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Research Article

Evaluation of the efficacy of esterified glucomannan, sodium bentonite, and humic acid to ameliorate the toxic effects of aflatoxin in broilers

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Abstract: A study was conducted to determine the efficacy of esterified glucomannan, sodium bentonite, and humic acid in counteracting the toxic effects of aflatoxin in a naturally contaminated diet fed to broilers. Seven-day-old chicks were randomly assigned to 1 of the 9 dietary treatments. The treatments were as follows: 1) control; 2) naturally contaminated diet with aflatoxin; 3, 4, 5, 6, and 7) naturally contaminated diet with aflatoxin supplemented with 0.2%, 0.4%, 0.6%, 0.8%, and 1.0% humic acid, respectively; 8 and 9) naturally contaminated diet supplemented with 0.5% sodium bentonite and 0.1% esterified glucomannan, respectively. Compared with the control, the naturally contaminated diet significantly increased feed consumption and resulted in poor feed efficiency. Increased relative weights of liver and decreased relative weights of bursa of Fabricius were observed in chicks fed the naturally contaminated diet. Further, feeding a contaminated diet was associated with significant decreases in serum albumin, total protein, uric acid, and cholesterol. Compared with the control, the naturally contaminated diet significantly increased the activities of γ-glutamyl transferase, lactate dehydrogenase, and aspartate amino transferase. Humic acid showed protective effects on feed efficiency and against liver and bursa of Fabricius damage, as well as some of the serum enzyme activities and serum biochemical changes associated with aflatoxin toxicity.

Key words: Aflatoxin, broiler, esterified glucomannan, humic acid, sodium bentonite

Introduction

Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur worldwide as contaminants of grain. Among the various mycotoxins identified to affect especially poultry, some occur significantly in naturally contaminated foods and feeds. They are aflatoxin (AF), ochratoxin A (OA), zearalenone, T-2 toxin, vomitoxin, and fumoninsin (1). AFB, a metabolite of fungus *Aspergillus flavus* and *Aspergillus parasiticus*, is an extremely hepatotoxic compound that frequently contaminates poultry feeds at low levels (2). Mycotoxins cause a wide variety of adverse clinical signs, depending on the nature and concentration of toxins in the diets, on the animal species, on their age, and on nutritional and health status at the time of exposure to contaminated feed (3). The United States Food and Drug Administration (FDA) has set regulatory levels for poultry feeds for 20 ppb AF (4). Fungi or mould growth in feedstuff is associated with

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the utilization of nutrients from the host. Consequently, alterations in the nutritional content of the feedstuff are expected. The extent of mould growth determines the degree of depletion in the nutrient content of the feedstuff. The germ of the grain is the main site for *Aspergillus* sp. development, which leads to a greater potential for the synthesis of AFB (5).

Numerous strategies for detoxification or inactivation of mycotoxin in contaminated feedstuff have been used. Most of these techniques are impractical or ineffective (6,7). One approach to the detoxification of mycotoxin is the use of nonnutritive absorptive materials in the diet to reduce the absorption of mycotoxins from the gastrointestinal tract (8). Sodium bentonite (SB), activated charcoal, polymers such as cholestyramine and polyvinylpyrrolidones, and esterified glucomannan (E-GM) have been extensively studied with promising, but varying, results (9). However, several adsorbents have been shown to impair nutrient utilization and mineral absorption, and lack binding effects against multiple mycotoxins of practical importance (4).

Humic substances are chemical compounds that derive from stabilized organic matter in the soil or humus and are formed during the decay of plant and animal material in soil (10). Humic acid (HA) has been used as an antidiarrheal, analgesic, immunostimulatory, and antimicrobial agent in veterinary practices in Europe (11). The composition of humate includes humus, humic acid, fulvic acid, ulmic acid, and trace minerals (10). Research has shown that HA has a strong affinity to bind with several compounds. A recent report by Jansen van Rensburg et al. (12) has described that humate, but not brewers dried yeast, could alleviate some of the toxic effects of AF in growing broilers. Results obtained during in vitro binding studies showed that humate has a high mycotoxin adsorption capacity. The protective effect of humate appears to involve sequestration of AF in the gastrointestinal tract and a reduction in bioavailability of AF. Thus, the objective of the present study was to determine the effects of diets containing moldy corn and AF (in naturally contaminated feed but not produced via fermentation of corn by *Aspergillus parasiticus* NRRL 3000), and the efficacy of SB, E-GM, and especially HA in ameliorating the toxic effects of AF in broiler chickens.

Materials and methods

Mycotoxin quantification and diet preparation

Individual feed ingredients and the finished experimental diets were analyzed and screened for AF content. AF was extracted according to Romer (13) and was quantified by thin-layer chromatography (TLC) as outlined by AOAC (14). The basal control diet was formulated and compounded to meet the nutritional requirements of commercial broilers (15) during the starter and grower period, presented in Table 1. The basal diet did not contain detectable levels of AF (<1 µg/kg diet). The maize was obtained from a private feed mill (that was naturally contaminated with mold) and stored in 20% moisture for 2 months to increase the mold growth. The naturally contaminated maize was discarded completely due to severe mold growth, and the presence of AF in the maize was confirmed by TLC. 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, 8% AFB2, 11% AFG1, and 2.4% AFG2 based on the total AF in the contaminated diet. During the experimental period, the control and contaminated diets were analyzed for AF. The detected 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.

Experimental design, bird, and data collection

A total of 500 one-day-old male broiler chicks were adapted for a 7-day period before commencement of the trial. During this period, the birds were submitted to conventional broiler chicken management and housed in floor pens in an environmentally controlled broiler house with litter floors. They received a commercial broiler starter diet, formulated to meet or exceed the nutritional requirements of broilers, as recommended by the NRC (15). This diet, as well as the basal diets used subsequently, was analyzed and tested negative for AF.

At 7 days of age, 432 chicks of similar weight were randomly assigned to 36 clean pens in the same broiler house used for the adaptation period. Birds were maintained on a 23L:1D schedule and allowed to consume feed and water ad libitum. The basal diet used throughout the study was 2-phase commercial corn and soybean meal based ration, formulated to meet or exceed nutritional requirement of broilers, as recommended by the NRC (15). The chicks were divided into 9 treatment groups, with 4 replicates per treatment and 12 chicks per replicate: 1) basal feed free of toxin (control), 2) diet naturally contaminated with AF, 3) naturally contaminated diet supplemented with 0.2% HA[1], 4) naturally contaminated diet supplemented with 0.4% HA, 5) naturally contaminated diet supplemented with 0.6% HA, 6) naturally contaminated diet supplemented with 0.8% HA, 7) naturally contaminated diet supplemented with 1% HA, 8) contaminated diet supplemented with 0.5% SB, 9) contaminated diet supplemented with 0.1% E-GM. Each kilogram of HA contained 160 mg of polymeric polyhydroxy acid (humic, fulvic, ulmic, and humatomelanic acids), 663.3 mg of SiO_2, 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).

Chicks were weighed at the end of the week, and feed consumption was recorded weekly. Mortalities were also recorded as they occurred. On day 35, all chicks were bled by puncture of the brachial vein. Blood samples were collected from all birds in tubes without anticoagulant. Serum was obtained from these samples and analyzed for serum albumin, total protein, uric acid, cholesterol, and the activities of γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), alanine amino transferase (ALT), and aspartate amino transferase (AST) using an automatic analyzer according to the recommendation of the manufacturer[2]. On day 35 of age, 8 birds in each treatment were killed by cervical dislocation, and the liver, heart, proventriculus, spleen, bursa of Fabricius, and gizzard were removed and weighed. The contents of the proventriculus and gizzard were removed before weighing to determine the stomach weight. The organ weights were expressed as g/100 g body weight. Data were analyzed with ANOVA for a complete randomized design, using the general linear models procedure of SAS software (16). The treatment means with significant differences at $P < 0.05$ were compared using Duncan's new multiple range procedure. All statements of differences were based on significance at $P \leq 0.05$.

Table 1. Effect of esterified glucomannan (E-GM), sodium bentonite (SB), and humic acid (HA) on body weight, feed intake, and feed conversion ratio (FCR) of broilers fed a naturally contaminated diet (35 days).

Treatment				Body weight (g)	Feed intake (g)	Feed:gain Ratio (g:g)
AF[1]	SB (%)	E-GM (%)	HA (%)			
-	-	-	-	1588.63^{ab}	2513.28^{b}	1.58^{c}
+	-	-	-	1537.95^{b}	2737.48^{a}	1.78^{a}
+	-	-	0. 2	1655.28^{a}	2801.98^{a}	1.69^{b}
+	-	-	0.4	1595.18^{ab}	2744.75^{a}	1.72^{ab}
+	-	-	0.6	1597.88^{ab}	2816.60^{a}	1.76^{ab}
+	-	-	0.8	1595.50^{ab}	2840.83^{a}	1.78^{a}
+	-	-	1	1584.35^{ab}	2845.73^{a}	1.79^{a}
+	0.5	-	-	1607.18^{ab}	2808.98^{a}	1.75^{ab}
+	-	0.1	-	1584.50^{ab}	2808.93^{a}	1.77^{ab}

[a-c] Values within a column with no common superscript differ significantly ($P < 0.01$).
[1] The AF composition consisted of 78.6% AFB1, 8% AFB2, 11% AFG1, and 2.4% AFG2 based on total AF (254 ppb) in the contaminated diet.

[1]Yayalar Mh. Sanayi Cd. No:29 Dolayoba-Pendik, İstanbul, Turkey.
[2]Boehringer Mannheim, Hitachi, Japan

Results

The effects of dietary treatments on chick performance from day 7 to 35 are presented in Table 1. When compared with the control, feeding the naturally contaminated diet resulted in a significant increase in feed intake (8.92%). Data presented in Table 1 demonstrate that addition of the adsorbents had no significant effect on the feed intake compared to the contaminated diet alone. There was no difference in BW gain observed for chicks fed AF + adsorbent diets compared to those fed the control diet.

When compared with controls, feeding the naturally contaminated diets resulted in a significant increase in the feed:gain ratio (kg of feed/kg of gain) ($P < 0.01$). The addition of 0.2% HA to the AF-containing diet significantly ameliorated the adverse effect of AF or moldy feeds on the efficiency of feed utilization when compared to the natural contaminated feed ($P < 0.01$). Mortality rate did not differ significantly among treatments. Data presented in Table 2 show the effects of dietary treatment on relative weight of organs (g/100 g BW). Consumption of the contaminated diet caused significant increases in the relative weights of liver and reduction in the relative weights of bursa of Fabricius. Supplementation of adsorbents to contaminated diets did not significantly diminish the effects of AF on the relative weights of liver and bursa of Fabricius, except for the bursa of Fabricius weight of the group fed 0.6% HA. None of the treatments induced changes in heart, spleen, gizzard, and proventriculus weights. The effects of different dietary treatments on serum enzyme activities and biochemical indicators are shown in Table 3. When compared with controls, feeding the naturally contaminated diet resulted in a significant increase in serum GGT, AST, and LDH activities and decrease in serum biochemical values at 35 days of age ($P < 0.05$). In this study, we did not measure the levels of AF in chicken muscle and organs, because when there are low levels of AF in the diet the remaining levels of AF are low as well. Moreover, 72 to 96 h after replacing the contaminated diet with the normal diet AF is metabolized and excreted from the body.

Discussion

When compared with the control, consumption of contaminated diets resulted in a significant increase in the feed intake of broilers. This result may be related to the reduction in the carbohydrates and fat concentrations, dry matter, protein retention, and metabolizable energy level of moldy feeds (17). Consumption of contaminated diet alone resulted in

Table 2. Effect of esterified glucomannan (E-GM), sodium bentonite (SB), and humic acid (HA) on organ weight of broilers fed a naturally contaminated diet (35 days).

Treatment				Organ (g/100 g BW)					
AF[1]	SB (%)	E-GM (%)	HA (%)	Liver	Heart	Proventriculus	Gizzard	Spleen	Bursa of Fabricius
-	-	-	-	2.319[b]	0.593	0.496	2.155	0.131	0.205[a]
+	-	-	-	2.766[a]	0.596	0.532	2.446	0.117	0.126[b]
+	-	-	0.2	2.402[ab]	0.597	0.489	2.205	0.134	0.183[ab]
+	-	-	0.4	2.376[ab]	0.612	0.512	2.349	0.131	0.157[ab]
+	-	-	0.6	2.587[ab]	0.623	0.525	2.364	0.136	0.196[a]
+	-	-	0.8	2.576[ab]	0.602	0.5	2.323	0.141	0.184[ab]
+	-	-	1	2.717[ab]	0.606	0.537	2.383	0.147	0.161[ab]
+	0.5	-	-	2.519[ab]	0.577	0.51	2.34	0.133	0.173[ab]
+	-	0.1	-	2.633[ab]	0.588	0.492	2.27	0.127	0.155[ab]

[a-b] Values within a column with no common superscript differ significantly ($P < 0.05$).
[1]The AF composition consisted of 78.6% AFB1, 8% AFB2, 11% AFG1, and 2.4% AFG2 based on total AF (254 ppb) in the contaminated diet.

Table 3. Effect of esterified glucomannan (E-GM), sodium bentonite (SB), and humic acid (HA) on serum enzyme activities and serum biochemical values of broilers fed a naturally contaminated diet (35 days).

Treatment				Biochemical values				Enzyme activities			
AF[1]	SB (%)	E-GM (%)	HA (%)	Albumin	T-Protein	Uric acid	Cholesterol	AST IU/L	LDH IU/L	ALT IU/L	GGT IU/L
-	-	-	-	1.41^{a}	3.289^{a}	7.521^{a}	209^{a}	211.4^{bc}	325^{b}	45	5.46^{c}
+	-	-	-	0.65^{c}	2.999^{b}	5.515^{b}	107^{b}	270.3^{a}	592^{a}	49	9.36^{a}
+	-	-	0.2	0.85^{b}	3.044^{b}	5.982^{b}	130^{b}	251.1^{ab}	540^{a}	46	7.87^{ab}
+	-	-	0.4	1.45^{a}	3.096^{ab}	6.437^{ab}	180^{a}	250.1^{ab}	511^{a}	47	7.79^{ab}
+	-	-	0.6	1.38^{a}	3.126^{ab}	6.663^{ab}	184^{a}	225.2^{abc}	533^{a}	45	5.74^{bc}
+	-	-	0.8	1.50^{a}	3.191^{ab}	6.443^{ab}	182^{a}	212.1^{bc}	501^{a}	47	5.53^{bc}
+	-	-	1	1.58^{a}	3.191^{ab}	7.730^{a}	187^{a}	211.4^{bc}	430^{b}	48	5.79^{bc}
+	0.5	-	-	1.43^{a}	3.125^{ab}	6.871^{ab}	180^{a}	267.2^{a}	492^{a}	49	8.10^{ab}
-	0.1	-	0.67^{bc}	3.113^{ab}	6.013^{b}	177^{a}	199.0^{c}	401^{b}	46	5.93^{bc}	

$^{a-b}$ Values within a column with no common superscript differ significantly ($P < 0.05$).
[1] The AF composition consisted of 78.6% AFB1, 8% AFB2, 11% AFG1, and 2.4% AFG2 based on total AF (254 ppb) in the contaminated diet.

reduction in BW gain and poorer feed efficiency when compared to the control diet and other treatments. Supplementation of adsorbents to the contaminated diets effectively improved BW gain and feed efficiency. Chicks fed on a contaminated diet with 0.2% HA performed significantly better (7.6%) than those on the contaminated diet alone. These effects of HA might be attributed to mycotoxin adsorption, ability to block colonization of pathogens in the gastrointestinal tract, inhibitory effect on liver antioxidant depletion, and growth promoter (12). AF has been shown to cause inhibition of protein synthesis (18). Albumin and total protein levels in the serum proved to be sensitive indicators of aflatoxicosis in broilers. Kubena et al. (19) reported that decreased serum total protein and albumin in broilers as a result of aflatoxicosis were not alleviated by hydrated sodium calcium aluminosilicate. Our results are consistent with the findings reported by Bailey et al. (20), who observed a decrease in serum uric acid and cholesterol in birds fed a contaminated diet. Kececi et al. (21) have shown that some serum biochemical changes could be ameliorated by bentonite administration to the diet at doses of 5 mg/kg in broiler chickens given 2.5 mg AF/kg diet. However, in our case, the biochemical parameters for broilers fed diets containing SB + AF did not completely return to normal values, showing an inhibition of protein synthesis. These results are parallel with the findings reported by Tung et al. (18), who observed a decrease in these parameters and an inhibition in protein synthesis during the aflatoxicosis in poultry. HA supplementation to a contaminated diet significantly improved albumin values, probably as a result of effective adsorption in the gut to reduce the amount of AF absorption by the body. E-GM did not improve the albumin values of the contaminated birds. The increased serum GGT, AST, and LDH activities observed by feeding naturally contaminated diets in the present study could be due to hepatic degeneration and subsequent leakage of enzymes into circulation. ALT levels remained unaltered when compared to the control diet.

Similar increases in the activities of GGT as observed in the present trial have been reported during aflatoxicosis in broiler breeder hens (22). Serum GGT enzyme activity is a sensitive indicator of liver disease, whether the disorder involves liver inflammation, lesions, or obstruction to the biliary tract (19). These observations are consistent with the studies in which an increase in GGT activity in the serum was reported (4). Neither HA nor E-GM supplementation counteracted the observed increase in GGT, AST, and LDH. Raju and Devegowda (23) and Aravind et al. (4) reported that no significant effects were noted for E-GM on GGT or AST activities in the serum of broilers. The feed contaminated with AF had no effect on the relative weight of the heart, gizzard, spleen, and proventriculus, but it caused a significant increase in liver relative weight and decrease in bursa of Fabricius relative weight, indicating a possibly higher sensitivity of the liver and bursa of Fabricius to AF contamination than the other organs (Table 2). AF has been shown to be hepatotoxic in poultry (24) and research by Smith and Hamilton (25) and Huff et al. (26) demonstrated that relative liver weights are increased by exposure of the chicks to low concentrations of AF. The additions of HA, SB, and E-GM to the contaminated diet prevented the enlargement of the liver and decrease of the bursa of Fabricius relative weight. Recent studies have demonstrated that HA, SB, and E-GM were effective for reducing of AF-toxicity by studying growth performance, hematological, serum biochemical, and macroscopic-histopathological analysis (4,12,27). It is important to consider that AF present in the naturally contaminated feed significantly depressed performance, organ morphology, and most of the serum biochemical parameters. It is because these levels can be found in broiler feed in field conditions and the low levels show no significant clinical signs in broilers during the short periods.

The HA added to a naturally contaminated diet, as an adsorbent, increased performance and serum biochemical values and decreased the activities of GGT, AST, and LDH. These results suggest that HA (from 0.2% to 0.4%) might be sufficient to counteract the adverse effects of AF.

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