Esterified-Glucomannan in Broiler Chicken Diets-Contaminated with Aflatoxin, Ochratoxin and T-2 Toxin: Evaluation of its Binding Ability (*in vitro*) and Efficacy as Immunomodulator**

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ABSTRACT : *In vitro* binding efficacy of esterified glucomannan (E-GM) (0.1%) on aflatoxin B_1 (AF) (300 ppb), ochratoxin A (OA) (2 ppm) and T-2 toxin (T-2) (3 ppm), when present alone or in combination, was evaluated in toxin-contaminated feed at pH 4.5 and 6.5. Esterified glucomannan showed significantly ($p<0.01$) higher binding with AF (81.6%), whereas those recorded with T-2 (27.8%) and OA (25.6%) were moderate. Binding of each toxin decreased as the number of toxins in feed increased. pH of medium showed no effect on mycotoxin binding ability of E-GM. A 2×2×2×2 factorial experiment of 5 week duration was conducted to study the effects of two dietary levels each of AF (0 and 300 ppb), OA (0 and 2 ppm), T-2 (0 and 3 ppm) and E-GM (0 and 0.1%) on the immune competence of a total of 960 day-old commercial broilers. Reductions in size of thymus (by AF and T-2) and bursa (by AF) and antibody titers against Newcastle disease and Infectious Bursal disease (by all the toxins) were noted. Additive and antagonistic interactions were seen among the toxins on certain parameters. Esterified glucomannan significantly ($p<0.01$) improved antibody titers and weights of bursa of Fabricius and thymus indicating its counteracting efficacy against immunosuppression in mycotoxicosis of multiple origin. (*Asian-Aust. J. Anim. Sci. 2002. Vol 15, No. 7 : 1051-1056*)

Key Words : Esterified-Glucomannan, Aflatoxin, Ochratoxin, T-2 Toxin, Broilers

INTRODUCTION

Aflatoxin (AF), Ochratoxin A (OA) and T-2 toxin (T-2), the chief secondary metabolites of *Aspergillus flavus/Aspergillus parasiticus, Aspergillus ochraceus and Fusarium sporotrichoides,* respectively, are commonly encountered in animal feed stuffs. These mycotoxins when consumed in combination may show greater negative effects on well being and productivity of broiler chicken than when consumed alone (Raju and Devegowda, 2000). Many approaches have been tried to counteract mycotoxicosis in chicken including chemical, nutritional and biological methods. Though some of these have proved effective on some mycotoxins, the search is still on for a simple, cost effective and field applicable solution for the problem of mycotoxicosis in poultry.

Saccharomyces cerevisiae was found to have beneficial effects in poultry during mycotoxicosis (Stanley et al., 1993). These beneficial effects observed with the yeast were later attributed to the mannanoligosaccharide present in its cell wall. Mannan, extracted from the cell wall of *Saccharomyces cerevisiae*[1026] and esterified with glucan, appears to have considerable binding ability over several commonly occurring mycotoxins (M. Sala, Aletheias-Feed Analysis and Research, Argentina, personal communication).

The present trials were conducted to study the *in vitro* binding ability of esterified glucomannan (E-GM) (a proprietary product of Alltech Inc., 3031 Catnip Hill Pike, Nicholasville, Kentucky 40356, USA), derived from the cell wall of *Saccharomyces cerevisiae*[1026], on AF, OA and T-2 and its efficacy in counteracting the effects of these mycotoxins on immune competence of broilers.

MATERIALS AND METHODS

In vitro trial

Mycotoxin binding efficacy of E-GM was evaluated in toxin-contaminated feed under simulated *in situ* gastrointestinal (GI) tract environment of chicken.

Experimental design : Aflatoxin B_1 (300 ppb), OA (2 ppm) and T-2 (3 ppm) were studied individually and in combination with and without E-GM (0.1%) (14 treatments, table 2). Each of these treatments was tested at two pH levels of 4.5 and 6.5 so as to simulate the toxin adsorption activity of E-GM in the fore and mid portions of GI tract of chicken on triplicate samples.

Production and quantification of mycotoxins : Aflatoxin, OA and T-2 were produced employing solid substrate fermentation as per the methods of Shotwell et al. (1966), Trenk et al. (1975) and Burmeister et al. (1971), respectively. The respective fungal cultures used were *A. parasiticus* NRRL 2999, *A. ochraceus* NRRL 3174 (Source: National Center for Agricultural Utilization Research, USDA, Peoria, Illinois 61604, USA) and *F. sporotrichoides* MTCC 1894 (Source: Institute of Microbial Technology,

Chandigarh 160 036, India).

Mycotoxin content of the culture materials was determined by thin layer chromatography as per AOAC (1995) in case of AF and OA, and Rukmini and Bhat (1978) and Romer et al. (1978) in case of T-2.

Experimental procedure : Compounded broiler starter feed, weighing 25 g, was taken in a 250 ml Erlenmeyer flask and the required quantity of culture material was added to arrive at the desired level of toxin. Esterified glucomannan was added at 0.1% level to the treated flasks whereas the feed in the control flasks of the respective treatment was left untreated. Citric acid-sodium phosphate buffer (100 ml) of the desired pH (4.5/6.5) was added to each flask and the contents were mixed on a horizontal shaker for 30 min. Flasks were incubated at 37°C for 3 h, contents were filtered and the residue was dried at 37°C for 2 h. The respective toxin was extracted from the residue and quantified.

The percentage difference in the toxin content between the beginning and end of the trials in the E-GM treated and control flasks was calculated. Percent binding of each toxin in different treatments was determined as follows by subtracting the per cent difference in toxin content of the control flasks from that of the treated flasks in the respective treatment.

$$\% \text{ Toxin adsorption} = \frac{[Bt-Et \times 100]}{Bt} - \frac{[Bc-Ec \times 100]}{Bc}$$

Where Bt=aflatoxin content at the beginning in the treated flask

Et=aflatoxin content at the end in the treated flask

Bc=aflatoxin content at the beginning in the control flask

Ec=aflatoxin content at the end in the control flask

In vivo trial

Experimental animals : A total of 960 day-old commercial broilers (Avian 34) were divided at random into 48 replicate groups of 20 chicks each having equal number of males and females. Each such group was housed separately in an open sided deep litter pen house (each pen: 1.5×1.2 M) and reared under uniform managemental conditions. Chicks were vaccinated against Newcastle Disease (ND) (7 d) and Infectious Bursal Disease (IBD) (10, 21 d) using F_1 and intermediate strain D 78, respectively.

Experimental design and test diets : Two dietary levels each of AF (0 and 300 ppb), OA (0 and 2 ppm), T-2 (0 and 3 ppm) and E-GM (0 and 0.1%) were tested in a 2×2×2×2 factorial manner. Basal diet was formulated and

compounded to meet the nutrient requirements of commercial broilers (BIS, 1992) during the starter (0-3 wk) and finisher (4-5 wk) phases (table 1). Required quantities of the culture materials and E-GM were added to the basal diet to prepare the different experimental diets. Each such diet was fed *ad lib* to three replicate groups of chicks from 1 d till 5 wk.

Data collection : Blood was collected at 3 and 5 wk of age from twelve birds in each treatment (six males and six females) by puncturing the brachial vein. Serum was collected and antibody titers against ND and IBD were determined employing ELISA technique using commercial test kits (Source:Kirkegaard and Perry Laboratories, Gaithersburg, Maryland 20879, USA). The plates were read at 405 nm on ELISA reader (Labsystems Multiscan MS, Labsystems, SF-00881 Helsinki, Finland).

At 5 wk, twelve birds from each treatment (six males and six females) were sacrificed by cervical dislocation. Bursa of Fabricius, thymus and spleen were collected and weighed. The weights of these lymphoid organs were adjusted to 1 kg live weight.

Table 1. Composition of basal diet

	Starter (0-3 wks)	Finisher (4-6 wks)
Physical		
Yellow maize, %	60.4	66.0
Soyabean meal, %	36.0	27.0
Sunflower extractions, %	-	4.0
Mineral mixture[1], %	3.6	3.0
Salt, %	0.3	0.3
AB_2D_3K[2], g/100 kg	12.5	12.5
B Complex[3], g/100 kg	20.0	20.0
Aurofac[4], g/100 kg	50.0	50.0
Coxistac 12%[5], g/100 kg	50.0	50.0
DL Methionine, g/100 kg	180.0	100.0
Choline chloride[6], g/100 kg	100.0	100.0
Chemical		
ME, kcal/kg	2,857	2,898
Crude protein, %	22.6	19.9
Ca, %	1.16	0.98
Non-phytin P, %	0.46	0.40
Lysine, %	1.19	0.98
Methionine, %	0.52	0.42

[1] Contained 29% Ca, 9% P, 0.44% Mn, 0.33% Zn, 150 ppm I, 2,000 ppm Fe, 250 ppm Cu and 45 ppm Se.

[2] Contained per g, 82,500 IU Vit. A, 12,000 IU Vit. D_3, 50 mg Vit B_2 and 10 mg Vit. K.

[3] Contained per 10 g, 40 mg Vit B_1, 80 mg Vit B_6, 400 µg Vit B_{12}, 200 mg Vit. E, 125 mg Calcium Pantothenate and 600 mg niacin.

[4] Contained 100 g aureomycin per kg.

[5] Contained 120 g salinomycin 350 (mycelial cake) per kg.

[6] Contained 60% choline chloride on vegetable carrier.

Statistical analysis

The data were subjected to ANOVA using General Linear Models procedure of SAS Institute (1994) under factorial design for the main effects and interactions and randomized block design for the treatment effects. Data collected in percentages were converted into arc sine angles prior to statistical analysis. Means were compared using Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

In vitro trial

Results on the percent binding of AF, OA and T-2 by E-GM, when present either alone or in combination, are presented in table 2. Significant (p<0.01) differences were noted in per cent binding of different mycotoxins among the dietary treatments. Among the diets containing the individual toxins, significantly higher binding was seen with AF than with OA and T-2. Though the exact mechanism of action of E-GM is not yet elucidated, it is expected to trap the mycotoxin in the glucomannan matrix, which is both positively and negatively charged. This effect may be influenced by the nature of the functional atomic groups, present on the mycotoxin molecule. Thus, it is evident that E-GM has broad-spectrum efficacy against the three mycotoxins tested and selectively binds certain mycotoxin molecules with greater affinity over others. Research conducted at this laboratory also indicated significant improvement in the performance of broiler chicken by dietary supplementation of E-GM during the mycotoxicosis of multiple origin (Raju and Devegowda, 2000). The other commonly used binding agents viz. aluminosilicates, activated charcoal, bentonite etc. have been found to have little or no effect on ochratoxicosis (Huff et al., 1992) and T-2 toxicosis (Edrington et al., 1997).

Aluminosilicates are reported to selectively bind only those mycotoxin molecules that have polar functional atomic groups (Pasteiner, 1997).

The binding values of the mycotoxins decreased significantly when they were present in combination. This reduction in per cent binding might have been due to the competitive saturation of the reactive sites of the E-GM molecule. The cumulative binding of the mycotoxins by E-GM in different diets was dependent on the mycotoxin present (table 3). Among the diets containing combination of two mycotoxins, the highest and lowest binding percentages were seen with the diets having AF+OA and OA+T-2, respectively.

In vivo trial

Immune system and antibody response : Relative weights of thymus, bursa of Fabricius and spleen were significantly affected by the dietary treatments (table 4). Thymus weight was significantly (p<0.01) lower in the groups fed AF, AF+T-2 and OA+T-2 and bursa was significantly (p<0.05) smaller in the group, received all the toxins in combination. Analysis for the main effects showed significant reduction in size of thymus by AF and T-2 and in size of bursa by AF, whereas no effect was seen with OA (figure 1). Spleen weight remained unaffected. Atrophy of lymphoid organs was also reported earlier with AF, OA and T-2 (Dwivedi and Burns, 1984; Devegowda et al., 1994). These reductions in size of these organs might have been due to necrosis and cellular depletion by the mycotoxins (Hoerr et al., 1981).

The three mycotoxins exerted potentiated depressing effect on bursa weight when fed in combination than in isolation, suggesting additive toxic effects among them on bursa (table 5). On the contrary, antagonistic interaction was seen between AF-OA and OA-T-2 for their effects on

Table 2. Per cent mycotoxin binding *in vitro* by esterified glucomannan (0.1%) at two pH levels in different dietary treatments in contaminated feed

Treatments	AF			OA			T-2		
Mycotoxin	pH			pH			pH		
	4.5	6.5	$\overline{X}$	4.5	6.5	$\overline{X}$	4.5	6.5	$\overline{X}$
AF	80.68	82.54	81.61[a]	-	-	-	-	-	-
OA	-	-	-	25.09	26.42	25.76[e]	-	-	-
T-2	-	-	-	-	-	-	27.54	27.96	27.75[e]
AF+OA	62.15	65.79	63.97[b]	10.76	11.02	10.89[g]	-	-	-
AF+T-2	46.24	47.67	46.96[c]	-	-	-	16.41	16.93	16.67[f]
OA+T-2	-	-	-	12.80	12.50	12.65[g]	17.72	18.84	18.28[f]
AF+OA+T-2	37.79	36.34	37.07[d]	3.35	4.62	3.99[h]	4.10	4.70	4.40[h]
$\overline{X}$ for each mycotoxin			57.40[x]			13.32[y]			16.77[z]
SEM			3.60			1.73			1.76

[a-h] Means of different mycotoxins in each treatment, bearing common superscript, do not differ significantly (p<0.01).
[x-z] Pooled means of each mycotoxin among the various treatments, bearing different superscripts differ significantly (p<0.01).
AF: Aflatoxin B_1 300 ppb, OA: Ochratoxin A 2 ppm, T-2: T-2 toxin 3 ppm.

Table 3. Cumulative binding of aflatoxin (AF), ochratoxin (OA) and T-2 toxin (T-2) by esterified-glucomannan (0.1%) in different treatments *in vitro*

Treatments	Cumulative binding, %		
	pH		$\overline{X}$
Mycotoxin	4.5	6.5	
AF	80.68	82.54	81.61[a]
OA	25.09	26.42	25.76[f]
T-2	27.49	28.00	27.75[ef]
AF+OA	72.91	76.81	74.86[b]
AF+T-2	62.62	64.62	63.62[c]
OA+T-2	30.45	31.41	30.93[e]
AF+OA+T-2	45.28	45.62	45.45[d]
$\overline{X}$	49.22[x]	50.77[x]	
SEM	4.96	4.96	3.41

[a-f/x] Cumulative means of dietary treatments/pH levels having common superscripts do not differ significantly (p<0.01).
AF: Aflatoxin B_1-300 ppb; OA: Ochratoxin A-2 ppm; T-2: T-2 toxin-3 ppm.
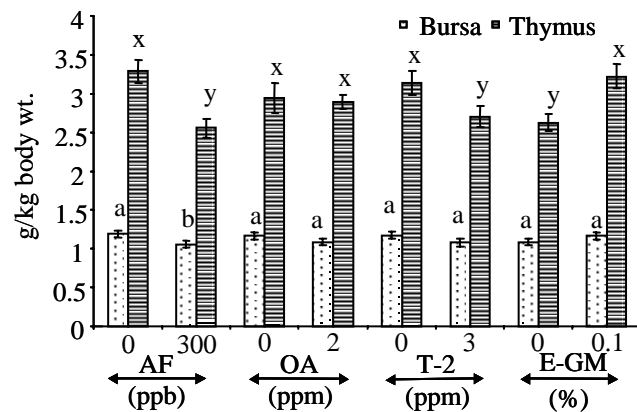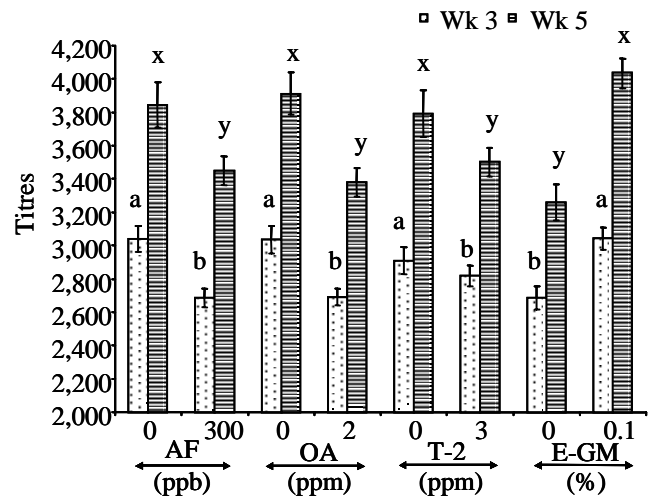

Figure 1. Main effects of aflatoxin B_1 (AF), ochratoxin A (OA), T-2 toxin (T-2) and esterified glucomannan (E-GM) on weights of bursa of Fabricius and thymus (g/kg body wt) in commercial broilers. a,b Mean values for each mycotoxin, having different superscript differ significantly (p<0.01). Y error bars on top of each bar represent SE of the respective mean.

thymus weight. Huff and Doerr (1981) reported no influence of combined feeding of AF and OA on bursa weight in broilers. Esterified glucomannan significantly increased weight of thymus (figure 1) while no effect was seen on bursa weight.

Antibody titers against Newcastle disease (ND) and Infectious bursal disease (IBD) were significantly (p<0.01) decreased in all the toxin fed groups (individual and combined) at both wk 3 and wk 5. All the three mycotoxins significantly (p<0.01) depressed the antibody titers against both the diseases (figures 2 and 3). Combined feeding of
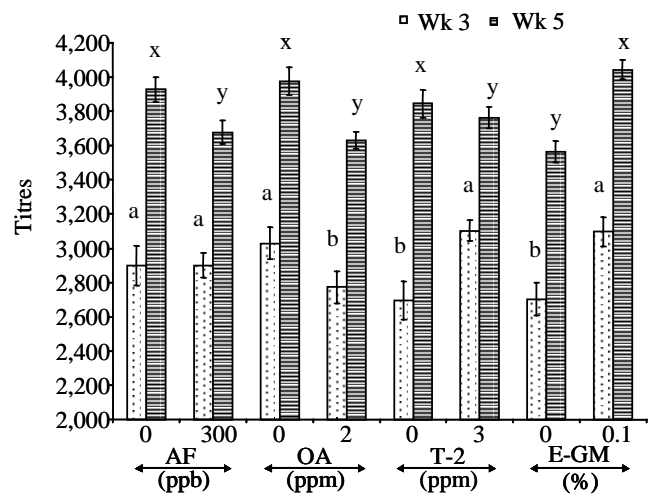
Figure 2. Main effects of aflatoxin B_1 (AF), ochratoxin A (OA), T-2 toxin (T-2) and esterified glucomannan (E-GM) on antibody titers against Newcastle Disease in commercial broilers. a,b Mean antibody titers for each mycotoxin at each age, having different superscript differ significantly (p<0.01). Y error bars on top of each bar represent SE of the respective mean.

Figure 3. Main effects of aflatoxin B_1 (AF), ochratoxin A (OA), T-2 toxin (T-2) and esterified glucomannan (E-GM) on antibody titers against Infectious Bursal Disease in commercial broilers. a,b Mean antibody titers for each mycotoxin at each age, having different superscript differ significantly (p<0.01). Y error bars on top of each bar represent SE of the respective mean.

mycotoxins did not show any further reduction in titers than that seen with individual toxins and antagonistic interaction was seen between OA and T-2 for their effects on ND titers at 3 wks (table 6). Combination of OA and AF is reported to reduce cell mediated immune response and HI titers against

Table 4. Effect of individual and combined feeding of 300 ppb aflatoxin B_1 (AF), 2 ppm ochratoxin A (OA) and 3 ppm T-2 toxin (T-2) either with or without esterified glucomannan (E-GM) on lymphoid organ weight and antibody titers in broilers

Experimental diets		Lymphoid organ weight (g/kg live wt)			Antibody (ELISA) titers			
					Newcastle disease		Infectious bursal disease	
Mycotoxin	E-GM 0.1 %	Bursa of fabricius*	Thymus	Spleen*	wk 3	wk 5	wk 3	wk 5
-	-	1.28[abc]	3.43[b]	1.4[ab]	3,484[ab]	4,293[b]	3,468[b]	4,754[b]
-	+	1.34[ab]	4.88[a]	2.03[ab]	3,573[a]	4,601[a]	3,825[a]	5,120[a]
AF	-	1.06[abcd]	2.34[def]	1.51[ab]	2,155[g]	3,263[hi]	2,481[gh]	3,135[fg]
AF	+	1.16[abc]	3.01[bcd]	1.72[ab]	2,598[ef]	4,161[bc]	2,561[gh]	4,055[cd]
OA	-	1.00[abcd]	2.85[bcde]	1.94[ab]	1,793[h]	3,401[ghi]	2,684[fg]	2,864[h]
OA	+	1.21[abc]	3.04[bcd]	1.59[ab]	2,178[g]	3,700[ef]	2,844[ef]	3,577[e]
T-2	-	1.14[abc]	2.95[bcd]	1.34[ab]	2,459[f]	3,655[efg]	2,958[de]	3,210[f]
T-2	+	1.14[abc]	3.36[bc]	1.78[ab]	3,374[abc]	4,153[bc]	3,116[cd]	3,864[d]
AF+OA	-	1.11[abc]	2.54[bcde]	1.70[ab]	2,834[d]	3,548[efg]	2,572[gh]	2,968[fgh]
AF+OA	+	1.26[abc]	3.05[bcd]	1.47[ab]	2,952[d]	3,793[de]	2,846[ef]	3,865[d]
AF+T-2	-	0.98[bcd]	1.57[f]	1.54[ab]	3,177[c]	3,500[fgh]	2,688[fg]	3,122[fg]
AF+T-2	+	1.23[abc]	2.05[ef]	2.18[ab]	3,404[ab]	4,195[bc]	3,197[c]	4,053[cd]
OA+T-2	-	1.37[a]	2.46[cde]	1.67[ab]	2,960[d]	3,640[efg]	2,448[h]	3,142[fg]
OA+T-2	+	1.06[abcd]	3.35[bc]	1.18[ab]	3,373[abc]	3,985[cd]	2,983[de]	4,226[c]
AF+OA+T-2	-	0.75[d]	2.89[bcde]	1.25[ab]	2,761[de]	3,208[i]	2,196[i]	2,886[gh]
AF+OA+T-2	+	0.92[cd]	3.01[bcd]	1.90[ab]	3,327[bc]	3,765[de]	2,970[de]	3,515[e]
SEM		0.033	0.106	0.068	67.6	51.6	51.9	84.2

[a,b,c,d,e,f,g,h,i] Means within each column, bearing common superscripts, do not differ significantly (p< 0.01). * p<0.05.

Table 5. Additive interaction among aflatoxin (AF), ochratoxin A (OA) and T-2 toxin (T-2) for their effects on bursa weight in commercial broilers at 5 wk of age

		OA				SEM
		0 ppm		2 ppm		
		T-2		T-2		
		0 ppm	3 ppm	0 ppm	3 ppm	
AF	0 ppb	1.31[ax]	1.14[ax]	1.10[ax]	1.22[ax]	0.04
	300 ppb	1.10[ax]	1.11[ax]	1.19[ax]	0.84[by]	0.05
	SEM	0.06	0.08	0.06	0.07	

[a,b] Means within each row having similar superscripts do not differ significantly (p<0.01).
[x,y] Means within each column having similar superscripts do not differ significantly (p<0.01).

sheep red blood cells in broilers (Verma et al., 1995).

Esterified glucomannan significantly (p<0.01) improved antibody titers against both the vaccines (figures 2 and 3). Similar improvements in immune response with mannanoligosaccharide supplementation were recorded earlier (Savage et al., 1996; Cotter and Weinner, 1997). This effect might have been due to its mycotoxin binding ability and/or its indirect effects on cellular immunity through activation of B cells, T cells and macrophages (Lyons, 1994). Since esterified glucomannan also has been reported to significantly improve performance of broilers, fed multiple mycotoxins (Raju and Devegowda, 2000) and also that it inhibited lipid peroxidation in liver of quails fed T-2

Table 6. Antagonistic interaction between ochratoxin A (OA) and T-2 toxin (T-2) for their effects on antibody titers against Newcastle disease in commercial broilers at 3 wk of age

		T-2		SEM
		0 ppm	3 ppm	
AF	0 ppb	2,952[ax]	3,104[ax]	93.9
	300 ppb	2,439[by]	3,105[ax]	93.4
	SEM	110.8	60.3	

[a,b] Means within each row having similar superscripts do not differ significantly (p<0.01).
[x,y] Means within each column having similar superscripts do not differ significantly (p<0.05).

toxin (Dvorska and Surai, 2001), its mycotoxin binding ability might have been primarily responsible for these beneficial effects noted on immune competence.

From the results of the study, it is concluded that E-GM has broad spectrum binding ability over aflatoxin, ochratoxin and T-2 toxin, present in broiler chicken diet either alone or in combination. The immune response of the broiler chicks, fed AF, OA and T-2, to ND and IBD vaccination was improved significantly by dietary inclusion of E-GM at 0.1% level.

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