Efficacy of Esterified Glucomannan to Counteract Mycotoxicosis in Naturally Contaminated Feed on Performance and Serum Biochemical and Hematological Parameters in Broilers

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ABSTRACT A study was conducted to determine the efficacy of esterified glucomannan in counteracting the toxic effects of mycotoxins in naturally contaminated diet (aflatoxin 168 ppb, ochratoxin 8.4 ppb, zearalenone 54 ppb, and T-2 toxin 32 ppb) fed to commercial broilers. One-day-old broiler chicks were randomly assigned to one of the four dietary treatments with five replicates of 14 chicks each. Four dietary treatments were 1) control; 2) esterified glucomannan, an adsorbent, tested at 0.05% of diet; 3) naturally contaminated diet; and 4) esterified glucomannan (0.05%) plus naturally contaminated diet. Body weight, feed consumption, feed efficiency, hematology, and serum biochemical and enzyme activities were evaluated. Compared with the control, the naturally contaminated diet significantly decreased body weight and feed consumption and resulted in poor feed efficiency. Esterified glucomannan effectively alleviated the growth depression caused by the naturally contaminated diet. Increased relative weights of liver and gizzard were observed in chicks fed the naturally contaminated diet. Further, feeding a naturally contaminated diet was associated with significant decreases in urea nitrogen and hematocrit values along with altered γ-glutamyl transferase activity; however, urea nitrogen concentration was improved with addition of esterified glucomannan. These findings suggest that addition of dietary esterified glucomannan is effective in counteracting the toxic effects of naturally contaminated feed with mycotoxins.

(*Key words*: broiler, esterified glucomannan, mycotoxicosis, naturally contaminated feed, performance)

2003 Poultry Science 82:571–576

INTRODUCTION

Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur worldwide as contaminants of grain. Among the various mycotoxins identified especially affecting poultry, some occur significantly in naturally contaminated foods and feeds. They are aflatoxin (AF), ochratoxin A (OA), zearalenone, T-2 toxin, vomitoxin, and fumonisin (Jelinek et al., 1989). Aflatoxin B_1, a metabolite of fungus *Aspergillus flavus* and *Aspergillus parasiticus*, is an extremely hepatotoxic compound that frequently contaminates poultry feeds at low levels (Rizzi et al., 1998). The United States Food and Drug Administration (FDA) has set regulatory levels for poultry feeds for 20 ppb AF (Julie Zimmerman, 2002). Another family of mycotoxins produced by *Penicillium* and *Aspergillus* genera is ochratoxins. OA, being the most potent toxin, adversely affects production parameters and the health of poultry (Gentles et al., 1999). Ingestion of ochratoxin causes severe kidney damage. The new European Union Legislation has fixed the maximum limits for OA in foods and feeds at 5 μg/kg (5 ppb) (European Union Legislation, 2002). T-2 toxin and zearalenone, important members of the trichothecene group of mycotoxins, are chiefly produced by *Fusarium tricinctum* and *Fusarium roseum*, respectively. T-2 toxin induces severe inflammatory reactions and neural disturbances in animals and humans (Ueno, 1983), whereas zearalenone appears to have no effect on poultry health and performance (Allen et al., 1981). The FDA has yet to establish advisory levels for T-2 toxin and zearalenone in poultry feeds. The toxicity and clinical signs observed in animals when more than one mycotoxin is present in feed are complex and diverse. Co-contamination of animal feedstuffs by AF and OA (Huff and Doerr, 1981), AF and zearalenone (Ravindran et al., 1996), T-2 with OA (Chandrasekaran, 1996), and T-2 toxin with other

Received for publication on February 4, 2002.

Accepted for publication November 21, 2002.

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Abbreviation Key: AF = aflatoxin; ALT = alanine amino transferase; AST = aspartate amino transferase; BUN = urea nitrogen; EG-M = esterified glucomannan; FDA = Food and Drug Administration; GGT = γ-glutamyl transferase; Hb = hemoglobin; OA = ochratoxin A; TEC = total erythrocyte count; TLC = thin-layer chromatography.

fusarium metabolites (Bata et al. 1983) has been reported in field conditions.

Practical methods to detoxify mycotoxin contaminated grain on a large scale and in a cost-effective manner are not currently available. At present, one of the more promising and practical approaches is the use of adsorbents. However, several adsorbents have been shown to impair nutrient utilization (Kubena et al., 1993) and mineral absorption (Chestnut et al., 1992) and lack binding effects against multiple mycotoxins of practical importance (Edrington et al., 1997).

The advent of biotechnology in the last decade has opened a new avenue for addressing problems of mycotoxicosis. A live yeast, *Saccaharomyces cereviciae*, was found to alleviate the adverse effects of aflatoxicosis in poultry (Stanley et al., 1993). These beneficial effects have been later attributed to esterified glucomannan (E-GM),[3] derived from cell wall of *Saccharomyces cerevisiae*[1026]. E-GM has shown considerable binding ability with several commonly occurring mycotoxins (Devegowda et al., 1998) and is also found more effective as a low-inclusion binder to bind AF present in contaminated poultry feed when compared with hydrated sodium calcium aluminum silicate (Mahesh and Devegowda, 1996). E-GM at 0.1% in feed beneficial in counteracting the adverse effects of dietary T-2 toxin in laying hens (Manoj and Devegowda, 2000). However, the ability of E-GM to alleviate the adverse effects of the several combinations of mycotoxins present naturally in feed on productivity and serum biochemical and hematological parameters remains yet to be explored. Thus, the objective of the present study was to determine the efficacy of E-GM at 0.05% to ameliorate the toxic effects of mycotoxins in naturally contaminated feed in broiler chickens.

MATERIALS AND METHODS

Mycotoxin Quantification and Diet Preparation

Individual feed ingredients and the finished experimental diets were analyzed and screened for mycotoxin content by thin-layer chromatography (TLC), employing the method appropriate for each toxin. AF was extracted according to Romer (1975) and was quantified by TLC as outlined by AOAC (1995). OA and zearalenone were extracted and quantified by column chromatography and TLC, respectively (AOAC, 1995). T-2 toxin was extracted as per the method of Romer et al. (1978) and was quantified by TLC as suggested by Rukmini and Bhat (1978).

The basal control diet was formulated and compounded to meet the nutritional requirements of commercial broilers (Bureau of Indian Standards, 1992) during the starter period (0 to 3 wk: maize, 60.4 kg; soybean meal, 36 kg; mineral mix, 3.6 kg; salt, 0.3 kg with other feed additives to make up 22.6% crude protein; 2.58% fat, 3.97% crude fiber, and 2,857 kcal/kg ME) and finisher period (4 to 5 wk: maize, 66 kg; soybean meal, 27 kg; sunflower extract, 4 kg; mineral mix, 3 kg; salt, 0.3 kg with other feed additives to make up 19.89% crude protein; 2.76% crude fat; 4.64% crude fiber, and 2,898 kcal/kg ME).

Feed ingredients used in formulating the control diet did not contain mycotoxins at detectable levels. The naturally contaminated maize was obtained from a private feed mill, which was discarded completely due to severe mold growth; the presence of mycotoxins in the maize was confirmed by TLC. The contaminated diet treatments were formulated by replacing mycotoxin-free maize with naturally contaminated maize. Upon analysis, the contaminated diet contained the following mycotoxins, 168 ppb of AF, 8.4 ppb of OA, 54 ppb of zearalenone, and 32 ppb of T-2 toxin.

Experimental Design, Birds, and Data Collection

One day-old broiler chicks were individually weighed, wing-banded, and randomly distributed to the different treatment groups (five replicates of 14 chicks per dietary treatment). Chicks were grouped based on the following dietary treatments: 1) basal feed free of toxin (control), 2) basal feed containing 0.05% E-GM, 3) diet naturally contaminated with mycotoxins, and 4) naturally contaminated diet supplemented with 0.05% E-GM. The chickens were reared under uniform management conditions with feed and water available ad libitum.

Chicks were weighed individually at the end of the week, and feed consumption was recorded weekly. On d 21 and 35 of age, 10 birds (five males and five females) in each treatment were humanely euthanized. Liver, kidney, and gizzard were collected, weighed, and calculated as a percentage of body weight. Blood was collected in nonheparinized tubes by brachial vein puncture. Serum was separated and stored at −20°C. The serum samples were analyzed for total protein, cholesterol, urea nitrogen (BUN), and the activities of γ-glutamyl transferase (GGT), alanine amino transferase (ALT), and aspartate amino transferase (AST) using automatic analyzer[4] according to the recommendation of the manufacturer. Total erythrocyte count (TEC), hemoglobin (Hb), and hematocrit were measured using auto analyzer.[5]

All experimental data were subjected to one-way ANOVA according to Snedecor and Cochran (1968). Least square means were compared by Duncan's multiple-range test. All statements of differences were based on significance at $P \leq 0.05$.

[3]A proprietary product of M/s Alltech Inc., Nicholasville, KY.
[4]Boehringer Mannheim, Hitachi, Japan.
[5]Syfmex K, Hitachi, Japan.

TABLE 1. Effect of esterified glucomannan (E-GM) on body weight, feed intake, and feed conversion ratio (FCR) of broilers fed a naturally contaminated diet (35 d)

Naturally contaminated diet	E-GM (%)	Body weight (g)	Feed intake (g)	FCR
–	–	1,391.2[b]	3,017.6[b]	2.17[b]
–	0.05	1,441.4[c]	2,994.0[b]	2.07[a]
+	–	1,258.8[a]	2,803.4[a]	2.22[c]
+	0.05	1,381.0[b]	2,952.6[b]	2.15[b]
SEM		7.25	20.01	0.015

[a–c]Means within a column with common superscripts do not differ significantly ($P \leq 0.05$).

RESULTS

The effects of dietary treatments on chick performance from d 1 to 35 are presented in Table 1. Consumption of contaminated feed resulted in significant reduction in BW gain (9.52%) as compared to the control diet. However, contaminated diet supplemented with E-GM significantly countered (8.84%) the growth-depressing effects of the contaminated diet. Feeding the contaminated diet also resulted in significant reduction in feed intake (7.11%) and poorer feed efficiency (2.3%). Further, supplementation of E-GM to the contaminated diet effectively improved feed intake (5.06%) and feed efficiency (3.25%). Chickens fed control diet with E-GM performed significantly better (3.48%) than those on the basal diet alone.

The responses of the relative weights of liver, kidney and gizzard, and serum protein and cholesterol concentrations to the different dietary treatments are summarized in Table 2. Consumption of the contaminated diet increased the relative weights of liver (24.9%) and gizzard (12.0%) compared to the control diet, whereas relative weight of kidney, total protein concentration, and cholesterol concentration remained unaltered. E-GM supplementation to the contaminated diet did not significantly diminish the effects of toxins on the relative weights of liver and gizzard.

The effects of different dietary treatments on serum enzyme activities and urea nitrogen are shown in Table 3. When compared with controls, feeding the naturally contaminated diet resulted in a significant increase in serum GGT activity (8.06%) and decreases in serum AST (11.47%) and ALT (20.8%) activities at 21 d of age; however, increased GGT activity (9.63%) was observed when compared to controls at 35 d of age. Supplementation of E-GM to the contaminated diet did not significantly improve the serum enzyme activities. Feeding a diet that was naturally contaminated significantly decreased urea nitrogen at 21 d (4.68%) and 35 d (24.25%) of age. E-GM supplementation to the contaminated diet significantly raised the urea nitrogen concentration (14.37%) at 35 d of age.

Data presented in Table 4 indicate the effects of dietary treatments on hematological values at 21 and 35 d of age. Consumption of contaminated feed significantly decreased hematocrit values (2.7%), whereas TEC and Hb values were unaltered. E-GM supplementation to the contaminated diet improved the hematocrit values at 21 d of age (3.7%), but no significant improvement was observed at 35 d of age.

DISCUSSION

The current study is first of its kind conducted to determine the effect of feeding diet naturally contaminated with mycotoxins on commercial broilers in India. Mycotoxins are a cause of concern in the poultry industry due to the potential for major health problems and economic losses as have been well documented (Council for Agricultural Science and Technology, 1989). These mycotoxins exist in feed ingredients long after the death of the mold, and there is a great possibility for feed ingredients to contain more than one mycotoxin by the time it is given to birds. Further, broilers are fed with compounded diets that are combination of several feed ingredients grown in different agroclimatic conditions. Thus, the use of multiple feed ingredients, contaminated with individual mycotoxins, when combined, may lead

TABLE 2. Effect of esterified glucomannan (E-GM) on organ weights (35 d of age), serum protein, and cholesterol content (at 21 and 35 d of age) of broilers fed a naturally contaminated diet

Naaturally contaminated diet	E-GM (%)	Organ weights (g/kg live weight)			Total proteins (g/100 mL)		Cholesterol (mg/dL)	
		Liver	Kidney	Gizzard	21 d	35 d	21 d	35 d
–	–	2.53[a]	0.54[a]	1.99[a]	2.45[a]	2.62[a]	112.2[b]	120.5[a]
–	+	2.63[a]	0.68[b]	1.95[a]	2.68[b]	2.76[b]	129.3[c]	129.7[b]
+	–	3.16[b]	0.55[a]	2.23[b]	2.42[a]	2.60[a]	102.2[a]	115.3[a]
+	+	3.02[b]	0.50[a]	2.14[b]	2.56[ab]	2.82[b]	116.2[b]	119.4[a]
SEM		0.004	0.004	0.005	0.03	0.01	1.39	1.89

[a–c]Means within a column with common superscripts do not differ significantly ($P \leq 0.05$).

TABLE 3. Effect of esterified glucomannan (E-GM) on serum enzyme activities (IU/L) and urea nitrogen (mg/dL) of broilers fed a naturally contaminated diet

Naturally contaminated diet	E-GM (%)	GGT[1] (IU/L)		AST[2] (IU/L)		ALT[3] (IU/L)		Urea nitrogen (mg/dL)	
		21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d
–	–	6.2^{a}	8.30^{a}	237^{bc}	235^{bc}	48^{b}	46^{a}	1.92^{b}	2.02^{c}
–	0.05	6.0^{a}	8.32^{ab}	237^{bc}	226^{ab}	42^{a}	48^{a}	2.14^{c}	2.13^{d}
+	–	6.7^{b}	9.1^{c}	210^{a}	236^{c}	38^{a}	49^{a}	1.83^{a}	1.53^{a}
+	0.05	6.1^{a}	8.8^{bc}	241^{c}	220^{a}	40^{a}	47^{a}	1.87^{a}	1.75^{b}
SEM		0.09	0.11	2.04	2.13	1.33	0.98	0.01	0.01

$^{a–d}$Means within a column with common superscripts do not differ significantly ($P \leq 0.05$).

to co-occurrence of all the mycotoxins present in the individual ingredients. Therefore, poultry producers are in need of methods to assist them in their protecting flocks from these toxic metabolites. Certain dietary additives have been developed that are purported to reduce the potential hazard to the chickens if the toxins do appear in the feed. (Harvey et al., 1993).

Addition of E-GM at 0.05% to basal diet resulted in improved performance. This observation was consistent with previous reports on the performance enhancing properties of mannanoligosaccharide in broilers (Kumprecht et al., 1997). Consumption of contaminated feed resulted in significant depression in the performance of broilers. This result may be due to the synergistic effect on growth rate from the combination of toxins presented in contaminated feed, as reported in previous studies (Huff et al., 1984). Addition of E-GM to contaminated feed effectively improved the performance of broilers. This effect of E-GM might be attributed to mycotoxin adsorption (Devegowda, 1997), ability to block colonization of pathogens in the gastrointestinal tract (Olsen, 1995), and its inhibitory effect on liver antioxidant depletion (Dvorska and Surai, 2001).

Although the precise mode of action of E-GM is not known, it is hypothesized that E-GM might trap the mycotoxin molecule in its glucomannan matrix and prevent toxin absorption from the gastrointestinal tract (Raju and Devegowda, 2000). Increases in the relative weights of liver and gizzard in birds fed contaminated feed as observed in the present study has been reported earlier with AF (Kubena et al., 1997). This result may be due to the hepatotoxic effect of AF, resulting in appreciable changes in the functioning and gross appearance of liver (Tung et al., 1973). The relative increase in gizzard weight is in accordance with earlier studies (Kubena et al., 1990), which may be due to the result of severe inflammation and thickening of the mucosal layer.

The increased serum GGT activity observed by feeding naturally contaminated diets in the present study could be due to hepatic degeneration and subsequent leakage of enzymes into circulation. Similar increases in the activities of GGT as observed in the present trial have been reported during aflatoxicosis in broiler breeder hens (Afzali and Devegowda, 1999). AST and ALT levels remained unaltered when compared to the control, although decreased activity occurred at an earlier age.

The BUN concentration was significantly lower in the birds fed naturally contaminated feed. Chicks synthesize a considerable amount of urea up to first 8 wk of life and later on shift to production of uric acid in place of urea. This shift may be explained by the presence of residual embryonic hepatic arginase, the level of which decreases as birds grow (Bell and Freeman, 1971). Therefore, the present depletion in BUN levels indicated the altered functional status of liver. Supplementatin of E-GM to the contaminated diet resulted in improved BUN concentration and decrease in the GGT activity. At 21 and 35 d of age, hematocrit values were significantly lower in birds fed the contaminated diet. Tung et al. (1975) suggested that the mechanism of reduction in hematocrit values during aflatoxicosis is related to destruction of red blood cells. However, the only apparent reduction was observed in TEC and Hb concentrations in birds fed contaminated feed. E-GM supplementation

TABLE 4. Effect of esterified glucomannan (E-GM) on hematological parameters in broilers fed naturally contaminated diet

Naturally contaminated diet	E-GM (%)	Erythrocyte count ($\times 10^6$/mm^3)		Hemoglobin (g %)		Hematocrit (%)	
		21 d	35 d	21 d	35 d	21 d	35 d
–	–	2.31	2.34	9.7	10.03	33.3^{b}	36^{b}
–	0.05	2.31	2.30	9.83	10.18	35.4^{c}	37.5^{b}
+	–	2.32	2.31	9.75	9.79	32.4^{a}	34.6^{a}
+	0.05	2.35	2.33	9.73	9.88	33.6^{b}	34.94
SEM		0.01	0.01	0.04	0.14	0.17	0.23

$^{a–c}$Means within a column with common superscripts do not differ significantly ($P \leq 0.05$).

to the contaminated diet did not significantly improve the hematocrit values, whereas H.V.L.N. Swamy (2001, University of Guelph, Ontario, Canada, personal communication) observed improvement in blood parameters with E-GM supplementation in broilers fed grains naturally contaminated with fusarium mycotoxins.

The results obtained in our study indicate that toxins present in the contaminated feed significantly depressed performance, organ morphology, and most of the serum biochemical parameters. The E-GM added to a naturally contaminated diet, as an adsorbent, increased performance and serum levels of BUN and decreased the activity of GGT. These results suggest that E-GM at 0.05% might be sufficient to counteract the adverse effects of mycotoxins.

ACKNOWLEDGMENTS

The authors thank Alltech Inc., USA and Venkateswara Hatcheries Private Limited, India, for providing E-GM (Mycosorb) and financial support, respectively, for this experiment.

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