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Effects of Two Different Types of Montmorillonite on Growth Performance and Serum Profiles of Broiler Chicks during Aflatoxicosis

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Abstract: Experiments were conducted to compare the efficacy of montmorillonite (M) and montmorillonite nanocomposite (MN) to reduce the toxicity of aflatoxin (AF) in broiler chicks from 0 to 42 days of age. A total of 240 chicks were assigned to 6 dietary treatment groups (0 g of adsorbents and 0 mg of AF/kg feed, 3 g of M/kg feed, 3 g of MN/kg feed, 0.11 mg of AF/kg feed, 0.11 mg of AF plus 3 g of M/kg feed, and 0.11 mg of AF plus 3 g of MN/kg feed). Compared to the control, AF alone significantly decreased ADG and feed efficiency. Marked improvements in performance by the addition of MN to AF diet demonstrated the ability of MN to reduce the toxic effects of AF. However, the addition of M can not diminish the growth inhibitory effects of AF. AF intake markedly increased relative weights of liver, kidney, spleen, and pancreas, and resulted in significant alterations of serum biochemical values and enzymatic activities. Chicks fed MN with AF diet had apparent recovery or restoration of AF-induced organ lesions and aberrations in serum profiles, whereas chicks fed M with AF diet had relative organ weights and serum profiles similar to those of chicks fed AF alone, which indicated beneficial effects of MN and nonprotective effects of M. These findings suggest that feeding MN can effectively prevent the adverse effects associated with aflatoxicosis in broiler chicks.

Key Words: Aflatoxin, montmorillonite nanocomposite, toxicity, broiler chicks

Introduction

Aflatoxins (AFs) are a group of extremely toxic chemicals produced by strains of *Aspergillus flavus* and *Aspergillus parasiticus*, and their main biological effects are carcinogenicity, mutagenicity, and teratogenicity (1). These toxins occur worldwide, contaminating corn, soybeans, wheat, and sorghum, which are normally used for poultry rations. Poultry are highly sensitive to the effects of AF, the frequent contamination of agricultural commodities with AF and the chronic exposure of poultry to these toxins greatly affect the profitability of poultry production, and cause severe economic losses in the poultry industry (2). Therefore, protection of feed and food from AF contamination is a critical need. A practical approach to detoxification is the use of sorbents in the diet that adsorb AF in the gastrointestinal tract of animals and reduce bioavailability and toxicity. Montmorillonite, bentonite, and hydrated sodium calcium aluminosilicate (HSCAS), as anticaking agent for animal feed, have been reported to prevent diseases associated with aflatoxicosis in animals, including chicks, turkey poults, and pigs (3,4). However, natural montmorillonite (M) is always congregated with some impurities, which makes its adsorptive effect difficult to exert and makes its addition level high. Via nanomodification of natural montmorillonite, a new sorptive additive-montmorillonite nanocomposite (MN) has been developed by Feed Science Institute of Zhejiang University. The purpose of the study was to evaluate the effectiveness of M and MN in reducing the adverse effects produced by AF in broiler chicks.

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Materials and Methods

Materials

AFs were produced via contamination of milled corn by *Aspergillus parasiticus* NRRL 2999. The AF content in the contaminated milled corn was 1.14 mg/kg (1.00 mg/kg AFB_1, 0.07 mg/kg AFG_1, 0.05 mg/kg AFB_2, and 0.02 mg/kg AFG_2) by HPLC determination (5). The contaminated milled corn was incorporated into the basal diet to provide the desired level of 0.11 mg AF/kg of diet.

M and MN were obtained from Feed Science Institute, Zhejiang University, Hangzhou, China. The montmorillonite sample was collected from Inner Mongolia, China. The sample was processed via separation and purification, and natural montmorillonite (M) was obtained with a purity above 98%. According to the method of Xu et al. (6), M was further prepared by depolymerization and dispersion, and nanoparticles were obtained in 10-60 nm sizes. These nanoparticles were modified by pillar composition, intercalation composition, and spot polymerization, and finally a new inorganic montmorillonite nanocomposite (MN) was constructed.

Animals and Diets

A total of 240 1-day-old Avian broiler chicks were individually weighed, wingbanded, and randomly distributed to 6 treatment groups by equal sex ratio (4 pens i.e. replicate per treatment with 10 chicks per pen). Chicks were fed for 42 days under standard management conditions with feed and water available ad libitum. All diets contained adequate levels of nutrients as recommended by the National Research Council (7). The composition and nutrient content of the basal diet are presented in Table 1. Experimental diets for each treatment were as follows: 1) control feed with 0 g adsorbents/kg and 0 mg AF/kg, 2) 3 g M/kg feed, 3) 3 g MN/kg feed, 4) 0.11 mg AF/kg feed, 5) 0.11 mg AF/kg plus 3 g M/kg feed, 6) 0.11 mg AF/kg plus 3 g MN/kg feed.

Measurements

Body weight and feed consumption were measured weekly and mortality was recorded. At the end of the trial, chicks were killed by cervical dislocation and

Table 1. The composition of normal and AF-contaminated corn/soybean diets.

Item	0-3 weeks [1]		4-6 weeks [2]	
	Normal diet	AF diet	Normal diet	AF diet
Uncontaminated corn	54.40	44.40	61.70	51.70
Contaminated corn [3]	0.00	10.00	0.00	10.00
Soybean meal	28.50	28.50	25.40	25.40
Corn gluten meal	8.00	8.00	5.00	5.00
Rapeseed oil	5.00	5.00	4.00	4.00
Limestone	1.60	1.60	1.50	1.50
Dicalcium phosphate	1.50	1.50	1.50	1.50
Salt	0.30	0.30	0.30	0.30
DL-methionine	0.20	0.20	0.10	0.10
Mineral premix [4]	0.30	0.30	0.30	0.30
Vitamin premix [5]	0.20	0.20	0.20	0.20

[1] Analyzed to supply 13.01 MJ/kg ME, 22.5% CP, 0.87% Methionine+cystine, 1.05% Lysine, 0.95% Ca, and 0.46 % available P.

[2] Analyzed to supply 12.92 MJ/kg ME, 19.4% CP, 0.70% Methionine+cystine, 0.96% Lysine, 0.91 % Ca, and 0.35 % available P.

[3] Contaminated corn contained 1.14 mg/kg of aflatoxin.

[4] Supplied per kg of diet: 12 mg of Cu; 98 mg of Fe; 50 mg of Zn; 80 mg of Mn; 0.35 mg of I.

[5] Supplied per kg of diet: 8000 IU of vitamin A; 200 IU of vitamin D_3; 40 IU of vitamin E; 4.5 mg of vitamin B_1; 9 mg of vitamin B_2; 9 mg of vitamin B_6; 0.04 mg of vitamin B_{12}; 85 mg of niacin; 27 mg of d-pantothenic acid; 0.4 mg of biotin; 1.2 mg of folic acid.

necropsied. Liver, kidney, spleen, and pancreas were removed and weighed. Serum concentrations of total protein (TP), albumin (ALB), globin (GLOB), urea nitrogen (UN), cholesterol (CHOL), triglycerides (TG) and activities of glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), -glutamyl-transferase (GGT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH), and cholinesterase (CHE) were determined on a biochemical autoanalyzer according to the manufacturer's recommended procedure (Beckman Instruments, Inc.).

Statistical Analysis

All data were subjected to statistical analysis using the General Linear Models Procedure of the Statistical Analysis System (8). The significance of the differences among the treatment groups with variable means was determined by Duncan's new multiple range test. All statements of significance were based on $P \leq 0.05$.

Results

The effects of M, MN, and AF on growth performance are shown in Table 2. Compared with control chicks, significant decrease in ADG and marked increase in feed/gain ratio were observed in chicks fed AF alone ($P < 0.05$). Chicks fed AF with MN had a higher ADG and lower feed/gain ratio than chicks fed AF alone (P 0.05), whereas no significant differences in ADG and feed/gain ratio were noted between chicks fed AF with M and chicks fed AF alone. All chicks fed adsorbents alone (i.e., M and MN) had ADG and feed/gain ratio similar to those of control chicks.

Data presented in Table 3 showed the effects of dietary treatments on relative organ weights. Compared with control chicks, there were increased relative weights of liver, kidney, spleen, and pancreas for chicks fed AF alone ($P < 0.05$). MN was effective in preventing these significant increases when added to AF diet ($P < 0.05$), whereas these increases were not prevented by the addition of M to AF diet. Generally, chicks fed adsorbents alone (i.e., M and MN) were not significantly different from control chicks for relative organ weights.

The effects of M, MN and AF on serum biochemical values are presented in Table 4. Levels of serum TP, ALB, UN, CHOL, and TG were markedly reduced in chicks fed AF alone compared with control chicks ($P < 0.05$). The addition of MN to AF diet significantly increased levels of these values compared with chicks fed AF alone (P <

Table 2. Effects of M and MN on growth performance of broiler chicks with and without AF administration.

Item	Control	M	MN	AF	AF+M	AF+MN
ADG (g)	42.84 ± 0.75 ^{a}	43.02 ± 0.68 ^{a}	43.15 ± 0.85 ^{a}	40.24 ± 0.66 ^{b}	40.60 ± 0.53 ^{b}	42.51 ± 0.78 ^{a}
ADFI (g)	83.10 ± 2.50	83.19 ± 2.58	83.28 ± 2.60	83.29 ± 2.79	83.36 ± 2.62	83.31 ± 2.47
Feed/gain ratio	1.94 ± 0.05 ^{c}	1.93 ± 0.03 ^{c}	1.9 ± 0.04 ^{c}	2.07 ± 0.07 ^{a}	2.05 ± 0.07 ^{ab}	1.96 ± 0.06 ^{bc}
Mortality (%)	0	0	0	2.5	0	0

Values within a row with the different superscript letters differ significantly ($P < 0.05$).

Table 3. Effects of M and MN on relative organ weights of broiler chicks with and without AF administration.

Item	Control	M	MN	AF	AF+M	AF+MN
Liver (%)	2.25 ± 0.13 ^{c}	2.23 ± 0.10 ^{c}	2.24 ± 0.13 ^{c}	2.37 ± 0.11 ^{a}	2.35 ± 0.07 ^{ab}	2.27 ± 0.08 ^{bc}
Kidney (%)	0.58 ± 0.06 ^{c}	0.57 ± 0.06 ^{c}	0.59 ± 0.07 ^{bc}	0.67 ± 0.06 ^{a}	0.65 ± 0.09 ^{ab}	0.60 ± 0.08 ^{bc}
Spleen (%)	0.13 ± 0.03 ^{b}	0.13 ± 0.02 ^{b}	0.14 ± 0.02 ^{b}	0.19 ± 0.04 ^{a}	0.18 ± 0.01 ^{a}	0.15 ± 0.03 ^{b}
Pancreas (%)	0.22 ± 0.02 ^{b}	0.23 ± 0.04 ^{b}	0.20 ± 0.04 ^{c}	0.27 ± 0.02 ^{a}	0.27 ± 0.01 ^{a}	0.23 ± 0.03 ^{b}

Values within a row with the different superscript letters differ significantly (P < 0.05).

Table 4. Effects of M and MN on serum biochemical values of broiler chicks with and without AF administration.

Item	Control	M	MN	AF	AF+M	AF+MN
TP (g/dL)	5.06 ± 0.06 [a]	5.01 ± 0.08 [ab]	4.98 ± 0.11 [ab]	4.61 ± 0.13 [c]	4.88 ± 0.09 [b]	4.89 ± 0.10 [b]
ALB (g/dL)	1.98 ± 0.13 [a]	1.97 ± 0.09 [a]	1.93 ± 0.15 [a]	1.60 ± 0.13 [b]	1.78 ± 0.10 [ab]	1.85 ± 0.17 [a]
GLOB (g/dL)	3.08 ± 0.11	3.04 ± 0.06	3.05 ± 0.08	3.01 ± 0.10	3.10 ± 0.13	3.04 ± 0.09
UN (mg/dL)	6.25 ± 0.30 [a]	6.17 ± 0.34 [a]	6.21 ± 0.31 [a]	4.56 ± 0.35 [c]	4.99 ± 0.28 [bc]	5.32 ± 0.27 [b]
CHOL (mg/dL)	125.10 ± 11.96 [a]	124.23 ± 10.04 [a]	131.38 ± 11.34 [a]	89.13 ± 9.79 [b]	98.58 ± 11.99 [b]	115.95 ± 9.82 [a]
TG (mg/dL)	71.07 ± 5.01 [ab]	72.52±5.10 [a]	76.08 ± 5.42 [a]	48.03 ± 5.57 [c]	54.45 ± 4.98 [c]	63.86 ± 5.99 [b]

Values within a row with the different superscript letters differ significantly ($P < 0.05$).

0.05), whereas these values in chicks fed AF with M were not significantly different from those in chicks fed AF alone except for TP level. There were no significant differences in these values between chicks fed adsorbents alone (i.e., M and MN) and control chicks. Serum GLOB levels were not affected by treatments.

Toxicity of AF was also expressed through significant changes in serum enzymatic activities (Table 5). Chicks fed AF alone had increased levels of Serum GPT, GOT, GGT, ALP, and LDH compared with control chicks ($P < 0.05$). Increased levels of these enzymes were prevented by the addition of MN to AF diet compared with AF diet alone ($P < 0.05$), whereas increased levels of these enzymes were not prevented by the addition of M to AF diet. These parameters in chicks fed adsorbents alone (i.e., M and MN) were not significantly different from control chicks. There were no differences in serum CHE levels.

Discussion

In the study, the toxic effects of AF were expressed as reduced ADG, higher feed/gain ratio, increased relative organ weights, and alterations in serum profiles. The toxic effects produced by AF were in general agreement with previous reports (9-11). Marked improvements in performance by the addition of MN to AF diet demonstrated the ability of MN to diminish the inhibitory effects of AF. Generally, there were no significant differences between chicks fed adsorbents alone (i.e., M and MN) and control chicks for most parameters evaluated, indicating the adsorbents were inert and nontoxic.

Aflatoxins have been known to irritate gastrointestinal tract and gut and cause dysfunction, thus increasing the relative weights of these organs, and also the target organs were liver and kidney (12). The relative weights of liver, kidney, spleen, and pancreas were significantly increased in chicks fed AF alone. The results obtained were in agreement with previous reports by Huff et al.

Table 5. Effects of M and MN on serum enzyme activities of broiler chicks with and without AF administration.

Item	Control	M	MN	AF	AF+M	AF+MN
GPT (U/L)	13.88 ± 0.50 [b]	13.70 ± 0.59 [b]	13.75 ± 0.40 [b]	15.37 ± 0.42 [a]	15.21 ± 0.39 [a]	14.20 ± 0.54 [b]
GOT (U/L)	106.12 ± 11.16 [c]	103.44 ± 12.13 [c]	101.93 ± 8.92 [c]	130.76 ± 13.27 [a]	127.48 ± 10.85 [ab]	113.03 ± 10.29 [bc]
GGT (U/L)	6.11 ± 0.42 [c]	6.24 ± 0.38 [c]	6.56 ± 0.34 [bc]	9.44 ± 0.46 [a]	8.90 ± 0.44 [a]	7.02 ± 0.35 [b]
ALP (U/mL)	11.43 ± 0.50 [c]	11.29 ± 0.49 [c]	10.78 ± 0.58 [c]	15.85 ± 0.46 [a]	15.76 ± 0.44 [a]	13.01 ± 0.42 [b]
LDH (U/L)	560.99 ± 45.59 [bc]	542.34 ± 35.13 [c]	535.52 ± 39.15 [c]	801.07 ± 56.38 [a]	767.29 ± 51.24 [a]	620.55 ± 41.94 [b]
CHE (U/mL)	26.06 ± 1.93	27.12 ± 2.20	25.57 ± 2.44	23.91 ± 2.72	24.84 ± 3.05	25.69 ± 2.61

Values within a row with the different superscript letters differ significantly ($P < 0.05$).

(13) and Kubena et al. (14). However, the increases in relative organ weights were prevented by the addition of MN to AF diet, which indicated that organ lesions induced by AF were ameliorated by MN addition, and apparently, MN had protective effects against the development of aflatoxicosis in chicks.

Aflatoxins have been reported to cause inhibition of protein synthesis. Hypoproteinemia is a common effect of aflatoxicosis, and reduced levels of serum TP, ALB, and UN are indictors of aflatoxicosis (15). Serum biochemical values and enzymatic activities of GPT, GOT, GGT, and ALP are sensitive serological indicators of liver and kidney toxicity (16). These parameters significantly altered in chicks fed AF diet alone, suggesting that AF caused a critical injury to these organs. Serum profiles in the trial were similar to those obtained by Kubena et al. (17,18), who reported that AF reduced indicators of protein synthesis such as serum TP, ALB, GLOB, and UN. The high levels of serum enzymes found in the study were consistent with the results of Kubena et al. (19,20) and Scheideler (21), who reported high levels of serum GOT, GGT, ALP, and LDH in chicks fed AF-contaminated diet. The addition of MN to AF diet reduced the high levels of the enzymes, showing the beneficial effects of MN on aflatoxicosis. The data agreed with previous results on the protective effects of MN on swine (22). A similar protection in poultry and rat by the addition of HSCAS, bentonite and zeolite to AF diet were also reported by Miazzo et al. (23) and Mayura et al. (24).

M and MN differed in their ability to recover AF-decreased performance, organ lesions, and aberrations in serum profiles. The performance data in the trial showed advantage for chicks fed MN with AF diet and disadvantage for chicks fed M with AF diet, which indicated beneficial effects of MN and nonprotective effects of M. The serum profiles reflected the growth performance data, chicks fed MN with AF diet had apparent recovery or restoration of serum profiles, whereas chicks fed M with AF diet had serum profiles similar to those of chicks fed AF alone. The variation in the ability of M and MN to prevent AF-induced changes in growth performance, relative organ weights and serum profiles may reflect the differing abilities of the adsorbents to bind toxins. The ability of the adsorbents to protect chicks from the toxic effects of AF may be influenced by a number of factors, including: 1) competition of the adsorbent with other ligands in the gastrointestinal tract, 2) binding capacity of the adsorbent (i.e., cation exchange capacities, plateau surface densities, porosities, predominant exchangeable cation) (25,26). These factors are important in predicting the efficacy of the adsorbent in vivo. Via nanomodification, MN possessed increased surface area, higher porosity, and stronger cation exchange activities along with more active sites, which, as a result, made its nanoparticle effect easy to exert and its efficacy of adsorption greatly enhanced. The presumed mechanism of action of MN was to adsorb the toxin in the gut and form stable sorption complex, then reduce gastrointestinal absorption of toxins and ameliorate the detrimental effects of AF on chicks (22). Effectiveness of different types of adsorbents for reducing the toxicity of AF in animals have been documented previously, but the results were discrepant, some had positive effects and the others had moderate or no effects at all, which is consistent with the findings in our study (27-30).

When diet containing AF were fed to broiler chicks, aflatoxicosis were induced and AF significantly affected overall broiler health and performance. There were reductions in growth rate, increases in relative organ weights and metabolic aberrations reflected in altered serum profiles. M and MN are not equal in their ability to protect against aflatoxicosis. The addition of MN can prevent the negative, toxin-induced effects; however, the addition of M can not diminish the adverse effects of AF. Whether the adsorbent can be incorporated into animal feed as anticaking agent to prevent diseases associated with aflatoxicosis, must be testified in vivo.

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