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Journal of Applied Animal Research

ISSN: 0971-2119 (Print) 0974-1844 (Online) Journal homepage: http://www.tandfonline.com/loi/taar20

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To cite this article: Ardeshir Mohaghegh, Mohammad Chamani, Mahmoud Shivazad, Ali Asghar Sadeghi & Nazar Afzali (2017) Effect of esterified glucomannan on broilers exposed to natural mycotoxin-contaminated diets, Journal of Applied Animal Research, 45:1, 285-291, DOI: 10.1080/09712119.2016.1174122

To link to this article: http://dx.doi.org/10.1080/09712119.2016.1174122

Published online: 25 Apr 2016.

JOURNAL OF APPLIED ANIMAL RESEARCH, 2017
VOL. 45, NO. 1, 285–291
http://dx.doi.org/10.1080/09712119.2016.1174122

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Effect of esterified glucomannan on broilers exposed to natural mycotoxin-contaminated diets

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ABSTRACT

The purpose of this study was to evaluate the effect of esterified glucomannan (E-GM) on performance, immunity, blood haematological and serum biochemical parameters in broilers exposed to diets naturally contaminated with mycotoxins. A total of 630 one-day-old male broiler chicks (Ross 308) were randomly assigned to 9 treatments and 5 replicates of 14 chicks based on factorial (3 × 3) arrangement in completely randomized design. The dietary treatments included 3 levels of substituting naturally contaminated corn (0%, 50% and 100%), three levels of E-GM (0%, 0.05% and 0.1%) and their interaction. Body weight gains (BWG), feed intake (FI) and feed conversion ratio (FCR) were evaluated from 7 to 49 days of age. Haematology, serum biochemical and enzyme activities were assessed. Antibody titre against Newcastle disease virus and infectious bursal disease was measured to evaluate the humoral immunity. In comparison to diets with no contamination, 50% and 100% naturally contaminated corn significantly decreased FI, BWG and FCR ($P < .05$). Supplementing 0.05% and 0.1% E-GM considerably improved the decreased BWG and FI ($P < .05$). However, only 0.1% binder ameliorated the negative impact of mycotoxins on FCR ($P < .05$). Replacement of contaminated corn remarkably diminished humoral immunity of chickens and increased liver enzyme activities which ameliorated by supplementing 0.05% and 0.1% of binder inclusion ($P < .05$). Results indicated that supplementing E-GM particularly at 0.1% level efficiently reversed the adverse effects of mycotoxins on broiler chickens.

ARTICLE HISTORY
Received 25 August 2014
Accepted 22 March 2016

KEYWORDS
Broiler chickens; Mycotoxin; Esterified glucomannan; performance; haematological parameters

1. Introduction

Mycotoxins are known as the secondary fungal metabolites that widely occur in animal feed and foods (Zhang & Caupert 2012). Due to the similar conditions that fungal species produce their second metabolites, existence of mycotoxins in the nature rarely manifests as a single contaminant. Additionally, several grain sources are the constituents of usual poultry diets which might be contaminated by different mycotoxins (Liu et al. 2011). Ingestion of mycotoxins causes unwanted biological effects inside animal and human organisms and leads to carcinogenic, oestrogenic and immunosuppressive condition. Furthermore, chronic mycotoxicosis resulted from minor levels of mycotoxin exposure, not necessarily occur accompanied by clinical symptoms. These chronic impacts affect animals' performance and immunity as well as increase toxin residues in poultry meat and egg (Resanović et al. 2009; Liu et al. 2011). Aflatoxin, ochratoxin (OTA) and deoxynivalenol (DON) are among the most important feed contaminants. Aflatoxin is an extremely toxic compound and is associated with liver damage, immune suppression; poor growth and feed conversion (Edds & Bortell 1983). OTA is a mycotoxin with adverse effect on production and health of poultry (Aravind et al. 2003). It has been reported that chickens can tolerate up to 15 mg/kg DON without any acute influence on their performance. On the other hand, DON can co-occur regularly with other mycotoxins with high values in the grains of all around the world (Placinta et al. 1999). Furthermore, consumption of mycotoxins in combination with other toxins may show greater synergism effects on broilers and consequently damage the productivity of chickens (Raju & Devegowda 2000).

Aluminosilicates, and esterified glucomannan (E-GM) derived from *saccharomyces cervisia* are two types of adsorbents which have been reported to decrease mycotoxicosis. Aluminosilicates efficiently absorb aflatoxin, but have not been able to effectively absorb trichotecenes (Phillips 1999). The effect of E-GM to improve the adverse consequences of mycotoxins on chickens performance, immunity, blood haematological and biochemical indices has been previously studied (Raju & Devegowda 2002; Aravind et al. 2003; Girish & Devegowda 2006; Zhao et al. 2010).

Regarding the mentioned experiments, crystalized toxin have been used to apply contamination in diets (Girish & Devegowda 2006; Zhao et al. 2010), but naturally mould-contaminated diet supplied in this experiment. Additionally, research on the use of high-performance liquid chromatography (HPLC) to detect mycotoxins at the levels of this trial is less investigated. This study also designed with special focus on the interactions of diet contamination and binder inclusion. Therefore, this experiment was carried out to evaluate the impact of feeding diets naturally contaminated with blend of

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mycotoxins, supplemented by different levels of E-GM on performance, immunity, blood haematological and serum biochemical parameters of broiler chicks.

2. Materials and methods

2.1. Experimental diets, animals and housing

Six hundred and thirty 1-day-old male broiler chicks (Ross 308) were individually weighed on arrival and assigned to 9 treatments and 5 replicates of 14 chickens based on factorial (3 × 3) arrangement in completely randomized design. Mycotoxin contamination was evaluated through replacing 3 levels of mould-contaminated corn (0%, 50% and 100%) in the diet. The mycotoxin contamination was obtained by water inclusion to uncontaminated corn until 20% moisture was reached. Afterward, a natural condition, 23–28°C temperature and 68–85% humidity was used to culture the wet corn until an obvious mildew appeared. Finally, the contaminated corn was air-dried naturally, mixed and sample was taken for subsequent analysis (Liu et al. 2011). The concentration of different toxins in corn and feed samples, including Aflatoxin (B1, B2, G1 and G2), DON, OTA and zearalenone, was determined using HPLC according to Yang et al. (2012). Growing and finisher diets were stored in an environmentally controlled place to avoid mycotoxin occurrence exceed the start of the experiment. Impact of mycotoxin binder was assessed using 0%, 0.05% and 0.1% E-GM (Mycosorb®); a toxin binder manufactured by Alltech, Inc. (Nicholasville, KY, USA) in the diet. Mycosorb® contained polymers of glucose that derived from the cell walls of saccharomyces *cerevisiae*. At the time of E-GM supplementation, sand flour was used as a filler to replace E-GM. Experimental diets were formulated to meet requirements of broilers over different periods according to NRC (1994) from day 8 to 21 (starter diet); 21 to 42 (grower diet) and 42 to 49 (finishing diet). The composition of experimental diets is presented in Table 1. Chickens had free access to water and feed throughout the trial. The ambient temperature was gradually decreased from 33°C to 25°C on day 21 and was then kept constant. All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Islamic Azad University, Science and Research Branch.

2.2. Performance and relative organ weights

Chickens were weighed and their body weight gains (BWG) and FI were measured throughout 7–49 days of age and the feed conversion ratio (FCR) was calculated. At the end of the experiment, three chickens from each pen were killed by cervical dislocation, and then liver, kidney, heart, spleen and bursa of Fabricius in all chickens were excised. Organ weights were expressed on a relative body weight basis.

2.3. Antibody titre against Newcastle and IBD viruses

Newcastle disease virus (NDV) vaccine (killed vaccine, Formosa Biomedical Inc., Taipei, Taiwan) was administered on day 11 after hatch and peripheral blood was collected from the brachial vein of 3 chickens on day 18. Antibody titres against NDV were determined by haemagglutination-inhibition test (Allan & Gough 1974). Vaccination against infectious bursal disease (IBD) virus (IBD,Polymed, Tabic) was administrated in drinking water of broilers at 16 days of age. Blood samples were collected from 3 chickens on day 49 to evaluate the IBD-ELISA antibody titres according the commercial kit manual (Biocheck® Inc., CA, USA).

2.4. Serum biochemical parameters and differential leukocyte counts

At the end of the experiment, blood samples were taken from three birds of each pen and collected in non-heparinized tubes by brachial vein puncture. Serum was separated via 2000×*g* centrifuge of blood samples for 15 min (SIGMA 4–15 Lab Centrifuge, Germany). Serum samples were analyzed for total protein (TP), albumin (ALB), uric acid (UA), alanine amino transferase (ALT) and aspartate amino transferase (AST) using an automatic analyser (Boehringer Mannheim, Hitachi, Japan) according to the recommendation of the manufacturer. Differential leukocyte numbers were determined according to Natt and Herrick's (1952) procedure. In this regard, blood samples were collected from the brachial vein using heparinized syringe-needle assemblies to avoid blood clot formation on day 49 of age.

2.5. Statistical analysis

Data were subjected to ANOVA using the general linear model procedure of SAS institute (2008) with the main effects of contaminated corn percentage in the diet, binder inclusion and the interaction between them. Tukey's test was used for multiple comparisons when a significant interaction was detected. All statements of significance were based on probability ($P < .05$). Where the interaction effect was significant, the effects of the main factors were not discussed.

3. Results

3.1. Dietary mycotoxin concentrations

The concentration of mycotoxins in the contaminated corn contained 427.2 µg/kg aflatoxin B1, 5.45 µg/kg aflatoxin B2, 2.23 µg/kg OTA and 206.31 µg/kg DON. It was found that aflatoxin was the major contaminant and after that, DON was the second and eventually OTA presented as a minor contaminant. The limits of detection were 0.50 µg/kg for aflatoxin G1, 0.05 µg/kg for aflatoxin G2 and 4.00 µg/kg for zearalenone. Chickens were chronically exposed to natural mycotoxins since experiment continued from day 7 to 49 days of age.

3.2. Performance and relative organ weights

The effects of different levels of contamination and E-GM inclusion on the performance of broilers are given in Table 2. The interaction of mycotoxin contamination and binder in different levels was significant during the whole production period. Binder inclusion did not induce any significant impact on FI, BWG and FCR when no contaminated corn was included

Table 1. Ingredients and composition of the diet.

	Starter (8–21)			Grower (21–42)			Finisher (42–49)		
UC	56.00	28.00	0.00	64.00	32.00	0.00	67.60	33.80	00.0
CC	00.00	28.00	56.00	00.00	32.00	64.00	0.00	33.80	67.60
Soybean meal	32.63	32.63	32.63	26.00	26.00	26.00	24.50	24.50	24.50
Fish meal	3.00	3.00	3.00	2.00	2.00	2.00	0.00	0.00	0.00
Soybean oil	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.26	1.26	1.26	1.13	1.13	1.13	1.34	1.34	1.34
Dicalcium phosphate	1.24	1.24	1.24	1.10	1.10	1.10	0.86	0.86	0.86
Sand flour	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
DL-Methionine	0.17	0.17	0.17	0.07	0.07	0.07	0.00	0.00	0.00
Vitamin premix[a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Calculated composition									
ME (kcal/kg)	2905.3	2905.3	2905.3	2891.6	2891.6	2891.6	2934.4	2934.4	2934.4
Crude protein (%)	20.88	20.88	20.88	18.07	18.07	18.07	16.51	16.51	16.51
Lysine (%)	1	1	1	1	1	1	0.85	0.85	0.85
Met + Cys (%)	0.9	0.9	0.9	0.72	0.72	0.72	0.6	0.6	0.6
Calcium (%)	1	1	1	0.9	0.9	0.9	0.8	0.8	0.8
Available phosphorous (%)	0.45	0.45	0.45	0.4	0.4	0.4	0.3	0.3	0.3

Note: **UC**, uncontaminated corn; **CC**, contaminated corn.

[a]Vitamin premix provided per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopheryl acetate), 18 mg; vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg.

[b]Mineral premix provided per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe), 50 mg; Mn (MnSO4.H2O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO4.5H2O), 10 mg; I (KI, 58% I), 1 mg; Se (NaSeO3, 45.56% Se), 0.2 mg.

in experimental diets. Inversely, FI and BWG were notably reduced via replacing the uncontaminated corn by contaminated one from 0 up to 100% which also negatively affected FCR ($P < .05$). Supplementing 0.05 and 0.1% binder along with dietary administration of 50 or 100% contaminated corn increased FI and BWG considerably ($P < .05$). Moreover in contrast to incorporation of 50% contaminated corn with no binder, FCR improved significantly with 0.1% E-GM compared to 0% of binder inclusion ($P < .05$). As shown in Table 3, mycotoxin contamination increased liver and bursa of Fabricius relative weights ($P < .05$). A significant interaction was observed between diet contamination and binder addition in liver and bursa of Fabricius relative weights. Although feeding of contaminated diet (50% and 100% contaminated corn) significantly increased liver weight ($P < .05$) when no binder added, supplementing 0.05% and 0.1% E-GM decreased the liver relative weight ($P < .05$). On the other hand, bursa of Fabricius weight which decreased ($P < .05$) in broilers fed on contaminated diet was increased significantly by 0.1% of binder inclusion ($P < .05$). No efficient impact of contaminated diet, binder or interaction of them observed on kidney, spleen and heart relative weights.

Table 2. Effect of diet contamination and E-GM supplementation on the performance of broilers chicks.

Treatments		Performance		
Corn (%)	E-GM (%)	Feed intake	BW gain	FCR
0	0	4719[a]	2736[a]	1.72[b]
0	0.05	4709.1[a]	2727.6[a]	1.72[b]
0	0.1	4727.8[a]	2751.6[a]	1.71[b]
50	0	4621.1[b]	2438.4[b]	1.89[ab]
50	0.05	4699.2[a]	2676[a]	1.75[b]
50	0.1	4720.1[a]	2698.8[a]	1.74[b]
100	0	4507.8[c]	2268[c]	1.98[a]
100	0.05	4695.9[a]	2478[b]	1.89[ab]
100	0.1	4730[a]	2678.4[a]	1.76[b]
SEM		32.49	50.12	0.09
Main effects				
Corn (%)				
0		4718.6[a]	2738.4[a]	1.72[c]
50		4680.1[b]	2604.4[b]	1.79[b]
100		4644.5[c]	2474.8[c]	1.87[a]
SEM		18.75	66.50	0.035
E-GM (%)				
0		4615.9[b]	2607.8	1.77[a]
0.05		4709.1[b]	2650.2	1.77[a]
0.1		4727.8[a]	2690.6	1.75[b]
SEM		8.85	21.01	0.005
Probability				
Mycotoxin		<0.0001	0.01	<0.0001
E-GM		0.001	0.07	0.002
M × E-GM		0.004	0.02	0.005

Note: **M**, mycotoxin contamination; **E-GM**, esterified glucomannan.
Values in the same column not sharing a common superscript differ significantly ($P < .05$).

3.3. Antibody titre against Newcastle and IBD viruses

Effects of diet contamination and binder inclusion on antibody titres against NDV and IBD virus are given in Table 4. Mycotoxin contamination significantly affected antibody titres against NDV and IBD virus ($P < .05$). Interaction results indicated that antibody titre against IBD and NDV reduced notably because of 50% and 100% replacement of contaminated corn in the diet, however, increased through addition of 0.05% and 0.1% E-GM ($P < .05$).

3.4. Serum biochemical parameters and differential leukocyte counts

As Table 5 indicates, there was an interaction between contaminated diet and mycotoxin binder in the case of serum biochemical parameters. E-GM inclusion did not affect serum biochemical parameters when no mycotoxin contaminations existed in the diet. Compared to 0% and 50%, substitution of 100% contaminated corn significantly reduced ALB

concentration which inversely increased after 0.05% and 0.1% of binder addition ($P < .05$). The concentration of TP also affected by 50% and 100% supplementation of contaminated corn but significantly enhanced using 0.05% and 0.1% binder ($P < .05$). Concentrations of ALT and AST considerably increased in response to 50% and 100% contaminated corn supplementation compared with uncontaminated diet ($P < .05$). Application of 100% contaminated corn obviously elevated these enzymes compared to 50% substitution ($P < .05$). Different levels of E-GM decreased the ALT and AST concentrations regardless the level of diets contamination ($P < .05$). The differential leukocyte counts are given in Table 6. The interaction of contamination and E-GM showed that replacing 50% contaminated corn with no binder inclusion tended to change the heterophile and lymphocyte counts, whereas 100% corn substitution considerably increased heterophile but decreased lymphocyte ($P < .05$). Compared with 0% binder inclusion in high-contaminated diet, consumption of dietary added binder (0.05% and 0.1 %) led to increased lymphocyte and decreased heterophile counts ($P < .05$). White blood cells (WBC) also significantly increased by all levels of mycotoxin contamination which improved by both levels of E-GM inclusion ($P < .05$). Diet contamination and binder inclusion did not induce any noticeable influence on monocyte and eosinophil counts.

4. Discussion

In the present study, diet samples were analysed by the HPLC technique. Lin et al. (1998) found no superiority of HPLC over the other techniques such as thin layer chromatography (TLC). Conversely, Rahmani et al. (2009) reported that TLC has some disadvantages, such as low sensitivity, high detection limit and lack of potential, for automation. Therefore it is almost replaced by HPLC. Additionally, Rodrigues (2014) explained that purified mycotoxins have no similar effects to those observed for naturally contaminated diets. It is probably because the existence of multiple, synergistically acting metabolites that occur in natural contamination. In this respect, the applied HPLC in this trial has been a development that is able to detect multiple mycotoxins simultaneously in a sample (Schumacher et al., 1997).

The reduced performance of chickens using contaminated corn without binder in this trial may be related to the decreased efficiency of feed utilization (Girish et al. 2008), OTA existence as a potential mycotoxin (Duff et al. 1987) and chronic exposure of chickens to aflatoxin (Resanović et al. 2009) which affected FI and subsequently decreased BWG. Similar reports were found in the research of Swamy et al. (2002, 2004) and Aravind et al. (2003). On the other hand, this performance decrease may be in part attributed to the synergetic effects between different mycotoxins (Huff et al. 1984). Interaction results of diet contamination and binder addition show that FI and BWG of chickens fed different levels of mycotoxin significantly modified with various levels of binder inclusion. These findings further support the idea that E-GMs can absorb mycotoxin and block the colonization of pathogens in gastrointestinal tract (Olsen 1995). It is also suggested that E-GM might trap the mycotoxin molecule in its glucomannan matrix and prevent the toxin from entering gastrointestinal tract (Raju & Devegowda 2000). Moreover, when diet contamination increased, 0.05% binder inclusion could ameliorate the negative effects of mycotoxins on FI, whereas better BWG was shown by 0.1% binder addition. It confirms that BWG is not solely influenced by FI under

Table 3. Effect of diet contamination and E-GM supplementation on body organs relative weight of broiler chicks.

Treatments		Body organs (% of live BW)				
Corn (%)	E-GM (%)	Liver	Spleen	Kidney	Heart	Bursa of Fabricius
0	0	2.51^{c}	0.21	0.63	0.57	0.23^{a}
0	0.05	2.47^{c}	0.19	0.66	0.60	0.24^{a}
0	0.1	2.60^{c}	0.21	0.63	0.58	0.24^{a}
50	0	3.62^{b}	0.23	0.69	0.58	0.18^{b}
50	0.05	2.63^{c}	0.22	0.71	0.61	0.23^{a}
50	0.1	2.68^{c}	0.20	0.68	0.61	0.23^{a}
100	0	3.98^{a}	0.21	0.70	0.60	0.16^{b}
100	0.05	2.71^{c}	0.23	0.72	0.65	0.19^{ab}
100	0.1	2.78^{c}	0.19	0.69	0.62	0.23^{a}
SEM		0.17	0.03	0.05	0.04	0.025
Main effects						
Corn (%)						
0		2.52^{b}	0.20	0.64	0.58	0.23^{a}
50		2.97^{b}	0.21	0.69	0.60	0.21^{a}
100		3.15^{a}	0.21	0.70	0.62	0.19^{b}
SEM		0.09	0.01	0.01	0.01	0.01
E-GM (%)						
0		3.17	0.21	0.67	0.58	0.22
0.05		2.60	0.21	0.69	0.62	0.21
0.1		2.68	0.20	0.66	0.60	0.19
SEM		0.04	0.01	0.01	0.01	0.01
Probability						
Mycotoxin		<0.0001	0.73	0.87	0.31	0.05
E-GM		0.59	0.23	0.15	0.56	0.14
M×E-GM		0.04	0.35	0.80	0.23	0.03

Note: **M**, mycotoxin contamination; **E-GM**, esterified glucomannan.
Values in the same column not sharing a common superscript differ significantly ($P < .05$).

Table 4. Effect of diet contamination and E-GM supplementation on antibody titre against Newcastle and IBD viruses in broiler chicks.

Treatments		Immunity	
Corn (%)	E-GM (%)	Newcastle (log_2)	IBD (log_2)
0	0	7^{a}	3003.5^{a}
0	0.05	6^{a}	2904.5^{a}
0	0.1	7^{a}	3099.9^{a}
50	0	5^{b}	2599.4^{b}
50	0.05	7^{a}	2832.7^{a}
50	0.1	6^{a}	2930.1^{a}
100	0	3^{c}	2125.6^{c}
100	0.05	6^{a}	2804.9^{a}
100	0.1	7^{a}	2733.3^{a}
SEM		0.4	183.2
Main effects			
Corn (%)			
0		6^{a}	3002.6^{a}
50		6^{a}	2787.4^{b}
100		5^{b}	2554.6^{b}
SEM		0.45	105.60
E-GM (%)			
0		5	2576.1
0.05		6	2847.3
0.1		6	2921.1
SEM		0.50	37.40
Probability			
Mycotoxin		0.0001	0.004
E-GM		0.08	0.09
M×E-GM		0.04	0.04

Notes: **M**, mycotoxin contamination; **E-GM**, esterified glucomannan; **IBD**, infectious bursal disease.
Values in the same column not sharing a common superscript differ significantly ($P < .05$).

Table 5. Effect of diet contamination and E-GM supplementation on biochemical parameters and serum enzyme activities of broiler chickens.

Treatments		Serum biochemical parameters				
Corn (%)	E-GM (%)	UA (mg/dl)	ALB (mg/dl)	TP (mg/dl)	ALT (U/L)	AST (U/L)
0	0	7.12	1.45^{a}	3.41^{a}	7.24^{c}	147.2^{c}
0	0.05	7.18	1.47^{a}	3.39^{a}	7.41^{c}	150.9^{c}
0	0.1	6.93	1.44^{a}	3.46^{a}	7.32^{c}	145.3^{c}
50	0	7.01	1.25^{ab}	2.82^{b}	10.10^{b}	170.1^{b}
50	0.05	7.16	1.30^{a}	3.30^{a}	7.63^{c}	159.8^{c}
50	0.1	7.02	1.42^{a}	3.40^{a}	7.23^{c}	156.6^{c}
100	0	6.98	1.01^{b}	2.25^{c}	13.33^{a}	201.2^{a}
100	0.05	7.09	1.31^{a}	3.27^{a}	7.85^{c}	161.1^{c}
100	0.1	7.08	1.40^{a}	3.37^{a}	7.13^{c}	160.7^{c}
SEM		0.13	0.11	0.09	1.05	9.12
Main effects						
Corn (%)						
0		7.07	1.45^{a}	3.42^{a}	7.32^{b}	147.8^{b}
50		7.06	1.32^{b}	3.17^{a}	8.32^{a}	162.1^{b}
100		7.05	1.24^{b}	2.96^{b}	9.43^{a}	174.3^{a}
SEM		0.005	0.06	0.21	0.45	5.60
E-GM (%)						
0		7.03	1.23	2.82	8.22	172.8
0.05		7.14	1.36	3.32	7.63	157.2
0.1		7.01	1.39	3.41	7.22	154.2
SEM						
Probability						
Mycotoxin		0.30	0.004	0.007	0.03	0.0001
E-GM		0.14	0.34	0.76	0.30	0.8
M×E-GM		0.31	0.004	0.005	0.04	0.005

Notes: M, mycotoxin contamination; E-GM, esterified glucomannan; UA, uric acid; ALB, albumin; TP, total protein; ALT, alanine amino transferase; AST, aspartate amino transferase.

Values in the same column not sharing a common superscript differ significantly ($P < .05$).

mycotoxicosis and therefore impact of different toxins on other factors such as nutrient absorption might indirectly affect BWG. This result is consistent with those reported by Marchioro et al. (2013) that aflatoxin increased digestive enzymes of broilers, but did not improve their growth performance, indicating that absorption is affected by existence of mycotoxins in the diet. The improving role of 0.05% E-GM to neutralize the deleterious effects of mycotoxicosis on performance has been previously reported by Aravind et al. (2003) and Modirsanei et al. (2004). On the contrary, Liu et al. (2011) failed to improve the chickens' performance by 0.05% E-GM supplementation.

Both liver and bursa of Fabricius relative weights were affected by mycotoxins contamination. In agreement with previous data (Aravind et al. 2003), liver relative weight increased, as aflatoxin elevated at the time of no E-GM supplementation. This might be due to hepatotoxic effect of aflatoxin, leading to significant changes of functioning and gross appearance of the liver (Tung et al. 1973). Bursa of Fabricius is a major part of humoral and cellular immunity and any regression in bursal development would appear in poor immune response of broilers (Qureshi et al. 1998; Ortatatli et al. 2005). In line with this experiment, bursa of Fabrisius weight was significantly decreased in broilers fed by aflatoxin and T-2 toxin-contaminated diet (Girish & Devegowda 2006). Reduced relative weight of lymphoid organs may be related to the necrosis and cellular depletion created by mycotoxins (Hoerr et al. 1981). Moreover, effects of fusarium-born mycotoxins like DON on liver and bursa of Fabrisius have been previously studied in broilers (Swamy et al. 2004; Li et al. 2012) and turkeys (Girish et al. 2008). In these studies as data showed contradictory results, authors concluded that organ weights might not be a proper indicator of fusarium mycotoxins toxicity and also effect of these toxins on organ weights is species, toxin and dosage dependent (Swamy et al. 2004; Li et al. 2012). The presented background indicates that the main reason for organ weight variations in this trial might be attributed to aspergillus-born mycotoxins especially aflatoxin. Binder

Table 6. Effect of diet contamination and E-GM supplementation on haematological parameters of broiler chickens.

Treatments		Haematological parameters				
Corn (%)	E-GM (%)	WBC (×10^{3})	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Heterophile (%)
0	0	4.3^{c}	61.5^{a}	0.5	2.5	34.5^{b}
0	0.05	4.4^{c}	62.7^{a}	0.6	2.7	34^{b}
0	0.1	4.7^{c}	63.3^{a}	0.6	2.6	33.5^{b}
50	0	5.6^{b}	57.2^{ab}	0.9	2.1	39.8^{ab}
50	0.05	4.5^{c}	60.5^{a}	0.8	1.9	36.8^{b}
50	0.1	4.8^{c}	60.19^{a}	0.7	2.3	36.9^{b}
100	0	6.5^{a}	50.7^{b}	0.5	1.7	47.1^{a}
100	0.05	4.4^{c}	59.3^{a}	0.6	2.1	38^{b}
100	0.1	4.9^{c}	61.6^{a}	0.8	2.4	35.2^{b}
SEM		0.3	3.25	0.27	0.55	3.65
Main effects						
Corn (%)						
0		4.4	62.5	0.56	2.6	34
50		4.9	59.2	0.80	2.1	37.8
100		5.2	57.2	0.63	2	40.1
SEM		0.10	1.55	0.90	0.30	1.20
E-GM (%)						
0		5.4	56.4	0.63	2.1	40.4
0.05		4.4	60.8	0.66	2.2	36.2
0.1		4.8	61.6	0.70	2.4	35.2
SEM		0.35	0.45	0.02	0.15	2.70
Probability						
Mycotoxin		0.40	0.30	0.80	0.35	0.40
E-GM		0.35	0.14	0.76	0.23	0.59
M × E-GM		0.04	0.03	0.30	0.15	0.04

Note: M, mycotoxin contamination; E-GM, esterified glucomannan.

Values in the same column not sharing a common superscript differ significantly ($P < .05$).

inclusion to diet may compensate the negative effect of mycotoxins on liver and bursa of Fabricius relative weights. Similarly, an ameliorating impact of binder inclusion on bursa of Fabricius relative weight against aflatoxin has been suggested earlier by Girish and Devegowda (2006) and also Raju and Devegowda (2002). This trial also showed that 0.1% binder inclusion reversed the compromising impact of toxins on bursa of Fabricius.

Increasing diet mycotoxin contamination deleteriously affected antibody titre against NDV and IBD. According to DÄnicke et al. (2003), serum anti-NDV titre after vaccination is a suitable indicator of immune system function to study the impact of fusarium mycotoxins on broiler chickens. A possible explanation for this effect might be the regression of bursa of Fabrisius due to the aflatoxin contamination. In a study, anti-NDV titre was not affected by feeding naturally contaminated diets (12.6 DON and 0.6 mg/kg zearalenone) to fusarium mycotoxins in broiler chickens (Yegani et al. 2006). Inversely, Li et al. (2012) indicated the reduction in anti-NDV titre of chickens fed diets contaminated with mycotoxins. In accordance with the present study, depressed antibody titres against NDV and IBD virus during the chickens exposure to diets contaminated with aflatoxin and T-2 toxin have been demonstrated (Girish & Devegowda 2006). Interaction results revealed that both levels of binder supplementation improved antibody titre against NDV and IBD in chickens exposed to contaminated diet. It seems possible that these results are due to the binder's ability to absorb the mycotoxins and also their indirect impact on cellular immunity through activation of B cells, T cells and macrophages (Girish & Devegowda 2006).

Administration of 100% contaminated corn also decreased serum ALB. Depression in serum concentration of TP and ALB and the increased serum AST are among the sensitive serological indicators of liver and kidney toxicity (Shi et al. 2006). The reduction in serum TP of this study might be due to the impaired protein synthesis (Tung et al. 1975) resulting from hepatotoxicity of aflatoxins (Kubena et al. 1990). Additionally, the increased serum ALT and AST could be explained by these enzymes leakage into blood because of mycotoxin damage to the liver tissue. Improved serum ALT and AST as well as reduced serum TP and ALB have been previously reported by Cao et al. (2010). Recently, Yang et al. (2012) observed no difference in the content of serum TP, ALB, AST and ALT by feeding corn naturally contaminated with aflatoxin B1 and B2 on day 42. These contradictory results might be related to the dosage and duration of mycotoxin exposure, however, age and species of the bird also should be considered. The 0.05 and 0.1% E-GM administration significantly reversed the mycotoxins influence on serum ALT, AST and ALB. In contrast to the present study, Aravind et al. (2003) stated no improving effect of E-GM on these serum indices.

Differential leukocyte count showed that high mycotoxin contamination (100% corn substitution) increased heterophile counts but decreased lymphocyte. An increase in WBC and heterophile counts in the present study shows that aflatoxin may elicit an inflammatory response in the chickens as Kececi et al. (1998) observed by 2.5 mg/kg aflatoxin application into diet. It has been also demonstrated that aflatoxin caused lymphoid cells depletion in the bursa of Fabricius and thymus (Kiran et al. 1998). In consistent with this experiment Keçeci et al. (1995) reported that aflatoxicosis caused lymphocytopenia and heterophilia in growing chickens. The impact of OTA on lymphoid organs also has been previously studied (Farshid and Rajan 1992). Actively dividing cells in bone marrow, lymph nodes, spleen and thymus can be strictly damaged through trichothecene mycotoxins like DON. Therefore, leukocytes and the immune system are one of the primary targets for trichothecens (Bondy & Pestka 2000). Because the high contamination of OTA (2.2 µg/kg) and DON (206.3 µg/kg) in the present study, it seems that mostly synergetic impact of them besides aflatoxicosis affected leukocyte counts. Interaction of mycotoxin contamination and binder inclusion showed that the adverse effect of high mycotoxin contamination on heterophile and lymphocyte counts was reversed using 0.1% E-GM, however, 0.05% binder was enough to improve WBC in both levels of contaminations. Similarly Swamy et al. (2004) indicated the boosted WBC and lymphocyte counts compared to basal diet supplemented with E-GM.

5. Conclusion

This study suggested that blend of natural mycotoxin contamination severely affected chickens performance, liver and bursa of Fabricius relative weights, some hematological and most of the serum biochemical parameters as well as antibody titre against NDV and IBD virus. Dietary E-GM supplementation efficiently improved acute impacts of toxin contamination. Although 0.05% E-GM in most of the cases could promote the negative effects of toxin contamination but it was not completely able to guarantee the chickens protection against higher than 50% administration of contaminated corn in the diet. Therefore 0.1% of E-GM made a better protection against natural-contaminated diets in higher contamination.

Disclosure statement

No potential conflict of interest was reported by the authors.

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