The control of micro-organisms and their metabolites is key in animal health and food safety
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Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminant of grains worldwide. *Aspergillus, Fusarium, Penicillium, and Claviceps* species of fungi are ubiquitous in nature and under ideal conditions often infect economically important crops and forages already in the field, during storage, transportation and processing.

The effects of mycotoxins in animal rearing are well recognised. The clinical response is both dose-dependent and time-dependent and can vary from acute to chronic. The response to known mycotoxins of clinical importance tends to be sub acute or chronic and symptoms often are subtle and vague. Many of these mycotoxins can cause serious health problems in livestock and their presence in agricultural products may result in serious economic losses. Measures used by the livestock industry to protect animals from the toxic effects of mycotoxins include the use of microbial inactivation, mould inhibitors, fermentation, physical separation, thermal inactivation, irradiation, ammoniating, ozone degradation and the use of adsorbents. Unfortunately, most of these methods are costly, time-consuming, and only partially effective. The most promising and practical approach has been the addition of adsorbents to contaminated feed to selectively bind the mycotoxin during the digestive process, allowing the mycotoxin to pass harmlessly through the animal.

Research indicates that a number of adsorbents are capable of binding aflatoxin and reducing or preventing its toxic effects, but nevertheless not all types of mycotoxins can be physically adsorbed. Therefore more specific products have been developed to neutralise the toxic effect of various mycotoxins by processes, such as bio-transformation.

**AFLATOXINS**

Aflatoxins represent a closely related group of heterocyclic metabolites synthesized predominantly by the fungi *Aspergillus* spp.

At present 18 different aflatoxins have been identified. However, aflatoxin B1, B2, G1 and G2 are the main naturally occurring compounds.

Aflatoxin B1 can be classified as a highly toxic compound for most animal species and as cancerogenic for men. It is extremely toxic for some species such as rainbow trout, cats and ducklings.

**TRICHOThECENS**

The Trichothecens mycotoxins (TCT) are a chemical group of fungal metabolites with the same basic structure, produced by various types of fungi growing on plants.

The reason for their name originates from the basic chemical ring described as tetracyclic 12,13-epoxytrichothecene skeletons.

At the cellular level, the main toxic effect of TCT mycotoxins appears to be a primary inhibition of protein synthesis.

TCT affect actively dividing cells such as those lining the gastrointestinal tract, the skin, lymphoid and erythroid cells. The toxic action of TCT results in extensive necrosis of the oral mucosa and skin in contact with the toxin, acute effect on the digestive tract and decreased bone marrow and immune function.
OCHRATOXINS

The Ochratoxins are a group of 7 fungal metabolites composed by an iso-coumarin moiety linked to the amino acid L-β-phenyl alanine.
Although the ochratoxin group comprises 7 compounds, only ochratoxin A (OA) has been found widespread as a natural contaminant of cereal grains such as barley, wheat, oats rye and Maize.
OA is the most toxic mycotoxin for domestic fowl. In terms of lethality, which is the simplest measure of toxicity, OA is more toxic than aflatoxin and comparable to the trichothecone mycotoxin DAS.

FUMONISINS

So far Six different fumonisins have been isolated and identified, namely fumonisins A1, A2, B1, B2, B3 and B4. Fumonisins of the A series are amides, while those of the B series have a free amine. Differing hydroxyl substitution account for different fumonisins within each series.
Fumonisins might interfere in some way with the biosynthesis of sphingolipids or sphingosine turnover because of the similarity of the polyhydric alcohol moiety.

ZEARALENONE

Zearalenone is a phenolic resorcylic acid lactone with potent estrogenic properties, produced primarily by Fusarium.
Zearalenone can induce signs of estrus in ovariectomised sows or in prepubertal gilts and dietary concentration as low as 1-5 ppm can cause vulvovaginitis in young female swine.
Even though zearalenone appears to be non-toxic for poultry species, the detection of this mycotoxin in poultry feed has been suggested to be used as a" biomarker" for other unknown Fusarium toxins.

Raw materials and often consequently animal feeds might be infected not only by moulds but also by other harmful micro-organisms. The EU Committee identified several food-borne zoonoses:
- Salmonella, Campylobacter, Verotoxigenic Escherichia coli, Listeria monocytogenes,
- Cryptosporidium, Echinococcus granulosus Trichinella spiralis Clostridia Perfringens as dangerous for causing toxicity of foodstuffs. Some of these micro-organisms are able to produce highly toxic metabolites as well.
It is important to avoid the invasion and adaptation in the digestive tract of these pathogenic micro-organisms. The control of the microbial conditions of feed ingredients and of manufactured feeds has to be monitored more and more today.

A safety programme specifically designed to identify and control critical points within the feed production chain is essential for a successful operation. The key points are:
1. Correct product
2. Correct application rate
3. Correct method of application
4. Correct point/location within the production process
5. Correct monitoring after application

The use of preservatives in conjunction with processing techniques which involve increased temperatures are very effective in controlling harmful micro-organisms. A specific range of products in liquid and dry form have been designed to control the contamination at various application points such as in storage.
facilities, silos of raw materials, oil crushing and protein meal production plants, feed mills, transport systems, storage facilities of feeds, etc.

Mould inhibition is essential to avoid spoilage caused by the development of moulds species, but also to reduce the risk for further generation of toxic during storage. It is not always possible to obtain all raw materials free of these toxic secondary metabolites as the crop can be infected already prior to harvest. The toxic level of single mycotoxins can vary from type to type. Aflatoxins are dangerous at extremely low levels (part per billion), whereas some of the Fusarium toxins are more tolerant up to concentrations in the part per million range. But as raw materials and animal feeds are often contaminated with various types of mould species it is inevitable that various types of mycotoxins are present at the same time.

When the feedstuff has been contaminated by more than one mycotoxin, the toxicological effects can be additive and, perhaps, even synergistic. Of the mycotoxin combinations that have been investigated in poultry and pigs, the interactions between aflatoxin and ochratoxin A and between aflatoxin and T-2 toxin are amongst the most toxic. Toxicological synergism between fusaric acid and vomitoxin was demonstrated when both toxins were fed to young pigs.

Zearalenone has a serious impact on the reproduction of pigs. It has oestrogenic activity. A lot of clinical signs such as: swollen red vulva, enlargement of the teats and mammary glands, infertility, pseudo pregnancy, anoestrus, rectal and vaginal prolapse, depressed piglet growth in utero, weak splay-legged piglets born, early embryo mortality, delayed repeat matings, etc., are caused by zearalenone.

The impact of zearalenone and the detoxificating effect TOXY-NIL Plus dry (TNP) has been investigated at the Laboratory of Animal Reproduction of Lithuanian Institute of Animal Science.(2001)

Lithuanian White boars of 10 months of age and 150-155 kg weight were used in the experiment and allotted to three groups:
- **Control group**: fed mycotoxin-free feeds.
- **Experimental 1**: fed compound feed containing 0.57 mg/kg of zearalenone.
- **Experimental 2**: fed zearalenone (0.57 mg/kg) containing feeds which, before feeding, were detoxicated with the product TOXY-NIL Plus dry (TNP) at a rate of 1 kg per 1000 kg of feed.

The experiment was divided into three periods.
- **Pre-experimental period.** Boars were fed high quality feeds, and trained to give semen by manual method. Qualitative and quantitative parameters of semen have been evaluated. (period – 10 days.)
- **Period of intoxication.** At this period, boars in the experimental groups were fed zearalenone containing feed (Experimental 1) and zearalenone contaminated feed treated with TOXY-NIL Plus dry (Experimental 2). Semen was collected manually once a week from the boars in all groups. Physiological responses of semen were determined during the period of 32 days.
- **Period of recovery.** Boars in all groups were fed only high quality feeds. Physiological responses of semen were evaluated. The length of the period was 21 days.

**Results and Discussion**
Three days after starting feeding zearalenone-containing feeds to boars, the volume of ejaculation has decreased by 40.8 % (P<0.001) compared with the control group and amounted to 141.0±12.59 ml (Fig1).

The volume of ejaculation remained similar to control in the Experimental group 2.
The volume of ejaculation was recovered in a week's time when non-contaminated feed was offered to the boars after the experimental phase.

During the intoxication period, the spermatozoa count per ejaculation in experimental group 1 reduced substantially within one week compared to the control and experimental group receiving the zearalenone and TOXY-NIL PLusdry (Fig 2).

Here as well when non-contaminated feed was offered after the trial phase the total spermatozoa count per ejaculation recovered within a week.

The lowest initial spermatozoa motility in the semen collected during intoxication was determined in the group of boars fed zearalenone contaminated feed (3.9±0.44 points, P<0.001). As soon as the contaminated feed was replaced by good quality feed the motility of spermatozoa has recovered within a few days and amounted to 7.0±0.15 points (Fig 3).
Lower motility of spermatozoa could be associated with either sperm membrane damage or metabolism disturbances in the cell.

The sperm motility of boars fed TOXY-NIL Plus dry treated feed was 7.0±0.16 points and did not differ significantly from the sperm motility of the control group (P>0.05).

Blood analysis indicated that neither TOXY-NIL Plus dry nor zearalenone had any effect on the quantity of macro- and microelements in the boar blood (Table 1).

**Table 1.** The level of macro- and microelements in the boar blood during the period of chronic intoxication

<table>
<thead>
<tr>
<th>Item</th>
<th>Control n=3</th>
<th>Experimental 1 (zearalenone) n=5</th>
<th>Experimental 2 (TPD) n=5</th>
<th>Physiological norm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium, mg%</td>
<td>10.29</td>
<td>10.19</td>
<td>10.55</td>
<td>10.0 -14.0</td>
</tr>
<tr>
<td>Phosphorus, mg%</td>
<td>5.04</td>
<td>5.29</td>
<td>4.86</td>
<td>4.0 - 6.0</td>
</tr>
<tr>
<td>Zinc, mg%</td>
<td>0.329</td>
<td>0.325</td>
<td>0.306</td>
<td>0.250 - 0.500</td>
</tr>
<tr>
<td>Copper, mg%</td>
<td>0.115</td>
<td>0.127</td>
<td>0.121</td>
<td>0.080 - 0.120</td>
</tr>
<tr>
<td>Manganese, µg%</td>
<td>10.72</td>
<td>11.90</td>
<td>11.95</td>
<td>5.0 - 12.0</td>
</tr>
<tr>
<td>Iron, mg%</td>
<td>41.22</td>
<td>41.27</td>
<td>37.86</td>
<td>35.0 - 50.0</td>
</tr>
</tbody>
</table>

**TOXY-NIL Plus mode of action on various mycotoxin.**

TOXY-NIL Plus main mode of action is by means of stimulating Bio-transformation. This action is the sum of processes by which a foreign chemical is subjected to chemical changes by living organisms or by their products.
Metabolites are formed by bio-transformation. The end products are chemically distinct from the original compound and are usually more polar (hydrophilic).
These metabolites with more water solubility characteristics reduce the ability for partition into biological membranes and therefore restrict its distribution to the various tissues. This decreases the renal tubular and intestinal re-absorption and ultimately promotes its excretion via the urine and faeces.

Bio-transformation reactions are accomplished by several enzyme systems located in the cytosol and smooth endoplasmic reticulum of many living cells.
The basic enzymatic reactions involved in the bio-transformation of toxicants are: reactions, which involve oxidation, reduction and hydrolysis (derivatives formed are more polar by adding or exposing functional groups e.g. hydroxyl, thiol, amino or carboxyl).

These groups allow the compound to undergo into another series of reactions, which consists of conjugation or synthetic reactions. These reactions involve covalent linkage to an endogenous molecule, producing a conjugate that is then eliminated.

Trichothecene mycotoxins are metabolised (Bio-transformed) by three major reactions: de-acylation (hydrolysis), hydroxylation (oxidation) and de-epoxidation (reduction). The reduction of the 12,13-epoxide ring is the most important detoxification reaction since the epoxide is considered essential for toxicity. The major identified bio-transformation reaction of Ochratoxin A is its hydrolysis into Oa and L-phenylalanine.

The basic enzymatic bio-transformation reaction of Aflatoxins are conceptually divided into: reactions consisting of Oxidation, reduction and Hydrolysis and reactions in which the metabolites produced in the first phase are conjugated with endogenous substances in order to facilitate excretion.

Zearalenone is transferred into α-zearalenol and further into β-zearalenol.

TOXY-NIL Plus dry has a wide spectrum of activity. It controls polar and less polar Mycotoxins such as: aflatoxin, ochratoxin, trichothecenes (T-2, TH-2), nivalenol, diacetoxyscirpenol, etc. The animal trial at the Laboratory of Animal Reproduction of Lithuanian Institute of Animal Science clearly indicates that TOXY-NIL Plus dry neutralises the toxicity of zearalenone in vivo circumstances. Furthermore, TOXY-NIL Plus dry has a specific activity of adsorbing and neutralising complex molecules such as Mycotoxins and does not influence the availability of simple molecules such as minerals.

The role of organic acid and mycotoxin binders in animal feed is very important in terms of food safety and efficient animal production. Often raw materials or even final products are contaminated with microorganisms bacteria such as: Salmonella, Campylobacter, Verotoxigenic Escherichia coli, Clostridia, etc., moulds and yeasts which produce specific secondary metabolites. Preservation of raw materials for both human and animal nutrition is a major challenge as the very nature of these materials makes them susceptible to microbial contamination. These can cause severe diseases or be toxic for animals and humans. Use of various organic acids and their salts is important to maintain good nutritional quality in stored materials.

Specific programmes are therefore established to diminish the risks, optimise the animal performance and assure the production of safe food.
Mycotoxins: Adsorbents: Myths and Truths


Goldblatt, 1971; Park et al., 1984; CAST, 1989

Mckenzie et al., 1997,
Masimanco et al., 1973; Phillips et al., 1990a,b
Phillips et al., 1988;
Harvey et al., 1993;
Kubena et al., 1990b, 1993;
Ledoux et al., 1999