Layer Charge of Clays and Aflatoxin Adsorption.

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ABSTRACT

Aflatoxins are toxic fungal metabolites produced by *Aspergillus flavus* and other fungi. Human and animal cancers and death can result from aflatoxin ingestion. Grain crops, such as corn and peanuts, are frequently contaminated with aflatoxins due to fungal infestations before or after harvest. Clay minerals when added to animal feeds have been shown to effectively bind aflatoxins and reduce aflatoxicosis. Aflatoxin binding to clays must occur during digestion because dry clays are mixed with dry feeds. The Langmuir-like form of aflatoxin adsorption isotherms suggest that aflatoxins adsorb to the clay surface. Clay products that effectively bind aflatoxins and reduce aflatoxicosis generally have low to intermediate layer charge. This suggests that layer charge affects aflatoxin adsorption by clays. N-alkylammonium exchange indicated that that the commercial feed additive, Novasil plus, is a low-charge montmorillonite comparable in charge to the CMS reference montmorillonite, SWy-2. Animal feeding studies have shown that Novasil plus prevents or reduces aflatoxicosis. Adsorption isotherms of aflatoxin B1 (AfB1) indicated that SWy-2 adsorbed AfB1 as effectively as Novasil plus, but high-charge CMS reference montmorillonite, SAz-1, was much less effective. AfB1 adsorption by natural expandable clays was as follows: hectorite > SWy-2 = Novasil plus > SAz-1 >> vermiculite. Reduced-charge SAz-1 montmorillonite samples were synthesized by Li exchange and heat treatment. The reduced-charge SAz clays adsorbed AfB1 more effectively than SAz-1 in proportion to the charge reduction. Low-charge expandable clays more effectively adsorb AfB1 and would likely be more effective feed additives to reduce aflatoxicosis.

INTRODUCTION

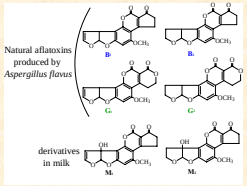
Aflatoxins are toxic fungal metabolites produced by *Aspergillus flavus* and other molds. The name aflatoxin was derived from the abbreviation A.flavus. The different toxins were named based on the fluorescent color and relative solubility. The B toxins have a blue fluorescence and the G toxins have a green fluorescence. The M toxins are derivatives found in milk.

The addition of clays to animal feeds has been shown to decrease aflatoxin toxicity. The FDA has approved up to 2% clays in feed as "anti-caking agents", but the claims of aflatoxin binding to clays have not been accepted. Aflatoxin toxicity is a world wide problem. Contamination can occur during crop growth and after harvest while in storage. Aflatoxin-contaminated grain is regulated in the U.S., but worldwide 4.5 billion people may suffer chronic sub-lethal exposure, especially in tropical countries (USAID study).

Some clay feed additives reduce aflatoxicosis, but others do not. Previous research has shown that commercial feed additives that effectively reduce aflatoxicosis are typically low- to intermediate-charge montmorillonites. Activated carbon removes aflatoxins from water, but it does not effectively reduce aflatoxicosis. Animal feeding studies are the ultimate proof of effectiveness, but are too expensive to screen potential clay additives. Layer charge might be a useful method to identify clays that can effectively reduce aflatoxicosis.

The objectives of this study were to measure the adsorption of aflatoxin B1 by clays from water and from aqueous corn meal and relate adsorption to layer charge. A variety of clays with a range in layer charge will be used. Mineral formula total charge, cation-exchange capacity, and N-alkylammonium expansion will be used as measures of layer charge.

Figure 1. The common aflatoxins.



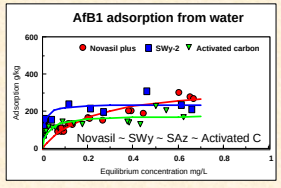
U.S. Regulations	Aflatoxin concentration	Fed animal
none, free circulation	≤20 µg/kg	humans, young animals, dairy cattle
labeling required	≤50 µg/kg	wildlife
labeling required	≤100 µg/kg	breeding livestock, poultry, sheep, goats
labeling required	≤200 µg/kg	finishing swine
labeling required	≤300 µg/kg	feedlot cattle
labeling / blending to reduce content	>300 µg/kg	depends on blended concentration
must be destroyed	>1000 µg/kg	crop plowed under, burned as fuel, or sent to toxic landfill
diary products	0.5 µg/kg AfM1	cow's milk for humans

ELISA aflatoxin measurements

ELISA – enzyme-linked immunoassay measurement of AfB1 concentrations. A competitive ELISA method for AfB1 modified from Asis et al. (2002) method.

Microplates coated with AfB1 conjugated to bovine serum albumin (BSA). Samples and standards diluted with 12% methanol /88% phosphate buffered saline. Rabbit anti-aflatoxin added, two hour incubation with shaking. Plate washed. Goat anti-rabbit antibody conjugated to horse-radish peroxidase enzyme added. One hour incubation with shaking. Plate washed. Substrate ortho-phenylenediamine dihydrochloride added. Optical density of wells read using microplate reader and concentrations calculated.

Figure 2. Aflatoxin adsorption to clays from water.



Materials

Clay Minerals Society reference clays.
SHCa -- California hectorite <2µm (low-charge)
SWy-2 -- Wyoming Na-montmorillonite <2µm (low-charge)
SAz-1 -- Arizona Ca-montmorillonite <2µm (high-charge)
IMt-1 -- Montana illite <2µm (high-charge)
KGa-2 -- Georgia kaolinite <2µm (no-charge)

Other clays and materials
Novasil plus -- HSCAS, Na,Ca-montmorillonite <2µm (low-charge)
VSC -- S Carolina vermiculite, hydrobiotite, biotite <2µm (high-charge)
0.35Li-250 SAz -- Reduced charge SAz-1 heated to 250 °C with 35% exchangeable Li, <2µm (low-charge).
0.50Li-250 SAz -- Reduced charge SAz-1 heated to 250 °C with 50% exchangeable LI, <2µm (low-charge).
Activated carbon – Fisher alkaline Norit-A decolorizing carbon

Biochemicals
Aflatoxin B1, Aflatoxin B1-BSA conjugate, OPD, antibodies, and other chemicals for ELISA obtained from Sigma.

Sorption methods

***Water:* Batch adsorption to clays from water using aflatoxin B1 concentrations below 15 mg/L. Overnight equilibration with shaking, centrifugation, and 0.2µm filtration. Direct ELISA measurement of AfB1 in diluted equilibrium solutions.**

***Corn meal/water*: Batch adsorption to clays from aqueous aflatoxin-contaminated corn meal. Overnight equilibration with shaking, extraction with 60% methanol / 40% 2M NaCl, centrifugation, and 0.2µm filtration. ELISA measurement of AfB1 in diluted extracts.**

***Standards*: Standard AfB1 concentrations by AOCS method from optical density of methanol solutions at 360 nm. Aliquots of known AfB1 content prepared by drying.**

Table 1. Charge and other properties of adsorbent materials.

Sample	N_2 Surface area	Unit cell charge	CEC	N-Alkylammonium expansion d_{001} spacing in nm		
	m^2/g	mol_c	$cmol_c/kg$	C8	C12	C18
Activated carbon	1010	0	-	-	-	-
KGa-2 <2µm	11	0	3	-	-	-
SHCa-1 <2µm	60	0.62	87	1.37	1.42	1.75
SWy-2 <2µm	29	0.68	87	1.36	1.45	1.77
Novasil plus <2µm	70	-	-	1.37	1.42	1.76
SAz-1 <2µm	76	1.14	130	1.43	1.77	2.02
0.35Li250-SAz <2µm	-	~0.67	~85	-	-	-
0.50Li250-SAz <2µm	-	~0.57	72	1.34	1.36	1.47
VSC <2µm	-	1.32	80	-	-	~2.60
IMt-1 <2µm	-	1.44	24	-	-	-

RESULTS AND DISCUSSION

The aflatoxin structures are illustrated in Figure 1. Aflatoxins are small enough to be readily sorbed into the interlayer region of smectites. Aflatoxin B1 (AfB1) was effectively removed from water by activated carbon and the montmorillonites, SWy-2, SAz-1, and Novasil plus (Figure 2). Aqueous AfB1 sorption was similar for all four materials. Some feed additives, such as activated carbon, that do not reduce aflatoxicosis have been shown to effectively remove aflatoxins from water. The AfB1 sorption isotherms for activated carbon, Novasil plus, SWy-2, and SAz-1 have a Langmuir-like form as reported by Grant and Phillips (1998) for Novasil. This suggests that AfB1 adsorbs to the clay surface.

Aflatoxin sorption from corn meal was 100 times lower than from water (Figure 3) and suggests that the methanol extraction removed weakly-sorbed AfB1. Previous work by Jaynes et al. (2007) suggests that AfB1 sorption measured using methanol extraction correlates with reduced aflatoxicosis. Activated carbon does not effectively retain AfB1 from corn meal after methanol extraction. SWy-2 and Novasil plus effectively retained AfB1 from corn meal . Jaynes and Boyd (1991) similarly found that high-charge clays were less effective sorbents than low-charge clays. Low-charge hectorite and SWy-2 effectively retained AfB1 from corn meal, whereas, high-charge SAz-1 did not (Figure 4). Zero-charge kaolinite and the very high-charge vermiculite and illite samples retained very little AfB1 (Figure 5). The reduced-charge SAz clays, 0.35Li250-SAz and 0.50Li250-SAz, retained AfB1 much more effectively than SAz-1 (Figure 6). Fewer exchange cations in the low-charge clays free more of the siloxane surface for AfB1 adsorption.

These data suggest that the layer charge of clays strongly affects aflatoxin adsorption. Layer charge measurements might be used to screen clays for possible use as feed additives.

Figure 3. Aflatoxin adsorption to clays from corn meal.

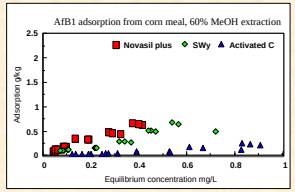


Figure 4. Layer charge and AfB1 adsorption to smectite clays.

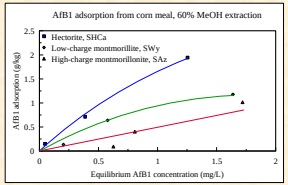


Figure 5. AfB1 adsorption to high-charge and zero-charge clays.

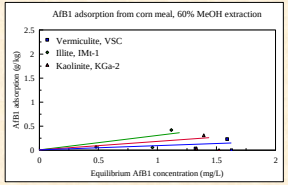
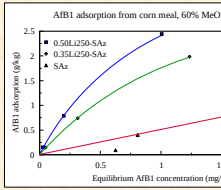


Figure 6. Effect of reduced layer charge on AfB1 adsorption to SAz.



CONCLUSIONS

Large amounts (>20%) of aflatoxin were adsorbed to a variety of materials from water. In contrast, aflatoxin adsorption from aqueous corn meal was 100 times less than from water. Soluble corn meal components and the 60% methanol extraction account for the much lower aflatoxin retention in the corn meal/clay experiments. However, aflatoxin adsorption measured from corn meal/methanol correlates with reduced aflatoxicosis. Activated carbon added to feed does not effectively reduce aflatoxicosis and does not effectively retain aflatoxins from corn meal/methanol. The commercial feed additive, Novasil plus, effectively reduces aflatoxicosis when added to aflatoxin-contaminated feed. Aflatoxin adsorption from corn meal/methanol by Novasil plus was comparable to the reference montmorillonite, SWy-2. Both Novasil plus and SWy-2 are low-charge montmorillonites. The low-charge trioctahedral smectite, hectorite (SHCa), more effectively retained aflatoxin than Novasil plus or SWy-2. High-charge clays, such as high-charge montmorillonite (SAz-1), vermiculite (VSC), and illite (Imt-1), retained far less aflatoxin. This suggests that the addition of SWy-2, SHCa, or other low-charge clays to animal feeds might also effectively reduce aflatoxicosis. Layer charge combined with aflatoxin adsorption from aqueous corn meal/methanol measurements could be used to screen for potential feed additives to effectively reduce aflatoxicosis.

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