

Effective control of mycotoxin contamination in pig feed

by U. Hofstetter, K. Griessler and I. Rodrigues, *BiomIn Holding GmbH, Industriestrasse 21, 3130 Herzogenburg, Austria.*

Mycotoxins are toxic secondary metabolites produced by a large variety of moulds mainly of *Fusarium*, *Aspergillus* and *Penicillium* species. The contamination of food and feedstuffs with mycotoxins is a serious threat and causes substantial economic losses in animal husbandry.

Global trade of agricultural commodities intensified the discussion about the mycotoxin hazard. Generally, the occurrence of mycotoxins in food and animal feed often shows a geographical pattern, as for example *Aspergillus* sp. find optimal conditions in tropical and subtropical regions, whereas *Fusarium* and *Penicillium* sp. are adapted to the moderate climate of North America and Europe.

However, worldwide trade of food and feed commodities has resulted in a worldwide distribution of mycotoxins. From the agricultural point of view, there are five important classes of mycotoxins namely trichothecenes, zearalenone, ochratoxins, aflatoxins and fumonisins.

Due to their diverse structure, mycotoxins cause a wide variety of different symptoms in animals. Prevention methods in the field, during harvest and storage are important but not sufficient to eliminate the risk of mycotoxin contamination completely.

According to the literature, adsorption of mycotoxins is mainly effective against aflatoxins but for example trichothecenes or zearalenone need to be deactivated by a different approach.

BiomIn's Mycofix product line represents

feed additives which combine adsorption and biotransformation. This is the most promising way to counteract different mycotoxins effectively. The efficacy against several mycotoxins was proven by in vitro adsorption or detoxification experiments and in vivo trials.

Problems caused

Mycotoxins may cause various toxic effects, so called mycotoxicoses. Diagnoses of mycotoxicoses are often very difficult as the effects of mycotoxins in animals are diverse, varying from specific to unspecific symptoms like immune suppression, diarrhoea, haemorrhages or reduced performance.

Symptoms due to mycotoxin contamination depend not only on the level and type of mycotoxin but also on several factors such as animal species, sex, environment, nutritional status and other toxic entities.

In addition, mycotoxicoses can already occur at toxin concentrations below the detection limit. Mycotoxins may be present in feedstuffs despite negative analytical findings. It is well understood that mycotoxins are not homogeneously dispersed in feedstuffs but usually occur in 'hot-spots'. This makes sampling difficult and mycotoxins may stay analytically undetected, even with perfect sampling procedures.

Mycotoxins may also be masked from analytical detection by small molecules (glycosides, glucuronides, fatty acid esters, and proteins) attached to the toxin thus giving a false negative result.

Consequently, these masked mycotoxins are not detectable with conventional analytical methods. However, the attached molecules may be removed during digestion,

releasing the mycotoxin to affect the animal.

Additionally, each plant might be affected by more than one fungus and each of them can produce several mycotoxins.

Consequently, there is a great probability that many mycotoxins occur simultaneously in feeds.

The combination of multiple mycotoxins in feedstuffs may cause more adverse effects than a single mycotoxin due to additive or synergistic interaction.

Synergistic effects can occur at low levels when the combined effects of two or more mycotoxins are much greater than the individual effects of each toxin alone.

For instance, aflatoxin B1 acts synergistically with ochratoxin A or T-2 toxin and fumonisin B1 with deoxynivalenol.

Counteracting mycotoxins

Prevention of fungal infections during plant growth (planting of more resistant grains, balanced fertilisation), harvest (appropriate date and equipment, ploughing of fields), storage (humidity, temperature and insect control) and distribution (good shipping conditions) is one effective way to avoid mycotoxin contamination in agricultural commodities.

So far, a number of physical and chemical approaches have been taken to counteract mycotoxins, but only a few have practical application. Investigated physical treatments include washing, polishing, mechanical sorting and separation, density segregation, flotation, autoclaving, roasting and microwave heating, UV irradiation, ultrasound treatment and solvent extraction.

The efficiency of these techniques highly depends on the grade of contamination and the distribution of mycotoxins throughout the grain. Subsequently, the results obtained are uncertain and often connected with high product losses.

Moreover, some of these physical treatments are relatively costly and may remove or destroy essential nutrients in feed.

Chemical methods require not only suitable reaction facilities but also additional treatments (drying, cleaning) that make them very time consuming and expensive.

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Table 1. The effects of deoxynivalenol and zearalenone on growth performance in piglets.

Groups	NC	PC	TG 1	TG 2
Initial weight (kg)	9.0	8.9	9.1	8.9
Weight at day 16 (kg)	16.2 ^a	15.2 ^b	15.9 ^a	15.9 ^a
Final weight (kg)	29.8 ^a	26.2 ^b	29.1 ^a	29.4 ^a
Daily feed intake (kg/d)	1.14 ^a	0.82 ^b	1.01 ^a	1.14 ^a

^{a,b} Different superscripts indicate significant differences (P<0.05)

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Nevertheless, various chemicals, including oxidising and reducing agents, acids, bases, salts and chlorinating substances have been tested for their ability to degrade mycotoxins in agricultural commodities. However, only a limited number turned out to be effective without diminishing nutritional value or palatability.

Treatment of contaminated feed with ammonia was the most attractive method in the past. Although this technique was apparently safe and effective in earlier studies, it has not been approved by the US Food and Drug Administration due to the potential toxicity and carcinogenicity of reaction products.

As the success of physical or chemical detoxification of mycotoxins is quite low there is a necessity for new and effective methods to counteract mycotoxins. One investigated method is a reduction of the toxin's bioavailability in the gastrointestinal tract by the use of various adsorption materials in the feed.

However, several studies have proven that adsorption of mycotoxins is mainly effective for aflatoxins. Adsorption of other mycotoxins is limited (for example zearalenone, ochratoxin A) or failed (for example trichothecenes) under field conditions.

Therefore, mycotoxin deactivation by biotransformation is seen as the most promising way to detoxify mycotoxins that cannot

be bound by adsorbents. This enzymatic or microbiological degradation of mycotoxins leads to non-toxic metabolites and offers a very specific, irreversible, efficient way of detoxification.

In the case of trichothecenes the 12,13-epoxide ring is responsible for their toxic activity, removal of this epoxide group results in a decreased toxicity. Continuous research led to microbial supplements able to detoxify all kinds of trichothecenes, zearalenone and ochratoxin A.

Binder et al. (1996) were the first who developed a feed additive, which was able to biotransform the epoxide group of trichothecenes by using an *Eubacterium* strain that was isolated out of bovine rumen fluid.

Thus, the detoxifying strain *Eubacterium* BBSH 797 was the first microbe used in a mycotoxin deactivating feed additive.

Subsequently, a novel yeast strain capable of degrading ochratoxin A and zearalenone was isolated and characterised. The strain named *Trichosporon mycotoxinivorans* detoxifies ochratoxin A by cleavage of the phenylalanine moiety from the isocoumarin derivate to ochratoxin α .

This metabolite has been described to be at least 500 times less toxic than the parent compound.

Zearalenone has no acute toxicity, but shows oestrogenic effects and therefore causes substantial fertility problems.

The biotransformation of zearalenone by *T. mycotoxinivorans* leads to a non-oestrogenic metabolite, which was proven in an in vitro E-screen assay at the University of Utrecht based on the cell proliferation of the human oestrogen-receptor-positive breast cancer cell line, MCF-7.

The Mycofix product line

The Mycofix product line represents specially developed feed additives that protect animal health by combining adsorption and biotransformation of mycotoxins.

The modular system – adsorption of mycotoxins by selective blends of minerals and biological degradation of less or non-adsorbable mycotoxins by patented biological components – are the basis of the mode of action. The efficacy against the most important mycotoxins was proven in several in vivo trials.

Weaning piglets

The effects of deoxynivalenol and zearalenone on growth performance, blood chemistry, immune response and the alleviating effect of Mycofix Plus were investigated.

A trial was conducted with 48 weaning piglets from a pseudorabies-free breeder farm. The animals were randomly divided into four treatment groups with six pigs per pen and each pen with a duplicate:

● NC: mycotoxin free, no Mycofix Plus.

Groups	NC	PC	TG 1	TG 2
Chemotactic index	4.24 ^a	2.07 ^b	4.42 ^a	4.20 ^a
Phagocytic macrophages (%)	27.6 ^a	20.8 ^{ab}	25.6 ^{ab}	25.2 ^{ab}

^{ab} Different superscripts indicate significant differences (P<0.05)

Table 2. The effects of deoxynivalenol and zearalenone on alveolar macrophage activities in pigs.

- PC: 1ppm deoxynivalenol, 250ppb zearalenone.
- TG 1: 1ppm deoxynivalenol, 250ppb zearalenone, 0.15% Mycofix Plus.
- TG 2: 0.15% Mycofix Plus.

Body weight and feed intake of pigs were measured at days 0, 16, 28 and at the end of the experimental period for the calculation of performance parameters. For additional evaluation of toxic effects blood chemistry parameters, immune response and histopathological examination were measured.

Elevated activities of serum enzymes such as alanine aminotransferase and aspartate aminotransferase are a sign of organ damage. In the positive control the level of total protein was decreased compared to the control group.

In the γ -glutamyltransferase, aspartate aminotransferase and alanine aminotransferase assay, the positive control demonstrated the highest enzyme activity in the serum and increased 2.3-2.6 fold in comparison to the negative control group.

The addition of Mycofix Plus diminished the negative effect on γ -glutamyltransferase and aspartate aminotransferase levels from the toxins.

Histopathological observations of the liver and other organs confirmed the liver damage indicated by the elevated aspartate aminotransferase and alanine aminotransferase. Macrophages represent the immune-competence of animals to respond to pathogenic invasion. The results of alveolar macrophage activity in pigs are shown in Table 2.

Only 49% of the chemotactic activity was found in the deoxynivalenol and zearalenone challenged group. Mycofix Plus improved the ability for chemotaxis and phagocytosis, with similar values to the negative control group and the percentage of phagocytic macrophages was not further elevated.

The chemotactic index is an important sign for the ability of macrophage movement toward pathogen invasion sites.

Pseudorabies vaccine was tested on day seven and 14 of the experiment and serum

was collected on the 14th and 28th day after vaccination. The antibody titers showed lower levels in the toxin challenged group than in the other groups.

Conclusions

For many reasons it is not possible to totally avoid mycotoxin contaminations in feed. Mycotoxins cause severe effects in animals as well as in humans.

Therefore it is important to avoid these hazardous compounds entering the food chain. A lot of research has been done to adsorb or deactivate these toxins in the intestinal tract of animals with products that are directly mixed into the feed. It turned out that some mycotoxins, like trichothecenes, cannot be adsorbed sufficiently.

Thus, enzymatic biotransformation to metabolites without pathogenic activity is the only way to avoid their negative effects on animals. The Mycofix product line combines different strategies to counteract mycotoxins. Several experiments proved the efficacy of the feed additives against the negative impacts of mycotoxins.

The negative impact on performance parameters as well as on clinical findings in pigs like swollen vulva and prepuce, rectum prolapse, increased urination, vomiting and diarrhoea was proven to be reduced by the addition of Mycofix to the feed. ■