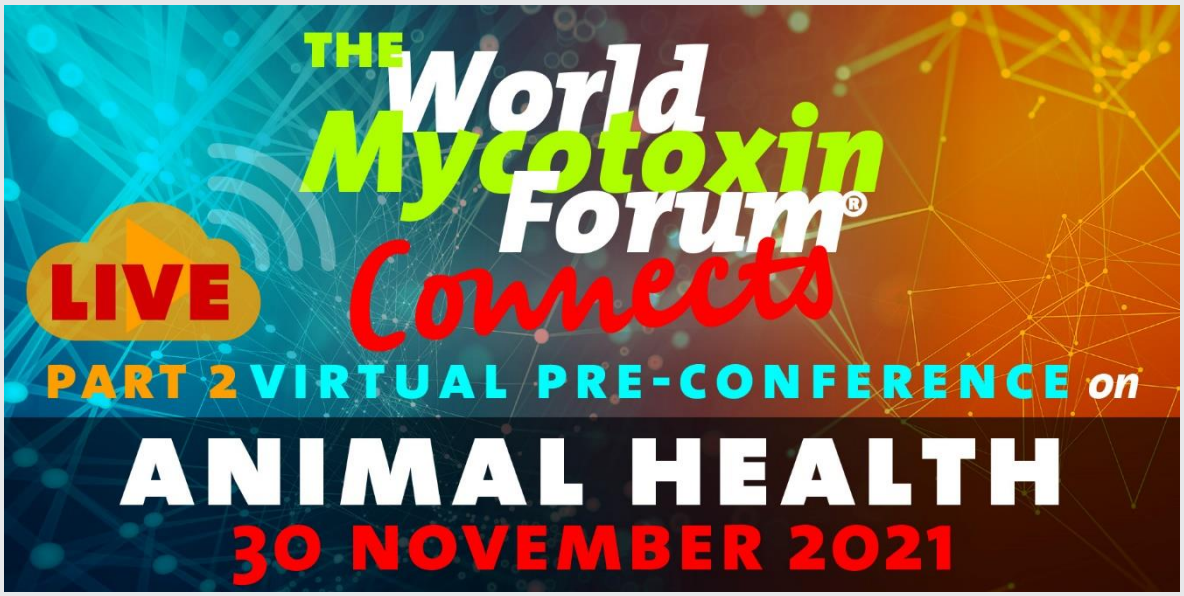
BOOK OF ABSTRACTS



Mycotoxins – ongoing issues of animal health and productivity

ANIMAL HEALTH, the second in the series of virtual pre-conferences preceding WMF*meets*ITALY, the in-person conference of The World Mycotoxin Forum®, 16-18 May 2022, Parma, Italy

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THE WORLD MYCOTOXIN FORUM® *CONNECTS*

The World Mycotoxin Forum® is the leading international meeting series on mycotoxins dedicated to assembling the world's best minds across the spectrum of integrated strategies ensuring the safety and security of the food and feed supply chain. **The World Mycotoxin Forum®** brings together a holistic conference programme covering the latest issues in mycotoxin management and is targeted at everyone working in the mycotoxin space - researchers, food and feed industry, laboratories, policy makers, and enforcement agencies from around the world.

Since the previous conferences of **The World Mycotoxin Forum®** in Belfast (October 2019) and in Bangkok (January 2020), the world has changed due to the COVID-19 outbreak. We haven't met each other for quite while but the "Times they are a- (hopefully) changin" (to paraphrase the legendary singer-songwriter Bob Dylan). Therefore, we are happy to announce that the 13th conference of **The World Mycotoxin Forum®** – WMF*meets*Italy – takes place IN-PERSON again. Mark your calendar: 16-18 May 2022, Parma, Italy.

What's happening in the meantime? In the run-up to the in-person conference, **The World Mycotoxin Forum®** will present virtually three one-day pre-conferences focusing on specific mycotoxin topics to keep you informed and connected:

- Human exposome, 12 October 2021
- Animal health, 30 November 2021
- Analysis, 1 February 2022

These three pre-conferences will be held on a highly interactive platform with great networking opportunities.

The General Conference Chairs – Prof. Rudolf Krska and Prof. Chris Elliott – and the members of the Steering Committee and the Advisory Committee are looking forward to getting you informed and connected.

See you in the cloud for the three pre-conferences and in Parma for the in-person event!

Rudolf Krska
Chris Elliott
General Conference Chairs

PROGRAMME

All times are in Central European Time (CET)

12:00 **The World Mycotoxin Forum® *Connects***
General conference chairs: Prof. Rudolf Krska, Department IFA-Tulln, BOKU Vienna, Austria and Prof. Chris Elliott, Institute for Global Food Security, Queen's University of Belfast, Northern Ireland

12:15 *Mycotoxins – ongoing issues of animal health and productivity*
Pre-conference chair: Prof. Isabelle Oswald, Toxalim Research Centre in Food Toxicology, France

12:25 *Advantages and limitations of biomarker monitoring in pigs in the assessment of animal exposure to mycotoxins*
Dr Piotr Jedziniak, Department of Pharmacology and Toxicology, National Veterinary Research Institute, Poland

12:50 *Mycotoxins in feed: impact on animal health and animal source foods in developing countries*
Dr Johanna F. Lindahl, International Livestock Research Institute, Kenya and Swedish University of Agricultural Sciences, Sweden

13:15 *The effects of mycotoxins on dairy cattle*
Dr Antonio Gallo, Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Italy

13:40 *Toxic effect of aflatoxins in dogs fed contaminated dry feed*
Prof. Arturo Gerardo Valdivia-Flores, Centro de Ciencias Agropecuarias, Universidad Autonoma de Aguascalientes, Mexico

14:05 *Deleterious effects of mycotoxins on the intestine of farm animals*
Dr Philippe Pinton, Toxalim Research Centre in Food Toxicology, INRAE, France

14:30 **EXHIBITION:** Visit the booths and live chat with our sponsors.
PIAZZA CONNECTS: Meet & Greet the chairs, the speakers and the WMF community.

15:00 Company pitches

- **R-Biopharm:** *Managing mycotoxins for petfood*
  Ronald Niemeijer, M.Sc.
- **Biomin:** *Cutting-edge science, absolute protection*
  Verena Starkl, M.Sc.
- **Adisseo:** *Mycotoxin management is not a betting game – Adisseo helps you identify the risks and adopt the best strategy*
  Dr Olga Averkieva
- **Trouw Nutrition:** *Trouw Nutrition – innovations driven by customers*
  Pedro Caramona
- **Alltech:** *The evolution of mycotoxin management strategies, and beyond*
  Dr Alexandros Yiannikouris
- **Phileo by Lesaffre:** *Yeasts fractions to mitigate mycotoxins in animal feed: new insights*
  Dr Virginie Marquis

15:30 *Ergotism in livestock – an insight on potential mechanisms*
Dr Ahmad Al-Dissi, Department of Veterinary Pathology, University of Saskatchewan, Canada

15:55 *Zearalenone and pigs: to reprotoxicity and beyond*
Dr Laura Soler-Vasco, Toxalim Research Centre in Food Toxicology, INRAE, France

16:20 *Mycotoxins in poultry – recent update on gut health and immunity*
Dr Swamy Haladi, Trouw Nutrition, India

16:45 *Effects of mycotoxins on fish – what do we know so far?*
Dr Constanze Pietsch, Aquaculture Group, Bern University of Applied Sciences, Switzerland

17:10 *Promising detoxification of Fusarium mycotoxins in maize silage: biological transformation of deoxynivalenol and zearalenone*
Dr Yan Zhu, Guelph Research & Development Centre, Agriculture and Agri-Food Canada, Canada

17:35 **EXHIBITION:** Visit the booths and live chat with our sponsors.
PIAZZA CONNECTS: Meet & Greet the chairs, the speakers and the WMF community.

17:50 Young scientist pitches

- *Emerging Fusarium mycotoxins disrupt homeostasis of bovine mammary cells by altering cell permeability and innate immune function*
  Ran Xu, Department of Animal Biosciences, University of Guelph, Canada
- *Novel mycotoxin binder from agricultural waste to enhance safety of agricultural products*
  Wipada Siri-anusornsak, Department of Food Science and Technology, Kasetsart University, Thailand
- *Mycotoxins in aquaculture: occurrence in feed ingredients and feed – effects on fish productivity and health*
  Vivi Koletsi, Aquaculture and Fisheries Group, Wageningen University & Research, the Netherlands

18:15 Presentation of the Pitch Award
Prof. Chiara Dall'Asta

18:20 Closing remarks and outlook for:

- Virtual pre-conference on Analysis (1 February 2022)
  Prof. Rudolf Krska and Prof. Chris Elliott
- WMF*meets*ITALY, 16-18 May 2022, Parma, Italy
  Dr Michele Suman and Prof. Chiara Dall'Asta

18:45 End of pre-conference

ABOUT THE SPEAKERS

Piotr Jedziniak
Dr Jedziniak is head of the Department of Pharmacology and Toxicology, National Veterinary Research Institute, Poland.

Johanna Lindahl
Dr Lindahl is a veterinary epidemiologist working on a joint appointment between ILRI and Swedish University of Agricultural Sciences. Her research is focused on zoonotic diseases in developing countries, mainly in Africa and Asia as well as on food safety, coordinating projects on aflatoxins.

Antonio Gallo
Dr Gallo is associate professor at the Università Cattolica del Sacro Cuore, Italy. His main research topics regard animal nutrition science, feed evaluation, feed safety, and modelling digestive and metabolic processes in the gastro-intestinal tract of animals.

Arturo Gerardo Valdivia-Flores
Prof. Valdivia-Flores is a researcher at the Centro de Ciencias Agropecuarias, Universidad Autonoma de Aguascalientes, Mexico. His research field includes animal health, animal production, and mycotoxins.

Philippe Pinton
At the Toxalim Research Centre in Toxicology, France, Dr Pinton contributes to the development of several methodologies used, including experimental approaches on animal models and cell culture of intestinal explants. He also leads a research project on the effects of deoxynivalenol and chemical derivatives on the intestinal tissue.

Ahmad Al-Dissi
Dr Al-Dissi's research focuses on studying the effects of toxins on animal and human health. He is currently investigating the effects of ergot alkaloids on the vasculature and the mechanisms of these effects. His research team utilises an arterial tissue bath system to study these effects in sheep after *in vivo* exposure.

Laura Soler-Vasco
Dr Soler-Vasco studies the toxic effects of (emerging) mycotoxins using a translational approach, from the molecular level to the whole animal, using the pig as the favourite research model. She is interested in combining traditional reductionist toxicology and molecular biology techniques with holistic approaches based in -omics.

Swamy Haladi
Dr Haladi is the global programme manager for mycotoxin risk management with Trouw Nutrition. Dr Haladi has obtained extensive knowledge of animal nutrition (monogastric and ruminant animals).

Constanze Pietsch
Dr Pietsch is researcher the Bern University of Applied Sciences, Switzerland. Her focus areas are fish nutrition, well-being and stress, immunology, toxicology, and effects of mycotoxins.

Yan Zhu
Dr Zhu's current research at the Guelph Research and Development Centre, one of Agriculture and Agri-Food Canada's network of 20 research and development centres, is in the field of mycotoxin detoxification and inactivation of foodborne pathogens.

Ran Xu
Ran Xu is a PhD candidate in Animal Nutrition and Toxicology in the Department of Animal Biosciences at University of Guelph, Canada. Her PhD research project is focused on the *in vitro* assessment on individual and combined toxicity of mycotoxins and their remediation with adsorbents.

Wipada Siri-anusornsak
Wipada Siri-anusornsak obtained a M.Sc. in Biotechnology at Chulalongkorn University, Thailand. Currently, she is a PhD candidate in Food Science at Kasetsart University, Thailand

Vivi Koletsi
Vivi Koletsi is a PhD candidate at Aquaculture and Fisheries Group, Wageningen University & Research, the Netherlands. Her project deals with the impact of mycotoxins in aquatic farmed fish species.

LECTURES

ADVANTAGES AND LIMITATIONS OF BIOMARKER MONITORING IN PIGS IN THE ASSESSMENT OF ANIMAL EXPOSURE TO MYCOTOXINS

Piotr Jedziniak and Agnieszka Tkaczyk

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Mycotoxins are common in feed, and pigs are susceptible to their toxic effects. They can negatively affect their health as well as reduce their productivity. Besides the analysis of mycotoxins in feed, analysis of relevant biomarkers in body fluids (biomonitoring) can be beneficial in the overall exposure of animals. *In vivo* studies in pigs to date have been limited mainly to the determination of deoxynivalenol (DON) and zearalenone (ZEA) biomarkers in urine and serum. The feed was most often artificially contaminated with mycotoxin standards, which does not reflect the real situation. In addition, there is limited data on biomarkers of other mycotoxins, e.g., ochratoxin A (OTA) or citrinin (CIT) and information on the kinetics of mycotoxins.

The study aimed to develop liquid chromatography coupled with a tandem mass spectrometer (LC-MS/MS) method for the determination of 35 toxins in the urine and serum of pigs and their application to the analysis of real samples from the experiment on pigs fed with feed naturally contaminated with mycotoxins (DON, ZEA – concentrations similar to the EU guidance values, OTA and CIT) for two weeks, and the mycotoxin-free feed for the next two weeks. Of the many methods tested for preparing urine samples, liquid-liquid extraction made it possible to clean the sample relatively inexpensively and sufficiently (low limits of quantification). Steps omitted in the previous methods were included: enzymatic hydrolysis as well as creatinine determinations. Serum preparation was based on extraction with an organic solvent. Both methods have been validated according to the guidelines of the European Medicines Agency. Levels of biomarkers of all analysed mycotoxins (DON, ZEA, CIT, and OTA) in biological matrices correlated well with the levels of mycotoxins in the feed. It showed that they are suitable biomarkers, and their concentration can be calculated from their content in the feed. DON, ZEA, and CIT biomarkers reach a constant level already on the first day after the administration of contaminated feed. They do not accumulate in pigs, and already two days after conversion from contaminated feed to mycotoxin-free feed, they could not be determined in urine or serum. On the other hand, OTA biomarkers (including the metabolite ochratoxin alfa (OTα) for the first time in pigs' urine) reached a constant level after four days. Even two weeks after replacing the feed contaminated with mycotoxins with zero feed, they were determined in significant concentrations. OTA biomonitoring can therefore be a very important tool in assessing the long-term impact of OTA on pig health.

The study has clearly shown the advantages but also the limitations of biomarker analysis in porcine body fluids. Analysis of urine and serum provides us with valuable information about current exposure to mycotoxins (DON, ZEA) and, in the case of OTA or CIT, also about exposures from a few or several days ago. Data on individual animal exposure to mycotoxins are also interesting. Significant limitations of biomarker analysis are the high cost of analysis, the lack of data on biomarkers of other mycotoxins than DON and ZEA, and the necessity to collect urine and serum for testing.

MYCOTOXINS IN FEED: IMPACT ON ANIMAL HEALTH AND ANIMAL SOURCE FOODS IN DEVELOPING COUNTRIES

Johanna F. Lindahl[1,2,3]

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[2] Swedish University of Agricultural Sciences, Sweden
[3] Uppsala University, Sweden

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Many mycotoxins have negative impacts on animal health and productivity, and in addition, aflatoxins can be transferred into animal-source foods. Our studies in Kenya and in Pakistan show high levels of aflatoxin M1 AFM1) present in different dairy products, and whenever studied AFM1 has been found in high levels in milk also elsewhere in Africa.

Animals can be protected from mycotoxins by preventing the occurrence in feed, but many countries do not have legislation for this, nor the means to implement regulations. Animals can also be protected from the harmful effects by using anti-mycotoxin additives, binder. Our studies in Kenya found that different binders are sold on the market, but of shifting quality and often in large volumes, unaffordable by poor farmers. Nine different mycotoxin binder types were used in the study areas. The binders are being sold by 8% (4/49) of agrovets and 33% (3/9) of feed processors (overall 12%; n=58) and are bought either by farmers formulating their own feeds or by feed processors, who mainly use it when they receive batches of raw materials that are suspected to be contaminated. Feed processors have to adhere to the maximum allowable limit of 5µg/kg for AFB1 in complete feeds. Inclusion of binders in animal feeds is not mandatory and there are no specific standards governing their use in Kenya. In the trials conducted among small-holder dairy farmers, the levels of aflatoxins were reduced while the milk production was perceived to increase in the trial farms compared to the control farms, even though a longer study would be necessary to evaluate long term effects. While farmers were interested in purchasing the binder used in the trial, they could not find it in affordable volumes on the market, and they were sceptical to purchasing from the local agrovets, since they are afraid of adulteration.

In conclusion, that aflatoxins are present in dairy products whenever searched for in Africa, and while binders are having good potential for use, there is little information to farmers, and little scope for buying effective binders.

THE EFFECTS OF MYCOTOXINS ON DAIRY CATTLE

Antonio Gallo

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Mycotoxins are a diverse group of secondary metabolites produced by filamentous fungi mainly belonging to genera *Aspergillus*, *Fusarium*, and *Penicillium* spp*.,* that can cause toxic responses when ingested by humans and other vertebrates. Mycotoxins are generally very stable and can be detected in animal feeds and homegrown forage. Among mycotoxins, those produced by *Fusarium* spp. are usually detected in several feeds because mycotoxigenic *Fusarium* moulds are widespread and able to contaminate field crops in the temperate and warm climate zones. The *Fusarium* toxins in farm animals cause a lot of toxicological effects. In particular, animals can develop numerous symptoms following consumption of feed contaminated with *Fusarium*-produced mycotoxin, such as deoxynivalenol (DON), including gastrointestinal problems, soft faeces, diarrhoea, immuno-suppression, and a general decrease of performance, probably due to feed refusal and low dry matter (DM) intake. The biological mechanisms underlying these responses are still not well understood, but dysbiosis in the rumen or gut milieu, increased permeability of the rumen or gut epithelia, and damage of gut epithelium are common signs. The fumonisins (FB) are another group of mycotoxins produced by *Fusarium* spp. that are cytotoxic, hepatotoxic, and nephrotoxic. Although their mechanism of action is uncertain, the animal gut absorbs FB and they can alter some metabolic pathways. Generally, an immunosuppressive condition is related to ingestion of diets contaminated by FB.

Gallo *et al.* (J. Dairy Sci. 103 (2020) 11314) investigated the effects of regular contamination levels of DON and FB in total mixed ration (TMR), meaning contamination levels that can be commonly detected in dairy feeds, on the performance, diet digestibility, milk quality, plasma liver enzymes and gene expression in dairy cows. This trial examined 12 lactating Holstein dairy cows using a 3-period × 3-treatment Latin Square design and the cows received one of 3 diets: (1) CTR (control) diet, TMR contaminated with 340.5 µg of DON/kg of DM and 127.9 µg FB/kg of DM; (2) MTX diet, TMR contaminated with *Fusarium* mycotoxins at levels higher than CTR but below US and European Union guidelines (i.e., 733.0 µg of DON/kg of DM and 994.4 µg of FB/kg of DM); or (3) MDP diet, which was MTX diet supplemented with a mycotoxin deactivator product (i.e., 897.3 µg of DON/kg of DM and 1,247.1 µg of FB/kg of DM; Mycofix, 35 g/animal per day). Milk production was significantly greater in the CTR group (37.73 kg/d) than in the MTX (36.39 kg/d) and the MDP (36.55 kg/d) groups. Regarding milk traits, curd firmness and curd firming time were negatively affected by the MTX diet compared with the other 2 diets. The activities of plasma liver transaminases were higher after the MTX diet than after the CTR and MDP diets. In another experiment, Gallo *et al.* (JDS Communications 2 (2021) 243) studied how these mycotoxins affect the fermentation kinetics and volatile fatty acid concentrations of different feeds incubated in rumen fluids sampled from lactating dairy cows ingesting contaminated diets (CTR, MTX, and MDP). Three experimental runs were used, corresponding to three intoxication periods of *in vivo* trial (Gallo et al., 2020). The presence of *Fusarium*-produced mycotoxins reduced rumen fluid fermentation potential and slowed the degradation dynamics of incubated feeds. The presence of a mycotoxin-deactivating product enabled the protection of the rumen environment.

Concluding, these results indicated that feed contaminated with regular levels of *Fusarium* mycotoxins adversely affected the performance, milk quality, diet digestibility, rumen fermentation profile, metabolic variables, and immunity of dairy cows, and that supplementation with mycotoxin deactivator product counteracted most of these negative effects, but not milk yield. Lastly, evidence suggests the greatest exposure to mycotoxin in ruminants are related to ingestion of contaminated forage. With the aim to provide information for a comprehensive risk assessment, results of a recently published survey (Gallo et *al.*, Toxins 13 (2021) 232) were presented regarding a deep characterization of regulated and emerging mycotoxins detectable in maize silage, an ingredient largely used in dairy cow diets. As a result, the maize silage resulted concomitantly contaminated by 23 to 43 mycotoxins.

TOXIC EFFECT OF AFLATOXINS IN DOGS FED CONTAMINATED DRY FEED

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Since its first patent (1897), commercial dry feed (CDF) for dogs has diversified its formulation to meet the nutritional needs of different breeds, age, or special conditions and establish a foundation for integration of these pets into urban lifestyles. The risk of aflatoxicosis in dogs has increased because the ingredients used to formulate CDF have also proliferated, making it difficult to ensure the quality required of each to achieve the safety of the entire CDF. Various agro-industrial by-products used as ingredients of CDF show high levels of aflatoxins (AF), which reveals the exposure of dogs to AF in their diet, causing clinically observable damage from low doses (60 µg/kg).

This review study highlights the extensive presence of *Aspergillus flavus* and its AF in CDF for dogs (Martinez *et al.*, Toxins 13 (2021) 65). Unfortunately, information on mycotoxins in dogs is scarce and scattered. The study contains a description of the fungi and aflatoxins detected in CDF and the ingredients commonly used for their formulation. The mechanisms of action and pathogenic effects of aflatoxins are outlined; as well as the clinical findings, and macroscopic and microscopic lesions found in aflatoxicosis in dogs. In addition, alternatives for diagnosis, treatment and control of aflatoxins (AF) in CDF are analysed, such as biomarkers of effect, improvement of blood coagulation, control of secondary infection, protection of gastric mucosa, reduction of oxidative stress, use of chemo-protectors, and maximum permitted limits, are also included. Therefore, this study serves as the foundation for the knowledge of the risk that dogs integrated into urban life have, in which the CDF contaminated by AF is ingested until all the feed contained within each bag is finished, suggesting an urgent need for quality control mechanisms.

DELETERIOUS EFFECTS OF MYCOTOXINS ON THE INTESTINE OF FARM ANIMALS

Philippe Pinton

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The gastrointestinal tract is the first barrier against food contaminants as well as the first target for these toxicants. Mycotoxins are secondary metabolites produced by certain strain of fungi, mainly Aspergillus, Penicillium and Fusarium. They are regarded as an important risk factor for human and animal health as a high percentage of the world's crop production may be contaminated. Due to the high amount of cereals ingested by farm animals, the effects of mycotoxins on the intestinal structure and function can be of concern.

The first part of the presentation focuses on the structure and main functions of the intestine as well as some models used to study the effects of food contaminants. Then, the main effects of the most common mycotoxins (deoxynivalenol, fumonisins, ergot alkaloids, ochratoxins, zearalenone, and aflatoxins) on the gut of different farm species (pig chicken, duck, cattle, and fish) are presented. Studies in pigs and chickens provide most of the data, mainly through histo-morphological analysis. Most of the mycotoxins presented can alter the structure of the epithelium, the cell cycle and the cells producing mucus. Alteration of barrier function by mycotoxin can contribute to increase of bacterial or viral infection as shown for pig or chicken. Meta-analyses confirm that mycotoxins reduce feed intake and weight gain in chickens and pigs. Finally, the consequences of the exposure of farm animals to mycotoxins on the enteric nervous system and the microbiota, more recently investigated, are evoked.

ERGOTISM IN LIVESTOCK – AN INSIGHT ON POTENTIAL MECHANISMS

Ahmad Al-Dissi

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Ergot alkaloids are produced by the fungus *Claviceps purpurea* and their levels are carefully monitored in animal and human diets due to their harmful effects and widespread contamination of cereal crops. Two studies are presented.

The first examined the vasoactive effects of acute ergot exposure in sheep. Twelve adult female sheep were randomly placed into control and exposure groups (n=6/group). Ergot sclerotia were collected, finely ground and administered to sheep via a stomach tube (600 µg/kg BW). Animals were euthanized 12 h after the treatment, and the pedal artery (dorsal metatarsal III artery) was collected and mounted on a tissue bath. The vascular contractile response to phenylephrine (PE) was compared between the two groups before and after terazosin (TE) treatment. Acute exposure to ergot alkaloids resulted in a 38% increase in vascular sensitivity to PE compared to control. TE treatment resulted in a significant dose-dependent increase in EC50 in both exposure and control groups ($P<0.05$ for all treatments). Surprisingly, TE effect was significantly more pronounced in the ergot exposed group compared to the control group at two of the three concentrations of TE. Similar to chronic exposure, acute exposure to ergot alkaloids results in increased vascular sensitivity to PE. The acute effects of ergot alkaloids may be related to the activation of adrenergic receptors.

The second study examined the vasoactivity potential (contractile response) of four (S)-epimers, namely ergocryptinine, ergocristinine, ergocorninine, and ergotaminine utilizing an *in vitro* arterial tissue bath system. Bovine metatarsal arteries were collected from healthy mixed-breed beef steers immediately after slaughter, cut into 3-mm arterial cross sections, and suspended in a tissue bath. A cumulative contractile dose–response curve was constructed by incubating arteries with increasing concentrations (1×10^{-11} to 1×10^{-6} M) of that (S)-epimers. Contrary to the widespread belief, all tested (S)-epimers were found vasoactive and produced a concentration-dependent arterial contractile response similar to what has been reported for the (R)-epimers. The levels of (S)-epimers should be carefully monitored in human and animal diets worldwide.

ZEARALENONE AND PIGS: TO REPROTOXICITY AND BEYOND

Laura Soler-Vasco

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Some of the mycotoxins can act as endocrine disruptors, and the most known is zearalenone (ZEA). The structure of the mycoestrogen ZEA resembles the endogenous oestrogen 17β-estradiol and is able to bind oestrogen receptors. ZEA is present in a range of food products including cereals, dried fruits, and spices. Due to its toxicity, a tolerable daily intake of 0.25 µg/kg body weight and maximum levels in food for human consumption (20-100 µg/kg) have been set by the European Commission by Regulation (EC) No 1126/2007. The well-known reprotoxic and endocrine disrupting activities of ZEA include infertility, hormonal dysfunctions, and reproductive tract hyperplasia.

ZEA is also toxic to other organs, especially those that are oestrogen responding, such as the liver, immune cells, and the intestine. The toxicity of this molecule can, however, change depending on the target organ (oestrogen-responding or not) and the exposure conditions (low or high dose). In the intestine, the endocrine disruptor activity of ZEA is able to regulate a cascade of highly inter-connected signalling events essential for the small intestinal crypt-villus cycle and immune status. These molecular mechanisms are also implicated in the onset and progress of intestinal immune disorders and cancer indicating that exposure to ZEA could play an important role in intestinal pathogenesis.

Many endocrine disruptors are also metabolic disruptors able to modulate energy balance and inflammatory processes in a process often involving a family of protein hormones known as adipokines. Our recent results indicate that ZEA has an impact in lipid and glucose metabolism that was different depending on the dose and time of exposure. In agreement with these changes, ZEA altered circulating concentrations of adipokines, inducing significant changes in adiponectin, resistin, and fetuin B. ZEA may function as a natural metabolism-disrupting chemical and the alterations in adipokines levels could be behind some of the reprotoxic effects of this mycotoxin, which is of relevance for public health and animal health.

MYCOTOXINS IN POULTRY – RECENT UPDATE ON GUT HEALTH AND IMMUNITY

Swamy Haladi

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Mycotoxicosis is a condition observed in poultry upon the ingestion of different concentrations of mycotoxins in the complete feed. Mycotoxins, a diverse group of toxic secondary metabolites of fungi, are a world-wide challenge in the poultry industry due to the increasing globe trading of raw materials as well as climate change and changing agricultural practices. Although poultry can be exposed to more than 600 different mycotoxins, most research has focused on six groups of mycotoxins (mentioned in a decreasing order of sensitivity): ochratoxins (OTA), T-2 toxin, aflatoxins (AF), deoxynivalenol (DON), fumonisins, and zearalenone (ZEN). Emerging mycotoxins, such as moniliformin and cyclopiazonic acid (CPA), are also known to cause toxicity in poultry. Although mycotoxins affect most organs and systems in poultry, some organs/systems are more vulnerable for a specific group of mycotoxins. Aflatoxins are known as potent hepatotoxins while ochratoxins are known to affect kidneys severely. Similarly, the gastrointestinal tract (GIT) is more susceptible to trichothecene mycotoxins (DON and T-2) while fumonisins affect sphingholipid metabolism in various organs. The one system that is affected by all these mycotoxins is the immune system and it can be affected even at very low concentrations of mycotoxins.

The gastrointestinal tract is the first site of contact for feedborne mycotoxins and. therefore, intestinal epithelial cells integrity is crucial to prevent the entry of toxins and pathogens into the blood circulation. In the last decade or so, there has been extensive research on this area not only to protect health and performance of poultry but also to prevent the potential of pathogens entering food chain through animal products. Mycotoxins affect gut health through various mechanisms: poor intestinal cell proliferation, cell death, decreased villi height, necrotic lesions in GIT, decreased mucin production, compromised intestinal immunity, the disruption of intestinal barrier function, increased intestinal permeability (lowered tight junction proteins), and altered intestinal nutrient absorption. The most common mechanism across mycotoxins seems to be the negative effect on tight junction proteins.

The immune system of poultry is under constant threat even with low mycotoxin concentrations in feed. Mycotoxins affect the immune system through various mechanisms: interference with DNA, RNA and protein synthesis, increased gene expression of IL-6, regression of lymphoid organs, reduced weight of thymus and bursa, lymphocyte depletion, leukopenia, reduced complement, and interferon production, suppressed macrophagic phagocytosis, suppressed delayed type hypersensitivity, poor antibody titres, and vaccination failures. The result of all these mechanisms is reduced resistance of birds to infections and subsequently poor health and performance. Poultry industry across the world measures antibody titres to understand the health status of flocks but antibody measurement (humoral immunity) represents only 40% of the immune response. The remaining 60% comprises of cell-mediated immunity and innate immunity, and hence the practical means of measuring these two arms of immune system is also critical.

Effective mycotoxin binding of aflatoxins and ergot toxins can be achieved through the addition of good quality bentonite clay in the mycotoxin mitigation product. But for other mycotoxins that are less optimally bound by binding agent at the intestinal level, there is a need for ingredients capable of improving immune responses and gut health. Additionally, poultry feed is usually contaminated with multiple mycotoxins and poultry producers are looking for a broad-spectrum solution to safeguard their business. The negative effects of many mycotoxins can be managed through strengthening the tight junction proteins (use of glucose biopolymer extracted from specific strain of yeast) and innate immunity (beta-glucans of specific yeast sources).

EFFECTS OF MYCOTOXINS ON FISH – WHAT DO WE KNOW SO FAR?

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The occurrence of mycotoxins in feedstuffs for animals is indisputable. However, their presence in fish feed and their subsequent effects on fish have not sufficiently been investigated due to the fact that the analyses are laborious and in most cases also very expensive. For a comprehensive analysis of the risks for fish in aquaculture, the exact composition of compound feed has to be known in order to calculate their potential contamination with different mycotoxins. Unfortunately, fish feed producers rarely publish the exact composition of their feeds. Therefore, a feed composition calculator was established based on the average nutrient content of different feed ingredients. Based on this, the percentage of ingredients in more than 97 commercially available fish feeds was estimated. The calculations revealed that especially for the mycotoxins deoxynivalenol, zearalenone and fumonisin B1 higher values in different fish feeds can be expected. The obtained data were compared to already published contamination values in commercial fish feeds and known critical effect concentrations in fish. Since the contamination of feed ingredients is variable from year to year and insufficient storage of feedstuffs also contributes to contamination of feeds before they are actually used for feeding of fish, this risk assessment remains incomplete due to a lack of exact data. Nevertheless, the assessment can result in recommendations of certain feed ingredients that show low mycotoxin contaminations in general, and thus, appear to be less problematic for fish than others.

The last part of the presentation focuses on potential measures against mycotoxin contamination and effects of mycotoxins in fish feeds. Feed producers are very actively looking for strategies to reduce the detrimental effects of mycotoxins on fish health, but feed additives can have different efficiencies or may have unwanted side effects so that further research is needed to optimise these.

PROMISING DETOXIFICATION OF *FUSARIUM* MYCOTOXINS IN MAIZE SILAGE: BIOLOGICAL TRANSFORMATION OF DEOXYNIVALENOL AND ZEARALENONE

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Silages are important components of ruminant diets. However, the mycotoxin contaminations in silages were widely reported all around the world. Deoxynivalenol (DON) and zearalenone (ZEA) are mycotoxins occurring in many silages and result in the decrease of feed value and the loss of livestock production. The toxicity of DON and ZEA is also a safety concern to the consumers. Among various control measures, the biological solution became the promising strategy to detoxify the mycotoxins in silage due to its efficient, specific, and environmental-friendly properties.

In this presentation, we will report our research work on the DON/ZEA detoxification using the micro-organisms isolated from the maize silage. In the work of DON detoxification, we found a bacterial consortium presenting the DON-biotransformation activity. The detoxification product was identified as DOM-1. The antibiotics treatment guided by DGGE analysis simplified the consortium made it possible to be used for DON detoxification in silage. For ZEA detoxification, we isolated a new ZEA biotransformation bacterium and identified a new detoxification pathway and a metabolic product through the phosphate conjugation. The bacterium is non-pathogenic and sensitive to antibiotics, which is a good candidate to apply in the ZEA detoxification in silage.

YOUNG SCIENTISTS PITCHES

EMERGING *FUSARIUM* MYCOTOXINS DISRUPT HOMEOSTASIS OF BOVINE MAMMARY CELLS BY ALTERING CELL PERMEABILITY AND INNATE IMMUNE FUNCTION

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High incidence of emerging *Fusarium* mycotoxins enniatin (ENB) and beauvericin (BEA) in cereal grains and silages can be a potential threat to feed safety and the health of ruminant species. The antimicrobial properties of ENB and BEA could result in the inadequate biodegradation of these mycotoxin by rumen microflora, thus leading to their circulatory transport to the target tissues such as mammary gland (MG). The bovine udder plays a pivotal role in maintaining milk yield and composition, thus human health. However, the toxic effects of ENB and BEA on the bovine MG have been rarely studied. In this study, the bovine mammary epithelial cell line (MAC-T) as an *invitro* model of bovine mammary epithelium to investigate effects of ENB and BEA on bovine MG homeostasis. Our results indicated that exposure to ENB and BEA for 48 h significantly decreased MAC-T cell viability in a concentration-dependent manner ($P<0.001$). Exposure to BEA at 2.5 μM for 48 h also decreased paracellular flux of FITC-40 kDa dextran ($P<0.05$), whereas neither of the mycotoxins affected transepithelial electrical resistance (TEER) after 48 h exposure. qPCR was performed to assess expression of Toll-like receptor 4 (TLR4) and various cytokines after 48 h of exposure. The qPCR results demonstrated ENB and BEA significant down regulated TLR4 ($P<0.05$), ENB markedly increased mRNA expression of cytokines IL-6 ($P<0.001$), TNF-α ($P<0$ .05) and TGF-β ($P<0$ .01), and BEA significantly down regulated TNF-α at 48 h of exposure ($P<0.001$). These findings suggest ENB and BEA can potentially disrupt MG homeostasis by inducing cell death, altering paracellular permeability and innate immune function.

NOVEL MYCOTOXIN BINDER FROM AGRICULTURAL WASTE TO ENHANCE SAFETY OF AGRICULTURAL PRODUCTS

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The occurrence of mycotoxins in agricultural commodities is a significant challenge for the food and feed industry. Poor post-harvest practices could create scenarios where mycotoxin exposures occur above the levels set by health regulatory bodies. These scenarios require effective methods to reduce mycotoxin in agricultural commodities providing protection of humans and animals from adverse health effects of mycotoxin exposure. The use of mycotoxin binders, or adsorbents, has been greatly applied for routine avoidance of exposure to mycotoxins. In Thailand, the farm and feed industry use some imported binders that appear to be useful in terms of protecting livestock from mycotoxin exposure, especially aflatoxin B1 (AFB1) commonly found in agricultural products. However, the imported binders are expensive. Therefore, the high costs of importing commercial binders can be avoided by developing local resources with a similar potential. The objective of this study was to evaluate a novel mycotoxin binder from young coconut husk, which is a common local byproduct from the food industry in Thailand. Acid-treated young coconut husk showed an AFB1 binding capacity that reached 86% *in vitro*, a relatively higher potential equivalent to the commercial mycotoxin binder employed in the study for reference purposes. Based on the physical structure of the acid-treated young coconut husk binder, a rough surface with an abundant mesoporous structure was reported. The adsorption data were fitted with two adsorption isotherm models, i.e., the Langmuir and Freundlich isotherm. In conclusion, the cheaper acid-treated young coconut husk has potential for use as a mycotoxin binder to minimize the exposure of aflatoxin to animals through contaminated agricultural raw materials.

MYCOTOXINS IN AQUACULTURE: OCCURRENCE IN FEED INGREDIENTS AND FEED - EFFECTS ON FISH PRODUCTIVITY AND HEALTH

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In the last decade, the aquafeed industry has made significant attempts to develop sustainable fish feeds by replacing traditionally used marine ingredients with novel ingredients derived from crops and their byproducts. However, crops are susceptible to fungal species and their secondary metabolites, mycotoxins. Fungal growth is climate-dependent, and with the undergoing climate change, the quantity of mycotoxin-producing fungi in the environment and the fungal community structure alter, raising concerns in the aquaculture industry for mycotoxin contamination in the fish feeds. Firstly, we aimed to identify the mycotoxin contamination patterns from a large data pool derived from wheat (n=857), maize (n=725), soybean meal (n=139), and fish feed (n=44) samples in European countries and based on sample analysis by liquid chromatography/tandem mass spectrometry (LC-MS/MS) over the period 2012–2019. Our results showed that a *Fusarium* mycotoxin, deoxynivalenol (DON) was present in maize (47% of the samples) > wheat (41%) > soybean meal (11%), and fish feeds (48%). The next step was to explore the impact of DON on fish performance and health. Firstly, we employed a meta-analysis to estimate to which extent DON affects feed intake and growth performance in fish. Prediction equations showed that each additional mg/kg of DON in fish feed would reduce feed intake and growth exponentially by 13.2 and 16.5%, respectively. Responses were more severe for rainbow trout (18.8 and 20.0%) and, threrefore, it was characterised as the most sensitive fish species, among others. Secondly, we carried out an *in vivo* experiment using rainbow trout fingerlings (8 g) as a model species to investigate the impact of realistic DON doses (up to 1.6 mg/kg) on fish. Results showed that restrictive exposure to DON for six weeks reduced retained protein in trout treated with the highest DON levels. By feeding DON-contaminated diets for two more weeks *ad libitum*, we also reported suppressed body weight gain and altered feed efficiency. Our histological assessment revealed severe hepatic damage, which was alleviated overtime during restrictive exposure and aggravated after *ad libitum* exposure. Overall, DON is an underrated and highly present feed contaminant that threatens rainbow trout productivity and health at levels even below the current EC recommended limit (5,000 mg/kg).

COMPANY PITCHES

MANAGING MYCOTOXINS FOR PETFOOD

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Companion animals, such as cats and dogs, are carnivores. Nevertheless, pet food may contain plant-based proteins from maize, soy, or wheat. These plant-based raw materials may be contaminated with mycotoxins. As cats and dogs are very sensitive towards mycotoxins, a proper mycotoxin risk management should be in place. There are several analytical methods available for monitoring the raw materials for the most common mycotoxins. Analysing pre-mixes and raw materials is, however, more complicated and requires a more effective sample clean-up. We present a solution for analysing mycotoxins with LC-MS/MS in complicated matrices: the 11+Myco MS-PREP® immunoaffinity column.

CUTTING EDGE SCIENCE, ABSOLUTE PROTECTION

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The contribution of BIOMIN to mycotoxin risk management.

BIOMIN (part of DSM) is a science-based company that benchmarks the most complete mycotoxin risk management. To manage mycotoxins, an integrated programme starting with the prediction of the toxin in the crops, the correct analysis and appropriate risk estimation in the feed, and ultimately a specific detoxifying solution is needed.

In mycotoxin risk management, the first step is to quantify or at least estimate the risk of mycotoxins. The BIOMIN Prediction Tool offers an estimation of the risk for crops in different regions already during growth of the crops. Additionally, raw materials as well as finished feed is being analysed since 2004 by the BIOMIN Mycotoxin Survey. Per year, more than 100,000 mycotoxin analyses of different feed materials from more than 70 countries are performed, giving the full picture about mycotoxin contamination worldwide

Due to a close collaboration with different universities and scientific institutes and more than 30 years of research, BIOMIN leads with highest technology and constant innovations in the Mycofix® product line. The specific ingredients of the Mycofix® product line are the only ones authorized in the European Union in the category of technological feed additives: substances for reduction of the contamination of feed by mycotoxins. The bentonite is authorized to adsorb aflatoxins, Biomin® BBSH® 797 to biotransform trichothecenes and FUM*zyme*®, a purified enzyme is authorized to cleave fumonisins. Currently a positive EFSA opinion and subsequent authorization for ZEN*zyme*®, a brand-new purified enzyme to deactivate zearalenone is expected.

BIOMIN is the best partner for mycotoxin risk management!

MYCOTOXIN MANAGEMENT IS NOT A BETTING GAME – ADISSEO HELPS YOU IDENTIFY THE RISKS AND ADOPT THE BEST STRATEGY

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Every year, buying new grain is like a betting game for the feed manufacturer. Animal producers also bet on feed quality because in most cases they do not know what they will get. The contamination of grain harvested in a single year, can differ from the mycotoxin patterns and levels of previous years in the same climatic region. The risk is therefore ever-present, and ever-changing. At Adisseo, we offer an integral approach by going through all the steps of the chain.

Adisseo has established a comprehensive mycotoxin risk management program, by going through all the steps of the chain from raw material procurement until animal consumption.

- At the arrival point, the feed or animal producer receives grain that already contains different mycotoxins before it is introduced into the storage silo. Therefore, buying the newly harvested grain is like a betting game for the producer of animal feeds who has to deal with unknown, 'on-fire' contamination. Our MycoMan range of services allows to identify risk from the raw materials to the animals:
    - MycoMan Predict (prediction of the pre-harvest grain quality of maize and wheat)
    - MycoMan Harvest Bulletin (assessment of the overall crop conditions and grain quality in maize and wheat)
    - MycoMan Test (Quick) (estimates the contamination of raw ingredients).
- There are several strategies that can be adopted to control growth and development of moulds and to reduce their effects on feed and feed material quality and animal performance. An important one is proper storage and treatment of raw materials with mould inhibitors
- One of the complementary strategies of mycotoxin risk management is to test mycotoxins presence in finished feeds. Indeed, by only testing some feed ingredients by rapid test kits we can miss some important feeding stuffs with inclusion rate below 5-10% and which can still cause significant contamination of finished feed. A good example are the secondary products of grain, such as as DDGS or bran, which are much more contaminated than the initial grain
- What if we implement all 3 of 4 steps of the Mycotoxin Management Programme – Forecast Crop Contamination, Secure Storage, and Screen Finished Feed – and still come out with multiple mycotoxin contamination of feed? In most cases we would not have any choice – feed should be fed to animals! Using mycotoxin sequestrants with efficacy proven in animals, is the only strategy available for now

TROUW NUTRITION – INNOVATIONS DRIVEN BY CUSTOMERS

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Trouw Nutrition, the animal nutrition division of Nutreco, has a 90-year history of dedication to innovation and deep experience in developing smarter and more sustainable ways of raising healthy farm animals and companion animals. Our team of 8,300 passionate people in 32 countries is driven to solve the challenges facing our feed-to-food chain and relentlessly help our customers Feed the Future. Our broad portfolio of feed specialties, feed additives, premixes and nutritional models and services, along with our ever-increasing expertise in feed, farm and health makes us a one-stop-shop with limitless opportunities to create tailored, integrated solutions suited to each customer's local situation. And we are everywhere farmers and home-mixers, feed producers, integrators and distributors need us, with a presence in 105 countries, and manufacturing plants in 22. We believe the power of nutrition and good farm management can transform our industry – and even our planet. So, we are challenging how the feed-to-food chain works today, to create a brighter future, from planet to plate.

Trouw Nutrition's integrated mycotoxin risk management programme enables feed producers to make data-based decisions and use mycotoxin control solutions more precisely to address this issue. This programme is proven to reduce overall mycotoxin risk by a 3D approach: risk identification, quality control, and application of solutions.

Trouw Nutrition's TOXO products provide a complete solution against a broad spectrum of mycotoxins, combining four main modes of actions, designed to support animal health and performance during mycotoxin exposure.

- Effective absorption ability through smectite-based bentonite clays has been proven by numerous *in vivo* and *in vitro* validation studies. These ingredients do not bind to other valuable nutrients in feed.
- Support for gut integrity through specific glucose biopolymers, reinforcing enterocyte tight junction protein complexes, which are damaged by inflammation during mycotoxin exposure.
- Immune system modulation through highly purified Beta glucans, which act as immune modulating agents with proven *in vivo* and *in vivo* efficacy.
- Anti-oxidative support through specific antioxidants and vitamins that promote the metabolism of mycotoxins in the liver.

ALLTECH®: THE EVOLUTION OF MYCOTOXIN MANAGEMENT STRATEGIES, AND BEYOND…

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Founded in 1980 by Irish entrepreneur and scientist Dr Pearse Lyons, Alltech is a cutting-edge technology company in a traditional industry, agriculture. Our products improve the health and nutrition of plants and animals, resulting in more nutritious products for people as well as less impact on the environment. With expertise in yeast fermentation, solid state fermentation, mycotoxins, and the sciences of nutrigenomics and metabolomics, Alltech is a leading producer of yeast additives, organic trace minerals, feed ingredients, premix, and feed.

Together, with more than 5,000 team members worldwide, we believe in "Working Together for a Planet of Plenty™." With the adoption of new technologies, the adaptation of better farm management practices and the ingenuity inherent in the human spirit, we believe a world of abundance could be ours. Alltech is a private, family-owned company, which allows us to adapt quickly to our customers' needs and stay focused on advanced innovation. Headquartered in Kentucky, USA, Alltech has a strong presence in all regions of the world commercially and scientifically with 4 Biosciences centers, and more than 20 Research Alliances with Academic partners, reuniting a network of more than 150 scientists. In 2019, we took a significant step toward in our sustainability goals and vision for a Planet of Plenty™ by signing on to nine of the United Nations' 17 Sustainable Development Goals (SDGs) that we felt we could impact most significantly and committed to the Science Based Targets initiative to reduce greenhouse gas emissions.

The Alltech Mycotoxin Management Program. Although mycotoxins are an unavoidable problem, some key management practices can help reduce the risk attached to their presence in animal feeds. Alltech believes that effective mycotoxin management is about seeing the whole challenge, from the farm to the feed mill and from risk assessment to feed management. To effectively manage the inevitability of feed mycotoxin contamination, it is crucial to understand the level of mycotoxin challenges so that the right steps can be taken to mitigate any adverse effects on animal performance, production efficiency and food safety. Using a combination of modern management tools, the Alltech® Mycotoxin Management Program provides a complete holistic solution to help producers take control of mycotoxin contamination and protect their businesses. The program is built around next-generation risk identification technology, data analysis and insights and mycotoxin binder solutions designed to reduce the damaging effects of mycotoxins on animal health and production potential. A robust research and development program has helped to maintain a strong scientific stewardship and leadership through in-depth interaction with key experts in the field. This allowed us to develop a multi-faceted exploration of mycotoxin's impact to animal systems and remediation, using in silico, in vitro, ex vivo, in situ and in vivo methodologies, pushing the frontiers in the search of successful mitigation strategies.

YEASTS FRACTIONS TO MITIGATE MYCOTOXINS IN ANIMAL FEED: NEW INSIGHTS

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At Phileo by Lesaffre, we are developing yeasts and bacteria probiotics, postbiotics and functional ingredients, including yeasts fractions to mitigate mycotoxins in animal feeds. Here are presented some results regarding a new yeast cell wall able to adsorb fumonisin B1 and reduce the systemic exposure of animals to this toxin.

LOOKING FORWARD TO SEEING YOU AGAIN!

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