Efficacy of Montmorillonite Clay (NovaSil PLUS) for Protecting Full-Term Broilers from Aflatoxicosis

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Primary Audience: Nutritionists, Producers, Veterinarians, Researchers

SUMMARY

Contamination of poultry feeds by aflatoxin is a problem faced by poultry producers at one time or another. Of the several strategies used to reduce the economic effect of aflatoxin-contaminated feeds, the inclusion of selective enterosorbents may be the most practical and cost effective. This study evaluated the effectiveness of montmorillonite clay (MC) supplied as NovaSil PLUS on the performance of full-term commercial type broilers. Eight hundred male commercial broiler chickens (all vaccinated for Marek's disease, Newcastle disease, and bronchitis) were randomly assigned, (20 each) to 40 floor pens and fed 1 of the 4 dietary treatments of industry-type corn-soy broiler diets. Treatments consisted of a control feed, control feed plus 0.5% MC (CMC), a feed containing ~4,000 ppb aflatoxin (AF), and a feed containing ~4,000 ppb aflatoxin plus 0.5% MC (AMC). The starter diet was fed for 3 wk, the grower for 2 wk and, the finisher for 1 wk. Selected birds were randomly retained for specific tissue analyses of blood serum; relative liver, spleen, and kidney weights; and histological examination of sampled organs.

The combined data showed that birds fed MC as NovaSil PLUS received significant protection against the effects of the aflatoxin for most parameters measured. This level of protection did not totally protect the birds from the effects of feeding extremely high concentrations of aflatoxin, however, as their performance was not as good as that of the control group. To our knowledge this is the first study addressing the protective effects of MC in full-term broilers fed steam-pelleted feeds more nearly approximating commercial production.

Key words: aflatoxin, detoxification, montmorillonite, NovaSil PLUS, broiler

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[2]Use of trade names in this publication does not imply endorsement of the product mentioned or criticism of similar products not mentioned.

DESCRIPTION OF PROBLEM

Growers of corn and cottonseed in the South and Southeast, particularly Texas and Louisiana, have repeatedly suffered severe economic losses from aflatoxin contamination. In 1998, the estimated loss ranged between $17 and $40 million in Texas alone [1].

Long-term solutions to the problem (e.g., breeding aflatoxin-resistant plant hybrids) without sacrificing other desirable aspects of the crop are just that—long term and may never be achieved.

With respect to aflatoxin there are at least 3 possible short-term solutions: displacement of toxin-producing *Aspergillus flavus* with nontoxic strains [2], ammoniation (effective, but facing regulatory and environmental objections), and use of selective montmorillonite clays (**MC**) in the diet. Although the use of up to 2% MC is approved to increase flowability or as carriers, these clays are not approved by the FDA for the purpose of minimizing the well-known effects of aflatoxin-contaminated feed on animal health and performance.

Numerous laboratory studies have shown the effectiveness of these calcium and sodium MC [3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23], but no one has developed a general set of specifications by which a user could select a specific MC. Nor have there been studies to determine whether laboratory results can be scaled up to provide commercial feed products.

The purpose of this study was to determine whether a calcium MC (NovaSil PLUS) incorporated into commercial broiler feeds and fed using a 3-phase feeding program over a 6-wk production period would diminish the adverse effects of a feed containing very high concentrations of aflatoxin.

MATERIALS AND METHODS

Birds and Housing

A total of 900 male Cobb × Cobb [24] commercial broiler chickens were purchased from an area hatchery, and 800 were randomly assigned to 40 pens (20 chicks / pen) so that the total chick weight for each pen was 708 ± 20 g (35.4 g / chick). All chicks had been vaccinated for Marek's, Newcastle disease, and bronchitis at the hatchery. Each pen measured 1.73 × 2.29 m and provided a total floor area of 3.96 m^2. Feed was provided ad libitum by a single Brower tube-type hanging feeder [25] (13.6-kg capacity; 0.114 m^2 base area) in each pen. Maximum bird density was thus 1 chick / 0.19 m^2. Water was provided by Ziggity nipple drinkers (6 nipples / 48-in. stick per pen) [26]. Bedding was provided using 7.5 ft^3 of fresh pine shaving litter to a depth of approximately 10 cm on concrete floors. Temperature was monitored continuously over the 6-wk experimental period using 4 ERTCO Temp101 2400 baud temperature recorders [27]. The 1-d-old chicks were placed in their pens on the morning of April 07, 2004. Chicks dying within the first 4 d of the study were replaced with chicks of similar weight from a pool of extra birds that were separately maintained on each of the 4 dietary treatments. All animal procedures were reviewed and approved by the University IAACC committee under the AUP protocol number 2003-170.

Experimental Design

The 4 dietary treatments were split among the 40 floor pens in a complete block design. A 3-phase feeding program was utilized in which the starter diet was fed for 3 wk, the grower for 2 wk, and the finisher for 1 wk. Treatments consisted of a control, a control plus 0.5% MC (**CMC**), NovaSil PLUS [28], aflatoxin (**AF**), and aflatoxin plus 0.5% MC (**AMC**). A total of 6 chicks per pen were randomly preselected for blood, organ, and histological examination at the end of the 6-wk study period. These birds were identified by spray painting a black spot on each of their backs. This group of birds was repainted twice during the 6-wk growing period to insure positive identification at the end of the rearing period. At the end of the 6-wk growing period, 3 of the 6 painted birds were randomly retained for specific tissue analyses of blood serum; relative liver, spleen, and kidney weights; and histological examination of sampled organs. If any pen contained fewer than 3 spray-painted birds, another bird was randomly selected from the pen to provide a complete set of 120 birds for specific tissue analysis.

Observations were made at least twice per day in the morning and evening, and a general necropsy was performed on all dead birds. A

Figure 1. Photograph of *Aspergillus parasiticus* cultured corn used to prepare the diets for this study.

total of 8 chicks were replaced during the 4-d replacement window and were not counted as treatment-related mortality. All birds and unconsumed feed were weighed on a pen basis at the end of each of the 3 feeding phases. The weight gains of the dead birds were used to calculate mortality adjusted feed conversion for each feeding period.

Industry-type corn-soy broiler diets were formulated using clean (<20 ppb aflatoxin) corn or aflatoxin corn (~7,000 ppb) and dehulled soybean meal. Basal diets were prepared for each phase based on the assayed CP content of clean corn and soybean meal. Aflatoxin-contaminated corn replaced the clean corn in the 2 aflatoxin treatments without regard to actual CP content, which was about 1.5% higher than the clean corn. Significant quantities of mold remained on the aflatoxin-contaminated corn to the point of it appearing blackish green in color (Figure 1). We believe this contaminating mold contributed to the higher protein concentration. All diets were mixed for a minimum of 30 min using a Weigh-Tronix SFM-2000 stationary feed mill [29] fitted with a 1/16-in. hammer mill screen. Mixing time to achieve homogeneity for the aflatoxin and MC content was established by using a microtracer and salt assay techniques in test feeds. Basal diets for each phase of feeding were divided in half and sent directly to the CPM 1100 pellet mill (control and AF treatments) or mixed an additional 30 min after addition of 0.5% MC (CMC and AMC treatments). Starter diets were crumbled, whereas grower and finisher feeds were fed as intact 11/64-in. diameter pellets.

Aflatoxin Corn Preparation. To ensure that physiological and histopathological effects could be determined, we formulated the diets to contain a minimum aflatoxin concentration of 3,600 ppb for all aflatoxin treatments. To achieve this level of contamination, we set a target aflatoxin concentration for the aflatoxin-contaminated corn at 6,000 ppb. This level of aflatoxin was achieved using a combination of naturally contaminated corn with an average aflatoxin concentration of 708 ppb, cultured rice powder (2.4×10^6 ppb) produced through fermentation of rice by *Aspergillus parasiticus* NRRL 2999 as described by Kubena et al. [11], and additional corn (7,830 ppb) produced by culturing with *Aspergillus parasiticus* under green house conditions. The contaminated corn was finely ground using a hammer mill fitted with a 1/16-in. screen. The cultured rice powder was added to the corn and mixed for a minimum of 40 min. Separately prepared batches were then remixed in a 1:1 ratio an additional 40 min to produce a single uniform batch of finely ground aflatoxin contaminated corn with average aflatoxin B_1 and B_2 concentrations assayed at 6,867 and 380 ppb, respectively. The ground corn preparation was further screened for 18 additional mycotoxins and found to contain 14 ppm fumonisin. No other mycotoxins were detected [30].

Analytical

Determination of CP. Corn and soybean meal were analyzed for crude protein by combustion nitrogen analysis (LECO) FP2000 [31] prior to diet preparation. Crude protein concentration of the clean and aflatoxin corns used for this study averaged 8.44 and 10.02%, respectively. The CP content of the soybean meal used in this study averaged 47.75%. Mixed feeds were further assayed for CP by combustion.

Determination of Aflatoxin. Feed ingredients and diets were assayed using both the Vicam Afla Test Kits [32] (AOAC method 972.26) and thin-layer chromatography (AOAC method 991.31) using a procedure developed by the Agricultural Analytical Services of the Office of the Texas State Chemist.

Determination of Mixing Times. Feed mixing time was established by using a microtracer

and by determining added salt on at least 7 samples obtained at various times by vertical grain probing within the mixer taking stream samples collected within the mixer while mixing or collecting stream samples upon load-out of test feeds.

Blood Serum Analysis. After the 6-wk rearing period, 3 of the 6 broilers from each pen that had been randomly selected and spray painted on d 1 were bled by cardiac puncture for serum biochemical analyses. Serum concentrations of uric acid, creatinine, urea nitrogen, glucose, calcium, inorganic phosphorus, total protein, albumin, cholesterol, triglycerides, and activities of alkaline phosphatase, alanine transferase, aspartate aminotransferase, glutamyltransferase, lactate dehydrogenase, and creatine kinase were determined on a clinical chemistry analyzer [33] according to the manufacturer's recommended procedures.

Tissue Histology. After blood samples were taken, the same broilers were killed by cervical dislocation, and the liver, kidney, and spleen were removed and weighed. Tissue samples (liver, kidney, and spleen) were fixed in formalin and evaluated for various lesions and other abnormalities. Liver tissue was evaluated for necrosis, bile duct proliferation, hemorrhage, hepatic lipidosis, fibrosis, inflammation, cytomegaly, and disruption of normal lobular architecture. Kidneys were evaluated for thickening of the glomerular basement membrane, and spleens were evaluated for lymphoid depletion. Tissues with no, minor, or major evidence of lesion or abnormality were given a score of 0, 1, or 2, respectively.

Safety

All personnel involved in this research attended a safety seminar given by experts in toxicology prior to being allowed to participate in this study. Personnel involved in the preparation of aflatoxin-contaminated corn and diets wore half-face respirators fitted with P100 particle filters, Tyvek-hooded coveralls with attached boots, eye protection, and latex and leather gloves. Those workers most likely to come into direct contact with dust during the feed preparation wore North 7600 full faceplate respirators (North Safety no. 7600-8A [34]). Workers were required to wear N95 particulate respirators (3M No. 9211 [34]), latex gloves, KomfortGuard disposable coveralls (Kimberly-Clark No. 40054 [34]) and bouffant caps whenever entering the rearing facility. Workers actually entering the pens wore half faceplate respirators fitted with P100 particle filters and leather gloves in addition to the other safety apparel.

Cleanup

Birds dying during the course of the study were disposed of by incineration after general necropsy. At the end of the 6-wk study period, all remaining birds were killed by cervical dislocation and buried together with used litter at a local landfill.

Flush corn was passed through the mixing and pelleting equipment in a series of 500-lb batches until the residual aflatoxin was determined to be less than 100 ppb. This contaminated flush corn was disposed of at the University toxic waste facility. All facilities and equipment were then washed with a solution of 2% household bleach delivered via high-pressure spray. The bleach solution was allowed to set approximately 1 h before rinsing with clean water. Care was taken to avoid hosing down critical electrical equipment.

Statistical Treatment

Data were subjected to 1-way ANOVA based on the complete block design. Block effects were not significant for any treatment variable; therefore, the data were analyzed as a simple 1-way ANOVA. Arcsin transformations of mortality data were performed prior to statistical analysis. Means showing significant differences in the ANOVA were separated using the Duncan's multiple range procedure. The pooled SEM were calculated by taking the square root of the ANOVA mean squares error term divided by the harmonic mean sample size (10 with respect to pen data). All data were analyzed using the GLM univariate procedures of SPSS Version 11.0 for Windows [34]. The threshold for statistical significance was $P \leq 0.05$.

RESULTS

Growth and Performance

Broilers receiving the aflatoxin-contaminated feed gained about half as much weight as

Table 1. Effects of montmorillonite clay (MC) on average gain, feed conversion, and mortality

Treatment[1]	Gain, g	FCR[2]	Mortality, %
Phase 1[3]			
Aflatoxin	330.1[d]	2.05[a]	16.5[a]
Aflatoxin + MC	380.7[c]	1.95[a]	13.0[a]
Control	732.6[a]	1.33[b]	3.5[b]
Control + MC	701.3[b]	1.33[b]	6.0[b]
PSEM	8.82	0.05	1.9
Phase 2			
Aflatoxin	566.4[c]	1.91[a]	15.2[a]
Aflatoxin + MC	759.8[b]	1.72[b]	6.9[b]
Control	1,134.9[a]	1.66[b]	1.6[c]
Control + MC	1,082.1[a]	1.68[b]	0.5[c]
PSEM	22.81	0.04	1.7
Phase 3			
Aflatoxin	318.9[c]	2.32[a]	9.2[a]
Aflatoxin + MC	424.5[b]	2.18[ab]	4.2[b]
Control	550.3[a]	2.08[ab]	0.5[c]
Control + MC	592.8[a]	1.98[b]	1.1[bc]
PSEM	21.94	0.09	1.2
6-wk cumulative			
Aflatoxin	1,215.5[c]	2.01[a]	36.0[a]
Aflatoxin + MC	1,565.0[b]	1.89[b]	22.5[b]
Control	2,417.8[a]	1.65[c]	5.5[c]
Control + MC	2,376.2[a]	1.65[c]	7.5[c]
PSEM	38.18	0.02	1.9

[a–d]Values within a column with different superscripts are significantly different ($P < 0.05$).
[1]PSEM = pooled SEM.
[2]Feed to gain ratio calculated on a per pen basis (kg/kg).
[3]Values represent the average of 10 pens.

those birds receiving the uncontaminated feeds during each phase of production (Table 1). During phase 1, broilers receiving the AMC diet gained about 50 g more than birds not receiving the supplement. Birds given the CMC diet gained about 31 g less than the nonsupplemented control treatment. Weight gains during phases 2 and 3, as well as 6-wk cumulative gain, were not different between the control and CMC groups, whereas birds receiving the aflatoxin-contaminated feed with 0.50% MC gained significantly more weight than the birds receiving the aflatoxin corn diet without MC. The control and CMC groups gained approximately 2.4 kg over the 6-wk growing period. The broilers receiving aflatoxin-containing feed gained approximately 1.2 kg, whereas the AMC group gained approximately 1.6 kg throughout the study.

The feed to gain ratio (**FCR**) of broilers receiving the aflatoxin-contaminated feed were significantly higher when compared with the birds receiving the control diet during phase 1 (Table 1). By phases 2 and 3 there was no difference in FCR between the AMC treatment and the control feed treatments. Cumulative FCR over the entire 6-wk growing period for the control feed treatments averaged 1.65, the AMC group averaged 1.89, whereas the A group averaged 2.01.

Mortality was significantly higher during phase 1 for broilers receiving the aflatoxin-contaminated feed vs. the control feed. During phase 2 there was significantly less mortality in the AMC group than in the AF group, although mortality in the AMC group was still significantly higher than the control and CMC treatments. During phase 3, mortality was once again significantly lower in the AMC group than in the AF group. There was no difference in mortality between the AMC treatment and the CMC treatment, but the control group experienced significantly less mortality than the AMC treatment. Overall, a total of 36% of the birds receiving the aflatoxin treatment died, whereas 22.5% of the birds receiving aflatoxin in combination with NovaSil PLUS died. Only 5.5 and 7.5% of the birds on the control treatment and the control plus NovaSil PLUS died (Table 1).

Relative Organ Weights

Aflatoxin had very deleterious effects on liver, kidney, and spleen as shown in Table 2. Relative weights (organ weight divided by body weight) were essentially twice that of control birds not receiving aflatoxin, which is very typical of aflatoxicosis.

The MC did not have any significant effects on the relative organ weights of the liver, kidney, or spleen in broilers receiving the noncontaminated control feed (Table 2). The relative weights of the liver and kidney were significantly lower in the AMC treatment vs. the AF treatment, indicating some protection by MC. The MC did not affect the relative spleen weight in birds receiving aflatoxin-contaminated feed.

Blood Serum Chemistry

Effects of MC on blood serum chemistry are shown in Table 3. In birds receiving the control feed, MC had little effect except that uric acid was significantly higher, whereas albumin and

Table 2. Effects of montmorillonite clay (MC) on relative organ weights

	Treatment[2]				
Organ variable[1]	AF	AMC	Control	CMC	PSEM
Relative liver weight, %	4.56[a]	4.00[b]	1.90[c]	1.96[c]	0.13
Relative kidney weight, %	1.50[a]	1.11[b]	0.56[c]	0.60[c]	0.04
Relative spleen weight, %	0.22[a]	0.22[a]	0.12[b]	0.12[b]	0.01

[a–c]Values within a row with different superscripts are significantly different ($P < 0.05$).
[1]Values represent the average of 30 birds.
[2]AF = aflatoxin; AMC = feed with aflatoxin plus 0.5% MC; CMC = control feed plus 0.5% MC; PSEM = pooled SEM.

cholesterol were significantly lower than the control. The MC did not significantly alter any of the serum variables within the birds receiving the aflatoxin-contaminated feed. There were no differences between the AMC and control treatments with respect to alkaline phosphatase, alanine aminotransferase, creatine kinase, glutamyltransferase, lactate dehydrogenase, triglycerides, or uric acid (Table 3). Aflatoxin effects were most notable with respect to total protein, albumin, glucose, calcium, cholesterol, phosphorus, and aspartate aminotransferase. For each of these serum variables, concentrations were significantly lower in birds receiving aflatoxin, irrespective of MC addition.

Tissue Histology

Histological analysis suggested there was significant damage to liver, kidney, and spleenic tissues in broilers receiving aflatoxin-contaminated feed (Table 4). Liver tissue from the aflatoxin treatments had significantly more bile duct proliferation, hepatic lipidosis, inflammation, and disruption of normal lobular architecture than tissue from the clean feed treatments. With respect to total liver lesions, the aflatoxin treatments resulted in significantly more lesions than the clean feed treatments. With respect to thickening of the kidney GMB, MC resulted in a significant reduction vs. the aflatoxin treatment but not as low as either of the clean feed treatments. When total scores were summed across all 3 tissues, we found a significant reduction in lesions in birds fed the MC-supplemented aflatoxin feed.

DISCUSSION

The primary purpose of this study was to evaluate the reported protective effects of MC

Table 3. Effects of montmorillonite clay (MC) on blood serum chemistry

	Treatment[2]				
Serum variable[1]	AF	AMC	Control	CMC	PSEM
Albumin, g/dL	0.37[c]	0.42[c]	1.10[a]	0.98[b]	0.04
Alkaline phosphatase, IU/L	2317[c]	2,944[bc]	3,995[ab]	4,467[a]	441
Alanine aminotransferase, IU/L	3.61	3.42	3.14	4.03	0.48
Aspartate aminotransferase, IU/L	136.6[b]	157.9[b]	235.1[a]	222.1[a]	10.0
Urea nitrogen, mg/dL	1.18	1.19	1.23	1.18	0.11
Calcium, mg/dL	7.82[b]	8.01[b]	9.47[a]	8.98[a]	0.20
Cholesterol, mg/dL	39.70[c]	45.30[c]	90.55[a]	79.13[b]	3.11
Creatine kinase, IU/L	6,719[c]	7,316[bc]	10,302[ab]	10,511[a]	1070
Creatinine, mg/dL	0.24	0.26	0.27	0.26	0.01
Glutamyltransferase, IU/L	17.97	15.30	15.76	14.83	1.04
Glucose, mg/dL	180.3[b]	185.3[b]	218.5[a]	209.6[a]	5.32
Phosphorus, mg/dL	4.99[b]	4.90[b]	5.71[a]	5.61[a]	0.18
Lactate dehydrogenase, IU/L	1174	1000	1172	1231	126
Total protein, g/dL	1.16[b]	1.20[b]	2.26[a]	2.07[a]	0.08
Triglycerides, mg/dL	18.8[c]	22.6[bc]	26.0[ab]	30.8[a]	1.98
Uric acid, mg/dL	3.13[b]	2.84[b]	3.66[b]	4.56[a]	0.10

[a–c]Values within a row with different superscripts are significantly different ($P < 0.05$).
[1]Values represent the average of 30 birds with the exception of the control treatment, which represents 29 birds.
[2]AF = aflatoxin; AMC = feed with aflatoxin plus 0.5% MC; CMC = control feed plus 0.5% MC; PSEM = pooled SEM.

Table 4. Effects of montmorillonite clay (MC) on liver, kidney and spleenic tissue score

	Treatment[2]				
Tissue variable[1]	AF	AMC	Control	CMC	PSEM
Hepatic bile duct proliferation	0.47[a]	0.37[a]	0.0[b]	0.0[b]	0.09
Hepatic lipidosis	1.03[a]	1.13[a]	.28[b]	.45[b]	0.09
Hepatic inflammation	1.13[a]	0.93[a]	0.48[b]	0.38[b]	0.08
Abnormal lobular architecture	0.70[a]	0.60[a]	0.0[b]	0.0[b]	0.06
Total Liver Score	3.47[a]	3.07[a]	0.76[b]	0.83[b]	0.19
Kidney GBM thickening	1.57[a]	0.83[b]	0.07[c]	0.14[c]	0.09
Spleenic lymphoid depletion	0.20[a]	0.13[ab]	0.03[b]	0.00[b]	0.05
Total tissue score	5.24[a]	4.03[b]	0.86[c]	0.97[c]	0.23

[a–c]Values within a row with different superscripts are significantly different ($P < 0.05$).
[1]Values represent the average of 29 to 30 birds. Only tissue scores for which there were significant differences are shown. Tissues were given a score of 0, 1, or 2 depending on whether no lesions, mild lesions or severe lesions were observed. GBM = glomerular basement membrane.
[2]AF = aflatoxin; AMC = feed with aflatoxin plus 0.5% MC; CMC = control feed plus 0.5% MC; PSEM = pooled SEM.

with respect to aflatoxin-contaminated feed in full-term broilers raised as closely as possible to an industry-type setting. This is important with respect to any future claims regarding the efficacy of MC for protection from aflatoxin. The MC was supplied as NovaSil PLUS at 0.5% of the diet. We had hoped to obtain naturally contaminated corn with aflatoxin concentrations high enough to achieve a final dietary concentration approaching 4,000 ppb but were unable to procure significant quantities with concentrations over 1,200 ppb. We felt it absolutely critical that we feed enough toxin to observe a significant reduction in performance. To achieve these objectives we had to use aflatoxin cultured greenhouse corn (7,830 ppb, Figure 1) as well as cultured rice powder containing aflatoxin (2.4×10^6 ppb) to obtain sufficient quantities of feed materials to conduct this experiment. Numerous studies in the literature suggest that 0.5% MC will diminish the adverse effects of aflatoxin on broiler performance. Most published studies were conducted using aflatoxin cultured rice powder or purified aflatoxin B1 supplemented to a mash feed over a 3-wk starter period. To our knowledge this is the first study to use substantial quantities of aflatoxin contaminated corn in combination with rice powder in steam-pelleted diets.

Based on published reports in the literature [5, 10, 11, 12, 14, 16] we had anticipated a higher level of protection than we ultimately observed. Those researchers observed approximately 40% to almost 100% protection against the adverse effects of aflatoxin. Although the birds in our study fed aflatoxin-contaminated diets with 0.5% MC performed significantly better than birds fed the toxin without the clay, they did not perform as well as the control group. The MC treatment reduced mortality from aflatoxin by 37.5% (36% for the aflatoxin alone treatment and 22% for the AMC treatment) and provided 15% protection in terms of body weight gain. Ideally, we would have liked higher levels of protection, as reported in previous studies. There may be several explanations for this unexpected performance including the presence of other mycotoxins in the feeds, sampling error with respect to our assays leading to higher than anticipated concentrations, or complex physical matrix effects due to 3 different sources of aflatoxin in combination with steam pelleting. The net effect was that we had a very complex matrix of contaminated ingredients unlike anything previously reported in the literature. The test feeds were analyzed for various specific mycotoxins, but none were detected other than 14 ppm fumonisin, which is relatively nontoxic to chickens. It should be noted that the aflatoxin-contaminated diets used in this study were literally green with mold and undoubtedly contained many other toxic compounds. Mycotoxin assays are always problematic because of localized areas of high concentration. Although we were careful in our sampling procedures, one is never sure of truly getting a representative sample with respect to aflatoxin analysis. There were clearly other unanticipated factors affecting this study,

including a possibility that we exceeded the capacity of the MC to bind all of the aflatoxin present in these highly contaminated feeds. This possibility is comparable with previous research in which researchers [12] observed less than expected protection against the toxic effects in chicks fed an aflatoxin plus T-2 toxin combination diet based on the protection against aflatoxin alone. These researchers suspected that there was sufficient aflatoxin remaining to interact synergistically with T-2 toxin (synergism had been previously shown), thus causing greater effects than would be predicted. Also, unlike most of the previous studies conducted in battery brooders, this study was conducted on the floor where the birds were in continual contact with their feces.

CONCLUSIONS AND APPLICATIONS

1. The data showed that 0.5% MC fed as NovaSil PLUS could provide a level of protection from over 4,000 ppb aflatoxin even in pelleted diets containing as much as 66% moldy corn.
2. Additional studies are warranted to further evaluate the effects of ingredient matrix, steam pelleting, and rearing environment on the aflatoxin binding capacity of MC.

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