Mycotoxin adsorption assays

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Research has shown that products and clay materials marketed as mycotoxin binders do not always show consistent in vitro activities when different sources of scientific and technical literature are compared.

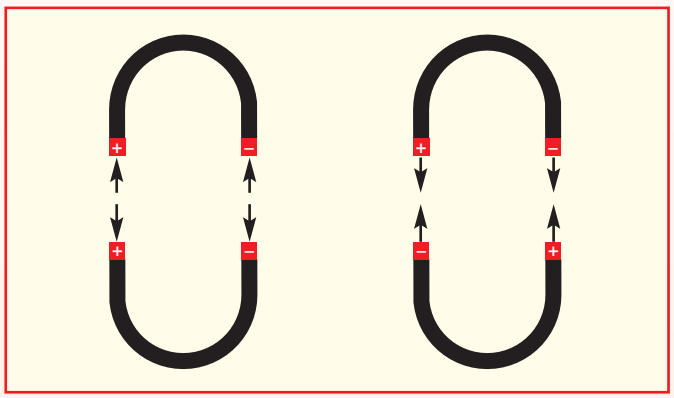
One of the most important reasons for this variation is the difference in the set-up of the assays that are being used to evaluate binders. Because most binders work on the principle of adsorption there are a few very important parameters that will influence the outcome of binding capacity measurements. It is important to understand the influence of these parameters and to design in vitro assays that are relevant for in vivo performance.

The adsorption mechanism

To date clay minerals appear to be the only group of binders that demonstrate consistent efficacy.

Although detoxification with micro-organisms has received a lot of attention, unfortunately this methodology seems only applicable to foods because of the difficulty to enable the micro-organisms to detoxify the gut contents in the beginning of the gastro-intestinal tract, before the absorption of mycotoxins occurs.

Fig. 1. Magnets with identical poles repel and unlike poles attract each other.



Also a lot of uncertainty still exists about the toxicity of the metabolites formed by the micro-organisms or enzymes. It is not yet known if these metabolic products are less harmful than the original mycotoxin.

Consequently, the main mode of action for mycotoxin removal remains the physical process of adsorption. Briefly summarised this process is caused by neutralisation of charges. Both the mycotoxin and the binder have a specific distribution of charges.

When these charges are able to neutralise each other, the toxin will be adsorbed on the surface of the binder. This process can be compared to the mechanism of a magnet where positive poles and negative poles attract each other.

Influence of pH

During the adsorption process the mycotoxin is not really bound to the surface of the clay.

The electrostatic forces that join the toxin with the binder are not permanent links, which means that the adsorption process is reversible.

A change of the surrounding environment of the mycotoxin binder (for example in the digestive tract) can have a dramatic effect on the binding efficacy.

The major parameter that has an influence is the pH of the environment. At a low pH there is an excess of positive charges due to the presence of acidic protons (H^+).

At a higher pH there are more negative charges (OH^-). These charges can change both the mycotoxin and the surface of the binder, causing a modification of the attraction between the two. Translating this principle to the 'magnet' model would mean that by changing the pH one of the magnets would, for example, become less strong. It will then be easier to separate the two magnets.

Exactly the same thing can occur in an animal, in the foregut the conditions of low pH may promote adsorption of mycotoxins, while further in the digestive tract the mycotoxin may be released again.

Efficacy over a broad pH range

The inconsistent efficacy data for toxin binders can be ascribed mostly to the different pH ranges that are used in the assays.

Theoretically there are three

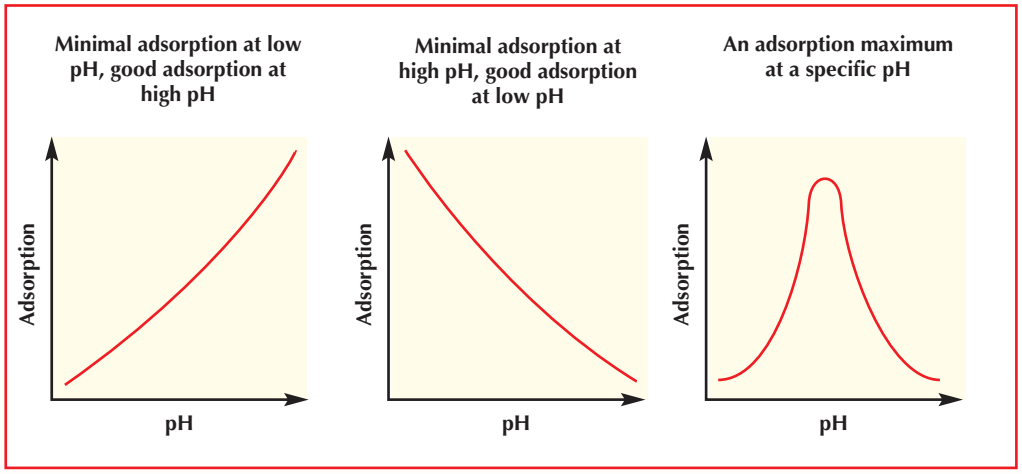


Fig. 2. Three possible scenarios to describe the influence of pH on toxin binding efficacy.

possible scenarios to describe the influence of pH on the efficacy of a toxin binder (Fig. 2).

It is clear from the graphs that there may be a considerable difference between results obtained at pH 3 compared to results at pH 8. It may be tempting to report the maximum adsorption that was observed.

Although this may be a valuable approach to compare different binders on a chemical basis, it is certainly not always relevant to the conditions found in the digestive tract of animals.

Mycotoxin concentration

Differences in mycotoxin concentration used in efficacy studies will not cause a major change in the observed activity of the binder.

In most tests systems, the toxins are used at a maximum concentration in the low ppm range.

Any application in practice will use mycotoxin binders in excess compared to the toxin concentration.

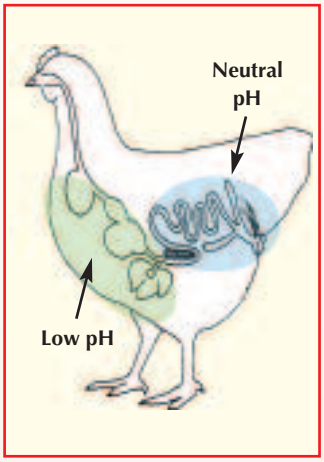
On the other hand sometimes relatively high concentrations of toxins are used for the evaluation of toxin binders.

The reason for this is often that the sensitivity of the equipment used is not good enough to allow detection of lower concentrations.

Kemin Agrifoods Europe uses sensitive methods that are able to detect the most harmful toxins at ppb (parts per billion) level.

The sensitivity of the measurements allows a better identification of the mycotoxin binding efficacy of newly developed products.

Fig. 3. Areas of different pH in the digestive tract in poultry.



Adapted model for evaluation

The foregoing observations indicate the need to redefine the method for evaluating the efficacy of mycotoxin binders.

Because of the vast effect of pH on the adsorption it is of primordial importance to use an in vitro system that mimics the change of the pH conditions along the gastrointestinal tract.

Kemin Europa developed Toxfin based on studies of the adsorption of mycotoxins in a two phase method. First the adsorption is measured at low pH (foregut conditions). Then the adsorbed mycotoxins are resuspended in a more neutral medium (hindgut conditions). Depending on the clay material the mycotoxin can be released again (desorption) at the neutral pH.

This new methodology allows a much more accurate evaluation of mycotoxin binders.

The method is also used in product development to improve mycotoxin binding formulations. It was possible to compensate for many observed desorption effects resulting in new formulations with improved broad spectrum activity.

When no desorption effects are observed, it may well be possible that the binder has the potency to bind even more toxins at higher pH conditions (see Fig.2, first graph). In that case more mycotoxins are expected to be bound in vivo along the path of the intestinal tract with increasing pH. The adsorption at the higher pH can be evaluated easily in a separate assay. ■

References

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