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22

Influence of Soluble Feed Proteins and Clay Additive Charge Density on Aflatoxin Binding in Ingested Feeds

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1. Introduction

Hartley et al. (1963) isolated and identified toxic metabolites of *Aspergillus flavus* as aflatoxins B1, B2, G1, and G2; named from the blue and green fluorescence of the compounds under ultraviolet light. The aflatoxins are a group of mycotoxins produced by *Aspergillus* fungi that are both toxic and carcinogenic to animals and humans (Murphy et al., 2006). Aflatoxin B1 (AfB1) and mixtures of B1, G1, and M1 are proven human carcinogens (IARC, 1993). Aflatoxin B1 is the most toxic (Figure 1), most abundant, and the most potent natural carcinogen known (Squire, 1981). An estimated 4.5 billion people living in developing countries are chronically exposed to uncontrolled amounts of aflatoxins (Williams et al., 2004). Iraq produced aflatoxins for use in biological warfare between 1985 and 1991, but the weapons had little military value (Zilinskas, 1997). After ingestion, aflatoxins are converted to the reactive 8,9-epoxide form that can bind to DNA and proteins. Aflatoxin consumption results in diseases that are loosely called aflatoxicoses. Chemically, aflatoxins are derivatives of difuranocoumarin (Bennett & Klich, 2003).

Various methods have been used to reduce the toxicity of aflatoxin-contaminated grains. Cleaning to remove damaged corn kernels is sometimes effective in reducing aflatoxin concentrations, but undamaged kernels can also contain high aflatoxin concentrations (Vincelli et al., 1995). Treatment with anhydrous ammonia can be used to detoxify grain that is to be used on the farm (Vincelli et al., 1995). Brekke et al. (1977) ammoniated trout feed contaminated with 180 µg/kg aflatoxins, which inactivated the aflatoxins and reduced the carcinogenicity to a level not significantly different than the control. Grove et al. (1981) examined the ammoniation products of aflatoxin model coumarins and determined that the keto group in the cyclopentene ring is required for ammonia-induced decomposition. Nixtamalization is an Aztec word that means lime-cooked corn (Herrera et al., 1986) and is an ancient method used to soften grain before it is used in foods. Nixtamalization also increases protein quality and niacin bioavailability (Sefa-Dedah et al., 2004). The strong alkalinity imparted by lime (CaO, $Ca(OH)_2$) might have a similar effect on aflatoxins as ammonia. Arrriola et al., (1988) examined the effect of nixtamalization on aflatoxin fate during tortilla preparation using 2-10% CaO. Nixtamalization decreased aflatoxin concentrations at even the lowest CaO concentrations. However, nixtamalization did not

reduce the 1360-1896 μg/kg initial aflatoxin concentrations down to the allowable value of 20 μg/kg. Ammoniation and other detoxifying methods, however, are not approved or sanctioned by the U.S. Food and Drug Administration (Sweets & Wrather, 2009).

Clays are colloidal or near-colloidal hydrous aluminum silicates that are more or less plastic when moist (Bates, 1969). Bentonites are natural materials that dominantly consist of clay minerals in the smectite group (Hosterman & Patterson, 1992). Smectites are a group of phyllosilicate minerals that include montmorillonite, beidellite, nontronite, saponite, and hectorite (Odom, 1984). Bentonite was the name given by Wilbur G. Knight in 1898 to deposits in the Benton Shale near Rock River, Wyoming (Hosterman & Pattterson, 1992). Bentonite deposits contain altered volcanic ash glass shards and field evidence suggests that bentonites formed from ash that fell into shallow lakes or seas (Bates, 1969). Smectites, vermiculites, talc, and pyrophyllite are structurally-related 2:1 clay minerals, but talc and pyrophyllite have zero layer charge and do not expand in water. Smectites and vermiculites characteristically expand in water along the crystallographic c-axis to form an interlayer region. Structurally, the 2:1 clay minerals consist of an octahedral aluminum or magnesium oxide sheet sandwiched between two tetrahedral silica sheets. Unlike talc and pyrophyllite, smectites and vermiculites have isomorphic chemical substitutions of Al^{3+} for Si^{4+} (tetrahedral charge) and Mg^{2+} for Al^{3+} (octahedral charge) that impart a negative charge to the mineral surface and a cation exchange capacity (CEC). Inorganic exchange cations, such as Na^{+} and Ca^{2+}, compensate for the negative charge on smectite and vermiculite surfaces. Smectite CECs range from 50 to 129 cmol/kg and vermiculite CECs range from 130 to 210 cmol/kg (Mermut & Lagaly, 2001; van Olphen & Fripiat, 1979). Bentonites are relatively pure, commercial deposits of smectites found throughout the world that can be mined, but smectites, vermiculites, and other clay minerals commonly also occur in soils and sedimentary deposits. In soils, clays retain exchangeable cations, such as Ca^{2+}, Mg^{2+}, and NH_4^{+}, which are essential plant nutrients. Vermiculite expansion (Ca-saturated) in water is limited to ~1.5 nm, but smectites (Ca-saturated) expand to 1.9 nm or more (McEwan & Wilson, 1984). Free expansion of smectites in water is almost unlimited. Completely dispersed smectite particles consist of single unit cells with no c-axis direction repeat distance (Eberl et al., 1998). Interlayer expansion of air-dried (32% relative humidity) samples of Na-smectite and Ca-smectite is illustrated in Figure 1 with characteristic basal spacings after McEwan & Wilson (1984). The single water layer and Na^{+} cations in Na-smectite interlayers (Figure 1) is ~0.25 nm and the water bilayer and Ca^{2+} cations in Ca-smectite interlayers is ~0.52 nm. Calcium- and magnesium-saturated smectites yield similar basal spacings. The replacement of inorganic exchange cations in smectites and vermiculites with organic cations can result in interlayer expansion. Jaynes & Boyd (1991) exchanged the organic cation, hexadecyltrimethylammonium (HDTMA), for the inorganic cations in a low-charge smectite, a high-charge smectite, and a vermiculite, which produced expanded interlayer basal spacings of 1.8, 2.3, and 2.8 nm, respectively. Polymer adsorption to clays can also produce interlayer expansion. Polyvinylpyrrolidone (PVP) expanded SAz high-charge montmorillonite to ~2.3 nm (Blum & Eberl, 2004). Smectite layer charge ranges from 0.2 to 0.6 per $O_{10}(OH)_4$ unit , whereas, vermiculite layer charge ranges from 0.6 to 0.9 (Bailey, 1980). Vermiculites are hydrous minerals that form by the weathering of micas and have a platey mica-like morphology (Newman & Brown, 1987). The name "vermiculite" is more commonly used for macroscopic heat-expanded (800 – 1100 °C) vermiculite particles that are used as a packing material, plant media, insulation, and

construction material. This heated vermiculite should not be be confused with the natural mineral because heat-treatment greatly alters the properties. Sepiolite, palygorskite, and the zeolites are structurally much different than smectites and vermiculites and do not have interlayers. Sepiolite and palygorskite are fibrous, non-expandable, hydrous magnesium aluminosilicates. There are some health concerns about the possible effects of inhaled fibrous minerals. Bellman et al. (1997) used intratracheal instillation studies in rats to evaluate the carcinogenic potential of sepiolites. A short-fiber sepiolite from Spain showed no evidence of carcingenic potential, but a long-fiber sepiolite from China had a more pronounced fibrotic response (Bell et al., 1997). Sepiolite (CEC = 20–40 cmol/kg) and palygorskite (CEC = 5-30 cmol/kg) contain internal channels with exchangeable cations and water (Singer, 2002). Zeolites (e.g. clinoptilolite, erionite, analcime, mordenite) are framework-structure, three-dimensional aluminosilicate minerals with interconnected channels and cages that contain exchangeable cations (CEC = 220-570 cmol/kg) and adsorbed water (Boettinger & Ming, 2002). The internal channels in sepiolite (0.37 x 1.06 nm), palygorskite (0.37 x 0.64 nm), and zeolites (0.26 x 0.26 to 0.74 x 0.74 nm) are too small to accomodate aflatoxins. Hence, aflatoxins can only adsorb to external sites on these minerals.

Commercial clay additives have been used to prevent caking and improve the physical properties of animal feeds. The decreased toxicity of aflatoxins observed for contaminated animal feed mixed with clay feed additives has stimulated research on clay additives to prevent mycotoxicosis. The commercial clay feed additives, Novasil, Novasil plus, Astra-Ben 20, and Astra-Ben 20A, are bentonites that primarily consist of the smectite group mineral, montmorillonite. Animal feeding studies have demonstrated that Novasil, Novasil plus, Astra-Ben 20, Astra-Ben 20A, Na-bentonite, zeolite, and sepiolite feed additives can effectively reduce or prevent the toxicity caused by feed contaminated with *Aspergillus* mycotoxins, such as AfB1 (Phillips et al., 1988, 1995; Scheideler 1993; Schell et al., 1993a, 1993b; Edrington et al., 1996; Abdel-Wahhab et al., 1999; Miazzo et al., 2000; Diaz et al., 2004; Pimpukdee et al. 2004; Bailey et al., 2006; Fairchild et al., 2008; Magnoli et al., 2008). Ruminant animals, such as cattle and sheep, can tolerate higher aflatoxin levels and longer low-level intake periods than simple-stomached animals (Vincelli et al., 1995). The adsorption of aflatoxins to ingested soil minerals might partly explain the greater aflatoxin tolerance of ruminants. Soil ingested by cattle averaged 14% of the dry weight of fecal matter and increased as forage availability decreased (Mayland et al., 1975). Soil ingestion by grazing sheep in March exceeded 30% of dry matter intake at 2 of the 11 sites in mid-Wales (Abrahams & Steigmajer, 2003). Winfree and Allred (1992) measured significant aflatoxin adsorption to bentonite from methanol/water, which is commonly used in the extraction and measurement of aflatoxins in contaminated feed. Gallo et al. (2010) developed a more aggressive extraction procedure using acetone rather than methanol to more accurately measure aflatoxins in feeds that contain feed additives. Deng et al. (2010) measured smectite interlayer expansion of >1.2 nm that was stable to 400 °C after AfB1 treatment, which demonstrated that AfB1 adsorbs to interlayer clay surfaces. Interlayer clay surfaces account for most of the ~800 m^2/g surface area of smectites, such as montmorillonite. From infrared spectroscopy, Deng et al. (2010) concluded that hydrogen bonds between AfB1 carbonyl groups and the hydration water of exchangeable cations in clays is the dominant bonding force under humid conditions. Aflatoxin adsorption from aqueous corn and peanut meal to feed additives was consistent with animal feeding studies

that used the feed additives, Novasil, Novasil Plus, Astra-Ben 20, Astra-Ben 20A, sepiolite, and activated (Norit-A) carbon (Jaynes et al., 2007; Seifert et al., 2010). Feed additives that effectively reduced or prevented aflatoxin toxicity in feeding studies adsorbed more AfB1 from aqueous corn and peanut meal.

Animal feedings studies (*in vivo*) are the surest way to identify effective feed additives, but are much too expensive for routine use. Hence, various approaches have been used for the *in vitro* evaluation of potential feed additives. Feed additive *in vitro* test methods should produce results that are consistent with animal feeding studies. Unfortunately, most animal feeding studies evaluated only one or a few feed additives, which makes comparisons of relative effectiveness difficult. This also frustrates efforts to identify feed additive properties related to effective aflatoxin toxicity reduction. In contrast, Schell et al. (1992a) evaluated a sodium calcium bentonite (Novasil), a calcium bentonite (Astra-Ben 20), a sodium bentonite (FD-181), a zeolite (Zeobrite), a palygorskite (Min-U-Gel), and a sepiolite (Sepiolgel UF). Phillips et al. (1988) measured AfB1 adsorption from water to aluminas, zeolites, silicas, phyllosilicates, a Mn-exchanged phyllosilicate, and an acid-activated phyllosilicate. Winfree & Allred (1992) measured a 70% reduction in AfB1 concentrations in methanol/water extracts of trout feed 1 hour after 10% bentonite was added to moistened feed. Grant & Phillips (1998) fitted AfB1 adsorption from water to Novasil data to the Langmuir and other isotherm equations to calculate adsorption capacities. Jaynes et al. (2007) measured AfB1 adsorption from aqueous corn meal after extraction with 60% methanol. Siefert et al. (2010) measured AfB1 adsorption to clays in aqueous peanut meal and total extractable aflatoxins in peanut meal/clay water extracts. Vekiru et al. (2007) measured AfB1 adsorption to bentonites and charcoal from acetate buffer, artificial gastric fluid, and from gastric fluid. Thieu and Pettersson (2008) measured AfB1 adsorption to zeolite and bentonite in simulated gastrointestinal fluids. Dixon et al. (2008) used X-ray diffraction, infrared spectroscopy, cation exchange capacity, and particle size to measure smectite purity and the Langmuir isotherm to measure aflatoxin adsorption from water. To identify feed additives that can effectively bind aflatoxins in ingested feed, *in vitro* tests should model the environment of ingested feed/feed additive as accurately as practical. Proteins are soluble ionic polymers of amino acids that can adsorb to clay surfaces. Living organisms, animal feed, and human food contain proteins. Lipson & Stotzky (1984) showed that the proteins chymotrypsin, ovalalbumin, and lysozyme adsorbed to montmorillonite and reduced adsorption of the Reovirus. Perez-Castells et al. (1985) puried collagen protein from calf skin and adsorbed 0.4 mg of collagen to 1 mg of sepiolite. Similarly, Garwood adsorbed 97 g of the enzymatic protein, glucose oxidase, to 100 g of Na-montmorillonite. Ralla et al. (2010) used a smectite clay to adsorb and separate proteins. Aflatoxin-contaminated peanuts are unsuitable as food or feed and are used to produce peanut oil (Siefert et al., 2010) and defatted peanut meal is a byproduct of peanut oil production. Siefert et al. (2010) used Astra-Ben 20A to bind aflatoxins in defatted peanut meal in the insoluble residue and produce an aflatoxin-free water-soluble protein extract. Addition of 0.2% Astra-Ben 20A to peanut meal decreased total aflatoxins in the soluble protein extracts from 50 to 4.8 μg/kg. The addition of 2% Astra-Ben 20A decreased total aflatoxins in the soluble fraction to 0 μg/kg, but decreased protein recovery by 37%. Soluble protein adsorption to clay additives in ingested feed might adsorb to clay feed additives and block aflatoxin adsorption. Other soluble compounds in feed, such as polysaccharides, might also adsorb to clay feed additives and block potential aflatoxin binding sites. Chenu et al. (1985) adsorbed ~400 mg of the polysaccharide, scleroglucan, to 1 g of Na-montmorillonite.

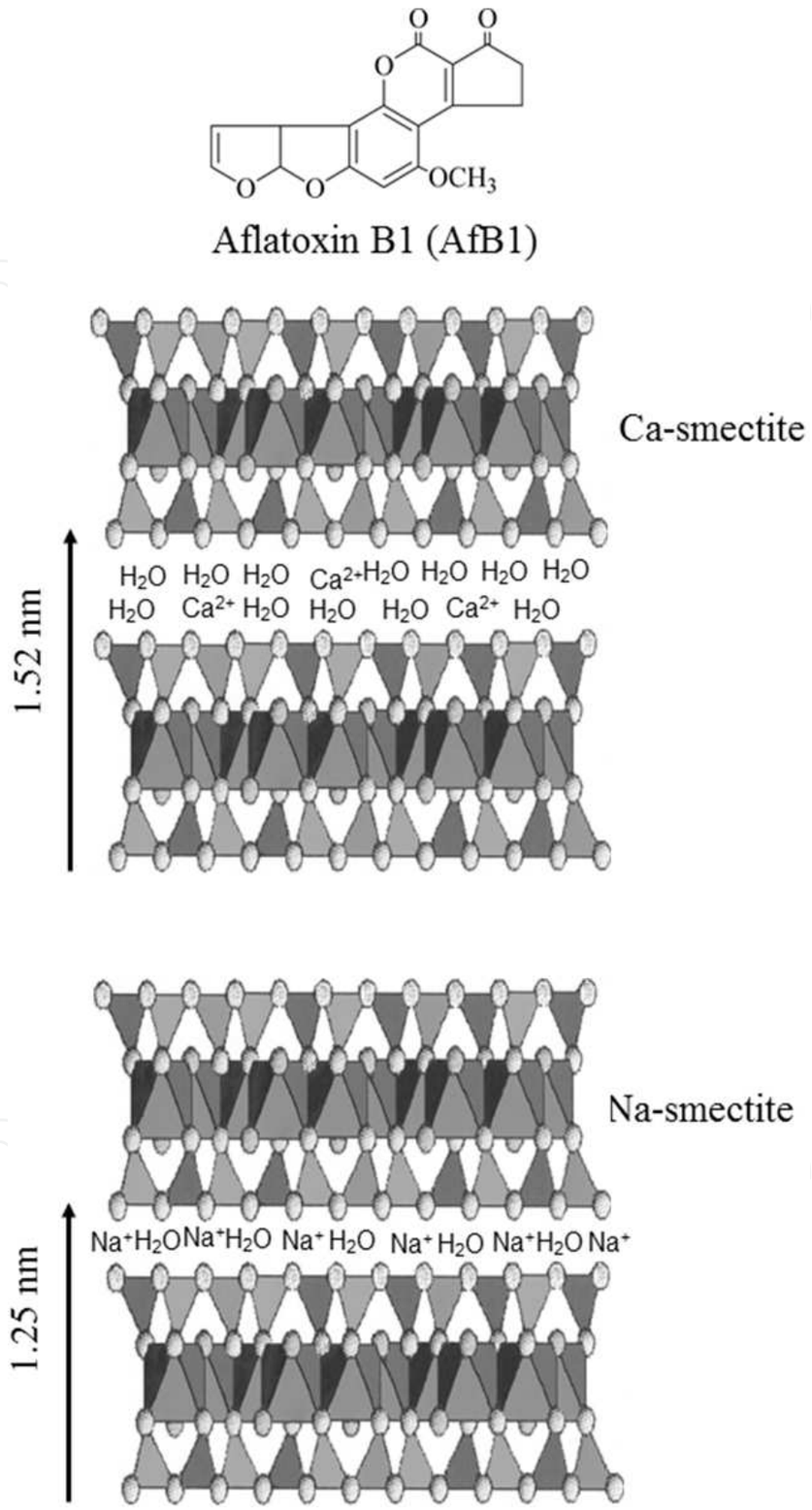


Fig. 1. Chemical structure of aflatoxin B1 and illustration of interlayer expansion of air-dried calcium-saturated and sodium-saturated smectites.

Decker (1980) measured the adsorption of 1 mg AfB1 to 100 mg of activated carbon (Norit-A) from aqueous media at pH 7 and suggested activated carbon might be used to prevent animal and human absorption of aflatoxins from contaminated foodstuffs. Early animal feeding studies by Hatch et al. (1982), Dalvi & Ademoyero (1984), and Dalvi & McGowan (1984) indicated that activated carbon can reduce aflatoxicosis. Similarly, bentonites and activated carbon reduced excretion of aflatoxin M1 in milk cows, turkey poults, and goats in feeding studies by Veldman (1992), Edrington et al. (1996), and Rao & Chopra (2001). However, animal feeding studies by Kubena et al. (1990), Bonna et al. (1991), Edrington et al. (1996), and Diaz et al. (2004) concluded that activated carbon does not effectively reduce aflatoxin toxicity to fed animals or is not as effective as clay additives. Diaz & Smith (2005) did not recommend routine inclusion of activated charcoal in diets after reviewing activated charcoal use in animal feeding studies. Vekiru et al. (2007) measured AfB1 binding by various sorbents and identified another drawback to activated carbon use as a feed additive; unlike the bentonites, activated carbon adsorbed the essential nutrients, vitamin B12 and biotin.

Clay feed additives are marketed and sold in the United States as anti-caking agents to improve the physical properties of feed because U.S. Food and Drug Administration (FDA) regulations do not permit feed additive companies to claim that feed additives can bind mycotoxins and reduce mycotoxicoses. Therefore, feed additive companies have little financial incentive to develop additives with improved mycotoxin binding. Feed additives are mixed with dry feeds and, hence, mycotoxin binding to clays must occur after ingestion. During digestion, pH, feed composition, and other factors can affect mycotoxin binding to feed additives. Mycotoxin adsorption to feed additives can sequester the toxins and limit absorption by animals or humans. However, feed additives that effectively remove mycotoxins from water might not prevent toxicity to animals from contaminated feed because the adsorption of soluble feed or digestive compounds might block mycotoxin adsorption to feed additives.

In this study, the effects of soluble feed compounds and clay layer charge on the adsorption of AfB1 to commercial feed additive clays, reference clays, and activated carbon will be examined. Adsorption of AfB1 to a variety of commercial feed additives and reference clays were measured from water and aqueous corn meal. Clays and activated carbon were treated with aqueous corn and peanut meal extracts to simulate the adsorption of soluble feed compounds to feed additives ingested with feed. The physical and chemical properties of materials treated with corn- and peanut-meal water extracts will be measured and related to aflatoxin adsorption. The adsorption of AfB1 to clays with a wide range in layer charge will be measured to determine layer charge effects on AfB1 adsorption.

2. Materials and methods

The reference clay samples, SWy-2 (SWy), SAz-1 (SAz), SepSp-1 (SepSp), and SHCa-1 (SHCa) were obtained from the Source Clay Repository of the Clay Minerals Society located at Purdue University (West Lafayette, IN). The SWy sample is a low-charge sodium montmorillonite from Wyoming and SAz is a high-charge calcium montmorillonite from Arizona. The SepSep sample is a sepiolite from Spain and SHCa is a hectorite from California. Sepiolite is a fibrous magnesium silicate clay mineral and hectorite is a low-charge magnesium layer silicate. Both montmorillonite and hectorite are expanding smectite-group minerals. Novasil and Novasil plus (low-charge montmorillonite) are products of Trouw Nutrition, which is a division of Englehard Corporation, Chemical

Catalysts Group (600 East McDowell Road, Jackson, MS). A vermiculite sample, VSC, from a mine in South Carolina was obtained from the W.R. Grace Company. Vermiculites have a higher CEC/layer charge than smectites. Clay mineral samples were Na-saturated by treatment with NaCl, <2 μm clay fractions were separated by centrifugation, and the <2 μm clay fractions were freeze-dried. Powdered activated carbon (alkaline Norit-A decolorizing carbon) was obtained from Fisher Scientific. Dispersions of the <2μm clays and activated carbon were prepared using an ultrasonic probe and a vortex mixer. Corn meal was purchased at a local grocery and defatted peanut meal was prepared by grinding and acetone-extraction of raw peanuts. Bovin serum albumin (BSA) protein, Bradford Reagent (Bradford, 1976) for total protein measurements, castor seed protein, and peanut protein (peanut agglutinin, PNA) were obtained from Sigma-Aldrich. Aflatoxin B1, rabbit anti-aflatoxin B1 antibody, aflatoxin B1-BSA conjugate, goat anti-rabbit horse radish peroxidase (HRP) conjugate, phosphate buffered saline with 0.05% Tween 20 (PBST), and o-phenylenediamine dihydrochloride (OPD) substrate tablets were obtained from Sigma-Aldrich. Stable AfB1 stock solutions were prepared in 95% toluene/5% acetonitrile and stored in a freezer (AOCS, 1999a). The aflatoxin stock solutions were calibrated by measuring the UV absorbance of AfB1 dissolved in methanol at 360 nm (AOCS, 1999a). A Shimadzu UV-1800 spectrometer and quartz glass cuvets were used to collect spectra of AfB1 solutions from 190 to 500 nm. Enzyme-linked immunoassay microplates were washed and read using a Bio-Tek ELx50 plate washer and a ELx800uv plate reader.

2.1 Preparation of reduced-charge clays

The cation exchange capacity (CEC) and layer charge of lithium-saturated montmorillonites decrease after heat treatment. Layer charge is a measure of the charge density of specific mineral particles, whereas, CEC is a bulk measure of the average charge density of all the mineral particles in a sample. Layer charge can be measured using the n-alkylammonium method of Lagaly & Weiss (1976). The CEC is directly related to layer charge in pure monomineralic samples, but not in samples with a mixed mineralogy. The decrease in montmorillonite CEC/layer charge by lithium/thermal treatment is termed the Hofmann-Klemen effect after Hofmann & Klemen (1950). Lithium charge reduction makes it possible to vary the CEC/layer charge of a particular clay sample and examine the effect of layer charge on other properties. Samples of <2μm SAz-1 montmorillonite with 35% Li/65% Na and 50% Li/50% Na on the cation exchange sites were prepared according to the method used by Jaynes & Bigham (1987). The 0.35Li,0.65Na-SAz and 0.50Li,0.50Na-SAz samples were heated at 250 °C in quartz glass crucibles to produce about 35% and 50% decreases in CEC. The reduced-charge SAz clays formed were designated 0.35Li-SAz and 0.50Li-SAz. The 0.35Li-SAz sample should have a CEC/layer charge comparable to SWy and the 0.50Li-SAz sample should have a CEC/layer charge about 20% less than SWy.

2.2 AfB1 adsorption from water

Batch adsorption isotherms (6 points in triplicate with 4 blanks) from water were prepared with an initial concentration of 1 μg AfB1/mL in 5-mL aqueous dispersions. The aqueous dispersions contained 10 to 180 μg of clay or activated carbon in 15-mL polypropylene centrifuge tubes. A stock solution containing 100 μg AfB1/mL in acetonitrile was prepared and aliquots of the stock solution were diluted with water and used to deliver aflatoxin to isotherm solutions (50 μL stock + 0.95 mL of water = 5 μg AfB1/mL). Blanks containing only AfB1 and water were prepared and samples and blanks were thoroughly mixed using a

vortex mixer. After overnight agitation on a reciprocating shaker, the tubes were centrifuged, and the supernatants were passed through 0.2-µm filters and collected in 20-mL plastic vials. The AfB1 adsorption data were fitted to the Langmuir equation and monolayer adsorption capacities (X_m) were calculated (Hiemenz, 1986). Grant & Phillips (1998) similarly used the Langmuir equation and other model equations to fit data from AfB1 adsorption to Novasil from water isotherms.

2.3 AfB1 retention from aqueous corn meal with 60% methanol extraction

Aflatoxin retention from aqueous corn meal dispersions after 60% methanol extraction was used as a more applied and more conservative measure of aflatoxin binding to feed additives. Batch adsorption isotherms from aquous corn meal (6 points in triplicate with 4 blanks) were prepared with an initial concentration of 1.5 µg AfB1/mL (3 µg AfB1/g corn meal) in 2-mL aqueous dispersions containing 1 to 20 mg (0.1 to 2%) clay or 5 to 100 mg (0.5 to 10%) of activated carbon. Blanks were prepared similarly using only AfB1, corn meal, and water. The 3 µg AfB1/g corn meal is comparable to highly-contaminated (3000 µg aflatoxins/kg) grain. The samples were thoroughly mixed using a vortex mixer. After overnight agitation on a reciprocating shaker, 8 mL of a 60% methanol/40% 2M NaCl extracting solution were mixed with the samples using a vortex mixer. The tubes were centrifuged and the supernatants passed through 0.2-µm filters and collected in 20-mL plastic vials. Aflatoxins are more soluble in methanol than in water. The 60% methanol extraction was modified from the Asis et al. (2002) procedure to extract and measure aflatoxins in peanuts. The AOCS method for aflatoxins in corn, cottonseed, peanuts, and peanut butter similarly uses an 80% methanol/20% water extraction (AOCS, 1999b). The modified procedure first equilibrates AfB1/corn meal/clay in water (more like the environment of ingested aflatoxins) prior to extraction with 60% methanol. This contrasts with the immediate 60% methanol extraction used by Asis et al. (2002). The AfB1 adsorption data were fitted to a line using least squares linear regression and the slopes or adsorption coefficients (K_d) were calculated to compare relative adsorption capacities. Three-point isotherms were used to measure the effect of layer charge on AfB1 retention by natural and reduced-charge clays.

2.4 AfB1 retention from water with 60% methanol extraction

Clay and activated carbon samples (2 mg) in 15-mL polypropylene centrifuge tubes were equilibrated in 2 mL of water containing 60 µg AfB1 and later extracted with 8 mL of 60% methanol to distinguish between the effects of aqueous corn meal and 60% methanol extraction on AfB1 adsorption. A control sample with 60 µg AfB1 in 2 mL of water was similarly extracted with 8 mL of 60% methanol. Blanks containing 2 mg of clay or activated carbon without AfB1 were used to compensate for minor UV absorbance at 360 nm caused by trace amounts of suspended clay. Samples, blanks, and control were centrifuged to sediment suspended clay/activated carbon after overnight equilibration on a shaker and the addition of 8 mL of 60% methanol. The amounts of AfB1 retained by the clays and activated carbon were measured using UV/visible spectroscopy based on differences in the absorbance of AfB1 at 360 nm between samples and the control.

2.5 ELISA AfB1 measurement

A modification of the Asis et al. (2002) enzyme-linked immunoassay (ELISA) method was used for measuring aflatoxins. The modified method is thoroughly described in Jaynes et al.

(2007). Briefly, 96-well polystyrene microplates were coated with AfB1-BSA conjugate, washed, and saved for later use. In the first step, aflatoxin standards, sample solutions, and anti-AfB1 antibody were added to AfB1-BSA coated plates. The method is a competitive ELISA technique because AfB1 in solution (from standards or samples) competes with AfB1-BSA bound to the microplates for rabbit anti-AfB1 antibody. The AfB1 in high AfB1-concentration samples or standards binds to most of the rabbit anti-AfB1 antibody, which is subsequently washed out of the microplates. The AfB1 in low AfB1-concentration samples or standards does not bind as much of the anti-AfB1 antibody as the AfB1-BSA attached to the plates and most of the anti-AfB1 antibody is retained in the microplate. Goat anti-rabbit-HRP antibody is then added to the microplates and binds to any rabbit anti-AfB1 antibody that is attached to the microplates. Any unattached goat anti-rabbit-HRP is subsequently washed from the microplates. In the final step, OPD substrate is added to the microplates and the horse radish peroxidase enzyme in the goat anti-rabbit-HRP that is attached to the plates catalyzes color development. The optical densities of the colored solutions that are produced are inversely proportional to AfB1 concentration. The high AfB1-concentration standards and samples are lightly-colored and the blanks are darkly-colored. The optical densities are measured using a microplate reader and AfB1 concentrations are calculated based on a plot of standard concentration versus optical density.

2.6 Treatment of clays and activated carbon with corn and peanut meal water extracts

Corn and peanut meal extracts from 100-g samples were used to treat 2-g clay and activated carbon samples to model feed samples ingested with 2% feed additive. Corn and peanut meal samples (100 g) were suspended in 800 mL of deionized water and agitated on a shaker for 1 hour. The corn and peanut meal suspensions were centrifuged and filtered to remove solids and mixed with samples (2 g) of Novasil plus, SWy, SAz, Astra Ben 20A, SepSp, and activated carbon. The clay and activated carbon samples that were treated with corn and peanut meal extracts were stirred for 1 hour, centrifuged, and washed with deionized water 3 times to remove unadsorbed material, frozen, and freeze-dried.

2.7 Basal spacings, organic carbon contents, and specific surface areas

Oriented clay samples were prepared by air-drying suspensions on glass slides. The SWy-PNA sample was prepared by treating 30 mg of SWy dispersed in 5 mL of deionized water with 10 mg of Sigma-Aldrich peanut agglutinin dissolved in pH 5 acetate buffer. The SWy-PNA sample suspension was washed three times with deionized water before drying on a glass slide. The glass slides were further dried in a vacuum dessicator over anhydrous $CaCl_2$ and stored until collection of X-ray diffraction patterns. X-ray diffraction patterns were collected for the oriented clays by scanning from 2 to 15 °2θ using CuKα radiation and a Philips or Rigaku diffractometer interfaced to a computer. Organic carbon contents were measured using a LECO carbon analyzer. Clay and activated carbon samples were outgassed at 120 °C and nitrogen specific surface areas were measured by the single-point method using a Micromeritics Flowsorb II model 2300 surface area meter using a 30% N_2 / 70% He carrier gas and liquid N_2. The N_2 surface areas of expandable clays, such as montmorillonite, are attributed to external surfaces because the interlayers collapse under the dry conditions required for measurement. Specific surface areas were also measured by ethylene glycol monoethyl ether (EGME) adsorption using the method of Carter et al. (1986). Samples were dried over P_2O_5 in a vacuum dessicator, weighed, and subsequently transferred to another vacuum dessicator with $CaCl_2$-EGME solvate. Three mL of EGME

were added to the (~1g) samples and excess unadsorbed EGME was removed under vacuum until constant weight was achieved. Surface areas were calculated using the weight of adsorbed EGME. Specific surface areas measured using EGME are attributed to total surface area because EGME is adsorbed into and expands the interlayers.

3. Results and discussion

3.1 AfB1 adsorption from water

The adsorption of AfB1 from water by activated carbon (X_m=179 g/kg) and the montmorillonites Novasil (X_m=248 g/kg), SWy (X_m=239 g/kg), and SAz (X_m=166 g/kg) were comparable, but SepSp (X_m=87 g/kg, sepiolite) adsorbed much less AfB1 (Figure 2). Monolayer AfB1 adsorption capacities (X_m) calculated from a fit of the data to the Langmuir model indicate relative AfB1 adsorption by the materials. The relative amounts of AfB1 adsorbed from water do not correlate with feeding study results. Animal feeding studies have shown that Novasil and sepiolite both effectively reduce or prevent aflatoxin toxicity, but activated carbon was not effective. Although Novasil and sepiolite were equally effective in a feeding study by Schell et al. (1992a), the sepiolite (SepSp) adsorbed far less AfB1 from water than Novasil. Simple water adsorption isotherms are clearly relevant to environmental contaminants, but not necessarily to ingested toxins. The relative amounts of aflatoxins that evidently must bind to feed additives in animal feeding studies seem unrelated to maximum adsorption from water.

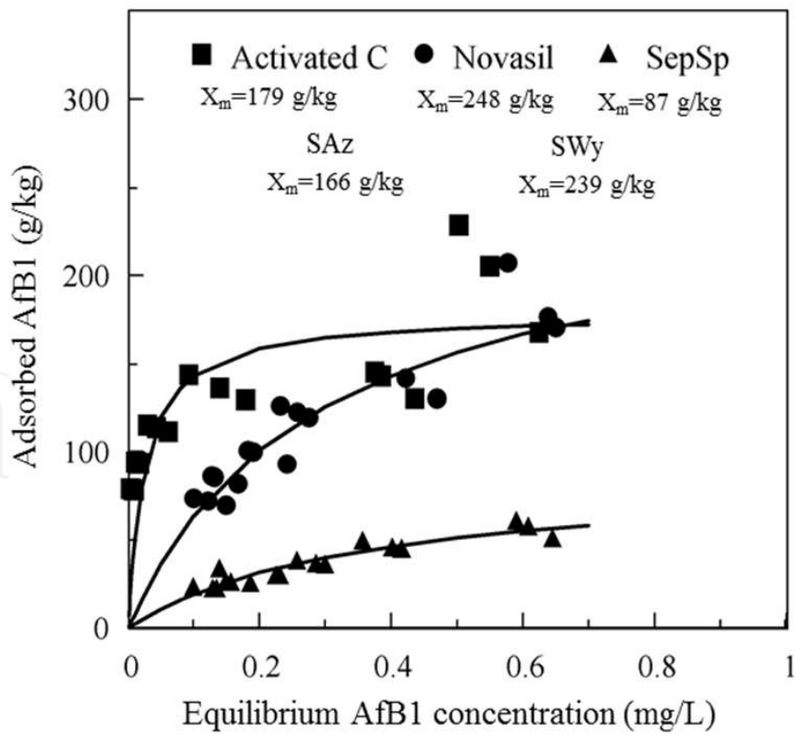


Fig. 2. Aflatoxin B1 adsorption to activated carbon, Novasil, SepSp, SWy, and SAz from water fitted to Langmuir model with monolayer adsorption capacities (X_m) calculated from Langmuir fit parameters.

3.2 AfB1 retention from aqueous corn meal after 60% methanol extraction

The amounts of AfB1 retained by the clays and activated carbon was 100 times less from aqueous corn meal extracted with 60% methanol (Figure 3) than from water (Figure 2); data were fitted to a linear model with adsorption coefficients (K_d) calculated from the linear fit.

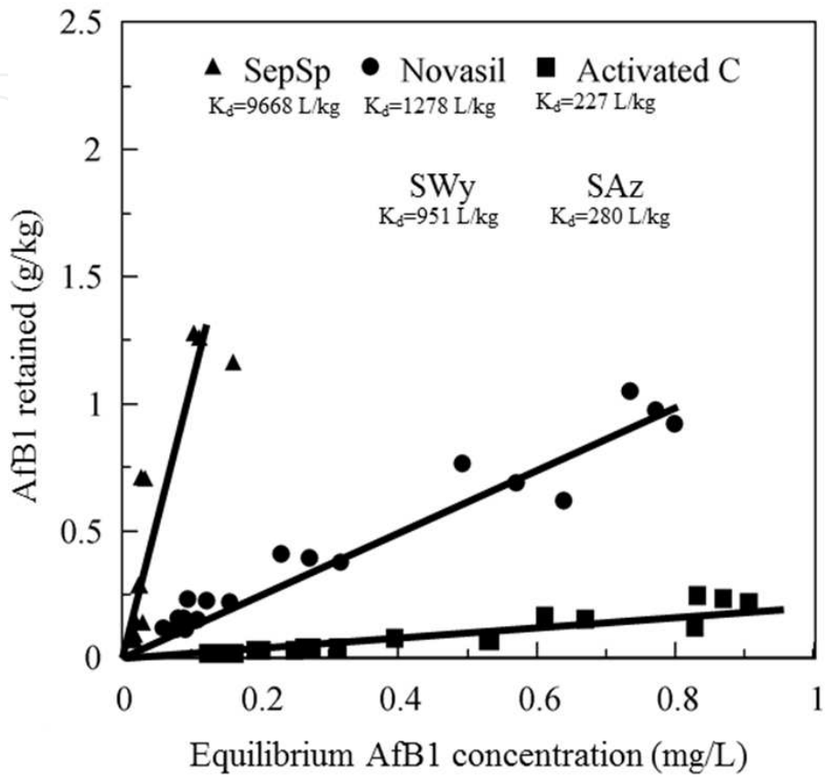


Fig. 3. Aflatoxin B1 retention from aqueous corn meal with 60% methanol extraction.

However, the relative amounts of AfB1 retained by Novasil (K_d=1278 L/kg), SepSp(K_d=9668 L/kg), and activated carbon (K_d=227 L/kg) are more consistent with animal feeding studies. Novasil and sepiolite effectively reduced aflatoxin toxicity in feeding studies and retained much more AfB1 from corn meal than activated carbon which was not effective. Aflatoxin adsorption from aqueous corn meal appears to model ingested aflatoxins more effectively than aflatoxin adsorption from water. The reference low- and high-charge montmorillonite samples, SWy (K_d=951 L/kg) and SAz (K_d=280 L/kg), retained significantly different amounts of AfB1. The SWy sample has a layer charge similar to Novasil and retained comparable amounts of AfB1. The high-charge SAz sample retained much less AfB1 that is comparable to the activated carbon. This suggests that high-charge montmorillonites would not reduce aflatoxin toxicity in ingested feed. However, no animal feeding studies have been identified that directly examined the effect of feed additive layer charge on toxicity reduction.

3.3 AfB1 retention from water after 60% methanol extraction

Both the soluble compounds in corn meal and extraction with 60% methanol probably contributed to the lower amounts of AfB1 retained by the clays and activated carbon in the

aqueous corn meal adsorption isotherms (Figure 3) relative to the water isotherms (Figure 2). Aflatoxins are more soluble in methanol than in water and methanol might desorb weakly-adsorbed AfB1. To separate the effects of corn meal and methanol extraction, AfB1 adsorption from water to Novasil plus, SepSp, and activated carbon with 60% methanol extraction were measured (Figure 4). The Control, Novasil plus, SepSp, and activated carbon samples initially contained ~60 µg AfB1 in 2 mL of water. After overnight equilibration and the addition of 8 mL of 60% methanol, the control AfB1 concentration was 6.07 µg/mL. Adsorption of AfB1 by Novasil plus, SepSp, and activated carbon decreased the AfB1 concentration and the absorbance at 360 nm. Activated carbon (26.1 g/kg) retained far more AfB1 than Novasil plus (6.7 g/kg) and SepSp (4.0 g/kg), which indicates that 60% methanol extraction affected AfB1 retention by activated carbon less than the clays. Therefore, aqueous corn meal must reduce AfB1 retention to activated carbon more than methanol extraction. Activated carbon is generally used late in wastewater treatment after most organic materials (e.g. proteins, natural organic matter) have been removed by secondary wastewater treatment (Manahan, 2000). Activated carbon can be fouled or preloaded by the organic matter in untreated wastewater, which can limit uptake of pollutants, such as herbicides. Aqueous corn meal or feed contains a variety of soluble compounds, such as proteins, that can adsorb to the surface of activated carbon and limit access to internal adsorption sites. Soluble feed compounds can also adsorb to clays and block potential aflatoxin adsorption sites, but might not reduce aflatoxin adsorption to some clays as much as to activated carbon.

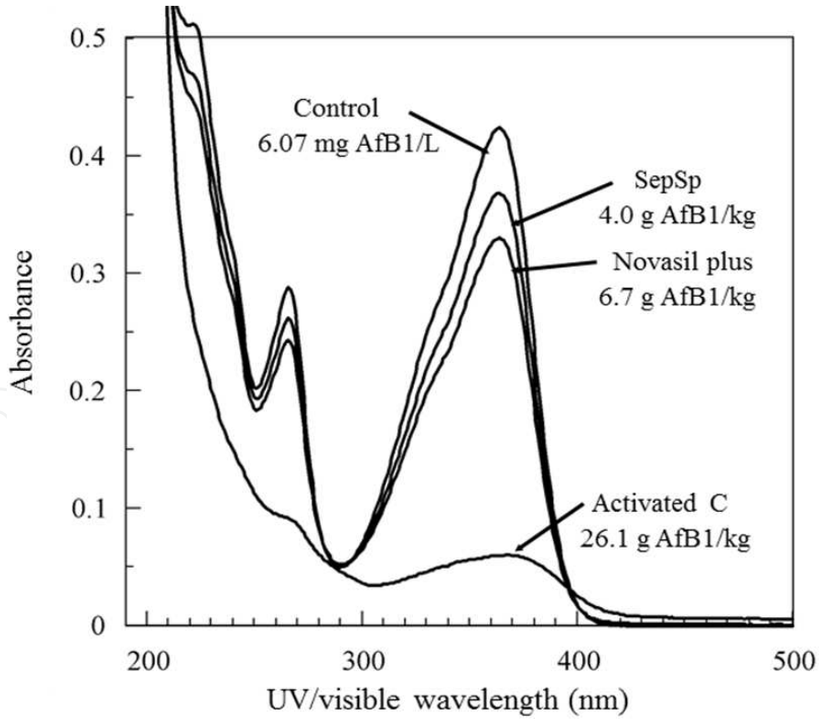


Fig. 4. Spectra of aqueous Control, Novasil plus, SepSp, and activated carbon samples (2 mg) after adsorption of aflatoxin B1 and extraction with 60% methanol. Maximum absorbance of aflatoxin B1 is at 360 nm. The amounts of aflatoxin adsorbed were calculated from the sample weights and absorbance differences from the control.

3.5 Corn and peanut meal water-extract-treated clay and activated carbon samples

The increased %C and %N contents after treatment with corn and peanut meal extracts indicate that soluble compounds were adsorbed to the clays and activated carbon (Table 1). The increased N contents suggest proteins were adsorbed. The higher protein content of defatted peanut meal (45 – 60%) relative to corn meal (8%) is reflected by the greater C and N contents of Novasil plus treated with peanut meal extract. Peanut agglutinin protein (PNA) is a 110,000 molecular weight lectin that contains four identical subunits. The pure PNA protein is readily available and is used to distinguish between normal and tumor tissues and for other medical uses (Vector Labs, 2011). X-ray diffraction patterns of SWy-Na, SWy-Mg, and SWy treated with PNA protein in Figure 5a are shown for comparison with the X-ray patterns of Novasil plus treated with corn meal and peanut meal extracts. Magnesium-and sodium-saturated SWy had basal spacings of 1.57 nm and 1.26 nm. Treatment with PNA protein greatly expanded the basal spacing to 2.65 nm, which indicates a ~1.65 nm interlayer thickness. Novasil plus (Figure 5b) has a basal spacing intermediate between SWy-Mg and SWy-Na, which indicates a mixture of Ca/Mg and Na. The Novasil plus sample was washed with NaCl, but apparently not enough to replace all of the Ca/Mg. Treatment with corn meal extract broadened, but did not appreciably shift the basal spacing of Novasil plus.

Sample	d001	C	N
	nm	%	%
Novasil plus	1.38	0.04	0.00
Novasil plus + corn extract	1.33	6.07	1.42
Novasil plus + peanut extract	2.56	22.10	5.83
SWy	nd	0.06	0.00
SWy + corn extract	nd	5.76	0.90
SWy-Na	1.26	nd	nd
SWy-Mg	1.57	nd	nd
SWy-HDTMA*	1.77	17.46	nd
SWy-PNA	2.65	nd	nd
SWy-ricin*	3.53	nd	nd
SAz	nd	0.07	0.00
SAz-HDTMA*	2.29	23.00	nd
SAz-PVP*	2.30	nd	nd
SAz + corn extract	nd	4.64	1.08
Astra Ben 20A	nd	0.04	0.01
Astra Ben 20A	nd	6.99	1.42
SepSp	na	0.12	0.00
SepSp + corn extract	na	4.27	0.83
Activated Carbon	na	78.40	0.68
Activated Carbon + corn extract	na	79.10	1.51

*na = not applicable, nd = not determined; SWy-ricin data from Jaynes et al., 2005; SWy-HDTMA and SAz-HDTMA data from Jaynes & Boyd 1991; and SAz-PVP data from Blum & Eberl 2004.

Table 1. Carbon and nitrogen contents from chemical analysis and d001 basal spacings from X-ray diffraction.

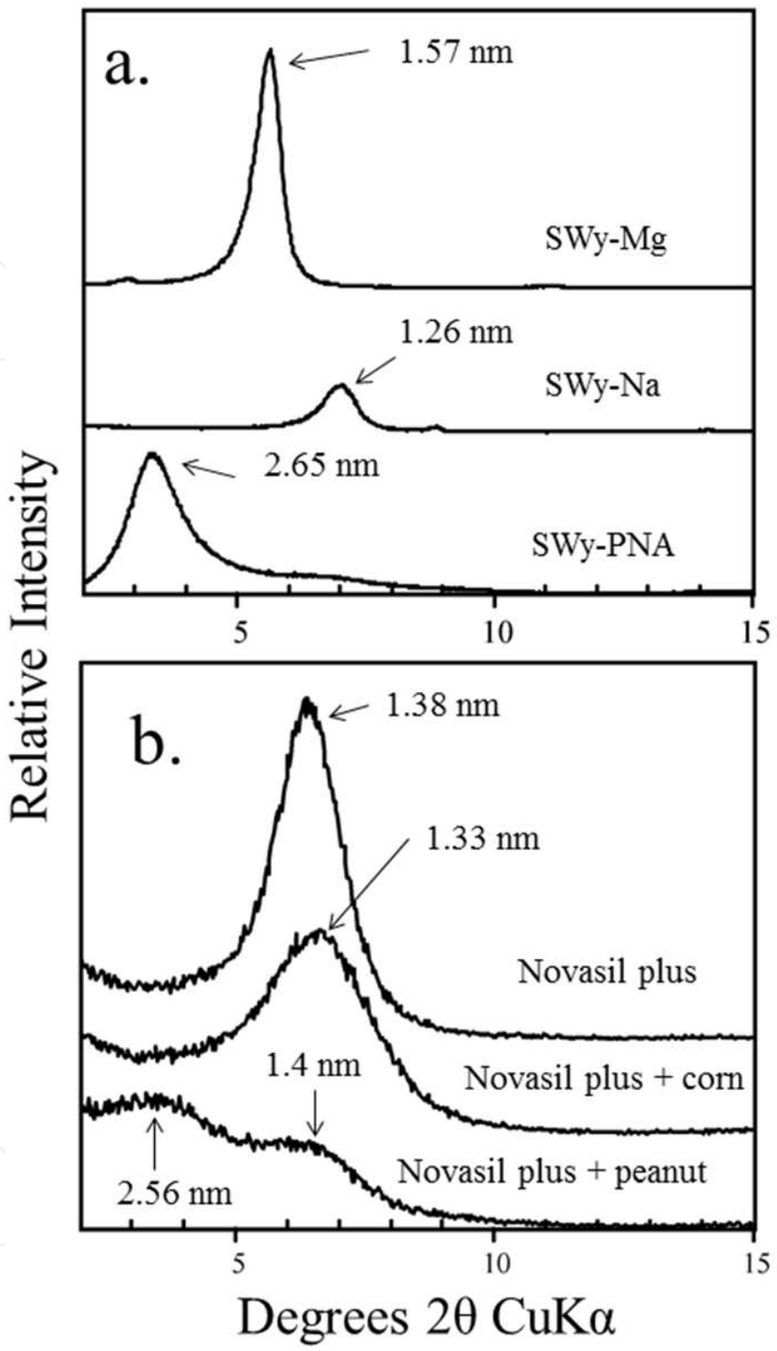


Fig. 5. X-ray diffraction patterns of a) SWy-Mg, SWy-Na, SWy-PNA; and b) Novasil plus, Novasil plus treated with corn meal extract, and Novasil plus treated with peanut meal extract.

Treatment with peanut meal extract yielded a broad peak centered at 2.56 nm similar to the 2.65-nm SWy-PNA peak. The peanut meal extract contains PNA protein, but also other proteins and soluble compounds. Adsorption of pure PNA protein yielded a sharp and intense 2.65 nm peak (Figure 5a), but adsorption of the mixture of soluble compounds in peanut meal

extract yielded relatively weak, broad peaks. Proteins are polymers of amino acids and amino acid side-chain properties differ greatly. The 20 common amino acids have acidic, basic, aromatic, and aliphatic side chains (Stryer, 1975). Clays, such as montmorillonite, have negative charge sites compensated by inorganic cations. Basic amino acid side chains in proteins are protonated at pHs below the isoelectric point and can impart a net positive charge to the protein molecule. At pHs above the isoelectric point, proteins have a net negative charge. Proteins that have a large proportion of basic amino acid residues might adsorb more strongly to clays. The toxic castor seed globular protein (ricin) has an isoelectric point of 7.1 and contains 529 amino acid residues of which 43 are the basic amino acids histidine, lysine, and arginine (Merck, 2001; Katzin et al., 1991; Rutenber et al., 1991). Jaynes et al. (2005) examined castor seed protein adsorption to clay minerals and showed that adsorption was pH dependent. Castor protein adsorption at pHs below the isoelectric point (pH 5 and 7) was much greater than at pH 10 (Figure 6). Similarly, Garwood et al. (1983) adsorbed ~970 g/kg of the protein, glucose oxidase, to a Na-montmorillonite at pHs below the isoelectric point, which expanded the basal spacing to ~4.4 nm. The adsorption of purified peanut PNA protein and castor seed protein at pH 5 increased the basal spacing of SWy from 1.2 nm to 2.6 and from 1.2 nm to 3.5 nm (Table 1), respectively. Aflatoxin adsorption to clays does not show much pH dependence. However, protein adsorption to clays is pH dependent and might secondarily result in pH dependent aflatoxin adsorption.

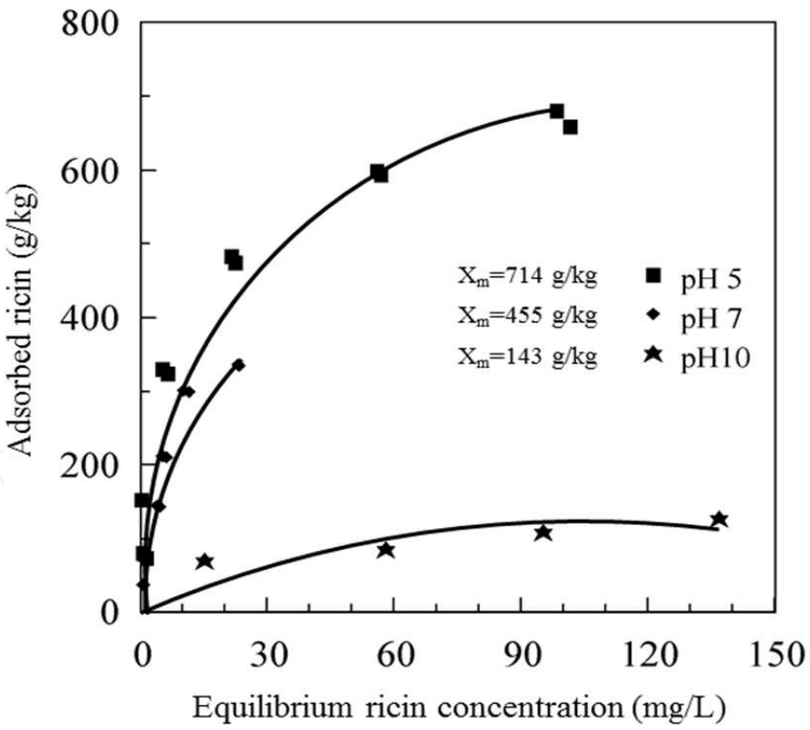


Fig. 6. Effect of solution pH on adsorption of castor protein (ricin) to SWy montmorillonite. Monolayer adsorption capacities (X_m) calculated from Langmuir model fit parameters.

The SepSp and activated C samples were not examined using X-ray diffraction because these samples are structurally different than montmorillonites and do not have basal spacings. The N_2 and EGME surface areas of the clays and activated carbon decreased after

treatment with corn and peanut meal water extracts (Table 2). The adsorption of BSA protein and AfB1 generally decreased after treatment with corn and peanut meal extracts, which suggests that potential BSA and AfB1 adsorption sites were blocked by compounds that were adsorbed from the water extracts.

3.6 Layer charge and AfB1 retention from aqueous corn meal

Greater AfB1 retention by low-charge SWy than by high-charge SAz (Figure 3) suggests that aflatoxin adsorption to clays might be related to layer charge. The natural clays SHCa, SWy, SAz, and VSC range from low-charge to very high charge. The retention of AfB1 from aqueous corn meal decreased as layer charge increased (Figure 7). Aflatoxin retention by the high-charge vermiculite (VSC) was negligible compared to the low-charge hectorite (SHCa). The layer charge of hectorite (0.34 eq/$O_{10}(OH)_2$ formula unit) is about one-half of the South Carolina vermiculite (0.66 eq) layer charge (Laird et al., 1989). The layer charge of SWy (0.32 eq) is about 2/3 of the SAz (0.47 eq) layer charge (Laird et al., 1989). The effect of layer charge on AfB1 adsorption can also be determined using reduced-charge samples of one clay sample. The retention of AfB1 by SAz and reduced charge SAz was inversely proportional to layer charge (Figure 8). The 0.50LiSAz sample retained much more AfB1 than SAz. Low-charge clays more effectively adsorb AfB1 than high-charge clays. Lithium charge reduction would not be practical for clay feed additives, but layer charge might be used to identify natural clays that can effectively bind aflatoxins in feed. Other clay mineral properties, such as exchangeable cation and charge location (octahedral vs. tetrahedral), might also affect AfB1 adsorption.

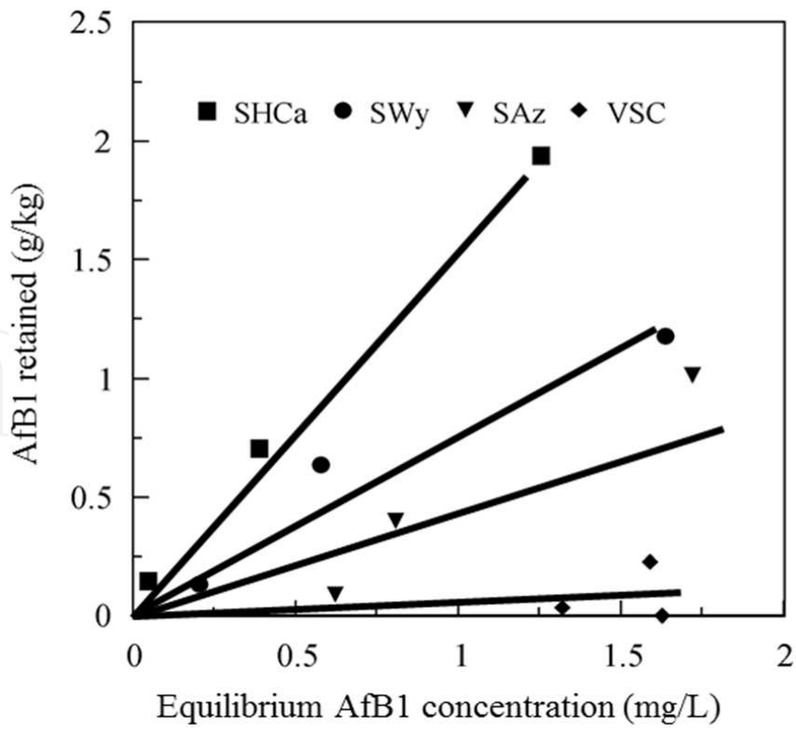


Fig. 7. Aflatoxin B1 retention from aqueous corn meal by natural clay samples after 60%methanol extraction with layer charge decreasing in the order VSC > SAz > SWy > SHCa.

Sample	N_2 surface area	EGME surface area	BSA adsorption	AfB1 adsorption
	m_2/g	m_2/g	g/kg	g/kg
Novasil plus	70	721	192	28
Novasil plus + corn extract	16	573	34	16
Novasil plus + peanut extract	8	167	11	14
SWy	29	691	192	29
SWy + corn extract	12	366	77	22
Astra Ben 20A	93	735	213	nd
Astra Ben 20A + corn extract	4	333	141	nd
SAz	63	715	212	nd
SAz + corn extract	55	112	152	nd
SepSp	250	460	53	6
SepSp + corn extract	92	385	77	3
Activated C	658	881	21	40
Activated C + corn extract	272	573	5	16
Activated C + peanut extract	180	494	11	10

Table 2. Nitrogen (N_2) and ethylene glycol monoethyl ether (EGME) surface areas, bovine serum serum (BSA) protein adsorption and aflatoxin B1 adsorption to clays and activated carbon. *nd = not determined.

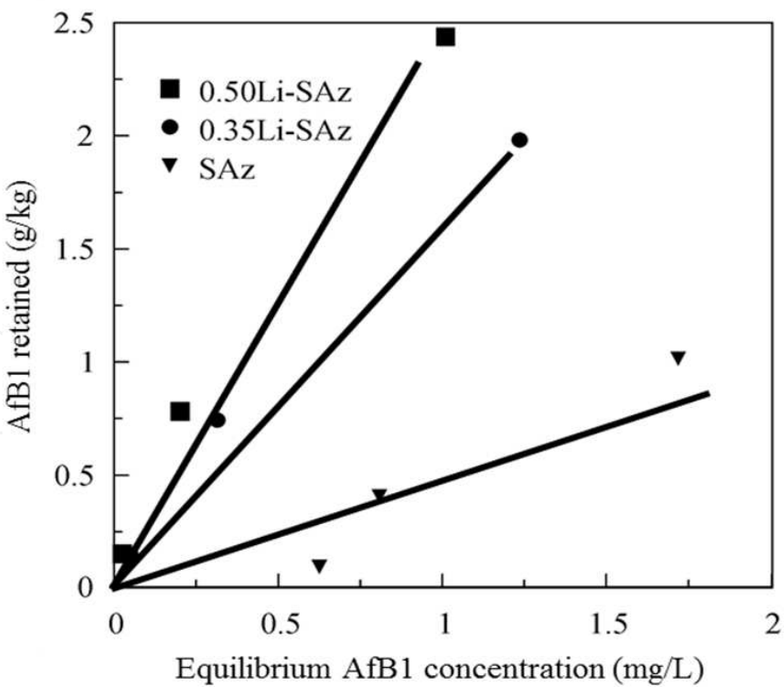


Fig. 8. Aflatoxin B1 retention from aqueous corn meal by natural (SAz) and reduced-charge SAz samples after 60% methanol extraction. Layer charge decreases in the order SAz > 0.35Li-SAz > 0.50Li-SAz.

4. Conclusions

The relative amounts of aflatoxin B1 retained from aqueous corn meal by clays and activated carbon after 60% methanol extraction were consistent with animal feeding study results, but animal feeding study results were not consistent with adsorption from water. Aflatoxin retention from aqueous feed seems to model aflatoxin adsorption to ingested feed additives more effectively than simple adsorption from water. Clay and activated carbon samples that were treated with aqueous extracts of corn and peanut meal had increased C and N contents and decreased surface areas. Smectite interlayer basal spacing was increased after treatment with the peanut extracts. The adsorption of bovine serum albumin protein and aflatoxin B1 also decreased after corn/peanut meal extract treatment. These data indicate that soluble compounds in corn and peanut meal extracts adsorbed to clay and activated carbon surfaces and adversely affected aflatoxin binding. Low-charge smectites, such as Novasil plus and reduced-charge SAz, retained more aflatoxin B1 from aqueous corn meal than higher charge minerals, such as vermiculite (VSC) and high-charge smectite (SAz). The selection of low-charge smectite feed additives might assure greater aflatoxin binding and toxicity reduction. The use of *in vitro* aflatoxin adsorption tests from aqueous feed might be used to identify feed additives that can effectively bind aflatoxins in ingested feed.

The correlation between animal feeding study results and *in vitro* aflatoxin adsorption from feed tests might be improved by use of gastrointestinal fluids or other factors to make the chemical environment of feed additives in adsorption tests more like ingested toxins. However, adsorption of soluble feed compounds to feed additives appears to have a strongly adverse effect on aflatoxin binding to ingested feed additives.

5. References

Abdel-Wahhab, M.A., Nada, S.A., & Amra, H.A. 1999. Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in rat. *J. Appl. Toxicol.* 19, 199-204.

Abrahams, P.W., & Steigmajer, J. 2003. Soil ingestion by sheep grazing the metal enriched floodplain soils of mid-Wales. *Environ. Geochem. Health* 25, 17-24.

AOCS, 1999a. Aflatoxin standards. Official method Aj 0-88. Sampling and Analysis of Vegetable Oil Source Materials. American Oil Chemists Society.

AOCS, 1999b. Total aflatoxins (B1, B2, and G1) in corn, cottonseed, peanuts, and peanut butter. Official method Aj 6-95. Sampling and Analysis of Vegetable Oil Source Materials. American Oil Chemists Society.

Arriola, M.C., Porres, E., Cabrera, S., Zepeda, M., & Rolz, C. 1988. Aflatoxin fate during alkaline cooking of corn for tortilla preparation. *J. Agric. Food Chem.* 36, 530-533.

Asis, R., Di Paola, R.D., & Aldao, M.A.J. 2002. Determination of aflatoxin B1 in highly contaminated peanut samples using HPLC and ELISA. *Food and Agricultural Immunology* 14, 201-208.

Bailey, C.A., Latimer,G.W., Barr, A.C., Wigle, W.L., Haq, A.U., Balthrop, J.E. & Kubena, L.F. 2006. Efficacy of montmorillonite clay (Novasil plus) for protecting full-term broilers from aflatoxicosis. *J. Appl. Poult. Res.* 15, 198-206.

Bailey, S.W. 1980. Summary of recommendations of AIPEA nomenclature committee. *Clays Clay Miner*. 28, 73-78.

Bates, R.L. 1969. Clay. pp. 117-155. In: *Geology of The Industrial Rocks and Minerals*. Dover, New York.

Bell, B., Muhle, H., & Ernst, H. Investigations on health-related properties of two sepiolite samples. *Environ. Health Perspect.* 105 (Suppl 5) 1049-1052.

Bennett, J.W., & Klich, M. 2003. Mycotoxins. *Clinical Microbiology Reviews* 16, 497-516.

Blum, A.E., & Eberl, D.D. 2004. Measurement of clay surface areas by polyvinylpyrrolidone (PVP) sorption and its use for quantifying illite and smectite abundance. *Clays Clay Miner*. 52, 589-602.

Boettinger, J.L., & Ming, D.W. 2002. Zeolites. pp. 585-610. In Dixon, J.B., & Schulze, D.G. (eds.) *Soil Mineralogy with Environmental Applications*. SSSA Book Series, no. 7. Soil Science Society of America, Madison, WI.

Bonna, R.J., Aulerich, R.J., Bursian, S.J., Poppenga, R.H., Braselton, W.E., & Watson, G.L. 1991. Efficacy of hydrated sodium aluminosilicate and activated charcoal in reducing the toxicity of dietary aflatoxin to mink. *Arch. Environ. Contam. Toxicol.* 20, 441-447.

Bradford, M.M. 1976. A refined and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248-254.

Brekke, O.L., Sinnhuber, R.O., Peplinski, A.J., Wales, J.H., Putnam, G.B., Lee, D.J., & Ciegler, A. 1977. Aflatoxin in corn: ammonia inactivation and bioassay with rainbow trout. *Appl. Environ. Microbiol*. 34, 34-37.

Carter, D.L., Mortland, M.M., & Kemper, W.D. 1986. Specific surface. pp. 413-423 In: *Methods of Soil Analysis: Part I. Physical and Mineralogical Methods*, 2nd edtiion (A. Klute, editor). Soil Science Society of America, Madison, WI.

Chenu, C., Pons, C.H., & Robert, M. 1985. Interaction of kaolinite and montmorillonite with neutral polysaccharides. In: Schultz, L.G., van Olphen, H., & Mumpton, F.A. (eds.) *Proceedings of the International Clay Conference 1985, Denver, CO.* pp. 375-381, The Clay Minerals Society, Bloomington, IN.

Dalvi, R.R. & Ademoyero, A.A. 1984. Toxic effect of aflatoxin B1 in chickens given feed contaminated with *Aspergillus flavus* and reduction of the toxicity by activated charcoal and some chemical agents. *Avin Dis.* 28, 61-69.

Dalvi, R.R. & McGowan, C. 1984. Experimental induction of chronic aflatoxicosis in chickens by purified aflatoxin B1 and its reversal by activated charcoal, phenobarbitol and reduced glutathione. *Poult. Sci.* 63, 485-491.

Decker, W.J. 1980. Activated charcoal adsorbs aflatoxin B1. *Vet. Hum. Toxicol.* 22, 388-389.

Deng, Y., Barrientos Velazquez, A.L., Billes, F., & Dixon, J.B. 2010. Bonding mechanisms between aflatoxin B1 and smectite. *Appl. Clay Sci.* 50, 92-98.

Diaz, D.E. & Smith, T.K. 2005. Mycotoxin sequestering agents: Practical tools for the neutralisation of mycotoxins. In Diaz, D.E. (editor) *The Mycotoxin Blue Book*. pp 323-340. Nottingham University Press.

Diaz, D.E., Hagler, W.M., Blackwelder, J.T., Eve, J.A., Hopkins, B.A., Anderson, K.L., Jones, F.T., & Whitlow, L.W. 2004. Aflatoxin binders II: reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia*, 157, 233-241.

Dixon, J.B., Kannewischer, Tenorio Arvide, M.G., & Barrientos Velazquez, A.L. 2008. Aflatoxin sequestration in animal feeds by quality-labeled smectite clays: an introductory plan. *Appl. Clay Sci.* 40, 201-208.

Eberl, D.D., Nüesch, R, Ŝuchá, V., & Tsipursky, S. 1998. Measurement of fundamental illite particle thicknesses by X-ray diffraction using PVP-10 intercalation. *Clays Clay Miner*. 46, 89-97.

Edrington, T.S., Sarr, A.B., Kubena, L.F., Harvey, R.B., & Phillips, T.D. 1996. Hydrated sodium calcium aluminosilicate (HSCAS), acidic HSCAS, and activated charcoal reduce urinary excretion of aflatoxin M1 in turkey poults. Lack of effect by activated charcoal on aflatoxicosis. *Toxicol. Lett.* 89, 115-122.

Fairchild, A.S., Croom, J., Grimes, J.L., & Hagler, W.M. 2008. Effect of Astra-Ben 20A on broiler chicks exposed to aflatoxin B1 or T2 toxin. *Int. J. Poult. Sci.* 7, 1147-1151.

Gallo, A., Masoero, F., Bertuzzi, T., Piva, G., & Pietra, A. 2010. Effect of the inclusion of adsorbents on aflatoxin B1 quantification in animal feedstuffs. *Food Additives and Contaminants* 27, 54-63.

Garwood, G.A., Mortland, M.M., & Pinnavaia, T.J. 1983. Immobilization of glucose oxidase on montmorillonite clay: hydrophobic and ionic modes of binding. *Journal of Molecular Catalysis*, 22, 153-163.

Grant, P.G. & Phillips, T.D. 1998. Isothermal adsorption of aflatoxin B1 on HSCAS clay. *J. Agric. Food Chem.* 46, 599-605.

Grove, M.D., Plattner, R.D., & Weisleder, D. 1981. Ammoniation products of an aflatoxin model coumarin. *J. Agric. Food Chem.* 29,1161-1164.

Hartley, R.D., Nesbitt, B.F., & O'Kelly, J. 1963. Toxic metabolites of *Aspergillus flavus*. *Nature* 198,1056-1058.

Hatch, R.C., Clark, J.D., Jain, A.V., & Weiss, R. 1982. Induced acute aflatoxicosis in goats: treatment with activated charcoal or dual combinations of oxytetracycline, stanozol and activated charcoal. *Am. J. Vet. Res.* 43, 644-648.

Herrera, M., Zyman, J., Pena, J.G., Segurajauregui, J.S., & Vernon, J. 1986. Kinetic studies on the alkaline treatment of corn (Zea mays) for tortilla preparation. *J. Food Sci*. 51, 1486-1490.

Hiemenz, P.C. 1986. *Principles of Colloid and Surface Chemistry*. Marcel Dekker, New York, 398-407.

Hofmann, U. & Klemen, R. 1950. Verlust der Austauschfahigkeit von Lithiumen an Bentonit durch Erhitzung: *Z. Anorg. Allg. Chem*. 262, 95-99.

Hosterman, J.W., & Patterson, S.H. 1992. Bentonite and fuller's earth resources of the United States: *U.S. Geological Survey Professional Paper* 1522. U.S. Government Printing Office.

IARC. 1993. Aflatoxins: Naturally occurring aflatoxins (Group 1), aflatoxins M1 . *Int. Agency Res. Cancer* 56, 245.

Jaynes, W.F. & Bigham, J.M. 1987. Charge reduction, octahedral charge, and lithium retention in heated, Li-saturated smectites. *Clays Clay Miner.* 35, 440-448.

Jaynes, W.F., & Boyd, S.A. 1991. Clay mineral type and organic compound sorption by hexadecyltrimethylammonium-exchanged clays. *Soil Sci. Soc. Am. J.* 55, 43-48.

Jaynes, W.F., Zartman, R.E., & Hudnall, W.H. 2007. Aflatoxin B1 adsorption by clays from water and corn meal. *Appl. Clay Sci*. 36, 197-205.

Jaynes, W.F., Zartman, R.E., Green, C.J., San Francisco, M.J., & Zak, J.C. 2005. Castor toxin adsorption to clay minerals. *Clays Clay Miner.* 53, 268-277.

Katzin, B.J., Collins, E.J., & Robertus, J.D. 1991. The structure of ricin A chain at 2.5 Å. *Proteins: Structure, Function, and Genetics*, 10, 251-259.

Kubena, L.F., Harvey, R.B., Phillips, T.D., Corrier, D.E., & Huff, W.E. 1990. Diminution of aflatoxicosis in growing chickens by the dietary addition of hydrated sodium calcium aluminosilicate. *Poult. Sci.* 69, 727-735.

Lagaly, G. & Weiss, A. 1976. The layer charge of smectitic layer silicates: In *Proc. Int. Clay Conf., Mexico City, 1975*, S.W. Bailey, ed., Applied Publishing, Wilmette, IL, 157-172.

Laird, D.A., Scott, A.D., & Fenton, T.E. 1989. Evaluation of the alkylammonium method of determining layer charge. *Clays Clay Miner*. 37, 41-46.

Lipson, S.M., & Stotzky, G. 1984. Effect of proteins on Reovirus adsorption to clay minerals. *Appl. Environ. Micro.* 48, 525-530.

MacEwan, D.M.C., & Wilson, M.J. 1984. Interlayer and intercalation complexes of clay minerals. pp. 197-248. In Brindley, G.W., & Brown, G. (eds.) *Crystal Structures of Clay Minerals and their X-ray Identification*. Mineralogical Society Monograph No. 5. Mineralogical Society, London.

Magnoli, A.P., Tallone, L, Rosa, C.A.R., Dalcero, A.M., Chiacchiera, S.M., & Torres Sanchez, R.M. 2008. Commercial bentontites as detoxifier of broiler feed contaminated with aflatoxin. *Appl. Clay Sci*. 40, 63-71.

Manahan, S.E. 2000. *Environmental Chemistry*. 7th edition. Lewis Publishers, New York. p. 237, 247.

Mayland, H.F.,, Florence, A.R., Rosenau, R.C., Lazar, V.A., & Turner, H.A. 1975. Soil ingestion by cattle on semiarid range as reflected by titanium analysis of feces. *J. Range Manage.* 28, 448-452.

Merck. 2001. *The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals.* Merck Research Laboratories Division of Merck & Co., Inc. Whitehouse Station, NJ. p. 8290.

Mermut, A.R., & Lagaly, G. 2001. Baseline studies of the Clay Minerals Society Source Clays: Layer-charge determination and characteristics of those minerals containing 2:1 layers. *Clays Clay Miner*. 49, 393-397.

Miazzo, R., Rosa, C.A.R., De Queiroz Carvalho, E.C., Magnoli, C., Chiacchiera, S.M., Palacio, G., Saenz, M., Kikot, A., Basaldella, E., & Dalcero, A. 2000. Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. *Poult. Sci.* 79, 1-6.

Murphy, P.A., Hendrich, S., Landgren, C., & Bryant, C. M. (eds.). 2006. Food mycotoxins: An update. *J. Food Sci*. 71, R51-R65.

Newman, A.C.D., & Brown, G. 1987. The chemical constitution of clays. In: Newman, A.C.D. (ed.) *Chemistry of Clays and Clay Minerals*. p. 56. Mineralogical Society Monograph No. 6, Longman Scientific & Technical, Essex, England.

Odom, I.E. 1984. Smectite clay minerals: properties and uses. *Phil. Trans. R. Soc. Lond. A* 311, 391-409.

Perez-Castells, R., Alvarez, A., Gavilanes, J., Lizarbe, M.A., Martinez Del Pozo, A., Olmo, N., & Santaren, J. 1985. Adsorption of collagen by sepiolite. In: Schultz, L.G., van Olphen, H., & Mumpton, F.A. (eds.) *Proceedings of the International Clay Conference, Denver, CO 1985*, pp. 359-362, The Clay Minerals Society, Bloomington, IN.

Phillips, T.D., Kubena, L.F., Harvey, R.B., Taylor, D.R., & Heidelbaugh, N.D. 1988. Hydrated sodium aluminosilicate: a high affinity sorbent for aflatoxin. *Poult. Sci*. 67, 243-247.

Phillips, T.D., Sarr, A.B., & Grant, P.G. 1995. Selective chemisorption and detoxification of aflatoxins by phyllosilicate clay. *Natural Toxins* 3, 204-213.

Pimpukdee, K., Kubena, L.F., Bailey, C.A., Huebner, H.J., Afriyie-Gyawu, E., & Phillips, T.D. 2004. Aflatoxin-induced toxicity and depletion of hepatic vitamin A in young broiler chicks: Protection of chicks in the presence of low levels of Novasil Plus in the diet. *Poult. Sci.* 83, 737-744.

Ralla, K., Sohling, U., Riechers, D., Kasper, C., Ruf, F., & Scheper, T. 2010. Adsorption and separation of proteins by a smectitic clay mineral. *Bioprocess Biosyst. Eng*. 33, 847-861.

Rao, S.B.N. & Chopra, R.C. 2001. Influence of sodium bentonite and activated charcoal on aflatoxin M1 excretion in milk of goats. *Small Ruminant Research* 41, 203-213.

Rutenber, E., Katzin, B.J., Ernst, S., Collins, E.J., Mlsna, D., Ready, M.P., & Robertus, J.D. 1991. Crystallographic refinement of ricin to 2.5 Å. *Proteins: Structure, Function, and Genetics*, 10, 240-250.

Scheideler, S.E. 1993. Effects of various types of aluminosilicates and aflatoxin B1 on aflatoxin toxicity, chick performance, and mineral status. *Poult. Sci.* 72, 282-288.

Schell, T.C., Lindemann, M.D., Kornegay, E.T, Blodgett, D.J., & Doerr, J.A. 1993a. Effectiveness of different types of clay for reducing the detrimental effects of aflatoxin-contaminated diets on performance and serum profiles of weanling pigs. *J. Anim. Sci*. 71, 1226-1231.

Schell, T.C., Lindemann, M.D., Kornegay, E.T., & Blodgett, D.J. 1993b. Effects of feeding aflatoxin-contaminated diets with and without clay to weanling and growing pigs on performance, liver function, and mineral metabolism. *J. Anim. Sci.* 71, 1209-1218.

Sefa-Dedeh, S., Cornelius, B., Sakyi-Dawson, E., & Afoakwa, E. O. 2004. Effect of nixtamalization on the chemical and functional properties of maize. *Food Chem*. 86, 317-324.

Seifert, L.E., Davis, J.P., Dorner, J.W., Jaynes, W.F., Zartman, R.E., & Sanders, T.H. 2010. Value-added processing of peanut meal: aflatoxin sequestration during protein extraction. *J. Agric. Food Chem*. 58, 5625-5632.

Singer, A. 2002. Palygorskite and sepiolite. pp. 555-586. In Dixon, J.B., & Schulze, D.G. (eds.) *Soil Mineralogy with Environmental Applications*. SSSA Book Series, no. 7. Soil Science Society of America, Madison, WI.

Squire, R.A. 1981. Ranking animal carcinogens: a proposed regulatory approach. *Science* 194, 877-880.

Stryer, R. 1975. Introduction to protein structure and function. Chapter 2 In: *Biochemistry*. W.H. Freeman & Company. San Francisco, CA.

Sweets, L.E., & Wrather, J.A. 2009. Aflatoxin in corn. University of Missouri, Delta Research Center. http://aes.missouri.edu/delta/croppest/aflacorn.stm.

Thieu, N.Q., & Pettersson, H. 2008. *In vitro* evaluation of the capacity of zeolite and bentonite to adsorb aflatoxin B1 in simulated gastrointestinal fluids. *Mycotoxin Research* 24, 124-129.

van Olphen, H., & Fripiat, J.J. 1979. Data Handbook for Clay Materials and other Non-metallic Minerals. p. 198. Pergamon Press, New York.

Vector Labs. 2011. Peanut agglutinin. www.vectorlabs.com/catalog.aspx? prodID=265.

Vekiru, E., Fruhauf, S., Sahin, M., Ottner, F., Schatzmayr, G., & Krska, R. 2007. Investigation of various adsorbents for their ability to bind aflatoxin B1. *Mycotoxin Research* 23, 27-33.

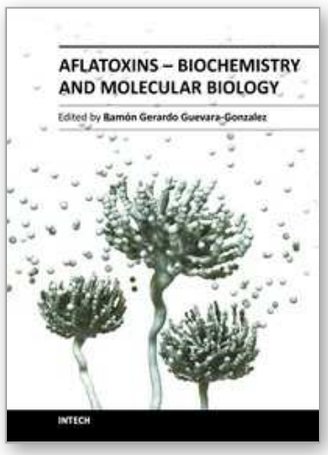
Veldman, A. 1992. Effect of sorbentia on carry-over of aflatoxin from cow feed to milk. *Michwissenschaft* 47, 777-780.

Vincelli, P., Parker, G., & McNeill. 1995. Aflatoxins in corn. Cooperative Extension Service, University of Kentucky. www.ca.uky.edu/agc/pubs/id/id59/id59.pdf.

Williams, J.H., Phillips, T.D., Jolly, P.E., Stiles, J.K., Jolly, C.M., & Aggarwal, D. 2004. Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *Am. J. Clin. Nutr*. 80, 1106-1122.

Winfree, R.A., & Allred, A. 1992. Bentonite reduces measurable aflatoxin B1 in fish feed. *The Progressive Fish-Culturist* 54, 157-162.

Zilinskas, R.A. Iraq's biological weapons: The past as future? *JAMA* 278, 418-424.



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Aflatoxins â€“ Biochemistry and Molecular Biology is a book that has been thought to present the most significant advances in these disciplines focused on the knowledge of such toxins. All authors, who supported the excellent work showed in every chapter of this book, are placed at the frontier of knowledge on this subject, thus, this book will be obligated reference to issue upon its publication. Finally, this book has been published in an attempt to present a written forum for researchers and teachers interested in the subject, having a current picture in this field of research about these interesting and intriguing toxins.

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