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Counteracting Effect of High Grade Sodium Bentonite during Aflatoxicosis in Broilers

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ABSTRACT

The effects of Aflatoxin (AF) (0.0 and 0.5m g kg^{-1}) and High Grade Sodium Bentonite (HGSB) (5, 7.5 and 10 g kg^{-1}) were tested in an *in vivo* study including 8 dietary treatments with three replicates of 14 birds per diet per replicate, using a total of 336 broiler chicks up to five weeks. Results showed that chicks receiving AF contaminated feed had significantly (P≤ 0.05) suppressed body weight gain, which improved significantly (P≤ 0.05) with addition of HGSB to AF contaminated diet. Supplementation of HGSB at 7.5 and 10 g kg^{-1} to the diets containing AF significantly (P≤ 0.05) improved feed consumption by 9.97 and 9.15 g kg^{-1}, respectively, compared to the control group. Efficiency of feed utilization decreased significantly (P≤ 0.05) with addition of 0.5 mg kg^{-1} AF and improved significantly (P≤ 0.05) in HGSB treated group. The relative weights of liver and kidney, which increased significantly (P≤ 0.05) with addition of 0.5 mg kg^{-1} AF (19.56 and 18.38 g kg^{-1}) compared to control group, were improved with dietary inclusion of 7.5 and 10 g kg^{-1} HGSB. Relative weights of gizzard and pancreas were not affected in AF fed and the control groups. The relative thymus and bursal weights were significantly (P≤ 0.05) lower at inclusion of 0.5 mg kg^{-1} of AF (38.99 and 31.36%) compared to the control group, but were not altered by supplementation of HGSB. The serum antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) vaccination, which were significantly (P≤ 0.05) depressed by AF, were restored with the inclusion of 7.5 and 10 g kg^{-1} HGSB. The serum concentration of uric acid and albumin in comparison with control group were not affected by treatment groups. The activity of serum gamma glutamyl transferase (GGT) significantly (P≤ 0.05) increased in AF fed group and the addition of HGSB did not show significant reduction in the activity of serum GGT (P≥ 0.05). Activity of serum alanine amino transferase (ALT) was not affected by the treatment groups. It was found that HGSB at 10 g kg^{-1} level is partially effective in counteracting the adverse effects of aflatoxin in broilers.

Keywords: Aflatoxin, Broilers, High Grade Sodium Bentonite, Performance.

INTRODUCTION

Cereal grains and associated by-products constitute important sources of energy for poultry. However, there is increasing evidence that global supplies of cereal grains for animal feedstuffs are frequently contaminated with mycotoxins (Manafi *et al.,* 2009a). Aflatoxins are secondary toxic metabolites, produced by certain strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species, of which aflatoxin B_1 (AFB_1), is the most toxic among all other aflatoxins i.e. AFB_1, AFB_2, AFG_1 and AFG_2. In poultry, aflatoxin ingestion leads to "Aflatoxicosis" syndrome that is characterized by retardation in growth, decreased feed consumption and feed conversion efficiency, immunosuppression and the increase of mortality (Mohamadi and Alizadeh, 2010). Co-contamination of cereal grains with other mycotoxins produced by different fungal genera, including *Fusarium* and *Aspergillus,* has

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been reported to increase the toxicity symptoms in poultry (Hagler *et al.,* 1984).

At present, one of the more encouraging approaches to make the feed harmless is the addition of non-nutritive and natural adsorbent materials to the contaminated feed in order to selectively bind the mycotoxin during the digestive process. The major advantages of these adsorbents include low cost, safety, and the ease of addition to animal feed. Layered amino silicates, such as sodium bentonite, have been found effective in counteracting mycotoxins (Smith and Ross, 1991; Hagler *et al.,* 1992; Santurio *et al.,* 1999; Rosa *et al.,* 2001; Eralsan *et al.,* 2005). However, the ability of bentonite to bind mycotoxins depends on pH, molecular arrangements, and its geographic region of origin (Vieira, 2003). Considering all these facts, the present study was undertaken to investigate the ability of graded levels of Ashafeed (property product of Ashapura Volclay Limited, Mumbai, India), a High Grade Sodium Bentonite obtained from mines of Kutch area of Gujarat, India, to counteract the toxic effects of aflatoxin in broilers.

MATERIALS AND METHODS

Experimental Animals and Design

Three hundred and thirty six unsexed one-day old commercial broiler chicks were wing banded, weighed, and treated with two levels of Aflatoxin (AF) (0 and 0.5 mg kg^{-1}) and four levels of High Grade Sodium Bentonite (HGSB) (0, 5, 7.5 and 10 g kg^{-1}) in a Completely Randomized Design manner, forming a total of 8 dietary treatments each with 3 replicates (14 chicks per diet per replicate).

Experimental Housing, Management and Diet

Each replicate group of chicks was housed in an independent floor pen in an open sided deep litter conventional house. Chicks in all the replicate groups were reared up to five weeks of age under uniform standard conditions throughout the study. Brooding was done until three weeks of age using incandescent bulbs. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided continuous light throughout the study.

Aflatoxin was produced using the pure culture of *Aspergillus parasiticus* MTCC 411 grown on potato dextrose agar. The harvested *Aspergillus* spores were inoculated on rice (*Oryza sativa*) to produce toxin culture material. Aflatoxin produced on rice was then extracted as described by Romer (1975) and quantified by thin layer chromatography (TLC) as described in AOAC (1995) and the quantity of revealed toxin in culture material was found to be 220 mg kg^{-1}.

Upon analyzing the basal diet for presence of aflatoxin B_1, the experimental diets were prepared by adding the calculated quantities of aflatoxin B_1, in the form of rice containing cultured materials, needed to arrive at the levels of 0 and 0.5 mg kg^{-1} of AF B_1 for each diet period (starter and finisher). To each of these diets, High Grade Sodium Bentonite (HGSB) was added at the rates of 0, 5, 7.5 and 10 g kg^{-1}. HGSB is a natural raw material obtained from the mines of Kutch area of Gujarat, India (property product of Ashapura Volclay Limited, Mumbai, India) claimed to possess high adsorption capacity due to high surface area, with 95% purity.

Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers during the starter (0-3 weeks) and finisher (4-5 weeks) phases. The ingredient composition and nutrient content of basal diets of broilers are presented in Table1. Chicks were provided *ad libitum* supply of feed and water throughout the study (0-5 weeks) and were vaccinated against Newcastle Disease (ND) on the 7^{th} day using F_1 strain (Ventri's Biologicals, Bangalore) and against Infectious Bursal Disease (IBD) on the 14^{th}

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Table1 . The ingredient composition and nutrient content of basal diets of broilers.

Ingredients	Starter (0-3 weeks)	Finisher (4-5 weeks)
Maize (%)	63	69
Soybean meal (%)	33.8	27.8
Mineral mixture[a] (%)	3.2	3.2
Salt (g)	300	300
DL-methionine (g)	170	100
Vit. A B_2 D_3 K[b] (g)	15	15
B complex[c] (g)	20	20
Coxistac[d] (g)	50	50
Oil (kg)	-	3.7
Zinc Bacitracin[e] (g)	35	35

[a] Mineral mixture provided per kilogram of feed: 30% Ca; 9% P; 0.4% Mn; 0.4% Zn; 100 mg kg^{-1} I; 2,000 mg kg^{-1} Fe; 500 mg kg^{-1} Cu, 23 mg kg^{-1} Se.

[b] AB_2D_3K provided per kilogram of feed: Vitamin A= 12,375 IU; Vitamin B_2= 7.5 mg; Vitamin D_3= 1,800 IU, Vitamin K= 15mg.

[c] B complex provided per kilogram of feed: Vitamin B_1= 0.8 mg; Vitamin B_6= 1.6 mg; Vitamin B_{12}= 8 µg; Vitamin E= 8 mg; Niacin= 12 mg, Calcium pantothenate= 8 mg.

[d] Coxistac: A propriety product of Pfizer Limited; Mumbai; containing 120 g of Salinomycin activity (Mycelial form) Per Kg.

[e] Zinc Bacitracin: A propriety product of Pfizer Limited.

day using intermediate strain (Ventri's Biologicals, Bangalore). Both vaccines were given via the ocular route.

Data Collection

At the end of the trials, body weight, feed consumption, and mortality were recorded and body weight gain and feed conversion efficiency were calculated. Upon obtaining the permission of Ethical Committee of the University, six birds (3 males and 3 females) from each replicate were randomly chosen and sacrificed by cutting the jugular vein at the end of the trial. Blood was collected in non-heparinized tubes by puncturing the brachial vein during the 5th week of age and stored at 4°C for 8 to 10 hours. Then, serum was separated as standard procedure (Calnek *et al.,* 1992) and stored at –20ºC for subsequent analysis. The individual serum samples were analyzed for serum total protein, albumin, uric acid and the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT), using an automatic analyzer (Boehringer Mannhein Hitachi 704 automatic analyzer, Japan). Antibody titers against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) were analyzed (ND and IBD test kits - Abbott) using the indirect analogous conventional enzyme-linked immunosorbent assay (ELISA-analogous Imperacer®). The weight of the scarified birds internal organs such as liver, kidney, gizzard, pancreas, spleen, thymus, and bursa were recorded and expressed as g kg^{-1} of body weight.

Statistical Analysis

The experimental data were analyzed statistically using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS®) software (SAS Institute, USA, 2000). Overall data were analyzed using one way ANOVA test with treatments compositions as shown in Table 2. The Duncan multiple range test was used to compare means at 5 percent of significance (Duncan, 1955).

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Table 2. Compositions of the treatments.

Treatments	AF (mg kg^{-1})	HGSB (g kg^{-1})
1 [a]	0	0
2	0.5	0
3	0	5
4	0	7.5
5	0	10
6	0.5	5
7	0.5	7.5
8	0.5	10

[a] Treatment No. 1 is control diet.

RESULTS

Body weight gain, feed consumption, feed conversion efficiency, and mortality data for broilers at fifth week of age are presented in Table3. Chicks receiving AF contaminated feed had significantly (P≤ 0.05) suppressed body weight gain, feed consumption and increased feed conversion efficiency compared to chicks fed the control diet. Supplementary feeding of HGSB at the rate of 7.5 and 10 g kg^{-1} to the diets containing AF significantly (P≤ 0.05) improved body weight gain and feed consumption when compared to the AF control diet. Feed conversion efficiency was improved with inclusion of 7.5 and 10 g kg^{-1} HGSB to AF contaminated diet. High mortality rate of 14.20% was observed in chicks fed with diet containing 0.5 mg kg^{-1} AF. Mortality percentage was reduced considerably in chicks supplemented with 5 and 7.5 g kg^{-1} HGSB and no mortality was observed in chicks fed with 10 g kg^{-1} HGSB.

The relative weights of various organs expressed as g kg^{-1} body weight are shown in Table 4. The AF showed significant (P≤ 0.05) increase in the relative weights of liver and kidney. Supplementation of 7.5 and 10 g kg^{-1} HGSB to AF containing diets significantly (P≤ 0.05) reduced the relative weights of liver and kidney with respect to AF group, to get a comparable weight to the control group.

The relative weights of pancreas, gizzard and spleen were not different from those of the control in all treatments. The relative weight of spleen numerically increased from

Table 3. The mean (±SE) of the growth performance parameters of broilers after five weeks feeding with Aflatoxin (AF) and High Grade Sodium Bentonite (HGSB).

AF (mg kg^{-1})	HGSB (g kg^{-1})	Body weight gain (g)	Feed consumption (g bird^{-1})	Feed conversion efficiency	Mortality (%)
0	0	1313.4±0.88^{a}	2513.1±6.07^{a}	1.9±0.00^{d}	4.70
0.5	0	1180.0±7.49^{c}	2306.0±2.92^{b}	2.0±0.00^{a}	14.20
0	5	1314.0±11.11^{a}	2505.0±10.3^{a}	1.9±0.00^{d}	0
0	7.5	1326.0±4.91^{a}	2501.0±8.13^{a}	1.8±0.00^{de}	0
0	10	1339.0±10.07^{a}	2495.0±14.5^{a}	1.8±0.00^{e}	0
0.5	5	1202.0±8.81^{c}	2305.0±2.89^{b}	2.0±0.00^{b}	7.10
0.5	7.5	1240.0±6.35^{b}	2517.0±14.63^{a}	2.0±0.00^{b}	4.70
0.5	10	1274.0±0.73^{b}	2536.0±7.77^{a}	1.9±0.00^{c}	0

Means bearing at least one common superscript in a column do not differ significantly at $P \leq 0.05$ (n= 336).

Table 4. The Mean±SE of relative weights of organs (g kg^{-1} body weight ±SE) in broilers at the fifth week of age.

AF (mg kg^{-1})	HGSB (g kg^{-1})	Liver	kidney	Gizzard	Pancreas	Spleen	Bursa	Thymus
0	0	27.6±0.76^{b}	8.1±0.16	24.5±0.6	5.1±0.17	1.5±0.28	1.6±0.02^{a}	4.3±0.21^{a}
0.5	0	33.0±0.57^{a}	9.6±0.16	25.6±1.20	4.6±0.33	1.6±0.16	1.1±0.16^{b}	2.6±0.33^{b}
0	5	27.6±0.42^{c}	8.3±0.16	25.7±0.52	4.1±0.03	1.5±0.16	1.6±0.03^{a}	4.0±0.12^{ab}
0	7.5	28.6±0.23^{bc}	8.1±0.16	25.9±0.21	4.6±0.26	1.5±0.16	1.6±0.04^{a}	4.0±0.40^{ab}
0	10	30.4±1.03^{abc}	8.3±0.16	24.6±0.69	4.8±0.30	1.5±0.16	1.8±0.04^{a}	4.1±0.06^{a}
0.5	5	31.3±0.33^{ab}	9.0±0.28	26.0±1.15	4.6±0.33	1.6±0.16	1.3±0.16^{ab}	2.6±0.33^{b}
0.5	7.5	30.0±0.57^{bc}	8.8±0.16	25.3±0.33	4.3±0.33	1.6±0.16	1.3±0.16^{ab}	3.3±0.33^{ab}
0.5	10	30.0±0.57^{bc}	8.3±0.16	24.6±0.88	4.3±0.33	1.6±0.16	1.5±0.02^{ab}	3.6±0.33^{ab}

Means bearing at least one common superscript in a column do not differ significantly at $P \leq 0.05$ (n= 6).

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that of the control in all AF fed groups. Feeding AF significantly ($P \leq 0.05$) reduced relative weights of bursa of fabricius and thymus when compared to the control. Thymus weights appeared to be more sensitive to the adverse effects of AF than the bursa of fabricius. Supplementation of different levels of HGSB to the control group and diets containing AF did not result in any significant differences in relative weights of bursa of fabricius and thymus as compared to AF control diet ($P \geq 0.05$).

The effect of HGSB supplementation of the diets containing AF on the antibody titers against New Castle Disease (ND) and Infectious Bursal Disease (IBD), serum protein, albumin, uric acid, the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) are presented in Table 5. A significant ($P \leq 0.05$) decrease in antibody titer values against ND and IBD vaccines was observed upon feeding AF. Addition of graded levels of HGSB alone (5 g kg^{-1}) to the control diet could reduce the antibody titers against ND and IBD at five weeks of age, but 7.5 and 10 g kg^{-1} HGSB increased those values compared to the control. However, addition of HGSB to diets containing AF significantly ($P \leq 0.05$) improved the antibody titers against ND and IBD vaccine compared to AF control diet.

The serum concentration of total protein, which was significantly ($P \leq 0.05$) decreased by AF, was elevated to the control level with the inclusion of 10 g kg^{-1} HGSB. Serum concentrations of uric acid and albumin were not affected in either AF fed group or HGSB supplemented groups.

The activity of serum GGT significantly ($P \leq 0.05$) increased in AF fed group. The addition of HGSB to AF containing diet did not show significant reduction in the activity of serum GGT ($P \geq 0.05$). Compared with the control group, activity of serum ALT was not affected in either AF fed group, the control, or HGSB supplemented groups.

Table 5. Effect of AF and HGSB on the Mean±SE of immune status and serum biochemical parameters in broilers fed aflatoxin, at fifth week of age.

AF (mg kg^{-1})	HGSB (g kg^{-1})	ND titer	IBD titer	Serum protein (g%)	Serum Albumin (g%)	Uric acid (µg dl^{-1})	GGT (µg l^{-1})	ALT (µg l^{-1})
0	0	4297.7 ± 17.05^{ab}	4281.0 ± 8.08^{a}	2.7 ± 0.18^{a}	1.2±0.17	647.9±7.54	9.53 ± 1.15^{d}	28.1±0.60
0.5	0	3204.0 ± 106.3^{e}	3149.0 ± 69.72^{d}	1.6 ± 0.15^{bc}	1.1±0.18	600.4±6.73	17.8 ± 1.72^{ab}	25.8±1.36
0	5	4018.0 ± 119.2^{bc}	4252.0 ± 21.79^{a}	2.4 ± 0.23^{abc}	1.2±0.06	610.6±0.69	11.6 ± 0.37^{bcd}	25.0±1.47
0	7.5	4305.0 ± 93.19^{ab}	4329.0 ± 25.48^{a}	2.5 ± 0.20^{ab}	1.2±0.07	629.0±2.02	11.6 ± 0.14^{bcd}	28.6±1.62
0	10	4418.0 ± 56.72^{a}	4378.0 ± 26.74^{a}	2.7 ± 0.15^{a}	1.3±0.06	653.6±3.01	10.6 ± 0.96^{cd}	29.6±2.34
0.5	5	3582.0 ± 30.19^{d}	3352.0 ± 73.59^{cd}	1.6 ± 0.11^{bc}	1.1±0.17	614±34.09	22.5 ± 2.16^{a}	27.7±0.34
0.5	7.5	3797.0 ± 10.73^{cd}	3694.0 ± 73.64^{bc}	1.6 ± 0.15^{c}	1.1±0.17	610.6±3.00	17.4 ± 2.25^{ab}	28.6±0.14
0.5	10	4225.0 ± 78.83^{ab}	4046.0 ± 182.3^{ab}	2.5 ± 0.22^{a}	1.2±0.37	636.3±6.98	13.7 ± 1.01^{bcd}	28.8±0.49

Means bearing at least one common superscript in a column do not differ significantly at $P \leq 0.05$(n= 6).

DISCUSSION

The decreased body weight and feed consumption and the increased feed conversion efficiency caused by AF in this study are consistent with the findings of Swamy and Devegowda, (1998); Raju and Devegowda, (2000); Arvind *et al.* (2003) and Girish and Devegowda, (2004). The growth depression effects of AF may be due to its inhibitory action on protein synthesis and nutrient utilization (Marquardt and Frohlich, 1992). Addition of HGSB at different levels (5, 7.5 and 10 g kg^{-1}) to the control diet did not affect body weight gain and feed consumption in broilers. Feed conversion efficiency was significantly reduced in birds given either 7.5 or 10 g kg^{-1} HGSB to control diet. The results indicated that the naturally occurring sorbent used in the study was inert and non toxic. Kurnick and Reid (1989) reported similar results. The aflatoxin is extremely caustic and its toxic effects have been described as radiomimetic (Ueno, 1977). The depression in growth upon aflatoxin feeding could be attributed to inhibition of protein synthesis, through the inactivation of inhibition and termination, possibly through its binding to ribosome (Ueno, 1977) and also impaired nutrient utilization. The results suggest a beneficial effect of addition of HGSB in the presence of AF on growth performance.

Significant increase in the relative weights of liver and kidney due to AF could be attributed to increased lipid deposition in the liver due to impaired fat metabolism (Shaline *et al.,* 1980). A reversal line in aflatoxicosis was concluded when addition of 7.5 and 10 g kg^{-1} HGSB to AF containing diets significantly reduced the relative weights of liver and kidney with respect to AF group, to get a comparable weight to the AF control group. The increase in liver and kidney weights was in accordance with the findings of Raju and Devegowda (2000); Arvind *et al.* (2003); Perozo and Rivera (2003); Girish and Devegowda (2004), and Miazzo *et al.* (2005). The protective effects of bentonite against AF induced organ weight increase in broilers were earlier observed by Barmase *et al.* (1990); Chaturvedi and Singh (2004) and Eralsan *et al.* (2005).

Relative weights of bursa of fabricius and thymus were significantly reduced by AF feeding when compared to the control group. Aflatoxins are known (Manafi *et al.,* 2009b) to cause immunosuppression in broilers and concomitantly decrease the relative weights of bursa and thymus responsible for immunological competence. Similar results were reported by Devegowda *et al*. (1994); Raju and Devegowda (2002); Perozo and Rivera (2003), and Miazzo *et al.* (2005). The increasing trend in weight of spleen observed in this study was in accordance with findings of Kubena *et al.* (1990), Girish and Devegowda, (2004), and Miazzo *et al.* (2005).

The depression in New Castle Disease (ND) and Infectious Bursal Disease (IBD) titer values is a clear indication of immunosuppression effects of AF on humoral antibody response. These findings agreed with the previous reports of Swamy and Devegowda (1998); Ibrahim *et al.* (2000); Kumar *et al.* (2002) and Gupta and Singh (2003). The reduction of antibody titers could be due to inhibition of DNA and protein synthesis by aflatoxin through impairment of amino acid transport and m-RNA transcription, which resulted in lowered level of antibody production (Thaxton *et al.,* 1974).

The results of HGSB addition to chickens diets containing AF at 5 weeks of age clearly demonstrated the protective effects of HGSB at 10 g kg^{-1} rate. These findings of present study were comparable to the reports of Daoud (2002).

Serum GGT activity significantly increased by AF and could not be restored by addition of HGSB to AF containing diet, whereas activity of serum ALT remained unchanged in all treated groups. Similar observations were reported by Miazzo *et al.* (2005) and Manafi *et al.* (2009b).

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It maybe concluded that HGSB is partially effective in counteracting the adverse effects of aflatoxin in broilers. Among the various levels of High Grade Sodium Bentonite, 10 g kg^{-1} showed the best protective effect against the aflatoxicosis in broilers.

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تأثیر استفاده از بنتونیت در کاهش میزان سمیت آفلاتوکسین در مرغان گوشتی

م. منافی

چکیده

به منظور تعیین تاثیرات میزان سمیت سم آفلاتوکسین (AF) (۰/۵ میلی گرم در کیلوگرم) و سطوح مختلف بنتونیت (HGSB) (۵، ۷/۵ و ۱۰ گرم در کیلوگرم) در جهت کاهش اثرات مضر آنها در جوجه های گوشتی تجارتی، آزمایشی با استفاده از ۳۳۶ جوجه گوشتی و با ۸ تیمار و ۳ تکرار و در هر تیمار ۱۴ پرنده برای هر تکرار تا سن پنج هفتگی انجام شد. نتایج آزمایش حاکی از کاهش معنی دار ($P< 0/05$) وزن بدن پرنده هائی بود که AF در جیره غذائی خود دریافت کرده بودند . این کاهش به صورت معنی داری ($P< 0/05$) با اضافه نمودن HGSB در جیره آنها جبران شد. در مقایسه با گروه شاهد، اضافه نمودن HGSB در سطوح ۷/۵ و ۱۰ گرم در کیلوگرم در جیره هائی که حاوی AF بود به صورت معنی داری ($P< 0/05$) مصرف غذا را بهبود بخشید. ضریب تبدیل غذائی با اضافه نمودن AFدر سطح ۰.۵ گرم در کیلوگرم به صورت معنی داری ($P<0/05$) افزایش یافت در صورتی که با اضافه نمودن HGSB به صورت معنی داری ($P< 0/05$) جبران گردید. اوزان نسبی کبد و کلیه که به صورت معنی داری ($P< 0/05$) با مصرف ۰/۵ میلی گرم در کیلوگرمAF در مقایسه با گروه شاهد زیاد شده بود، به صورت معنی داری ($P< 0/05$) با اضافه نمودن HGSB در سطوح ۷/۵ و ۱۰ گرم در کیلوگرم بهبود یافت (به ترتیب ۱۹/۵۶ و ۱۹/۳۸ گرم/کیلوگرم). هیچگونه تفاوتی در اوزان سنگدان و طحال طی مصرف AF و HGSB در مقایسه با گروه شاهد مشاهده نشد. با اضافه نمودن ۰/۵میلی گرم در کیلوگرمAF، اوزان غده تیموس و بورس فابریسیوس به صورت معنی داری ($P< 0/05$) پایین تر از گروه شاهد بود که با مصرف سطوح ۷/۵ و ۱۰ گرم در کیلوگرمHGSB به صورت معنی داری ($P< 0/05$) جبران گردید (به ترتیب ۳۸/۹۹ و ۳۱/۳۶ درصد). تیتر آنتی بادی بر علیه بیماریهای نیوکاسل و گامبورو که در مقایسه با گروه شاهد با مصرف AF کاهش معنی داری ($P<0/05$) یافته بود، با مصرف سطوح ۷/۵ و ۱۰ گرم در کیلوگرم HGSB جبران گردید. غلظتهای اسید اوریک و سرم آلبومین در مقایسه با گروه شاهد تحت تاثیر هیچ کدام از تیمار ها تحت تاثیر قرار نگرفتند. فعالیت آنزیم گاما گلوتامیل ترانسفراز (GGT) به صورت معنی داری ($P< 0/05$) با مصرف AF افزیش یافت و افزودن HGSB به جیره قادر به تغییر فعالیت این آنزیم به صورت معنی داری نگردید. فعلیت آنزیم آلانین آمینو ترانسفراز (ALT) سرم تحت تاثیر هیچ کدام از تیمارها قرار نگرفت. در نتیجه می توان گفت که اضافه نمودن HGSB در سطح ۱۰ گرم در کیلوگرم می تواند به صورت نسبی باعث کاهش سمیت آفلاتوکسین در مرغان گوشتی شود.

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