In-feed aflatoxin control

Evaluating recent *in-vitro* and *in-vivo* research

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Mycotoxins are co-products of mould growth that contaminate an estimated 25% of the world's food crops, including the major feedstuffs crops. Aflatoxin B_1—one of the most toxic in food and feed—occurs frequently in yellow maize or corn, which is a principal ingredient in manufactured poultry and pig diets in many countries and also a component of many cattle diets. Certain mined clay minerals are effective as feed additives to 'bind' or sequester aflatoxin B_1 in feeds following intake by the animal, thereby reducing or controlling aflatoxicosis.

However, aflatoxin binding efficacy is a complex process, involving a combination of porosity characteristics, surface acidity and distribution of exchangable cations. Recent research shows that *in-vitro* aflatoxin binding experiments alone only serve to screen potentially useful materials. *In-vitro* tests should be augmented by *in-vivo* experiments designed to demonstrate both safety and efficacy.

In 1988, T.D. Phillips and colleagues at the University of Texas in the USA published what many consider the seminal paper on using a high affinity mineral adsorbent for *in-vitro* and *in-vivo* aflatoxin binding. Since that time, there have been reports on the use of this and related mineral adsorbents to bind aflatoxin and ameliorate its *in-vivo* effects in a number of animal species. In 1988, Phillips and colleagues, in 1990 L.F. Kubena and colleagues, in 1992 M. Araba and associates and again in 1992 R.D. Wyatt and colleagues all studied aflatoxins in poultry diets. In 1989, R.B. Harvey and colleagues and in 1990 M.D. Lindemann and associates studied aflatoxicosis in swine. In 1990, Harvey and E. Davee studied aflatoxins in dairy cows. Then in 1991 Harvey and colleagues studied aflatoxins in sheep. These early studies used the various inorganic silicate minerals against an aflatoxin challenge, but by 1990 researchers had begun to test other mycotoxins.

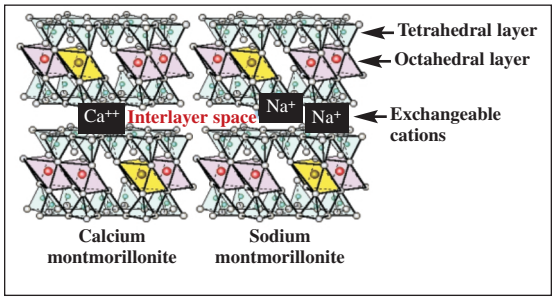

Figure 1. Molecular structure of calcium and sodium montmorillonite.

Note: Montmorillonite and similar bentonite compounds are naturally occurring clay minerals of the type called hydrated sodium calcium aluminosilicates (HSCAS).

Characterising binders

The mineral adsorbent first reported by Phillips and colleagues was described as hydrated sodium calcium aluminosilicate (HSCAS). Subsequently others have used this nomenclature as well. Because HSCAS is a generic description, it does not uniquely define the material of use.

In 1999, I characterised the mineralogy, chemistry and *in-vitro* aflatoxin-binding characteristics of 21 commercially available mycotoxin binding products from around the world. The majority—about two-thirds—are classified as montmorillonite. These products are also sometimes called bentonite, after the name of the ore that bears this mineral as its major constituent. Montmorillonite is a clay mineral that has an extensive list of other commercially important applications.

My studies found that no single physical or chemical property correlated well with *in-vitro* aflatoxin binding. Most likely, a combination of porosity characteristics, surface acidity and distribution of exchangeable cations was involved in a complex way to bind aflatoxin (Figures 1 and 2).

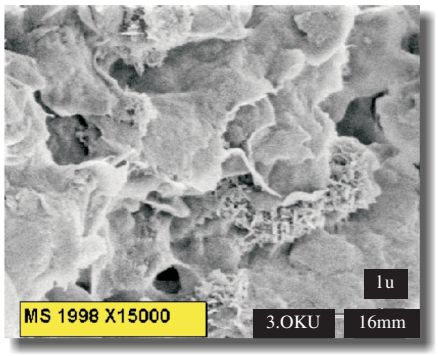

Figure 2. Scanning electron micrograph of commercially available HSCAS aflatoxin binder.

Note: Stacked, interconnecting pores in the range of 0.01-1.0 µm provide the greatest *in-vitro* aflatoxin binding capacity.

Other clay minerals—besides montmorillonite and zeolites—also are sold as commercial mycotoxin binding products. However, research indicates that they are rarely among the best binders. Even some montmorillonite binders are not always the best binders. Two of the 14 montmorillonite binders identified in my 1999 study were only about half as effective as 'top-tier' montmorillonite binders.

An *in-vivo* binding experiment

A study—conducted at the Federal

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University of Santa Maria (Brazil) under the direction of Drs. C.A. Mallmann and J.M. Santurio, which employed 540 one-day old Cobb chicks—was completed last year. This trial can be used to illustrate a fairly standard, but adequate experimental design.

Researchers speculate that aflatoxin binding results from a combination of HSCAS porosity characteristics, surface acidity and distribution of exchangeable cations.

The experimental design included aflatoxins at either 0 parts per million (ppm) or 3 ppm and a commercially available HSCAS binder included at 0%, 0.25% or 0.5% of the diet. There were 15 birds in each test group, which consisted of six treatments with six repetitions—three male and three female. The test groups were separated in 30 experimental pens on a bed of wood chips (Table 1). The researchers contaminated the diets with a mixture of aflatoxins from rice fermented by *Aspergillus parasiticus* (stock NRRL 2999). The aflatoxin mixture contained 83.6% B_1, 8.3% B_2, 5.0% G_1, 3.1% G_2 based on HPLC analysis.

The study showed that the negative control group weighed more and experienced significantly better feed conversion than the positive control group (Table 2). Results also showed that the binder itself is safe and does not bind nutrients or vitamins because bird performance was not impaired. Birds that ate no toxin, but had 0.25% and 0.5% binder in the diet—treatments 3 and 5, respectively—were not statistically different from the negative control.

The trial results demonstrated *in-vivo* product efficacy against aflatoxin contamination. Feed conversion at 0.5% binder inclusion with 3 ppm toxins—treatment 6—is statistically better than the positive control. Also at the 0.5% inclusion level with 3 ppm toxins, weight and feed conversion are not statistically different from the negative control, though they are numerically lower. The birds that ate aflatoxin contaminated diets with either 0.25% or 0.5% binder—treatments 4 and 6, respectively—improved their weight performance, though the improvement was not statistically significant relative to positive control.

Sensitive males

The results of this study show that the male birds are more sensitive to the effects of the aflatoxins than the female birds (Tables 3 and 4). Note that the weight difference is greater for the male positive control (treatment 2) than the female positive control (-19.2% versus -17.0%, respectively). However, because the male birds are more sensitive to the aflatoxin mixture than the female birds, they benefit more from the protection offered by the binder. In particular, at the 0.5% level, the improvement in both weight and feed conversion over the positive control is statistically significant and feed conversion is indistinguishable from the negative control.

Including 3 ppm aflatoxins in the diet is far above levels encountered under normal industry growing conditions. But, the high toxin level used in this trial is appropriate because the number of birds is small. The high aflatoxin inclusion level is also appropriate because normal stress conditions like high bird density and lack of ventilation can increase the negative effects of toxins. These stresses are not found in the controlled environment of the experimental pens. The researchers also boosted the aflatoxin levels because chickens are relatively more resistant to the effects of aflatoxicosis than many other species.

The trial showed significant mortality losses for treatment 2, the positive control group (Table 4). Although mor-

Table 1.
***In-vivo* HSCAS aflatoxin binder test treatments with chickens.**

Treatment	Binder (%)	Aflatoxins (ppm)
1 (negative control)	0	0
2 (positive control)	0	3
3	0.25	0
4	0.25	3
5	0.5	0
6	0.5	3

Note: HSCAS aflatoxin binder—ConditionAde™ 200 HPC from Oil-Dri Corporation USA.

Table 2.
***In-vivo* performance of HSCAS aflatoxin binder in lot of mixed gender chickens.**

Treatment	1-42 days Consumption (kg)	Weight (kg)	Weight difference	Feed conversion
1	4.507 ± 0.263^{a}	2.463 ± 0.218^{a}	—	1.829 ± 0.071^{ab}
2	3.904 ± 0.364^{b}	2.014 ± 0.166^{b}	-18.2%	1.939 ± 0.096^{b}
3	4.498 ± 0.243^{a}	2.453 ± 0.204^{ab}	-0.40%	1.833 ± 0.061^{ab}
4	3.868 ± 0.329^{b}	2.072 ± 0.153^{b}	-15.9%	1.867 ± 0.080^{ab}
5	4.496 ± 0.306^{a}	2.449 ± 0.214^{ab}	-0.6%	1.836 ± 0.040^{ab}
6	3.812 ± 0.220^{b}	2.120 ± 0.192^{ab}	-13.9%	1.798 ± 0.080^{a}
CV (%)	10.11	11.79	—	4.35

Note: Averages in columns followed by different letters—statistically significant to the level of 5% using the Tukey test. HSCAS aflatoxin binder in treatments 3-6—ConditionAde™ 200 HPC from Oil-Dri Corporation USA.

Table 3.
***In-vivo* performance of HSCAS aflatoxin binder in male chickens.**

Treatment	1-42 days Consumption (kg)	Weight (kg)	Weight difference	Feed conversion
1	4.732 ± 0.134^{a}	2.661 ± 0.028^{a}	—	1.778 ± 0.043^{a}
2	4.227 ± 0.124^{b}	2.149 ± 0.044^{c}	-19.2%	1.968 ± 0.088^{b}
3	4.717 ± 0.051^{a}	2.638 ± 0.031^{a}	-0.9%	1.789 ± 0.039^{ab}
4	4.136 ± 0.154^{b}	2.202 ± 0.067^{bc}	-17.2%	1.880 ± 0.118^{ab}
5	4.772 ± 0.043^{a}	2.639 ± 0.057^{a}	-0.8%	1.809 ± 0.029^{ab}
6	3.998 ± 0.073^{b}	2.295 ± 0.012^{b}	-13.7%	1.742 ± 0.036^{a}
CV (%)	7.64	9.43	—	5.26

Note: Averages in columns followed by different letters—statistically significant to the level of 5% using the Tukey test. HSCAS aflatoxin binder in treatments 3-6—ConditionAde™ 200 HPC from Oil-Dri Corporation USA.

Table 4.
***In-vivo* performance of HSCAS aflatoxin binder in female chickens.**

Treatment	1-42 days Consumption (kg)	Weight (kg)	Weight difference	Feed conversion
1	4.282 ± 0.059[a]	2.265 ± 0.016[a]	—	1.891 ± 0.037
2	3.580 ± 0.049[b]	1.879 ± 0.109[b]	-17.0%	1.910 ± 0.113
3	4.279 ± 0.026[a]	2.268 ± 0.012[a]	0.1%	1.887 ± 0.021
4	3.599 ± 0.177[b]	1.941 ± 0.056[b]	-14.3%	1.853 ± 0.038
5	4.219 ± 0.042[a]	2.259 ± 0.052[a]	-0.3%	1.868 ± 0.025
6	3.626 ± 0.108[b]	1.945 ± 0.011[b]	-14.1%	1.864 ± 0.061
CV (%)	8.86	8.75	—	2.81

Note: Averages in columns followed by different letters—statistically significant to the level of 5% using the Tukey test. HSCAS aflatoxin binder in treatments 3-6—ConditionAde™ 200 HPC from Oil-Dri Corporation USA.

tality numbers from trials with small numbers of birds under controlled conditions are often substantially different from normal industry conditions, they can give an added measure of assurance that the binder is providing protection.

Mortality clearly improves as the level of binder in the diet is increased. At the 0.5% level, the binder provides a statistically significant improvement in mortality versus the positive control and is statistically indistinguishable from the negative control.

Table 5.
HSCAS aflatoxin binder versus mortality in lot of mixed gender chickens.

Treatment	Mortality over 1-42 days
1 (negative control)	0.00 + 0.00[a]
2 (positive control)	15.56 ± 10.04[b]
3 (0 ppm aflatoxins, 0.25% binder)	0.00 ± 0.00[a]
4 (3 ppm aflatoxins, 0.25% binder)	6.67 ± 7.30[ab]
5 (0 ppm aflatoxins, 0.5% binder)	0.00 ± 0.00[a]
6 (3 ppm aflatoxins, 0.5% binder)	3.33 ± 5.58[a]
CV (%)	180.04

Note: Averages in columns followed by different letters—statistically significant to the level of 5% using the Tukey test. HSCAS aflatoxin binder in treatments 3-6—ConditionAde™ 200 HPC from Oil-Dri Corporation USA.

In-vivo versus *in-vitro* research

Choosing a feed additive aflatoxin binder can be difficult: Binders vary widely in their ability to bind aflatoxin and aflatoxin binding cannot be predicted on the basis of fundamental physicochemical properties or mineral type. How then can one decide whether a particular product might provide some protection against the deleterious effects of aflatoxicosis caused by feeding a contaminated diet?

In the absence of supporting *in-vivo* data, many mycotoxin binder manufacturers supply only *in-vitro* data. They propose that demonstrated significant *in-vitro* binding capacity correlates directly with significant *in-vivo* efficacy. Unfortunately, *in-vitro* binding data can be manipulated—or inadequately reported—in ways that make it difficult, if not impossible, to adequately judge the merits of the research. Binding conditions can be chosen so as to yield substantial binding for practically any mycotoxin simply by increasing the amount of binder relative to toxin.

In-vitro binding experiments are conducted with low levels of aflatoxin dissolved in water. By contrast, *in-vivo* studies typically use 0.5% to 1% binder to ameliorate the effects of aflatoxicosis in diets contaminated with a few tenths to 3-4 ppm aflatoxin. Because conditions in the lab are so different than in the animal, it is difficult to establish any direct correspondence between *in-vivo* and *in-vitro* experiments.

Testing assumptions

In fact, the assumption of a correspondence between *in-vitro* and *in-vivo* binding should be thoroughly tested. For example, although both charcoal and HSCAS bind aflatoxin *in-vitro*, it has been shown (Phillips, 1990) that only the latter provides protection against aflatoxicosis *in-vivo*.

It seems reasonable to suggest that companies selling aflatoxin-binding feed additives, regardless of type, should conduct and be able to produce results from *in-vivo* trials proving both efficacy and safety. In 1998, poultry nutrition Professor Nick Dale (University of Georgia, USA) called for more rigorous evaluation of these substances prior to making binding efficacy claims and I support his position. While the experimental design will vary from one study to another, a key element should be the incorporation of both positive and negative controls.

In evaluating commercially available aflatoxin binders, animal nutritionists and feed formulators should keep in mind that these products are made from naturally variable montmorillonite or bentonite clays and that their *in-vitro* binding efficacy can vary considerably—even by a factor of two. *In-vitro* aflatoxin binding experiments, while they have value as a screening tool, should be augmented by *in-vivo* experiments designed to demonstrate both safety and efficacy for animal production. **fi**

Complete references for this article are available directly from the author or from FEED INTERNATIONAL by e-mail (e-mail to gill@wattmm.com with subject line 'F092 aflatoxin references').