Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: An introductory plan

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Abstract

Over the past two decades the use of smectite to suppress aflatoxin has been demonstrated for many farm animals; yet commercial marketing of smectite as an aflatoxin binder has been stymied and blending of grains containing aflatoxin persists. The suppression of aflatoxin depends of the adsorption capacity of the smectite clay binders but effectiveness is influenced by several factors. Our research indicates that smectite clays can be evaluated by the following methods: X-ray diffraction for smectite identification, Langmuir isotherm for aflatoxin binding effectiveness, structural composition by Fourier transform infrared, and cation exchange capacity for smectite quantification. Particle size determined by laser diffraction is a useful and convenient measure of smectite potential that accounts for 65% of the variability in aflatoxin adsorption by most of the clays investigated. Also, pH and Al influence are poorly understood and subjects that need further research. The organic matter content influences smectite quantitative estimates and may reduce aflatoxin adsorption. This introductory plan is intended to expedite the use of aflatoxin binders for grain while improving the performance of animals by sequestering aflatoxin permitted by regulatory action levels for aflatoxin in animal feeds.

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1. Introduction

Animal scientists have demonstrated the effectiveness of certain smectite clays as binders of aflatoxin in animal feeds thus presenting an opportunity to utilize clay science to determine the effectiveness of various smectite clays to sequester aflatoxin. We have shown a ten-fold difference in the effectiveness of smectite binders of aflatoxin and recently that the particle size and structural composition of smectites influence their effectiveness as binders of aflatoxin.

The recent surge in aflatoxin production in corn grown in Texas stimulates action to alleviate the costly reduction in the value of corn as an animal feed ingredient. Also, this occurrence is a reminder that blending of infected grains is practiced in the manufacture of animal feed products with...
aflatoxin below regulatory action levels. As a result, animals and humans are subject to the direct and indirect effects of low levels of the mycotoxins in animal feeds and in certain human food products. Blending of grains infected with aflatoxin raises the risk of liver cancer and other diseases.

This document describes the potential of smectite clays to alleviate a serious health threat and to utilize an abundant resource that is likely to be affordable when bentonite clays of the world have been investigated as sequestering agents of aflatoxin. Establishing the distribution of effective clay adsorbents on a wide geographic basis has tremendous potential for international control of diseases caused by mycotoxins in animals and in humans.

Since the early 1960s when aflatoxin was discovered there have been numerous research articles, three books (Barug et al., 2004; Goldblatt, 1969; Eaton and Groopman, 1994), and multiple large research reviews published on aflatoxin and other mycotoxins yet no strategy has been found to prevent their production on crop plants. In spite of the historical and scientific information on the subject no accepted plan for prevention of aflatoxin problems in animals is available for the immediate future (Table 1). Direct control is necessary to alleviate the problem when aflatoxin occurs.

Smectite clay has been used in the diet of animals to form better pellets in animal feed and to improve animal growth and health for many years (Grim, 1962). Bentonite has been added to flour to reduce staling of bread, to reduce the amount of yeast required, and to increase loaf volume. In Germany montmorillonitic clay is sold and taken internally to control digestive disorders including diarrhea. The adsorption properties of clays including calcium montmorillonite (a smectite) were

<table>
<thead>
<tr>
<th>Animal subject</th>
<th>Type of clay in animals’ diet</th>
<th>Amount of clay in diet (wt.%)</th>
<th>Aflatoxin in diet (ppb)</th>
<th>Author and year</th>
<th>Improvement</th>
</tr>
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<tbody>
<tr>
<td>Broiler chickens</td>
<td>Ca-Montmorillonite (from Ca-bentonite) HSCAS</td>
<td>0.5</td>
<td>200</td>
<td>Desheng et al., 2005</td>
<td>Significant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>3500</td>
<td>Kubena et al., 1993a</td>
<td>Total</td>
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<td></td>
<td></td>
<td>0.5</td>
<td>5000</td>
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<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>2500</td>
<td>Kubena et al., 1993b</td>
<td>30 to 90%</td>
</tr>
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<td></td>
<td>HSCAS (NovaSil, Engelhard Corp.)</td>
<td>0.25</td>
<td>40,000</td>
<td>Doerr, 1989</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.125</td>
<td>20,000</td>
<td></td>
<td></td>
</tr>
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<td>0.25</td>
<td>20,000</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>20,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sorbents of primarily smectite</td>
<td></td>
<td></td>
<td>Reduced AfM1 in milk</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>University, personal communication.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reduced AfM1 in milk 34 to 48%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HSCAS (Engelhard Corp.) Bentonite (Ain-Shams</td>
<td>0.5</td>
<td>2500</td>
<td>Abdel-Wahabet et al., 1999.</td>
<td>Prevented maternal developmental</td>
</tr>
<tr>
<td></td>
<td>University, Cairo)</td>
<td></td>
<td></td>
<td></td>
<td>effects of AfB1</td>
</tr>
<tr>
<td>Pregnant rats</td>
<td>HSCAS (Engelhard Corp.)</td>
<td>0.5</td>
<td>500</td>
<td>Kubena et al., 1990.</td>
<td>68% decrease in mortality</td>
</tr>
<tr>
<td></td>
<td>Bentonite (Ain-Shams</td>
<td></td>
<td>1000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>University, Cairo)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turkey poults</td>
<td>HSCAS</td>
<td>0.5</td>
<td>34</td>
<td>Bonna et al., 1991.</td>
<td>Prevented mortality, reduced lesions in liver</td>
</tr>
<tr>
<td>Mink</td>
<td>HSCAS</td>
<td>0.5</td>
<td>102</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weanling pigs</td>
<td>Ca bentonite (HSCAS)</td>
<td>0.25, 0.5, 1.0 and 2.0, 0.5</td>
<td>800</td>
<td>Schell et al., 1993.</td>
<td>Improved weight gain</td>
</tr>
<tr>
<td>Broiler chicks</td>
<td>HSCAS (Myco-Ad)</td>
<td>0.25</td>
<td>7500</td>
<td>Casarin et al., 2005a</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1000 ppb, T-2 toxin</td>
<td>Casarin et al., 2005b</td>
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</tr>
</tbody>
</table>
utilized for the cleaning of wool, silk, and other materials since antiquity (Robertson, 1986).

The state regulatory authority, Office of the Texas State Chemist (OTSC) has set action levels for aflatoxin in grains for the diets of particular animals (http://otsweb.tamu.edu/). Currently OTSC is drafting a policy levels on the selection and approval of clay binders for use in animal feeds containing aflatoxin below action levels. This development was stimulated by the occurrence of aflatoxin at action levels in 35% of the corn grown in Texas in 2006 and analyzed by the OTSC.

Also, mycotoxin levels in foods have been established in other countries e.g. in 16 South American and Caribbean countries (Pineiro, 2004). The action levels provide an upper limit for marketing contaminated grains (e.g. 100 ppb). Grains with aflatoxin below these levels can be amended with smectite to improve animal performance (Feed Industry Memorandum 5–12, April 26, 2006). Thus the user can decide how effective approved smectite clays are at improving animal health and performance. Since commercial bentonites are processed in large batches sampling, analysis, and regulatory approval or rejection can be performed before the need occurs. Once labeled, the smectites can be marketed to feed manufacturers for inclusion in their feeds.

Several researchers have pursued a direct strategy for reduction of the influence of aflatoxin in animal feeds by applying smectite clay adsorbents primarily NovaSil™ (a smectite clay) (Phillips et al., 1995). Smectite clay has consistently suppressed the impact of aflatoxin B1 in the diets of many types of farm animals (CAST, 2003). Smectite clays with demonstrated effectiveness as adsorbents of aflatoxin (AfB1) can be added to those feeds that contain aflatoxins below the action level established by OTSC or other regulatory agency for the specific types of animals to consume the feeds.

Natural clays occur in large deposits yet they are impure and are sometimes diverse in composition and properties. We have analyzed many smectites from the United States and Mexico and found a ten-fold range in sorption maxima (Kannewischer et al., 2006a). Thus thorough analysis and adsorption tests are required before they can be approved as feed additives. As we learn the controlling factors for aflatoxin adsorption we anticipate reduction in the number of tests that will be required for confirmation of the performance of the smectite as an adsorbent of this mycotoxin.

Recently we tested twenty smectite clays for adsorption capacity of aflatoxin, three of them were as effective as NovaSil™. There is clear evidence that use of smectite clay in animal feeds can reduce or eliminate aflatoxin that enters the human food chain and thus reduce the incidence of liver cancer in humans and farm animals.

1.1. Goal

The goal of this plan is to initiate the use of adsorbent smectites as dietary amendments of farm animals to suppress aflatoxin (AfB1) in Texas and elsewhere under regulations. It is based on the premise that the ultimate success of the smectite adsorbent is acceptance by the users who observe its effectiveness. We propose that each batch of clay offered by dealers be tested and approved by the appropriate regulatory body before it is listed for sale as an aflatoxin adsorbent. The approved and labeled clay can then be sold to feed manufacturers for amending their products containing aflatoxin below the action level. Then the user will have the opportunity to evaluate the health and performance of the animals that consume the amended feed.

Objectives:

1. Develop a set of quality criteria for smectite clay as a binder of aflatoxin.
2. Illustrate the use of selection criteria for a set of bentonite samples and industrial products submitted to sequester aflatoxin.

1.2. Scientific basis for use of smectite adsorbents of aflatoxin

The effectiveness of smectite clays as adsorbents of aflatoxin has been investigated and found successful for many farm animals over the past 20 years (Table 1). In young broiler chickens with a diet containing 2000 to 4000 ppb AfB1 HSCAS (a smectite) produced 50% to 100% improvement in body weight although it did not fully protect against liver and spleen weight changes (Doerr, 1989). In mink smectite prevented mortality and eliminated histopathological lesions in the liver (Bonna et al., 1991). Feeding experiments with 60 lactating dairy cows showed that four of the eight adsorbents gave statistically significant reduction of feed-to-milk transfer of mycotoxin as aflatoxin M1 were smectite clays. Four of the poorest adsorbent performers contained abundant C and a 5th was very disordered (Lindemann et al., 1993). Suppression of AfB1 in the diet of rainbow trout by smectite clay was effective when 2% clay was included in the feed. In each case smectite
clay reduced the impact of the AFB1 as measured by various parameters: weight gain, toxin in the milk, or toxin in the animal. Quality smectite clays have been effective adsorbents of mycotoxins in animals and these results provide a foundation for public utilization of them as adsorbents (Bluthgen and Schwertfeger, 2000; CAST, 2003; Grim, 1962). Smectite clay alone had no negative effects on young broiler chicks or chickens (Kubena et al., 1998; Casarin et al., 2005a,b) or pregnant rats (Abdel-Wahab et al., 1999).

The concentration range of AFB1 in the in vivo research (Table 1) was generally greater than the action levels that can be legally sold as animal feeds and they are higher than the average levels in corn i.e. 20 and 30 μg kg⁻¹ in the USA (CAST, 2003, p. 40). Even at these high levels e.g. 100 to 2000 ppb AFB1 the smectite clays were effective in sequestering the mycotoxin.

Smectite clays are most efficient at sequestering AFB1 in the action level range where the adsorption curves are steep (Kannewischer et al., 2006a). Thus the use of clay binders below action levels as recommended in this report will have a greater depressing influence on the unbound AFB1 because the data fit the Langmuir curve and equilibrium solution levels at low concentrations are very small. In practical terms sequestration may eliminate the presence of the mycotoxin in the digestive track of the animal.

AFB1 has a relatively planar molecule and we have determined that AFB1 molecules can occupy most of the interlayer space in smectites based on adsorption of AFB1 and the crystal structure (Kannewischer et al., 2006a, 2007). Evidence for that conclusion is the resistance of the layers to closure on heating to remove interlayer water. We calculated 84% coverage based on the density of AFB1 in crystal form, 0.5 mol kg⁻¹ adsorbed assuming each molecular layer is in contact with two clay layers and clay surface area is 800 m² g⁻¹ (van Soest and Peerdeman, 1970). This interpretation is in agreement with our findings that the more orderly layer stacking in smectite increases the amount of mycotoxin adsorbed.

The chemical, physical, and mineralogical properties of smectite clays are known (Brindley and Brown, 1980; Dixon and Weed, 1989). Smectite clays are sold internationally for industrial and agricultural uses. Commercial bentonite (smectite) clays are prepared for market in batches of many tons that can be sampled by the regulatory authorities before they are approved (or rejected) as feed additives based on their adsorption properties. Thus labeled smectite clays that are reliable adsorbents of aflatoxin can be obtained by feed manufacturers for amending animal feed.

2. Materials and methods
Clays are identified by the symbol for the state of origin in the USA and from Mexico as MX. Industrial clay products are identified by a letter and number designated provided by OTSC to preserve the confidentiality of the source. Clays were analyzed as received without fractionation or chemical treatment.

Aflatoxin sequestration evaluation was based on the structural dimensions of smectite clay by X-ray diffraction (XRD), adsorption of aflatoxin molecules as a monolayer indicated by the Langmuir equation, clay structural composition of the octahedral sheet indicated by infrared absorption (FTIR), and quantitative smectite calculation by cation exchange capacity (CEC), clay particle size by laser diffraction, and the negative effects of acidity and Al dissolution and total C% as a contributor to CEC and possible occupant of clay surfaces (Kannewischer et al., 2006a; Tenorio Arvide et al., 2006). The infrared method is a diffuse reflectance powder methods in which the sample is diluted with potassium bromide by mixing in a Wig-L-Bug (Lyons, Ill 60534) mixer and scanned in an infrared instrument (Perkin-Elmer System 2000 FTIR) with controlled humidity atmosphere.

3. Results
Infrared spectra (FTIR) indicate the displacement of water by AFB1. FTIR patterns for samples 16MX and 1MX illustrate the reduction in adsorbed water shown by decreased absorbance in water bands near 1600 and 3400 cm⁻¹ (Fig. 1). This pair of smectites also illustrated differences in the adsorption of AFB1 0.06 mol kg⁻¹ for 16MX versus 0.52 mol kg⁻¹ for 1MS. Further research is needed to understand the reason for their contrasting performance as adsorbents of AFB1.

The wide range of smectite performance illustrated in Fig. 2 indicates the need for analyses and performance ratings for clays submitted as binders of aflatoxin. These data serve as a performance scale for selection of smectite adsorbents as presented in Table 2. These clays are predominantly members of the smectite structural group and their sequestration range is 10-fold. Clays with similar adsorption characteristics have properties that can be evaluated by the selection criteria proposed. Clays of other structural groups (kaolinite, palygorskite, etc.) are unlikely to be effective adsorbents of aflatoxin because they lack the charge, hydration, high surface area, and variable expansion characteristics of smectites.

3.1. Selection criteria
1. Identification: X-ray diffraction to identify smectite clay on the basis of structural properties: first order
peak near 1.3 nm and expansible to ~1.7 nm when glycerated or glycolated. This is a required test.

2. Adsorbent effectiveness: Langmuir adsorption isotherm was used by for determining the amount of aflatoxin sorbed by smectite clay (Grant and Phillips, 1998). The Langmuir isotherm is an established method of evaluating the adsorption behavior of solids where monolayer adsorption occurs as indicated by our X-ray diffraction data (Kannewischer et al., 2006a). A Langmuir adsorption isotherm for AfB1 adsorption maximum is required.

3. Quantitative evaluation: Cation exchange indicates the amount of smectite present in clays and soils (van Olphen and Fripiat, 1979; Borchardt, 1989).

4. Laser diffraction particle size analysis: Thus far 65% of the variability of the adsorption data are accounted for by the particle size distribution by LDPSA for dispersion in Na hexametaphosphate solution minus dispersion in water for most of the clays investigated. We only have a partial data set on this criterion.

5. Structural composition: Fourier transform infrared (FTIR) analysis of the clay is a recommended
Table 2
Smectite clays adsorption properties arranged in decreasing aflatoxin adsorption order (Kannewischer et al., 2006a,b)

<table>
<thead>
<tr>
<th>Sample</th>
<th>XRD</th>
<th>Sorption</th>
<th>CEC</th>
<th>Particle size Δ</th>
<th>FTIR Mg</th>
<th>FTIR Fe</th>
<th>pH</th>
<th>Organic C</th>
<th>Total score</th>
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<tr>
<td>8TX</td>
<td>30</td>
<td>30</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>85</td>
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<tr>
<td>1MS</td>
<td>30</td>
<td>30</td>
<td>5</td>
<td>10</td>
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<td></td>
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<tr>
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<tr>
<td>16MX</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>35</td>
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</tr>
</tbody>
</table>

a Minimum score for approval 70.

b Above correlation line, subject of further research.

c No data.

Table 2 Smectite clays adsorption properties arranged in decreasing aflatoxin adsorption order (Kannewischer et al., 2006a,b)

Based on the above criteria index values can be assigned to accommodate the selection of promising bentonite clay products. Those products with 70 index points or more may be approved for labeling by OTSC.

4. Discussion

There are several observations that suggest that the adsorption of aflatoxin molecules is an orderly process that fits a geometric model suggesting the development of a well-bonded association between smectite clay surfaces and the organic molecules. The fit of the adsorption data to the Langmuir equation indicates an association between the mycotoxin molecules and the clay surfaces shown by Phillips et al. many years ago. The spacings of the aflatoxin-saturated smectites are consistent with a molecule that is parallel with clay layers (Kannewischer et al., 2006a,b).

The relative ratings of 14 natural reference clays indicate need for more information on the influence of acidity e.g. would 17TX perform better if more of its exchange ions were Ca rather than Al in various forms (Table 2). More samples are needed to answer some of these questions. Is the difference in absorbance in the 800 to 1000 cm⁻¹ FTIR reliable? The weak signal makes quantitative analysis of Fe and Mg in the clay structure difficult (Fig. 2). We need a wider data base to develop more confidence in the particle size measurements with the laser diffraction particle size instrument. Thus far it performs well.
The occupancy of the interlayer surfaces has been calculated (see earlier) to be about 84% based on the size and shape of the molecule and the amount of aflatoxin adsorbed i.e. relatively complete. The better adsorbent smectites have a more orderly stacking of the layers and poorly ordered smectites adsorb less of the mycotoxin e.g. Na saturated smectites.

The oxygen atoms on the mycotoxin molecule localize the negative charge on both sides of the molecule: the coumarin ring on one side and the two furan rings on the other. Carbonyl oxygens are suggested bonding sites with the clay between clay layers (Phillips et al., 1995; Tenorio Arvide et al., 2007). Once the aflatoxin molecules are sequestered to the clay they resist removal by washing.

These observations suggest the hypothesis that sequestered aflatoxin in smectite clay is shielded from reaction with the solution around it and is likely to pass on in the waste stream of the host animal. Coincidentally our laboratory experience with aflatoxin suggests bacterial destruction of the molecule when stored in water solution which seems a likely scenario for the aflatoxin molecules deposited in moist soil that contains abundant bacteria of many types.

5. Summary

Performance data for 20 smectites and for several commercial clay products have been developed for comparative purposes.

LDPSA has been tested as an additional method for evaluating smectites as aflatoxin adsorbents shows an important correlation.

Structural properties of smectites influence their sequestration capability e.g. octahedral composition by FTIR.

Morphology observed by TEM provides some descriptive information that relates to adsorption capability.

The OTSC is developing a policy statement on the use of smectite clay binders to sequester aflatoxin in grains for animal feeds marketed in Texas.

Kannewischer et al. (2006a,b) in narrated form (English, Spanish, and Chinese) is now available on mycotoxin website of http://otscweb.tamu.edu in mycotoxin presentations and in text form on this departmental website.

The properties of smectite clays influence the sequestration of aflatoxin provide added insight into the behavior of smectites as a group of reactive clays.

The results of these investigations provide new insight into land-use quality where wild or domestic poultry are fed on the ground.

Acknowledgements

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