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Amelioration of Aflatoxicosis in Rats by Volclay NF-BC, Microfine Bentonite

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ABSTRACT

Addition of sequestering agents to feeds and foods has been proposed as a protective strategy against mycotoxins. To investigate the efficacy of Volclay, a bentonite clay, to protect against aflatoxicosis, rats were fed peanut butter (50% wt/wt)-based diets containing 1,500 ppb aflatoxin (AF), 1,500 ppb aflatoxin with 0.1% Volclay supplementation (AF-LD), or 1,500 ppb aflatoxin with 1% Volclay supplementation (AF-HD) for 8 weeks. The control group was fed a peanut butter-based diet without aflatoxin or Volclay supplementation and a fifth group was fed the control diet with 1.0% Volclay supplementation (VC). No differences in appearance, behavior, or selected hematological and serum chemical variables were observed. Decreased weight gain, decreased food consumption, and liver lesions consistent with hepatic aflatoxicosis were found in AF-fed rats. Weight gain and food consumption of the AF-HD group were comparable to the control and VC groups and were significantly increased compared to AF-fed rats, even though weekly aflatoxin ingestion of AF-HD rats equaled or exceeded that of the AF group. Body weight and food consumption of the AF-LD group were slightly increased compared to AF group and decreased compared to the control, VC, and AF-HD groups, but the differences were not statistically significant. Liver lesions were found in all AF and AF-LD rats. Lesions were also detected in eight of 10 AF-HD-fed rats, but were subtle and significantly less extensive than in AF and AF-LD rats. The data suggest that Volclay is nontoxic and may be an efficacious sequestering agent for residual aflatoxin in peanut butter.

Aflatoxins are a class of compounds produced by *Aspergillus* spp., particularly *Aspergillus flavus*. These mycotoxins cause a variety of effects, including liver cancer, in virtually all domestic and laboratory animal species tested (*1*). Aflatoxin has been epidemiologically linked to liver cancer in humans (*1,5,13,15*), and although debate whether or not aflatoxin is a human carcinogen continues, it is considered by the International Agency for Research on Cancer as a probable human carcinogen. Consequently, aflatoxin residues in feeds and food have been regulated (*5*).

Aflatoxins can occur naturally in a variety of agriculturally important commodities including peanuts and, consequently, may be found in peanut butter. Addition of sequestering agents such as activated charcoal (*2*) and hydrated sodium calcium aluminosilicate (HSCAS) (*12*), which bind aflatoxin and decrease its bioavailability, to animal feeds has been proposed as a detoxification strategy. In vivo investigations have shown that HSCAS reduces the toxicity of aflatoxin (*2,6-11*).

Sodium bentonite, another proposed sequestering agent, likewise diminished the effects of aflatoxin in growing pigs (*11*) when used as a dietary supplement. Volclay NF-BC is a bentonite clay which is generally recognized as safe for human consumption and is currently allowed for use in feeds and in food, including peanut butter, as a processing aid. We investigated the ability of a Volclay NF-BC to ameliorate aflatoxicosis in rats fed peanut butter-based diets naturally contaminated with aflatoxin.

MATERIALS AND METHODS

Diets

Five diets were prepared by blending certified rodent ration (RMH 3000, Agway, Waverly, NY) containing <1.0 ppb aflatoxin, Volclay (Volclay NF-BC Microfine Bentonite, American Colloid Company, Arlington Heights, IL), control (uncontaminated) peanut butter, and/or peanut butter naturally contaminated with aflatoxin (Table 1) in a vertical cutter mixer. All diets contained 50% wt/wt

TABLE 1. *Diet composition.*

Diet[a]	Aflatoxin (ppb)	Volclay (% wt/wt)
Negative control (control)	None	None
Volclay control (VC)	None	1.0
Aflatoxin control (AF)	1500	None
Aflatoxin plus low-dose Volclay (AF-LD)	1500	0.1
Aflatoxin plus high-dose Volclay (AF-HD)	1500	1.0

[a] All diets contained 50% wt/wt peanut butter and 49-50% wt/wt basal feed.

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peanut butter. Aflatoxin concentration of the formulated diets was confirmed by high-performance liquid chromatographic analysis (*3*).

Experimental design

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), 4 weeks of age, were acclimated and randomly divided into five groups of 10 rats each. Animals were individually housed in stainless steel cages placed in an environmentally controlled room. Food and fresh water were provided ad libitum, except during fasting, at which time food was withheld. The groups were fed the negative control (control), Volclay control (VC), aflatoxin control (AF), aflatoxin plus low-dose Volclay (AF-LD) or aflatoxin plus high-dose Volclay (AF-HD) diets for 8 weeks. They were observed daily. Body weights and food consumption were measured weekly. Daily and total aflatoxin ingestion of each rat was estimated from food consumption, body weight, and dietary aflatoxin concentration data.

Blood was drawn from the periorbital sinus for serum chemistry (after 4 and 8 weeks) and hematological (after 8 weeks) evaluations. Serum alanine and aspartate amino-transferase, alkaline phosphatase, lactate dehydrogenase, and gamma-glutamyltranspeptidase activities; total bilirubin concentration; total leukocyte, erythrocyte, and platelet counts; hemoglobin concentration; hematocrit; and erythrocyte indices were done using previously described methods (*14*).

The animals were killed after 8 weeks and examined by necropsy for gross lesions. The adrenal glands, brain, heart, kidneys, liver, lungs, spleen, and testes were excised, weighed, and preserved in Carson's Buffered Formalin. Hematoxylin and eosin-stained liver sections of all animals were microscopically examined. Lesions were scored as absent (0), minimal (1), mild (2), moderate (3), or severe (4).

Statistics

Results were statistically analyzed using appropriate parametric and nonparametric tests using the scheme of Gad and Weil (*4*).

RESULTS

Mortality did not occur. Clinical appearance and behavior of all groups were unremarkable. Weight gain (percent of initial body weight) of rats fed AF was significantly less than that of rats fed the control, AF-HD, or VC diets (Fig. 1). Weight gain of rats fed AF-LD was significantly decreased compared to the controls and AF-HD group. Weight gain of the AF-LD group was also slightly greater than that of the AF group and was slightly less than that of the VC group, but the differences were not statistically significant.

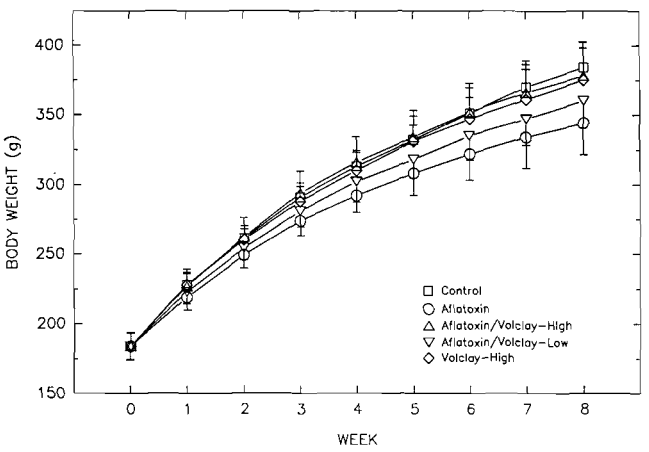

Figure 1. *Body weight curves of rats fed control or aflatoxin-contaminated (1,500 ppb) diets with and without Volclay NF-BC supplementation for 8 weeks. Each point represents the mean, n = 10; error bars denote standard deviation.*

Food consumption of the control, AF-HD, AF-LD, and VC groups was comparable. Food consumption of rats fed AF was consistently decreased relative to the other groups, averaging 90-95% of the control values. Aflatoxin consumption (mg per rat) of rats fed AF-HD was significantly greater than those fed AF (Table 2). When prorated for body weight (μg aflatoxin per kg body weight per day), aflatoxin consumption of the AF-HD group was significantly greater than that of the AF group during weeks 1 through 3. Thereafter, aflatoxin consumption of these groups was comparable. Aflatoxin consumption of rats fed AF-LD did not differ significantly from rats fed the AF or AF-HD diets.

No significant differences among groups were found for any hematological or serum chemistry variable measured. Relative (percent body weight), but not absolute (g), liver weight of rats fed AF, AF-HD, or AF-LD was significantly increased (Table 3). Relative brain weight of the AF and group and relative spleen weight of the AF-LD group were also increased.

Necropsy findings were infrequent and unrelated to aflatoxin exposure. Histopathological findings were confined to the AF, AF-LD, and AF-HD groups. Lesions were characterized by multiple foci of slightly smaller, round to oval hepatocytes, some of which had distinct cellular membranes and vacuolated, rarefied, and/or slightly basophilic cytoplasm. Peripheral compression was rarely seen in association with these foci and, when present, was minimal. These foci varied in both size, some consisting of only a few cells, and the number present per specimen. Other differences between affected and normal livers were less distinct. Affected livers had increased variability of cellular and nuclear size, slight cytomegaly, and/or focally thickened or atypical hepatic cords (more than two cells thick). Bile duct proliferation was not observed. Lesions were present in all rats fed AF or AF-LD and in eight of 10 rats fed AF-HD. Lesions found in the latter group were subtle and less extensive than the obvious histopathological changes observed in the former groups; mean histopathological scores for the AF, AF-LD, and AF-HD groups were 1.7, 1.7, and 0.8, respectively.

DISCUSSION

Rats fed a diet (AF) prepared from peanut butter naturally contaminated with aflatoxin incurred significantly decreased weight gain and food consumption without developing clinical, hematological, serum chemical, or gross pathological effects. Aflatoxin concentration of the AF diet, 1,500 ppb, was sufficient to induce histopathological liver lesions, and therefore, this protocol provided a scheme by which the protective potential of Volclay was rigorously evaluated.

Addition of 1.0% Volclay (AF-HD) to the AF diet successfully prevented decreased body weight and food consumption, while addition of 0.1% Volclay (AF-LD) diminished these effects. The ameliorative effect could not be attributed to reduced mycotoxin ingestion as food and aflatoxin consumptions of the AF-LD and AF-HD groups equaled or exceeded that of rats fed AF. The high-dose Volclay

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TABLE 2. *Aflatoxin consumption*[a].

Diet	Total during Weeks 1-8 (mg)	µg/kg Body weight/Day during week							
		1	2	3	4	5	6	7	8
AF	1.24[b] [0.080]	105[b] [5.52]	96.2[b] [6.43]	83.6[b] [3.76]	80.4 [4.81]	76.7 [3.51]	72.0 [3.64]	68.7 [3.21]	62.4 [2.41]
AF-LD	1.31[bc] [0.125]	110[bc] [7.74]	96.5[b] [6.65]	86.7[bc] [8.78]	84.1 [7.53]	77.0 [5.46]	74.2 [4.58]	68.2 [4.30]	63.3 [1.95]
AF-HD	1.39[c] [0.073]	115[c] [3.77]	103[c] [5.40]	89.5[c] [5.27]	83.0 [4.44]	78.7 [3.51]	76.3 [4.81]	69.2 [4.74]	62.0 [2.81]

[a] Values indicate group mean with standard deviation denoted in [ ], n = 10; Control and VC groups not included.
[b,c] Where indicated, groups in columns without shared superscript are significantly different, $p < 0.05$.

TABLE 3. *Relative organ weights* [a].

Diet	Final body weight (g)	Relative weights (% Body weight)							
		Adrenals	Brain	Heart	Kidneys	Liver	Lungs	Spleen	Testes
Control	355[b] [15.5]	0.05 [<0.01]	0.50[c] [0.03]	0.40 [0.03]	0.73 [0.03]	2.80[c] [0.10]	0.70 [0.07] (9)	0.22[bc] [0.02]	1.04 [0.04]
VC	351[b] [15.5]	0.05 [0.01]	0.50[c] [0.04]	0.39 [0.04]	0.75 [0.05]	2.81[c] [0.15]	0.74 [0.07]	0.22[bc] [0.01]	1.03 [0.11]
AF	324[c] [54.3]	0.06 [0.01]	0.55[b] [0.05]	0.37 [0.02]	0.78 [0.03]	3.05[b] [0.10]	0.74 [0.05]	0.24[bcd] [0.04]	1.10 [0.06]
AF-LD	338[bc] [15.5]	0.05 [<0.01]	0.52[bc] [0.03]	0.38 [0.03]	0.73 [0.04]	3.15[b] [0.21] (9)	0.76 [0.10]	0.24[d] [0.02]	1.07 [0.05]
AF-HD	354[b] [21.7]	0.05 [<0.01]	0.51[c] [0.03]	0.38 [0.04]	0.76 [0.05]	3.02[b] [0.17]	0.71 [0.07]	0.23[bd] [0.02]	1.05 [0.05]

[a] Values indicate group means with standard deviation given in [ ], n = 10 except where indicated in ( ).
[b,c,d] Where indicated, groups in columns without shared superscript are significantly different, $p < 0.05$.

supplement provided significant protection against hepatotoxicity, as both the extent and severity of liver lesions found in rats fed AF-HD were reduced compared to lesions found in rats fed AF or AF-LD. Although aflatoxicosis was not completely prevented under the conditions (1,500 ppb aflatoxin: 1.0% Volclay) of this study, our findings indicate that Volclay can potentially provide meaningful protection against aflatoxin in peanut butter, other foods, or animal feeds.

Up to 20 ppb total aflatoxin is currently allowed in peanut butter and other foods, milk excepted, in the United States. It cannot be assumed that addition of 0.010-0.015% Volclay, a level providing a Volclay-to-aflatoxin ratio approximating that of our AF-HD diet, to food containing 20 ppb aflatoxin would provide a degree of protection similar to that observed in the AF-HD group. Additional investigations are clearly needed to find a Volclay level which, under conditions of continuous, long-term exposure to dietary aflatoxin concentrations normally encountered (≤20 ppb) in food, is efficacious as well as organoleptically and nutritionally acceptable.

HSCAS is the most thoroughly studied sequestering agent. It has been shown to ameliorate the clinical effects of aflatoxin in chickens (*8-10*), lambs (*7*), mink (*2*), and pigs (*6*). However, HSCAS did not protect against development of aflatoxin-induced liver lesions in some studies (*2,6,7*) but did prevent lesion development in other investigations (*6*). Species-specific differences in response to aflatoxin, differences in the severity of lesions induced by the requisite aflatoxin treatments, and/or differences in the ratios of sequestering agent to mycotoxin in the feed may account for the differing results.

Lindemann et al. (*11*) reported that addition of another sodium bentonite preparation, Volclay FD-181, to aflatoxin-contaminated swine rations diminished toxicity and that its efficacy was similar to that of HSCAS. However, the efficacy of that bentonite preparation to inhibit liver lesion development was not reported. In the present study, both liver weight effects and microscopic lesions were found in rats which were fed aflatoxin-contaminated diets supplemented with Volclay NF-BC and which did not exhibit clinical or serum chemical signs of aflatoxicosis. Therefore, although tissue

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aflatoxin levels were not measured, it can reasonably be concluded that neither 1.0% nor 0.1% Volclay completely prevented aflatoxin absorption. Future studies should incorporate both histopathological endpoints, and at dosages which do not elicit a histopathological response, molecular dosimetric techniques, such as aflatoxin-guanine adduct formation, for judging efficacy of Volclay, other bentonite preparations, and nonbentonite sequestering agents.

In summary, our results indicate that Volclay was nontoxic and can provide significant protection against aflatoxicosis. Because bentonite materials such as Volclay are currently recognized as safe for use in food processing, are used in pharmaceutical preparations, and have been safely added to unmedicated animal feeds for various purposes at levels up to 2.5%, Volclay warrants consideration as a food additive/aflatoxin sequestering agent for use in peanut butter and other foods.

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