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Aflatoxin B_1 adsorption by clays from water and corn meal

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Abstract

Aflatoxins are toxic compounds found in grains and other food crops infested by *Aspergillus* fungi. Aflatoxins B_1 and M_1 are recognized carcinogens for animals and humans. Clay additives have been used to pelletize and improve the flow characteristics of animal feeds. Reduced aflatoxicosis in animals is an extra benefit of clay additives. Clay additive use has also been examined for reducing human aflatoxicosis. In this study, aflatoxin B_1 (AfB1) adsorption by reference clays and activated carbon (AC) will be compared to a commercial clay additive, Novasil, that lessens aflatoxicosis in animals. The *n*-alkylammonium expansion identified Novasil as a low-charge montmorillonite. AC and the montmorillonites, Novasil, SWy-2, and SAz-1 adsorbed ~200 g/kg AfB1 from water, whereas, sepiolite (SepSp-1) adsorbed only ~60 g/kg. For AfB1 adsorption from aqueous corn meal, a 60% methanol extraction was used. Retention of AfB1 from corn meal by all samples was much less (<1.5 g/kg) than from water and suggests that methanol might remove weakly-adsorbed AfB1. Low-charge montmorillonites, Novasil and SWy-2, retained ~0.7 g AfB1/kg from corn meal, but high-charge montmorillonite (SAz-1) and AC only retained ~0.1 g/kg. SepSp-1 adsorbed less AfB1 from water than AC or montmorillonite, but retained more AfB1 (1.3 g/kg) from corn meal at a lower equilibrium concentration. A plot of AfB1 extracted from corn meal versus % clay suggests SepSp-1 is far more effective than the montmorillonites. Methanol extraction is a more cautious estimate of AfB1 binding than simple aqueous adsorption and might better correlate to reduced aflatoxicosis in animals and humans.

Keywords: Feed additives; Clay minerals; Montmorillonite; Sepiolite; Activated carbon; Adsorption; Aflatoxins; Mycotoxins; *n*-alkylammonium expansion; ELISA

1. Introduction

Mycotoxins are toxic compounds that are produced by many species of the *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps*, and *Alternaria* genera of fungi (Huwig et al., 2001). The toxins are secondary metabolites produced by the fungi after infesting grain and other food crops. Crops may be contaminated by mycotoxins in two ways: as parasites on living plants and during storage of harvested crops (Huwig et al., 2001). Six groups of mycotoxins are produced by the *Aspergillus*, *Penicillium*, and *Fusarium* genera of fungi (Yiannikouris and Jouany, 2002). Mycotoxins have a diversity of chemical structures which accounts for different biological effects. Mycotoxins can be carcinogenic, mutagenic, teratogenic, oestrogenic, neurotoxic, or immunotoxic. Aflatoxins B_1 and M_1 have been demonstrated to be carcinogenic to animals and humans (Yiannikouris and Jouany, 2002).

Caporael (1976) argued that convulsive ergotism may have been a physiological cause for the Salem witchcraft crisis in 1692. Ergot (*Claviceps purpura*) is a

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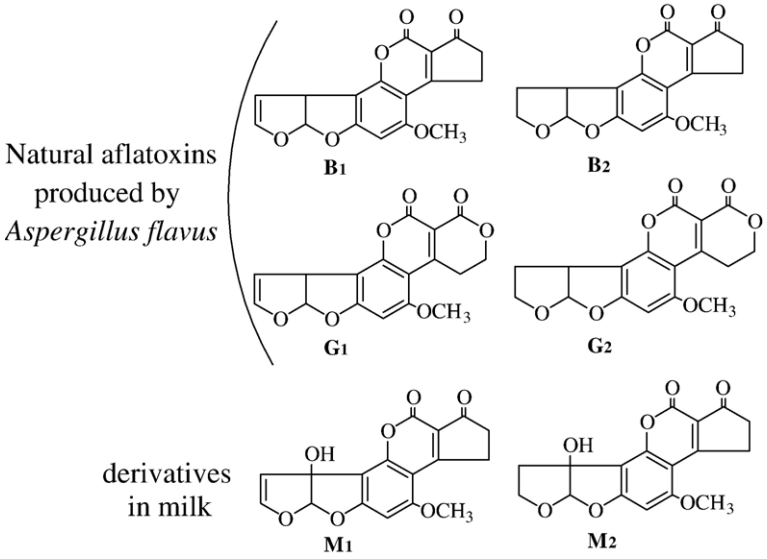

Fig. 1. Chemical structures of aflatoxins.

parasitic fungus that grows on a variety of cereal crops, especially rye. Fusiform sclerotia replace individual grains on the host plant. The scerotia contain large numbers of ergot alkaloids which are potent pharmacologic agents. One of the most potent alkaloids is lysergic acid amide which has 10% of the activity of LSD (lysergic acid diethylamide).

The first demonstrated case of mycotoxicosis was in Great Britain in 1960 where more than 100,000 turkeys died of severe liver necrosis and biliary hyperplasia caused by aflatoxins in feed contributed by *Aspergillus*-contaminated peanut meal imported from Brazil (Sargeant et al., 1961). Hartley et al. (1963) examined fluorescence and other properties of the toxic metabolites of *Aspergillus flavus* previously identified as aflatoxins B and G. They identified aflatoxins B_1, B_2, G_1, and G_2 based on the blue or green fluorescent color of the compounds in ultraviolet light and on differences in melting point (Fig. 1). They noted that naturally-occurring aflatoxins consisted mainly of B_1 (AfB1) with some B_2. When cows are fed aflatoxin-contaminated feed, a hydroxylated derivative of AfB1 is found in the milk named aflatoxin M_1 (AfM1). Veldman et al. (1992) examined the carry-over of aflatoxin from feed (AfB1) into cow's milk (AfM1) and concluded that maximum levels of AfB1 in feed should be set to limit AfM1 in milk.

Food contaminated with very small quantities of aflatoxins can render it unfit for animal or human consumption. Milk is not permitted to contain more than 0.5 μg AfM1/kg (The Texas A&M Aflatoxin Resource, 2006). According to United States Food and Drug Administration (FDA) guidelines, grain crops for human or animal consumption must generally contain <20 μg aflatoxins/kg (FSRIO, 2005). However, feed for sheep and cattle (not dairy cows) can contain as much as 300 μg aflatoxins/kg. Grain crops containing >1000 μg aflatoxins/kg must be destroyed.

Ruminants, such as cattle and sheep, are more resistant to mycotoxins than most animals. This suggests that the microbial population of the rumen plays a role in detoxification. Many bacteria, however, are completely inhibited by <10 μg AfB1/mL and this suggests that the toxin might disturb the growth and metabolic activity of rumen microorganisms (Yiannikouris and Jouany, 2002). Grazing animals probably ingest more soil materials than other animals. The clays present in ingested soil materials might contribute to the reduced risk that mycotoxins pose to ruminants.

Although mycotoxins threaten food supplies, these compounds are strongly retained by soil materials and probably do not pose a long-term environmental risk. Dust from grain harvesting equipment, however, can contain high concentrations of mycotoxins. Dust collected near a combine in Georgia contained from 2.03 to 41.2 mg aflatoxins/kg (The Texas A&M Aflatoxin Resource, 2006). Goldberg and Angle (1985) examined the leaching and adsorption potential of aflatoxins in soil and reported that 80 to 92% of total applied aflatoxin was retained in the upper 2.5 cm of soil columns. All of the aflatoxin was retained within the upper 20 cm of all tested soil types and no aflatoxin was detected in any of the soil