Selected activated clays for broad-spectrum & strong mycotoxin binding

Rick Carter, PhD, Kemin Animal Nutrition and Health, Asia Pacific

Food contamination scandals continue to reinforce consumer concerns over how food is produced which adds to the pressures applied to primary producers. This may have contributed to the remarkable increase in scientific publications on mycotoxins over the last 15 years.

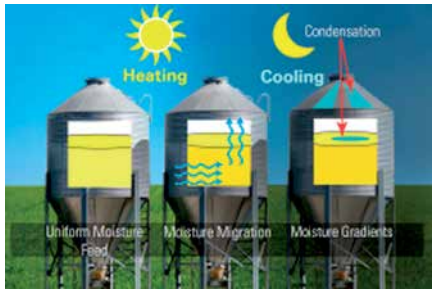
Mycotoxins are produced from mould and so the prevention of mould growth would ensure the prevention of mycotoxin formation.

Mould growth & mycotoxin formation

Mould growth on stored feed ingredients or feed can be prevented by ensuring moisture levels are below critical levels – the highest value measured being the important reading rather than the average moisture content as mould growth will start in spots of higher moisture. Aerated silos and the use of organic acid based mould inhibitors (Myco CURB®) also play a significant part in safe storage. This is also important due to moisture migration within silos due to day-night temperature differences (fig. 1) that will lead to moisture condensation causing clumping and flow problems, and of course, mould growth which reduces palatability, creates potential mycotoxin problems for animals along with the undesirable possibility of people inhaling mould spores when handling the affected material. These problems are made worse if insects are present because they produce heat and moisture and damage grain, all adding to the risk of mould growth which also produces heat and moisture. The increasing tendency for grain to be stored on-farm will increase the potential for mould and mycotoxin issues.

Of course mould can grow on a standing crop prior to harvest with the possibility of mycotoxins being present on grain at harvest. Mould inhibitors can be used to prevent further mould growth during storage but strategies will be needed to counter any presence of mycotoxins, particular for young pigs and breeding stock. Whilst laboratory testing for mycotoxins can be done, sample-to-sample variation can be very high due to the uneven growth of mould and therefore mycotoxin production.

Figure 1: Condensation in silos arising from moisture migration due to day-night temperature differences



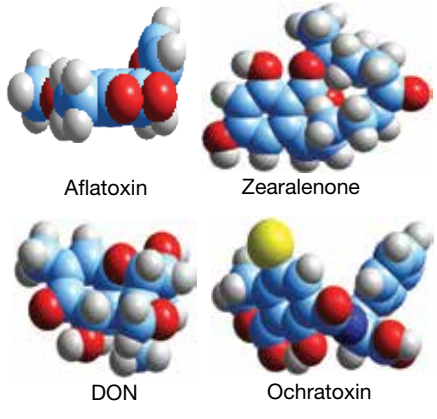
Effects of mycotoxins on pigs

Whilst there are 'text-book lists' of signs and symptoms of mycotoxins, the reality can be complicated not only by the level of one particular ingested mycotoxin, but also by the ingestion of multiple toxins. However, examples of specific mycotoxin symptoms are shown in table 1. The negative impacts of health challenges not caused by mycotoxins may become worse when coinciding with mycotoxin ingestion from feed. This has been shown in chickens with E.coli or Salmonella infections. Mycotoxin ingestion by food producing animals may also pose a food safety risk, eg. aflatoxin M1 in milk and ochratoxin in meat and other organs.

Activated clays for strong and broad spectrum binding

Binding of mycotoxins to certain clays is made possible due to areas of negative charge on the toxin compound which binds to areas of positive charge on the clay and vice versa. The distribution of positive and negative charges over each type of mycotoxin is different as shown in figure 2.

Figure 2: Mycotoxin structures and areas of positive and negative charge



Red = oxygen (negative) White = Hydrogen (positive)

Table 1. Some signs and symptoms of toxic effects of mycotoxins in pigs

Zearalenone	Aflatoxin	DON (vomitoxin)	Ochratoxin
• Vulval swelling • Uterine prolapse • Reproductive problems • Early embryoni deaths	• Rough hair coat • Pale skin • Reduced appetite • Reduced growth rate • Lethargy	• Diarrhoea • Vomiting • Feed refusal • Weight loss • Reduced immunity • Reduced milk production	• Increased water intake • Increased urine output • Reduced growth rate • Kidney lesions

Fortunately, mycotoxins can be adsorbed on to specific materials included in the feed. This binding of mycotoxins occurs in the digesta in the gut of the animal and thus prevents the toxin from being absorbed across the gut wall and into animal tissues.

Aflatoxin is easily bound due to its relatively flat structure enabling it to fit into the layered structure of clay as well as the area of negative charge (red oxygen atoms) being mostly at one end of the compound allowing for ease of opposite charge attraction. It's important to note

however, that all mycotoxins have areas of positive and negative charge and therefore have the potential to be bound by clays, even though the separation of these charges may not be as clear as with aflatoxin (which may be described as more 'polar' compared with zearalenone for example).

The lack of clear charge separation and the more complex physical structure of mycotoxins other than aflatoxin make them more difficult to bind to clays.

However, specific physical and chemical processing of natural clays solves this problem as was found when Kemin® screened many types of clays from different origins and different physical and chemical processing. This physical and chemical processing is called 'activation'. This activation process is also applied to charcoal which enables it to successfully bind the toxin from the Oleander plant – this toxin having a very complicated structure.

Activated clays have a range of applications including bleaching colour and odours from oils prior to use in soap manufacture. Activated clays are far more efficient at removing impurities that the non-activated clay. The conditions of the activation process can be specifically altered to change the clay's physical and chemical properties which then change its capacity to bind impurities and contaminants. The features of the activation process which benefit the binding process of mycotoxins are shown in table 2.

Successful binding of zearalenone to a specific activated clay has been reported in the literature - zearalenone being one of the most difficult mycotoxins to successfully bind.

Systematic screening of a large number of different activated clays for their

Table 2: Features of 'activation' processes applied to clay & benefits of these processes to mycotoxin binding

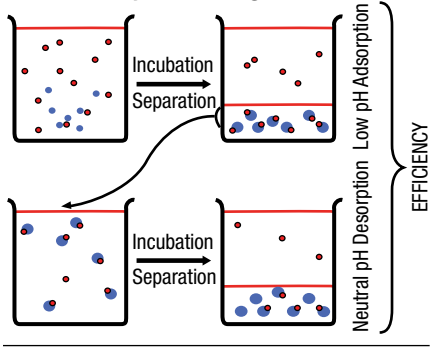
Feature	Benefit
• Grinding which increases the surface area of the clay	• Increases area over the clay where mycotoxins can be bound
• High temperature heating which stabilises the physical structure which is otherwise quite 'elastic' in the original clay	• Ensures the altered physical structure won't change
• Increasing the depth of the layers (ie. pore size) in the clay by exchanging the naturally occurring ions within the clay layers with other ions introduced by specific chemical treatment - which can be altered depending on the final application for the activated clay	• Enables larger, more complex mycotoxin structures to fit into and onto the clay
• Replacing the exchangeable ions in the clay with different ons also modifies the clay surface, i.e. can increase or decrease the areas of positive and negative charge	• Enables binding of mycotoxins with less clear areas of positive and negative charges, ie. less 'polar' structures

capacity to bind different mycotoxins enabled Kemin to isolate specific activated clays that not only bound the toxins tightly, but also bound a wide range of mycotoxins.

Laboratory screening & comparative testing

An important part of this screening process was the method used. Whilst mycotoxins can be bound in the acidic conditions of the stomach, they can become un-bound in the intestines. The screening method was conducted at body temperature with toxin adsorption measured firstly at an acidic pH with the bound toxins then re-suspended in solution at a neutral pH to simulate conditions of the intestine in order to measure any desorption of toxins (fig. 3). The desorption result is subtracted from the adsorption result to give a net toxin binding efficiency. This method ensures a more physiologically meaningful result than simply measuring adsorption at a single pH.

Figure 3. Diagrammatic representation of laboratory screening method.



The activated clays that resulted in the broadest range of bound mycotoxins and the highest net binding efficiency were selected for inclusion in TOXfINTM Dry. This product formulation was then tested against other commercial products using the same laboratory method as shown in figure 3. All products were used at same dose rate with the highest binding result for each toxin given a score of 100 with the remaining products scored relative to this result. The results are shown in table 3.

This comparison showed that TOXfIN's relative binding values were greater than 50 for all toxins and had the highest overall average mycotoxin binding for the 7 toxins tested at the levels shown in table 3.

This result is important as it clearly shows that a product based solely on clays can effectively bind a wide range of mycotoxins. The key to this success is the use of activated clays that were selected from a large number of different clays subjected to different activation processes after a careful and systematic screening process which quantified their net binding efficiency using the bi-phasal pH method against a range of myocotoxins.

Table 3: Relative binding of mycotoxins for TOXfIN Dry compared with other products using the bi-phasal pH laboratory method

Mycotoxin	Relative mycotoxin binding value						
	TOXfIN	C1	C2	C3	C4	C5	C6
Fumonsin B1: 300ppb	100	41	8	46	80	7	0
T 2: 500ppb	100	72	76	32	16	64	40
Ochratoxin A: 200ppb	81	100	80	73	55	36	11
Deoxynivalenol (DON): 2,500ppb	91	59	100	23	0	59	55
Zearalenone: 300ppb	58	93	75	100	46	37	3
Aflatoxin G1: 20ppb	99	100	98	60	95	85	80
Aflatoxin B1: 20ppb	100	99	98	65	98	95	97
Average	90	81	76	57	56	55	41

Binding in the gut

Successful binding of a range of mycotoxins has also been demonstrated for TOXfIN in an experiment using broiler chickens by measuring mycotoxins in excreta compared with the quantity of toxin added to the bird's feed (table 4, Tamil Nadu University, India, 2005). The quantity of toxin added to the feed was 250ppb of each mycotoxin.

Table 4: Mycotoxin excretion in broiler chickens: excreta levels as % of feed addition levels

	Aflatoxin	Ochratoxin	T2	Citrinin
Control diet	36%	24%	32%	28%
Control diet + TOXfIN	80%	72%	76%	80%
Difference	2.2x	3.0x	2.4x	2.9x

If a mycotoxin binder is effective at binding toxins in the gastro-itestinal tract, then the levels in the excreta should be much higher than when no binder is used. This experiment showed excreta toxin levels were from 2.2-3.0 times greater with TOXfIN in the diet.

Results from pig production units in the field have also shown positive outcomes.

On-farm pig results

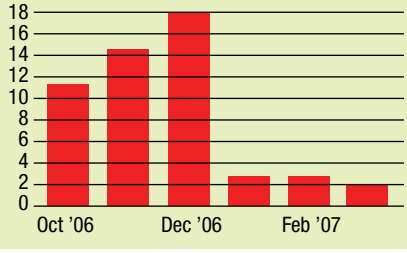
• A clear, consistent improvement in return to service % in breeding sows was recorded on a unit in New Zealand after TOXfIN was included in sow feed at 1kg/tonne from the end of December (figure 4)

• Another N.Z. producer changed from an alternative product to TOXfIN and observed an improvement in skin and coat condition in sows which can be adversely affected by mycotoxins.

• Ergot alkaloids were suspected of causing 25% pre-weaning mortality and 70% of new-born piglets exhibited tail necrosis on a unit in Holland. The health of new-born piglets improved several days after an 'emergency' inclusion in feed of 5kg/tonne of TOXfIN. No problems were seen after 1 month of TOXfIN use.

• Mycotoxins were found in feed on a unit in Bulgaria with symptoms including cannibalistic behaviour, skin lesions and prolapsed vulvae and rectums. These problems started to disappear several days after the inclusion of 2kg of TOXfIN per tonne of feed. ■

Figure 4: Return to service % in breeding sows before and after TOXfIN Dry inclusion in diets.



Key conclusions:

1. Activated clays can bind a range of mycotoxins including toxins with more complex structures and with less clear charge polarity.

2. The key differences in clays are clay type and mine location, plus the activation methods applied to the clay.

3. Both physical and chemical activation processes effect the toxin binding capacity.

4. Careful and systematic screening enabled the selection of activated clays to achieve both broad spectrum and strong mycotoxin binding.

5. The laboratory method used accounted for adsorption at stomach acidity levels and any desorption at pH neutral intestinal conditions.

6. TOXfIN Dry is based on selected activated clays.

7. Excreted mycotoxin levels are increased when TOXfIN is in the diet.

8. Successful results have been achieved with TOXfIN on pig production units around the world.

9. The outcome is a cost-effective contribution to feed hygiene and food safety.