Effect of dietary esterified glucomannan on performance, serum biochemistry and haematology in broilers exposed to aflatoxin

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ABSTRACT: The amelioration of aflatoxicosis in broiler chickens was examined by feeding two concentrations of yeast component (esterified glucomannan; EG). EG, incorporated into the diet at 0.5 and 1 g/kg, was evaluated for its ability to reduce the detrimental effects of 2 mg total aflatoxin (AF; 82.72% AFB_1, 5.50% AFB_2, 10.20% AFG_1 and 1.58% AFG_2) in diet on growing broiler chicks from 1 to 21 d of age. A total of 240 male broiler chicks (Ross-308) were divided into 6 treatment groups [control, AF, EG (0.5 g/kg), AF plus EG (0.5 g/kg), EG (1 g/kg), and AF plus EG (1 g/kg)]. Compared to the control, AF treatment significantly decreased body weight gain from week 2 onwards. AF treatment also caused significant decreases in serum total protein, albumin, total cholesterol, triglyceride, glucose, inorganic phosphorus, creatinine levels and alanine-aminotransferase (ALAT) activity but increased the aspartate-aminotransferase (ASAT) activity. Red blood cell, haematocrit, haemoglobin, thrombocyte, and lymphocyte counts and tibial crude ash levels were significantly reduced by AF treatment, while significant increases were seen in heterophil counts. The addition of EG (1 g/kg) to an AF-containing diet significantly improved the adverse effects of AF on haematological parameters, total protein, albumin values and ASAT activity. EG (1 g/kg) also partially improved body weight gains (59%) and the other biochemical parameters influenced by AF treatment. The addition of EG (both 0.5 and 1 g/kg) to the AF-free diet did not cause any considerable changes in the investigated values. These results clearly indicated that EG (1 g/kg) addition effectively diminished the adverse effects of AF on the investigated values. Also, the higher dietary concentration of EG (1 g/kg) was found more effective than the lower concentration (0.5 g/kg) against the adverse effects of AF on the variables investigated in this study.

Keywords: aflatoxin; esterified glucomannan; broiler; prevention

Aflatoxins (AF), potent mycotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, are a major concern in poultry production. AF contamination causes reduced feed quality and reduced animal efficiency either through poor conversion of nutrients or problems such as reproductive abnormalities (Oguz and Kurtoglu, 2000; Ortatatli *et al.*, 2002). Aflatoxicosis in poultry also causes listlessness, anorexia with lowered growth rate, poor feed utilization, decreased egg production and increased mortality (Miazzo *et al.*, 2000). Additionally, anaemia (Oguz *et al.*, 2000), reduction of immune function (Oguz *et al.*, 2003), hepatotoxicosis, haemorrhage (Ortatatli and Oguz, 2001), teratogenesis (Sur and Celik, 2003), carcinogenesis and mutagenesis are associated with aflatoxicosis.

Significant changes in serum biochemical and haematological parameters are seen in aflatoxicosis cases and these can assist in the diagnosis of toxication (Huff *et al.*, 1986). AF toxicity in broilers may be manifested by decreased serum concentrations of total protein, albumin, total cholesterol (Rosa

et al., 2001), uric acid (Oguz *et al*., 2000), inorganic phosphorus and calcium (Harvey *et al.,* 1993). Broiler chicks given 2.5 to 3.5 AF/kg diet showed not only decreased amount of haemoglobin, haematocrit and thrombocyte values, percentage of lymphocyte and monocyte counts (Kececi *et al.,* 1998) and bone ash values (Scheideler, 1993) but also increased percentage of heterophils (Oguz *et al.,* 2000).

Prevention of feed and feedstuffs from possible mould growth and AF contamination is very important. When contamination cannot be prevented, decontamination of AF is needed before using these materials. Practical and cost-effective methods for detoxification of AF-containing feed and feedstuffs are in great demand. At the beginning of the 1990s, the adsorbent-based studies were performed for removing AF from contaminated feed and minimizing the toxicosis of AF in poultry (Huwig *et al*., 2001; Peraica *et al*., 2002). However, some researchers are concerned about the possible disadvantages of adsorbents such as required high inclusion rates and negative interactions with feed nutrients (Parlat *et al*., 1999; Miazzo *et al.,* 2000; Rosa *et al*., 2001).

Previous studies suggested that the best approach to decontamination should be degradation by biological materials giving a possibility of AF removal under moderate conditions, without using harmful chemicals and without significant losses of the nutritive value and palatability of detoxified feed and feedstuffs (Bata and Lasztity, 1999). Recent biotechnological progress has opened new approaches to tackle this problem. Live yeast *(Saccharomyces cerevisiae;* SCE), initially used as a performance promoter in the early 1990s, was found to have beneficial effects on weight gain and immune response in broilers exposed to AF (Stanley *et al*., 1993). Recent studies made with SCE also showed significant improvements in aflatoxicosis cases in quail chicks (Parlat *et al*., 2001; Yildirim and Parlat, 2003), while no beneficial effects were reported in aflatoxicosis in broilers (Marin *et al*., 2003).

The beneficial effects of SCE have been attributed to mannan in the cell wall of SCE. Mannan was then extracted and esterified with glucan. Esterified glucomannan (EG) showed considerably high binding ability (80–97%) with AF (Mahesh and Devegowda, 1996; Diaz *et al.*, 2002), and it has been preferred for detoxification of AF in poultry species. The studies performed with EG (0.5 and 1 g/kg) at different concentrations of AF (0.05 to 5 mg/kg) in broilers (Raju and Devegowda, 2000; Aravind *et al*., 2003; Santin *et al.,* 2003) showed that EG partially and/or completely reversed the adverse effect of AF on performance, biochemistry-haematology and immune responses of birds. EG was also used for detoxification of other mycotoxins such as zearalenone (Swamy *et al*., 2003) and aurofusarin (Dvorska and Surai, 2001).

The main objective of this study was to develop a practical method for AF detoxification. Therefore, the toxic effects of AF (2 mg/kg) on growth performance, serum biochemistry, haematology and bone parameters of broiler chicks were examined and the preventive effects of two different dietary concentrations of EG (0.5 and 1 g/kg) were evaluated and compared.

MATERIAL AND METHODS

Chickens and diet

Two hundred forty 1-d-old male unvaccinated broiler chicks (Ross-308) were obtained from a commercial hatchery. Individually weighed chicks were divided at random into 6 groups. There were 4 replications of 10 broiler chicks for each dietary treatment, totalling 240 chicks. The chicks were housed in floor pen. Basal diet was prepared and formulated to contain National Research Council (1994) requirements of all nutrients, without antibiotics, coccidiostats, or growth promoters (maize and soybean meal diet providing 231.5 g protein, 12.97 MJ ME/kg; Table 1). To maintain accurate and safe control the diets containing the various treatments were placed in plastic feed containers with lids in the growing house. Feed and water were always available and lighting was continuous. The basal diet was also tested for possible residual AF before feeding (Howel and Taylor, 1981) and there were no detectable levels present (detection limit 1 μg/kg feed, recovery of the extraction method 95%).

Experimental design

The experimental design consisted of 6 dietary treatments. (1) Control: basal diet; (2) AF: basal diet plus 2 mg AF (total aflatoxin; the composition given below)/kg diet; (3) EG (0.5 g/kg): basal diet plus 0.5 g EG (esterified glucomannan)/kg diet;

Table 1. Composition of the experimental diet fed to broilers[1]

Ingredients	g/kg
Yellow maize	520.00
Soybean meal	200.00
Full fat soybean	200.00
Fish meal	50.00
Vegetable (sunflower) oil	5.00
Limestone flour	9.50
Dicalcium phosphate	7.00
Salt	3.00
Vitamin premix[2]	2.50
Mineral premix[3]	1.00
Methionine	2.00
Calculated analysis	
Crude protein	231.50
Metabolisable energy (MJ/kg)	12.97
Calcium	9.90
Available phosphorus	4.40
Lysine	13.70
Methionine	6.20
Composition, analysed	
Crude matter	900.50
Crude protein	230.10
Crude ash	53.00
Crude fibre	31.60
Ether extract	65.70
Starch	358.00
Sugar	30.00
Calcium	10.30
Total phosphorus	6.50
Metabolisable energy* (MJ/kg)	13.01

*value calculated

[1]batches of diet were prepared weekly and stored (in a plastic container) in a dry and cool place

[2]provides per kg of diet: vitamin A 10 000 IU; vitamin D_3 1 000 mg; vitamin E 25 mg; vitamin K_3 3 mg; vitamin B_1 2 mg; vitamin B_2 6 mg; vitamin B_6 4 mg; vitamin B_{12} 0.015 mg; niacin 20 mg; calcium-D-pantothenate 8 mg; folic acid 0.8 mg; choline chloride 300 mg

[3]provides per kg of diet: Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.15 mg

(4) AF + EG (0.5 g/kg): basal diet plus 2 mg AF plus 0.5 g EG/kg diet; (5) EG (1 g/kg): basal diet plus 1 g EG; (6) AF + EG (1 g/kg): basal diet plus 2 mg AF plus 1 g EG/kg diet. EG (Mycosorb^{TM}) was provided from Alltech, K.Y., USA.

Aflatoxin

The aflatoxin (AF) was produced from *Aspergillus parasiticus* NRRL 2 999 culture (USDA, Agricultural Research Service, Peoria, IL) via fermentation of rice by the method of Shotwell *et al*. (1966) with minor modifications by Oguz (1997). Successfully fermented rice was then steamed to kill the fungus, dried and ground to a fine powder. The AF content in rice powder was analysed by the method of Shotwell *et al.* (1966) and measured on Thin Layer Chromatography (TLC)-fluorometric densitometer (Camag-II, Basel, Switzerland) on a TLC spots (Plates from Merck, other equipment from Desega). The AF in the rice powder consisted of 82.72% AFB_1, 5.50% AFB_2, 10.20% AFG_1 and 1.58% AFG_2 based on total AF in the ground rice powder (detection limit 1 μg AF/kg rice powder, recovery of the extraction method 92%). The rice powder was incorporated into the basal diet to provide the required amount of 2 mg AF/kg feed.

Performance parameters

During the experiment the body weights of chickens were assessed at 7, 14 and 21 days of age and mortality was recorded as it occurred. Feed was weighed on the same days above to evaluate the feed consumption (FC) and feed conversion ratio (FCR).

Feed analysis

The standard technigues of the Proximate analysis were used to determine the nutrient concentrations in the experimental diet (Nauman and Bassler, 1993). The experimental diet was analysed also for starch, sugar, calcium and total phosphorus according to VDLUFA method (Naumann and Bassler, 1993). Metabolisable energy content of the diet was calculated based on chemical composition (Anonymous, 1991).

Serum biochemical and haematological analysis

When the chicks reached 3 weeks of age, the feeding trial was terminated and 10 broilers from each treatment were selected at random and bled

by cardiac puncture. Serum concentrations of total protein, albumin, total cholesterol, triglycerides, urea nitrogen, glucose, calcium, inorganic phosphorus and activity of creatinine, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined on a clinical chemistry auto analyser (Tokyo Boeki, TMS, 1024, Japan) with commercial test kits (Spinreact, Spain). The red blood cell (RBC), white blood cell (WBC) and thrombocyte counts were determined by a haemocytometer method using Natt-Herrick solution; haematocrit values were measured by microhaematocrit method. Haemoglobin (HBG) amounts were determined by Sahli's haemometer; the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated; differential leukocyte counts were determined as described by Yilmaz (2000).

Tibia samples from each broiler (10 broilers/each treatment) were excised to determine tibial crude ash at 3 weeks of age. Tibial crude ash was determined after heating in a muffle furnace at 550°C for 16 hours.

Statistical analysis

When the chicks reached 21 d of age, the feeding trial was terminated. The data on FC, body weight gain (BWG), FCR, serum biochemical, haematological and bone parameters were grouped and expressed as mean ± pooled standard errors of means. The obtained results were statistically analysed using Duncan's multiple range test (SPSS, 1999). Differences were considered to be significant based on the 5% level of probability.

RESULTS

Data presented in Tables 2 to 6 show the effects of dietary treatments on performance, serum biochemical, haematological and bone parameters. Feeding AF alone caused significant decreases in BWG (7.2% to 14.3%) from week 2 onwards compared with the control (Table 2). The decreasing effect of AF on FC was not statistically different, but it was about 8% to 11% during the experiment. The addition of EG (1 g/kg diet) to an AF containing diet partially improved the adverse effect of AF on BWG and FC. However, the ameliorating effect of EG (0.5 g/kg) on BWG was not as great as EG (1 g/kg). Mortality was not statistically significant between the groups.

Feeding AF alone also caused significant decreases in serum total protein, albumin, total cholesterol, triglyceride, glucose, inorganic phosphorus and creatinine values and ALAT activity, while a significant increase was found in ASAT activity (Tables 3 and 4). AF also caused significant changes in haematological and some bone parameters. Compared to control, RBC, haematocrit, haemoglobin, thrombocyte, lymphocyte counts and tibial

Table 2. Effect of esterified glucomannan (EG) on body weight gain in broiler chicks on diet containing 2 mg total aflatoxin (AF)/kg diet at 1 to 21 days of age*

Treatment			Body weight gain (g)				Change from control (%)
AF	EG addition		days of age				
	0.5 g/kg	1 g/kg	1–7	8–14	15–21	1–21	
–	–	–	86.01 ± 5.41^{ab}	195.27 ± 6.90	308.65 ± 6.01^{ab}	589.93 ± 16.0^{a}	0
+	–	–	74.85 ± 4.29^{b}	181.28 ± 2.97	264.75 ± 8.29^{c}	520.86 ± 15.01^{b}	–11.71
–	+	–	86.79 ± 4.48^{ab}	200.08 ± 9.77	288.32 ± 14.24^{bc}	575.19 ± 26.86^{ab}	–2.50
+	+	–	82.88 ± 2.59^{ab}	185.73 ± 1.79	268.88 ± 8.07^{c}	535.23 ± 8.40^{ab}	–9.27
–	–	+	90.43 ± 6.14^{a}	195.21 ± 6.32	329.39 ± 12.23^{a}	590.02 ± 28.26^{a}	+0.01
+	–	+	92.65 ± 3.35^{a}	193.30 ± 5.41	275.39 ± 12.39^{c}	561.58 ± 17.18^{ab}	–4.80

$^{a–c}$values in columns with no common superscripts are significantly different ($P < 0.05$) according to multiple range tests;
*values represent the $\bar{x}$ ± SEM of six groups of 40 broiler chicks (four replications of 10 broilers each) per treatment

Table 3. Effect of esterified glucomannan (EG) on serum biochemical values in broiler chicks on diet containing 2 mg total aflatoxin (AF)/kg diet at 1 to 21 days of age*

Treatment			Total protein (g/100 ml)	Albumin (g/100 ml)	Total cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	Urea nitrogen (mg/100 ml)	Glucose (mg/100 ml)
AF	EG addition							
	0.5 g/kg	1 g/kg						
–	–	–	2.92 ± 0.12^{a}	1.25 ± 0.05^{a}	143.30 ± 5.93^{a}	119.30 ± 5.17^{a}	2.50 ± 0.40^{a}	141.20 ± 10.71^{a}
+	–	–	1.72 ± 0.14^{d}	0.74 ± 0.06^{d}	112.20 ± 4.27^{cd}	91.10 ± 4.96^{b}	2.10 ± 0.31^{a}	110.10 ± 4.95^{c}
–	+	–	2.63 ± 0.08^{ab}	1.22 ± 0.06^{a}	137.10 ± 3.16^{ab}	116.30 ± 3.77^{a}	2.30 ± 0.30^{a}	138.30 ± 5.15^{ab}
+	+	–	1.81 ± 0.09^{d}	0.84 ± 0.06^{cd}	106.10 ± 5.70^{d}	93.00 ± 6.24^{b}	2.20 ± 0.33^{a}	119.60 ± 5.14^{bc}
–	–	+	2.52 ± 0.13^{bc}	1.09 ± 0.07^{ab}	134.20 ± 5.69^{ab}	112.30 ± 4.40^{a}	2.30 ± 0.30^{a}	139.20 ± 4.24^{ab}
+	–	+	2.21 ± 0.16^{c}	0.96 ± 0.08^{bc}	126.10 ± 5.47^{bc}	105.20 ± 5.56^{ab}	2.50 ± 0.43^{a}	124.30 ± 6.44^{abc}

$^{a–d}$values in columns with no common superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range tests; *values represent the $\bar{x}$ ± SEM of six groups of 10 broiler chicks each per treatment

Table 4. Effect of esterified glucomannan (EG) on serum Ca, P and creatinine values and enzyme activities in broiler chicks on diet containing 2 mg total aflatoxin (AF)/kg diet at 1 to 21 days of age*

Treatment			Calcium (mg/100 ml)	Inorganic phosphorus (mg/100 ml)	Creatinine (mg/100 ml)	ASAT (IU/l)	ALAT (IU/l)
AF	EG addition						
	0.5 g/kg	1 g/kg					
–	–	–	8.53 ± 0.42^{ab}	6.82 ± 0.34^{a}	0.32 ± 0.04^{a}	169.20 ± 8.37^{b}	7.80 ± 0.73^{ab}
+	–	–	7.90 ± 0.17^{b}	5.60 ± 0.24^{b}	0.23 ± 0.03^{b}	217.20 ± 9.59^{a}	5.30 ± 0.52^{c}
–	+	–	8.62 ± 0.17^{ab}	6.75 ± 0.25^{a}	0.18 ± 0.02^{b}	184.30 ± 5.68^{b}	7.50 ± 0.54^{ab}
+	+	–	8.11 ± 0.19^{ab}	5.95 ± 0.16^{b}	0.20 ± 0.02^{b}	191.10 ± 9.02^{b}	5.70 ± 0.40^{c}
–	–	+	8.87 ± 0.28^{a}	6.84 ± 0.28^{a}	0.25 ± 0.03^{ab}	173.10 ± 4.14^{b}	8.30 ± 0.47^{a}
+	–	+	8.29 ± 0.18^{ab}	6.13 ± 0.24^{ab}	0.17 ± 0.03^{b}	187.70 ± 7.71 ^{b}	6.50 ± 0.65^{bc}

$^{a–c}$values in columns with no common superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range tests; *values represent the $\bar{x}$ ± SEM of six groups of 10 broiler chicks each per treatment

Table 5. Effect of esterified glucomannan (EG) on some haematological parameters in broiler chicks on diet containing 2 mg total aflatoxin (AF)/kg diet at 1 to 21 days of age

Treatment			RBC[1] (10^6 mm^3)	Haematocrit (%)	MCV[2] (μm^3)	Haemoglobin (g 100/ml)	MCH[3] (pg)	MCHC[4] (%)
AF	EG addition							
	0.5 g/kg	1 g/kg						
–	–	–	2.17 ± 0.11^{ab}	33.60 ± 1.67^{a}	123.99 ± 3.15^{a}	8.81 ± 0.36^{a}	32.66 ± 0.95^{ab}	26.41 ± 0.75^{ab}
+	–	–	1.94 ± 0.13^{c}	23.40 ± 1.50^{c}	120.88 ± 1.67^{a}	6.47 ± 0.42^{b}	33.48 ± 0.68^{a}	27.71 ± 0.49^{a}
–	+	–	2.63 ± 0.20^{ab}	32.40 ± 2.34^{ab}	123.63 ± 1.64^{a}	8.69 ± 0.58^{a}	33.31 ± 0.61^{ab}	26.95 ± 0.39^{ab}
+	+	–	2.29 ± 0.12^{bc}	28.20 ± 1.19^{bc}	123.99 ± 2.68^{a}	7.56 ± 0.34^{ab}	33.15 ± 0.58^{ab}	26.81 ± 0.55^{ab}
–	–	+	2.78 ± 0.13^{a}	34.20 ± 1.30^{a}	123.52 ± 1.84^{a}	8.83 ± 0.29^{a}	31.94 ± 0.49^{ab}	25.88 ± 0.34^{b}
+	–	+	2.51 ± 0.17^{ab}	30.90 ± 2.11^{ab}	123.51 ± 3.26^{a}	7.84 ± 0.51^{a}	31.37 ± 0.49^{b}	25.46 ± 0.57^{b}

$^{a–c}$values in columns with no common superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range tests; *values represent the $\bar{x}$ ± SEM of six groups of 10 broiler chicks each per treatment; [1]red blood cell; [2]mean corpuscular volume; [3]mean corpuscular haemoglobin; [4]mean corpuscular haemoglobin concentration

Table 6. Effect of esterified glucomannan (EG) on thrombocyte, heterophil, lymphocyte and tibial ash levels in broiler chicks on diet containing 2 mg total aflatoxin (AF)/kg diet at 1 to 21 days of age*

Treatment			Thrombocyte ($\times 10^3$/mm^3)	Heterophil (%)	Lymphocyte (%)	Tibial crude ash (%)
AF	EG addition					
	0.5 g/kg	1 g/kg				
–	–	–	34.20 ± 2.52^{a}	37.90 ± 0.86^{c}	56.10 ± 0.80^{a}	35.18 ± 1.15^{a}
+	–	–	24.70 ± 2.24^{b}	48.50 ± 1.78^{a}	46.40 ± 1.41^{c}	32.59 ± 0.70^{bc}
–	+	–	33.70 ± 2.48^{a}	39.10 ± 1.86^{c}	55.20 ± 2.04^{a}	34.63 ± 0.72^{ab}
+	+	–	29.70 ± 2.99^{ab}	45.90 ± 2.16^{ab}	48.50 ± 2.34^{bc}	32.09 ± 0.52^{c}
–	–	+	33.90 ± 3.57^{a}	38.40 ± 1.96^{c}	56.00 ± 1.79^{a}	32.28 ± 0.82^{bc}
+	–	+	30.30 ± 2.73^{ab}	41.70 ± 1.93^{bc}	52.90 ± 1.86^{ab}	33.21 ± 0.59^{abc}

$^{a–c}$values in columns with no common superscripts are significantly different ($P < 0.05$) according to Duncan's multiple range tests; *values represent the $\bar{x}$ ± SEM of six groups of 10 broiler chicks each per treatment

ash levels were decreased, but heterophil counts were significantly increased by AF (Tables 5 and 6). The addition of EG (1 g/kg) to an AF-containing diet significantly improved the adverse effects of AF on the haematological parameters investigated, total protein, albumin values and ASAT activity. Other biochemical parameters influenced by AF were moderately recovered by the addition of EG (1 g/kg). The dietary addition of EG (both 0.5 and 1 g/kg) to the AF-free diet did not produce any significant changes in the investigated values compared with the control, except the decline in total protein values in EG (1 g/kg)-alone and creatinine levels in EG (0.5 g/kg)-alone treatments (Tables 3 and 4). No significant differences were found between control and AF-treated group in the other biochemical and haematological parameters analysed in this study.

DISCUSSION

The most prevalent symptoms of aflatoxicosis in poultry and livestock are reduced growth rate and poor performance. The failure in BWG will lead to economic losses and also severe AF-dependent diseases in poultry flocks. In this study, chickens consuming 2 mg/kg AF-containing diet had significantly poor body weight gains ($P < 0.05$). This adverse effect of AF was progressively seen from week 1 onwards. The adverse effects of AF on FC and BWG were due to anorexia, listlessness, inhibition of protein synthesis and lipogenesis (Oguz and Kurtoglu, 2000; Oguz *et al.,* 2000). Impaired liver functions and protein/lipid utilization mechanisms may also have affected the growth performance and general health (Ortatatli and Oguz, 2001). The detrimental effects of AF on growth performance in this study agreed with the previous studies, for example Kubena *et al.* (1993) and Miazzo *et al.* (2000) reported that FC and BWG decreased by 10% to 20% in broiler chicks given AF (2.5 mg/kg) for 3 weeks ($P < 0.05$).

Chronic and sub-clinical aflatoxicosis cases may be diagnosed by determining changes in serum biochemical and haematological parameters before major symptoms become apparent (Kececi *et al.*, 1998). These parameters are sensitive indicators of toxic effects of AF on the target organs. The biochemical and haematological toxic effects of AF are a well-investigated and well-known subject. These toxic effects were also clearly observed in the present study. Liver and kidney are regarded to be target organs for AF. The decreased serum total protein, albumin, total cholesterol, triglyceride and glucose values and increased ASAT activity observed in the present study ($P < 0.05$; Tables 3 and 4) were due to the hepatotoxic effects of AF characterized by the inhibition of protein synthesis and impairment of carbohydrate and lipid metabolism (Rosa *et al.*, 2001). The decrease in serum inorganic phosphorus and creatinine values ($P < 0.05$) may be related to the nephrotoxic effects of AF in agreement with other studies (Glahn *et al.*, 1991; Harvey *et al.,* 1993).

Significant gross and histopathological lesions were also reported in the haemopoietic and immune system organs in aflatoxicosis cases (Ortatatli and Oguz, 2001). In the present study, significant

decreases were observed in RBC, haematocrit, haemoglobin, thrombocyte, lymphocyte counts ($P < 0.05$; Tables 5 and 6). These findings agreed with the other reports that explain the suppressive effects of AF on haematopoiesis and immune responses (Huff *et al.*, 1986; Oguz *et al.*, 2003). The increase in heterophil counts suggested that the toxin was eliciting inflammatory response of chicks (Kececi *et al.*, 1998). Bone ash levels of chicks consuming AF were found significantly lower compared to control as indicated by Scheideler, (1993), who used 2.5 mg/kg AF in broilers.

Feeds contaminated with AF pose a health risk to animals and, as a consequence, may cause high economic losses due to the lower efficiency of animal husbandry. Producers and scientists aim to develop an effective decontamination technology dealing with this feed-borne toxin. Decontamination procedures have focused on degrading, destroying, inactivating or removing AF by physical, chemical and biological methods. Recently, the researchers have directed towards the effective biological degradation process for AF. In this context, live yeast (SCE) was used to control the severity of AF and provided significant improvements (Stanley *et al.*, 1993; Parlat *et al.*, 2001). The beneficial effects of SCE have been attributed to mannan and the researches then extracted this complex sugar from the cell wall of SCE and modified (Aravind *et al.*, 2003).

As seen in Tables 3 to 6, the addition of EG (1 g per kg) to an AF-containing diet significantly ameliorated the adverse effects of AF on haematology and total protein, albumin values and ASAT activity ($P < 0.05$). The findings of the other biochemical parameters in AF plus EG (1 g/kg) treatment were intermediate between control and AF groups including BWG (Table 2). Similarly, the cumulative BWG was reduced by 11.71% among the chicks consuming 2 mg/kg AF-containing diet without EG, but by only 4.80% for the birds fed 2 mg/kg AF plus EG (1 g/kg) diet. Khajarern and Khajarern (1999) provided significant improvements in biochemical parameters by the addition of EG (1 g/kg) and the reported EG (1 g/kg) was more effective than EG (0.5 g/kg) on growth performance and biochemistry of broilers fed with diet containing AF (0.1 to 0.3 mg/kg) for 6 weeks. The significant improvements that were achieved by the inclusion of EG (1 g/kg) in this study agreed with the previous reports performed by high (2.5 to 5 mg/kg) and low (0.05 to 0.4 mg/kg) AF doses in broilers fed for 3 to 5 weeks (Stanley *et al.*, 1996; Rizzi *et al.*, 1998; Raju and Devegowda, 2000).

The beneficial counteraction of EG with AF molecules in the gastrointestinal tract was clearly seen in our study as predicted. The roles of EG in AF detoxification were attributed first to have selective binding capacity for AF molecules (Devegowda, 1997; Diaz *et al.*, 2002), second to modulate the immune response (Fernandez *et al.,* 2002; Shashidhara and Devegowda, 2003) and third to provide nutrients to beneficial gut flora and to improve animal production (Newman, 1994; Fritts and Waldroup, 2003; Santin *et al.*, 2003). It was hypothesized that EG might trap the AF molecule in its glucomannan matrix and prevent toxin absorption from the gastrointestinal tract (Raju and Devegowda, 2000). Single additions of EG to an AF-free diets did not produce any negative changes compared to control. This was also supported that EG was inert and non-toxic such as SCE in terms of performance and biochemical-haematological parameters.

In conclusion, the growth performances and serum biochemical-haematological values were significantly affected by AF (2 mg/kg) treatment; the addition of EG (1 g/kg) to the AF-containing diet significantly recovered the adverse effects of AF on performance, biochemical-haematological values of broilers; the protective effect of 1 g/kg EG was higher than that of 0.5 g/kg EG against the toxic effects of AF; and these improvements should contribute to a solution of AF problem in broiler chickens on the basis of biological detoxification.

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Received: 04–04–16
Accepted after corrections: 04–09–17

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