

## Effects of Montmorillonite on Growth Performance and Immune Function of Broiler Chickens Fed Moldy Peanut Meal-Based Diets

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### Abstract

This experiment aimed to investigate the effects of montmorillonite on growth performance and immune function in broiler chickens fed diets containing naturally aflatoxin B1 (AFB1)-contaminated peanut meal, and to determine the feeding efficacy of montmorillonite as a mycotoxin adsorbent. A total of 288 one-day-old healthy Arbor Acres (AA) male chicks were selected and randomly divided into 4 groups with 6 replicates per group and 12 birds per replicate. The positive control group was fed a corn-soybean meal basal diet, the negative control group diet replaced part of the soybean meal with naturally moldy peanut meal (containing AFB1 at 1,284.21  $\mu\text{g}/\text{kg}$ ; added at 8.8% and 12.0% in the starter and grower diets, respectively), and the treatment groups were supplemented with 2.0 g/kg of natural or modified montmorillonite in the negative control diet. The experimental period lasted 42 days. The results showed: 1) Compared with the positive control group, the moldy peanut meal significantly decreased average daily gain (ADG), average daily feed intake (ADFI), breast muscle percentage and thigh muscle percentage at 42 days of age, and plasma protein content ( $P < 0.05$ ), and significantly increased feed conversion ratio and immune organ index ( $P < 0.05$ ). 2) Compared with the negative control group, both natural and modified montmorillonite significantly increased ADG, ADFI, and plasma protein content ( $P < 0.05$ ), and significantly decreased immune organ index ( $P < 0.05$ ). 3) Compared with the natural montmorillonite group, modified montmorillonite significantly increased ADG and plasma total protein and albumin content ( $P < 0.05$ ), and significantly decreased thymus index ( $P < 0.05$ ). In conclusion, AFB1-contaminated moldy peanut meal can reduce growth performance and immune function in broiler chickens, montmorillonite can alleviate the adverse effects caused by AFB1, and modified montmorillonite demonstrates superior efficacy.

Full Text

## Effects of Montmorillonite on Growth Performance and Immune Function of Broiler Chickens Fed Moldy Peanut Meal-Based Diets

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**Abstract:** This experiment was conducted to investigate the effects of montmorillonite on the growth performance and immune function of broiler chickens fed peanut meal-based diets contaminated with naturally occurring aflatoxin B1 (AFB1), and to determine the feeding efficacy of montmorillonite as a mycotoxin adsorbent. A total of 288 one-day-old healthy male Arbor Acres (AA) broilers were selected and randomly divided into 4 groups, with 6 replicates per group and 12 chickens per replicate. The positive control group was fed a corn-soybean meal basal diet; the negative control diet used naturally moldy peanut meal (containing AFB1 1 284.21  $\mu\text{g}/\text{kg}$ ; replacing part of the soybean meal at 8.8% and 12.0% in the starter and grower diets, respectively); and the experimental groups were supplemented with 2.0 g/kg of natural or modified montmorillonite, respectively, in the negative control diet. The experimental period was 42 d. The results showed that: 1) Compared with the positive control group, moldy peanut meal significantly reduced the average daily gain (ADG), average daily feed intake (ADFI), 42-day-old breast muscle percentage and thigh muscle percentage, and plasma protein content of broilers ( $P<0.05$ ), and significantly increased the feed-to-gain ratio and immune organ indices ( $P<0.05$ ). 2) Compared with the negative control group, both natural and modified montmorillonite significantly increased the ADG, ADFI, and plasma protein content of broilers ( $P<0.05$ ), and significantly reduced immune organ indices ( $P<0.05$ ). 3) Compared with the natural montmorillonite group, modified montmorillonite significantly increased broiler ADG and the contents of total protein and albumin in plasma ( $P<0.05$ ), and significantly decreased the thymus index ( $P<0.05$ ). In summary, AFB1-contaminated moldy peanut meal can reduce the growth performance and immune function of broiler chickens; montmorillonite can alleviate the adverse effects caused by AFB1, with modified montmorillonite showing a better effect.

**Key words:** montmorillonite; aflatoxin B1; broiler chicken; growth performance; immunity

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In recent years, contamination of feed ingredients by mycotoxins has been relatively widespread, causing enormous economic losses to the breeding industry[1]. As a raw material for meal-type feeds, peanut meal contains nutrients such as protein, vitamins, and minerals (such as phosphorus, iron, and calcium), has a high protein content (about 50%) and good palatability; however, its imbalanced amino acid profile and severe contamination with aflatoxin B1 (aflatoxin B1, AFB1) have seriously affected its extensive use in feed[2]. AFB1 is highly toxic and was classified by the World Health Organization (WTO) in 1993 as a Group 1 carcinogen. AFB1 has strong hepatotoxicity and carcinogenicity, and adversely affects the digestive function and immune system of livestock and poultry, leading to reduced immunity, reproductive capacity, and feed conversion rate, damage to parenchymal organs, and growth

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...is impaired, and broiler chickens are among the sensitive animals<sup>[3-5]</sup>. At present, measures for the prevention and control of mycotoxins mainly include two approaches: natural adsorption and biodegradation; among them, natural adsorption is relatively low in cost<sup>[6]</sup>. Montmorillonite belongs to the natural aluminosilicate minerals and has a large specific surface area and strong adsorptive capacity, showing good adsorption of mycotoxins; modified aluminosilicate minerals have better selective adsorption and detoxification effects<sup>[7]</sup>. At present, many experiments use reagent-grade pure mycotoxins as test materials to study the adsorption of mycotoxins by different types of montmorillonite and their effects on livestock and poultry, whereas relatively few studies have used naturally mold-contaminated raw materials commonly present in production as materials. Therefore, in this experiment, naturally moldy peanut meal was used as the source of AFB<sub>1</sub> to study the adsorption effects of natural and modified montmorillonite and to evaluate their effects on the growth performance and immune function of broiler chickens.

## 1 Materials and Methods

### 1.1 Experimental Materials

Natural and modified montmorillonite products were both commercially available products, numbered SDNZ and SDAD, respectively. After X-ray diffraction

(XRD) testing, the physicochemical properties of the two montmorillonites are shown in Table 1.

**Table 1 Physical and chemical properties of montmorillonite**

Items	Montmorillonite content/%	Ethylene blue adsorbed/%	pH	Spacing of layers/nm
SDNZ	89.5	40.3	7.49	1.01
SDAD	91.0	43.9	9.37	1.51

The AFB<sub>1</sub> originated from moldy peanut meal (containing AFB<sub>1</sub> 1 284.21  $\mu\text{g}/\text{kg}$ ). The AFB<sub>1</sub> contents in the feeds of the positive control group, negative control group, natural montmorillonite group, and modified montmorillonite group during the early and later periods were 0.57, 112.51, 110.92 and 113.62  $\mu\text{g}/\text{kg}$ , and 0.60, 154.04, 153.86 and 155.50  $\mu\text{g}/\text{kg}$ , respectively. AFB<sub>1</sub> was determined by enzyme-linked immunosorbent assay (ELISA). The Chinese *Hygienical Standard for Feeds* (GB 13078–2001) stipulates that the AFB<sub>1</sub> content in broiler chicken feed should be  $\leq 10.0 \mu\text{g}/\text{kg}$  in the early period and  $\leq 20.0 \mu\text{g}/\text{kg}$  in the later period.

With reference to the *Chicken Feeding Standard* (NY/T 33–2004), corn-soybean meal type diets were formulated (Table 2) and cold-pressed into pellets. The feed for the positive control group was a corn-soybean meal type diet, whereas in the feeds for the negative control group and the experimental groups, part of the soybean meal was replaced with naturally moldy peanut meal.

**Table 2 Composition and nutrient levels of basal diets (air-dry basis) %**

Items	1 to 21 days of age		22 to 42 days of age		
	Control group	Experimental group	Control group	Experimental group	
		Control group	Experimental group	Control group	Experimental group
原料	Ingredients				
玉米	Corn	55.200	54.550	59.188	60.912
豆粕	Soybean meal	36.992	29.200	31.900	19.800
花生粕	Peanut meal		8.800		12.000

		Control group	Experimental group	Control group	Experimental group
豆油	Soybean oil	3.705	3.050	5.150	3.100
磷酸氢钙	CaHPO <sub>4</sub>	1.955	1.965	1.698	1.715
石粉	Limestone	1.190	1.220	1.095	1.160
食盐	NaCl	0.350	0.350	0.350	0.350
DL-蛋氨酸	DL-Met	0.268	0.288	0.248	0.272
L-赖氨酸盐酸盐	L-Lys · HCl	0.020	0.192	0.034	0.273
苏氨酸	Thr		0.065	0.017	0.098
维生素预混料	Vitamin premix <sup>1)</sup>	0.020	0.020	0.020	0.020
矿物质预混料	Mineral premix <sup>2)</sup>	0.200	0.200	0.200	0.200
氯化胆碱	Choline chloride	0.100	0.100	0.100	0.100
合计	Total	100.000	100.000	100.000	100.000
营养水平	Nutrient levels <sup>3)</sup>				
代谢能	ME/(MJ/kg)	12.56	12.56	13.19	13.19
粗蛋白质	CP	22.00	22.00	20.00	20.00
钙	Ca	1.00	1.00	0.90	0.90
总磷	TP	0.70	0.70	0.64	
有效磷	AP	0.45	0.45	0.40	0.40
赖氨酸	Lys	1.15	1.15	1.05	1.05
蛋氨酸	Met	0.62	0.63	0.50	0.51
蛋氨酸 + 半胱氨酸	Met+Cys	0.91	0.91	0.76	0.76
苏氨酸	Thr	0.81	0.81	0.72	0.72
色氨酸	Try	0.24	0.24	0.21	0.21

<sup>1)</sup> Vitamin premix provided the following per kg of diets: VA 12 500 IU, VD<sub>3</sub> 2 500 IU, VE 15 IU, VK<sub>3</sub> 2.65 mg, VB<sub>1</sub> 2 mg, VB<sub>2</sub> 6 mg, VB<sub>12</sub> 0.025 mg, biotin 0.35 mg, folic acid 1.25 mg, calcium pantothenate 12 mg, niacin 50 mg.

<sup>2)</sup> The mineral premix provided the following per kg of diet: Cu (as copper sulfate) 8 mg, Zn (as zinc sulfate) 75 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 80 mg, I (as potassium iodide) 0.35 mg, Se (as sodium selenite) 0.15 mg.

<sup>3)</sup> Nutrient levels were all calculated values.

## 1.2 Experimental Animals and Experimental Design

A total of 288 healthy 1-day-old Arbor Acres (AA) male broiler chicks were selected and randomly divided into 4 groups, with 6 replicates per group and 12 chickens per replicate. In view of the fact that peanut meal is prone to contamination by aflatoxins during production, and that it is difficult to obtain peanut meal raw material completely free of AFB<sub>1</sub>, in this experiment the positive control group was fed a corn-soybean meal basal diet; the negative control diet used naturally moldy peanut meal to replace part of the soybean meal, with the starter and grower diets containing 8.8% and 12.0% peanut meal, respectively; the 2 experimental-group diets were prepared by adding 2.0 g/kg natural or modified montmorillonite, respectively, to the negative control diet. The experimental period was 42 d.

## 1.3 Feeding Management

Broilers were reared in the same chicken house in 3-tier cages, with 24 h lighting, free access to drinking water, routine immunization, and feeding twice daily.

## 1.4 Measurement Indices and Methods

**1.4.1 Growth Performance** Birds were weighed after overnight fasting at 1, 21, and 42 days of age. Feed was withdrawn at 22:00 on the preceding day, with free access to drinking water, and weighing was conducted at 08:00 the next day using the replicate as the unit. Feed consumption of each replicate of experimental chickens at 1-21 days of age and 22-42 days of age was recorded, and average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) were calculated.

**1.4.2 Carcass Composition** At 21 and 42 days of age, respectively, one broiler whose body weight was close to the mean body weight of the replicate was selected from each replicate. Blood was collected from the wing vein, and the bird was slaughtered. The breast muscle and thigh muscle were separated and weighed, and the eviscerated yield, breast muscle percentage, thigh muscle percentage, and abdominal fat percentage were calculated. The calculation formulas were as follows:

$$[ \text{Eviscerated yield ;(\%)} = \text{eviscerated weight;(kg)} / \text{live weight;(kg)} \times 100; ]$$

$$[ \text{Breast muscle percentage ;(\%)} = \text{breast muscle weight;(kg)} / \text{eviscerated weight;(kg)} \times 100; ]$$

$$[ \text{Thigh muscle percentage ;(\%)} = \text{thigh muscle weight;(kg)} / \text{eviscerated weight;(kg)} \times 100; ]$$

$$[ \text{Abdominal fat percentage ;(\%)} = \text{abdominal fat weight;(kg)} / [\text{eviscerated weight;(kg)} + \text{abdominal fat weight;(kg)}] \times 100. ]$$

**1.4.3 Organ Indices** At 21 and 42 days of age, respectively, 1 broiler in each replicate whose body weight was close to the replicate mean was selected and slaughtered. The thymus, spleen, and bursa of Fabricius were excised, weighed, and the organ indices were calculated. The formula was as follows:

$$[\text{Organ index}(\%) = \text{organ weight (g)} / \text{live body weight (kg)} \times 100. ]$$

**1.4.4 Blood biochemical indices** At 21 and 42 days of age, respectively, 1 broiler in each replicate whose body weight was close to the replicate mean was selected. Blood was collected from the wing vein into anticoagulant tubes, centrifuged at 3 000 r/min for 10 min, and the supernatant was aliquoted and stored at  $-20^{\circ}\text{C}$ . Plasma total protein (TP), albumin (ALB), and globulin (GLB) contents were determined using an automatic biochemical analyzer; the reagent kits were purchased from Shanghai Kehua Bio-Engineering Co., Ltd.

## 1.5 Statistical analysis

After preprocessing in Excel, the data were subjected to one-way analysis of variance (one-way ANOVA) using SPSS 19.0. Multiple comparisons were performed using Duncan's method. Differences were considered significant at ( $P < 0.05$ ), and the results are expressed as means  $\pm$  standard deviations.

## 2 Results and analysis

### 2.1 Growth performance

As shown in Table 2, from 1 to 21 days of age: compared with the positive control group, moldy peanut meal significantly reduced broiler body weight, ADG, and ADFI ( $P < 0.05$ ), and significantly increased F/G in the negative control group and the natural montmorillonite group ( $P < 0.05$ ); compared with the negative control group, dietary supplementation with montmorillonite significantly increased broiler body weight ( $P < 0.05$ ), significantly increased ADG in the modified montmorillonite group ( $P < 0.05$ ), and F/G tended to decrease, with F/G in the modified montmorillonite group being significantly reduced ( $P < 0.05$ ); compared with the natural montmorillonite group, ADG was significantly increased in the modified montmorillonite group ( $P < 0.05$ ), while the other indices did not differ significantly ( $P > 0.05$ ).

From 22 to 42 days of age: compared with the positive control group, the negative control group had significantly lower broiler body weight, ADG, and ADFI ( $P < 0.05$ ), and significantly higher F/G ( $P < 0.05$ ); compared with the negative control group, the natural and modified montmorillonite groups had significantly higher body weight, ADG, and ADFI ( $P < 0.05$ ), and F/G was significantly reduced in the modified montmorillonite group ( $P < 0.05$ ); compared with the natural montmorillonite group, the modified montmorillonite group had significantly higher ADG ( $P < 0.05$ ) and significantly lower F/G ( $P < 0.05$ ).

From 1 to 42 days of age: compared with the positive control group, moldy peanut meal significantly reduced broiler ADG and ADFI ( $P < 0.05$ ) and significantly increased F/G ( $P < 0.05$ ); compared with the negative control group, the natural and modified montmorillonite groups both had significantly higher ADG and ADFI ( $P < 0.05$ ) and significantly lower F/G ( $P < 0.05$ ); compared with the natural montmorillonite group, the modified montmorillonite group showed no significant differences in any index ( $P > 0.05$ ).

Table 2 Growth performance of broilers

Table 2 Growth performance of broilers

Items	Positive control group	Negative control group	Montmorillonite group	Modified montmorillonite group
1 to 21 days of age				
BW/g	851.67 $\pm$ 24.87 <sup>a</sup>	750.44 $\pm$ 27.12 <sup>c</sup>	810.00 $\pm$ 28.03 <sup>b</sup>	829.93 $\pm$ 16.88 <sup>ab</sup>
ADG/g	38.28 $\pm$ 2.73 <sup>a</sup>	34.04 $\pm$ 2.86 <sup>b</sup>		

In the same row, values with different small letter superscripts mean significant difference ( $P < 0.05$ ), while with the same or no letter superscripts mean no significant difference ( $P > 0.05$ ). The same as below.

## 2.2 Carcass composition

As can be seen from Table 3, there were no significant differences in carcass composition among groups for broilers at 21 days of age ( $P > 0.05$ ). At 42 days of age, compared with the positive control group, moldy peanut meal significantly reduced the breast muscle percentage and leg muscle percentage of broilers ( $P < 0.05$ ), and had no significant effect on the eviscerated yield and abdominal fat percentage ( $P > 0.05$ ); compared with the negative control group, the breast muscle percentage and leg muscle percentage in the natural and modified montmorillonite groups were significantly increased ( $P < 0.05$ ); there were no significant differences in any indices between the 2 montmorillonite groups ( $P > 0.05$ ).

Table 3 Carcass composition of broilers %

Items	Positive control group	Negative control group	Montmorillonite group	Modified montmorillonite group

	Positive control group	Negative control group	Montmorillonite group	Modified montmorillonite group
21 days of age				
Dressing percentage	70.78±1.38	67.59±1.64	68.52±1.46	69.62±2.78
<i>Breast muscle percentage</i>	24.44±0.66	23.80±0.8		

### 2.3 Immune organ indices

As shown in Table 4, at 21 days of age, compared with the positive control group, moldy peanut meal significantly increased the thymus index, spleen index, and bursa of Fabricius index of broilers ( $P < 0.05$ ). Compared with the negative control group, the natural montmorillonite and modified montmorillonite groups had significantly lower thymus index and bursa of Fabricius index ( $P < 0.05$ ), and the spleen index in the modified montmorillonite group was significantly lower ( $P < 0.05$ ). Compared with the natural montmorillonite group, the modified montmorillonite group had a significantly lower thymus index ( $P < 0.05$ ), while the spleen index and bursa of Fabricius index showed no significant differences ( $P > 0.05$ ).

At 42 days of age, compared with the positive control group, the negative control group had significantly increased thymus index, spleen index, and bursa of Fabricius index in broilers ( $P < 0.05$ ). Compared with the negative control group, the natural montmorillonite and modified montmorillonite groups had significantly lower thymus index, spleen index, and bursa of Fabricius index ( $P < 0.05$ ). Compared with the natural montmorillonite group, the modified montmorillonite group had significantly lower thymus index and spleen index ( $P < 0.05$ ), while the bursa of Fabricius index showed no significant difference ( $P > 0.05$ ).

Table 4 Immune organ indices of broilers %

Items	Positive control group	Negative control group	Montmorillonite group	Modified montmorillonite group
21 days of age				
Thymus index	1.40±0.07 <sup>d</sup>	2.37±0.04 <sup>a</sup>	2.21±0.05 <sup>b</sup>	1.75±0.01

Items	Positive control group	Negative control group	Montmorillonite group	Modified montmorillonite group
Spleen index	0.58 $\pm$ 0.04 <sup>c</sup>	0.71 $\pm$ 0.03 <sup>a</sup>	0.66 $\pm$ 0.08 <sup>ab</sup>	0.62 $\pm$ 0.03 <sup>bc</sup>

## 2.4 Plasma protein contents

As shown in Table 5, at 21 days of age, the plasma TP, ALB, and GLB contents of broilers in the negative control group were significantly lower than those in the other groups ( $P < 0.05$ ). Compared with the negative control group, the plasma ALB and GLB contents in the natural montmorillonite group were significantly increased ( $P < 0.05$ ), whereas the TP content showed no significant difference ( $P > 0.05$ ); the plasma TP, ALB, and GLB contents in the modified montmorillonite group were significantly increased ( $P < 0.05$ ). Compared with the natural montmorillonite group, the plasma TP and ALB contents in the modified montmorillonite group were significantly increased ( $P < 0.05$ ), whereas the GLB content showed no significant difference ( $P > 0.05$ ).

At 42 days of age, compared with the positive control group, moldy peanut meal significantly reduced the plasma TP, ALB, and GLB contents of broilers ( $P < 0.05$ ). Compared with the negative control group, the plasma TP and ALB contents in the natural montmorillonite group were significantly increased ( $P < 0.05$ ), whereas the GLB content showed no significant difference ( $P > 0.05$ ); the plasma TP, ALB, and GLB contents in the modified montmorillonite group were significantly increased ( $P < 0.05$ ). Compared with the natural montmorillonite group, the plasma TP and ALB contents in the modified montmorillonite group were significantly increased ( $P < 0.05$ ), whereas the GLB content showed no significant difference ( $P > 0.05$ ).

**Table 5 Plasma protein contents of broilers mg/mL**

Items	Positive control group	Negative control group	Montmorillonite group	Modified montmorillonite group
21 days of age				
TP	30.79 $\pm$ 1.48 <sup>a</sup>	27.13 $\pm$ 1.12 <sup>b</sup>	28.65 $\pm$ 1.31 <sup>b</sup>	30.42 $\pm$ 2.07 <sup>a</sup>

Globulin GLB | 23.74 $\pm$ 0.74<sup>a</sup> | 20.87 $\pm$ 0.76<sup>c</sup> | 22.22 $\pm$ 0.64<sup>b</sup> | 21.68 $\pm$ 0.70<sup>bc</sup>

### 3 Discussion

#### 3.1 Effects of Moldy Peanut Meal on the Growth Performance of Broiler Chickens

Moldy peanut meal can reduce feed intake, daily weight gain, and feed conversion efficiency in poultry. AFB<sub>1</sub> at 100  $\mu\text{g}/\text{kg}$  can reduce ADG in yellow-feather broilers by 5.09%, increase F/G by 4.42%, and decrease ADFI by 0.85%<sup>1</sup>; 100.0 and 150.0  $\mu\text{g}/\text{kg}$  AFB<sub>1</sub> can reduce body weight, markedly reduce ADG and ADFI, and increase F/G<sup>2</sup>; feeding moldy rice (containing AFB<sub>1</sub>) significantly reduced ADG and ADFI and significantly increased F/G in meat ducks<sup>3</sup>; naturally moldy corn can significantly reduce body weight, ADG, and ADFI in meat ducks, increase F/G, and increase mortality<sup>4</sup>. In the present experiment, moldy peanut meal was used to replace soybean meal, and ADG and ADFI of broiler chickens were significantly decreased, whereas F/G was significantly increased, consistent with the above findings. Thus, both naturally moldy raw materials and added AFB<sub>1</sub> can affect the growth performance of broilers. Because synergistic effects exist among mycotoxins, it is generally believed that the toxic effects of naturally moldy feed are greater than those of a single added toxin<sup>5</sup>.

#### 3.2 Effects of Moldy Peanut Meal on Immune Indices of Broiler Chickens

Immune organ indices are important indicators for evaluating immune capacity in poultry. This study showed that the effects of moldy peanut meal on immune function in broilers were manifested as increases in the thymus index, spleen index, and bursa of Fabricius index. He Jian et al.<sup>6</sup> reported that when moldy corn was used to replace normal corn at 0, 50%, and 100%, respectively, the spleen index and bursa of Fabricius index of meat ducks at 14 and 35 days of age increased significantly with increasing replacement proportion. When 8% moldy cottonseed meal was used to replace normal cottonseed meal, the thymus index, spleen index, and bursa of Fabricius index of meat ducks at 35 days of age were significantly increased<sup>7</sup>.

This study showed that moldy peanut meal reduced the contents of TP, ALB, and GLB in the plasma of broilers. AFB<sub>1</sub>-contaminated feed can likewise significantly reduce serum protein (TP, ALB, and GLB) contents in meat ducks<sup>8</sup>; moldy corn significantly reduced serum TP, ALB, and GLB contents in meat ducks at 14 and 35 days of age<sup>9</sup>; 2 500  $\mu\text{g}/\text{kg}$  aflatoxin (containing 83.06% AFB<sub>1</sub>)

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<sup>1</sup>8

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<sup>4</sup>11

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<sup>8</sup>15

<sup>9</sup>16

significantly decreased serum TP and ALB contents in broiler chickens<sup>10</sup>. When poultry consume AFB<sub>1</sub>-contaminated feed, the spleen index, thymus index, and bursa of Fabricius index increase, whereas plasma protein (TP, ALB, and GLB) contents decrease. This may be because AFB<sub>1</sub> causes damage to immune organs such as the thymus, spleen, and bursa of Fabricius in broilers, leading to reduced synthesis of immunoglobulins in the body; it may also be that aflatoxin binds to nucleic acids and inhibits protein synthesis, thereby causing incomplete development of immune organs in broilers and suppressing the production of immune factors, ultimately affecting immune function in poultry.

### 3.3 Alleviating Effect of Montmorillonite on AFB<sub>1</sub> Toxicity

The use of montmorillonite to reduce the harm caused by aflatoxin in poultry production has already been applied in practice. Montmorillonite is a type of aluminosilicate; because it possesses unsaturated negative charges and ion-exchange capacity, it can capture, adsorb, and immobilize toxins, reduce intestinal absorption of toxins, and lessen toxic effects<sup>11</sup>. Adding 3.0 g/kg sodium-based modified ...

montmorillonite can significantly alleviate the effects of aflatoxin on growth performance, immune organ indices, and serum biochemical indices in broilers<sup>[19]</sup>; adding 5.0 g/kg montmorillonite to feed contaminated with aflatoxin reduced broiler mortality by 37.5% and increased body-weight gain by 28.8%<sup>[20]</sup>; adding 2.0 g/kg montmorillonite to moldy feed significantly increased ADG, ADFI, and plasma protein content in meat ducks, and significantly inhibited the increase in immune organ indices<sup>[14]</sup>; adding 2.5 and 5.0 g/kg calcium-based montmorillonite, respectively, to feed contaminated with 3 000  $\mu\text{g}/\text{kg}$  AFB<sub>1</sub> increased 42-day body weight of broilers by 13.3% and 22.7%, increased ADFI by 9.7% and 24.7%, and improved feed conversion rate by 53.0% and 66.5%, respectively<sup>[21]</sup>; adding bentonite to feed contaminated with 2 500  $\mu\text{g}/\text{kg}$  AFB<sub>1</sub> alleviated the negative effects of AFB<sub>1</sub> on growth performance and plasma ALB in broilers<sup>[17]</sup>. The present study showed that 2.0 g/kg natural or modified montmorillonite could effectively alleviate the growth suppression and immune-organ enlargement in broilers caused by moldy peanut meal in the diet, and this result is consistent with previous studies.

The Si-OH and Al-OH groups on the surface of montmorillonite act as hydrogen-bond donors, while the two carbonyl groups in the molecular structure of AFB<sub>1</sub> act as hydrogen-bond acceptors. These may be spatially complementary, thereby forming double hydrogen bonds, enabling montmorillonite to selectively adsorb AFB<sub>1</sub>. Some studies have suggested that there is no significant difference in the adsorption capacity of natural and modified montmorillonite for AFB<sub>1</sub>; however, after modification of montmorillonite, one end of some modifier molecules carrying positive charges can adsorb onto the

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<sup>10</sup><sub>17</sub>

<sup>11</sup><sub>18</sub>

outer surface of montmorillonite or enter the interlayer space of montmorillonite, while the alkyl end remains at the edge of the montmorillonite. This increases the number of hydrogen-bond donors at the edges of montmorillonite, increases the opportunities for double hydrogen-bond formation between them, strengthens the adsorption stability of AFB<sub>1</sub>, and reduces the desorption rate of AFB<sub>1</sub>[<sup>22</sup>-23]. In diets for male broilers, adding 1.5 g/kg montmorillonite and copper-loaded montmorillonite, respectively, showed that adding copper-loaded montmorillonite could increase broiler ADG and reduce the number of intestinal *Escherichia coli*, whereas adding natural montmorillonite alone had no significant effect[<sup>24</sup>]. Other studies have shown that the adsorption rate of modified montmorillonite for zearalenone reaches 92.44%, with a desorption rate of 5.31%, indicating that modified montmorillonite has a relatively high adsorption detoxification rate for zearalenone and that the complex formed after zearalenone adsorption has relatively good stability[<sup>25</sup>]. The present study showed that 2.0 g/kg natural and modified montmorillonite could both effectively alleviate the effects of moldy peanut meal on growth performance and immune indices in broilers, and that modified montmorillonite was more effective than natural montmorillonite.

#### 4 Conclusion

Moldy peanut meal significantly reduced growth performance and immune function in broilers. Adding montmorillonite effectively alleviated the adverse effects of AFB<sub>1</sub> on broilers, and the adsorption effect of modified montmorillonite on AFB<sub>1</sub> was superior to that of natural montmorillonite.

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## Montmorillonite Affected Growth Performance and Immune Function of Broilers Fed Diets

### Containing Mouldy Peanut Meal

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**Abstract:** This experiment was conducted to investigate the effects of montmorillonite on growth performance and immune function of broilers fed diets containing peanut meal naturally polluted with aflatoxin B1 (AFB1), with an aim to determine the feeding results of montmorillonite as an adsorbent. A total of 288 one-day-old Arbor Acre (AA) male broiler chicks were randomly divided into 4 groups with 6 replicates containing 12 birds each. The four diets formulated included a corn-soybean meal basal diet (positive control group), a negative diet with naturally mouldy peanut meal (AFB1 content was 1 284.21  $\mu\text{g}/\text{kg}$ , with 8.8% and 12.0% for starter and grower period) to replace part of soybean meal (negative control group), and two contaminated diets supplemented with 2.0 g/kg natural or modified montmorillonite based on the negative diet. The experiment lasted for 42 days. The results showed as follows: 1) compared with the positive control group, AFB1 contaminated peanut meal diet significantly decreased average daily gain (ADG), average daily feed intake (ADFI), percentage of breast muscle and leg muscle of broilers at 42 days of age, and plasma protein contents ( $P<0.05$ ), while significantly increased the ratio of feed to gain and immune organ indices of broilers ( $P<0.05$ ). 2) Compared with the negative control group, the natural or modified montmorillonite significantly increased ADG, ADFI and plasma protein contents ( $P<0.05$ ), and significantly inhibited immune organ indices of broilers ( $P<0.05$ ); 3) Compared with the natural montmorillonite group, the modified montmorillonite significantly increased ADG and contents of total protein and albumin in plasma ( $P<0.05$ ), and significantly decreased thymus index of broilers ( $P<0.05$ ). In conclusion, AFB1 contaminated peanut meal decreases the growth performance and immune functions of broilers, and dietary supplementation of natural or modified montmorillonite can ameliorate the negative effects of AFB1, and the modified montmorillonite gets the better effects.

Key words: montmorillonite; aflatoxin B<sub>1</sub>; broilers; growth performance; immune

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