, Wageningen, The Netherlands

Bio-based screening includes both cell based effect assays (in-vivo and in-vitro bioassays) and a variety of immuno based detection methods (biosensors). In-vitro bioassays have been developed and applied in routine screening of feed and animal products. Well known examples are bacterial inhibition assays for antibiotics and mammalian or yeast cell-based receptor induced transcriptional activation assays for different steroid classes and dioxins and planar PCBs. Furthermore, in-vitro bioassays to detect marine biotoxins in fish and shellfish have been developed to replace a mouse bioassay, where a mouse is injected with a shellfish extract and death is the endpoint recorded. The suitability of the animal-friendly neuro-2a bioassay is now evaluated for the detection of marine biotoxins in seaweed and fish meal. The main advantage of the mentioned bioassays is their ability to detect both the known regulated compounds and the possible presence of yet unknown bioactive compounds. Therefore, bio-based screening is a valuable tool in risk based monitoring. Finally, it is noticed that sometimes animals die or people get ill without any known cause or link. Besides the above mentioned bioassays, toxicity to a whole organism, e.g. the water flea (mobility *Daphnia*), can be of use for the broad screening of acute toxic substances.

Biosensors are often based on antibody-antigen interactions to either detect a specific compound or a group of structurally related compounds. A Lateral Flow Device (LFD (dipstick)) is the most common format to detect a specific compound, e.g. a LFD to detect ampicillin in milk. Besides speed, ease of use and low costs, the main advantage of the LFD is its on-site applicability. However, nowadays clients ask for multi-analyte analysis. A novel immunoassay format based on paramagnetic beads enables rapid and cheap (portable) on-site multiplex analysis, e.g. detecting over 70 various antibiotics in over 60 samples within 90 minutes. Moreover, this technology allows multi-multiplex analysis, e.g. detecting both antibiotics and mycotoxins in milk at the same time.

The above mentioned bioassays and biosensors will be addressed and their added value for routine monitoring demonstrated by examples from real practice.

Keywords: bioassays, biosensors, contaminants, residues, screening, toxins

P21. Selenium speciation in fish feed by HPLC-ICPMS

Sele V.¹, Sloth J.J.², Ørnsrud R.¹, Berntssen M.H.G.¹, Amlund H.¹

¹*Institute of Marine Research (IMR), Bergen, Norway*

²*National Food Institute, Technical University of Denmark (DTU), Kgs. Lyngby, Denmark*

Fish feed can contain relative high levels of the essential, but also toxic, trace element selenium (Se). Selenium can be present in different chemical forms, or Se species, and the bioavailability and toxicity of Se depend on the physiochemical properties of the Se species present.

The levels of Se in fish feed depend on the levels of Se in the feed ingredients, e.g. natural high levels of Se in the fish meal causes high levels of Se in the complete feed. Addition of Se to feed is limited to a maximum concentration of 0.5 mg/kg feed [1], and the addition of organic selenium (Se-yeast) is limited to a maximum concentration of 0.2 mg/kg feed [2]. To control whether feed comply with the legislation there is a need for an analytical method that can discriminate between organic and inorganic selenium species in feed.

Two methods have been developed for the determination of Se species using High Pressure Liquid Chromatography coupled to Inductively Coupled Plasma Mass Spectrometry (HPLC-ICPMS). For selenium speciation analysis anion-exchange HPLC-ICPMS for analysis of inorganic Se species and cation-exchange HPLC-ICPMS for analysis of organic Se species, were applied. Also, different extraction procedures were applied for the extraction of inorganic and organic Se species. Results from analysis of fish feeds and salmon muscle tissue will be presented, and discussed with regards to analytical challenges.

[1] EC, Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on additives for use in animal nutrition. Official Journal of the European Union L268/29 (2003).

[2] EFSA, Scientific Opinion on the safety and efficacy of selenium in the form of organic compounds produced by the selenium-enriched yeast *Saccharomyces cerevisiae* NCYC R645 (SelenoSource AF 2000) for all species. The European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), EFSA J. 9 (2011) 15.

Keywords: selenium, feed, speciation analysis, HPLC, ICP-MS

P22. Novel antibody-based mass spectrometry method for the detection of banned animal proteins in feed

Niedzwiecka A.¹, Steinhilber A.², Zagon J.¹, Poetz O.³, Roeder M.⁴, Weber W.⁴, Braeuning A.¹, Lampen A.¹

¹German Federal Institute for Risk Assessment, Dept. Food Safety

²NMI Natural and Medical Sciences Institute at the University of Tuebingen, Germany

³Signatope GmbH, Germany

⁴Institute for product quality GmbH, Germany

The authentication of food and feed poses a challenge to the inspection of animal by products and processed proteins thereof. Since the increased number of bovine spongiform encephalopathy and the subsequent release of EU regulations in 2001, processed animal proteins (PAP) were banned as feed ingredients with only few exceptions (Regulation (EC) No 999/2001). After the reintroduction of PAP from porcine and poultry origin in 2013 (Regulation (EC) No 56/2013), new feed formula posed new analytical requirements on methods to distinguish between species as well as tissues. Analytical requirements are only partly met by the current official methods microscopy and polymerase chain reaction, where macromolecular structures like bones or muscle fibers can be detected and species can be differentiated in harshly processed PAPs. The methods reach detection limits of 0.1% (w/w) [1]. However, the methods are not capable of differentiating legal ruminant milk products from banned ruminant PAP. To cover this analytical gap, new analytes like proteins and peptides must be taken into account.

In the presented approach protein analysis is the key to detect banned animal proteins. Protein enrichment via immunoprecipitation followed by a mass spectrometric analysis allows identifying protein sequences from PAP and differentiating their originating tissue and species. Marker peptides are considered to detect different farm animals. Additionally, markers should be suitable to represent tissues like meat, bone, blood and milk. The mass spectrometric approach should be as sensitive as official methods and applicable for a variety of matrices.

Tissue specific proteins like haemoglobin and alpha-2-macroglobulin (blood meal and spray dried plasma) or myosin-7 and matrilin-1 (meat and bone meal) were used as markers. Species specific peptide sequences can distinguish material from different animal origin like cattle, pig, sheep/goat, horse, and poultry. With this approach adulterations of different feeds with bovine spray dried plasma, bovine blood meal and bovine meat and bone meal were detected and quantified down to a range of 0.05% to 1% (w/w) with different mass spectrometric technologies.

The MS-based immunoassays meet the requirements for future methods to detect PAPs and can overcome the limitations of the official methods.

Keywords: processed animal proteins, BSE, LC-MS/MS

P23. Isolation method for processed animal proteins from insects in feed and their identification by microscopy

Veys P., Baeten V.

Food and Feed Quality Unit, Valorisation Department, Walloon Agricultural Research Centre CRA-W, Gembloux, Belgium.

Insect processed animal proteins (PAPs) constitute a new alternative source of proteins in feed. In 2017, a closed list of insect species was authorized on the European market for use in aquafeed production. Authenticity and contamination controls will have to be set up by authorities and feed actors and supported by adequate detection methods, which are lacking.

The lecture presents an original isolation and detection protocol for insect material. The protocol, based on sedimentation by a mixture of petroleum ester and tetrachloroethylene to concentrate insect particles, was developed and tested on a series of ten different aquafeeds fortified at 1 % w/w with four different commercially available insect meals (from *H. illucens*, *T. molitor*, *G. assimilis* and *A. diaperinus*). The results showed that this sedimentation protocol combined with light microscopic observation was adequate for insect detection and more efficient than the current official method. Morphological key features for reliable characterization of insect PAPs were also investigated. Structural details of cuticular fragments, such as sensilla and tracheolar structures, combined with patterns of muscle fibers, were found to constitute robust identification keys to establish the insect origin of particles. The prospective use of these markers for lower taxonomic ranking, at order level, was also addressed. Finally, the value of the markers proposed was discussed in terms of their ability to distinguish insect PAP from other types of invertebrate meal, such as that produced from marine arthropods, but also within the global framework of controls for the enforcement of the legal feed ban.

Keywords: insects, feed, control, detection, microscopy

P24. Validation of a screening method for pesticides and veterinary drug residues in feed by LC-HRMS

Wegh R.S., Bolck Y.J.C., Bor G., Zomer P., Zuidema T., Mol H.J.G.J.

RIKILT – Wageningen University & Research, Wageningen, The Netherlands

Feed and feed ingredients comprise an extremely wide variety of matrices such as cereals, by-products from food and biofuel industry and compound feeds. During production, storage and processing, a wide variety of substances may enter the feed chain. For example, environmental contaminants, mycotoxins, plant toxins and residues of pesticides and veterinary drugs. Dedicated methods exist for regulated compound/matrix combinations, but these are far from comprehensive and undesirable substances may go unnoticed. The aim of this work was to develop a chemical screening method capable of detection of a very wide variety of substances in representative feed materials. For this, existing methods and approaches were (re)considered to select the optimum method (best compromise) to achieve a comprehensive screening method using high resolution mass spectrometry (LC-Q-Orbitrap).

Full scan acquisition was done using multiple scan events to have a truly non-targeted measurement with retrospective data analysis possibilities while still obtaining data on precursor ions and fragment ions which facilitates selective detection and (tentative) identification. The combined screening method for pesticides and veterinary drugs is fully validated for most components at relevant levels. Based on these results, the approach can be expanded to cover other groups of relevant compounds e.g. contaminants and mycotoxins.

Keywords: screening, pesticides, veterinary drugs, animal feed

P25. Determination of urea in animal feed with liquid chromatography-mass spectrometry (LC-MS)

Wegh R.S., Berentsen R. , Bolck Y.J.C., Zuidema T., de Jong J.

RIKILT – Wageningen University & Research, Wageningen, The Netherlands

Urea is a feed additive that is used as a source of nitrogen for the production of protein by rumen microbes through the production of ammonia and carbon dioxide. The use of urea as a feed additive is only allowed for ruminants with a functional rumen with a maximum content of 8800 mg/kg complete animal feed. When too much ammonia reaches the liver, the liver can no longer process it and toxic effects and even death can occur. With these rules, it is important to be able to check the real urea content in an animal feed. The aim was to develop a robust method for the analysis of urea in animal feed with a high specificity In order to effectively monitor urea use in animal feed.

A method is developed to determine the concentration of urea in animal feed using ultra-high performance liquid chromatography (UHPLC) coupled to tandem mass spectrometry (MS/MS) with atmospheric-pressure chemical ionization (APCI). The method is suitable for label control of urea in animal feed. Confirmation of the identity of urea should be performed with HRMS since only one fragment ion is present. The method was fully validated according to Commission Decision 2002/657/EC using a wide range of different animal feed samples. The developed method and validation characteristics will be presented.

Keywords: urea, animal feed, LC-MS

P26. True untargeted workflow for identification of antimicrobial compounds in animal feed using bioassay-directed screening in combination with liquid chromatography-high resolution mass spectrometry

Wegh R.S., Berendsen B.J.A., Driessen-van Lankveld W.D.M., Pikkemaat M.G., Zuidema T., van Ginkel L.A.

RIKILT – Wageningen University & Research, Wageningen, The Netherlands

When a compound causing microbial growth inhibition in an effect based assay is not within the scope of the targeted LC-MS/MS method, used for confirmation, e.g. if it is a disinfectant or a substance not registered for veterinary application, it will not be detected. Non-targeted analysis is needed to identify such 'unknown' antimicrobial compounds.

A true untargeted workflow is reported for the isolation and identification of antimicrobial active compounds using bioassay-directed screening and LC coupled to high-resolution MS. Suspect samples are extracted using a generic protocol and fractionated using two different LC conditions (A and B). The behaviour of the bioactive compound under these different conditions yields information about the physicochemical properties of the compound and introduces variations in co-eluting compounds in the fractions, which is essential for peak picking and identification. The fractions containing the active compound(s) obtained with conditions A and B are selected using a microbiological effect-based bioassay. The selected bioactive fractions from A and B are analysed using LC combined with high-resolution MS. Selection of relevant signals is automatically carried out by selecting all signals present in both bioactive fractions A and B, yielding tremendous data reduction. The method was assessed using two spiked feed samples and subsequently applied to different feed samples containing an unidentified compound showing microbial growth inhibition. In all cases, the identity of the compound causing microbiological inhibition was successfully confirmed.

Keywords: untargeted HRMS, antimicrobials, bioassay, animal feed

P27. LC-MS/MS method for the determination of mycotoxins in animal feed and raw materials

González Estadella M., Serret Puig R., Rodríguez Martínez P., Medina Sala M.

Government of Catalonia, Spain

Mycotoxins are secondary metabolites produced by species of the genera *Aspergillus*, *Penicillium* and *Fusarium*. They are resistant to decay during digestion, so they remain in the food chain. Mycotoxins can cause immunosuppressive, hepatotoxic, mutagenic, carcinogenic, or estrogenic effects in mammals.

A LC-MS/MS method for analyzing 12 mycotoxines in animal feed and raw materials has been developed based in the method proposed by EURL "Determination of Multiple Mycotoxins in Feed Materials and Compound Feed by LC-MS/MS. Method for the Determination of Deoxynivalenol, Aflatoxin B1, Fumonisin B1&B2, T-2 & HT-2 toxins, Zearalenone and Ochratoxin A by LC-MS/MS". These natural toxins were legislated in Directive 2002/32/CE and Commission Regulation (EU) 401/2006 and 1881/2006. To identify and confirm the presence of the analytes the criteria established in the document "SANTE/12089/2016 Guidance document on identification or mycotoxins in food and feed" has been followed.

The method consists of an extraction with ACN/Water/Formic mixture, followed by a clean-up with Oasis PRiME HLB (Waters) which is a SPE pass through. An aliquot is added with ¹³C isotopically labelled standards (isotopic dilution), then evaporated and rediluted with ACN/Water mixture. The extract is analysed by UHPLC system coupled with Xevo TQ-XS, using a column CORTECS® UPLC t3 (100 mm x 1,6 µm x 2,1 mm) in 15 minutes.

The most critical point of the analysis is the composition of the matrix. Some analytes can be masked by the effect of it or by ion suppression, due to this problem, we made the decision of introducing Prime cartridges (which retain a large part of the phospholipids and of the pigments) and isotopic dilution (that minimizes the matrix effect and the ionization problems).

Excellent linearity and good sensitivity were achieved in the quantification method validation. Recoveries between 73-149% and the lowest quantification limit was 1 µg/Kg for aflatoxins (which means a 10 µg/L standard) and the highest quantification limit was 451 µg/Kg (which means a 361 µg/L standard) for fumonisins.

67 % positives of various feed matrix from agriculture production in Catalonia were obtained during 2018. The positive mycotoxins were AfB1, DON, OTA, FB1, FB2, FB3 and HT-2.

Keywords: mycotoxins, LC-MS/MS, feed, raw materials

P28. Zinc speciation in fish feed – analytical challenges in sample preparation and compound characterization

Silva M.^{1,2}, Merel S.¹, Sele V.¹, Araujo P.¹, Amlund H.¹, Sloth J.J.³

¹*Institute of Marine Research, Bergen, Norway*

²*Department of Biological Sciences, University of Bergen, Bergen, Norway*

³*National Food Institute, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark*

Zinc (Zn) is an element essential to all living organisms and it has an important role as a cofactor of several enzymes. In fish, Zn deficiency has been associated with skeletal abnormalities and reduced activity of various Zn metalloenzymes. Fish meal and fish oil traditionally used in fish feed are being increasingly replaced by plant-based ingredients. This shift in feed composition can cause a change in the Zn level and Zn species present in the feed. Moreover, Zn additives are supplemented to fish feed to ensure adequate Zn levels for the farmed fish. Fish feed contains different Zn chemical species (i.e. contribution from ingredients and additives) and this influences the Zn availability in fish.

The present study aims to chemically characterize unknown Zn species found in fish feed. This study includes:

- optimization of an extraction method by use of fractional factorial design;
- use of a mild extraction conditions to keep Zn chemical species intact;
- characterization of Zn-containing compounds by size exclusion chromatography (SEC) coupled simultaneously with inductively coupled plasma mass spectrometry (ICP-MS) and electrospray ionization mass spectrometer (ESI-MS).

The combination of size exclusion chromatography-inductively coupled plasma mass spectrometry (SEC-ICP-MS) and electrospray ionization-mass spectrometry (ESI-MS) techniques was used to achieve on the one hand the detection of Zn-containing compound in feed and on the other hand the suggestion of potential chemical formula based on their mass to charge ratio.

Keywords: zinc species, fish feed, speciation analysis

P29. Analysis of selenoproteins in Atlantic salmon (*Salmo salar*) fed inorganic and organic selenium supplemented feeds

Sele V.¹, Perez M.², Raab A.², Mariotti M.³, Krupp E.², Feldmann J.², Sloth J.J.⁴, Ørnstrud R.¹, Berntssen M.H.G.¹, Rasinger J.D.¹

¹*Institute of Marine Research, Bergen, Norway*

²*TESLA, University of Aberdeen, Department of Chemistry, Scotland, UK*

³*Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, USA*

⁴*National Food Institute, Technical University of Denmark, Lyngby, Denmark*

Selenium (Se) is an essential micronutrient for vertebrates and fish, including Atlantic salmon (*Salmo salar*). Se is central to the functioning of selenoproteins, which play key roles in many biological functions including antioxidant defense, hormone metabolism and fish growth performance. The number and expression levels of selenoproteins vary between different animal species, where teleost fish features a higher number of selenoproteins compared to vertebrates. While selenoproteomes have been well described for many species, a comprehensive analysis of selenoproteins in Atlantic salmon has yet to be performed.

In the present study we applied high performance liquid chromatography (HPLC) coupled to both ICP-MS and high-resolution tandem electrospray mass spectrometry (HR-ESI-MS) for the direct analysis of selenoproteins in salmon liver tissues. Furthermore, we set out to combine the analytical work with theoretical in-silico predictions of selenoproteins, using computational tools, with aim for a comprehensive analysis of the selenoproteome of salmon. Liver tissues of salmon fed inorganic and organic selenium supplemented feeds have been analysed in respect to selenoproteins, and the results and analytical challenges will be presented.

Keywords: selenium, selenoproteins, salmon, HPLC-ICPMS-HR-MS, in-silico predictions

P30. Metabolomics approaches for origin verification of grain maizeSchütz D.¹, Achten E.², Horn B.², Fischer M.¹

¹University of Hamburg, Hamburg School of Food Science, Institute of Food Chemistry, Hamburg, Germany

²German Federal Institute for Risk Assessment, Department Safety in the Food Chain, Berlin, Germany

The authentication of the geographical origin of food as well as of feed is becoming more and more important. Currently, this verification is predominantly done by checking freight documents. The country of origin is to be included in reports to the Rapid Alert System for Food and Feed (RASFF, European Union) and is handled as an important part of the risk evaluation. The quality origin of corn in terms of fungal infestations is highly dependent on the climate conditions of the growing area and might be associated with a risk of the occurrence of mycotoxins. Thus, the assignment of the geographical origin is a very important issue in this context.

Metabolomics approaches are promising to find biomarkers determining the origin of feed. The metabolome is closest to the phenotype and therefore the most variable part of an organism (more than the proteome, the transcriptome, or the genome). Due to its heterogeneity in quality and quantity, a non-targeted approach, which at first just has the aim to hypothesis free maximize the coverage of metabolites, seemed reasonable. Therefore we extracted non-polar metabolites from grain maize samples collected around the world. The extract was fractionated using ultrahigh performance liquid chromatography (UHPLC) prior to on-line detection by an electrospray ionisation quadrupole-time-of-flight mass spectrometer (ESI-Q-TOF-MS). We found that especially the lipid profile was promising to differentiate the samples along their origin. The detected triglycerides, diglycerides and phospholipids were subjected to multivariate data analysis, to obtain the most promising biomarkers for origin verification. Current work is now focused on the development of targeted methods, to obtain an absolute quantitation of the obtained biomarkers. Also the reproducibility of the results along different harvest years and different maize varieties will be accessed.

Keywords: maize, authentication, metabolomics, lipidomics, LC-MS

P31. Microscopic methods and computer image analysis for distinguishing fish meals containing pelagic and farmed fish vs sea mammals (no target species)

Ottoboni M., Tretola M., Pinotti L.

*Department of Health, Animal Science and Food Safety,
University of Milan, Milano, Italy.*

The aim of this study was to investigate whether microscopic methods and computer image analysis are useful in distinguish and classify marine/fish meals containing pelagic and farmed fish (e.g. salmon) vs sea mammals (no target species). Accordingly, eight samples of controlled origin were used, namely: fishmeal (4 samples) and sea mammal meals (4 reference samples). Specifically, two fish meals were from pelagic catch (FM Latin America and FM Scandinavia), one was Mackerel meal and one Salmon meal. Sea mammal meals (i.e no target species) were obtained from Dolphin, Porpoise, Seal or Whale carcass provided by the Walloon Agricultural Research Centre - CRA-W, Belgium, European Reference Laboratory for Animal Proteins. Samples were analyzed by the microscopic method, according to Annex VI of Regulation 152/2009. Sediment fractions of each sample were observed with a compound microscope at X40. Bone fragment lacunae (n. 625) images from 8 samples (FM Latin America, FM Scandinavia, Mackerel, Salmon, Dolphin, Porpoise, Seal or Whale) were recorded and processed through an IA software. Accordingly, on each lacunae 30 geometric variables have been obtained and measured. The geometric variables have been grouped in two main families, namely: size descriptors and derived shape descriptors. Considering the size descriptors, 11 of them have shown higher mean values in pelagic and farmed fish samples (FMs, mackerel and salmon) than in sea mammal pure meals. By contrast axis minor, diameter min, radius min, size width, ferret min were lower in other fish than in sea mammals. Of note, some differences within the group of fish (FM Latin America, FM Scandinavia, mackerel and salmon) have been also observed: three size descriptors referred to lacunae area were higher in salmon lacunae than in the other fish meals. With regard to the shape descriptor, with the exception of perimeter ratio and solidity, all variables were diverse in fish and sea mammal. Therefore, it can be concluded that combining microscopy and image analysis can contribute in distinguishing fish material vs marine mammal's materials. Among fish, salmon have shown different bone lacunae size features.

Keywords: fish meals, sea mammals, advance microscopy

P32. Validation of a LC-MS/MS method for the detection of peptides from bovine-specific muscle proteins in animal feed

Leporati M.¹, Altomare A.², Degani G.², Morello S.¹, Gili M.¹, Giovannini T.¹, Amato G.¹, Aldini G.², Casalone C.¹, Marchis D.¹

¹*Istituto Zooprofilattico Sperimentale Torino*

²*Università degli studi Milano*

In the past, meat by-products have been associated to the exposure to the agent of the bovine spongiform encephalopathy. After the total feed ban of 2001, the reintroduction of not ruminant PAPs in aquaculture caused a lot of feed samples not compliant for ruminant DNA.

Regulation EU 51/2013 lays down the official methods for determination of constituents of animal origin for the official control of feed. According to it, banned PAPs can be detected by light microscopy and polymerase chain reaction (PCR). Nevertheless, even combined, these methods do not allow to discriminate between forbidden and allowed ingredients, such as milk and milk products. Mass-spectrometry can be helpful in identifying peptides from specie-specific muscle proteins, as PCR cannot distinguish the tissue origin of DNA. We identified three peptides from specie-specific muscle proteins of bovines by means of high resolution mass spectrometry (HRMS). Then a specific LC-MS/MS method for their identification in feed has been developed and validated as qualitative method according to EC/2002/657 Decision. The method can identify bovine PAP in feed at a contamination level up to 0.1% w/w. Parameters evaluated were specificity, selectivity, ruggedness and stability.

All samples used for the validation process were tested with PCR analysis, in order to exclude the presence of any constituents of bovine origin. Specificity was evaluated on twenty feeds and PAPs samples for different species to check the absence of interfering peaks. These samples were then spiked with a 100% bovine PAP 0.1% w/w and the capability of the method for identifying correctly the analytes was evaluated. Ruggedness was tested introducing seven small changes in the operating parameters (Youden approach) and assessing their influence on the results. Extracts' stability was verified after one week of storage in appropriate conditions.

The method showed to be specific for all peptides and selective for one out of three peptides; in fact, only one analyte was still detectable at the contamination level tested. It appeared to be rugged to the slight changes applied and the extracts resulted stable also after a week.

Keywords: PAPs, peptides, LC-MS/MS

P33. Monitoring bird feed for the presence of undesired and possibly viable seeds harmful for the animals or the environment

Frick G., Pradervand N., Boschung H.

Agroscope, Switzerland

For years, feed control authorities have been well aware of the environmental hazard posed by alien seeds (ex. *Ambrosia* spp.) contaminating bird feeds.

Indeed, those seeds may have retained their ability to germinate and may easily disseminate in the environment (either by the out-door disposal for wild birds or the composting of the dung). These risks applies to all the seeds in a bird feed, including desired component species. Knowing that some of the bird seed mixes and / or their components are produced in North America, the importance of checking these feeds also for the presence of GMO was admitted.

Among the recurring components found in bird feeds are the Brassicacea seeds (*Brassica napus* as well as *Brassica rapa*) and both have known GMO cultivars produced in North America. As well, small amounts of *Brassica* spp. often contaminate other seed lots, for example wheat. GM *Brassica* spp. are considered to present a high risk of colonization in Switzerland and of crossing with native wild species through pollination.

This presentation describes how *Brassica* and *Ambrosia* seeds were microscopically recognized and sorted manually, isolating 500-1000 *Brassica* seeds (3.5 g) per sample in case of a component, or a minimum of 50 seeds in case of a contaminant. *Brassica* seeds DNA were then investigated by PCR for known GM *Brassica* events. *Ambrosia* seeds were isolated from 500 g. of the feed sample and quantified.

In the course of three campaigns (spring 2017, fall 2017, spring 2018), GM-*Brassica* seeds (all authorized for feeds in the EU) were found in at least one third of the samples at a low percentage. In some cases, more than one GMO event was detected per sample. *Ambrosia* spp. monitoring is performed since more than 10 years, with a set maximum content (0.005%). The analyses performed in 2017 and 2018 show results in line with those of previous years with a few contaminated samples, rarely overstepping the maximum content.

Keywords: bird feed; ambrosia; GMO-*Brassica*; dissemination

P34. L-selenomethionine – a new feed resource for pigs with beneficial effects on the antioxidant system

Falk M.¹, Bernhoft A.², Framstad T.³, Salbu B.⁴, Wisløff H.², Kortner T.M.⁵, Kristoffersen A.B.², Oropeza-Moe M.¹

¹*Department of Production Animal Clinical Sciences, Faculty of Veterinary Medicine, Norwegian University of Life Sciences (NMBU), Sandnes, Norway*

²*Norwegian Veterinary Institute, P.O. Box 750, Sentrum, NO-0106, Oslo, Norway*

³*Faculty of Veterinary Medicine, Department of Production Animal Clinical Sciences, Campus Adamstuen, NMBU, Oslo, Norway*

⁴*Department of Environmental Sciences/CERAD CoE, Campus Ås, NMBU, Ås, Norway*

⁵*Department of Basic Science and Aquatic Medicine, NMBU, Oslo, Norway*

Modern pigs grow faster and have leaner muscle tissue, possibly causing increased levels of oxidative stress. In the last years, diseases associated with selenium (Se)/vitamin E deficiency seem to have re-emerged in feed efficient, fast-growing pigs. The aim of this study was to evaluate selenomethionine as source of organic Se in the feed of modern, fast growing, feed efficient pigs and compare its effects to those of a non-supplemented control diet and a diet supplemented with other Se sources.

Growers, LYDD (n=24, ~ 25 kg body weight (BW)) were allocated into four groups. Feed was either supplemented with Se at a level of 0.3 mg Se/mg feed from sodium selenite (Na₂SeO₃), Se-yeast, (Sel-Plex®, Alltech), selenomethionine (SeMet, Excellent Selenium4000®, Orffa) or without Se-supplementation (control). All feeds were added 100 mg VitE /kg feed. Twelve of the pigs received a single LPS-injection (2 µg/kg BW) after 6 weeks (appr. 70 kg BW). From the other twelve pigs, biopsies were obtained from the M. longissimus dorsi (LD) during the trial. Samples from LD were also collected after slaughtering. Gene expression studies were conducted on whole blood samples and all LD samples taken during the study.

In LD, the selenogenes SelenoW and H were higher expressed whereas the genes coding for interferon gamma and cyclooxygenase 2 were lower expressed in pigs fed Se-supplemented diets compared with control. In whole blood samples prior to LPS, SelenoN, SelenoS and thioredoxin reductase 1 were higher expressed in pigs fed NaSe supplemented feed compared with the other groups. After LPS exposure glutathione peroxidase 1 and SelenoN were stronger downregulated in pigs fed NaSe compared with pigs fed organic Se.

Consistent with previous reports, our results indicate that dietary Se at adequate levels can support the body's antioxidant system and that muscle fibers of pigs fed organic Se are less vulnerable to oxidative stress compared with the other groups. Currently, 0.2 mg/kg of organic Se can be added to complete pig feed. Our study and other recent reports should encourage and stimulate a discussion on Se levels and combinations of Se sources in feed for pigs.

Keywords: fast growth, pigs, gene expression, selenium

P35. Exploiting seaweed and Black Soldier Fly for sustainable salmon farming- identifying the risks of pathogen transfer within the production chain

Swinscoe I.¹, Oliver D.¹, Gilburn A.¹, Lunestad B.², Lock E.J.²,
Ørnstrud R.², Quilliam R.¹

¹*Biological and Environmental Sciences, University of Stirling, UK*

²*Institute of Marine Research, Bergen, Norway*

The global aquafeed industry traditionally relies on fishmeal for protein in the diets of farmed fish. Concerns about overfishing have seen a shift towards greater inclusion of vegetable ingredients, leading to competition with human food resources. Recent times have seen growing emphasis on sustainable feed ingredients. The Aquafly project led by the Institute of Marine Research, Norway, is exploring the commercial feasibility of rearing Black Soldier Fly larvae (BSF) on a marine macroalgae supplement, and feeding the larvae to sustainably farmed Atlantic salmon. Both stranded and cultivated seaweed represent an abundant organic material which would provide a source of Omega-3 for the larvae and therefore the marine fish diets. However, mass production of seaweed-fed insects as feed for fish in the EU is currently limited due in part to major knowledge gaps relating to potential pathogen contamination acquired from the environment, and whether these pathogens pose a risk to both fish and, ultimately, to human consumers. A feed production trial was conducted, during which an assessment of potential points of entry for, and risks of persistence of, environmental pathogenic bacteria and FIOs, was undertaken, at all stages from the harvest of raw materials to fish feed pellet production. Freshly harvested brown, red and green seaweed was free of pathogens, reflecting the high water quality standards of the harvesting site, and the seaweed powder was FIO and pathogen-free when fed to the BSF larvae. *Listeria* spp. was frequently detected in other raw feed ingredients, and was a recurrent environmental contaminant of finished products. However, larvae products and the feed pellets at the point of production were effectively decontaminated as a result of processing. This study establishes that seaweed powder manufactured from seaweed harvested at a site of 'Excellent' water quality is a microbiologically safe feed substrate for BSF larvae. Several Critical Control Points for the control of microbiological hazards in this novel insect feed are identified, which will contribute to development of Good Agricultural Practices and Good Manufacturing Practices throughout the production chain, to ultimately ensure microbial safety of seaweed feed.

Keywords: novel insect feed, microbiological safety, CCP

P36. Exploring the safety of novel feed ingredients for Black Soldier Fly larvae - temperature dependent survival of environmental pathogens on dried seaweed

Swinscoe I.¹, Oliver D.M.¹, Gilburn A.S.¹, Lunestad B.², Lock E.J.², Ørnsrud R.², Quilliam R.S.¹

¹*Biological and Environmental Sciences, University of Stirling, UK*

²*Institute of Marine Research, Bergen, Norway*

Concerns about overfishing and deforestation have prompted a shift towards sustainable aquafeed ingredients for farmed fish. Thus, the ability of insects such as black soldier fly (BSF; *Hermetia illucens*) to convert a wide range of organic substrates to valuable sources of protein and lipid resources has led to renewed interest in using insect larvae as aquafeed. Seaweed represents an abundant organic material and supplementing the diet of BSF larvae with seaweed provides a natural source of Omega-3 for the insects, and subsequent transfer to farmed marine fish, such as Atlantic salmon. However, mass production of seaweed-fed insects in the EU is limited by uncertainties relating to potential environmental pathogen contamination, which may pose a risk to fish and, ultimately, human consumers. In this study, a mixture of freshly harvested brown, red and green seaweeds were contaminated with *E. coli*, *E. coli* O157:H7, *Listeria monocytogenes* and *Vibrio parahaemolyticus*. The seaweed was subsequently hand washed, dried at a range of temperatures (20 °C, 40 °C, 50 °C, 60 °C), and stored for 72 h at ambient temperature to simulate industrial seaweed processing methods currently used for producing livestock feed. Hand washing was ineffective at removing pathogens from seaweeds, and only the 60 °C thermal treatment led to inactivation of pathogens below detection levels within 24 h, with the exception of *L. monocytogenes* which survived better at 60 °C than 50 °C. Drying for long-term storage cannot be relied on as a method of microbial disinfection of seaweed destined for use as insect feed.

Keywords: novel feed, microbiological safety, thermal challenge

P37. Tolerance and dose-response assessment of subchronic dietary ethoxyquin exposure in Atlantic salmon (*Salmo salar* L.)

Bernhard A.¹, Rasinger J.D.¹, Betancor M.B.², Caballero M.J.³, Berntssen M.H.G.¹, Lundebye A.-K.¹, Ørnstrud R.¹

¹ *Institute of Marine Research, Bergen, Norway*

² *University of Stirling, UK*

³ *University of Las Palmas de Gran Canaria, Spain*

Ethoxyquin (EQ; 6-Ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline) is one of a number of technological feed additives, which have routinely been used as an antioxidant in fish meal used in aquaculture feed production. However, to date the possible risks of EQ used in aquafeed for fish health have not yet been characterized. Taking a systems toxicology approach, the present study investigated the toxicity and dose-response of subchronic dietary EQ exposure at doses ranging from 41 to 9666 mg EQ/kg feed in Atlantic salmon (*Salmo salar* L.).

Feed at concentrations higher than 1173 mg EQ/kg were rejected by the fish, resulting in reduced feed intake and growth performance. No mortality was observed in fish exposed to any of the doses.

As a primary screening, metabolomic and proteomic profiling was performed in salmon livers, in order to explore possible treatment-related changes and metabolic pathways. The identified target pathways were subsequently investigated using traditional physiological and biochemical measures to consolidate their significance for the development of potentially adverse outcomes. The integration of the multi-omic data indicated an effect of dietary EQ on bioenergetics pathways and hepatic redox homeostasis in fish fed concentrations above 119 mg EQ/kg feed. Increased energy expenditure, associated with an activation of hepatic fatty acid β -oxidation and induction and carbohydrate catabolic pathways, was confirmed by a dose-dependent depletion of intracytoplasmic lipid vacuoles in liver histological sections, decreasing whole body lipid levels and altered purine/pyrimidine metabolism. Increased levels of GSH and TBARS in the liver indicated a state of oxidative stress, which was associated with activation of the NRF2-mediated oxidative stress response and glutathione-mediated detoxification processes. However, no indications of oxidative DNA damage were observed. In order to characterize the risk from the dietary intake of EQ in Atlantic salmon, experimental data of toxicological responses were analysed using benchmark dose models.

As manifestation of altered energy metabolism, the depletion of liver intracytoplasmic lipid vacuoles was considered the critical endpoint for benchmark dose assessment, and a BMDL10 of 243 mg EQ/kg feed was derived as a safe upper limit of EQ exposure in Atlantic salmon.

Keywords: feed additives, feed safety, benchmark dose

P38. Predictive modelling the transfer of dietary ethoxyquin and one of its main metabolites, ethoxyquin dimer, to the fillet of farmed Atlantic salmon (*Salmo salar* L.)

Berntssen M.H.G.¹, Hoogenveen R.², Bernhard A.¹, Lundebye A.K.¹, Ørnstrud R.¹, Zeilmaker M.J.²

¹ *Institute of Marine Research, Bergen, Norway*

² *RIVM, National Institute for Public Health and the Environment, Bilthoven, The Netherlands*

Ethoxyquin (ETQ; 1,2-dihydro-6-ethoxy-2,2,4-trimethylquinoline) is an antioxidant supplemented to feed ingredients, mainly fish meal, to animal feeds. ETQ is partly metabolized into several metabolites of which the ethoxyquin dimer (EQDM) is one of the dominant ones that accumulate in the fillet. The feed-to-fillet transfer of dietary ETQ in Atlantic salmon was investigated, and a predictive transfer model was established that can be used to link feed ETQ levels and upper legal limits with food safety assessments. The uptake and elimination rate kinetics were determined in seawater adapted Atlantic salmon (initial weight 213 ± 35 g) fed a ETQ enriched diet (119 mg kg^{-1}) for 90 days, followed by a 90 days depuration period with feeding on control diets. A two-compartmental model, based on a fat and central compartment with and intake and metabolism of ETQ into EQDM was developed. Model calibrations showed a good fit with measured values during overall uptake and elimination period, and the model was verified by using another dataset on ETQ and EQDM accumulation in Atlantic salmon. The model showed a good verification fit in fish fed ETQ in the range of 18 to 108 mg kg^{-1} , however when fed excessive levels (1800 mg kg^{-1}) the model over predicted the actual EQDM and ETQ uptake. The model was used to simulate the long term (>16 months) ETQ EQDM feed-to fillet transfer in Atlantic salmon under realistic farming conditions such as the seasonal fluctuations in feed intake, growth, and fillet fat deposition. The model predictions showed that initial EQDM levels in juvenile fish are the driving factor in final levels found in food producing animals. The model can be used to link feed EQ supplementation and legal limits for food safety assessments.

Keywords: ethoxyquin, salmon transfer model

P39. Fur animal botulism – an outbreak report

Kukier E.¹, Goldsztejn M.¹, Kozieł N.¹, Dębski J.², Kwiatek K.¹

*¹Department of Hygiene of Animal Feedingstuffs,
National Veterinary Research Institute, Pulawy, Poland*

*²Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics,
Polish Academy of Sciences, Warsaw, Poland*

The reservoirs of botulinum neurotoxin-producing Clostridia are fishes, birds, mammals and their slaughter by-products, what pose potential threat for carnivores. As yet, the fur animal botulism has been reported due to botulinum toxin (BoNT) type A, B, C, CD and E. The present paper reports the investigation of mink botulism outbreak. In summer 2016 botulism was suspected in five farms in Poland. All minks were immunized against BoNT type C and all of these farms were supplied with feed by the same feed processing plant. The feed contained poultry slaughter by-products, fish by-products, extruded cereals, dried haemoglobin, fishmeal, meal of pork, acidifier and preservative. The mink feed as a ready-to-use product was unpasteurised. Laboratory tests included animal sera, internal organs and implicated feed. Presence of BoNT was confirmed by mouse bioassay in serum (farm A and B), liver extract (farm A, B, C and D) as well as feed extract (farm B and E). The serotyping of positive serum revealed presence of BoNT type E and F in farm A and BoNT type C and F in farm B. Moreover, the presence of BoNT protein in pooled serum from farm B was detected by LC-MS/MS method, where non-toxic non-haemagglutinin protein of C. botulinum was revealed. The animal botulism has been diagnosed in Poland since 10 years, but these mink botulism were the largest laboratory confirmed outbreaks so far. To the best of our knowledge, this is the first reported mink botulism outbreak due to BoNT type F.

Keywords: mink botulism, BoNT, MBA, LC-MS/MS

P40. Diagnosis of an outbreak of bovine botulism

Kukier E., Goldsztejn M., Kozieł N., Kwiatek K.

*Department of Hygiene of Animal Feedingstuffs,
National Veterinary Research Institute, Pulawy, Poland*

Animal botulism is life-threatening flaccid paralysis caused by ingestion of botulinum neurotoxin (BoNT) or botulinum neurotoxin-producing Clostridia spores. The reservoirs of the pathogen are fishes, birds and mammals as well as decaying carcasses occasionally occurred in poultry litter. Crops fertilization with poultry litter can induce botulism of animals due to contaminated grass, hay or silage. This paper reports the diagnosis of two cattle botulism outbreaks, where totally more than 350 animals have died. In summer 2017 the disease was suspected in two farms (A and B) in the Mazovia region of central Poland. Animals were not immunized against botulism and fields of corn crops were fertilized with poultry litter, which was both utilised in farm biogas plant and obtained digestate was repeatedly applied on fields with silage plants. The animals were fed with corn silage, grain corn silage, grass silage, rapeseed meal, protein concentrate as well as mineral-vitamin premix.

For laboratory diagnostics were collected animal serum, liver, spleen, kidney, lumen, content of small intestine, rumen content as well as implicated feeds. Official veterinary control involved milk powder, cream powder, butter and swabs from dairy plant processing implicated milk. Presence of BoNT was confirmed by mouse bioassay in serum and liver extract of farm A animals and culture of intestinal content of farm A and B animals. The positive samples revealed presence of BoNT type C and D. The animal botulism has been diagnosed in Poland since 10 years, and these bovine botulism were the largest laboratory confirmed outbreaks so far.

Keywords: cattle botulism, BoNT, MBA

P41. Application of pesticide Regulation (EC) No 396/2005 to animal feed materials

Meijer N., Adamse P., van Egmond H., Bikker P.

RIKILT - Wageningen University & Research, Wageningen, The Netherlands

Regulation (EC) No 396/2005 lays down Maximum Residue Limit (MRLs) for pesticides in or on food and feed materials/products of plant and animal origin. The MRLs in this Regulation apply to whole products or specific parts of the products. The application of this legislation to animal feed materials is complex. In many cases, feed materials are a processed form of a raw product. In principle, these feed materials are covered by the respective MRLs if they are derivative of the part of the product to which MRLs apply, as described in the pesticide Regulation. It was therefore assessed for the most relevant feed materials from the Catalogue of feed materials whether they are covered by the specification of the part of the product to which pesticides MRLs apply. Several products were identified for which this was not the case.

Subsequently, the legal framework for processing factors for feed materials was analysed. Since processing may concentrate or dilute the concentration of pesticides in derivative products compared to the original unprocessed form, processing factors should be applied. Although a legislative procedure for the inclusion of a list of processing factors in Annex VI of the Regulation is provided for; this list has thus far not been created. This makes harmonized enforcement difficult. Although published lists of processing factors are available, they appear to be primarily oriented on food rather than feed. The practical applicability of these processing factors to feed materials was assessed by comparing them to derivative products of three representative feed commodities (soy, wheat, sugar beets).

Keywords: pesticides, processing factors, legislation, animal feed

P42. Occurrence of *Alternaria* and *Fusarium* toxins in grains used in Slovenia in 2014–2016

Babič J., Grandič M., Pavšič-Vrtač K., Tavčar-Kalcher G., Jakovac-Strajn B.

University of Ljubljana, Veterinary Faculty, Ljubljana, Slovenia

Mycotoxins are naturally occurring secondary fungal metabolites, which can contaminate various food and feed commodities, such as grain and grain-based products, fruits, vegetables, etc. Besides other well-known mycotoxins, also less studied *Alternaria* mycotoxins gain more and more interest due to their possibility to induce toxic effects for animal and public health concern [1].

Cereal grains samples were collected in cooperation with Slovenian farms and agriculture cooperatives. Six cereal commodities (wheat, barley, triticale, rye, spelt and oat) were included in three-year study between 2014 and 2016. Using a quantitative LC-MS/MS method, 13 *Alternaria* and *Fusarium* toxins were determined: alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), tentoxin (TTX), deoxynivalenol (DON), 3-acetyl DON, 15-acetyl DON, diacetoxyscirpenol (DAS), HT-2 and T-2 toxins, fumonisins (FB1 and FB2), and zearalenone (ZEA). The aim of the survey was to inform Slovenian farmers and feed industry about occurrence of mycotoxins in feed samples and to identify contamination level and the co-occurrence of different mycotoxins.

Among 433 tested samples, a total of 265 (61%) samples were contaminated with at least one mycotoxin. Positive sample occurrence rate over total sample number was 26% in 2014, 19% in 2015 and 16% in 2016. Regarding co-occurrence, 137 samples contained one, 75 samples contained two and 53 samples contained three or more mycotoxins. Furthermore, the most represented cereal grain samples, wheat, barley and triticale, were mainly contaminated by four *Alternaria* toxins, DON, and ZEA, whereas DAS, HT-2, T-2, FB1, and FB2 were not detected. The maximum determined concentrations of *Alternaria* toxins TeA, TTX, AOH and AME were 1053 µg/kg (barley, 2015), 61 µg/kg (wheat, 2016), 150 µg/kg (triticale, 2014) and 115 µg/kg (triticale, 2014), respectively. The maximum determined concentrations of DON and ZEA were 4082 µg/kg (wheat, 2015) and 300 µg/kg (triticale, 2014), respectively.

[1] European Food Safety Authority (EFSA), 2011. Scientific opinion on the risk for animal and public health related to the presence of *Alternaria* toxins in feed and food. EFSA J 9: 1–97.

Keywords: mycotoxins, cereals, occurrence, LC-MS/MS, Slovenia

P43. Development of a method for quantification of quinolizidine alkaloids in lupines and lupine products

Fry H., Klis V., Kolrep F., Schwieters M., Preiß-Weigert A.

German Federal Institute for Risk Assessment, Berlin, Germany

Lupines belong to the family of legumes grown for animal and human consumption and as green manure crops. In nutrition they are a resource for proteins and an alternative to soybean. Sweet lupines, dehulled sweet lupines and lupine hulls, pulp, middlings, protein and protein meal can be used as feed material for pigs, cattle, sheep, chickens and fishes. All lupines contain as secondary plant metabolites quinolizidine alkaloids (QA), piperidine alkaloids and indole alkaloids. Only limited data on the toxicity of QA both in animals and humans are available, but the toxicity in humans can be assumed to be higher than in animals.

The quinolizidine alkaloids are biosynthesized in the green tissues of plants and stored in all organs, especially seeds are rich in QA. Up to now around 200 QA-structures are identified and the content of alkaloids in for their bitter taste so-called “bitter lupines” can reach a level up to 30.000 mg/kg. Of approximately 450 lupine species only four are suitable for consumption. By genetic selection alkaloid reduced sweet varieties of the white lupine (*Lupinus albus*), blue lupine (*Lupinus angustifolius*), yellow lupine (*Lupinus luteus*) and pearl lupine (*Lupinus mutabilis*) were obtained.

The pattern of QA in the different species is well characterized and varies significantly between species. The major alkaloids in the white and blue lupine are lupanine, 13 α -hydroxylupanine, angustifoline and α -isolupanine, and in the yellow lupine, lupinine and sparteine. In all lupine species a number of minor alkaloids were identified.

In cultivars the QA content depends on the geographical origin and environmental factors. Identification of the different QA is performed primarily by gas-chromatography mass spectrometry. Since pure standard substances of the major alkaloids are only recently commercially available so far quantification is primarily based on relative concentrations. In this project the applicability of methods for analysis using GC-MS and LC-MS/MS was investigated. The quantification of lupanine, 13 α -hydroxylupanine, isolupanine, lupinine, sparteine and cytisine was performed by means of external standardization.

Keywords: quinolizidine alkaloids, lupine, LC-MS/MS

P44. The susceptibility of different maize varieties for the fusarium mycotoxins

Reiter E.V., Mechtler K., Felder H., Kuchling S., Strnad I.

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The fusarium mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and fumonisins (FUM) are known to be the most abundant mycotoxins in Austria. Despite, the visual susceptibility for cob surface rot which was already comprised in the registration procedure of maize varieties the contents of mycotoxins were not considered until 2011. Within a three-year project (KOFUMA) a methodology was developed to evaluate the susceptibility to ear fusariosis by both namely the affected cob surface and the fusarium mycotoxins contents. In total, about 1000 maize samples (from existing varieties and candidates) are taken annually at over 33 different locations in Austria belonging to the three different climatic areas. The samples are analysed for DON, ZEA and FUM content by ELISA. In addition, the visual susceptibility is assessed on all trial sites.

Out of the results relative values on ear fusariosis and mycotoxin content are calculated and transferred into a 9-stage scale. The final grade of each maize variety is calculated from the cumulative grades of each criterion and location. The present methodology is applied on the classification of maize varieties since in 2011.

Keywords: mycotoxins, maize, variety testing, feed safety

P45. Risk based monitoring of mycotoxins in animal feed

van der Fels-Klerx H.J., Adamse P., Punt A., de Jong J., van Asselt E.D.

RIKILT Wageningen University & Research, Wageningen, the Netherlands

Following legislation, European Member States should have control programs for contaminants, such as for heavy metals and mycotoxins, in feed. These programs need to be risk-based implying the checks are regular and relative to the (estimated) risk for animal and human health.

The objective of this study was to rank feed ingredients for deoxynivalenol and aflatoxin B1 monitoring in the Netherlands. Historical mycotoxin monitoring results from the period 2007-2016 were used, and combined with data from other sources. Occurrence data on these mycotoxins were used for trend analyses of mycotoxin levels in ingredients and compounds feeds over time. These occurrence data were also used as input in a risk model to prioritize the ingredients based on animal and human health risks related to the occurrence of the mycotoxin in the particular ingredient.

Results showed that, based on occurrence data, groundnuts had high priority for aflatoxin B1 monitoring; some feed materials (maize and maize products and several oil seed products) and complete/complementary feed excluding dairy cattle and young animals had medium priority; and all other animal feeds and feed materials had low priority. For monitoring deoxynivalenol, high priority was given to maize by-products, whereas complete and complementary feed for pigs had medium priority, and all other feed and feed materials had a low priority. Also including health consequences showed that feed materials that ranked highest for aflatoxin B1 monitoring included sunflower seed and palmkernel expeller/extracts and maize. For deoxynivalenol monitoring, maize products were ranked highest, followed by various small grain cereals (products); all other feed materials were of lower concern. Results of this study are used in setting up the annual risk based control program for mycotoxins in animal feed and feed materials in the Netherlands.

Keywords: risk based monitoring, feed safety, contaminants

P46. Systems toxicological assessment of methylmercury neurotoxicity and the influence of selenium in wild type and OCTN1 knockout zebrafish

Rasinger J.D.¹, Lie K.K.¹, Ellingsen S.^{1,2}, Imamura S.³, Seko T.³, Yamashita M.³, Lundebye A.K.¹, Amlund H.¹

¹*Institute of Marine Research, Bergen, Norway*

²*Department of Biology, University of Bergen, Bergen, Norway*

³*National Fisheries University, Shimonoseki, Japan*

Methylmercury (MeHg) is a toxicant of concern for aquatic food chains. The toxicity of MeHg, including neurotoxicity, is well documented, and the ameliorating effects of selenium (Se) have been described; yet the molecular mechanisms underlying this contaminant nutrient interaction still remain largely uncharacterized. The present study used a combination of element analysis, high throughput multi-omics and bioinformatics to describe mechanisms of action of MeHg neurotoxicity in the presence and absence of Se using zebrafish (ZF; *Danio rerio*) as a model. Effects of MeHg were investigated both in wild type (wt) and organic cation transporter (OCTN1) knockout (ko) fish. OCTN1 plays an important role in cholinergic neurotransmission and has been implicated in MeHg toxicity and Se-MeHg interactions. In total 252 fish (126 wt and 126 OCTN1 ko) fish were exposed for two, eight and 12 weeks to MeHg (5 mg kg⁻¹ or 10 mg kg⁻¹) or an unspiked control. Brains of fish (both wt and OCTN1-ko) from the eight week exposure groups were subjected to multi-omics analyses to assess the role of OCTN1 in MeHg neurotoxicity. In a follow up experiment, OCTN1-ko fish (n=226) were exposed to MeHg (10 mg kg⁻¹) and Se (5 mg kg⁻¹) in a full factorial design for two, eight and 12 weeks to investigate the influence of dietary Se on the accumulation and toxicity of MeHg in brain in the absence of OCTN1. Similar to the first trial, multi-omics tools were used to study the mechanisms underlying MeHg induced neurotoxicity and their modulation by Se. The data obtained in the present work will contribute to the understanding of the physiological role of the organic cation transporter OCTN1 in MeHg neurotoxicity and provide molecular explanations for the observed protective role of dietary Se on MeHg toxicity.

Keywords: mercury, selenium, element analysis, multi-omics

P47. Trichothecenes and ergot alkaloids in Norwegian cereal grains of potential concern for animal health

Bernhoft A., Christensen E., Ivanova L., Er C., Torp M.

Norwegian Veterinary Institute

The Norwegian Veterinary Institute analyses and reports results on mycotoxins and fungi in cereal grains for the Norwegian Food Safety Authority's yearly surveillance programme. In 2017, oats, barley, wheat and rye were analysed. Oats had higher levels of trichothecene mycotoxins than barley and wheat, and in rye these mycotoxins were generally not detectable. Deoxynivalenol (DON) and T-2 toxin (T-2) + HT-2 toxin (HT-2) were the major trichothecenes detected. However, the level of DON was at the lower end and that of T-2 + HT-2 within the range measured in cereal grains during the last 16 years. Also certain levels of DON-related compounds were found, and these compounds showed significant positive correlation with DON. Exposure to trichothecenes causing gastrointestinal signs such as reduced feed intake and growth rate are well documented in farmed animals. T-2 and HT-2 have similar but potentially stronger toxic effects than DON, in causing gastrointestinal lesions as well as immune suppression. Ergot alkaloids are of increasingly considerable interest in EU and their occurrence data are desired. They show moderately acute toxicity with neurotoxic properties, and may inhibit blood circulation and interfere with hormonal levels. Ergot alkaloids were hardly detected in the oats but were detected in some samples and at a similar levels in barley, wheat and rye. The maximum concentrations of ergot alkaloids may be worth attention but no legislated levels exist currently. The producer of ergot alkaloids, *Claviceps purpurea*, was detected in most samples of rye and about half of the wheat samples. However, no significant correlations were found between ergot alkaloids and *C. purpurea* in the cereal samples. Thus, the legislative control of ergot alkaloid levels instead of on *C. purpurea* sclerotia would improve the risk management.

Keywords: trichothecenes, ergot alkaloids, cereals, animal health

P48. Detection of artificial fibres in fish meal

Marchis D.¹, Mandrile L.², Rossi A.M.², Giovannini T.¹, Amato G.¹, Ferrari A.¹, Abete M.C.¹, Cicero C.³, Mattalia G.⁴, Scasciamacchia S.⁴, Veys P.⁵

¹*Istituto Zooprofilattico Sperimentale Torino*

²*Istituto Nazionale Ricerca Metrologica*

³*Ministero della Salute Roma*

⁴*PIF Genova*

⁵*European Union Reference laboratory on animal proteins in feed, Gembloux*

The EU feed production relies for most of the Member States on fish meal import. Six different fish meals, coming from Chile and from Mauritius island were sampled at the Genova Border Inspection Post to be officially analyzed for detection of terrestrial animal constituents. Fibres of different length uniformly distributed into the fish meal were visible by eye all the six samples, and two of them were so heavily contaminated that balls of those fibres could be seen into the container. The analyses were performed with official microscopy method according to Regulation (EU) 51/2013. All samples were found to be seriously contaminated by fibres which showed to be highly birefringent, with regular diameter, no medulla, and absence of scales; when structural surface details appeared to be present, they looked too regular for being from natural origin. Some of those fibres looked brightly coloured. In order to identify the nature of the fibres detected, FTIR in Attenuated Total Reflectance mode was applied. IR technology can in fact provide complete information on the chemical constituents in a sample scanned, it is thus a convenient tool for characterising food and feed materials (Andrés et al. 2007; Wu et al. 2008) in terms of very specific fingerprint. Unknown fibres were analysed in medium infrared spectroscopy using IN10 spectrometer (ThermoFisher Scientific) with a DTGS detector, a room temperature, spectral resolution 4 cm⁻¹, exposure time 12 seconds for 64 accumulation scan. Spectra were recorded in the spectral range 4000–400 cm⁻¹. Fibres were mechanically removed from the fish meal, then samples were placed under 10X objective and connected with the Ge ATR tip. A qualitative analysis was carried out. The spectra collected were then compared to those of reference materials. Distinctive IR signals revealed those fibres as polyester. The reason why those fish meals, coming from two different part of the globe, were found so heavily contaminated with polyester fibres is unknown. It was supposed fish was processed together with fishing nets.

Keywords: fish meal; artificial fibres

P49. A first monitoring of cobalt in Italian feed

Capra P., Brizio P., Stella C., Rizzi M., Gavinelli S., Colombero G.,
Squadrone S., Abete M.C.

Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino

Cobalt (Co) is the core element of cobalamin (vitamin B12) and it is essential for certain animal species (mainly ruminants and horses and, to a lesser extent, pigs and poultry) which can synthesize this vitamin in the digestive track.

Co containing compounds have been authorized as feed additives for ruminants, equines, lagomorphs, rodents, herbivore reptiles and zoo mammals by the European Commission with a maximum content of 1 mg kg⁻¹ of complete feed with a moisture content of 12%. Moreover, permitted tolerances with respect to the compositional labelling has been set by Regulation (EC) No 767/2009.

As established by Regulation (EC) No 178/2002 and Regulation (EC) No 882/2004, the Italian Ministry of Health draw up a National Animal Feed Plan (PNAA) in order to control the entire feed chain and ensure human, animal and environmental safety. This plan includes the research of elements allowed in animal nutrition as feed additives at maximum and/or minimum content (Iron, Copper, Manganese, Zinc, ...); Co has been included in the list of metals to be monitored only in 2018.

The National Reference Centre for the Surveillance and Monitoring of Animal Feed (C.Re.A.A.), which is also National Reference Laboratory (NRL) for Heavy Metals in Feed and for Feed Additives Authorization, has developed, validated and accredited an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) method for quantification of Co in feed, in order to establish compliance to European legislation.

The first nine months of activity will be presented and discussed, including several cases of non-compliance to tolerances and maximum limit.

Keywords: Cobalt, Italy, ICP-MS

P50. Development of an extraction method for selenium speciation in feed

Brust H., Frankhuizen S., Van der Lee M.

RIKILT Wageningen UR, Wageningen, The Netherlands

Selenium is an essential nutrient for both humans and animals, however at higher concentrations selenium can be toxic. Organic selenium species, incorporated in proteins, are more effectively taken up by the body than inorganic selenium. Therefore, selenized yeast is often added to feed, providing a source of organically bound selenium.

Addition of selenium to feedingstuffs is limited to a maximum concentration of 0.5 mg total Se/kg feed and a maximum of 0.2 mg organic Se/kg feed (EU 2015/489). To control whether feedingstuffs comply with this legislation there is a need for a method to discriminate between different selenium species. Elemental speciation is commonly performed by online coupled HPLC-ICP-MS.

This approach was used for selenium speciation as well. We have developed a method for the separation of the most common selenium species using HPLC-ICP-MS. The anion exchange method separates the organic species selenomethionine, selenocysteine and seleno-DL-cystine. The inorganic species selenite and selenate are included in the method as well. Sensitive detection of the selenium species was achieved by operating the ICP-MS in DRC mode using methane as reaction gas.

The challenge with selenium speciation is the extraction of the organic species from the matrix. The protein-bound selenium species are usually extracted from the matrix using enzymes, but issues with recoveries and reproducibility have been reported. We have tested several methods for the extraction of selenium species from feed samples. The inorganic selenite and selenate usually show recoveries close to 100%. For selenomethionine, the most common organic selenium species, recoveries in reference materials using enzymatic extraction are about 80%. However, the type of matrix influences the enzymatic extraction, shown by highly variable recoveries of selenomethionine when mixing the reference material with different feed samples. The different extraction methods will be compared and challenges will be discussed.

Keywords: selenium speciation, feed, method development

P51. Sensitive analysis of veterinary drugs in feedstuff on high resolution mass system

Macaluso A., Giaccone V., Cicero A., Pantano L., Calabrese I.,
Lo Dico G., Camilleri G., Ferrantelli V.

Istituto Zooprofilattico Sperimentale della Sicilia, Palermo, Italy

Determination of veterinary drug residues in feed is essential to safeguard animal welfare and to avoid consumer health risks. Appropriate analytical methods are essential to support the enforcement of regulations. The use of liquid chromatography coupled to high-resolution accurate mass spectrometry (HRAM) has emerged as a successful alternative for the multiclass/multianalyte analysis in food and feed safety and environmental control. However, the complexity of biological matrices makes it difficult to guarantee a high sensitivity. Even though data-dependent acquisition (DDA) is a powerful technique, contaminants and interference may cause an increase in background noise and an overall reduction in sensitivity. Selective/Multiple reaction monitoring (SRM/MRM) methods mainly on triple-quadrupole (QqQ) mass spectrometers are the common strategies that have been applied for the identification and detection of veterinary drugs. However, in a SRM/MRM assay, only a limited number of transitions are monitored for each analyte and the interferences may confound the data analysis. More recently, the parallel reaction monitoring (PRM) assay has emerged as an alternative method of targeted quantification. Studies using SRM and PRM have shown that both these targeted methods are comparable in performance. But, PRM-based targeted mass spectrometry provides high selectivity, high sensitivity and high performance of quantification. In this study, we have investigate the performance of PRM on HRAM for routine analysis of veterinary drugs in feed. In these complex matrices, the co-eluting ions were present in high abundance and they interfere with the signal from the analyte. Since, in SRM, only a limited number of transitions are monitored, the interferents make it difficult the analysis. For this reason, we tried to apply the PRM mode in a second determination on HRAM. In PRM analysis, limited information about the analytes are necessary, which precursor ion m/z and elution time range but it is need to optimize some instrumental parameters. The present results indicate how monitoring procedure in PRM mode can be adapted to maximize sensitivity or selectivity of determination methods also of veterinary drugs with highly reliable qualitative and quantitative results. The PRM performed on quadrupole-Orbitrap mass spectrometers offers a clear advantage over the conventional SRM measurements executed on same instrument.

Keywords: veterinary drugs, feedstuff, HRMS

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List of Participants

Participant

Achten, Elisabeth
 Albrektsen, Sissel
 Amlund, Heidi
 Ardizzone, Michele
 Atkinson, Angela
 Baeten, Vincent
 Belghit, Ikram
 Bernhoft, Aksel
 Berntssen, Marc
 Biancarosa, Irene
 Brust, Hanneke
 Capra, Pierluigi
 Dahm, Georges
 Danielsen, Elin
 de Jong, Jacob
 Dillon, Gerry
 Dunn, Greg
 Fournier, Annie
 Frick, Geneviève
 Fry, Hildburg
 Fumiere, Olivier
 Gilburn, Andre
 Gjerde, Jennifer
 Gruven, Kåre
 Haaland, Herborg
 Haldorsen, Randi Nordstoga
 Hansen, Ann-Cecilie

Institution

Federal Institute for Risk Assessment (BfR), Germany
 Nofima, Norway
 Institute of Marine Research, Norway
 European Food Safety Authority
 Nuproxa Switzerland
 Walloon Agricultural Research Centre
 Institute of Marine Research, Norway
 Norwegian Veterinary Institute
 Institute of Marine Research, Norway
 Institute of Marine Research, Norway
 RIKILT – Wageningen University & Research
 Istituto Zooprofilattico Sperimentale PLVA
 Laboratoire National de Santé, Luxembourg
 Institute of Marine Research, Norway
 RIKILT Wageningen University & Research
 Alltech
 BDC AGRI
 Canadian Food Inspection Agency
 Agroscope
 Federal Institute for Risk Assessment (BfR), Germany
 Walloon Agricultural Research Centre
 University of Stirling
 Institute of Marine Research, Norway
 NRS ASA
 Cargill Aqua Nutrition
 Marine Harvest
 Norwegian Food Safety Authority

Helland, Ellinor	BioMar AS
Hemre, Gro-Ingunn	Institute of Marine Research, Norway
Hinterberger, Thomas	Official Swiss Feed Control
Hosotani, Guilherme	Cargill
Koch, Marianne Wethe	Cargill
Koeleman, Emmy	Proagrica (All About Feed)
Kousoulaki, Katerina	Nofima
Krogdahl, Åshild	Norwegian University of Life Sciences (NMBU)
Kukier, Elzbieta	National Veterinary Research Institute, Poland
Kvamme, Elin	Alltech Norway AS
Larsen, Natalia	Institute of Marine Research, Norway
Larsen, Øystein	Alltech Norway AS
Lecrenier, Marie-Caroline	Walloon Agricultural Research Centre
Leporati, Marta	Istituto Zooprofilattico Sperimentale Torino, Italy
Lie, Kai	Institute of Marine Research, Norway
Lien, Kirsten Skogan	SYNLAB AS Stjørdal
Lundebye, Anne-Katrine	Institute of Marine Research, Norway
Macaluso, Andrea	Istituto Zooprofilattico Sperimentale della Sicilia, Italy
Medina Sala, Mirea	Laboratori Agroalimentari -DARP, Spain
Meijer, Nathan	RIKILT Wageningen University & Research
Merel, Sylvain	Institute of Marine Research, Norway
Miladinovic, Dejan	Norwegian University of Life Sciences (NMBU)
Mosies, Charlotte	Nederlands Normalisatie-instituut (NEN)
Niedzwiecka, Alicia	Federal Institute for Risk Assessment (BfR), Germany
Nordbø, Elisabeth	Fiskå Mølle
Ørnstrud, Robin	Institute of Marine Research, Norway
Oterhals, Åge	Nofima, Norway
Ottoboni, Matteo	University of Milan, Italy
Pinotti, Luciano	University of Milan, Italy
Prabhu, Antony	Institute of Marine Research, Norway
Rasinger, Josef	Institute of Marine Research, Norway

Richardson, Finlay	University of Stirling
Samuelson, Tor Andreas	Nofima, Norway
Sebastien, Dubois	Agroscope
Sele, Veronika	Institute of Marine Research, Norway
Silva, Marta	Institute of Marine Research, Norway
Sloth, Jens	DTU Food
Søfteland, Liv	Institute of Marine Research, Norway
Strachan, Fiona	Institute of Aquaculture, UK
Strnad, Irmengard	AGES GmbH - Institut for Animal Nutrition and Feed
Tavčar-Kalcher, Gabrijela	Veterinary Faculty, University of Ljubljana, Slovenia
Tienstra, Marc	RIKILT – Wageningen University & Research
Tretola, Marco	University of Milan, Italy
van Macklenbergh, Odette	Glencore Agriculture BV
van Mil, Job	Alltech Coppens
van Raamsdonk, Leo	RIKILT – Wageningen University & Research
Venemans, Annemieke	CEN/TC 327
Veys, Pascal	Walloon Agricultural Research Centre
von Holst, Christoph	JRC Geel
Waagbø, Rune	Institute of Marine Research, Norway
Weesepeel, Yannick	RIKILT – Wageningen University & Research
Wegh, Robin	RIKILT – Wageningen University & Research
Whatmore, Paul	Institute of Marine Research, Norway



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