The biotechnological detoxification of mycotoxins

by Árpád Bata, Dr Bata Ltd, 2364 Ocsa, Bajcsy-Zs. ut 139, Hungary.

Mycotoxin contamination of feeds poses a serious problem to farmers all over the world. According to data of the mycotoxin literature, 20-25% of the world's grain crop is contaminated by mycotoxins each year, which means millions of tons of mycotoxin contaminated feed is fed each year.

Mycotoxin prevention

The production of feeds free from mycotoxins or containing mycotoxins at levels under the tolerance limit requires the application of a mycotoxin prevention program that involves a successive series of actions consisting of multiple elements.

The program starts with the selection of suitable grain varieties and ends with the adsorption or biodegradation of mycotoxins in the gastrointestinal system of animals.

Components of the mycotoxin prevention program include:

- Selection of mycotoxin resistant grain varieties.
- Strict compliance with all elements of good agricultural practice, with particular attention to the chemical control of mould contamination during the grain production period.
- Good harvesting practice: preventing the mechanical damage of kernels, starting the harvest at an optimum time when the grain has relatively low moisture content.
- Post-harvest quality preservation actions such as removing the broken and damaged kernels; using clean transport and storage containers; ensuring proper grain storage conditions, for example preventing leakage or increases in grain moisture content due to other causes; preventing the multiplication of fungi during grain storage by fungicidal treatment.
- Using a mycotoxin elimination treatment during the feed manufacturing process, to minimise the effect of, or eliminate, the mycotoxins present in the raw materials. For this purpose, the biotechnological detoxification can be considered the most powerful option.

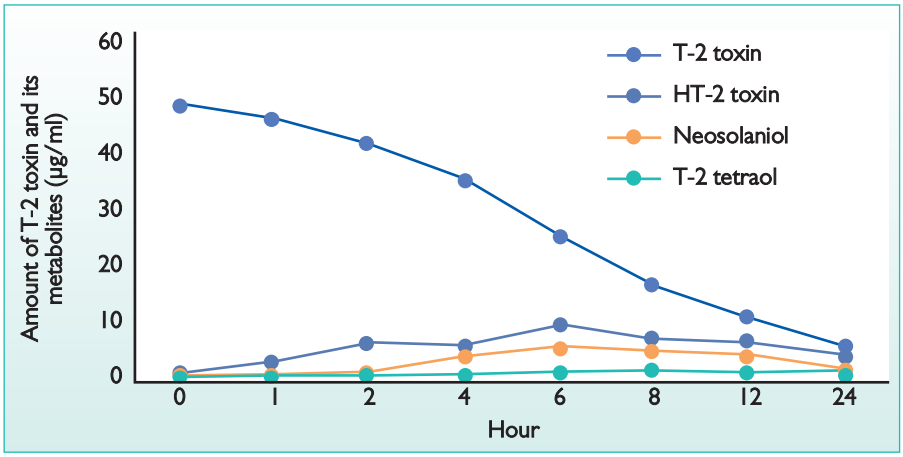

Fig. 1. Biotransformation of T-2 toxin by rumen fluid (Bata, 1989).

Detoxification

After the discovery of mycotoxins, researchers started feeding trials on laboratory animals to study their harmful effects.

These experiments brought conflicting results and were not reproducible, which was attributed to the variable mycotoxin dose administered to the animals via the artificially mycotoxin contaminated feed.

However, the results of repeated experiments ensuring the administration of identical mycotoxin doses were still inconsistent.

Some researchers attributed that to the presence of mycotoxin metabolites and premycotoxins in the test feedstuff; this hypothesis, however, did not prove valid.

Repetition of the trials with purified mycotoxins to determine the biological effects already gave reliable results but revealed that there were large differences in the individual mycotoxin sensitivity of animals in the groups treated.

This scientific discovery triggered studies on the fate of mycotoxins in the animal organism. The species- and individual-specific differences in the reactions of mammals to mycotoxins can be attributed to differences in the biodegradation and absorption of mycotoxins. The individual-specific degradation of mycotoxins can be explained by the endo- and exo-epoxidase enzyme activity of the liver.

The interaction of the mycotoxin aflatoxin B1 (AFB1), with hepatic endo- and exo-epoxidase enzymes results in a metabolic compound, which after conjugation with enzymes (glutathione-S-transferases and glucuronyl transferases), forms an adduct that is eliminated via the urine at a rate depending on the speed of the above reaction,

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Table 1. Treatments of the groups and the daily T-2 toxin intake.

	Dose of T-2 toxin (mg/kg)	Detoxa Plus (kg/t)	Average daily T-2 intake (mg/pig)	Average daily T-2 intake (mg/kg BW)
Group 1 (negative control)	0	0	0	0
Group 2	0.3	0	0.21	0.0115
Group 3	0.3	1	0.23	0.0128
Group 4	0.5	0	0.32	0.0186
Group 5	0.5	1	0.41	0.022
Group 6 (positive control)	0	1	0	0

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which thus determines the level of individual mycotoxin resistance.

Species-specific sensitivity is illustrated by the example of zearalenone (ZEA). ZEA is metabolised to α-zearalenol and β-zearalenol by the hepatic microsomes. α-zearalenol is four times more bioactive than β-zearalenol. The hepatic microsomes of pigs produce a higher amount of α-zearalenol than β-zearalenol, whereas those of chickens produce more β-zearalenol than α-zearalenol.

Both ZEA and its α- and β-metabolites are eliminated from the mammalian organism after conjugation with glutathione-S- and glucuronyl transferases. As α-zearalenol has much higher biological activity than β-zearalenol, animal species in which a higher amount of the α-metabolite is formed will be more sensitive to ZEA than species with a dominance of the β-metabolite.

In summary, we can state that it is possible to diminish the effect of mycotoxins in the animal organism. This can theoretically be achieved by decreasing the absorption of mycotoxins, by influencing their activation or deactivation within the body, and by accelerating their elimination. However, the current standard of science does not allow us to use any of the above approaches in practice. Thus, we can conclude that, in order to reduce the impact of mycotoxins, we should find a solution that renders them harmless before they are absorbed.

In vitro degradation

The microbiological decomposition of mycotoxins was first reported in the second half of the 1960s. Subsequently, an increasing number of micro-organisms were demonstrated to be capable of metabolising mycotoxins into non-toxic metabolites.

The first result of potential use in practice was the recognition that rumen bacteria can metabolise mycotoxins (Fig. 1).

The researchers found that molecules of trichothecene structure were metabolised into other, less toxin trichothecene molecules or into compounds of non-trichothecene structure. Some years later Fleisch and Voight-Scheuerman (1994) isolated two microbiological decomposition metabolites of T-2 toxin. These are derivatives of the original trichothecene molecule but do not contain the so-called 12,13-epoxy ring responsible for the toxicity of trichothecenes.

Later, cytotoxicologic studies conducted by Ericsen et al. (2004) demonstrated that derivatives lacking the epoxy ring are 50-60 times less toxic than trichothecene toxins containing it.

Kollarczik et al. (1994) reported that some bacteria forming part of the digestive tract microflora of pigs were capable of metabolising deoxynivalenol (DON) and ZEA in vitro. Micro-organisms from the caudal segments (caecum, colon and rectum) of the gut were capable of reducing the toxicity of trichothecene compounds but bacteria from the cranial segments (duodenum, jejunum) could not transform the mycotoxins tested. Bacteria from the caudal part of the intestine hydrolysed ZEA to α-zearalenol and an unknown metabolite.

In an in vitro test, Young et al. (2007) found that bacteria of the digestive tract reduce the toxicity of trichothecene mycotoxins primarily by deacylation of the trichothecene structure. Deacylated trichothecenes have 10-50 times lower toxic activity than the original molecules.

Recent studies have demonstrated that de-epoxidation is probably a more important mechanism of detoxification by bacteria originating from the chicken gut. It can be stated that some micro-organisms reduce the toxicity of mycotoxins decisively by the deacylation of the trichothecene structure, while others are capable of modifying the groups responsible for the toxic effect of certain mycotoxins.

Other authors achieved good results in the bioconversion of ochratoxin A into the practically non-toxic metabolite ochratoxin α by the use of Rhizopus isolates.

The most recent achievement of in vitro research comes from the study of Wang et al. (2011), who found that manganese peroxidase can reduce the mutagenic activity of aflatoxin B1 by more than 80% after 48-hour incubation. This is the first direct demonstration of the existence of a specific enzyme-mycotoxin interaction.

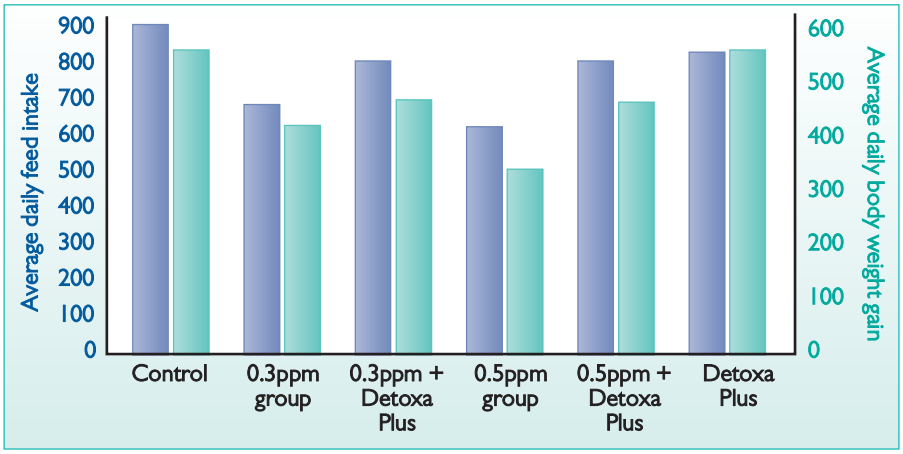

Fig. 2. Average daily feed intake and body weight gain during the experiment.

In vivo studies

Results of mycotoxin biodegradation observed in vitro could successfully be reproduced in vivo in several cases. Already in 1994, Kollarczik et al. (1994) reported the successful confirmation of their in vitro findings in an experiment conducted on pigs in vivo. Rafai et al. (2011) have recently reported the results of an extensive in vivo study. Sixty six-week-old pigs, were allocated into six groups of 10 animals each on the basis of body weight and conformation, and reared for three weeks on a commercially available starter feed. The experiment lasted for 28 days and consisted of two periods.

A one-week period of adaptation was followed by a three week experimental period during which the groups received the treatments shown in Table 1. Neither the control nor the experimental groups showed clinical signs attributable to T-2 toxin treatment. The average daily feed intake was significantly lower in the groups fed the toxin-containing diet than in the control groups or in the toxin-treated groups receiving the Detoxa Plus feed supplement (Fig. 2). The daily T-2 toxin intake followed the tendency of the feed intake (Table 1).

As a consequence, the groups receiving Detoxa Plus supplementation ingested higher amounts of T-2 toxin than the groups used for comparison.

The daily weight gain was typical of the breed and age of the experimental animals. The weight gain followed the trend expected on the basis of the feed intake data (Fig. 2). Fig. 2 shows that both the feed intake and the weight gain of groups receiving T-2 toxin added to the feed were significantly lower than those of the groups not fed T-2 toxin. Detoxa Plus supplementation significantly improved the feed intake, weight gain and feed conversion results of Groups 3 and 5.

Conclusions

The expanding knowledge of the biological metabolisation processes of mycotoxins enables the development of new control tools collectively designated as biotechnological methods. These new procedures are expected to provide multiple benefits over the traditional methods. The biotechnological techniques are regarded as natural methods, the efficacy of which is demonstrated by an increasing number of reports. Adverse effects need not be reckoned with. The price of such products is expected to decrease or remain unchanged. ■

References are available from the author on request