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Ameliorative effect of esterified glucomannan, sodium bentonite, and humic acid on humoral immunity of broilers during chronic aflatoxicosis

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Abstract: A study was conducted on the impact of aflatoxin (AF) and mycotoxin binders on immunization against Infectious Bursal Disease (IBD) and Infectious Bronchitis (IB) in chickens. Broiler chicks (7-day-old) were randomly assigned to 9 dietary treatments with 4 replicates of 12 chicks each. Treatments were 1) control; 2) diet containing corn naturally contaminated with AF (NCD); 3) NCD + 0.2% Farmagülatör DRYTM humate (FH); 4) NCD + 0.4% FH; 5) NCD + 0.6% FH; 6) NCD + 0.8% FH; 7) NCD + 1% FH; 8) NCD + 0.5% sodium bentonite (SB); and 9) NCD + 0.1% esterified glucomannan (E-GM). Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software. Compared to control diet; the antibody titers of IB and IBD were significantly lower in the 254 ppb aflatoxin fed chicks from 28 to 35 days of age. The addition of E-GM, SB, and FH to the aflatoxin-containing diet significantly ameliorated the adverse effects of aflatoxin on IBD and IB antibody titers, but FH supplementation to the contaminated diet with aflatoxin proved to be much more effective in the amelioration of the adverse effect of aflatoxin on humoral immunity against IB.

Key words: AFB₁, esterified glucomannan, sodium bentonite, humic acid, broiler chicks, humoral immunity

Introduction

Aflatoxins are a group of related toxic metabolic byproducts produced by *Aspergillus flavus* and other species of aspergilli. *A. flavus* produces several aflatoxins, but aflatoxin B₁ is the most important of the toxins (1). Aflatoxin (AF) (as mean total

aflatoxins) is a relatively low molecular-weight, lipophilic molecule that appears to be absorbed rapidly (2) and completely (3) from the gastrointestinal tract. Aflatoxin is the best-known mycotoxin for its ability to impair reticuloendothelial activity (4), primary immune response (5), phagocytic

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activity of leukocytes, and alveolar macrophages (6). AF causes a dose-related regression in the size of both the thymus and bursa of Fabricius, which are the primary determinants of immunocompetence, depletion of functional cells (7) and decrease in antibody production (8). Prophylactic immunization against several infectious diseases in poultry, such as Infectious Bursal Disease (IBD), Infectious Bronchitis (IB), and Newcastle Disease (ND) is crucial to avoid these infections (9,10). Studies have shown that aflatoxin is immunosuppressive and its ingestion has resulted in decreased immunity in vaccinated birds (9,11). The inhibitory effect of aflatoxin on antibody production has also been demonstrated in chicks (12) and turkeys (13).

A variety of chemical, physical, and biological techniques for mycotoxin decontamination of feeds have been used, but they have had limited success (14). Adsorbents used to prevent the gastrointestinal absorption of mycotoxins must form a strong complex and also have a high capacity to prevent saturation (15). Aluminosilicates, activated charcoal, and yeast products have been extensively studied with promising, but varying, results (16). According to Decker and Corby (17), activated charcoal adsorbed at a rate of 10 mg of AFB₁/g whereas a gram of montmorillonite silicate was able to adsorb only about 1 mg of AFB₁ at pH 7 (15). The term 'humus' has been known to science for years; it is a transformation product of animal and plant organisms. Humate or humic acid (HA) is a class of compounds resulting from decomposition of organic matter and are natural constituents of drinking water, and solid and lignite disintegrated compounds particularly from plants. They inhibit bacterial and fungal growth, thus decreasing levels of mycotoxins in feed (12). Its beneficial effects include stress management, immune system, anti-inflammatory activity, antiviral properties as well as prevention of intestinal diseases, mainly diarrhea in humans and animals (12). The use of HA and related products in feed improved gut health for better nutrient utilization as well as improved the health status by working against pathogens developing immunity (12). Routine use of HA in feed improved growth of broilers by increasing the digestion of protein and improved trace element utilization. Research on humate includes humus,

humic acid, fulvic acid, ulmic acid, and trace minerals (18). In recent years, it has been observed that humates included in the feed and water of poultry promoted growth. According to Jansen van Rensburg et al. (19) humic acid was able to adsorb about 10.3, 7.4, and 11.9 mg of AFB₁/g of oxihumate at pH 3, 5, and 7, respectively. In contrast, the maximum AFB₁ adsorption capacity of sodium bentonite from southern Argentina was estimated to be 45 mg/g at pH 2 (20). A recent report by Jansen van Rensburg et al. (19) described that humic acid, but not brewers' dried yeast, could alleviate some of the toxic effects of aflatoxin in growing broilers. Therefore, the purpose of the present study was to evaluate the possible preventive role of dietary adsorbents on the humoral immunity of broilers given AF (254 ppb) in 1 broiler period.

Materials and methods

Experimental design, bird, and data collection

Five hundred 1-day-old broiler chicks (Ross308) of both sexes were adapted for a 7-day period prior to commencement of the trial. During this period, the birds were maintained with conventional broiler chicken management and housed in floor pens on litter in an environmentally controlled broiler house. They received a commercial broiler starter diet (similar to the negative control diet in the respective experiments) formulated to meet or exceed the nutritional requirements of broilers as recommended by the NRC (21). This diet as well as basal diets used subsequently were analyzed for aflatoxin and were negative. At 7-day of age, 432 chicks were individually weighed, wing-banded, and were randomly assigned to 36 pens in the same broiler house used for the adaptation period. Chicks were maintained on a 23L:1D schedule and allowed to consume feed and water ad libitum. The basal diet used throughout the study was a 2-phase commercial corn and soybean meal based ration, formulated to meet or exceed nutritional requirement of broilers, as recommended by the NRC (21). A completely randomized experimental design was used, and chicks were divided into 9 treatment groups, with 4 replicates per treatment and 12 chicks per replicate. Treatments were 1) basal feed free of aflatoxin (control), 2) diet

containing corn naturally contaminated with 254 ppb aflatoxin, 3) naturally contaminated diet with aflatoxin supplemented with 0.2% Farmagülatör DRY™ humate¹ (FH), 4) naturally contaminated diet with aflatoxin supplemented with 0.4% FH, 5) naturally contaminated diet with aflatoxin supplemented with 0.6% FH, 6) naturally contaminated diet with aflatoxin supplemented with 0.8% FH, 7) naturally contaminated diet with aflatoxin supplemented with 1% FH, 8) naturally contaminated diet with aflatoxin supplemented with 0.5% Na-bentonite, and 9) naturally contaminated diet with aflatoxin supplemented with 0.1% esterified glucomannan. Each kilogram of humate contained 160 mg polymeric polyhydroxy acids (humic, fulvic, ulmic, and humatomelanic acids), 663.3 mg SiO₂, and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.5 mg; and Al, Na, K, Mg, and P in trace amounts).

Mycotoxin quantification and diet preparation

Individual feed ingredients and the finisher diet were analyzed and screened for AF content. AF was extracted according to Romer (22) and was quantified by thin-layer chromatography (TLC) as outlined by AOAC (23). The basal control diet was formulated and compounded to meet the nutritional requirements of commercial broilers (21) during the starter and grower period. The basal diet did not contain detectable levels of AF (<1 µg/kg diet). Naturally contaminated maize that had been rejected due to severe mold growth was obtained from a private feed mill; the presence of aflatoxins in the maize was confirmed by TLC. The contaminated maize was stored in 20% moisture for 2 additional months to increase mold growth. The contaminated diet treatments were formulated by replacing aflatoxin-free maize with naturally contaminated maize. Upon analysis, the contaminated diet contained 254 ppb AF (detection limit: 1 µg/kg diet). The AF composition consisted of 78.6% AFB₁ (200 ppb), 8% AFB₂, 11% AFG₁, and 2.4% AFG₂ based on total AF in the contaminated diet. During the experimental period, the control and contaminated diet were analyzed for AF. The levels of AF in the

control diet were below the detection limits. Levels of AF in the contaminated diet ranged from 278 to 285 µg/kg.

Vaccination

Before chicks (1-day-old) were separated into groups, blood samples were taken and maternal antibody titers against IB and IBD were measured by the hemagglutination-inhibition test (HI). All chicks were vaccinated by aerosol-spray on the 1st day of age with bronchitis vaccine (Intervet International B.V. Boxmeer, Holland). On the day 14 chicks were vaccinated with IB vaccine administered in drinking water (Nobilis® IB 4/91 Intervet International B.V. Boxmeer, Holland). Chicks in all groups were vaccinated twice at days 18 and 24 with attenuated live IBD vaccine (Nobilis® Gumboro D78, Intervet International B.V. Boxmeer, Holland).

Blood samples were taken individually from all chickens in all groups, at days 7, 14, 21, 28 and 35 via wing vein puncture. Serum was separated by centrifugation at 1000 rpm for 15 min, inactivated at 56 °C for 30 min and stored at -20 °C until tested. IB and IBD antibody titers were determined using commercial ELISA kits (IDEXX Corb, Portland, ME, USA). The kits included both negative and positive control samples. An automated IBM computerized reader was used (24). Two readings/samples were obtained for each serum dilution and mean antibody titers log₁₀ were calculated according to the equation provided with the kit:

$$\text{Log}_{10} \text{ titers} = 1.09(\log_{10} \text{ sample mean} / \text{positive mean}) + 3.36$$

$$\text{S/P ratio} = \text{Sample mean} - \text{negative control} / \text{positive control} - \text{negative control}$$

The mean antibody titers of the groups were compared to that of the negative control provided with the kit.

Statistical analysis

The feeding trail was terminated when the chicks reached 35 days of age. Data are expressed as mean and were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software (24). The treatment means

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with significant differences were compared using Duncan's new multiple range test (25). All statements of differences were based on significance at $P \leq 0.05$.

Results

The effects of dietary treatments on antibody production against IB and IBD in broilers from day 7 to day 35 are presented in Tables 1 and 2. On day 7 of the study, there was no statistically significant change in the antibody titers of experimental groups. Consumption of aflatoxin contaminated feed resulted in significant reduction in antibody titers against IB as compared to the control diet at 28 and 35 days of age. The addition of FH (0.4, 0.6, 0.8, and 1.0 percent) to the AF-containing diet significantly ameliorated the adverse effect of AF on antibody production against IB in chicks at 35 days of age. Sodium bentonite and esterified glucomannan

supplementation to the contaminated diet improved the antibody production against IB at 28 days of age, but no significant improvement was observed at 35 days of age. The feeding of AFB₁ at a level of 254 ppb in the ration reduced the antibody production against IBD in broilers from 28 to 35 days of age. Addition of 0.4, 0.8, and 1.0 percent of FH to the AF-containing diet significantly ameliorated the adverse effect of AF on the antibody production against IBD in broilers at 35 days of age. The addition of sodium bentonite and esterified glucomannan to the AF-containing diet significantly ameliorated the adverse effect of AF on the antibody production against IBD in broiler from 28 to 35 days of age.

Discussion

Survey studies showed that the AF levels in broiler feed ranged from 5 to 100 ppb in Turkey (26,27) and

Table 1. Effect of sodium bentonite (SB), esterified glucomannan (E-GM), and Farmagülatör DRY™ humate (FH) on IB antibody titers for broiler chicks fed an aflatoxin contaminated diet from 7 to 35 days of age*.

AF	Treatment			Antibody titers				
	SB (%)	E-GM (%)	FH (%)	Day 7**	Day 14	Day 21	Day 28	Day 35
-	-	-	-	576.5	456.5	462	681.00 ^{ab}	631.50 ^a
+	-	-	-	605.1	440	455.75	497.50 ^c	439.25 ^c
+	-	-	0.2	575.25	459	452.75	550.75 ^{bc}	494.00 ^{bc}
+	-	-	0.4	588.75	451	463.75	740.00 ^a	569.75 ^{ab}
+	-	-	0.6	563.25	456.75	455	545.50 ^{bc}	550.00 ^{ab}
+	-	-	0.8	590.75	464.5	505.25	624.75 ^{abc}	567.75 ^{ab}
+	-	-	1	576.25	456.75	504.75 504.25	687.75 ^{ab}	643.00 ^a
+	0.5	-	-	577	463.23	480.75	697.50 ^{ab}	521.50 ^{bc}
+	-	0.1	-	619.25	465.25		599.75 ^{abc}	528.00 ^{bc}
SEM				23.13	11.17	23.17	54.77	32.18
P-Value				0.8003	0.8497	0.4672	0.0339	0.0011

^{a-c} values within a column with no common superscript differ significantly.

* Each value represents the mean of 4 replicates with 12 chicks per replicate.

** Maternal antibody titers were measured before all chicks (1-day-old) were separated into groups.

Table 2. Effect of sodium bentonite (SB), esterified glucomannan (E-GM), and Farmagülatör DRY™ humate (FH) on IBD antibody titers for broiler chicks fed an aflatoxin contaminated diet from 7 to 35 days of age*.

AF	Treatment			Antibody titers				
	SB (%)	E-GM (%)	FH (%)	Day 7**	Day 14	Day 21	Day 28	Day 35
-	-	-	-	1702	574.00 ^{ab}	535.25 ^{ab}	1347.00 ^a	2513.8 ^{ab}
+	-	-	-	1721	589.25 ^{ab}	474.50 ^{ab}	459.75 ^d	1566.0 ^c
+	-	-	0.2	1688.75	723.75 ^a	465.0 ^b	1162.75 ^{ab}	1881.5 ^{cde}
+	-	-	0.4	1675.75	536.25 ^{ab}	472.0 ^{ab}	634.50 ^{cd}	2309.0 ^{abc}
+	-	-	0.6	1706.25	486.75 ^b	617.0 ^a	561.25 ^{cd}	1768.5 ^{de}
+	-	-	0.8	1688.5	461.25 ^b	564.25 ^{ab}	557.00 ^{cd}	2126.3 ^{bcd}
+	-	-	1	1705.5	498.50 ^{ab}	482.25 ^{ab}	997.75 ^b	2538.3 ^{ab}
+	0.5	-	-	1701.5	458.00 ^b	585.50 ^{ab}	1368.25 ^a	2766.3 ^a
+	-	0.1	-	1702	451.00 ^b	454.50 ^b	694.25 ^c	2478.3 ^{ab}
SEM				45	22.33	44.11	61.13	55.95
P-Value				0.9994	0.1837	0.04395	0.0001	0.0001

^{a-c} values within a column with no common superscript differ significantly.

* Each value represents the mean of 4 replicates with 12 birds per replicate.

** Maternal antibody titers were measured before all chicks (1-day-old) were separated into groups.

in several other countries (20,26). The immunotoxic effects of AF in poultry have been well-documented. Therefore, we particularly aimed to assess the impact of 254 ppb AF, which naturally occurred in field conditions, on antibody production against IB and IBD in the present study. Intake of aflatoxin from naturally contaminated feed at 200 ppb has been shown to reduce or suppress protection in chickens already immunized against fowl cholera (27). In our study, ingestion of aflatoxin contaminated feed significantly lowered antibody titers in chickens immunized against IB and IBD compared to control groups. This was similar to the results in other studies (8,28) that showed the immunotoxic effects of AF at 100 to 2500 ppb AF in the diet. It is important to consider poor humoral immunity in chicks caused by this AF-level (254 ppb) in the present study, because this level of AF can be found in broiler feed in field

conditions without showing significant clinical signs in broilers during the rearing period. The immune-suppressive effect of aflatoxin has been related to its direct inhibition of protein synthesis including those with specific functions, such as immunoglobulins IgG, IgA, inhibition of migration of macrophages, interference with the hemolytic activity of complement, reduction in the number of lymphocytes through its toxic effect on the bursa of Fabricius, and impairment of cytokines formation by lymphocytes (4,5). Our study agrees with previous findings by Giambrone et al. (1) who reported that infected chicks fed aflatoxin produced lower antibodies than uninfected chicks. The higher potency of aflatoxin might be due to the fact that it is hepatotoxic and inhibits nucleic acid and protein syntheses that reduce the concentrations of various serum proteins (28).

Recent studies showed that sodium bentonite (20,29), esterified glucomannan (30), and humic acid (19) were effective in reducing AF-toxicity in terms of growth performance, hematological-serum biochemical, and macroscopic-histopathological analyses. Our findings show that FH significantly ameliorated the adverse effect of AF on the humoral immunity against IB and IBD. These effects of FH might be attributed to mycotoxin adsorption, ability to block colonization of pathogens in the gastrointestinal tract, and inhibitory effect on liver antioxidant depletion (19). Humic acid has been shown to bind the AF molecules in the gastrointestinal tract and can alleviate the toxicity of AF in poultry. In this study, FH proved to be much more effective in the amelioration of aflatoxicosis in broiler chicks than the E-GM. Jansen van Rensburg et al. (19) showed that humic acid, but not E-GM, could alleviate some of the toxic effects of aflatoxin in growing broilers. The decrease in IBD antibody titers caused by the consumption of contaminated diet was diminished by the addition of E-GM to the diet. Although the best response was obtained when Na-bentonite and humic acid were added in the diet, it is

reported that using Na-bentonite up to 0.6% in the diet is effective in reducing aflatoxicosis in chickens (31). In the present study the addition of Na-bentonite was effective in ameliorating the negative effect of AF on antibody production. The ameliorative effect of dietary Na-bentonite on the reduced antibody titers caused by AF could be attributed to the role of Na-bentonite as a sequestering agent of AF in the gastrointestinal tract. These results clearly demonstrated that the 254 ppb AF-treatment significantly affected the humoral immunity against IB and IBD, and the simultaneous addition of humic acid to the AF-containing diet provided significant reduction on the immunotoxic effects of AF. These improvements should contribute to a solution of the AF problem in broiler chickens, when used with mycotoxin management practices.

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