Where next with mycotoxin control?

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Mycotoxins are secondary metabolic products of fungi (moulds) and they are toxic to animals or humans. It is estimated that there are about 300 mycotoxins harmful to humans or animals. With improvements in analytical methods, the list is certainly going to be increased in the future.

Mycotoxins are responsible for diseases called mycotoxicoses. The toxicity of these compounds depends on the amounts ingested, time span of exposure, type of animal, their breed, age, sex and health status, but also other parameters such as density of animals, diseases and temperature

The fungi responsible for the production of mycotoxins grow on plants and commodities. Mycotoxins can be produced before harvest (by so-called field fungi) or during storage (by the storage fungi). They can also be produced on the finished feed when storage conditions are not correct. The fungi not only produce mycotoxins, they also damage the crop. Food and Agricultural Organisation (FAO) estimates that about 25% of the world's crops contain mycotoxins.

Prevention and control

If favourable growth conditions are met for the fungi, it is very difficult to avoid the production of mycotoxins. However, effective prevention strategies will certainly limit the incidence of mycotoxins. Prevention can be implemented before harvest with a good management of preceding crop residues, a correct crop rotation, the selection of seeds (quality of seeds, resistant varieties), an appropriate plant density, the correct use of fertilisers and of course the prevention of insects and fungi. Harvesting at the right time, in good conditions to avoid damaging grains and removing spoilt and moist grains, then storing good grain as soon as possible, will certainly help. Finally, during storage, the control of temperature, humidity, insects and rodents and the use of effective mould inhibitors will help to prevent mycotoxins.

It must be noted, however, that prevention does not remove existing mycotoxins! Many methods have been tested to remove mycotoxins from commodities. The problem is that they are costly, usually generate high losses and can reduce the palatability and the nutritional value of the raw materials.

Among the methods that have been experienced, the following can be mentioned: treatment with ammonia, together with heat and pressure (effective against aflatoxins and to a lesser extent fumonisins, but generates toxic compounds), treatment with ozone, chlorine gas, ammonium hydroxide, hydrogen peroxide, hydrochloric acid and sulphur dioxide gas (against DON), formaldehyde (against zearalenone), roasting, heating (useful against DON), colour sorting with UV (against aflatoxins). Dehulling, polishing and sieving have also been experimented with.

Today, however, most of these methods are not used because of their drawbacks.

Use of mycotoxin binders

About 20 ago, use of so-called 'mycotoxin binders' has given a new perspective to the control of mycotoxins. One of the first scientific studies on binding properties of clays is the one published by Phillips et al. (1988).

They tested 38 different adsorbents from the major chemical class of aluminas, silicas and aluminosilicates, and showed that a type of phyllosilicate clays, called hydrated sodium calcium aluminosilicates (HSCAS) have high affinity for aflatoxin B1.

Indeed, the good stability of the aflatoxin-HSCAS complexes over a wide pH range (2-10) and up to 37°C supports the in vivo efficacy of such binders. Further studies have demonstrated that HSCAS can be very helpful to prevent aflatoxicosis in different species including chickens and turkeys.

However the efficacy of HSCAS seems to be only partial against zearalenone and ochratoxin A, while they appear totally ineffective to tackle mycotoxins from the group of trichothecenes (for example, T-2 toxin, diacetoxyscirpenol or deoxynivalenol, also known as vomitoxin).

Zeolites, another type of hydrated aluminosilicates, have given inconsistent results against aflatoxins. While some in vitro studies have been promising, high levels of zeolite in feed have given disappointing results.

Ramos et al. (1996) also studied the possible benefits of sodium bentonite, a natural sealant used to treat porous soils. It is also used as a binding agent when producing pelleted feeds. Based on their findings, as well as data from other researchers, it appeared that bentonite is not effective against zearalenone, ochratoxin A or nivalenol while contradictory results have been obtained for aflatoxins.

Research has also been performed on the use of activated carbon, an insoluble powder formed by pyrolysis of different kinds of organic materials. Although activated carbon has proven to be effective at binding mycotoxins in vitro, for example, fumonisin B1 or ochratoxin A, it did not show clear positive effects when tested in vivo. Additionally, the concern is that activated carbon can indiscriminately bind other dietary components, such as vitamins, minerals and drugs.

Cholestyramine is a resin used to lower high cholesterol levels in the blood. It works by binding to bile acids in the intestine, which results in cholesterol being converted to bile acids in the liver. Ramos et al. (1996) observed that this resin is able to bind zearalenone while other researchers demonstrated a positive effect against fumonisins.

However, relatively large quantities are needed (for instance more than 10 kg/MT of feed in the case of zearalenone) which makes its use economically prohibitive.

Finally, polyvinylpyrrolidone (a vinyl polymer), used at 2kg per ton of a swine feed contaminated with deoxynivalenol, did not improve the situation.

Saccharomyces cerevisiae

Stanley et al. (1993) reported that Saccharomyces cerevisiae was helpful in the case of aflatoxin contamination, and their conclusion was that the cell wall was binding with the mycotoxins. Santin et al. (2003) studied the effects of yeast cell wall against ochratoxin in broilers. Their results indicate that ochratoxin impaired feed intake, weight gain and feed conversion of the birds. The yeast cell wall could not improve these parame-

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ters. Yiannikouris et al. (2004) studied the interaction of yeast cell wall with zearalenone in vitro. Their conclusion was that weak non-covalent bonds are involved in the complex forming mechanisms, and that the chemical interactions are therefore more of an adsorption type than a binding type.

Limitations of binders

Based on the different publications available, we can observe that the main limitations of the 'mycotoxin binders' are:

- **Their efficacy is limited to a few mycotoxins.**

Generally speaking, binders are effective against so-called polar mycotoxins, such as aflatoxins. This is due to the fact that these mycotoxins have a chemical structure which allows an efficient binding. In the case of other mycotoxins, such as trichothecenes, binding efficacy is generally very poor, if not zero.

- **Their efficacy in vitro does not guarantee their performance in vivo.**

Because in vitro tests are performed under specific and rather simple conditions, they are not representative of what happens in the digestive tract. When parameters such as pH variation or interaction with feed or enzymatic secretions are not taken into account, the risk is to draw false conclusions. Indeed, when weak non-covalent bonds are formed between the binder and the mycotoxin, a change in the conditions of the 'environment' can lead to a release of the mycotoxin.

- **Some of them are not specific to mycotoxins.**

In such a case, the binder will interact with other dietary components, such as vitamins, minerals and drugs. This will limit the efficacy against the mycotoxin(s) and also affect the performance of the animals.

Biotransformation

Therefore, binding of mycotoxins is a reversible process, the efficacy of which depends on the conditions of the media. Its practical application is also limited to a few mycotoxins. As a consequence, other strategies had to be found.

Recent research indicates that the biotransformation of mycotoxins, using live micro-organisms or enzymatic preparations, gives promising results.

Shima et al. (1997) have for example reported the case where a bacterium belonging to the Agrobacterium-Rhizobium group was able to transform deoxynivalenol into a less toxic compound called 3-keto-deoxynivalenol, and suggested that the biotransformation was caused by an extracellular enzyme excreted by the organism.

Similarly, Völkl et al. (2004) observed that a mixed culture of micro-organisms was able to transform deoxynivalenol into two chromatographically separable products, the main one being identified as 3-keto-deoxynivalenol.

Again, they stated that an extracellular enzyme was involved. Other trichothecenes such as 15-acetyl- deoxynivalenol, 3-acetyl-deoxynivalenol and fusarenon-X were also transformed.

Zearalenone can be converted into a far less oestrogenic product, called 1-(3,5-dihydroxyphenyl)-10'-hydroxy-1'-undecen-6'-one. The enzyme responsible for the detoxification appears to be a hydrolase that cleaves the lactone ring. Zearalenone affects the reproduction cycle of animals when it interferes with oestrogen receptors. Since the structure of the mycotoxin is modified by the enzymatic reaction, it loses its toxic effect.

The application of such enzymatic transformations to the feed sector gives new opportunities. Indeed, enzymes can have a specific action and their reaction, compared to binding, is not reversible. With this new approach, we can talk about 'mycotoxin eliminators' in contrast to 'mycotoxin binders'.

The combination of mycotoxin binders and enzymes is of course possible. In the development of a product to counteract the effects of mycotoxins in feed, Belgian company Impextraco screened many products, including binders and enzymes, in a system designed to simulate the digestive tract.

Gut simulation model

Simple measurements in the feed (in vitro) are not sufficient to reveal the real binding or inactivation of mycotoxins, since it is not clear whether reactions in the animal itself would influence the binding or the enzymatic reaction. For example, if the toxin is bound in the feed, but later released in the animal, the binder is not effective. Similarly, if the product only binds the toxin in the animal, but not in the feed itself, it is effective, but efficacy will be difficult to verify.

Analysing the toxin inactivating effect in live animals is very difficult. Most of the studies look at performance of the animals.

However, this parameter is influenced by many other factors difficult to control, so large and expensive tests are necessary. Some studies measure the serum levels. This is not possible for all mycotoxins and the serum levels are not always a good indicator of the amount of mycotoxin absorbed. Most toxins are rapidly metabolised or stored in the animal. Consequently, the serum levels drop very fast.

The time between absorption in the gut and sampling of the animal is very important, but differs due to individual variations like feed intake and retention time in the gut. Also other factors like genetics, bodyweight and water intake will differ between the individual animals and influence the serum levels.

A perfect in vitro model of the animal would eliminate individual variation and control all other factors. Of course, there is no such thing as a perfect model, but the digestive tract has been well studied and several factors are easy to simulate.

Dr H. Clarijs's research group at the HAS in the Netherlands has developed a small intestinal model that can be applied to mycotoxin tests.

The following factors are simulated: anaerobic environment, constant (body) temperature, several subsequent environments at different pH, retention times and the correct subsequent addition of bile, pepsin and gut enzymes and the correct moisture : feed (digestive bolus) ratio.

The gut simulator mimics the digestive tract and allows interactions between feed, mycotoxins and mycotoxin-deactivating substances to be studied in 'real' conditions.

This offers a clear advantage when compared to the classical in-vitro tests where only pH is controlled and other parameters are not taken into account.

Immense differences were found between the classical in vitro tests and the gut simulation model.

The best toxin binder for aflatoxin B1 that emerged from this screening (a combination of several special types of HSCAS) was then combined with several enzymes which were capable of detoxifying a variety of mycotoxins.

Another substance selected was chitosan, a biopolymer derived from the exoskeleton of insects and crustaceans. This biopolymer was selected for its mycotoxin binding properties, but also because it has been proven to have antibacterial effects.

Helander et al. (2001) stated that chitosan appears to bind to the outer membrane of Gram negative bacteria and disrupts the barrier properties of the said membrane.

A combination of HSCAS, chitosan and enzymes was then obtained (Elitox, Impextraco NV, Belgium).

Conclusion

Mycotoxins are harmful to animals and can greatly affect their performance and productivity. Because there is a wide range of different mycotoxins, with different chemical structures, a simple approach cannot efficiently solve the problem.

Prevention is important but cannot guarantee the absence of mycotoxins.

When commodities are contaminated, the use of several strategies is required. A correct combination of mycotoxin binders with toxin degrading enzymes and a biopolymer gives a new approach and can be defined as a 'mycotoxin eliminator'. ■

References are available from the author upon request