Mycotoxin binders – evaluation of efficiency by the in vitro method

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Mycotoxins can be considered as natural products synthesised as secondary metabolites by certain filamentous fungi which cause a toxic response – mycotoxicosis – when administered into the animal's system by a natural route in low concentrations.

Mycotoxins are probably the most unfamiliar and least studied of the disease causing substances that affect both humans and domestic animals.

Wide range of species

Although some of the mycotoxins are produced only by certain species of fungi, there are many others which are produced by a wide range of species from different genera.

In fact, many of the foodborne and feedborne fungi are capable of producing one or more mycotoxins.

Aflatoxins, ochratoxin and zearalenone are a few of the more common mycotoxins found in animal feed. The presence of mycotoxins in animal feeds poses adverse effects including a decrease in feed intake, retarded growth and adverse animal performance, making the control of mycotoxins critical to the feed manufacturers and livestock producers.

Field studies have also confirmed the synergistic effects of different mycotoxins in causing more severe toxicosis in animals.

The risk of mycotoxicosis can be reduced by adopting an integrated mycotoxin management system incorporating the use of mould inhibitors and toxin binders in feed production and animal nutrition.

An extensive range of toxin binders containing adsorbents, such as clays and other ingredients like yeast extracts and enzymes, have been used to prevent toxicosis in birds.

The two main mechanisms of action proposed for toxin binders include binding to mycotoxins and the chemical or enzymatic conversion of mycotoxins to harmless derivatives.

In vitro methods

There are numerous ways to demonstrate the in vivo activity of mycotoxin binders. However, animal trials are costly and time consuming. Although a variety of in vitro methods are being used to evaluate the efficacy of mycotoxin binders, inconsistent toxin binding performance was observed.

One of the most important reasons for this variation could be due to the set-up of the assays used to evaluate the toxin binders.

Since adsorption is the mechanism of action of majority of the toxin binders, it is very important to understand the influence of factors that influence the adsorption of toxins by the binders and to design in vitro assays that could simulate their in vivo performance.

The adsorption mechanism

The most important and effective toxin binders that demonstrate consistent efficacy are the clay minerals and hence the key mode of action for mycotoxin removal is by the physical process of adsorption.

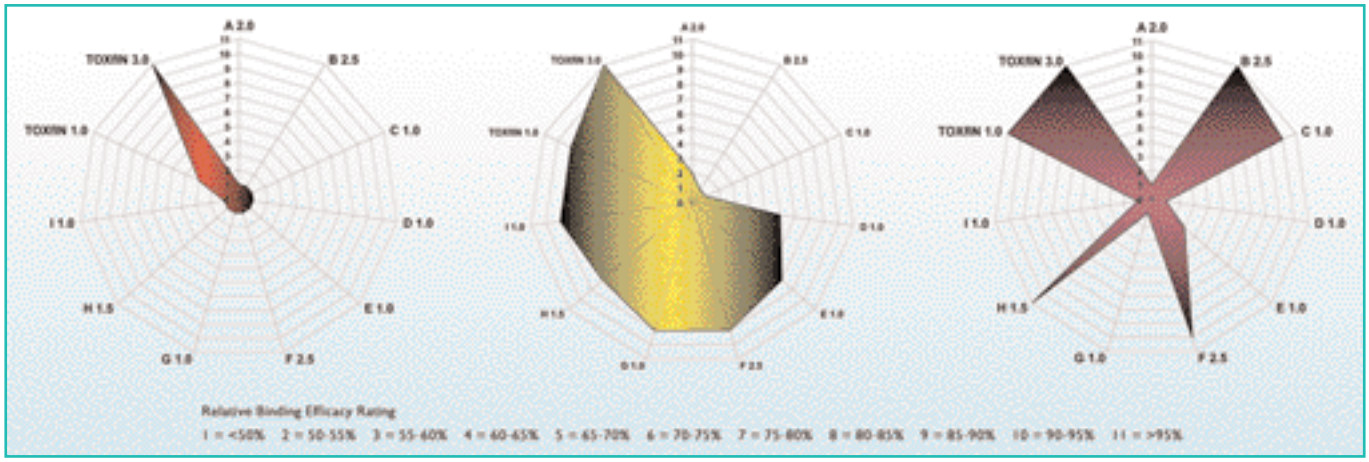
In clay minerals, the adsorption process occurs because of the charged nature of both the mycotoxins and the toxin binders.

Adsorption of mycotoxin by the toxin binder will take place when these charges neutralise each other.

However, this adsorption process is reversible and sensitive to pH changes. At low pH conditions similar to that existing in the foregut, an excess of positive charge is created by the presence of acidic protons (H^+) and at higher pH conditions, which

Continued on page 15

Fig. 2. Relative binding efficacy of various toxin binders for ochratoxin A (left), zearalenone (centre) and aflatoxin B1 (right). The length of the ray is proportional to the relative binding efficacy. Mycotoxin challenge levels used: aflatoxin B1 20ppb, ochratoxin A 100ppb, zearalenone 300ppb.



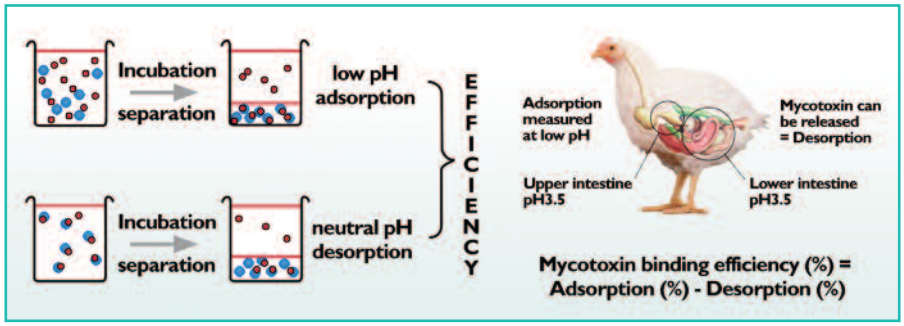


Fig. 2. In vitro biphasal method simulating the gastrointestinal conditions of animals.

occur at the lower part of the intestine, there are more negative charges (OH-).

These changes in the environment can influence both the mycotoxin and the surface of the toxin binder, modifying the interaction between the two.

This explains the inconsistency in the observed performance of the toxin binders using in vitro assays which are not designed to mimic the physiological pH changes occurring in vivo and the influence of these pH changes on the mycotoxin/binder interaction.

Two phase binding assay

The above mentioned factors necessitates the design of an in vitro method for evaluating the efficacy of mycotoxin binders based on the adsorption mechanism and the effect of pH on adsorbent/adsorbate interactions.

Hence, an in vitro two phase binding assay was developed by Kemin that mimics the change of the pH conditions along the gastrointestinal tract of animals.

First, the adsorption of mycotoxins by the toxin binder is measured at a low pH of 3 mimicking the foregut condition. This is followed by incubation in a more neutral medium (pH 6.8) mimicking the hindgut conditions (Fig. 2).

The overall binding efficacy is dependent on both the adsorption and desorption of the mycotoxins.

This in vitro two phase test method provides more accurate evaluation of mycotoxin binders since an important parameter which determines the efficiency is the mycotoxin retention ability of the toxin binders until excreted from the bird's body.

Kemin developed TOXfIN brandDry with broad spectrum mycotoxin adsorption efficiency based on the in vitro two phase binding assay using a range of mycotoxins.

The product consists of a synergistic blend of specific clay minerals which were modified and activated to assure superior mycotoxin binding activity and enhance broad spectrum mycotoxin adsorption.

Challenge study

The in vitro two phase test method was used to evaluate different toxin binders including TOXfIN brandDry for their mycotoxin binding efficacy. The toxin binders used for the study consisted of a wide range of active ingredients including magnesium sodium-calcium alumino-silicate base, yeast extracts, anti-fungal compounds, enzymes, phytobiotics and phycophytic substances.

Evaluation of the binding efficacy of the different toxin binders was performed by selecting three toxins based on their potency and occurrence in animal feeds.

These include aflatoxin B1, ochratoxin A and zearalenone.

The challenge level of the mycotoxins was selected based on the sensitivity and detection limit of the analysis.

The binding efficacy of the toxin binders evaluated using the in vitro two phase method demonstrated the broad spectrum toxin binding efficiency of TOXfIN brandDry (see Fig. 1 on the previous page).

Its unique formulation enhances the binding of the three molecularly different mycotoxins – aflatoxin B1, ochratoxin A and zearalenone.

Furthermore, TOXfIN brandDry especially at 3.0 kg/tonne, exhibited exceptional binding efficacy for ochratoxin A, for which the majority of the competitor's products failed to perform.

Outstanding efficiency

The outstanding binding efficiency of TOXfIN brandDry is attributed to its exceptionally high toxin adsorption at pH 3 and low desorption at pH 6.8, thus demonstrating its capacity to sequester mycotoxins from the gastrointestinal tract before absorption occurs in the small intestine.

Animal studies have indeed proven the broad-spectrum mycotoxin binding efficacy of TOXfIN brandDry which establishes the reliability of the in vitro two phase test method as a useful tool to evaluate the performance of toxin binders.

As illustrated by our results, the in vitro method based on the mechanism of toxin binding provides the livestock industry with a reliable method to critically evaluate the efficiency of the mycotoxin binders.

Livestock owners must be aware of the significance of using the in vitro test to evaluate toxin binders for their broad spectrum mycotoxin binding capacity since feeds are often infected with different varieties of fungi producing a wide range of mycotoxins. ■