A new approach to managing mycotoxins

Controlling mycotoxins is not an easy task. A number of approaches have been tried, but because of the large number of toxins and their interactions, a single solution to the problem may not exist. Research shows that combining selected adsorbants with bio-transformation methods will ensure effective protection in mycotoxin-contaminated feeds.

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Figure 1. Biotransformation of trichothecenes to non-toxic metabolites

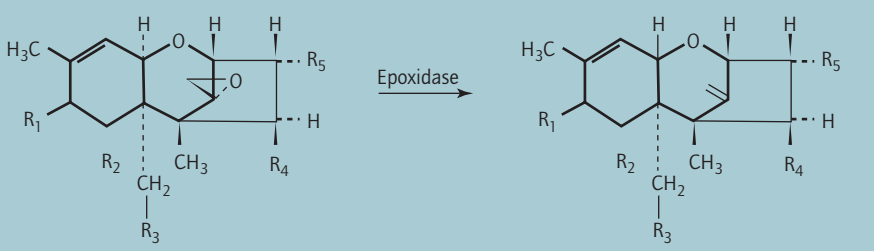


Table 2. Detoxification of trichothecenes by Eubacterium strain BBSH 797 (Fuchs, 1999)

Toxin	*Intermediate product*	*Transformation product*
DON		DOM-1
3-AcDON	DON	DOM-1
15-AcDON	DON	DOM-1
Nivalenol		De-epoxy-nivalenol
Fusarenon X	Nivalenol	De-epoxy-nivalenol
T-2 toxin	HT-2 toxin	De-epoxy-HT-2 toxin
HT-2 toxin		De-epoxy-HT-2 toxin
T-2 triol	T-2 tetraol	De-epoxy-triol, de-epoxy-tetraol
T-2 tetraol		De-epoxy-tetraol
Scirpentriol		De-epoxy-scirpentriol

Figure 2. Electron micrograph of BBSH 797

Mycotoxins are highly toxic secondary metabolic products of various moulds, mainly those belonging to the genera *Fusarium, Aspergillus* and *Penicillium*. It has been estimated that at least 300 of these fungal metabolites are potentially toxic to animals and humans. However, the most notorious- from the agricultural point of view- and thus extensively investigated mycotoxins are aflatoxin B_1, zearalenone, deoxynivalenol (DON, "vomitoxin"), T-2 toxin, ochratoxin A and fumonisin B_1. Their global occurrence is considered to be a major risk factor. According to the Food and Agriculture Organization (FAO) as much as 25 % of the world's crops are affected annually.

The individual toxicity of mycotoxins is extremely variable. It not only depends on the physical and chemical properties of each toxin, but also on the level of intake, the duration of exposure, the animal species, sex, age, breed and physiological status, nutritional status, environmental conditions (including hygiene, temperature, air conditioning, humidity and production density) and the synergy which can occur between mycotoxins simultaneously present in feed. Mycotoxins are reported to be carcinogenic, genotoxic, teratogenic, dermato-, nephro- and hepatotoxic. However in general, decreased performance, reproductive disorders and immune-suppression, resulting among other things in a higher susceptibility to disease, are of major concern.

Several studies have shown that economic losses due to mycotoxins occur at all levels of food and feed production, including crop and animal production, processing and distribution. Even during favourable climatic periods, millions of dollars are lost as a consequence of crop contamination.

No easy answers

Unfortunately, there are no easy answers to the mycotoxin dilemma, as:

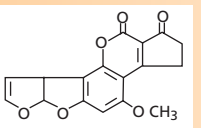
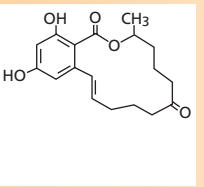
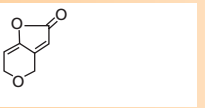
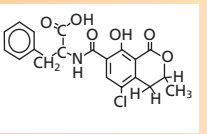
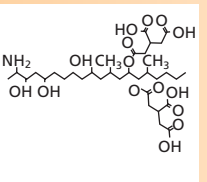
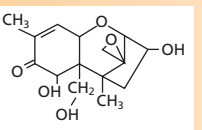
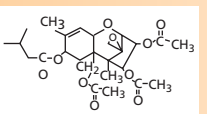
- These compounds often occur in very low concentrations that may be difficult to detect.
- Analysis may not give a true assessment of the situation, because the methodology is not sufficiently developed. "Masked" mycotoxins may also be present.
- Clinical symptoms are often not obvious or unique. Typical observations, such as lethargy, decreased feed intake, poor performance or increased susceptibility to infection could also be caused by a number of other health or management factors.
- There is no typical dose-response relationship with mycotoxins.
- Interactions between individual mycotoxins are not well characterised.
- Since contaminated feed usually contains a number of different mycotoxins, some of which might not yet be identified or well-known, correct interpretation of the situation is difficult.

Mycotoxins despite strenuous prevention efforts

Prevention of fungal infections during plant growth, harvest, storage and distribution would seem the most rational and efficient way to avoid mycotoxins in agricultural commodities. Common practical measures include planting of more resistant strains of cereals, selection of high quality seeds, avoiding high plant densities, balanced fertilisation, preventive management towards insect infestations as well as a suitable management of crop residues that are often the primary inoculum of mycotoxigenic fungi. Careful selection of harvest date, equipment and harvesting procedures to minimise crop damage and removal of damaged crops and high moisture plant parts may reduce mould infections. Immediate storage in good storage facilities (moisture, temperature, humidity and insect control) and the addition of antifungal agents may also diminish fungal growth but cannot detoxify contaminated feedstuffs.

Contamination of agricultural products with mycotoxins still occurs despite the most strenuous prevention efforts. Their economic impacts are felt by crop and animal producers as well as by food and feed processors.

Table 1. Bio-transformation of mycotoxins by isolated microorganisms

Microorganism	*Investigated Mycotoxins*		*References*
• Flavobacterium aurantiacum	Aflatoxins		Ciegler *et al.*, 1966
Aspergillus flavus	Aflatoxins		Hamid and Smith, 1987
• Gliocladium roseum NRRL 1859	ZON		El-Sharkawy and Hajj, 1988
Rhodococcus erythropolis	ZON		Rood and Duvick, 1998
Norcardia globulera	ZON		Rood and Duvick, 1998
• Saccharomyces cerevisiae	Patulin		Stinson *et al.*, 1978
Paecilomyces sp	Patulin		Anderson *et al.*, 1979
• Phenylobaterium immobile	OTA		Wegst and Lingens, 1983
Acinetobacter calcoaceticus	OTA		Hwang and Draughon, 1994
• Exophiala pinifera	Fumonisins		
Rhinocladiella atrovirens	Fumonisins		Duvick *et al.*, 1998
Bacterium ATCC 55552	Fumonisins		
• Agrobacterium sp.	DON		Shima *et al.*, 1997
Eubacterium sp.	DON		Binder *et al.*, 1998
• Butyrvibrio fibrisolvens	T-2 toxin		Westlake *et al.*, 1987
Selenomonas ruminantium	T-2 toxin		Westlake *et al.*, 1987
Anaerovibrio lipolytica	T-2 toxin		Westlake *et al.*, 1987
Curtobacterium sp.	T-2 toxin		Ueno *et al.*, 1983

Physical and chemical decontamination

The ever-increasing number of reports on the presence of mycotoxins in foods and feeds dictates the necessity for practical and economical detoxification procedures. A number of physical and chemical approaches have already been taken to counteract mycotoxins, though only a few have real practical application.

Physical treatments include washing, polishing, mechanical sorting and separation, density segregation, flotation, autoclaving, roasting and microwave heating, UV irradiation, ultrasound treatment and solvent extraction. However, the efficiency of these techniques depends on the level 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 can make them time consuming and expensive. 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. Only a limited number of these are effective without diminishing the feed's nutritional value or palatability. Treatment of contaminated feed with ammonia was once the most attractive method. Although early studies shoed this technique to be safe and effective, ammoniation has not been approved by the US Food and Drug Administration due to the potential toxicity and carcinogenicity of the reaction products.

Adsorbants successful against aflatoxicosis

The high costs and limitations of physical and chemical treatment of feed prompted a search for other solutions to the mycotoxin hazard. Consequently, techniques based on deactivation of mycotoxins directly in the gastrointestinal tract of animals (*in vivo*) have been investigated.

Up to now, the most widely investigated method in this field is the addition of *chemisorbents* with the capacity to tightly bind and immobilise mycotoxins in the gastrointestinal tract of animals, resulting in a major reduction in toxin bioavailability. In several studies, hydrated sodium calcium aluminosilicates (HSCAS) have proven to be the most promising adsorbents. However, while good and scientifically explained results were obtained for counteracting aflatoxins, adsorption of other mycotoxins was limited (e.g. zearalenone, ochratoxin A) or even failed under field conditions (e.g. trichothecenes such as DON).

Microbial detoxification – brand-new and proven

For the less- and non- adsorbable mycotoxins, an alternative practical detoxification method had to be found. Enzymatic or microbial degradation of mycotoxins ("biotransformation") has already been the subject of research for more than 30 years. A great deal of literature is available concerning the biotransformation of trichothecenes, among the world's most important agricultural toxins. It is well known that the 12,13-epoxide ring is responsible for tricothecene's toxicity and that reductive de-epoxidation caused by either enzymes or live microbes entails a significant loss of toxicity (*Figure 1*).

Although several microorganisms with mycotoxin degradation activity have been isolated in the past (*Table 1*), Binder *et al.* (2001) were the first to develop a mycotoxin deactivating feed additive based on live microbes. A bacterial strain originally isolated from bovine rumen contents was found to have trichothecene-detoxifying activity and was named *BBSH 797* after the research team that discovered it in July 1997: Binder, Binder, Schatzmayr and Heidler. Belonging to the genus *Eubacteria* this strictly anaerobic microbe is a gram-positive, non-motile, non-spore forming, irregular rod shaped bacterium (*Figure 2*). During its manufacture, BBSH 797 is stabilised by freeze-drying and embedding in protective substances (mainly organic polymers), to guarantee sufficient stability against environmental conditions during storage and to make its passage intact through the acidic gastric tract of animals to reach its site of action (is the intestine unharmed.

During its metabolilsm BBSH 797 produces enzymes (de-epoxidases) that degrade trichothecenes by selective cleavage of their toxic 12,13-epoxy group. This detoxification was investigated for the most important trichothecenes. The reaction products obtained (*Table 2*) were confirmed by high performance liquid chromatography mass spectroscopy (HPLC-MS), gas chromatography mass spectroscopy (GC-MS) and nuclear magnetic resonance spectroscopy (NMR). Apart from de-epoxidation, hydrolysation of ester groups into corresponding hydroxyl groups as well as de-acetylation could be detected in some cases.

Monogastric applications

Studies using *in vitro* models with pig and chicken intestine revealed that this bacterium exhibits activity in the gastrointestinal tract of monogastric animals and can therefore be used as feed additive to detoxify trichothecenes in the gut. In order to prove the *in vivo* efficacy of BBSH 797, scientific feeding trials were conducted under the surveillance of the University for Veterinary Medicine in Vienna and with the approval of the Austrian Ministry for Agriculture. In broiler trials, mycotoxin concentrations higher than those found under field conditions were used (10.5 ppm DON; 0.2 ppm AcDON) in order to obtain sufficient reduction in performance. However, even at such high levels of contamination, addition of BBSH 797 reduced mortality from 19.4 to 5.5 % and had a significantly (P<0.5) positive influence on the weight development of the birds (*Table 3*).

Ochratoxin A detoxification

A very recent development has been made in the fight against ochratoxin A (OTA), a mycotoxin with nephrotoxic-, hepatotoxic-, carcinogenic- and immunosuppressive-properties. OTA is mainly produced by specific species of *Penicillium* and *Aspergillus* during grain storage, but its occurrence has also been indicated in grapes, coffee beans and other commodities.

During animal production OTA causes economic losses mainly through a decrease in weight gain. However, it was the risk of residues in animal tissue and hence the possible transfer to humans through consumption of contaminated meat that finally led to an intensified search for counteracting measures.

As stated before, mycotoxin "binders" do not work efficiently enough against OTA, so a project was launched by the Austrian company Biomin with the aim of finding microorganisms with the capability to deactivate ochratoxins in the intestinal tract of animals. Based on the results of several studies conducted during the past years, natural bacterial habitats such as rumen fluid, intestinal contents and soil were screened for OTA-detoxifying microbes. Although many authors have reported that protozoa are mainly responsible for the detoxification of OTA in the rumen fluid of cattle and sheep, Schatzmayr *et al.* (2002) clearly demonstrated that bacteria also play an important role. By applying several enrichment methods and isolation procedures, two bacterial species (related to *Clostridium sporogenes* and *Lactobacillus vitulinus*) were isolated from rumen fluid which were able to cleave OTA into the non-toxic metabolite ochratoxin α and the amino acid phenylalanine (*Figure 3*).

OTA and ZON detoxifying yeasts

Besides bacteria, Schatzmayr *et al.* (2002) have investigated yeasts of the genera *Trichosporon*, *Rhodotorula* and *Cryptococcus* for their ochratoxin A-detoxifying activity. At the end of a very comprehensive selection process a new yeast species belonging to the genus of *Trichosporon* came out on top (*Figure 4*).

Figure 4. Light microscopic picture of ochratoxin A- detoxifying yeast strain *Trichosporon* sp. Nov

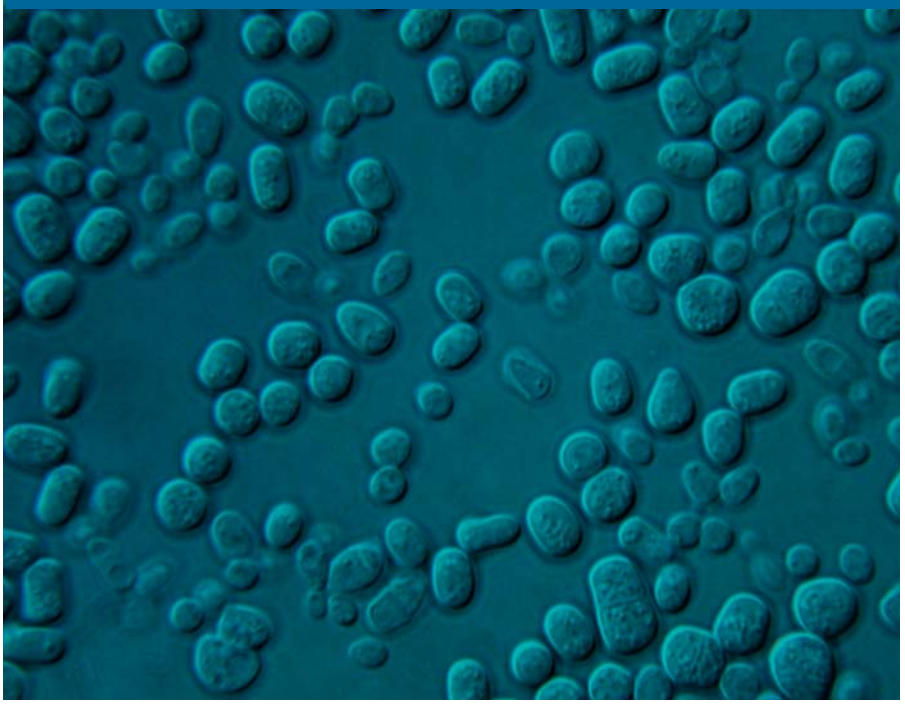
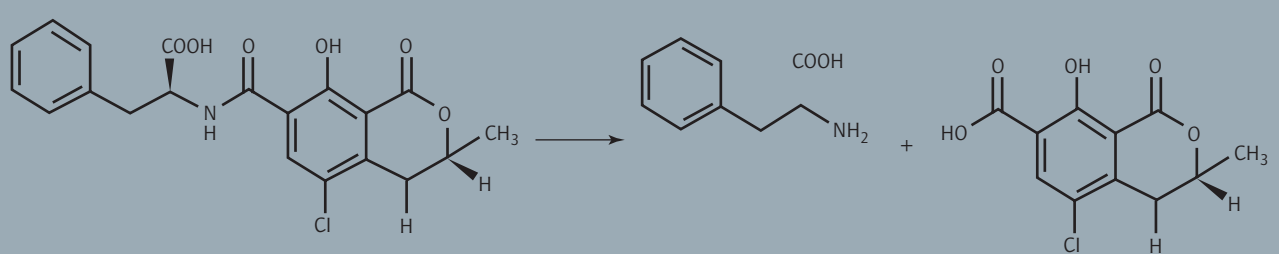


Table 3. Weight development of broilers fed a diet contaminated with 10.5 ppm DON and 0.2 ppm AcDON

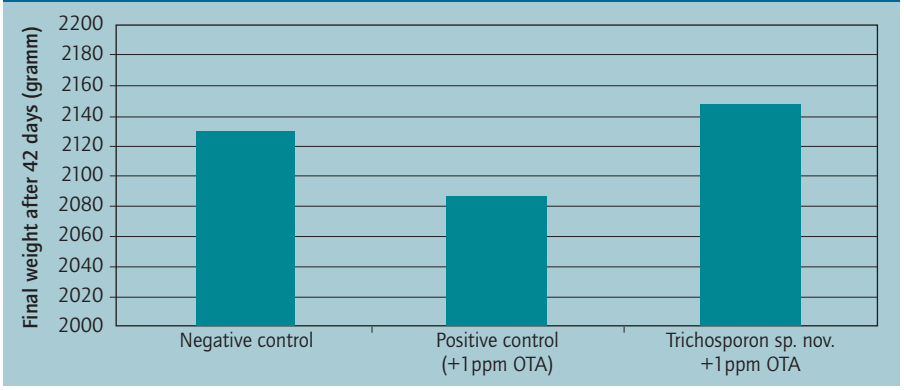
Day	*Control*	*BBSH 797*
1	36.4	36.4
8	60.8	95.5
15	161.9	217.1
22	384.3	473.1
29	817.3	972.2
36	1257.8	1437.2
ADWG (g)	35.94	41.06
FCR	1.919	1.871
Mortality (%)	19.4	5.5

Figure 3. Detoxification of ochratoxin A by cleavage of a phenylalanine moiety



Ochratoxin A | Phenylalanine | Ochratoxin alpha

Figure 5. Final weight of broiler chickens in a trial evaluating *Trichosporon* sp. nov. for *in vivo* detoxification of OTA



During selection, features like detoxification velocity, pathogenicity, ability to work under gastrointestinal conditions, the possibility of industrial scale fermentation and stabilisation as well as the requirements for the subsequent registration in the European Union were considered.

A feeding trial conducted at the University of Maribor in Slovenia revealed that the negative influence of high doses of ochratoxin A on the performance of broilers could be neutralised by the addition of stabilised *Trichosporon* cells. The final weight of the group receiving 1 ppm OTA and yeast was on average 61 g higher than that of the positive control group (1 ppm OTA without additive) and even better than the negative controls (*Figure 5*).

Incubation experiments with this new yeast strain (*Trichosporon* spp.) showed that zearalenone (ZON) could also be successfully degraded. The pathway of the detoxification process could not yet be determined, as metabolites neither showed fluorescence nor did absorb UV-light. Tests with animal cell cultures carried out at the University of Utrecht in the Netherlands showed that cells incubated with the yeast strain and zearalenone for a certain period of time no longer showed oestrogenic effects. Further *in vitro* and *in vivo* experiments will have to be undertaken to establish whether this yeast strain will be of practical use for deactivation of zearalenone in animal feed applications.

Conclusion

The isolation and characterisation of microorganisms that are able to biotransform mycotoxins could possibly be the breakthrough for the practical application of biotechnology in respective decontamination processes taking place directly in the intestinal tract of chickens. The biological methods described above may become the technology of choice, as enzymatic reactions offer a specific, irreversible, efficient and environmentally friendly way of detoxification that leaves neither toxic residues nor any undesirable by-products. □

References

Schatzmayr et al. (2002). Proceedings of the XVII Congreso Centroamericano y del Caribe de Avicultura, Havana, Cuba. 1-4 October.
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