

Assessment of serum biochemical parameters and pathological changes in broilers with chronic aflatoxicosis fed glucomannan-containing yeast product (Mycosorb) and sodium bentonite

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Received: June 05, 2014

Accepted: June 17, 2015

Abstract

The purpose of this study was to evaluate the efficacy of yeast glucomannan (YG) and sodium bentonite (SB) in reducing the toxicity in broilers fed a diet naturally contaminated with aflatoxin. Three hundred 7-day-old Ross 308 strain broilers were chosen and randomly assigned to 10 dietary treatments. Serum biochemical parameters and pathological changes in the liver were investigated at 42 d of age. Chickens fed a diet containing 250 ppb of aflatoxin displayed a decrease in uric acid, cholesterol, and triglycerides, and an increase in serum activities of AST and ALT when compared to control group. There were considerable gross and histopathological hepatic lesions ($P < 0.05$) in the form of small to moderate hydropic and/or fatty degeneration, bile duct hyperplasia, periportal fibrosis, cells infiltration, and congestion, in chickens fed the 250 ppb aflatoxin-containing diet. The addition of YG and SB to the aflatoxin-containing diet partially reduced the negative effects of aflatoxin. The 0.1% YG supplementation to the aflatoxin-contaminated diet significantly prevented the pathological effect of aflatoxin on serum biochemical parameters and liver, and was found to be more effective than other treatments.

Keywords: broiler chickens, aflatoxin, yeast glucomannan, biochemical parameters, pathological changes.

Introduction

Aflatoxins, strong mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are a main concern in poultry production and for public health because of critical economic losses and health problems. Various symptoms are associated with aflatoxicosis in poultry. The most common ones are apathy, anorexia with lowered growth rate, poor feed utilisation, decreased weight gain, decreased egg weight and production, and increased mortality (21).

The toxic effects of aflatoxin in poultry have been thoroughly studied by determining their teratogenic, carcinogenic, mutagenic, and growth inhibitory effects (26). The biochemical, haematological (4, 22), immunological (17), and pathological (24) effects of aflatoxin have also been described in details. Aflatoxin can also cause significant gross and microscopic alterations in the liver, such as hepatomegaly, paleness,

hydropic and fatty degeneration, bile duct hyperplasia, and periportal fibrosis (10, 25).

Preventing mold growth and aflatoxin contamination in feed and feedstuffs is very essential, but when contamination is inevitable, aflatoxin-decontamination is required before the use of these materials. Producers and researchers seek an efficacious prevention management and decontamination technologies to minimise the toxic effects of aflatoxin in animal production. In addition to prevention strategies, other novel methods, including physical, chemical, and biological treatments to detoxify aflatoxin-contaminated feeds and feedstuffs, have been applied (19, 27).

Since the early 1990s, surveys on adsorbent-based strategies have been carried out to eliminate mycotoxins from contaminated feed, and minimise their toxic effects on poultry health (21, 23). Zeolites (12, 16), bentonites (7, 11, 26), and yeast/esterified

glucomannan (1, 4, 6, 15) were opted for because of their effect on reducing aflatoxin absorption from the gastrointestinal tract. Some studies have showed that the best method for decontamination is biological degradation by yeast and yeast components, which can remove aflatoxin under mild conditions, without using harmful chemicals or causing noticeable losses in nutritive value and palatableness of the feed (21, 30).

The objective of this study was to evaluate the toxic effects of aflatoxin on serum biochemical parameters and gross and microscopic lesions in the liver, as well as to determine the preventive effects of dietary glucomannan-containing yeast product and sodium bentonite.

Material and Methods

Chickens and diet. A total of 325 1-day-old Ross 308 strain male broiler chickens were obtained from a commercial broiler producer. The chickens were conformed for 7 d before commencement of the trial. Subsequently, the chosen birds were kept in floor pens with litter floors in a broiler house where environmental conditions, such as temperature, were under control.

Three hundred chickens of similar weights were randomly assigned to 30 clean pens in the same broiler house used for the adaptation period. They were fed a commercial feed starter (maize and soybean-based, 20.84 % CP, 2900.29 ME) up to day 21, and from the day 21 on, a grower diet (19.68% CP, 3150 ME) up to day 42; they had access to feed and water *ad libitum* from 1 to 42 d of age. In addition, the birds were inspected daily and any health-related problems were recorded. The basal diet was supplemented with amino acids, minerals, and vitamins at levels approved by the National Research Council (20), without added antibiotics, coccidiostats, or growth promoters. Lighting was provided for 23 h/d.

Experimental design. The birds (three replicates of 10 chickens each) were allocated to the following treatment groups: 1) positive control diet, basal diet without additives; 2) negative control diet, diet naturally contaminated with aflatoxin (NCD, 250 ppb); 3) NCD supplemented with 1.5% sodium bentonite (SB); 4) NCD supplemented with 3% SB; 5) NCD supplemented with 0.05% yeast glucomannan (YG); 6) NCD supplemented with 0.1% YG; 7) NCD supplemented with 1.5% SB + 0.05% YG; 8) NCD supplemented with 1.5% SB+ 0.1% YG; 9) NCD supplemented with 3% SB + 0.05% YG; and 10) NCD supplemented with 3% SB + 0.1% YG.

Aflatoxin quantification and diet preparation. Individual feed ingredients were analysed and screened for aflatoxin content. Aflatoxin was extracted (7) and quantified by thin-layer chromatography (TLC). The basal control diet was formulated and increased to fulfil the nutritional needs of commercial broilers during the

starter and grower periods (20). The basal diet did not have noticeable levels of aflatoxin (below 1 µg/kg diet; 0.5 ppb). The maize which was obtained from a feed mill (having already been contaminated with mold) was stored at 20% moisture for two months to increase the mold growth. TLC indicated the presence of aflatoxin in the maize. Aflatoxin-free maize was replaced with naturally contaminated maize and this led to the formulation of contaminated diet treatments. The samples were randomly selected from four different portions of all the samples. The analysis showed that the contaminated diet contained 250 ppb of aflatoxin (detection limit - 1 ppb). The aflatoxin composition in the contaminated diet was 84.72% of AFB1, 5.50% of AFB2, 8.20% of AFG1, and 1.58% of AFG2. In the experimental period, the control and contaminated diets were analysed for aflatoxin and other mycotoxins. The levels of aflatoxin in control diet were below the detection limits. The levels of aflatoxin in the contaminated diet ranged from 240 to 250 ppb. The presence of other mycotoxins in the diets was not found.

Serum biochemical analysis. When the chickens reached 42 d of age, the trial was stopped and 10 broilers from each treatment were randomly selected and bled by wing vein for biochemical analysis. Blood was centrifuged at $1400 \times g$ at 8°C for 30 min (Sorvall, RC 3 B plus) and serum was separated and preserved at -20°C until biochemical analyses. Serum concentrations of cholesterol, uric acid, triglycerides, and activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using an auto-analyser (Tokyo Boeki, TMS, 1024, Japan) with commercial test kits (Spinreact, Spain).

Pathological examination. After bleeding, selected birds were weighed and euthanized for pathological examination. The liver was removed, cleaned, and weighed. The relative liver weights (organ weight; g/100 g live body weight) were calculated. Liver samples were fixed in 10% neutral buffered formalin, dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Sections were cut at 10 µm and stained with haematoxylin and eosin (H&E; Thermo Shandon, USA).

Grading of liver lesions. The following grading system was used: degree 1 (slight) - mild hepatocellular swelling because of hydropic degeneration as well as fatty changes only in centrilobular areas; degree 2 (moderate) - clear hepatocellular swelling and changes in both centrilobular and midzonal areas; degree 3 (severe) - dispersed and severe hepatocellular swelling and changes in other areas.

Other chickens in the groups were used to gauge the performance and immunological variables. The results have been published elsewhere (17).

Statistical analysis. The experiment was performed as a completely randomised design with three replicates of 10 chickens assigned to each of 10 dietary treatments. Data were subjected to statistical

analysis using the general linear models procedure of SAS software (29). The treatment means showing considerable differences in the one-way ANOVA were compared using Duncan's multiple-range test. To compare the histopathological lesions between treatment groups, chi-square statistical methods were used. All the statements of significance were based on the 0.05 level of probability.

Results

Biochemical changes. The effects of dietary treatments on serum biochemical parameters are showed in Table 1. Feeding aflatoxin alone caused considerable decreases in uric acid (52.4%) and triglyceride (54.22%) concentrations, as well as increases in AST (32.96%) and ALT (34.82%) activity ($P < 0.05$), when compared to the control group. A decrease in whole cholesterol (29.75%) levels in aflatoxin alone group was not statistically different ($P > 0.05$) from the control group.

The addition of YG (0.1%) to aflatoxin containing diet (group 6) significantly alleviated the adverse effects of aflatoxin on uric acid, triglycerides, AST, ALT, and relative liver weight ($P < 0.05$). Other adsorbent treatments (groups 3-5, 7-9, 10) provided partial amelioration on the parameters influenced by aflatoxin treatment ($P > 0.05$).

Pathological changes. Aflatoxin caused a significant increase in relative liver weight (5.1 versus 2.3; 121.7% ($P < 0.05$, Table 1). Microscopically, no significant lesions were observed in liver tissue of control birds (Table 2).

The liver of birds fed the aflatoxin containing diet revealed more significant lesions ($P < 0.05$), in comparison to the positive control group, including small to moderate hydropic degeneration and fatty vacuoles in hepatocytes in centrilobular and midzonal areas (8 in 10 cases; Fig. 1). In this group, bile duct proliferation (7 in 10 cases) in portal areas (Fig. 2), periportal fibrosis (5 in 10 cases), cell infiltration (4 in 10 cases; Fig. 3), and a slight hyperaemia (3 in 10 cases) were observed. There were also histopathological changes (slight to moderate) in the liver of other treated animals.

The addition of YG (groups 5 and 6) and YG+SB (group 10) to the aflatoxin containing diet significantly reduced the number of affected broilers and/or the severity of lesions (Table 2). These decreases in the severity of hydropic degeneration and other changes in the liver were considerable. Relative liver weight was also recovered by YG (0.05% and 0.1%) and YG (0.1%) + SB (3%) treatments (groups 5, 6, and 10; $P < 0.05$). The smallest histopathological lesions (slight hydropic degeneration) were observed after treatment

with 0.1% YG (group 6) as compared to other treatments, except positive control group.

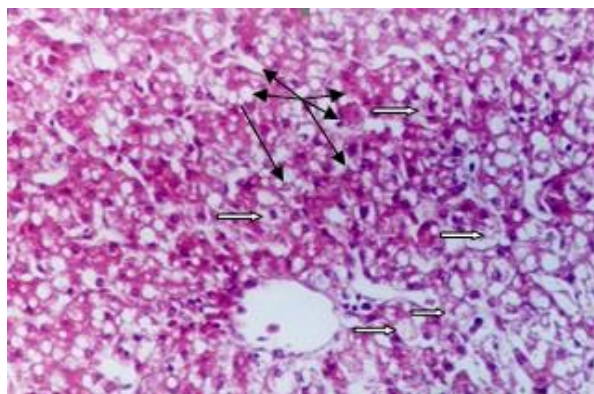


Fig. 1. Microscopic image of the liver from chickens fed aflatoxin-containing diet (250 ppb). Substantial fatty changes and moderate hydropic degeneration in hepatocytes (H & E, 300 $\times$)

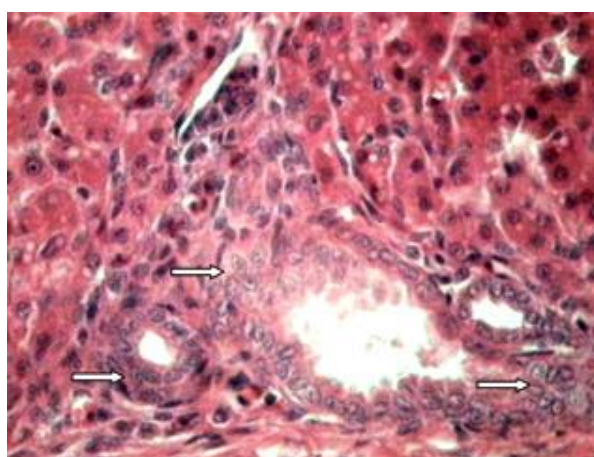


Fig. 2. The liver from 250 ppb aflatoxin-treated chickens. Bile-duct hyperplasia (H & E, 300 $\times$)

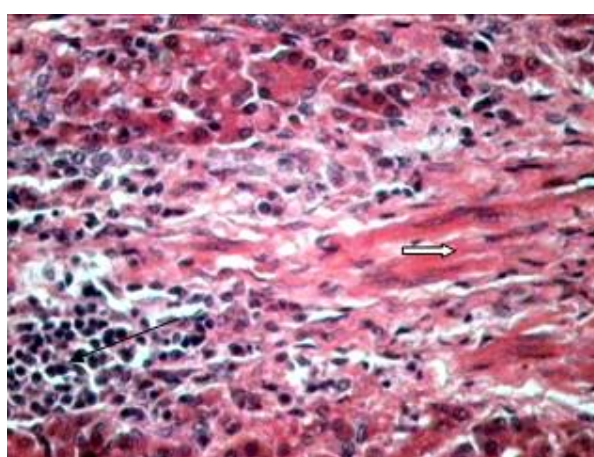


Fig. 3. The liver from 250 ppb aflatoxin-treated chickens. Mononuclear cell infiltration in the portal triad and proliferation of connective tissue (H & E, 300 $\times$)

Table 1. Effect of aflatoxin-contaminated diet, sodium bentonite (SB), and yeast glucomannan (YG) on serum biochemical parameters and relative liver weight in broiler chickens fed aflatoxin-contaminated feed from 7 to 42 d of age

Treatments	42 days of age					
	Uric acid (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	AST (iu/l)	ALT (iu/l)	Relative liver weight (g)
1	7.68 ± 2.20 ^a	165.16 ± 1.75	159.09 ± 44.85 ^a	241.82 ± 32.92 ^b	38.25 ± 2.52 ^b	2.3 ± 1.2 ^c
2	3.65 ± 0.27 ^b	116.02 ± 8.79	72.83 ± 58.80 ^c	321.53 ± 25.24 ^a	53.10 ± 7.95 ^a	5.1 ± 1.3 ^a
3	4.08 ± 1.36 ^b	140.83 ± 10.72	98.07 ± 10.03 ^{bc}	309.93 ± 34.05 ^{ab}	47.92 ± 5.64 ^{ab}	4.2 ± 0.8 ^{ab}
4	3.98 ± 2.20 ^b	132.42 ± 14.48	79.50 ± 26.90 ^c	303.27 ± 29.93 ^{ab}	48.17 ± 3.34 ^{ab}	4.5 ± 0.7 ^{ab}
5	4.30 ± 0.54 ^{ab}	147.11 ± 9.56	81.16 ± 5.75 ^c	305.39 ± 40.72 ^{ab}	44.13 ± 6.45 ^{ab}	3.5 ± 0.9 ^{bc}
6	6.35 ± 0.60 ^a	175.93 ± 9.45	144.89 ± 29.20 ^a	265.42 ± 18.63 ^b	41.72 ± 1.98 ^b	2.9 ± 0.5 ^c
7	4.24 ± 0.44 ^{ab}	131.16 ± 3.40	91.81 ± 14.64 ^{bc}	306.63 ± 52.48 ^{ab}	45.49 ± 7.20 ^{ab}	4.3 ± 1.1 ^{ab}
8	4.12 ± 0.52 ^b	142.50 ± 6.26	83.63 ± 21.86 ^{bc}	307.91 ± 60.04 ^{ab}	46.65 ± 4.35 ^{ab}	4.4 ± 1.2 ^{ab}
9	4.36 ± 0.47 ^{ab}	143.16 ± 17.92	82.05 ± 12.21 ^{bc}	301.60 ± 72.83 ^{ab}	46.12 ± 9.43 ^{ab}	3.7 ± 1.4 ^{abc}
10	4.68 ± 2.05 ^{ab}	141.92 ± 13.32	119.71 ± 20.25 ^{ab}	294.52 ± 81.46 ^{ab}	43.32 ± 8.16 ^{ab}	3.5 ± 1.1 ^{bc}
Sig.	**	NS	**	**	**	**

a-b: means presented in a column with different superscripts differ significantly ($P < 0.05$). 1 - positive control diet, basal diet without additives (control); 2 - negative control diet, diet naturally contaminated with aflatoxin (NCD); 3 - NCD supplemented with 1.5% SB; 4 - NCD supplemented with 3% SB; 5 - NCD supplemented with 0.05% YG; 6 - NCD supplemented with 0.1% YG; 7 - NCD supplemented with 1.5% SB+ 0.05% YG; 8 - NCD supplemented with 1.5% SB+ 0.1 % YG; 9 - NCD supplemented with 3% SB + 0.05% YG; and 10 - NCD supplemented with 3% SB+ 0.1% YG. NS - not significant, ** ($P < 0.05$)

Table 2. Effect of aflatoxin-contaminated diet, sodium bentonite (SB), and yeast glucomannan (YG) on microscopic changes in the liver in broiler chickens fed aflatoxin-contaminated feed from 7 to 42 d of age

Lesions	Treatments									
	1	2	3	4	5	6	7	8	9	10
Hydropic degeneration and/or fatty changes	0/10 ^b	8/10 ^a	8/10 ^a	7/10 ^a	4/10 ^{ab}	3/10 ^b	6/10 ^a	7/10 ^a	7/10 ^a	4/10 ^{ab}
Bile-duct hyperplasia	0/10 ^b	7/10 ^a	4/10 ^a	5/10 ^{ab}	1/10 ^b	0/10 ^b	5/10 ^a	4/10 ^{ab}	4/10 ^{ab}	2/10 ^b
Periportal fibrosis	0/10 ^b	5/10 ^a	4/10 ^a	3/10 ^{ab}	0/10 ^b	0/10 ^b	3/10 ^{ab}	3/10 ^{ab}	2/10 ^b	1/10 ^b
Cell infiltration	0/10 ^b	4/10 ^a	2/10 ^{ab}	2/10 ^{ab}	0/10 ^b	0/10 ^b	2/10 ^{ab}	1/10 ^b	2/10 ^{ab}	0/10 ^b
Hyperaemia	0/10 ^a	3/10 ^a	2/10 ^a	1/10 ^a	0/10 ^a	0/10 ^a	2/10 ^a	0/10 ^a	0/10 ^a	0/10 ^a

^{ab} Different superscripts in the same row show significant difference ($P < 0.05$) between treatments for each parameter.

The values represent the number of chickens showing histopathological changes/number of chickens examined in each treatment group.

1 - positive control diet, basal diet without additives (positive control); 2 - negative control diet, diet naturally contaminated with aflatoxin (NCD); 3 - NCD supplemented with 1.5% SB; 4 - NCD supplemented with 3% SB; 5 - NCD supplemented with 0.05% YG; 6 - NCD supplemented with 0.1% YG; 7 - NCD supplemented with 1.5% SB+ 0.05% YG; 8 - NCD supplemented with 1.5% SB+ 0.1 % YG; 9 - NCD supplemented with 3% SB + 0.05% YG; and 10 - NCD supplemented with 3% SB+ 0.1% YG. NS - not significant ** ($P < 0.05$)

Discussion

Aflatoxins are of major concern to the poultry industry due to their toxicity and frequency of occurrence in feedstuffs. Aflatoxin contaminated feed causes decreases in the activities of several enzymes important in digestion of carbohydrates, proteins, lipids, and nucleic acids in broiler chickens (21). Chronic and sub-clinical aflatoxicosis cases may be diagnosed by determining alterations in levels of serum biochemical and haematological parameters before major symptoms become apparent. These parameters are sensitive indicators of aflatoxicosis (11).

The biochemical and haematological toxic effects of aflatoxin have been investigated in detail and are a well-known subject. Some biochemical toxic effects were also clearly visible in the present study. Lower amounts of uric acid, cholesterol, and triglycerides, and higher AST and ALT activities were observed in chickens fed aflatoxin alone (negative control group) as compared to positive control group, and were in agreement with previous reports on the biochemical toxic effects of aflatoxin (4, 13, 18). Elevation in activities of AST and ALT and decreased cholesterol and triglyceride values in group 2 may result from hepatic damage and leakage of enzymes into the blood stream, as well as impairment of carbohydrate and lipid metabolism (18).

The gross and histopathological effects of aflatoxin on poultry are well known (24). The liver is considered to be the primary target organ for aflatoxin (25). Thus the aim of our study was to assess the impact of 250 ppb aflatoxin, a level which has been

shown to occur under field conditions, on pathological changes in the liver.

In poultry, the histopathological hepatic changes caused by lower levels of aflatoxin are more expressed than those of other organs (25). Hepatomegaly, hydropic and fatty changes, and acinar arrangements in hepatocytes, periportal fibrosis, and bile duct proliferation were noted. They were also observed in previous studies on aflatoxin (10). In the present study, relative liver weight was significantly increased in negative control group as compared to positive control group.

Removing aflatoxin from contaminated feed and feedstuffs is still a major problem and there is a desperate need for efficacious decontamination technology (21, 28). One of the methods is the use of non-nutritive and inert adsorbents and biological matter produced by microorganisms such as bacteria or yeast in the birds' diets to bind aflatoxin and decrease the absorption of aflatoxin from the gastrointestinal tract. To be effective, these compounds should not be absorbed from the gastrointestinal tract. They should also have the ability to connect physically with chemical substances and prevent their absorption (5). In this study, YG and SB were selected as the adsorbents for decreasing the aflatoxin absorption from the gastrointestinal tract, and to reduce the effect of aflatoxin on changes in biochemical parameters and in the liver.

In experimental trials, YG and/or SB were added to aflatoxin containing diet to see if they were effective in decreasing aflatoxin toxicity in terms of performance, haematology, serum biochemistry,

immunology, and pathomorphology of broilers (2, 7, 10, 13, 28) and duckling (3). In most previous reports on the effects of YG and SB, aflatoxin was added to the diet at different levels (from 200 to 2000 ppb). The present experiment was conducted to study the effects of SB and YG (Mycosorb) in a diet naturally contaminated with 250 ppb of aflatoxin regarded as the level similar to that produced under field conditions.

When compared to negative control group, the addition of 0.1% YG to a diet containing aflatoxin (250 ppb) (group 6) significantly decreased the adverse effects of aflatoxin on AST, ALT, and relative liver weight and liver pathological changes. Other adsorbent treatments (groups 3-5, 7-9, 10) provided partial amelioration of the parameters influenced by aflatoxin treatment. It means that differences in biochemical values and histopathological examination results were found between positive and negative control groups; according to the adsorbed aflatoxin molecules in the gastrointestinal tract by feed additives. It is predicted that more aflatoxin molecules adsorbed by feed additives in the gastrointestinal tract cause less negative effects in the target organs and biochemistry. These findings are in agreement with other reports that noted significant improvements by addition of YG (2, 5, 8).

The beneficial counteraction of YG with aflatoxin molecules in the gastrointestinal tract has been clearly seen in our study. The adsorbing effects of dietary YG are attributed to its ability for binding selectively to aflatoxin molecules. Cell wall of *S. cerevisiae* consists of a network of β -1,3 glucan back bone with β -1,6 glucan side chains, which is in turn attached to highly glycosylated mannoproteins, which form the external layer. The proteins and glucans provide numerous easily accessible binding sites with different binding mechanisms such as hydrogen bonding, ionic or hydrophobic interactions. The binding of aflatoxin to yeast cell surface has been attributed to cell wall glucans (30).

When compared to negative control, the preventive efficacy of 1.5% and 3% SB in aflatoxin containing diet was not significant (groups 3, 4) in contrast to the results of other authors reporting a significant amelioration after addition of SB to the diet containing aflatoxin (200 to 2500 ppb) (6, 13, 15). The beneficial effect of SB in above studies might be attributed to its sequestration action against aflatoxin in feeds based on the mechanism of adsorption and decrease in the aflatoxin bioavailability in the gastrointestinal tract. The differences between our results and their findings might have either been caused by the difference in the sodium bentonite or aflatoxin levels in feed, or poultry species.

Our previous study on the toxic effect of aflatoxin (250 ppb) and the ameliorative efficacy of dietary Mycosorb and SB on performance and antibody production against Newcastle disease in broilers were evaluated with the same experimental design (17). The amelioration in biochemical values and the liver in the

aflatoxin plus 0.1% YG group in the present study is paralleled with our previous findings in which 0.1% YG was proved to be much more useful in the amelioration of the negative effect of aflatoxin on performance and humoral immunity against ND.

In conclusion, the serum biochemical parameters and the liver were significantly affected by aflatoxin (250 ppb). The addition of YG and SB, alone or in combination, to the aflatoxin-containing diet reduced the negative effects of aflatoxin; however, supplementation with 0.1% YG-alone turned out to be much more effective than other treatments in ameliorating the adverse effects of aflatoxin.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Animal Rights Statement: The experiment procedures were approved by the Commission of Ethics and Animal Welfare of the Islamic Azad University.

Acknowledgement: The authors are thankful to Dr. H. Ghahri from the Faculty of Veterinary Medicine, Urmia Branch of Islamic Azad University, Iran, for his technical help and Dr. A.A. Farshid from the Faculty of Veterinary Medicine, University of Urmia, Iran, for kindly providing figures. The authors gratefully acknowledge the assistance of Prof. H. Oguz from Faculty of Veterinary Medicine, Selcuk University, Campus, Konya, Turkey, for reviewing of the manuscript.

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