

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 Diet¹

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ABSTRACT Aflatoxin contamination of foods and livestock feeds is an ongoing problem. In this research, NovaSil PLUS (NSP), a calcium montmorillonite clay that is commonly used as an anticaking agent in feeds, was evaluated for its ability to bind aflatoxin B₁ (Afb₁) in vitro and to prevent the onset of aflatoxicosis and vitamin A depletion in broiler chicks in vivo. Isothermal analyses were conducted with NSP and Afb₁ to quantitate and characterize critical sorption parameters at equilibrium, i.e., ligand saturation capacities, affinity constants, and thermodynamics of the sorption process. In vitro results indicated that Afb₁ was tightly sorbed onto the surface of NSP, which provided a high capacity and high affinity for the ligand. Thermodynamics favored sorption of Afb₁

to NSP. The process was exothermic and spontaneous with a mean heat of sorption equal to approximately -50 kJ/mol, suggesting chemisorption (or tight binding). In addition to the in vitro studies, the effectiveness of NSP as an aflatoxin enterosorbent to attenuate the onset of aflatoxicosis in broiler chicks was determined at 3 different inclusion levels in the diet (0.5, 0.25, and 0.125%). NSP alone was not toxic to chicks at a level as high as 0.5% in the total diets (based on body and organ weights, feed intake, and hepatic vitamin A levels). NSP in the diet significantly protected chicks from the effects of high-level exposure to aflatoxins (i.e., 5 mg/kg) and preserved hepatic vitamin A levels, even at lower dietary intake of clay.

(Key words: aflatoxin, sorption, detoxification, isotherm, NovaSil PLUS)

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INTRODUCTION

Aflatoxins represent a group of similar chemicals produced by the common molds *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are acutely toxic, carcinogenic, mutagenic, teratogenic, and immunosuppressive to most mammalian species. In production agriculture, aflatoxin contamination in livestock feeds often results in poor growth and feed conversion efficiency, increased mortality rates, and a greater susceptibility to diseases (Smith and Hamilton, 1970; Jones et al., 1982; and Huff et al., 1986). The impact of aflatoxins on micronutrient status is also a major concern. Harvey et al. (1994) reported that exposure to aflatoxin in the diets of growing

barrows reduced serum vitamin A levels; they suggested that aflatoxin exposure may exacerbate vitamin A and E deficiencies. In poultry, immune responses and disease susceptibility have been linked to vitamin A deficiencies. In fact, there is a current interest in the relationship between vitamin A status or availability and overall health of poultry (Aye et al., 2000 a,b; Dalloul et al., 2002). Recent studies have shown that a vitamin A deficiency in the diets of coccidiosis-challenged broilers resulted in compromised immune defenses as reflected in lymphocyte profiles, oocyst shedding, and interferon- γ levels (Dalloul et al., 2002). Of particular interest is the association between dietary aflatoxin exposure, vitamin A deficiencies, and susceptibility to disease.

Several strategies for the reduction or inactivation of aflatoxins have been previously reviewed and include diverse physical, chemical, and biological methods (Phillips et al., 1994, 2002; Phillips, 1999). One of the strategies of current interest is the inclusion of nonnutri-

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Abbreviation Key: Afb₁ = aflatoxin B₁; HSCAS = hydrated sodium calcium aluminosilicate; NSP = NovaSil PLUS; UV = ultraviolet.

tive enterosorbents in contaminated feeds for the inactivation of aflatoxins. This approach is considered to be practical and cost effective for the detoxification of contaminated feedstuffs on a large scale. Many studies have demonstrated that hydrated sodium calcium aluminosilicate (HSCAS) clays commonly used as anticaking agents for animal feeds (e.g., calcium and sodium montmorillonites) significantly diminish the adverse effects of aflatoxins in poultry and other animal species (Davidson et al., 1987; Phillips et al., 1987, 1988, 1990, 1991, 1994, 1995; Kubena et al., 1988, 1990a,b, 1991, 1993a,b; Harvey et al., 1989, 1991a,b, 1993, 1994; Lindeman et al., 1993; Voss et al., 1993; Ledoux et al., 1999). However, to date, the minimal effective dose of clay required for protection of broiler chicks from aflatoxin contaminated feeds as well as the impact of aflatoxin on hepatic vitamin A status have not been reported in the scientific literature.

Thus, the major objectives of this study were to characterize the *in vitro* sorption of aflatoxin B₁ (Afb₁) onto the surface of NovaSil PLUS (NSP) and to determine the minimal effective dose required to protect broiler chicks from aflatoxin and depletion of hepatic vitamin A levels.

MATERIALS AND METHODS

Sorption Studies

The NSP³ was evaluated for its effectiveness of Afb₁⁴ sorption at pH 7. A 100- μ g sample of the sorbent was added to a 5-ppm solution of Afb₁ in a total volume of 5 mL and then agitated at 1,000 rpm in an incubator at 25°C for 5, 10, 15, and 30 min and 1, 2, 4, and 24 h. After shaking, samples were centrifuged at 2,500 rpm for 10 min. The supernatant of samples was analyzed for Afb₁ content by measuring ultraviolet (UV) absorbance (362 nm; $\epsilon = 21,865 \text{ M}^{-1}\text{cm}^{-1}$) with a scanning UV-visible spectrophotometer.⁵ Additionally, sorption studies were conducted at pH 2, 7, and 10. Purified water was adjusted to pH 2 by adding HCl and to pH 10 by adding NaOH before preparing the Afb₁ stock solutions. All treatments and final concentrations were adjusted to the corresponding pH with HCl or NaOH. The samples and controls were agitated at 1,000 rpm in an incubator at 25°C for 2 h. After shaking, the samples were centrifuged at 2,500 rpm for 10 min to separate the sorbent from the supernatant. Supernatants were then analyzed for Afb₁ content by UV-visible spectrophotometry (as indicated previously).

Isotherm Studies

Isothermal analyses were carried out according to methods previously established in this laboratory

(Grant, 1998; Grant and Phillips, 1998; Lemke et al., 1998; Huebner et al., 1999; Pimpukdee, 2000). A stock solution of Afb₁ (8 μ g/mL) was prepared in purified water and verified by UV-visible spectrophotometry. Test samples were prepared in triplicate and contained increasing concentrations of Afb₁, ranging from 0.4 to 8 μ g/mL, with 20 μ g/mL sorbent in a total volume of 5 mL. These were agitated at 1,000 rpm for 24 h at 25°C in the dark and then centrifuged at 2,000 rpm for 30 min at 25°C. The concentration of Afb₁ remaining in solution was determined from the resulting supernatants by UV-visible spectrophotometry as previously described. These same experiments were repeated at 15 and 37°C to estimate the enthalpy of sorption (ΔH_{ads}) of Afb₁ on the surface of the test sorbent.

Data Calculations and Curve Fitting

The UV-visible absorption data were used to calculate the amount of Afb₁ left in solution and the amount sorbed for each treatment. Isothermal data were transferred to Table Curve 2D software⁶ and fit with appropriate models (Langmuir, Freundlich, generalized Freundlich, and Toth) to characterize the sorption of Afb₁ to sorbent. The equation that exhibited the best combination of shape, r^2 and standard deviation was chosen to model the experimental data. Estimates of the capacity values (Q_{max}), and the distribution constants (K_d) for sorbents were then calculated with the fitted equation (Grant and Phillips 1998; Lemke et al., 1998; Huebner et al., 1999). ΔH_{ads} (enthalpy) of sorption was determined with the van't Hoff equation by comparing individual K_d values at different temperatures.

Preparation of Poultry Diets

Aflatoxins were produced through fermentation of rice by *Aspergillus parasiticus* NRRL 2999 as described by Kubena et al. (1990a). Briefly, fermentation was carried out in 2.5-L Fernbach flasks containing 50 g of white rice (free of preservatives). Twenty-five milliliters of water was added to each flask; the rice was autoclaved, then shaken to break up clumps. Each flask was inoculated with 1 mL of an *Aspergillus* spore suspension then shaken on a platform shaker at 220 to 240 rpm at 15°C for 24 h. Ten milliliters of water was added at 48 h. The initial temperature of the rice fermentation was increased from 15 to 21°C after 24 h of incubation and then to 28°C after 48 h. Incubation was continued for a total of 5 d, and during the fermentation process grains were kept separated to prevent excessive hyphae formation. After incubation, the rice was autoclaved, dried, and then ground into a powder. The total aflatoxin (Af) content of the rice powder was initially measured by spectrophotometry (Nabney and Nesbitt, 1965) as modified by Wiseman et al. (1967) and consisted of approximately 79% Afb₁, 16% AfG₁, 4% AfB₂, and 1% AfG₂. The rice powder was incorporated into a corn-soybean meal basal diet (free of detectable mycotoxin contamination)

³Engelhard Corporation, Jackson, MS.

⁴Sigma Chemical Co., St. Louis, MO.

⁵Model DU-65, Beckman (www.beckman.com).

⁶Version 3, Genesis Technologies, Inc., Austin, TX.

TABLE 1. Effects of dietary NovaSil PLUS (NSP) inclusion alone or in combination with aflatoxin B₁ (AfB₁) on temporal BW gain in broiler chicks

Treatment group		BW gain ¹ (g)				Percentage change from control (21 d)
AfB ₁ (mg/kg of feed)	NSP (%)	1–7 d	8–14 d	15–21 d	1–21 d	
0	0	145 ± 2.9 ^a	265 ± 13.0 ^{ab}	446 ± 13.2 ^a	866 ± 12.7 ^a	0
0	0.500	132 ± 5.9 ^a	287 ± 11.0 ^a	418 ± 26.1 ^a	839 ± 42.6 ^a	-3
5	0	126 ± 9.6 ^a	229 ± 18.5 ^b	338 ± 22.7 ^b	699 ± 38.5 ^b	-19
5	0.125	138 ± 4.7 ^a	264 ± 8.9 ^{ab}	390 ± 31.2 ^{ab}	791 ± 43.5 ^{ab}	-9
5	0.250	144 ± 6.1 ^a	284 ± 6.9 ^a	427 ± 12.0 ^a	854 ± 22.7 ^a	-1
5	0.500	136 ± 3.4 ^a	290 ± 8.9 ^a	385 ± 30.5 ^{ab}	816 ± 37.6 ^a	-6

^{a,b}Mean values with no common superscript differ significantly ($P \leq 0.05$).

¹Data are reported as the mean ± SE of 4 replicate pens of 3 broiler chicks each per treatment group.

to provide 5 mg AfB₁/kg diet as confirmed by HPLC methods (Hutchins and Hagler, 1983). The basal diet contained no antibiotics, coccidiostats, growth promoters, or inorganic sorbents and met or exceeded the levels of critical nutrients recommended by the National Research Council (1994).

Experimental Design

One-day-old male broiler chickens (Peterson × Hubbard) were obtained from a commercial hatchery. Chicks were reared in wire-floored brooding cages with continuous fluorescent illumination and forced ventilation at the USDA-ARS, Southern Plains Agricultural Research Center (College Station, TX). Chicks were individually weighed, wing-banded and randomly distributed among the different treatment groups consisting of 4 replicates of 6 chicks per dietary treatment group (pen). Chicks were grouped by the following dietary treatments: 1) basal feed free of toxins (control), 2) basal feed containing 0.5% NSP, 3) basal feed containing 5 mg of AfB₁/kg, 4) basal feed containing 0.125% NSP + 5 mg of AfB₁/kg, 5) basal feed containing 0.25% NSP + 5 mg of AfB₁/kg, and 6) basal feed containing 0.5% NSP + 5 mg of AfB₁/kg. Broilers were provided with water and feed ad libitum for the duration of the study (3 wk).

Measurements

Body weights of chicks and feed consumption were measured weekly, and chick mortalities were recorded as they occurred. After termination of the study, liver and kidney weights were recorded and were evaluated as relative organ weight changes (g/100 g of BW). Random liver samples (3/pen) were also collected and stored at -75°C in preparation for liver vitamin A level determination.

Hepatic Vitamin A Determination

In brief, a portion of liver sample (1 g) was mixed with 100 mg of butylated hydroxytoluene and 25 g of

silica gel. The mixture was ground in a mortar then extracted with 30 mL hexane:acetone (1:1) for 30 min with agitation. Afterwards, 10 mL of deionized water was added to the mixture, which was then vortexed for 30 s and centrifuged at 1,000 rpm for 15 min (4°C). Ten milliliters of the hexane fraction was removed from the mixture and dried under nitrogen. The oily residue was resuspended in saturated potassium hydroxide (3 mL), incubated for 30 min at 65°C, then re-extracted with hexane:water (5:1), and centrifuged at 1,000 rpm for 15 min (4°C). The resulting hexane fraction was again removed, dried under nitrogen, and resuspended in HPLC mobile phase (methanol:ammonium acetate buffer) (9:1, pH 5.6) prior to analysis.

Quantitative measurements of vitamin A were performed by HPLC with a photodiode array detection method (325 nm) by using an ODS column⁷ (4.5 × 150 mm) maintained at 30°C. The mobile phase consisted of a gradient elution of 40 mM ammonium acetate buffer (pH 5.6):methanol (40:60) for 12 min, followed by 100% methanol elution for 4 min, and then ammonium acetate buffer:methanol (40:60) for 9 min (flow rate of 1 mL/

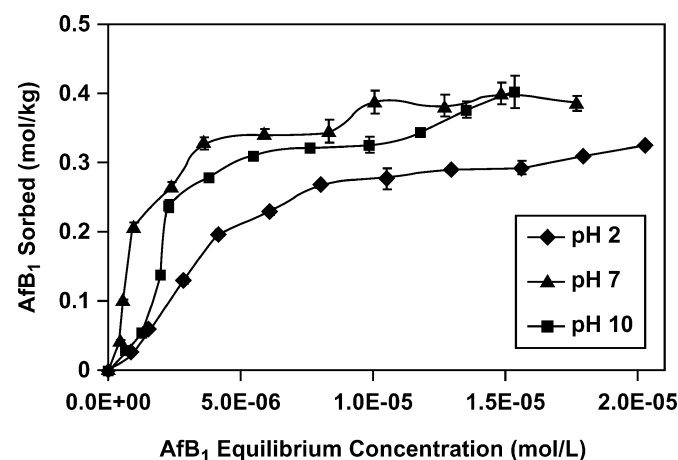


FIGURE 1. Isotherm plots for aflatoxin B₁ (AfB₁) sorption onto NovaSil PLUS (NSP) at pH 2, 7, and 10. Treatments were adjusted to the corresponding pH by adding HCl or NaOH (see methods for details). Samples and controls were agitated at 1,000 rpm in an incubator at 25°C for 2 h. Supernatants of samples were analyzed for AfB₁ with ultraviolet-visible spectrophotometry. Data represent the means ± standard deviations from 3 independent experiments.

⁷Waters Corporation, Milford, MA.

TABLE 2. Effects of dietary NovaSil PLUS (NSP) inclusion alone or in combination with aflatoxin B₁ (Afb₁) on 21-day BW gain in broiler chicks

Treatment group	BW gain ¹ (g)
Control feed	866 ± 12.7 ^a
0.5% NSP	839 ± 42.6 ^a
Afb ₁ (5.0 mg/kg feed)	699 ± 38.5 ^b
0.125% NSP + Afb ₁	791 ± 43.5 ^{ab}
0.25% NSP + Afb ₁	854 ± 22.7 ^a
0.5% NSP + Afb ₁	816 ± 37.6 ^a

^{a,b}Mean values with no common superscript differ significantly ($P \leq 0.05$).

¹Data are reported as the mean ± SE of 4 replicate pens of three broiler chicks each per treatment group.

min). Injection volume was 20 μ L with a total run time of 25 min. Vitamin A (retinol) was quantified on the basis of retention time, and peak area as compared with an external retinol standard.⁴

Statistical Analysis

Data were analyzed using SAS software (SAS Institute, 1982). Data were subjected to ANOVA using the general linear models procedure to establish differences between means. Means showing significant differences in ANOVA were compared using Fisher's protected least significant difference procedure (Snedecor and Cochran, 1967). All statements of differences were based on a significance of $P < 0.05$.

RESULTS

Initial sorption studies indicated that Afb₁ binding onto NSP surfaces occurred rapidly within 5 min; an equilibrium state was reached in 2 h (data not shown). Adsorption isotherms at 25°C under varied pH conditions are shown in Figure 1. The results indicated that NSP exhibited higher levels of sorption at pH 7 and pH 10 than at pH 2. Experimental data for Afb₁ sorption onto NSP at varied temperatures (pH 7.0) were fitted to the Langmuir model to allow for a quantitative comparison of capacities and affinity constants for test sorbents. The Q_{\max} (capacity) values for Afb₁ sorption onto NSP at 15, 25, and 37°C averaged 0.420 ± 0.013 , 0.456 ± 0.006 , and 0.379 ± 0.001 mol/kg, respectively (Figure 2A). Similarly, the average K_d (affinity) constants for Afb₁ sorption onto NSP at 15, 25, and 37°C were determined to be 1.18×10^6 , 7.48×10^5 , and 3.61×10^5 , respectively. Results also indicated that Afb₁ sorption to the surface of NSP was exothermic and spontaneous with an average enthalpy equal to -49.2 kJ/mol (Figure 2B).

Results from the in vivo study suggested that Afb₁ at 5 mg/kg of the diet can significantly affect broiler health and production. The effects of dietary NSP inclusion alone or in combination with aflatoxins on BW gain are shown in Table 1. Over the course of the study, BW gains were significantly reduced after the first week in chicks fed diets containing 5 mg/kg of Afb₁. The per-

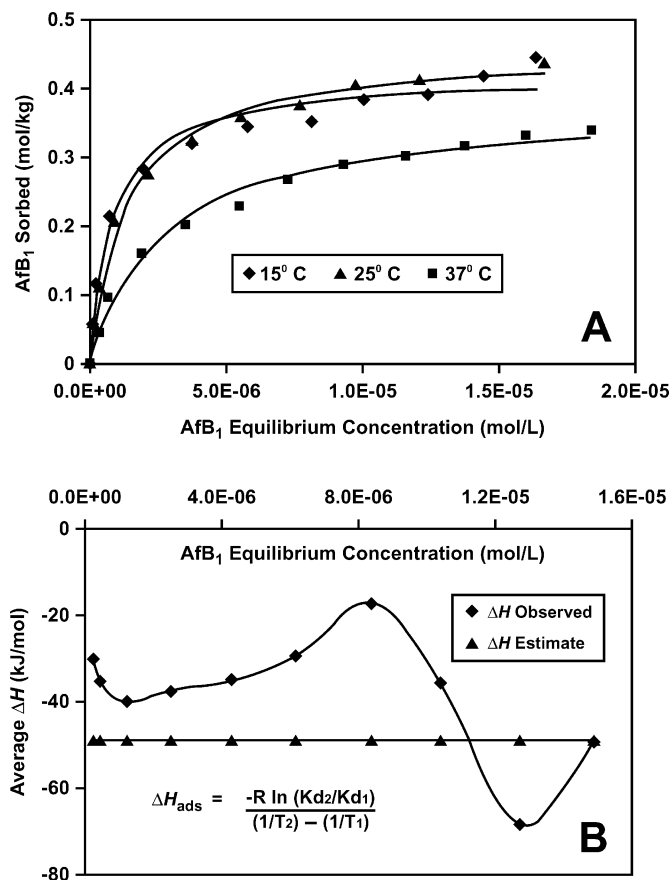


FIGURE 2. Isothermal analysis and enthalpy of sorption plots for aflatoxin B₁ (Afb₁) on NovaSil PLUS (NSP) surfaces. A. Isotherm plots for Afb₁ sorption to NSP at 15, 25, and 37°C. Isotherm data were fitted with the Langmuir model. Curve fits are displayed as solid lines, whereas experimental data are shown as average points. B. Enthalpy of sorption plots based on isothermal analysis at 15, 25, and 37°C. The enthalpy of sorption was calculated from experimental data using the van't Hoff equation (see inset). Data are shown as average enthalpy values derived from the observed experimental data and from estimated values based on the Langmuir modeled data.

centage reduction in BW produced by aflatoxin alone at 21 d was 19%. The BW gains of birds fed diets containing 0.5% NSP free of aflatoxins were not significantly different from those of controls. Beginning with the first period (1 to 7 d) and continuing through the third period (15 to 21 d), chicks consuming diets containing aflatoxins without NSP had lower gain in BW, which was significant compared with the chicks consuming the control diet. Inclusion of NSP in the diets prevented the growth inhibitory effects produced by aflatoxins with varying levels of effectiveness. At the end of the experiment (i.e., 21 d), BW of birds fed diets containing NSP at all levels of clay plus aflatoxins were not significantly different from controls (Table 2, Figure 3). However, BW gains of birds fed the diet containing NSP (0.125%) and aflatoxins were not significantly different from the aflatoxin control treatment group. Overall, the highest percentage protection from change in BW loss was observed in birds treated with 0.25% dietary NSP (Table 2, Figure 3).

Results showing the effects of dietary NSP and aflatoxins on relative organ weights are shown in Table 3.

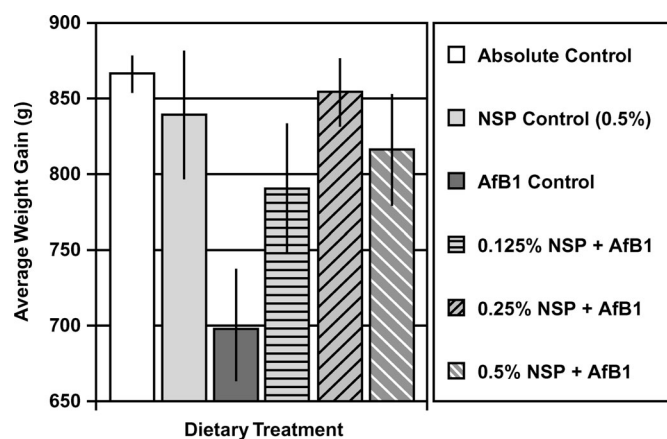


FIGURE 3. Dose-response effects of dietary NovaSil PLUS (NSP) additions on average body weight gains of 21-d-old broiler chicks consuming aflatoxin B₁ (AfB₁) (5 mg/kg) in the diet. Data are shown as the means \pm standard errors of 4 replicate pens of 3 broiler chicks each per treatment group.

When compared with controls, the relative weights of liver and kidney were significantly increased for the chicks treated with the aflatoxin control diet. The relative organ weights of chicks fed diets containing NSP (0.5%) alone were not significantly different from the control diet. Relative liver weights of chicks maintained on diets containing 0.5 and 0.25% NSP with aflatoxins were comparable with the control group, whereas liver weights of chicks consuming diets containing 0.125% NSP with aflatoxins were not significantly different from those of the aflatoxin control group. A similar trend in protection against the change in relative kidney weights was observed in birds treated with 0.5% NSP and aflatoxins. In general, the percentage protection from change in relative organ weights was dependent on clay level (Table 3).

The results pertaining to the feed:gain ratio and feed consumption are shown in Table 4. There were no significant differences among treatment groups and controls. However, the feed consumption of birds treated with the aflatoxin control diet was significantly lower than that of the absolute control, NSP control, and NSP (0.25 and 0.5%) plus aflatoxin diets. Figure 4 depicts the dose response effects of dietary NSP inclusion on hepatic vitamin A levels in chicks consuming aflatoxins in the

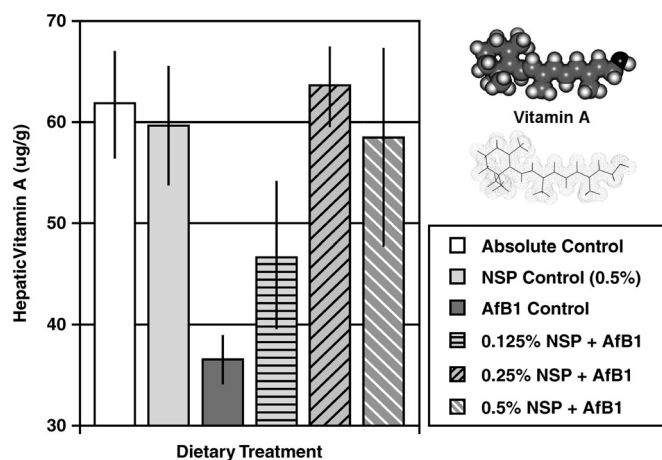


FIGURE 4. Dose-response effects of dietary NovaSil PLUS (NSP) addition on vitamin A levels in the livers of broiler chicks. Quantitative measurements of vitamin A in liver were performed by HPLC with photodiode array detection methods (325 nm). Data are shown as the means \pm standard errors of 4 replicate pens of 3 broiler chicks each per treatment group. AfB₁ = aflatoxin B₁.

diet. Results indicated that vitamin A levels in the livers of chicks consuming aflatoxin control diets were significantly lower than all other treatment groups (Figure 4 and Table 5). In addition, the hepatic vitamin A levels in chicks consuming diets treated with the aflatoxins and NSP (0.125, 0.25, and 0.5%) were not significantly different from those of the absolute control and NSP control diets (Table 5). At the conclusion of the poultry study, control birds were evaluated via necropsy by scientists and an attending veterinarian for underlying diseases. No evidence of pre-existing problems was reported.

DISCUSSION

In the present study, isothermal analysis was used to evaluate and characterize AfB₁ sorption by NSP. Overall, the *in vitro* data suggest that NSP provides the capacity and affinity for AfB₁ equivalent to, or greater than, that previously reported for HSCAS (Grant and Phillips, 1998). Also, the thermodynamics of the reaction favored a strong sorption of AfB₁ to the surface of NSP. The estimated enthalpy of sorption for NSP (i.e., -49.2 kJ/mol) was higher than values previously reported for

TABLE 3. Effects of dietary NovaSil PLUS (NSP) inclusion either alone or in combination with aflatoxin B₁ (AfB₁) on relative organ weights in broiler chicks

Treatment group	Mean liver weight ¹ (g/100 g of BW)	Mean kidney weight ¹ (g/100 g of BW)
Control feed	2.99 \pm 0.15 ^b	0.441 \pm 0.034 ^{bc}
0.5% NSP	2.82 \pm 0.14 ^b	0.371 \pm 0.023 ^c
AfB ₁ (5.0 mg/kg feed)	3.87 \pm 0.49 ^a	0.589 \pm 0.055 ^a
0.125% NSP + AfB ₁	3.54 \pm 0.32 ^{ab}	0.547 \pm 0.046 ^{ab}
0.25% NSP + AfB ₁	3.12 \pm 0.03 ^{ab}	0.503 \pm 0.024 ^{ab}
0.5% NSP + AfB ₁	2.81 \pm 0.16 ^b	0.449 \pm 0.024 ^{bc}

^{a-c}Mean values with no common superscript differ significantly ($P \leq 0.05$).

¹Data are reported as the mean \pm SE of 4 replicate pens of 3 broiler chicks each per treatment group.

TABLE 4. Effects of dietary NovaSil PLUS (NSP) inclusion either alone or in combination with aflatoxin B₁ (Afb₁) on average feed consumption and feed:gain ratios in broiler chicks

Treatment group		Feed:gain ¹ (kg/kg)	Feed consumption ¹ (g)
Afb ₁ (mg/kg)	NSP (%)		
0	0	1.628 ± 0.058 ^a	1369.0 ± 45.7 ^a
0	0.500	1.535 ± 0.029 ^a	1275.8 ± 38.5 ^a
5	0	1.480 ± 0.117 ^a	957.8 ± 183.5 ^b
5	0.125	1.483 ± 0.084 ^a	1153.8 ± 103.4 ^{ab}
5	0.250	1.460 ± 0.050 ^a	1235.0 ± 54.2 ^{ab}
5	0.500	1.538 ± 0.073 ^a	1191.5 ± 22.9 ^{ab}

^{a,b}Mean values with no common superscript differ significantly ($P \leq 0.05$).

¹Data are reported as the mean ± SE of 4 replicate pens of 3 broiler chicks each per treatment group.

HSCAS (i.e., -40 kJ/mol) (Grant and Phillips, 1998) suggesting tight binding to the clay. The enthalpy of aflatoxin binding to potential enterosorbents is an important parameter for predicting efficacy *in vivo*. Based on ΔH_{ads} , the adsorption of chemicals to a surface can be categorized as either a weak association (physisorption) or a chemical reaction or sharing of electrons between the chemical and surface (chemisorption) (Gatta, 1985). Physisorption is characterized by enthalpies <20 kJ/mol, whereas chemisorption is defined by enthalpies >20 kJ/mol (Gu et al., 1994). The *in vitro* results of this study predict that NSP chemisorbs aflatoxins and will act as an enterosorbent in animals, reducing their bioavailability to target organs and subsequent toxicity. The variability of ΔH_{ads} in these experiments may reflect multiple binding mechanisms or sites and the heterogeneous nature of clay minerals.

In the poultry study, the toxic effects of Afb₁ were expressed as reduced BW gains, increased relative organ weights (liver and kidney), and lower feed consumption. The toxic effects produced by Afb₁ were in general agreement with those published in previous reports (Huff et al., 1986; Kubena et al., 1990a, 1993b). The addition of NSP at a level of 0.25% in the diet was the most effective, reducing the negative impact of Afb₁ on BW gains by nearly 95%. Also, NSP at 0.5 and 0.125% reduced the toxic effects of Afb₁ on BW changes by 68

TABLE 5. Effects of dietary NovaSil PLUS (NSP) inclusion alone or in combination with aflatoxin B₁ (Afb₁) on hepatic vitamin A levels in broiler chicks

Treatment group		Vitamin A level ¹ ($\mu\text{g/g}$ liver)
Afb ₁ (mg/kg)	NSP (%)	
0	0	61.9 ± 5.4 ^a
0	0.5	59.8 ± 5.9 ^a
5	0	36.6 ± 2.5 ^b
5	0.125	46.9 ± 7.3 ^a
5	0.25	63.6 ± 4.0 ^a
5	0.5	58.6 ± 8.9 ^a

^{a,b}Mean values of vitamin A levels with no common superscript differ significantly ($P \leq 0.05$).

¹Data are reported as the mean ± SE of 4 replicate pens of 3 broiler chicks each per treatment group.

and 53%, respectively. The feed consumption of birds fed diets containing NSP, even at a level of clay as low as 0.25% with Afb₁, was comparable to the control group. Overall, the rank order of clay for protection from Afb₁ at 5 mg/kg in the diet was 0.25% NSP > 0.5% NSP > 0.125% NSP.

The NSP alone did not produce any adverse effects in chicks even when as high as 0.5% in the total diets. There were no significant differences between controls and chicks fed diets containing clay (without Afb₁) for the parameters evaluated in this study. Results indicated that the relative liver weights were significantly higher in chicks consuming Afb₁ without clay. The liver is considered to be a target organ for aflatoxin in broiler chickens, and the effects are typically expressed as increases in relative liver weights (Huff et al., 1986). The relative liver weights of birds treated with NSP (0.5 and 0.25%) plus 5 mg/kg Afb₁ were not only comparable with controls but were also significantly lower when compared with birds treated with Afb₁ alone. These data confirm the efficacy of NSP in protecting chicks from the hepatotoxicity of Afb₁, even at levels as low as 0.25%. A similar protective effect by HSCAS against Afb₁ was observed on relative kidney weights and was consistent with previously published reports (Kubena et al., 1990a, 1993b).

Vitamin A (consisting of retinol and its active metabolites) is vital for vision; controlling the differentiation program of epithelial cells in the digestive tract and respiratory system, skin, bone, nervous system, and immune system; and for hematopoiesis (Gursu et al., 2002). Vitamin A also induces lymphoproliferation, which results in a stimulated immune system; a deficiency can decrease specific antibody production, the number of circulating lymphocytes, and lymphocyte proliferation (West et al., 1991). Importantly, this study shows that no significant alteration in the hepatic concentration of vitamin A was observed at all NSP levels (0.125, 0.25, and 0.5%) tested against 5 mg Afb₁/kg. These results suggest that the overall health and immune defenses of aflatoxin-challenged broilers would be maintained and not compromised by a vitamin A deficiency.

Dietary interventions with clay-based enterosorbents may be used to block, retard, or diminish aflatoxin exposure, associated risks, and toxic residues in food of animal origin. This clay-based technology, when used in combination with other good management practices, may be a valuable tool for the development of an integrated approach to the preventive management of mycotoxin-contaminated commodities. Future research is needed to identify other sorbents to effectively sorb other mycotoxins found in food and feed. Most importantly, all dietary enterosorbents should be evaluated for safety *in vivo* before use in animal feeds.

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