

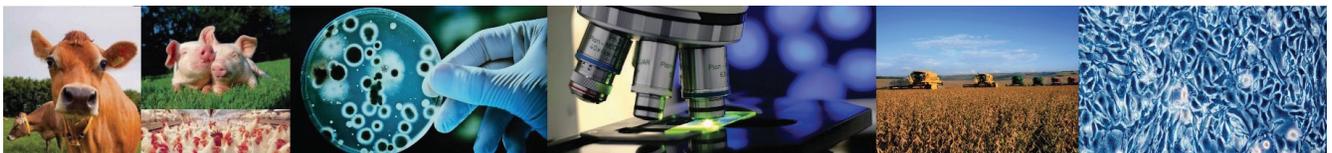


## Conference Proceedings



# International Forum on Mycotoxins in Animal Production

3 - 5 December, 2018. Curitiba, Brazil



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## Summary

### Lectures

Mycotoxins and poultry immunity .....	S1
The adverse effects of mycotoxins in aquaculture .....	S2
Mycotoxin mixtures: a challenge in risk assessment .....	S3
Biomonitoring of mycotoxins in production animals .....	S4
Mycotoxins in pig production .....	S5
Immunological methods in the analysis of mycotoxins in food and feed .....	S6
Ultra-rapid method for the management of mycotoxins in agribusiness .....	S7
Innovative tools for the control of toxigenic fungi and related mycotoxins in the field .....	S8
Mycotoxin risk management .....	S9
Can biodetoxification be an effective strategy for mitigating deoxynivalenol in the agri-food value chain? .....	S10
Mycokey approaches to mitigate mycotoxins in feed: efficacy assessment of physico-chemical treatments in improving the performances of bentonite as multi-mycotoxin binders .....	S11-12

### Session I - Impact of mycotoxins in animal performance

Aflatoxin and fumonisin residues in liver of broilers fed aflatoxin, fumonisin and turmeric powder ....	S13
Contamination of poultry litter by toxigenic fungi and its relation with mycotic pododermatitis .....	S14
Effect of a feed naturally contaminated with low levels of fumonisins with and without mycotoxins eliminator additive on immune cells of broiler chickens .....	S15
Effect of deoxynivalenol on oxidative stress in intestine, liver and kidney from broilers chickens ....	S16-17
Effects of deoxynivalenol on the liver of broilers chickens: <i>in vivo</i> assay .....	S18-19
Efficacy of mycotoxin eliminating agent against don contamination in weaned piglets .....	S20-21
Evaluation of growth performance and health parameters of <i>Rhamdia quelen</i> fed with aflatoxin B <sub>1</sub> contaminated feed .....	S22
Occurrence of mycotoxins in Brazilian fish and feed followed by risk assessment through deterministic approach .....	S23-24

### Session II - Recent approaches to evaluate the risk of mycotoxin in animal health

Aflatoxin B <sub>1</sub> residues in meat of swine fed AFB <sub>1</sub> contaminated diet and efficacy of HSCAS to reduce the contamination levels .....	S25-26
Antioxidant capacity of <i>Lactobacillus plantarum</i> metabolites exposed to deoxynivalenol in pigs intestinal tissue .....	S27
Assessment of Fumonisin B <sub>1</sub> in pig hair as an exposure biomarker .....	S28-29
Determination of aflatoxin B <sub>1</sub> in <i>Rhamdia quelen</i> tissues and water through LC/MS-MS .....	S30
Effect of feeding diet containing deoxynivalenol contaminated wheat on the feed consumption, weight gain and testis of young rats .....	S31-32
<i>In vitro</i> adsorption of mycotoxins: a comparative study between methods .....	S33
Urinary metabolites of aflatoxin to evaluate the HSCAS efficacy in pigs fed an aflatoxin B <sub>1</sub> contaminated diet .....	S34-35

### Session III - Detection of regulated mycotoxins in animal feed-low-cost and high-tech methods

Analysis of mycotoxins in Tunisian feed: study of the natural co-presence .....	S36-37
Cell-free supernatants of lactic acid bacteria cultures against mycotoxigenic fungi growth .....	S38
Correlation between mycotoxin content in maize silage and silo coating associated with ambient temperature in Brazilian farms .....	S39
Effect of gaseous ozone on toxigenic fungi decontamination of dehydrated fish .....	S40
Validation of UHPLC-MS/MS method for Aflatoxin B <sub>1</sub> and its metabolites in pig urine .....	S41-42

### Session IV - Novel strategies to mitigate the occurrence of mycotoxins in animal feed

Antifungal activity of Natamycin, against <i>Aspergillus</i> , <i>Fusarium</i> and <i>Penicillium</i> spp. ....	S43
Antimycotoxigenic action of the phenolic extract against the <i>Fusarium graminearum</i> strain in maize .....	S44
Correlation between mycotoxin content and maize silage management in Brazilian dairy farms .....	S45
Development of toxigenic fungi in fish products at different water contents .....	S46
Effects of exposure to cold plasma on toxigenic fungi species of the genus <i>Aspergillus</i> , <i>Fusarium</i> and <i>Penicillium</i> .....	S47
Innovative solution to reduce the growth of the mycotoxigenic fungi in cereals during storage .....	S48
Lactic acid bacteria as bio control agents against corn mycotoxigenic fungi .....	S49
Ozone technology as mycotoxin reduction strategy in cereal grains and their products .....	S50
Reduction of aflatoxins B <sub>1</sub> and B <sub>2</sub> in corn, corn flour and peanuts by gaseous AITC .....	S51-52
The use of essential oil of mustard to control aflatoxigenic species of <i>Aspergillus</i> .....	S53
Toxigenic fungi decontamination of commercial poultry eggshell by ozone gas treatment .....	S54
Transcriptional activation of mitochondrial Krebs cycle-related genes is associated with fumonisin production in <i>Fusarium</i> spp. ....	S55-56
Use of Allyl isothiocyanate to reduce levels of zearalenone in grains and maize flour .....	S57-58
Use of Allyl isothiocyanate to reduce levels of zearalenone in wheat flour and <i>in natura</i> .....	S59-60

### Session V - Novel feed additives

Dichloromethane and ethyl acetate extraction of <i>Lactobacillus plantarum</i> crude metabolite protect intestinal tissue exposed to deoxynivalenol in pigs .....	S61
Effect of the use of Elitox® against aflatoxins from feed in dairy cows .....	S62
Screening of environmental microorganisms for zearalenone biodegradation .....	S63

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LECTURE ABSTRACTS

## Mycotoxins and poultry immunity

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The state of immunity has received increasing attention in poultry farming. In a highly tech-nized industry such as this, changing the functioning of defense mechanisms has a significant impact on productivity, not just on animal health. Mycotoxins can have numerous effects on the organism, and thus there are many mechanisms by which the defense barriers of production poultry are modified by these fungal products. Fungal toxins can interact directly with immune cells, preventing their maturation or, on the other hand, inducing it. In addition, the immune system cannot be considered as an autonomous entity, since it naturally responds to modifications in other systems. Therefore, by affecting the microbiota, for example, mycotoxins also exert indirect effects on leukocyte responses. Although poorly used, the evaluation of microbiota parameters, intestinal integrity and cellular immunity may reveal subclinical effects that are little considered in mycotoxins. As an example, several toxins appear to alter the activation of cytotoxic lymphocytes (CD8) in the circulation. Since immunity takes priority over energy distribution in stress situations, changes in these small defense compartments end up significantly impacting other organ systems.

**Keywords:** Cellular immunity. Immune system. Leukocyte responses.

## LECTURE ABSTRACTS

## The adverse effects of mycotoxins in aquaculture

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Aquaculture is defined as the farming of aquatic organisms in marine or continental environments, with interventions to improve production. Fish farming contributes significantly with the continental aquaculture and the increasing use of plant ingredients in fish feed formulations has intensified the risk for mycotoxicosis due to the chance of contamination of these ingredients with mycotoxins. The growth of toxigenic fungi is highly dependent on climatic conditions on growing and storage sites, especially humidity and temperature. Therefore, the environment of fish farming becomes suitable to the feed contamination. Mycotoxins have a substantial impact on aquaculture production, causing diseases with high mortality and reduced fish quality, representing a concern in aquaculture systems. Some species of fish are sensitive to the effects of mycotoxins, with consequences in physiology, such as hepatic and hematological alterations, and in performance, such as reduction in growth or weight gain, worse feed conversion and increased susceptibility to diseases and mortality. Aflatoxins are the most studied mycotoxins in aquaculture. The deposition of aflatoxins in the fish occurs in a residual and cumulative way, and residues in the muscle and liver can be observed. The difference in the sensitivity of different fish species to aflatoxins is due to the different metabolism among species. Even with the knowledge that there are differences in the sensitivity and metabolism of aflatoxins among different animals and within fish species, global regulations establishing tolerance limits for aflatoxins in animal feeds do not distinguish species. In this way, the contamination of fish feed by aflatoxins can lead to productive losses due to poor performance of the animals and consuming fish may constitute a hazard to humans because of the possibility of accumulation of these toxins in the edible parts.

**Keywords:** Fish. Hepatic alterations. Hematological alterations.

## LECTURE ABSTRACTS

## Mycotoxin mixtures: a challenge in risk assessment

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Food is contaminated by multiple contaminants, mycotoxins being the most frequently occurring natural ones. Mycotoxins co-contamination is confirmed on the one hand by the co-occurrence of these toxins in food and feedstuff and on the other hand by co-exposure monitoring survey. The co-occurrence of mycotoxins in food and feed is explained by three different reasons: (i) most fungi are able to simultaneously produce several mycotoxins, (ii) commodities can be contaminated by several fungi simultaneously or in quick succession, and (iii) the complete diet comprised different commodities. In practice, the co-occurrence of mycotoxins represents the rule and not the exception. Besides mycotoxins, food can be contaminated with other contaminant such as heavy metals. The toxicity of combinations of contaminant cannot always be predicted based upon their individual toxicities. The data on the combined toxic effects of mycotoxins are limited and therefore, the health risk from exposure to a combination of mycotoxins is incomplete. Most of the studies concerning the toxicological effect of contaminant have been carried out taking into account only one compound. A synergistic effect between trichothecenes mycotoxins was observed both for intestinal cytotoxicity and inflammatory response and the synergy was already seen at low doses. The combined exposure to DON and Cadmium was also studied in several human cell lines and interactions were specific to the target organ. The importance of microbiota in intestinal health is gaining interest, with this aim the interaction between DON and microbiota was investigated. We demonstrated that DON exacerbated the intestinal DNA damages induced by *Escherichia coli* stains producing colibactin raising questions about the synergism between food contaminants and gut microbiota. Altogether, recently obtained data demonstrated that mycotoxin cocktails can lead to synergistic interaction and that mycotoxin contamination should be taken in the global context of all food contaminants and the host intestinal microbiota.

**Keywords:** Co-exposure. Co-occurrence. Health risk.

## LECTURE ABSTRACTS

## Biomonitoring of mycotoxins in production animals

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Since the aflatoxin discovery in the early 1960s, the assessment of negative effects of mycotoxins on production animals has been based on the observation of signs and symptoms of intoxication including decreased performance parameters, combined with the mycotoxin contamination data in feed and/or ingredients. In spite of several existing mycotoxicosis diagnosis criteria, these classical approaches are associated with important limitations such as the variability of individual susceptibility to mycotoxins and the heterogeneous distribution of mycotoxins in feed. Exposure biomarkers have been proposed for improving the direct exposure assessment to dietary mycotoxins in humans. A biomarker of exposure refers to the quantification of the specific compound, its metabolite(s) or interaction products in a body compartment or fluid, which indicates the presence and magnitude of exposure to the agent. The first mycotoxin biomarker used in production animals was the aflatoxin M<sub>1</sub> in milk of lactating animals fed rations containing aflatoxin B<sub>1</sub> (AFB<sub>1</sub>). However, the available data on toxicokinetics of several mycotoxins in animal models indicate that exposure to mycotoxins can be accurately measured by biomarkers in several bio-specimens, especially in serum. For example, serum aflatoxin B<sub>1</sub>-lysine (AFB<sub>1</sub>-lys), a digest product of AFB<sub>1</sub>-albumin used for human biomonitoring of aflatoxin exposure has been tested as a marker of aflatoxicosis in broilers and piglets. Thus AFB<sub>1</sub>-lysine has potential as an AFB<sub>1</sub> specific biomarker for diagnostic purposes, and for evaluating the efficacy of chemo protective interventions such as mineral adsorbents in production animals. For fumonisin B<sub>1</sub> (FB<sub>1</sub>), experimental studies indicate that plasma and urinary FB<sub>1</sub> are good biomarkers of early exposure of pigs to low dietary FB<sub>1</sub> levels, although plasma is recommended to assess prolonged exposure (> 14 days). In recent years, the liquid chromatography tandem mass spectrometry (LC-MS/MS) based on the multi-analyte approach has been successfully introduced into the field of mycotoxin analysis, opening new perspectives for the evaluation of suitable biomarkers for mycotoxins mixtures. Therefore, further validation studies will be required to provide physiologically based toxicokinetics of serum biomarkers of individual and combined mycotoxins in production animals.

**Keywords:** Mycotoxins mixtures. Serum biomarkers. Toxicokinetics.

## LECTURE ABSTRACTS

## Mycotoxins in pig production

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Pork is the most consumed animal protein around the globe. As the world population increases, a rise in pig production efficiency is required. In the last year, 108.2 million tons of pork was produced in the world. China, the European Union, the United States, Brazil and Russia are the main producers, supplying 86% of the total production worldwide. Mycotoxins are secondary metabolites produced by different fungi species that commonly contaminate the food and feed chain. Acute and chronic toxic effects are known in pigs. In general, mycotoxins induce a decrease in daily feed intake and weight gain (~21%), deteriorating the feed conversion ratio. The concentration of mycotoxins in feed and the age of the animals are the main parameters influencing the weight gain. Young animals and male are the most affected. Deficits in zootechnical parameters are mainly a consequence of changes in animal health. The intestine is the first system to have contact with feed contaminants and it is considered a target tissue for the action of mycotoxins. Intestinal epithelial cells chronically exposed to low levels of mycotoxins present a disruption in paracellular permeability allowing the passage of luminal antigens to the intestinal lamina propria. This event plays a pivotal role in promoting intestinal inflammation, followed by an increase in the production of reactive oxygen species and a lower antioxidant response. The consequences include villi fusion and atrophy, enterocyte flattening, necrosis of apical enterocytes and bacterial adhesion in the areas of necrosis. These changes in intestinal morphology and homeostasis result in an impaired absorption of nutrients and contribute to systemic diseases. In conclusion, ingestion of feed contaminated with mycotoxins affects animal health causing economic losses in the pig production chain.

**Keywords:** Animal health. Economic losses. Natural contaminants.

## LECTURE ABSTRACTS

## Immunological methods in the analysis of mycotoxins in food and feed

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Mycotoxins are toxic substances widely distributed in food destined to human or animal consumption. Nowadays, over 400 different mycotoxins are known, and 10 to 15 are considered to be of commercial interest. In this way, mycotoxin analysis is essential to evaluate the extent of mycotoxin contamination, determine risk analysis, confirm the diagnosis of a mycotoxicosis and for monitoring mycotoxin mitigation strategies. The competitiveness in the food and feed industry involves major cost reduction, with no extensive use of labor and immediate results. Fast methods have achieved more importance, due to the lower cost, ease of use and the possibility of in loco analysis. In general, the expression fast method refers to a method faster than the reference method, and has the objective to promote it. The development of suitable analytical techniques for the correct estimation of this variety of metabolites present in the wide range of food and feed where these substances are present, is a challenging task. Appropriate sampling is the first point to be observed. Then, sample preparation procedures, such as extraction, must allow mycotoxins analysis without the interference of major food components, as starch, protein, fat, pigments. In general, separation, identification and quantification are carried out with methods as Liquid Chromatography, coupled to a variety of detectors. Immunoassays were developed based on the specificity of the reaction between antigen and antibody. The high specificity of antibodies to the mycotoxins for which they were produced, allows the reliable determination in samples without extensive purification, with low use of solvents, and analysts with relatively low training. Immunoassays are used mainly to clean up, or directly to quantitative analysis. Immunoaffinity columns (IAC) are used in the clean up step, produced with monoclonal antibodies highly specific and sensitive. It contains anti-mycotoxin antibody that is immobilized onto a solid support such as agarose gel in phosphate buffer, all of which is contained in a small plastic cartridge. Enzyme linked immunosorbent assay (ELISA) kits are used to quantitative analysis. ELISA has low sample volume requirements and often less sample extract clean up procedures compared to conventional methods.

**Keywords:** Cost reduction. Mitigation strategies. Mycotoxicosis.

## LECTURE ABSTRACTS

## Ultra-rapid method for the management of mycotoxins in agribusiness

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The effects of mycotoxins on the production animals are well established, especially the economic ones, which significantly impact the agribusiness. Historically, control of raw materials has time of analysis as a limiting factor. However, such process may be enhanced by using tools of fast diagnosis, which is thus interpreted through a set of factors. Those are then translated by means of an algorithm and generate the Mycotoxins Risk graph. An ultra-rapid and handy tool for the diagnostic of mycotoxins via Near-Infrared Spectroscopy - NIR has recently been developed. The NIR technology is a highly precise tool that emits electromagnetic radiation. The energy absorption results from the organic compounds which are present in the sample, and may be used to obtain a direct or indirect estimation of the concentration of a substance. The technic comprehends the integration of a database attained through traditional methodologies and the spectral evaluations. This information is subjected to chemometric methods, and the prediction equations are then produced. When compared to other methods, important advantages of the use of NIR for control and monitoring are the easiness in sample preparation and analysis. Other aspects as positivity, prevalence of more than one mycotoxin in the raw material, animal species and sex, and production phase are extremely relevant and translate into the risk that each mycotoxin may produce along the production chain. Thus, modern solutions with a broad vision of science in mycotoxins may be of great aid for agribusiness. The NIR technology for mycotoxins analyses encompasses several technical benefits, besides allowing for the management of the Mycotoxins Risk and the decision-making. The present form of the Mycotoxins Risk undeniably represents the state of art of the complex system of mycotoxins management.

**Keywords:** Analyses. Chemometric methods. Control. Monitoring.

## LECTURE ABSTRACTS

## Innovative tools for the control of toxigenic fungi and related mycotoxins in the field

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Among the emerging issues in food safety, the increase of plant diseases associated to the occurrence of mycotoxigenic fungal species is of major importance. As a result of their secondary metabolism, these fungi can produce mycotoxins, which are low-molecular-weight toxic compounds, provided of a broad range of biological activities. The consumption of mycotoxin-contaminated food can have multiple consequences on both human and animal health worldwide. Mycotoxins occur naturally and are the most prevalent source of food related health risks in field crops of high agro-food interest. Many of these crops, such as cereals, can suffer of a devastating worldwide diseases, often caused by a complex of fungal toxigenic species producing a wide range of mycotoxins, that can be accumulated in the final products. Management of good agricultural practices in the pre-harvest is a key issue for minimizing the risk of mycotoxin accumulation in crops before the harvest. Such practices can involve traditional tools such as crop rotation, tillage, proper fertilization and distribution of fungicides, variety selection, timely planting and harvest, and innovative tools such as the use of predictive models, new fungicides with low impact, biological control agents, in situ early and easy DNA-based detection tools, and new ICT tools for real time management of the diseases. Along the 7th Framework Large collaborative project of European Union "MycoRed" "Novel integrated strategies for worldwide mycotoxin reduction in food and feed chains", and the Horizon 2020 European Union project "Integrated and Innovative Key actions for mycotoxin management in the food and feed chain" "MycoKey", an integrated and innovative management of pre-harvest practices for reducing the risk of contamination in crops has been and is in development. Moreover, further studies to evaluate the role played by the so called "masked" mycotoxins, in the final toxicity of food products are also deeply carried out. An update of the main scientific activities performed in both Projects will be provided, to define the main scenarios characterizing the work in progress on the mycotoxins in Europe and at worldwide level.

**Keywords:** Animal health worldwide. Human health worldwide. Masked mycotoxins.

## LECTURE ABSTRACTS

## Mycotoxin risk management

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Quality starts in the field. It is critical that when discussing mycotoxin intervention methods, the issues should start being handled where they are mostly produced, which is still in the field. The most common diseases faced by corn producers are caused by fungi. So, understanding the development of those fungi, their life cycles, and optimal growth conditions allow the industry to create alternatives to control the fungi growth and the possible production of mycotoxins. The selection of fungi and insect resistant hybrids, soil management, application of fungicides, proper harvest maturity, drying and storage process, management of the feed mills, using mycotoxin detoxifying agents, and setting correct risk levels are only a few steps to better manage the risks. Research has shown that insects and fungi resistant hybrids will be less contaminated with mycotoxins, the same effect is seen when hybrids are properly selected for the region they will be grown. Soil management plays an important role on the disease life cycle as most of the fungi can use residues of previous crops to survive and contaminate the following crop. The correct use of fungicides and bio-control methods can have a direct impact on the contamination levels, helping to reduce the risks. After harvest, it is recommended a well-designed sampling method at the reception of the grains, this will allow the companies to determine how to handle the ingredient during storage and at the final feed. Proper storage of the grains will not fix previous problems however will be critical to avoid further contamination and deterioration. Using safe drying methods, applying fungi inhibition products and properly aerate the grains throughout the storage period are a few risk management tools. On the feed mill it is imperative that the equipment is kept cleaned and out of debris, avoiding an increased contamination, also the use of mycotoxin detection methods can help on making the decision of what kind and how much to use of detoxifying agents. The future is arriving with the use of new technologies to better predict problems and provide valuable information to grains and animal producers.

**Keywords:** Bio-control methods. Detoxifying agents. Fungicides methods.

## LECTURE ABSTRACTS

## Can biotransformation be an effective strategy for mitigating deoxynivalenol in the agri-food value chain?

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The presence of mycotoxins in agricultural commodities is outspreading worldwide in recent years; one of the most significant mycotoxins associated with animal production is deoxynivalenol (DON) because of its high prevalence in grains and toxicity to farm animals. Many research teams around the globe have been involved in exploring environmentally-friendly and cost-efficient methods to combat such mycotoxin, and the biological detoxification is a very promising one due to its specificity, acceptance by consumers, and possible utilization under mild processing conditions. This presentation will reveal the development of the mycotoxin biotransformation strategy, provide an understanding of the DON epimerization system and demonstrate various potential applications of the biotransformation for mitigating mycotoxins in the agri-food value chain. The most recent isolation and characterization of *Devosia mutans* 17-2-E-8, a novel gram-negative bacterium capable of transforming DON to 3-epi-DON under aerobic conditions led to a new approach for DON mitigations. The bacterial transformation undergoes a two-step bio-catalysis. DON is initially oxidized to 3-keto-DON, that is sequentially reduced to 3-epi-DON (epimerization). The abrogation of toxicity by epimerization was confirmed using different human cell lines and mouse models and is predicted to result from the isomeric changes that influence 3-epi-DON ability to form hydrogen bonds within the peptidyl transferase center of ribosomes. The two enzymes responsible for DON epimerization have been identified and characterized, and performed great effectiveness when tested *in vitro*. It is demonstrated that the enzymes are stable at moderate high temperatures (50 - 60 °C) and show great potential to be incorporated into the processing of cereals or feed matrices. The nature of the epimerization suggests that the process can be optimized and exploited for the development of feed additives and/or genetically engineered crops, or used during ethanol fermentation process, in order to reduce foodborne exposure of consumers and farm animals to the mycotoxin.

**Keywords:** Bio-catalysis. DON. Mitigating mycotoxins.

## LECTURE ABSTRACTS

## MycoKey approaches to mitigate mycotoxins in feed: efficacy assessment of physico-chemical treatments in improving the performances of bentonite as multi-mycotoxin binders

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Mycotoxins contamination and co-occurrence of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), zearalenone (ZEA), ochratoxin A (OTA) and fumonisin B<sub>1</sub> (FB<sub>1</sub>) in animal feed are frequently observed and can impact animal health also at low doses. The addition of binders to contaminated diets (feed additives) is considered a promising dietary approach to reduce toxic effects of mycotoxins. The main drawback in the use of feed additives is that most mycotoxin adsorbents appears to bind to only a limited group of mycotoxins while showing very little or no binding to others. A variety of substances have been investigated as potential mycotoxins adsorbing agents, which includes aluminosilicates, activated carbons, complex indigestible carbohydrates and synthetic polymers. Aluminosilicates (e.g., smectites) are the largest class of mycotoxin sequestering agents and most studies on the alleviation of mycotoxicosis have focused on them. The purification and physicochemical modifications of pure smectites have a great importance to prepare some high technology materials such as Na-exchanged bentonites, acid activated clays and organoclays. The aim of this work was the development of a new bentonite-based material showing multi-mycotoxin adsorption efficacy. A specific surface-modification of Na/Ca smectites, comprising activation with an inorganic acid and functionalization with an organic non-toxic compound, was developed to enhance the adsorption features of the native smectites (untreated samples). The process was optimized at lab level, and optimal conditions (geological origin and physico-chemical properties of smectites, type and concentration of chemical agents, temperature, time of contact) were identified. The new additive developed by this study is an innovative bio-organoclay sequestering at low dosages (0.25 - 0.5% w/v) more than 95% of AFB<sub>1</sub>, FB<sub>1</sub>, OTA, and ZEA (from a 1 µg/mL multi-toxin solution), and in a large range of pH values (3 - 9). The *in vivo* efficacy of the bio-organoclay in

reducing the intestinal absorption of mycotoxins was confirmed by experiments with rodents and target animal species. This product can be considered safe, as it has been obtained using reagents that are listed in the European Union Register of Feed Additives (EC Regulation, No.1831/2003).

**Keywords:** Feed additives. Mycotoxins. Mycotoxin detoxification. Multi-mycotoxin detoxifying agents.

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SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Aflatoxin and fumonisin residues in liver of broilers fed aflatoxin, fumonisin and turmeric powder

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The presence of mycotoxins in feed and food products is extremely important in terms of human and animal health, and many of these toxins may result in serious economic losses. The objective of the research was to determine the residue concentration of aflatoxin (aflatoxins B<sub>1</sub>, G<sub>1</sub>, B<sub>2</sub>, and aflatoxicol) and fumonisin (fumonisins B<sub>1</sub> and B<sub>2</sub>) in the liver of broiler chickens fed aflatoxin culture material (AF), fumonisin culture material (FB) and turmeric powder, alone or in combination, using the following treatments (T): T1 (control): 0 AF + 0 FB + 0 Turmeric; T2: 0 AF + 0 FB + 222 mg/kg Turmeric; T3: 0 AF + 20 mg/kg FB + 0 Turmeric; T4: 0 AF + 20 mg/kg FB + 222 mg/kg Turmeric; T5: 0.5 mg/kg AF + 0 FB + 0 Turmeric; T6: 0.5 mg/kg AF + 0 FB + 222 mg/kg Turmeric; T7: 0.5 mg/kg AF + 20 mg/kg FB + 0 Turmeric; T8: 0.5 mg/kg AF + 20 mg/kg FB + 222 mg/kg Turmeric. A completely randomized design was used with a 2 x 2 x 2 factorial. Data were analyzed with SAS Mixed procedure, using Fisher's protected least significant difference ( $p \leq 0.05$ ). The residues of fumonisin B<sub>2</sub> in the liver were significant ( $p = 0.0001$ ) for the interaction of all the factors (aflatoxin, fumonisin, and turmeric). All residues of aflatoxin and fumonisin were significant for the aflatoxin factor. The results showed no effect of turmeric powder in reducing the residue concentration of aflatoxin and fumonisin in the liver of the broilers.

**Keywords:** Antioxidant. Mycotoxins. Poultry.

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SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Contamination of poultry litter by toxigenic fungi and its relation with mycotic pododermatitis

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Contamination by living organisms in poultry litter may occur throughout the breeding stages of poultry. The presence of mycotoxins in poultry feed or poultry litter can affect poultry health and the safety of meat products. Pododermatitis is an injury to the plantar surface of the feet of poultry caused by nutritional factors and inadequate handling of poultry litter. The present work identified genera of filamentous fungi that produce mycotoxins, present in the poultry litter and its relation with tegument tissue of the chickens feet. As expected, the physical-chemical characteristics of the poultry litter as pH, moisture (mc) and water activity ( $a_w$ ) ranged from 6.3 to 8.7, 9.7 to 40.6% and 0.74 to 0.98, respectively. These conditions are ideal for the proliferation of fungi toxigenic to produced mycotoxins. Through scanning electron microscopy, it was possible to identify fungi of the genus *Trichoderma*, *Aspergillus*, *Fusarium* and *Penicillium* that were present in all stages of breeding. Most isolated fungi can produce mycotoxins (aflatoxins, fumonisins/deoxinivalenol/zearalenone and ochratoxin A, respectively). Tissue fungal infections (meat) can lead to possible contamination by fungal toxins, since the feet of the poultry are in direct contact with the aviary bed. This dermal contact with mycotoxins compromises the quality of the poultry foot that is currently a product of export to several countries and also the health of poultry.

**Keywords:** *Aspergillus*. Fungi. Mycotoxins. Poultry litter. *Trichoderma*.

SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Effect of a feed naturally contaminated with low levels of fumonisins with and without mycotoxins eliminator additive on immune cells of broiler chickens

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Fumonisin are mycotoxins produced by *Fusarium*, a fungi commonly found in corn and derivatives. Mycotoxins induce toxic effects impairing animal health by suppressing the immunity of the animal. Therefore, the objective was to determine the effect of fumonisins on the immune response of broiler chickens and to evaluate the efficacy of a mycotoxin eliminator additive (MEA) to counteracting these effects. 90 male Cobb® 500 one-day-old chicks were divided into three groups: NEG - negative control, POS - positive control (fumonisins at 17 ppm) and MEA - treated group (T2 + 0,2% of MEA). The diet of POS and MEA was prepared with corn naturally contaminated with fumonisins (28 ppm: 69% of FB<sub>1</sub> and 31% of FB<sub>2</sub>). The following parameters were evaluated: activated CD8 T-lymphocytes (CD8<sup>+</sup>CD28<sup>-</sup>), helper T-lymphocytes (CD4<sup>+</sup>TCRVβ1<sup>-</sup>), mucosal T-lymphocytes (CD4<sup>+</sup>TCRVβ1<sup>+</sup>) and phagocytic monocytes (Kul<sup>+</sup>MHCII<sup>+</sup>) in peripheral circulation by flow cytometry. In addition, intestinal T-lymphocytes (CD3<sup>+</sup>) were quantified using immunohistochemical analysis of jejunum tissue. Data were submitted to Analysis of Variance (ANOVA) followed by *post hoc* Fisher's LSD test ( $p \leq 0.05$ ). Results show immunologic alterations in blood biochemistry due to the ingestion of toxins starting at 3 days of exposure. The inclusion of MEA to the diet inhibited the suppression of phagocytic monocytes, resulting in an improved unspecific early immune response increasing the disease resistance of the animal. At day 7, addition of MEA prevented the activation of CD8 T-Lymphocytes, and thereby avoiding excessive inflammation and metabolic waste. Moreover, MEA supplementation also reduced the negative impact of the toxin on the circulating CD4 T-Lymphocytes. Fumonisin affect the intestinal integrity, indicated by the presence of lymphocytes in mucosa. T-lymphocytes count in jejunum tissues was superior in group POS at 7 days, in agreement with the increase of circulating mucosal CD4 T-lymphocytes. Supplementation of MEA enhanced the intestinal barrier as indicated by a reduced T-lymphocyte count. To conclude, fumonisins cause quick immunologic alterations at both systemic and intestinal level, thereby compromising the birds' immunity. The inclusion of MEA in the diet totally compensated for the negative impact of fumonisins.

**Keywords:** Broilers. Flow cytometry. Immunohistochemical. Immune response. Mycotoxins.

SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Effect of deoxynivalenol on oxidative stress in intestine, liver and kidney from broilers chickens

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Brazil is the world's largest exporter of chicken meat. Mycotoxins are metabolites produced by filamentous fungi and negatively affect the performance and immune response of animals. Deoxynivalenol (DON) is a contaminant that often occurs naturally in cereals and its derivatives. The aim of this study was to evaluate the effect of ingestion of diet contaminated with DON on oxidative stress in jejunum, ileum, liver, and kidney from broiler chickens. Broiler chickens (n = 8), Ross, male, with one day of age (DA), were housed, received water and food *ad libitum* and heating according to the physiological requirement. The animals were divided into two treatments: T1 - negative control (n = 4, uncontaminated diet); T2 - Exposed group (n = 4, contaminated diet with DON 10 mg/kg). At 7 DA, T2 group started to eat the contaminated feed. At 20 DA, the chickens were euthanized; jejunum, ileum, liver and kidney fragments were collected, stored in microtubes at -80 °C until the tests were performed: FRAP, ABTS, NBT, and TBARS. The data were submitted to the T-test, significance level of 5%. DON intake induced a significant increase in levels of FRAP (jejunum; ileum and kidney); ABTS (kidney); NBT (liver and kidney) and TBARS (jejunum; liver and kidney) in relation to the control. Most of DON metabolism and excretion occurs in liver and kidneys. The increase in FRAP antioxidant activity in jejunum, ileum, and kidney in T2 is probably a consequence of the stimulus to the production of antioxidant factors due to the ingestion of DON which is an inducer of oxidative stress. In the kidney and liver from DON exposed birds there was

an increase in reactive oxygen species (ROS); these organs tend to be more affected because they are responsible for their metabolism and excretion. In jejunum there was an increase of lipid peroxidation (TBARS) in T2, the hypothesis to justify the increase only in this segment is because it is the place who has the highest intestinal absorption rate of these compounds. With this study it can be concluded that DON induces oxidative stress in the analyzed organs, mainly affecting liver and kidney.

**Keywords:** Free radical. Gut health. Metabolism. Mycotoxin. Poultry.

SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Effects of deoxynivalenol on the liver of broilers chickens: *in vivo* assay

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Brazil is a centerpiece in world poultry farming. Mycotoxins control is a challenge, since these metabolites are resistant to heat treatment. Deoxynivalenol (DON) is one of the most frequent mycotoxins in feed. The aim of this study was to evaluate the histologic alterations in broiler chickens' liver fed with DON. Broiler chickens (n = 24), Ross, male, with one day of age (DA), were housed, received water and food ad libitum and heating according to the physiological requirement. The chickens were divided into two treatments: T1 - negative control (n = 12, uncontaminated diet); T2 - Exposed group (n = 12, contaminated diet with DON 10 mg/kg). At 7 DA, T2 group started to eat the contaminated feed. At 14 and 20 DA, six animals per treatment were euthanized, liver fragments were collected and fixed in 10% buffered formalin. The liver was processed and stained by HE technique. The slides were examined according to the following criteria and degrees of severity (DS): trabecular disorganization (DS1), inflammation (DS1), cytoplasmic vacuolization (DS1), nuclear vacuolization (DS1), megalocytosis (DS2), apoptosis (DS2) and necrosis (DS3). The results were submitted to T-test, significance level of 5%. A significant increase (p = 0.03) in the lesion score in the animals exposed to DON was observed at 14 DA (5.66) in relation to the control (1.5). However, at 20 DA, no difference was observed between treatments (2.2 and 2.55 for control and DON, respectively). DON metabolism occurs in two phases. Phase I is characterized by metabolism by the intestinal microbiota and enterocytes; in phase II DON and its derivatives are metabolized by liver and kidneys. In the liver the main route of metabolism is conjugation with glutathione and biliary excretion. In this study, it was observed the occurrence of hepatic lesions in the birds only at 14 DA,

probably due to the acute phase of exposure and the inability/immaturity of the chicken liver in the initial phase to detoxify these compounds. In conclusion, the supply of DON (10 mg/kg) contaminated diet leads to significant changes in the liver morphology of broiler chickens at 14 DA and may interfere with animal health.

**Keywords:** Animal health. Detoxification. Mycotoxins. Poultry. Systemic effect.

SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Efficacy of mycotoxin eliminating agent against don contamination in weaned piglets

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Deoxynivalenol (DON), is the most frequently-occurring type B trichothecene produced by several field fungi, and is commonly found in cereals and grains, in areas with a moderate climate. There are obvious species variations in the susceptibility to DON, and pigs show the highest sensitivity to DON. Moreover, weaning piglets seems to be much more susceptible compared to growing and finishing pigs. The toxic effects induced by DON are mainly on affecting the immune system, feed consumption, growth, and intestinal function. Exposure of intestinal epithelial cells, highly dividing cells, to DON may alter their capacity to proliferate and to insure a proper barrier function, leading to impaired absorption of nutrients. It alters the barrier function of intestinal cells directly or indirectly by modulating the expression of tight junction proteins. A trial was setup to investigate zootechnical losses and damage to the intestinal tract induced by mixing a natural contaminated corn batch into the diet and to evaluate the efficacy of a mycotoxin eliminating agent (MEA). In total 90 piglets were housed in groups of 10 animals in floor pens for 42 days from weaning age onwards. They were allocated to one of the treatments: negative control group (NEG), positive, DON contaminated group (DON) and a DON contaminated group supplemented with MEA. At day 28 of the trial 4 piglets with the most average body weight of each pen were euthanized and intestinal samples were taken for gut morphology, gut barrier functioning and endogenous oxidative status. A significant drop in growth in the DON contaminated treatment compared to the control at the end of the trial was observed. The MEA could compensate for this loss. Also intestinal morphology at day 28, villus height and crypt depth, was improved by the addition of MEA, since the intestinal integrity is seriously affected by DON. No significant differences were found in the protein expression of the most important tight junction proteins. However, Claudin-1 and ZO-1 were numerically lowered by the presence of DON in the feed. Addition of MEA could counteract these numerical effects. Finally, cellular

oxidative stress is one of the non-specific responses of cells to toxic or inflammatory injury. DON has the capacity to induce oxidative stress, whereas the MEA improves the antioxidant capacity of the intestine. To conclude, the MEA was able to compensate for the negative impact of DON in weaned piglets on zootechnical performance and gut integrity by supporting the immune system stimulating villi growth, tight junction expression and the antioxidant capacity of the intestine.

**Keywords:** Flow cytometry. Immunohistochemical. Immune response. Mycotoxin. Piglets.

SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Evaluation of growth performance and health parameters of *Rhamdia quelen* fed with Aflatoxin B<sub>1</sub> contaminated feed

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In fish, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) contamination may trigger a number of nonspecific clinical signs, it has been associated with low animal growth, behavioral abnormalities, inhibition of immunity, necrotic hepatocytes and reproductive damage. Hematological, biochemical and histopathological parameters are considered important diagnostic tools to evaluate metabolic and cellular processes in these species. Thus, the objective of this study was to determine the possible deleterious effects of diets providing different levels of AFB<sub>1</sub> within a period of 56 days, through production performance parameters, hematological, biochemical and histopathological in silver catfish (*Rhamdia quelen*). 624 silver catfish fingerlings were distributed into four different groups (G1, G2, G3 and G4 containing 0, 45, 90 and 180 µg.kg<sup>-1</sup> AFB<sub>1</sub> in feed, respectively), the groups were randomly divided into 24 aquariums (n = 26 fingerlings/aquarium) and fish fed for 56 consecutive days with pelleted feed. Zootechnical indexes, hematological, serum biochemistry and histopathological parameters were evaluated on days +28, +42 and +56 from the experimental period. There were no significant differences (p > 0.05) in the zootechnical index parameters when comparing the groups exposed to AFB<sub>1</sub> with the control group. AFB<sub>1</sub> was able to induce hematological and biochemical alterations consistent with metabolic disorders in the silver catfish. It was not possible to show alterations in liver, gill and renal tissue compatible with AFB<sub>1</sub> exposure. The different concentrations of AFB<sub>1</sub> do not affect the zootechnical performance of silver catfish in a linear way, nor do they cause histological alterations in the evaluated organs. However, the animals exposed to AFB<sub>1</sub> presented alterations in hematological and biochemical parameters, consistent with immunosuppression and hepatotoxicity, both reported in aflatoxicoses in animals.

**Keywords:** Daily Weight Gain. Fish. Hepatic Enzymes. Leukocytes. Mycotoxin.

SESSION I  
IMPACT OF MYCOTOXINS IN ANIMAL PERFORMANCE

## Occurrence of mycotoxins in Brazilian fish and feed followed by risk assessment through deterministic approach

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Animal feed carry on various fungi species, these fungi can produce multiples mycotoxins. Even with frequently feed monitoring, mycotoxins may be present in seafoods after contaminated feeds been ingested by animals. The objective was to evaluate the possible contamination of mycotoxins in fish (*Oreochromis niloticus*) commercialized in Curitiba, Paraná, Brazil, as well as to evaluate the possible contamination of these mycotoxins in feed of these animals through high performance liquid chromatography coupled to a mass spectrometry detector (LC-MS/MS-LIT). Were collected 160 fish samples and 160 samples commercial fish feed were investigated for the presence of 21 legislated mycotoxins, by means of liquid LC-MS/MS-LIT. With the results of chromatographic analysis, the Estimated Daily Intake (EDI) of mycotoxins by fish in  $\mu\text{g.kg}$  of body weight. $\text{day}^{-1}$ . The mycotoxins detected in commercial fish feed were: aflatoxin B<sub>2</sub> (AFB<sub>2</sub>), fumonisins B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub> (FB<sub>1</sub>, FB<sub>2</sub>, FB<sub>3</sub>), zearalenone (ZEA), ochratoxin A (OTA), enniatin B (ENB) and enniatin B<sub>1</sub> (ENB<sub>1</sub>). The frequency of contamination with AFB<sub>2</sub> was 10,63% of total samples, with concentrations ranging from 1.67 to 6.98  $\mu\text{g.kg}^{-1}$ . ZEA was detected in 115 out of the 160 samples studied (71.88%), and its average concentration was 66.88  $\mu\text{g.kg}^{-1}$ . Among all emerging mycotoxins tested, only enniatins (ENs) were found in sampled commercial fish feed. The frequency of contamination with ENs (ENB and ENB<sub>1</sub>) was 81.88% and 25.00%, respectively. Already in fish samples, only ENB was detected. The frequency of contamination of total samples was 86.87% with concentrations ranging from 0.1 to 1.63  $\mu\text{g.kg}^{-1}$ . Regarding to EDI, among all the mycotoxins tested, the FB<sub>2</sub> is the one that had the highest EDI value (790.72  $\mu\text{g.kg}$  of bw. $\text{day}^{-1}$ ), whereas the lowest EDI value was for AFB<sub>2</sub> (1.96  $\mu\text{g.kg}$  of bw. $\text{day}^{-1}$ ). In general, at least one mycotoxin was found in each feed sample. It demonstrates the importance of

monitoring and implementing regulations for safe levels of these mycotoxins in feed. The presence of ENB in fish meat samples demonstrates the possibility that some mycotoxins have to be accumulated in the musculature and be a source of contamination for humans.

**Keywords:** Animal. Contamination. Humans. LC-MS/MS-LIT. Multi-mycotoxins.

SESSION II  
RECENT APPROACHES TO EVALUATE THE  
RISK OF MYCOTOXIN IN ANIMAL HEALTH

## Aflatoxin B<sub>1</sub> residues in meat of swine fed AFB<sub>1</sub> contaminated diet and efficacy of HSCAS to reduce the contamination levels

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Aflatoxins are toxic secondary metabolites produced mainly by *Aspergillus flavus* and *A. parasiticus*. One of the most critical aspects of aflatoxins in animal production is the presence of AFB<sub>1</sub> residues and its metabolites in animal products. In this way, mycotoxin binders can be added to feedstuffs to prevent the intestinal absorption of mycotoxins and allow fecal excretion of adsorbent-toxin complex. Among the adsorbents, hydrated sodium calcium aluminosilicate (HSCAS) is one of the most commonly used anti-mycotoxin additive to reduce the bioavailability of aflatoxins. The objective of this study was to evaluate the HSCAS efficacy to reduce the levels of residual AFB<sub>1</sub> in meat samples from swine fed with AFB<sub>1</sub> contaminated diet. Twenty-four crossbred barrows (average age 28 days) were allocated at individual cages. After 21 days of adjustment period, animals were randomly distributed in four treatments in a 2 x 2 factorial design, corresponding to two levels of AFB<sub>1</sub> inclusion in diets (0 and 1.1 mg.kg<sup>-1</sup>) and two levels of HSCAS incorporation (0 and 0.5%). The intoxication was maintained for 42 days. The efficiency of the adsorbent to reduce the AFB<sub>1</sub> residues in meat was assessed by a validated ultra-performance liquid chromatography coupled to a tandem mass spectrometer (UPLC-MS/MS) method. Residues of AFB<sub>1</sub> were not detected in meat samples from pigs fed on the basal diet (BD) and BD+HSCAS. Two pigs from the BD+AFB<sub>1</sub> group died during the experiment and are not included in the results. The concentration of residual AFB<sub>1</sub> in pigs fed with BD+AFB<sub>1</sub> were 4.71 ± 2.11 ng.g<sup>-1</sup> of meat. Supplementation of HSCAS significantly reduced (p < 0.05) the AFB<sub>1</sub> levels in meat for 0.56 ± 0.41 ng.g<sup>-1</sup>, decreasing meat

contamination by 83%. In conclusion, our results showed that addition of 0,5% of HSCAS can reduce the amount of residual AFB<sub>1</sub> in meat of intoxicated pigs, even in a high level of AFB<sub>1</sub> exposure.

**Keywords:** Adsorbent. Mycotoxin. Muscle. Pigs. Pork meat.

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SESSION II  
RECENT APPROACHES TO EVALUATE THE  
RISK OF MYCOTOXIN IN ANIMAL HEALTH

## Antioxidant capacity of *Lactobacillus plantarum* metabolites exposed to deoxynivalenol in pigs intestinal tissue

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Deoxynivalenol is a *Fusarium* toxin that causes a variety of toxic effects in several systems such as lymphoid, intestinal and reproductive. Generation of reactive oxygen species (ROS) are recognized as a plausible mechanism for DON-induced toxicity. Therefore, mechanisms that reduce oxidative stress in DON mediated cellular damage are needed. On the other hand, the nitroblue tetrazolium (NBT) is a qualitative test that undergoes reduction by O<sub>2</sub><sup>-</sup> (superoxide radical anion) to form diformazan, a dark blue insoluble precipitate. NBT is susceptible to reduction by several tissue reductases. The purpose of this study was to investigate with a NBT test, the potential modulation of *Lactobacillus plantarum* (Lp) metabolites in oxidative stress when exposed to DON using an ex vivo model. Cell free supernatant were obtained from two strains of *Lactobacillus plantarum* culture, one a comercial strain (ATCC 14917) (strain 1) and the other isolated from wheat grain (strain 2). Both Lp culture supernatant were followed by extration in water immiscible solvents dichloromethane and ethyl acetate to obtain the bacteria metabolites. Intestinal explants were obtained from jejunum of four animals and incubated with the bacteria metabolites for two hours; explants were exposed to DON one hour after the metabolites exposure. After incubation, explants were processed for oxidative stress assay (NBT). Intestinal explants from piglets were exposed to the following treatments: culture medium (control), DON (10 µM), bacteria metabolites of both strain of Lp extracted with dichloromethane and ethyl acetate alone or associated with DON. Superoxide radical anion production was measured by the NBT assay after two hours exposure. DON differed statistically from control (p = 0.000002) showing an important ROS generation. On the other hand, explants incubated with bacteria metabolites of both strains of Lp and obtained with the two solvents, alone or combined with DON remained statistically similar to control. With this results we could conclude that *L. plantarum* metabolites act as an antioxidant and ROS scavenger alone or combined with DON.

**Keywords:** *Lactobacillus plantarum*. Metabolite. Oxidative stress.

SESSION II  
RECENT APPROACHES TO EVALUATE THE  
RISK OF MYCOTOXIN IN ANIMAL HEALTH

## Assessment of Fumonisin B<sub>1</sub> in pig hair as an exposure biomarker

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Fumonisin B<sub>1</sub> (FB<sub>1</sub>) is a common contaminant of corn (*Zea mays* L.) that causes pulmonary edema in pig with high mortality. Clay products have been used as absorbent of FB<sub>1</sub> to reduce its effects. FB<sub>1</sub> contamination in pig is difficult to detect and swine hair may be used as a non-invasive biomarker for the presence of this toxin. The aim of this study was to evaluate the presence of fumonisin B<sub>1</sub> in swine hair in swine groups exposed to FB<sub>1</sub> with clay. Male swine were fed with standard laboratory chow, tap water and housed indoors under controlled conditions for 21 days. They were divided in four groups of 8 pigs: control (Group A), 15 mg of fungal culture kg<sup>-1</sup> (Group B), 15 mg of fungal culture kg<sup>-1</sup> and 0.15% of experimental clay (Group C), 15 mg of fungal culture kg<sup>-1</sup> and 0.20% of experimental clay (Group D) and evaluated in the 3<sup>rd</sup> and 6<sup>th</sup> weeks. Hair samples were stirred in ethanol for 15 minutes and dried at 50 °C for 3 hours, cut into small pieces. 0.5 g of swine hair samples were extracted with 20 mL of methanol overnight at 50 °C. The extract was filtered, and 15 mL was dried, resuspended in 20 mL of methanol: water (70:30), added 8 mL of water and filtered again. The solution was washed with 5 mL of hexane, centrifuged at 3000 rpm and top layer was discarded. pH was adjusted to 6. An aliquot of 20 mL of the extract was passed through the SAX column and eluted with 14 mL of methanol: acetic acid (0.5%). The eluted was dried, resuspended in 300 µL of water: acetonitrile (90:10), centrifuged and injected in HPLC. On the 3<sup>rd</sup> week, fumonisin B<sub>1</sub> was detected in levels of 20.9, 11.4 and 18.5 ng.g<sup>-1</sup> on group B, C and D, respectively. On the 6<sup>th</sup> week, toxin was identified only on group B at a level of 1.3 ng.g<sup>-1</sup> and in the

both weeks, it was not possible to identify FB<sub>1</sub> on the control samples. Swine hair can accumulate FB<sub>1</sub>, however clay showed an interference in the toxin absorption, decreasing it.

**Keywords:** Clay. Exposure. FB<sub>1</sub>. Hair.

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SESSION II  
RECENT APPROACHES TO EVALUATE THE  
RISK OF MYCOTOXIN IN ANIMAL HEALTH

## Determination of Aflatoxin B<sub>1</sub> in *Rhamdia quelen* tissues and water through LC/MS-MS

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The presence of aflatoxins in the raw materials used in animal feed may result in residues in the final product. The ingestion of this contaminated products may present a risk to human health. This study aimed to investigate the possible accumulation of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) in muscle, organs, intestinal content, plasma and water of *Rhamdia quelen* that ingested animal feed contaminated with AFB<sub>1</sub>. Six hundred and twenty four *Rhamdia quelen* fingerlings were divided in four different feeding groups (G1, G2, G3 and G4 containing 0, 45, 90 and 180 µg.kg<sup>-1</sup> of AFB<sub>1</sub>, respectively) and randomly divided into 24 aquariums (n = 26 fingerlings by aquarium) and fed for 56 consecutive days with pelleted feedstuffs. In order to quantify AFB<sub>1</sub> in water, 2 mL samples were collected from each aquarium on day 0 and then weekly onwards. Each week, all samples from each group were composed into a single sample per group per week. At the end of the trial period, there were a total of 36 samples. In order to measure the concentration of AFB<sub>1</sub> in muscle, organs (liver, kidneys, gills and intestine), intestinal content and plasma, 12 animals per group were sampled and samples were pooled at days +28, +42 and +56, totalling 12 pooled samples at the end of the trial period. The samples were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS). The chromatographic determination was performed with reverse phase column, Gemini NX C<sub>18</sub> column (150 x 2.0 mm, 5 µm). The presence of AFB<sub>1</sub> was not detected in organs, muscle or water analyzed. AFB<sub>1</sub> was detected only in plasma samples from G3 and G4. AFB<sub>1</sub> levels were 0.87, 0.61, 3.79 and 6.22, 5.81 and 7.08 µg.kg<sup>-1</sup> of in G3 and G4, on day +28, +42 and +56, respectively. AFB<sub>1</sub> was absorbed and distributed throughout the organism since it was detected in plasma, but there was no accumulation in the viscera or muscles. The different levels and times of consumption do not present a linear or increasing trend in the quantities of AFB<sub>1</sub> absorbed and accumulated by the body of *Rhamdia quelen*.

**Keywords:** Aflatoxicol. Bioaccumulation. Fish. Human health. Mycotoxins.

SESSION II  
RECENT APPROACHES TO EVALUATE THE  
RISK OF MYCOTOXIN IN ANIMAL HEALTH

## Effect of feeding diet containing deoxynivalenol contaminated wheat on the feed consumption, weight gain and testis of young rats

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Puberty in rats encompasses the period from weaning (postnatal day, PND, 21) to early adulthood (PND 60). During this period, there is continued growth of the testis with an increase of tubular diameter. Therefore, the adolescence is a large window of vulnerability to toxic agents, which may lead to temporary or permanent damage in the reproductive system. On the other hand, the reproductive toxicity of deoxynivalenol (DON) on the male reproductive system of young animals remain unreported. Thus, the present study aimed to evaluate the effects of DON on the animal performance and testis of young rats. Twelve 30-day-old male Wistar rats were exposed to a control diet (mycotoxin-free diet) or to a diet contaminated with DON (9.4 mg DON/kg). Weekly, body weight, weight gain and food consumption were recorded. After 28 days of treatment, the animals were euthanized and the right testis of each animal was analysed with HE stain. One hundred random tubular sections per animal were classified into four stages of the seminiferous epithelium cycle (I-VI, VII-VIII, IX-XIII and XIV) and the frequency of tubules according to these stages was obtained. In addition, the seminiferous tubule diameters, seminiferous epithelium height and the number of Sertoli and Leydig cells were quantified. In the animals exposed to DON-contaminated diet a significant reduction in body weight ( $p = 0.01$ ), weight gain ( $p = 0.006$ ) and food intake ( $p = 0.05$ ) was observed compared to control animals. No effect of DON was observed in the testicular weight, diameter of the seminiferous tubules, and height of the germinal epithelium. However, DON induced a significant reduction in the number of Sertoli cells ( $p = 0.04$ ) and in the Leydig cell population ( $p = 0.04$ ). Furthermore, the frequency of seminiferous tubules in stage XIV (meiosis) was significantly lower in animals exposed to DON ( $p = 0.02$ ), indicating a

reduction in the cell division rate. In conclusion, DON induces losses in animal growth and reproductive toxicity in testis of young animals. Since observed changes may compromise male fertility, additional studies evaluating the mechanisms of DON on the testis of young animals are needed.

**Keywords:** Animal performance. Deoxynivalenol. Reproductive toxicity. Spermatogenesis kinetics. Testicular morphometry.

SESSION II  
RECENT APPROACHES TO EVALUATE THE  
RISK OF MYCOTOXIN IN ANIMAL HEALTH

## ***In vitro* adsorption of mycotoxins: a comparative study between methods**

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Mycotoxins adsorbing agents (MAA) are widely used in livestock production as an additive in animal feed to reduce the risk of mycotoxicosis. In general, adsorption capacity of MAA is firstly evaluated by an *in vitro* test, which enables to screen materials in a shorter period with lower cost and under controlled experimental conditions. However, most of adopted and published *in vitro* methodologies tend to use a very simple test composition, where two pH values are used, without the addition of constituents of gastric or intestinal juice, and without considering the dynamics elevation of gastric to intestinal pH. Among the authors, there is no consensus regarding the composition of the medium and the methodology used, interfering in comparison of results and reliability of test for later *in vivo* application. Therefore, the present study brings forward a comparison between methodologies that consider in its protocols test solutions made up only with buffers or with the addition of components of gastric and intestinal juice and that consider or not the dynamics of pH conditions along the gastrointestinal tract. For the comparisons of the methodologies, the adsorption of mycotoxins Aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), Deoxynivalenol (DON), Fumonisin B<sub>1</sub> (FB<sub>1</sub>), and Zearalenone (ZEA) was evaluated with simultaneous presence of them in the test tube and using a commercial activated charcoal (CA) sample as adsorbent model. The mycotoxin concentrations were evaluated by liquid chromatography with fluorescence detection (AFB<sub>1</sub>, FB<sub>1</sub> and ZEA) and diode array (DON). Significant differences ( $p < 0.05$ ) were observed between the methodologies, mainly for the evaluation of adsorption of FB<sub>1</sub> and DON, reinforcing the need to consider more variables in *in vitro* methodologies evaluations for a better characterization of the products and avoiding to make a wrong estimate of adsorption.

**Keywords:** Adsorbent. *In vitro* methods. Multimycotoxins.

SESSION II  
RECENT APPROACHES TO EVALUATE THE  
RISK OF MYCOTOXIN IN ANIMAL HEALTH

## Urinary metabolites of aflatoxin to evaluate the HSCAS efficacy in pigs fed an Aflatoxin B<sub>1</sub> contaminated diet

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The exposition of swine to aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) - secondary metabolites produced mainly by *A. flavus* and *A. parasiticus* - occurs through the ingestion of contaminated feed. AFB<sub>1</sub> cause many adverse effects and promote economic losses in animal production. Moreover, due to high physiological similarities to humans, swine are one of the major animal species used in translational toxicology research. Hepatic metabolism of AFB<sub>1</sub> includes hydroxylation, hydration, O-dimethylation, epoxidation and reduction reactions, generating several metabolites. The AFB<sub>1</sub>-8,9-epoxide (AFBO) can bond covalently to DNA guanine residues and form AFB<sub>1</sub>-N<sup>7</sup>-guanine. Although urinary AFB<sub>1</sub> biomarkers are found for a shorter period than in blood, it is preferred for biomonitoring the AFB<sub>1</sub> exposure because sample collection is not invasive and easy to handle. The objectives of present study was to evaluate the occurrence of AFB<sub>1</sub>-N<sup>7</sup>-guanine, AFM<sub>1</sub>, AFQ<sub>1</sub>, AFP<sub>1</sub>, aflatoxicol and unchanged AFB<sub>1</sub> in urine of pigs fed AFB<sub>1</sub> diets, and *in vivo* efficacy of a hydrated sodium calcium aluminosilicate (HSCAS) in reduce these metabolites. Twenty-four 49-day-old crossbred barrows were maintained in individual cages and allowed *ad libitum* access to feed and water. A completely randomized design was used with six animals assigned to each of four dietary treatments for 42 days as follows: (A) basal diet (BD), (B) BD + 0.5 % HSCAS, (C) BD + 1.1 mg/kg AFB<sub>1</sub>, and (D) BD + 0.5 % HSCAS + 1.1 mg/kg AFB<sub>1</sub>. Urine samples were collected weekly. The yield of each metabolite was calculated through the equation [(pg AF metabolite/mg creatinine)/(μg B<sub>1</sub>/kg BW)]. The AFP<sub>1</sub> was not detected in any urine sample of intoxicated animals, and aflatoxicol levels were below

the quantification limit. HSCAS was able to reduce ( $p < 0.05$ ) the urinary levels of AFM<sub>1</sub>, AFQ<sub>1</sub>, AFB<sub>1</sub>-N<sup>7</sup>-guanine and unchanged AFB<sub>1</sub>. The decrease of metabolite yield in urine ranged from 46.8% to 75.5% for AFM<sub>1</sub>, 34.5% to 91.1% for AFQ<sub>1</sub>, 57.2% to 94.2% AFB<sub>1</sub>-N<sup>7</sup>-guanine and from 70.0 to 99.5% for AFB<sub>1</sub>. In conclusion, AFM<sub>1</sub>, AFQ<sub>1</sub>, AFB<sub>1</sub>-N<sup>7</sup>-guanine and unchanged AFB<sub>1</sub> urinary, but not AFP<sub>1</sub> and aflatoxicol, have potential as AFB<sub>1</sub>-specific biomarkers for diagnostic purposes and for evaluating the efficacy of chemoprotective interventions in pigs.

**Keywords:** Adsorbent. Aflatoxin B<sub>1</sub>-N<sup>7</sup>-guanine. Aflatoxin M<sub>1</sub>. Biomarker. Swine.

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SESSION III  
DETECTION OF REGULATED MYCOTOXINS IN  
ANIMAL FEED-LOW-COST AND HIGH-TECH METHODS

## Analysis of mycotoxins in Tunisian feed: study of the natural co-presence

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Mycotoxins exposure in animal feeds are linked by contamination of raw materials and different feed formulations. The European Union (EU) has established recommendations on raw materials and feedstuffs for animal comprised in: 2002/32/EC, 2006/576/EC and 2003/100/EC. Tunisian feed sector has been developed due to the raw material availability. Control and performance of analytical procedures are required to ensure the quality of such products and to avoid the mycotoxin contamination. To develop an analytical procedure to evaluate the presence and co-presence of ENs, BEA, AFBs, OTA, AME, AOH, TENT, DON, 3ADON, 15ADON, NIV, NEO, DAS, T-2 and HT-2 toxin in Tunisian animal feed for the first time. Different types of animal feed including poultry, cattle, rabbit, sheep and horse were considered on the performance method. A validated QuEChERS extraction was used to analyse 122 Tunisian market feed samples distributed in poultry (n = 43), cattle (n = 35), rabbit (n = 12), sheep (n = 16) and horse (n = 16). Liquid chromatography and gas chromatography, both coupled to tandem mass spectrometry, were used for the analyses. The developed method complied with the analytical requirement of the EU Commission Decision, 2002/657/EC. 85% of analysed feed samples were positive for at least one mycotoxin. Cereal ingredients in samples were: wheat, maize and soya for poultry feed; barley, wheat, oat and legumes for rabbit and sheep feed; wheat bran, maize, barley and soya for cattle feed; and oat for horse feed. Even though all of them contained high quantities of cereal, the most contaminated samples were poultry and rabbit feeds. None positive sample exceeded the recommended maximal amounts of EU regulations for animal feed. DON was detected at levels ranging from 16 to 250 ng/g for cattle and chicken feed, respectively. It was observed a natural mycotoxins co-occurrence in all positive samples with at least three different mycotoxins (21%). A relatively high rate of feed contamination especially by *Fusarium* mycotoxins was also registered (up to 8 mycotoxins). Even if no toxicological concern was revealed, the

contamination of feed is evidenced and especially the co-contaminated samples might exert not expected adverse effects due to the mycotoxins potential interactions.

**Keywords:** Co-presence. Determination. Feed. Mycotoxins. QuEChERS.

SESSION III  
DETECTION OF REGULATED MYCOTOXINS IN  
ANIMAL FEED-LOW-COST AND HIGH-TECH METHODS

## Cell-free supernatants of lactic acid bacteria cultures against mycotoxigenic fungi growth

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The contamination of feed grains, forages and silages by mycotoxins is of major concern in the animal production sector. Lactic acid bacteria (LAB) are reported to show antifungal, antimycotoxigenic and detoxifying effects, supporting their potential as biocontrol agents for mycotoxin reduction. Besides the lactic acid, other metabolic products may contribute to such effects. Thereon, the objective of this study was to evaluate the inhibitory and fungicidal activities of cell-free supernatants (CFSs) obtained from 16 different LAB cultures against the mycotoxigenic fungi *Aspergillus sclerotioniger* CECT 20583, *Aspergillus parasiticus* CECT 2681, *Fusarium graminearum* CECT 2150 and *Fusarium verticillioides* CECT 2983. Tubes containing 7 mL of De Man, Rogosa and Sharpe (MRS) broth were inoculated with 100 µL of LAB fresh culture, incubated for 48 h at 37 °C, centrifuged (5,000×g, 10 min) and syringe-filtered (0.22 µm). The minimum inhibitory concentrations (MICs) of the 16 produced CFSs were determined by microdilution for the four fungal species. The tested concentrations were 50% of each CFS serial diluted up to 3.12% in tryptic soy broth (TSB). Minimal fungicidal concentrations (MFCs) were also determined by plating the content of wells which had no visual growth. All the 16 different CFSs have demonstrated similar effects in both inhibition and fungicidal effect. The most resistant species was *A. sclerotioniger*, which was only inhibited with the 50% treatment, while the other 3 species required 25% doses. Fungicidal effect was only achieved against *F. verticillioides* at 50%, supporting potential application of CFSs for control of this fumonisin-producing species. Further assays shall be carried out to evaluate their antimycotoxigenic action.

**Keywords:** Antifungal metabolites. Biocontrol. Fermentation product.

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SESSION III  
DETECTION OF REGULATED MYCOTOXINS IN  
ANIMAL FEED-LOW-COST AND HIGH-TECH METHODS

## Correlation between mycotoxin content in maize silage and silo coating associated with ambient temperature in Brazilian farms

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Hygiene during the production and use of silages are important managements which avoid filamentous fungi in silages. In anaerobiosis and low pH, most fungi have stagnant growth. However, the input of oxygen enables the development of these microorganisms and their metabolites, especially at favorable temperatures. The presence of fungi involves losses of nutrients and risks of the contamination of animals. The objective of this study was to evaluate the correlation between silo coating (concrete x soil) and ambient temperature with the appearance and quantification of mycotoxins in maize silages. One hundred and nine maize silage producing farms that had large-scale trench silos, distributed in four regions (Paraná, Santa Catarina, Minas Gerais, and Goiás), were assessed. All farms were categorized by silo type. Only 35 farms fit the pre-defined parameters for correlation analysis. Three silage samples of approximately 1.1 kg were collected at points with different temperatures (higher, medium and low) on the exposed side of the silo, for content evaluation of the mycotoxins: zearalenone (ZEN); ochratoxin (OT); deoxynivalenol (DON) and fumonisins B<sub>1</sub>, B<sub>2</sub>, and total (FB<sub>1</sub>, FB<sub>2</sub>, and FT), by Liquid Chromatography – Mass Spectrometry. Completely randomized design was used. Each silo represented the experimental unit and each farm represented the repetition. Results means were submitted to the Pearson correlation test at 5% of significance. When correlated the concrete-coated silo and canvas management, it was observed a weak positive correlation (0.32) for FB<sub>2</sub>, but it was not significant. When correlated the soil-coated silo and ambient temperature with mycotoxin content, there was a moderate positive correlation ( $p < 0.05$ ) for OCR (0.58) and strong positive ( $p < 0.01$ ) for DON (0.79). When correlated the soil-coated silo and canvas management, it was observed a weak negative correlation (0.36) and (0.30) for OT and DON, respectively, which were not significant, and a weak positive correlation (0.31) for FB<sub>2</sub>, which also showed no significance. It was observed that soil-coated silos, considering the relation between ambient temperature and canvas management, presented higher presence and content of mycotoxins when compared to concrete-coated silos.

**Keywords:** Fumonisin. Fungi. Maize. Silage. Zearalenone.

SESSION III  
DETECTION OF REGULATED MYCOTOXINS IN  
ANIMAL FEED-LOW-COST AND HIGH-TECH METHODS

## Effect of gaseous ozone on toxigenic fungi decontamination of dehydrated fish

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Dehydration is one of the oldest known food preservation methods. It can be done naturally or artificially. Drying reduces the moisture content (mc) of the product to levels appropriate for its preservation. An effectively animal dry product is one in which the residual mc is less than 25%, while a partially dehydrated product is that of 50% residual mc. Ozone gas ( $O_3$ ) has already been used by the food industry and has proved to be a very promising technique regarding microorganisms and toxic compounds degradation. The aim of this work was to observe the  $O_3$  effect on toxigenic fungi contamination in dehydrated fish. Gas was applied (50  $\mu\text{mol/mol}$  and flow: 5 L/min) at different exposure times (GT I, II, III - 10, 20 and 30 min, respectively) in dehydrated fish ( $n = 3$ ) samples inoculated with the major toxigenic fungi genera spores (*Aspergillus*, *Fusarium*, *Penicillium* - aflatoxins, fumonisins, deoxynilenol, zearalanone, ochratoxin A producers, respectively). In parallel, two portions were kept as controls (no fungi spores/ $O_3$  - GC1 & with fungi spores/no  $O_3$  - GC2) and incubated at 25 °C. Colonies development was followed for seven days. As expected, the GC1 samples (no fungi spores) showed no growth (NG) over the whole period. The opposite happened with the GC2 (with fungi spores). Showed fungal proliferation since Day 2. Regarding the GT ( $O_3$  treated), 30 min presented the fungi reduction. The treated samples submitted to  $O_3$  for 30 min had the fungal growth reduction ( $\geq 30$  min.). In conclusion that  $O_3$  gas applied for longer time is more effective for the fish sample studied. Regarding the GTs ( $O_3$  treated), the GTIII samples submitted to  $O_3$  for 30 min had the final growth totally inhibited. In conclusion, that  $O_3$  concentration applied for longer time ( $> 30$  min.) showed to be more effective for the fish samples studied.

**Keywords:** Contamination. Fungal proliferation. Mycotoxins. Soft method.

SESSION III  
DETECTION OF REGULATED MYCOTOXINS IN  
ANIMAL FEED-LOW-COST AND HIGH-TECH METHODS

## Validation of UHPLC-MS/MS method for Aflatoxin B<sub>1</sub> and its metabolites in pig urine

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Pigs are commonly fed with vegetable energy source such as maize and other grains susceptible to aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) contamination. After oral uptake, AFB<sub>1</sub> is absorbed and metabolized mainly by cytochrome P450 (CYP) enzymes, converting AFB<sub>1</sub> to AFB<sub>1</sub>-8,9-epoxide (AFBO), aflatoxin M<sub>1</sub>, Q<sub>1</sub> and P<sub>1</sub>. Moreover, a reduced form of aflatoxin - aflatoxicol - also can be produced. AFBO can covalently bound to DNA forming aflatoxin AFB<sub>1</sub>-N<sup>7</sup>-guanine. In order to assess the urinary excretion of AFB<sub>1</sub> unaltered and aflatoxin (AF) metabolites, a method of urinary AFM<sub>1</sub>, AFP<sub>1</sub>, AFQ<sub>1</sub>, AFB<sub>1</sub>-N<sup>7</sup>-guanine and aflatoxicol by ultra-performance liquid chromatographic tandem mass spectrometric (UPLC-MS/MS) was validated. The UPLC-MS/MS system consisted of a Waters Acquity Class-1 UPLC system coupled to a Xevo TQ-S MS triple quadrupole (Waters®) operated in the positive ionization electrospray mode. Chromatographic separation was performed using a BEH C18 (2.1 × 50 mm, 1.7 μm) column. A gradient (5.0 min) was applied using a mobile phase consisted of (A) H<sub>2</sub>O/0.1% formic acid and (B) acetonitrile/0.1% formic acid. AFB<sub>1</sub> and its metabolites were obtained by a simple urine dilution 1:10 with Milli-Q water, and filtration with PTFE 0,22 μm filter. The validation parameters evaluated were linearity, limit of determination (LOD), limit of quantification (LOQ), recovery and signal suppression/enhancement (SSE) due to matrix effects. The LOQs ranged from 14 pg.mL<sup>-1</sup> for AFB<sub>1</sub>-N<sup>7</sup>-guanine to 78 pg.mL<sup>-1</sup> for AFB<sub>1</sub> and AFM<sub>1</sub>. The SSE effects varied between 28% for AFP<sub>1</sub> and 121% for AFB<sub>1</sub>-N<sup>7</sup>-guanine. In this way, a matrix-matched

calibration was used for quantification. The calibration curves were prepared in triplicate at the level of 14 to 15,000 pg.mL<sup>-1</sup> for AFB<sub>1</sub>-N<sup>7</sup>-guanine and 78 to 80,000 pg.mL<sup>-1</sup> for AFM<sub>1</sub>. The recoveries were in a range of 95.0% for AFM<sub>1</sub> to 100.3% for AFQ<sub>1</sub>, with relative standard deviation between 2.6 - 7.7% for all compounds. Then, the validated method was applied to urine samples collected from barrows exposed to diets contaminated with 1.1 mg.kg<sup>-1</sup> of AFB<sub>1</sub>. The method was efficient to evaluate AFB<sub>1</sub> unaltered, AFM<sub>1</sub>, AFQ<sub>1</sub> and AFB<sub>1</sub>-N<sup>7</sup>-guanine in pig urine, while aflatoxicol and AFP<sub>1</sub> were lower than LOQ and LOD, respectively, showing that both are minor products of metabolism in pigs.

**Keywords:** Derivatives. LC-MS/MS. Mycotoxin. Swine. Urinary biomarkers.

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SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Antifungal activity of Natamycin, against *Aspergillus*, *Fusarium* and *Penicillium* spp.

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Mycotoxins are highly toxic secondary metabolites synthesized mainly by fungi of the genus *Aspergillus*, *Penicillium* and *Fusarium*. These microorganisms affect in all the steps of the food chain production, so the presence of these toxic substances ranges from foodstuffs to feed. Natamycin is a polyene macrolide produced by *Streptomyces natalensis*. In the present study, the antifungal activity of natamycin was evaluated against fungi of the genus *Aspergillus*, *Penicillium* and *Fusarium*. First, qualitative assay was performed in solid medium (PDA plates). Then, Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) were determined quantitatively employing 96-well plates, with a concentration of natamycin ranging to 0.4 - 200 µg/mL. MIC was established visually at 72 h of incubation at 25 °C. MFC was determined placing doses higher of the MIC on PDA plates and incubating 48 h at 25 °C. Natamycin was effective against all the fungi tested, particularly to the strains of the genus *Aspergillus* and *Fusarium* (MFC oscillated between 1.6 - 25 µg/mL). In contrast, fungi of the genus *Penicillium* showed greater resistance to natamycin, with a MFC varying between 6.2 - 200 µg/mL. The results obtained in the study allow us to conclude that natamycin can be a substance of interest for use in the treatment of crops, in order to prevent fungal contamination and the consequent production of mycotoxins.

**Keywords:** Antifungal activity. Fungi. Natamycin.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Antimycotoxigenic action of the phenolic extract against the *Fusarium graminearum* strain in maize

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Maize is a versatile food of great economic value prone to fungal contamination in the field, after harvest, and also during storage and may lead to the production of mycotoxins. In this study we verified the effect of phenolic extract obtained from rice bran cultivated with *Rhizopus oryzae* as a way to mitigate the production of deoxynivalenol (DON) produced by *Fusarium graminearum* strain during storage of maize (*Zea mays* L.). Rice bran was subjected to solid-state fermentation with *R. oryzae* CCT 7560 at 30 °C for 24 h, using an initial population of  $4 \times 10^6$  spores/g. The phenolic compounds present in the fermented biomass were extracted with methanol, clarified and lyophilized. Then, the extract was resuspended in water and total phenolic compounds were quantified spectrophotometrically by the Folin-Ciocalteu method. The phenolic extract was applied as a spray in the concentrations of 0.25%, 0.5% and 1%. After drying the extracts the  $1 \times 10^3$  spores/g of *F. graminearum* were added. The experiment was conducted for 15 days at room temperature (25 °C). The extraction of DON was performed with methanol in Ultraturax and the quantification in LC-MS/MS. DON was found at the concentration of 570 ng/g in the control treatment. There was a significant reduction in its content with the treatment with 0.25% phenolic extract resulting in 248 ng/g. The maximum inhibition of 56% production of DON occurred in the treatment with phenolic extract of 1.0%. The antimycotoxigenic potential of this phenolic extract was observed, becoming a promising alternative way to better seize crops and preserve the environment.

**Keywords:** Deoxynivalenol. *Fusarium graminearum*. Solid state fermentation.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Correlation between mycotoxin content and maize silage management in Brazilian dairy farms

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Silage contamination by fungi is determined by several factors and the development of these microorganisms compromises silage sanitary quality. The main problem may be associated to the occurrence of mycotoxins that cause diseases to the animals which results in considerable economic losses. The objective of this study was to correlate ambient temperature, silo's wall coating (concrete x soil) and canvas management after opening (up x down) with the incidence and quantification of mycotoxins in maize silages. One hundred and nine dairy farms that used maize silage were assessed. All farms were categorized by silo type and management. Only 35 farms fit the pre-defined parameters for correlation analysis. Three silage samples of approximately 1.1 kg were collected at points with different temperatures on the exposed side of the silo for evaluation of the mycotoxins: zearalenone (ZEN); ochratoxin (OT); deoxynivalenol (DON) and fumonisins B<sub>1</sub>, B<sub>2</sub>, and total (FB<sub>1</sub>, FB<sub>2</sub>, and FT), by Liquid Chromatography - Mass Spectrometry. Completely randomized design was used. Each silo represented the experimental unit and each farm represented the repetition. Results means were submitted to the Pearson correlation test at 5% of significance. When all data were correlated, it was observed that the ambient temperature presented a moderate positive correlation ( $p < 0.01$ ) for DON (0.58) and the silo's wall coating presented a weak positive correlation ( $p < 0.05$ ) for FB<sub>2</sub> (0.40) and FT (0.36). Canvas management showed no correlation with any evaluated mycotoxin. Maize is an ideal substrate for fungi growth, since it has a large amount of starch present in the structure of its grains. High temperatures also favor the development of fungi. In addition, the soil is an important contaminant of silages and represents a way of introducing microorganisms into silage which may results in the production of mycotoxins. These results show that the ambient temperature is related not only to the development of filamentous fungi, but also to the presence and content of mycotoxins, as well as the silo coating.

**Keywords:** Canvas. Coating. Fungi. Temperature. Zearalenone.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Development of toxigenic fungi in fish products at different water contents

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Fish is susceptible to microbiological deterioration due to the amount of free water, chemical composition and pH variation. Fish changes occur due to either, their conditions when they were caught (physical exhaustion, lack of oxygen, excessive manipulation) or in the final products exposed commercially (fungi spores and bacteria). This work evaluated the development of different toxigenic fungal genotypes in fish products (dried and dry-salted, apart from *in natura*, with 49.4, 55 and 72% of moisture content – mc, respectively). Portions (50 g, n = 3) of each fish Type were inoculated with toxigenic fungi spores of *Fusarium*, *Aspergillus* and *Penicillium* genus and incubated (7 days at 25°C) followed by microculture. By means of stereo and light microscopies observation, it was possible to register that *Aspergillus* (aflatoxigenic) predominantly grew in all of fish Types (despite of the mc) and no growth registered on the others, including *in natura*. Also, no toxin was detected in any of the fish samples evaluated. In conclusion, despite of the rather high samples mc, it seems the fish substrate was not adequate and did not allow some of the genera to grow, or it occurred *Aspergillus* spores competition.

**Keywords:** Fish. Mycotoxins. Moisture. Toxigenic.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Effects of exposure to cold plasma on toxigenic fungi species of the genus *Aspergillus*, *Fusarium* and *Penicillium*

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Fungi are responsible for deterioration in grains and food. They cause countless economic losses because they are associated to nutrient reduction, loss of germination and discoloration of food. When exposed to optimum environmental conditions, some species of toxigenic fungi can produce mycotoxins. Cold plasma (CP) is a green technology used as a decontaminant of microorganisms in grains and foods. This gas can perform the activity at low temperature without altering flavor, odor and grain structure, in addition to leaving no residue. Thus, the objective of this work was to explore the *in vitro* antifungal effects of CP on different fungal species of the genus *Aspergillus*, *Fusarium* and *Penicillium*, where the growth of colonies and changes in hyphae such as morphology, mortality and reactive oxygen species (ROS) were highlighted by the application of scanning electron microscopy and fluorescence. The CP was formed by plasma jet reactor in dielectric barrier discharge (DBD), alternating current, voltage of 8.5 kV and power of 240 W, which effectively inhibited the growth of the fungal colonies of *A. flavus* and *F. verticillioides* at 10 min exposure time and *P. citrinum* for 20 min. In the tested parameters, CP exposure was able to inhibit fungal growth, caused morphological changes in hyphae and ROS production in all fungi tested, due to oxidative stress. This type of treatment proved to be effective in fungal inhibition.

**Keywords:** Cold plasma. Decontamination. Fungal inhibition. Toxigenic fungi.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Innovative solution to reduce the growth of the mycotoxigenic fungi in cereals during storage

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Isothiocyanates (ITCs) are bioactive substances characteristic of the plants of the *Brassicaceae* family. The antifungal activity of the ITCs is due to the strong electrophilic properties of these compounds and also they can react easily with nucleophiles such as amines, amino acids, alcohols, water, and sulfites during food treatment and under physiological conditions and also with several functional groups of many mycotoxins. The aims of this study were to evaluate the antifungal properties of the bioactive compound allyl isothiocyanate (AITC) against *Aspergillus flavus* (8111 ISPA) AFs producer and *Penicillium verrucosum* (D-01847 VTT) OTA producer on corn, barley and wheat. The experiments were carried out initially in a simulated silo system at lab scale composed of glass jars (1L) containing 300 g of cereals. Barley and wheat were contaminated with  $1 \times 10^4$  spores/g of *P. verrucosum* (OTA producer) and corn with  $1 \times 10^4$  spores/g *A. flavus* (AFBs producer). The cereals were treated with a gel of 12% hydroxyethylcellulose in water containing gel 500  $\mu$ L of AITC and the system was hermetically closed and incubated for 30 days at 21 °C. The cereal control group did not receive any treatment. After that we moved to small-scale experiments in silos of 100 L capacity containing 70 kg of cereals. Barley and wheat were contaminated under the same conditions as the previous trial. They were treated with a gel of 12% hydroxyethylcellulose in water containing 5 mL of AITC. The incubation has been carried out during 90 days at 21 °C. The fungal growth of the inoculated fungi and the reduction in the formation of AFs and OTA were determined respectively. The AITC gel reduced completely the fungal growth at 30 days. In corn, the amount of AFB<sub>1</sub> detected in the control and treated samples was of 8.07 and 0.12 ppb respectively. Likewise, in barley, the amount of OTA present in the control and treated samples was of 0.28 and 0.09 ppb, respectively. Wheat samples did not show a significant reduction of the OTA.

**Keywords:** Allyl isothiocyanate. *Aspergillus*. Cereals. Mycotoxins. *Penicillium*.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Lactic acid bacteria as bio control agents against corn mycotoxigenic fungi

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Cereal grains are the major source of food for most humans and domesticated animals. It is estimated that 5 to 10% of the world's food production is wasted due to fungal deterioration and mycotoxins content. Biopreservation is a biotechnological application that promotes shelf life extension and food safety using microorganisms or their metabolic products. Some LAB strains are able to produce low molecular weight compounds related to phenolic acids with important antifungal activities. Furthermore, provided LAB are food grade organisms and comply with the "Qualified Presumption of Safety" (QPS) introduced by the "European Food Safety Agency" (EFSA). In this study, seven lactic acid bacteria (LAB) strains were tested for antifungal activity against ten food toxigenic fungi belonging to the genus *Aspergillus* and *Fusarium*. LABs were grown on MRS broth during 48 h at 37 °C in anaerobic conditions. After that, the cell free supernatant (CFS) were tested for the antifungal properties using diffusion agar method. Also, was determined the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of each of the CFS in 96-well microplates. On the other hand, CFS was also used as a natural biopreservation agent in the conservation of corn and corn ears inoculated with *A. flavus* and *F. graminearum*, respectively. All LABs tested produced growth inhibition of the ten fungi in solid medium. The minimal inhibitory concentrations and minimum fungicidal concentrations calculated were 4-125 g/L and 8-250 g/L respectively. After the storage period of corn, the treatment with CFS showed a averaged reduction of 99% in the production of AFB<sub>1</sub> compared to the control.

**Keywords:** Antifungal activity. Biopreservation. Corn. Lactic acid bacteria. Mycotoxins.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Ozone technology as mycotoxin reduction strategy in cereal grains and their products

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Mycotoxins remain a world problem for food and feed production even adopting good production practices in the field, storage and distribution chain. Ozone technology has been presented as an alternative for decontamination of mycotoxins with advantage over other studied technologies. This technology does not generate toxic waste and is classified as a green technology. The aim of our study was to evaluate the reduction of zearalenone (ZEN) and deoxynivalenol (DON) contamination in cereals, their derivatives and co-products. The effect of ozone technology was studied on the following naturally contaminated products: wheat kernels, whole wheat flour, wheat bran, wheat wet milling effluent, whole corn flour and cereal pellets. Analyses were performed using HPLC-DAD for DON and HPLC-FLD for ZEN. Ozone treatment was performed at a flow rate ranging from 0.5 - 1.0 L min<sup>-1</sup>; O<sub>3</sub> concentration in the gas stream was 48-62 mg.L<sup>-1</sup> and up to 240 min at 25 °C depending on the product. The mean reduction of ZEN concentration was about 60% in wheat bran and whole maize flour. The reduction of DON concentration ranged from 20-70% in whole wheat flour and it was improved with increasing moisture content. It was also observed a reduction in DON concentration up to 82% in wet milling effluent. For Wheat bran processed at commercial moisture content DON, the reduction was about 32%. No significant DON or ZEN reduction was observed in wheat kernels and pellets on the studied conditions. Depending on the process, product (kernel or coproduct) and sample (moisture) conditions, the ozone technology can be a mycotoxin mitigation strategy. However, further studies are needed to evaluate the effects of ozone technology on the nutritional, functional and technological properties of the ozonated products as well in grains and large particle size products.

**Keywords:** Cereal. Deoxynivalenol. Ozone Technology. Zearalenone.

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SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Reduction of aflatoxins B<sub>1</sub> and B<sub>2</sub> in corn, corn flour and peanuts by gaseous AITC

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Aflatoxins (AFs), mainly produced by *Aspergillus* species, are a class of mycotoxins of major importance due to their high toxicity for humans and animals. Among them, group B is the most prevalent, being extensively associated to liver damage and carcinoma development. Natural antimicrobials application for fungal control has gained interest in the recent years since they present good acceptance and safety for consumers. Allyl isothiocyanate (AITC) is one of the most potent plant-derived antimicrobial, being capable of inhibiting the growth of several yeast, filamentous fungi and bacteria. Besides, AITC contains in its structure a highly electrophilic carbon which may react with different molecules, demonstrating AITC potential as detoxifying agent for mycotoxins. Therefore, the objective of this study was to evaluate the AITC effect on the reduction of AFB<sub>1</sub> and AFB<sub>2</sub> levels in contaminated corn, corn flour and peanut intended for animal feed. For this, samples of food products were inoculated with the mycotoxigenic mold *Aspergillus parasiticus* CECT 2681 and the mycotoxins were naturally produced. After seven days of incubation at 25 °C, the material was autoclaved, and the AFBs levels were determined by LC-MS/MS after extraction. Thereafter, two bottoms of Petri dishes received 5 g of the samples and placed into 1 L hermetic glass jars containing a 4 x 4 cm filter paper with different AITC concentrations (0, 50, 100 and 500 ppm). After 48 h of incubation at 25 °C, the jars were opened and left for 1 h at rest to the remaining AITC escape. Samples were processed and the AFBs levels were once again measured. For both AFBs, greater reductions were achieved at higher concentrations. Contamination levels in peanut treated with 50 ppm had no reduction. However, at the stronger treatments the reduction varied from 24.5 to 50.1%. Reductions were subtler in corn, varying from 1.0 to 33.4%, and in corn flour - 19.4 up to 40.3% (50 and 500 ppm, respectively). These results support the applicability of AITC as the active compound in a large-scale fumigation system for grains, oilseeds and derivatives intended for animal feed.

**Keywords:** Detoxification. Essential oil. Fumigation.

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SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## The use of essential oil of mustard to control aflatoxigenic species of *Aspergillus*

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Brazil is one of the biggest grain producers worldwide. Grain production faces some losses due to fungi growth and mycotoxins contamination, secondary metabolites produced by filamentous fungi and considered hazard for human's health. Among the known mycotoxins, aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is considered carcinogenic to humans. The use of natural compounds has been used as an option to decrease the fungi contamination and consequently, decrease mycotoxins presence in the grains. Among these natural compounds, allyl isothiocyanate, an essential oil extracted from mustard, has shown antimicrobial action. The aim of this work was to evaluate the use of allyl isothiocyanate (AITC) as an alternative to mitigate fungi growth. Three species of *Aspergillus* known as AFB<sub>1</sub> producers (*Aspergillus parasiticus*, *Aspergillus flavus* and *Aspergillus nomius*) were used in this work. For minimum inhibitory concentration (MIC), serially diluted doses (2x) of AITC, from 0.977 to 500 µL/L, were added to spore solutions (2.10<sup>4</sup> spores/mL) and incubated for 2 days at 25 °C. After, it was observed the partial growth, compared to the control, and considered as MIC 50%. For halo inhibition test, PDA plates were inoculated with 2.10<sup>4</sup> spores/mL, conditioned in hermetic bottles with AITC in concentration from 0.00312 to 0.500 µL/L and incubated for 5 days at 25 °C. After incubation, halos were measured with a pachymeter and compared to the control. MIC (50%) was 31.25 µL/L for the three species of *Aspergillus* and halos were completely inhibited from 0.5 µL/L of AITC for all species. AITC can be used as an alternative to reduce fungi growth and due to its high volatility, it was more efficient in gaseous form.

**Keywords:** AITC. *Aspergillus*. Coffee beans. Food safety. Mycotoxins.

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SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Toxigenic fungi decontamination of commercial poultry eggshell by ozone gas treatment

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Eggshell quality is essential for the preservation of commercial egg product properties. Poultry eggshells are fundamental for the growth and development of the embryo, and any quality alteration can lead to the death of the embryo and consequent damages to the production of chickens. In addition, in the Northeastern Brazilian region, the eggshell is consumed dry and crushed as a source of calcium for children. Contaminant fungi can penetrate eggshell through their microscopic hyphae, affecting its quality. Since the environment is conducive to this contamination, several eggshell decontamination methods have been studied. Ozone ( $O_3$ ) is a strong oxidant, with antimicrobial action, which spontaneously decomposes into  $O_2$ . It is classified as GRAS (Generally Recognized as Safe) by USDA and FDA. Emerging technology is explored by the food safety area for the inactivation of pathogens and degradation of chemical contaminants. The greatest effectiveness of  $O_3$  is observed at low temperature. In this work, 25 g of commercial eggshells were stored at 8 °C for 7 weeks, being ground, homogenized and divided into two portions of 12.5 g. Control Group (CG) was separated and inoculated directly, being exposed only by air stream, and the second one, Treated Group (TG) was submitted to treatment with  $O_3$ . The samples were transferred to glass chambers (70 x 50 mm), and the  $O_3$  applied at concentration of 15 mg of  $O_3$  for 1 kg of sample (15 ppm). The gas flow was maintained for 20 min, and then aseptically inoculated into PDA culture medium. The results were 6 UFC/g for CG and NG (no growths) CFU/g for TG. The decontamination of the commercial eggshells with  $O_3$  was effective, however, more studies are required regarding the exposure time and efficiency, including interaction with temperature, whether effects the internal physic-chemical properties of the egg and consumer acceptability.

**Keywords:** Decontamination. Eggshell. Fungi. Ozone.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Transcriptional activation of mitochondrial Krebs cycle-related genes is associated with fumonisin production in *Fusarium* spp.

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Fumonisin are a group of naturally occurring family of toxic, neurodegenerative and carcinogenic mycotoxins. Primarily produced by different *Fusarium* spp., the genetic regulation of fumonisin synthesis remains unclear, and few studies have associated mycotoxin-producing factors with specific cellular pathways. Some of these factors involve fructose metabolism, carbon source and increased levels of maltose. Coincidentally, these mycotoxin-producing factors are also directly associated with the mitochondrial Krebs cycle, that is known to play key roles in cellular respiration, growth and division. Krebs cycle, as in fumonisin production, is also activated during stress condition, and it might be also possible that these two cellular processes are directly associated. To investigate novel genes that are mis-regulated in Krebs cycle that might be associated with mycotoxin biosynthesis in *Fusarium fujikuroi* subjected to fumonisin-producing media (FP). The wild-type fungi *F. fujikuroi* strain MO409 transcriptome (RNA-seq) data was produced by Guruge and Uegaki (2018), available at BioProject code PRJDB6333. Briefly, the fungi were growth FP and non-FP culture conditions. Over the RNA-seq, we applied bioinformatics protocols including Cytoscape and R Bioconductor packages for genetic screening of fumonisin-related genes. Several genes indirectly involved in mitochondrial Krebs cycle were found to be up-regulated in FP samples compared to non-FP. The gene that codes for L-lactate dehydrogenase (cytochrome) (c3740\_g1\_i2) was found with an increased fold-change of 41.8 in FP compared to non-FP. It was also found high expression of the putative aldehyde reductase (c9193\_g1\_i1), with an increased fold-change of 97.4 in FP compared to non-FP. This protein catalyzes the reduction of several molecules, such as glucose, and is used for Krebs cycle. Furthermore, sugar transport protein STL1 (c6604\_g1\_i1) and lipase (c11112\_g1\_i1) were also found to be up-regulated, with a fold-change increase

of 80 and 12.8 in the fumonisin-producing samples, respectively. Lipases break down triglycerides into free fatty acids and glycerol, that are also used by Krebs cycle. Our work presented several overexpressed genes associated with mitochondrial Krebs cycle activation that could be influencing fumonisin B production in *Fusarium fujikuroi*. Finally, we also hypothesize that the activation of mitochondrial Krebs cycle are a necessary pathway for fumonisin production, both influenced by stress conditions.

**Keywords:** Fumonisin biosynthesis. *Fusarium fujikuroi*. Krebs cycle. Transcriptome modification.

SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Use of Allyl isothiocyanate to reduce levels of zearalenone in grains and maize flour

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Zearalenone (ZEA) is an estrogenic toxin produced by fungi of the genus *Fusarium*, especially by *Fusarium graminearum*, also known as *Gibberella zeae*, reproductive form of the fungus. In Brazil, the Tolerated Maximum Limit (TML) of this toxin present in maize for processing is limited to 400 µg/kg, and after processing the limit is 150 µg/kg. When consumption exceeds the TML, damage can be caused, from uterine fibrosis, breast cancer, endometrial carcinoma, uterine hyperplasia and decreased fertility in humans to vulvovaginitis, enlargement of the mammary gland, anestrus and abortion in animals. The objective of this work is to evaluate the potential of allyl isothiocyanate (AITC) to mitigate ZEA in naturally contaminated corn kernels and flour. For this, corn kernels and flour were contaminated with *Fusarium graminearum* CECT 2150, incubated for seven days at 25 °C. After, the matrices were autoclaved and added concentrations of 50, 100 and 500 µL/L of AITC, beyond the control group, without AITC. The toxin was extracted by methanol (MetOH) solubilization, with 5 g of the matrices homogenized in ultraturrax with 25 mL of MetOH, and mass spectrometry was used to analyze the presence of ZEA. The untreated group had a contamination of 147.9 µg/kg and 163.3 µg/kg in the grains and in flour, respectively. The concentrations of 50, 100 and 500 µL/L had reduction of 0%, 0% and 14.36 ± 20.92% and 0.10 ± 29.91%, 3.89 ± 32.29% and 0% in the grains and in flour, respectively. The AITC showed good potential to reduce ZEA in a concentration of 500 µL/L, evidenced by a reduction of 14.36%. This result can be explained by the AITC's ability to penetrate grains described in the literature. Once the AITC penetrates the grain, it will have a residual concentration more available to act on the toxin. It was not possible to neutralize the

ZEA present in maize flour using AITC. Previous studies have shown that the compound may inhibit toxin production, but the results indicate that once formed, the compound cannot reduce it on maize flour, so other decontamination methods should be investigated.

**Keywords:** Allyl isothiocyanate. Corn kernels. *Fusarium*. Maize flour. Zearalenone.

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SESSION IV  
NOVEL STRATEGIES TO MITIGATE THE OCCURRENCE  
OF MYCOTOXINS IN ANIMAL FEED

## Use of Allyl isothiocyanate to reduce levels of zearalenone in wheat flour and *in natura*

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Zearalenone (ZEA) is an estrogenic toxin produced especially by *Fusarium graminearum*. The toxin in question when present in animal feed can lead to miscarriages, reduced food intake, decreased zootechnical indexes, vaginitis, uterine and vulvar enlargement, ovulation decline and along estrus cycles. After a single oral dose of ZEA administered to pigs, more than 85% of ZEA is absorbed by the intestinal lumen in less than 30 minutes, necessitating the development of fast methods for detoxification of animal feed products. The objective of this work is to evaluate the potential of allyl isothiocyanate (AITC) to mitigate ZEA in naturally contaminated wheat *in natura* and in flour. For this, corn kernels and flour were contaminated with *Fusarium graminearum* CECT 2150, incubated for seven days at 25 °C. After, the matrices were autoclaved and added concentrations of 50, 100 and 500 µL/L of AITC, beyond the control group, without AITC. The toxin was extracted by methanol (MetOH) solubilization, with 5 g of the matrices homogenized in ultraturrax with 25 mL of MetOH, and mass spectrometry was used to analyze the presence of ZEA. The untreated group had a contamination of 532.2 µg/kg and 704.8 µg/kg, *in natura* and in flour, respectively. The concentrations of 50, 100 and 500 µL/L had reduction of 10.17 ± 6.94%, 17.77 ± 7.41% and 44.67 ± 29.52%, and 15.71 ± 5.34%, 26.95 ± 9.78% and 24.02 ± 23.06%, *in natura* and in flour respectively. The AITC showed a good potential to reduce ZEA in wheat *in natura* at concentrations from 100 µL/L, evidenced by a reduction of 17.77 ± 7.41%. There was no significant difference between the natural contamination and the reduction caused at the dose of 50 µL/L. Data from the literature demonstrate the effectiveness of AITC in flour-based products. The results presented here

show the possibility of the incorporation of the antifungal in the raw material. The data shows too the efficiency of AITC as a mitigate in wheat flour from the concentration of 50  $\mu\text{L}/\text{L}$ . This corroborates that, in addition to the possibility of incorporation *in natura*, it is possible to use AITC already in processed wheat.

**Keywords:** Allyl isothiocyanate. *Fusarium*. Wheat flour. Wheat *in natura*. Zearalenone.

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SESSION V  
NOVEL FEED ADDITIVES

## Dichloromethane and ethyl acetate extraction of *Lactobacillus plantarum* crude metabolite protect intestinal tissue exposed to deoxynivalenol in pigs

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Deoxynivalenol (DON) is a secondary fungal metabolite of the trichothecene family produced by *Fusarium graminearum* and *Fusarium culmorum* affecting animal and human health. Worldwide surveillance studies indicate its occurrence in 57% of cereal samples, representing the most prevalent mycotoxin in cereals with high average content. Therefore, effective detoxification and protective methods in animals would be useful in counteracting this problem. Biotransformation of mycotoxins and preservation of intestinal tissue by acid lactic bacteria is a promising way to minimize the deleterious effects of this toxin. The purpose of this study was to investigate the potential modulation of *Lactobacillus plantarum* (Lp) metabolites in DON toxicity using an ex vivo model. Crude cell free supernatant were obtained from two strains of *Lactobacillus plantarum* culture, one a commercial strain (ATCC 14917) (strain 1) and the other isolated from wheat grain (strain 2). Both Lp culture supernatant were followed by extraction in water immiscible solvents dichloromethane and ethyl acetate to obtain the bacteria metabolites. Intestinal explants were obtained from jejunum of four animals and incubated with the bacteria metabolites for two hours; explants were exposed to DON one hour after the metabolites. After incubation, explants were processed for morphological score. Intestinal explants from piglets were exposed to the following treatments: culture medium (control), DON (10  $\mu$ M), bacteria metabolites of both strain of Lp extracted with dichloromethane and ethyl acetate alone or associated with DON. DON induced significant intestinal lesions as apical necrosis, decreased villi height and altered enterocyte morphology ( $p = 0.0005$ ). Meanwhile, explants treated only with metabolites extracted with ethyl acetate of both culture supernatants (strain 1 and strain 2) showed and increased villi height, preserved columnar epithelial morphology and reduction of apical necrosis when compared to DON treated explants, therefore remained similar to control maintaining intestinal integrity ( $p = 0.008$ ,  $p = 0.002$ , respectively). When exposed to DON a significant reduction in morphological lesions was also achieved ( $p = 0.02$ ) in explants treated with crude metabolites of strain 2 extracted with ethyl acetate. This results demonstrated that *L. plantarum* metabolites improve intestinal integrity when exposed to DON and could represent a suitable protection and a solution against fungal degraded stuff.

**Keywords:** *Lactobacillus plantarum*. Metabolite. Toxicity.

SESSION V  
NOVEL FEED ADDITIVES

## Effect of the use of Elitox<sup>®</sup> against aflatoxins from feed in dairy cows

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The metabolization of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), present in the feed of dairy cows, into aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) is a public health challenge as it is a toxic and carcinogenic metabolite excreted in milk. Based on this, the objective was to evaluate the efficacy of the product Elitox<sup>®</sup> in reducing AFM<sub>1</sub> levels in milk of dairy cows fed with AFB<sub>1</sub> contaminated diets. Nine late lactating 6-year-old Holstein cows from a commercial dairy farm in the South region of Brazil were divided into three groups of three animals each, in a 3 x 3 latin square experimental design, according to daily milk production. Each group was assigned to one of the three treatments during a period of 21 days followed by a 7-days adaptation period, then randomly reassigned to the other treatments. Feeding consisted of corn silage and pasture *ad libitum*, and corn and soybean feed after milking, and differed according to the treatment as follows: T1) Negative control; T2) Positive control (150 ppb of AFB<sub>1</sub> in feed); T3) Treated group (150 ppb of AFB<sub>1</sub> and 15 g/cow/day of Elitox<sup>®</sup> in feed). Cows were milked twice daily (morning and afternoon), and milk production was recorded to calculate mean daily milk production per animal in each week. At the seventh day of each week, two individual milk samples (from morning and afternoon milkings) were collected per animal, thoroughly mixed, frozen at -20 °C, and posteriorly sent to Samitec Institute (Santa Maria, Brazil) to quantify AFM<sub>1</sub> levels by HPLC-FLD (High Performance Liquid Chromatography with fluorescence detection). Animals from group T1, even without receiving AFB<sub>1</sub> contaminated feed, showed detectable AFM<sub>1</sub> levels in milk (0.011 µg/L). Animals fed with AFB<sub>1</sub> contaminated feed presented AFM<sub>1</sub> levels in milk of 0.863 µg/L, while those receiving with the same contaminated feed added with Elitox<sup>®</sup> showed significantly lower ( $p \leq 0.05$ ) AFM<sub>1</sub> levels in milk (0.664 µg/L), reflecting in a reduction of, up to 23%. Milk production was not significantly affected ( $p > 0.05$ ) by the inclusion of either AFB<sub>1</sub> or Elitox<sup>®</sup>, however tendencies were found for a drop in milk production, fat and lactose level in the contaminated group. In conclusion, Elitox<sup>®</sup> at 15 g per cow per day was able to effectively reduce milk AFM<sub>1</sub> levels from cows fed with AFB<sub>1</sub> contaminated diet and recover for economical losses in milk drop and milk composition.

**Keywords:** Aflatoxin M<sub>1</sub>. Mycotoxins. Milk. Public health.

SESSION V  
NOVEL FEED ADDITIVES

## Screening of environmental microorganisms for zearalenone biodegradation

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Zearalenone (ZEA) is a mycotoxin produced by fungi under the *Fusarium* genus. The molecular structure of ZEA is analog to that of estrogen, causing damage to the reproductive system of humans and other animals, especially in swine. In view of Brazil's excellent climate for the development of mycotoxins, which harms the economy and food safety, it is essential to develop an effective process to decontaminate zearalenone. Among the decontamination methods, one that presents itself as potentially effective is biodegradation, which is the rupture of the compound's molecular structure. This study aimed to isolate microorganisms that are able to cause the biodegradation of ZEA in TSB broth, swine feed and food matrix. The microorganisms were isolated from maize and wheat fields. The isolated strains went through an esterase activity test in order to select the microorganisms with the greatest potential for degradation. The selected microorganisms were cultivated in TSB broth containing 1 µg/mL of ZEA and after 24h the mycotoxin was extracted for quantification in High Performance Liquid Chromatography (HPLC). The extraction methodology was validated by the analysis of the recovery percentage. The results indicate that from 196 isolated microorganisms, 6 were selected by the esterase test. The strains tested for ZEA biodegradation presented degradation of  $99 \pm 0.11\%$ ,  $78 \pm 0.31\%$ ,  $64 \pm 0.71\%$  and approximately 30% for the remaining strains. The validation of the ZEA extraction methodology was indicated by the recovery of  $97 \pm 0.03\%$ . In conclusion, the esterase activity test is an effective assay to screen microorganisms for zearalenone biodegradation.

**Keywords:** Biodegradation. Esterase activity. HPLC. Zearalenone.

# Sponsorship

