Newsletter

Vol. 3, No. 22, January 2005

> EDITORIAL

According to a FAO study 25% of grain worldwide is contaminated with mycotoxins despite all preventive methods. The formation of mycotoxins highly depends on the moisture conditions starting during the growth of crops and ending at the day when they are fed to the animal. Since we faced a very wet spring and summer in 2004 in Central and Northern Europe the conditions were ideal for the field growth of Fusarium fungus and hence contamination with trichothecene- mycotoxins such as deoxynivalenol (DON) and T-2 toxin. Fusarium graminearum is a widespread colonizer of crop residues, where the pathogen survives during winter. Wheat stubble and corn stalks are major inoculum sources in North America and Europe, while rice stubble is the major cause of inoculum in China. Airborne release of ascospores peaks during and after rainy periods.

Just to give you an idea: From all analyzed wheat samples in Austria 2004, 50% were contaminated up to 500ppb and 45% were contaminated higher than 500ppb. The maximum contamination found was 10 000ppb! Without including this extreme value the average contamination level was 655ppb, which is very high in field conditions. Especially in combination with other mycotoxins this contamination leads to depression of the immune-system, decreased performance and thus severe economic losses in animal production.

According to LUFA (Landwirtschaftliche Untersuchungs- und Forschungsanstalt) in Germany every 5th sample of wheat was contaminated with more than 1000ppb DON. In more than 50% of the analyzed samples DON contamination was higher than 100ppb. When continuously fed, already this low contamination leads to a negative impact on the animals!

In 26% of Norwegian oat and barley samples (www.mattilsynet.no) a low trichothecene contamination (< 250ppb) was observed, 33% were contaminated between 250 and 1000ppb and 15% were contaminated to a level higher than 1000ppb!

Several competiters products claim to be able to counteract trichothecenes. This newsletter was written to give you some more information on trichothecenes and to compare the main mode of action of available products.

Enjoy reading!

Verena Starkl

Mycofix® Plus product line

Mycotoxins are known to pose a great risk to animal health. In general they vary widely in their chemical structure and therefore it is impossible to deactivate all of them via adsorption (binding). However, some of them, like aflatoxins, can be adsorbed by minerals, but others e.g. trichothecenes need to be deactivated using a different strategy. Specific enzymatic degradation is a sophisticated approach that already proved its efficacy in various university and field trials. Although trichothecenes do not have the same toxic potential as aflatoxin (carcinogenic), they pose the greatest risk to animals' health especially in Europe and Northern America.

Unfortunately, trichothecenes are non-adsorbable, neither by minerals nor by yeast products. As outlined below, trichothecenes can only be deactivated by enzymatic transformation to a non-toxic metabolite.

Trichothecenes – How can you counteract these non-adsorbable but harmful mycotoxins?

What are Trichothecenes?

Trichothecenes are a group of approximately 170 (Weidenbörner, 2001) structurally related mycotoxins. They are mainly produced by various, ubiquitously present fungi of the *Fusarium* genus. Each trichothecene-mycotoxin possesses a conjugated double

ring system on which an epoxide ring is located (see left molecule Figure 1). The difference in a side chain of the molecule is used to subdivide this huge group into A- and B-trichothecenes. Type A trichothecenes include mainly T-2 toxin, HT-2 toxin and diacetoxyscirpenol (DAS), which are generally 10x more acute toxic than Type B trichothecenes such as deoxynivalenol (DON, also called vomitoxin), nivalenol and fusarenon X.
Deoxynivalenol is common in North American and European cereal grains, and of the trichothecenes poses the greatest problems to animal health. Pigs exhibit the greatest sensitivity to DON, while chickens and turkeys, followed by ruminants appear to have higher tolerance.

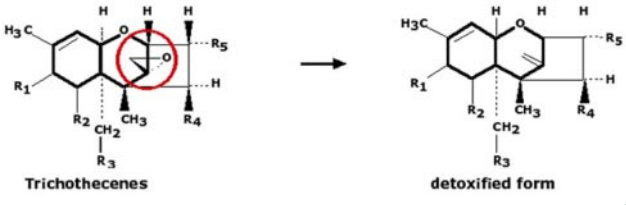

Figure 1: Trichothecenes share the same basic structure. The so-called "epoxide ring" (red ring!) is considered as being the main toxic structure. Enzymes, de-epoxidases, detoxify trichothecenes by reduction to a simple double binding.

Toxicity of Trichothecenes

Many of the toxic effects of trichothecenes stem from their capacity to inhibit DNA and protein synthesis. General signs of trichothecene toxicity in animals include weight loss, decreased feed conversion, feed refusal, vomiting, bloody diarrhea, severe dermatitis, hemorrhages, decreased egg production, abortion, and death.
Histologic lesions consist of necrosis and hemorrhages in proliferating tissues of the intestinal mucosa, bone marrow, spleen, testis, and ovary.
The most common signs of acute DON exposure in pigs are abdominal distress, increased salivation, and malaise, however vomiting has been reported at higher dietary concentrations. In pigs extensive lesions are not typically documented in field cases, due to the fact that pigs regulate the toxin ingestion by adjusting their feed intake (Friend et al. 1986). The extent of DON effects in pigs relates to age and sex as well as the contamination source - *F. graminearum* produces many metabolites besides DON, therefore mycotoxicosis may be caused by multiple toxins. Unidentified/bound toxins, conjugated mycotoxins or toxic agents of other origin might contribute substantially to the response.
In broiler chicken widespread hemorrhaging, urate deposition, neural toxicity, and upper GI tract irritation are considered as primary signs.

IMMUNOLOGIC EFFECTS

The capacity of trichothecenes to inhibit protein synthesis apparently contributes to their potential to modulate immune function. Acute exposure to trichothecenes results in severe damage to actively dividing cells in tissues such as bone marrow, lymph nodes, spleen, thymus, and intestinal mucosa. General effects on function of immunocompetent cells, host resistance, and immunoglobulin production at lower doses using oral and other routes of exposure have been reported and reviewed extensively.

EFFECTS ON CELLULAR IMMUNITY

- Chemotactic migration of neutrophils decreased
- Phagocytosis by alveolar macrophages decreased
- Mitogen-induced blastogenesis of lymphocytes inhibited
- Cytotoxic to lymphocytes
- Platelet function inhibited

EFFECTS ON INFECTIOUS DISEASES

Increased susceptibility to…

- *Candida*
- *Cryptococcus*
- *Listeria*
- *Salmonella*
- *Mycobacterium*
- *Herpes simplex Type 1*

Macrophages, lymphocytes, and erythrocytes may be decreased with prolonged exposure to trichothecenes. Furthermore trichothecenes also induce erythrocyte hemolysis blood clotting.

How can you counteract trichothecenes???

Trichothecenes are mainly produced by so-called field fungi during the growth of the plant. Mould inhibitors are only useful to stop the growth of so-called storage fungi such as *Aspergillus* and *Penicillium* which are mainly producers of aflatoxin and ochratoxin. Mould inhibitors in general cannot decrease mycotoxin contamination. Since trichothecenes belong to the most prevalent mycotoxins worldwide, there was a strong need for sustainable counteraction. In general, products based on the following three mechanisms are used to counteract mycotoxins during ingestion.

1. ALUMINOSILICATES

Already years ago it was found that trichothecenes cannot be deactivated by minerals and adsorption (Shehata, 2000). Biomin also performed extensive studies in this field, and its research confirmed that specific adsorption cannot take place

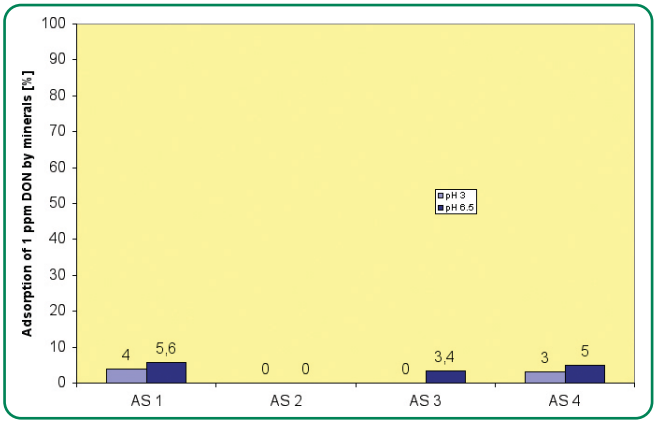

*Figure 2: In-vitro **adsorption** [%] of 1 ppm deoxynivalenol comparing 4 different **minerals** at ph 3 and pH 6.5. Institute of Geology, University of Vienna, Austria.*

(see figure 2). Most probably minerals adsorb mycotoxins via binding of suitably located polar groups. Definitely, trichothecenes do not possess polar groups in the right position to specifically bind to minerals.

2. MANNANOLIGOSACCHARIDES (MOS)

Mannanoligosaccarides are parts of yeast cell walls. Producers promote adsorption of various mycotoxins due to high specific surface. They promote that 500g of this yeast cell wall product have a surface of 1 hectare which is equivalent to 20 m^2/g.
But please consider, the mineral part of Mycofix® Plus has a proven specific surface of 103 m^2/g!
Biomin tested the adsorption of deoxynivalenol and T-2 toxin to MOS at two pH levels and found no adsorption (see figure 3).

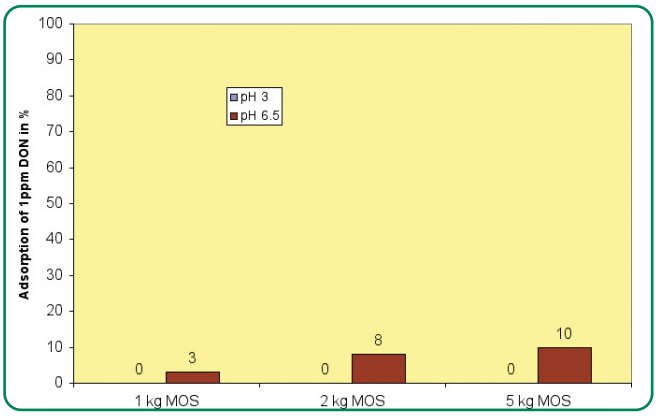

*Figure 3: In-vitro **adsorption** of 1ppm deoxynivalenol comparing 1, 2 and 5kg inclusion rate of **MOS**. Institute for Agrobiotechnology, IFA-Tulln, Austria.*

3. BIOTRANSFORMATION

The main toxic structure of all trichothecenes is the epoxide ring, and therefore this ring is the main target structure for a successful deactivation of these detrimental mycotoxins. Special enzymes, so called de-epoxidases produced by a unique micro-organism, detoxify the epoxide ring to a simple double binding (see figure 1). In vitro trials proved that in this way approx. 95% of trichothecenes can be deactivated (see figure 4)!

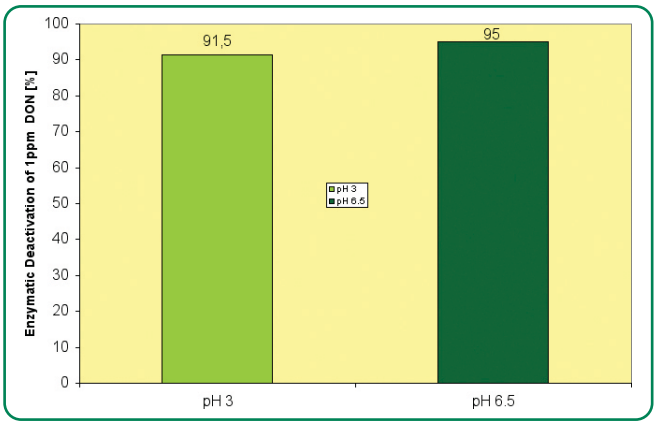

*Figure 4: In-vitro **deactivation** of 1ppm deoxynivalenol by **enzymes** present in Mycofix® Plus at pH 3 and pH 6.5. Institute for Agrobiotechnology, IFA-Tulln, Austria.*

Trial

Aluminosilicates, MOS and biotransformation were compared in an *in vivo* trial at the University of Colombia in Bogota under the supervision of Professor Dr. Gonzalo Diaz. 180 day-old male chickens were challenged to investigate the effect of 2ppm T-2 toxin and the capacity of different products to counteract this challenge. Detailed trial set-up can be taken from table 1.

Table 1. Trial set-up. Mycofix® Plus, MOS, and two aluminosilicates used in broiler diets to compare their effect on a 2ppm challenge with T-2 toxin.

Group	Feed Additive	IR	T-2 toxin
		[kg/t]	*ppm*
1	0	0	0
2	0	2	2
3	Mycofix® Plus	2	2
4	MOS	2	2
5	Aluminosilicate 1	2.5	2
6	Aluminosilicate 2	3.0	2

Results which confirm the capacity of Mycofix® Plus to decontaminate trichothecenes by biotransformation can be taken from figure 5. For detailed information on this trial please see Newsletter Vol. 2, No. 17, August 2004 "University Trial proves efficacy of Mycofix® Plus". Further Trials showing the efficacy of Mycofix® Plus to counteract trichothecenes are available on request.

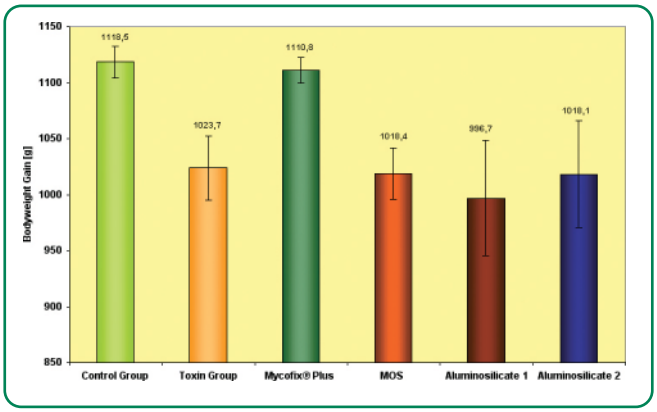

Figure 5: Results of a broiler-trial comparing different products on their capacity to counteract T-2 toxin. MOS, (adsorption by yeast cell wall), Mycofix® Plus (biotransformation), two different aluminosilicates (adsorption to minerals), National University of Colombia, Bogota, Colombia.

Conclusion

In non-tropical countries trichothecenes are the most important toxins. Several trials have shown that adsorption either by minerals or by mannanoligosaccharides is impossible.
A solution to the adverse effects of ubiquitously present trichothecenes such as DON and T-2 toxin can only be guaranteed when specific enzymes (Mycofix® Plus product line) are used.

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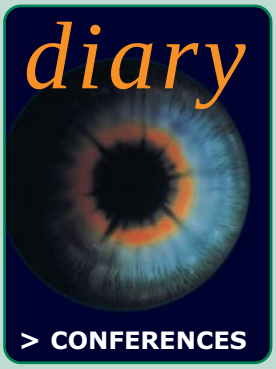

Australian Poultry Science Symposium, February 7th - 9th 2005, Sydney, Australia

North Carolina Pork Conference and Expo, February 16th – 17th, Greenville, North Carolina, USA

Western Dairy Management Conference, March 9th – 11th 2005, Reno, Nevada, USA

VIV Bangkok, March 16th – 18th, Bangkok International Trade & Exhibition Center, Bangkok, Thailand

Central Plains Dairy Expo and Conference, March 30th – 31st, Sioux Falls, South Dakota, USA

BSAS British Society of Animal Science April 4th – 6th 2005, Exhibition Centre, University of York, United Kingdom

> LITERATURE

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> IMPRESSUM

Newsletter is published by the export department of Biomin Innovative Animal Nutrition GmbH
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