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Protective Effects of *Bacillus sp.* MBIA2.40 and Gallipro on Growth Performance, Immune Status, Gut Morphology and Serum Biochemistry of Broiler Chickens Feeding by Aflatoxin B1

Abdolmaleki M[1], Saki AA[1] & Alikhani MY[2]

[1]Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
[2]Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran

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Corresponding author
Aliasghar Saki
alisaki34@yahoo.com

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Abstract

A total of 540 one-day old male broilers Ross 308 were allocated to 6 treatments in a completely randomized designed with a 2×3 factorial arrangement (aflatoxin B1 (AFB1) and probiotics) with 6 replicates and 15 birds in each. Factors include aflatoxin B1 (control, 500 ppb) and probiotics (control, 10^8 cfu/mL *Bacillus sp.* MBIA2.40, 0.2 g Gallipro per kg). Results showed that adding 500 ppb AFB1 to broiler diets resulted in a significant decrease of body weight gain and feed intake ($P < 0.05$). The highest of feed conversion ratio was observed in AFB1 group ($P < 0.05$). Average body weight gain was significantly increased while feed conversion ratio decreased by *Bacillus sp.* MBIA2.40 and Gallipro compared with control group ($P < 0.05$). The supplementation of *Bacillus sp.* MBIA2.40 and *Gallipro* in contaminated diets relieved the negative effects of aflatoxin on performance. Increased serum aspartate amino transferase, alanine amino transferase, alkaline phosphatase and lactate dehydrogenase were observed in diet contaminated with AFB1 compared with other groups ($P < 0.05$). The lowest antibody production against Newcastle disease and sheep blood cells and also the lowest skin response to phytohemagglutinin were observed in the AFB1 group ($P < 0.05$). Villus height and width were significantly increased by probiotics diets without AFB1 in compared with others ($P < 0.05$). The crypt depth was significantly higher by birds fed AFB1 rather than that others ($P < 0.05$). Improving villus height, width and crypt depth by the diets containing probiotics + AFB1 were found as similar as the control diet ($P > 0.05$). In general, the results of this study showed that adding of *Bacillus sp*. MBIA2.40 and Gallipro in contaminated diets reduce negative effects of AFB1 and consequently these probiotics (especially *Bacillus sp.* MBIA2.40) have a protective effect against aflatoxicosis in broiler chickens.

Introduction

Aflatoxins (AFs) are the common name for a group of natural toxins which are produced as secondary metabolites by fungi *Aspergillus flavus, Aspergillus nomius, Aspergillus parasiticus, Aspergillus ochraceoroseus, Aspergillus pseudotamarii, Aspergillus bombycis* and *Aspergillus tamari* (Chen *et al.,* 2005; Corassin *et al.,* 2013). They are considered as natural contaminant of animal feeds and human foods (Binder *et al.,* 2007; Kana *et al.*, 2013). It was reported that AFs cause clinical diseases and death in animals and humans due to their teratogenic, carcinogenic, mutagenic, and immunosuppressive effects (Guan *et al,* 2008; Yunus *et al.,* 2011). Aflatoxin B1 has the most biological activity and the most potent naturally occurring mutagens and carcinogens (IARC, 2002). The accumulation of AFB1 in feeds and foods can decrease growth performance and immune response ability, injure intestine, alter blood biochemical chemistry, and damage liver and kidney tissues in broilers (Kermanshahi *et al.*, 2009; Magnoli *et al.,* 2011). The

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investigations revealed that aflatoxins are difficult or inconceivable to be eliminated completely from grains and organisms; their defense mechanism is changed the chemical structure of mycotoxins (Berthiller *et al.,* 2013). Aflatoxins can decrease blood glucose levels which led to reduced feed consumption or impaired carbohydrate metabolism (Zhao *et al.,* 2010). Immune suppression by aflatoxin has been reported in poultry flocks and lead to economic losses in poultry industry (Monson *et al.,* 2015a, 2015b). Due to the harmful effects of aflatoxins on poultry industry, researchers described methods including physical, chemical and biological treatments (Topcu *et al.,* 2010). One of the most promising method is used microorganisms especially probiotic specious and their metabolites to detoxifying AFB1. Probiotics are live microbial feed supplements (direct fed microbial) in animal feeds (Manafi, 2015) which exert positive effects on animal health by improving its environment gastrointestinal, intestinal microbial balance and defending against enteropathogen adhesion and invasion (Dalloul *et al.,* 2003; Vila *et al.,* 2009; Mountzouris *et al.,* 2010). It was documented that polysaccharides and bacterial cell wall peptidoglycans of lactic acid bacteria, *Saccharomyces cerevisiae*, *bacillus genes* and others could bind with mycotoxins and reduce their adverse effects (Li *et al.,* 2010; Cao *et al.,* 2011). It was reported that some specious of *Bacillus genus* such as *bacillus* subtilis (Farzaneh *et al.,* 2012; Yu *et al.,* 2015) and *Bacillus licheniformis* (Petchkongkaew *et al.,* 2008) were effective in aflatoxin degrading. Our lab screened probiotic bacteria *Bacillus* sp. MBIA2.40 (92.98% identification) from gut broilers which exhibited antimicrobial activities against *Escherichia coli*, *Salmonella typhimurium*, and *Salmonella entritidis,* provided resistance to the simulated gut condition, produced strong biofilm, showed properties of hydrophobicity, aggregation and coaggregation and produced extracellular enzymes digestive. In addition, it had a strong ability to detoxify AFB1 (up to 75%) rather than Galiipro (up to 34%) *in vitro* condition (Data are not published). Current research was conducted to evaluating the ability of *bacillus* sp. MBIA2.40 and Galiipro on performance, immune status ability, gut morphology and biochemical chemistry in diets contaminated with AFB1 in broiler chickens.

Materials and methods

Aflatoxin production

Aspergillus parasiticus PTCC-5286 was purchased from the Iranian Research Organization for Science and Technology. Aflatoxin was produced from growing fungus on rice grains. The content of AFB1 was measured by the method of Shotwell *et al.* (1966). Briefly, fermented rice was steamed, dried at 70 ºC and ground to a fine powder. Chloroform, methanol and acetone were used for extracting of aflatoxin from rice powder. Rice powder was analyzed for aflatoxin B1 content through Thin Layer Chromatography method (TLC) (AOAC, 1995). In final, 6.7 g contaminated rice powder per kg of diet (containing 500 ppb AFB1) was added to the basal diet.

Table 1. Ingredients and chemical composition of based diet.

Ingredients	Starter (0-10 days)	Grower (11-24 day)	Finisher (25-42 day)
Corn grain	51.04	51.39	54.62
Soy bean-meal	35.60	36.77	34.61
Corn gluten	5.00	2.00	0.00
Sunflower oil	3.03	5.15	6.50
Oyster shell	1.11	1.00	0.92
Dicalsium phosphate	1.98	1.73	1.49
Vitamin premix[a]	0.25	0.25	0.25
Mineral premix[b]	0.25	0.25	0.25
Salt	0.40	0.40	0.40
L-Lysine	0.28	0.10	0.03
DL- Methionine	0.29	0.26	0.25
L- Threonine	0.10	0.03	0.01
Sand	0.67	0.67	0.67
ME (kcal/kg)	3000	3100	3200
Protein (%)	23	21.5	19.5
Lysine (%)	1.28	1.15	1.02
Methionine+ Cystine (%)	0.95	0.87	0.80
Calcium (%)	0.96	0.87	0.78
Available phosphorous (%)	0.48	0.43	0.39
DCAD[c]	207.84	221.49	215.39

[a]Vitamin premix supplied per kg of diet; Vit A 8800IU, Vit D3 2500IU, Vit E 11IU, Vit B1 1.5 mg, Vit B2, 4.0 mg, Vit B3 (Calcium panthotenate) 8 mg, Vit B5 (Niacin) 35 mg, Vit B6 2.5 mg, Vit B12 0.01 mg, Biotin, 0.15 mg, Folic Acid 0.48 mg, Cholin Chloride 400 mg, Vit K3 2.2 mg.
[b]Mineral premix supplied per kg of diet; Manganese 75 mg, Iron 75 mg, Zinc 64.8 mg, Copper 6.0 mg, Iodine 0.87 mg, Selenium 0.2 mg. [c]DCAD: Dietary Cation-Anion Balance.

Husbandry, diets, and experimental design
A total of 540 one- day–old male broiler chicks (Ross 308) were randomly assigned into a 6 treatment groups with 6 replicates and 15 birds in each. The experimental groups were followed as: control (without any additive or AFB1); AFB1 (500 ppb); *Bacillus sp.* MBIA2.40 (10^8 CFU/ml); Galiipro (0.2 g/kg diet); AFB1 + *Bacillus sp*. MBIA2.40 (500 ppb + 10^8 CFU/ml); AFB1 + Galiipro (500 ppb + 0.2 g/kg diet). *Bacillus sp*. MBIA2.40 (10^8 CFU/ml) and Gallipro (0.2 g/kg diet) were added to drinking water and basal diet respectively. A standard corn-soy bean meal diet was formulated to meet nutrient requirements of Ross 308 international recommendation (2014) (Table 1).

Performance parameters
Body weight (BW) and feed intake (FI) of each replicate were measured weekly and then body weight gain (BWG) and feed conversion ratio (FCR) .calculated

Serum biochemical
At the 42 days of age, two birds were randomly selected per replicate, and then 3 mL of blood was collected from wing vein. Serum samples were separated (centrifuged 1027 g for 15 min) and stored at -20ºC for analysis. The total protein, albumin, glucose, cholesterol, triglyceride, urea, uric acid, calcium and phosphorus content and activities of marker hepatic enzymes in serum including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkalinphosphatase (ALP) and lactate dehydrogenase (LDH) were determined using the clinical chemistry analyzer (commercial kit, Bionic Co, Tehran, Iran).

Humoral immune response to sheep red blood cell (SRBC) and Newcastle disease (ND)
On day 7, birds were vaccinated against Newcastle disease (ND) virus using an eye dropper (Live B1 strain; Vetrina; Zagreb, Croatia). The antibody titer produced against ND was evaluated by hemagglutination inhibition test on serum given on day 14. Sheep red blood cell (5%, 1 mL/bird) was injected into breast muscle on day 27. Blood samples were collected at days 32 and 37. The serum was separated to determine antibody produced against SRBC by Micro-hemagglutination test as described by Peterson et al (1999).

Cellular immune response
The *in vivo* cell mediated immune response to phytohemagglutinin (PHA-P, Gibco, 10ml) was assessed by the method of Corrier and Deloach (1990). On day 41, 0.1 mg/bird PHA-P was injected to the right toe web of 2 birds in each replicates. Then, 0.1 ml sterile phosphate buffer saline was injected intra-dermally left toe web of the same bird. The increase of toe web thickness was measured 24 h after injection.

Tissue sample and measurement
At 42 day of ages, 2 birds in each replicates were slaughtered by cervical dislocation. About 2-3 cm segment from midpoint of the ileum was removed. This segment was fixed in a 10 % buffered formalin solution and processed for measurement of villus characteristics (Uni *et al,* 1995).

Statistical analysis
Data were analyzed with AFB1 levels and feed additive levels as 2 × 2 factorial using a completely randomized design by the GLM procedure (SAS, 2013). Treatment means were compared with Tukey's multiple range tests. All differences were considered significant at P <0.05. There was evaluated the normal distribution of data using Shapiro-Wilk test.

Results
Growth performance
The effects of Bacillus MBIA2.40 and Gallipro on growth performance of contaminated chicken's diets with AFB1 are shown in Table 2. Body weight gain and FI were affected by treatments ($P < 0.05$). Birds fed *Bacillus sp.* MBIA2.40 and Gallipro had significantly increased body weight gain compared to those fed basal and AFB1 diets ($P < 0.05$). Broilers fed diet contained AFB1 had higher FCR rather than others ($P < 0.05$). Feed conversion ratio was significantly higher in birds fed control diet in compared to those fed probiotic diets ($P < 0.05$).

Serum biochemistry and enzymes activities
The serum total protein, albumin, glucose and triglyceride concentrations significantly raised in control group compared with AFB1 group ($P < 0.05$). *Bacillus sp*. MBIA2.40 had higher serum triglyceride compared to control and Gallipro groups ($P < 0.05$). Calcium, phosphorous and cholesterol levels were decreased significantly in birds fed 500 ppb AFB1 compared with others ($P < 0.05$). Birds fed 500 ppb had the highest value of serum urea and uric acid ($P < 0.05$, Table 3). Serum AST, ALT, ALP and LDH increased significantly in treatment inclusion AFB1 compared to others ($P < 0.05$). Adding *Bacillus sp.* MBIA2.40 and Gallipro to the contaminated diets did improve and restore the elevated activity of AST, ALT, and LDH and these treatments had no significant difference with control treatment ($P > 0.05$). The serum activity of ALP did not improve by adding probiotics (Table 4).

Table 1. Effects of *Bacillus sp*. MBIA2.40 and Gallipro in diets containing AFB1 on growth performance of broiler chicks at 0-42 day.

Groups	Feed intake (g/bird)	Body weight gain (g/bird)	Feed conversion ratio
AF (ppb)			
0	5668.49	2577.43	2.20[b]
500	5153.68	2224.44	2.32[a]
SEM	45.549	12.878	0.019
Pro			
0	5118.01	2184.77	2.35[a]
B	5619.31	2531.48	2.22[b]
G	5495.95	2486.55	2.21[b]
SEM	55.787	15.772	0.024
Treat			
C	5639.0[ab]	2454.54[b]	2.29
AF	4597.1[c]	1914.99[d]	2.40
B	5679.9[a]	2642.26[a]	2.15
G	5686.6[a]	2635.49[a]	2.16
AF+B	5558.7[ab]	2420.70[bc]	2.29
AF+ G	5305.3[b]	2337.62[c]	2.27
SEM	78.875	22.305	0.034
P-value			
Pro	<0.0001	<0.0001	0.0006
AF	<0.0001	<0.0001	0.0002
AF× Pro	<0.0001	<0.0001	0.8023

Means with common superscripts in same column are not significantly different ($P < 0.05$). *SEM*: Standard error means; AF: Aflatoxin B1; 500 ug/kg; Pro: Probiotic; B: *Bacillus sp.* MABI.A2.40; G: Gallipro;

Table 2. Effects of *Bacillus sp*. MBIA2.40 and Gallipro on serum biochemistry of broilers fed diets contaminated with 500 ppb AFB1

Groups	TP	Alb	Glc	TG	CL	Urea	Uric acid	Ca	P
AF (ppb)	(g/dL)	(g/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(mg/dL)	(g/dL)	(g/dL)
0	2.68[a]	1.24[a]	191.17[a]	162.00[a]	152.50	3.57	2.48	9.76	7.56
500	2.04[b]	0.94[b]	166.50[b]	107.72[b]	107.17	4.69	3.68	8.01	7.17
SEM	0.184	0.061	7.524	7.525	5.705	0.136	0.102	0.112	0.097
Pro									
0	2.26	0.96	169.67	113.17[b]	113.67	4.87	3.52	7.82	6.94
B	2.63	1.21	187.67	167.17[a]	149.58	3.67	2.58	9.39	7.55
G	2.19	1.09	179.17	124.25[b]	126.25	3.84	3.14	9.45	7.60
SEM	0.226	0.075	9.215	9.217	6.987	0.167	0.125	0.138	0.119
Treat									
C	2.73	1.18	194.00	146.67	154.00a	3.67[b]	2.54[b]	9.92[a]	7.61[a]
AF	1.79	0.74	145.33	79.67	73.33[b]	6.07[a]	4.49[a]	5.72[b]	6.27[b]
B	2.72	1.29	190.33	190.83	151.83[a]	3.62[b]	2.43[b]	9.63[a]	7.55[a]
G	2.59	1.24	189.17	148.50	151.67[a]	3.42[b]	2.47[b]	9.74[a]	7.53[a]
AF+B	2.54	1.14	185.00	143.50	147.33[a]	3.73[b]	2.74[b]	9.16[a]	7.56[a]
AF+ G	1.79	0.93	169.17	100.00	100.83[b]	3.42[b]	3.82[a]	9.17[a]	7.67[a]
SEM	0.319	0.106	13.032	13.034	9.881	0.236	0.177	0.195	0.169
P-value									
Pro	0.3475	0.0767	0.3962	0.0006	0.0037	<0.0001	<0.0001	<0.0001	0.0006
AF	0.0200	0.0016	0.0274	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0072
AF× Pro	0.4566	0.4031	0.2551	0.7018	0.0022	0.0001	0.0003	<0.0001	0.0002

Means with common superscripts in same column are not significantly different ($P < 0.05$). *SEM*: Standard error means; TP: Total protein; Alb: Albumin; Glc: Glucose; TG: Triglyceride; CL: Closterole; Ca: Calcium; P: Phosphorus. AF: Aflatoxin B1; 500 ug/kg; Pro: Probiotic; B: *Bacillus sp.* MABI.A2.40; G: Gallipro;

Table 3. Effects of *Bacillus sp*. MBIA2.40 and Gallipro on enzymes activity in AFB1- contaminated diets in broiler chickens.

Groups	AST	ALT	ALP	LDH
		(IU/L)		
AF (ppb)				
0	188.72	3.46	7503.1	1893.3
500	265.78	4.12	8483.1	2850.1
SEM	9.536	0.149	275.566	89.436
Pro				
0	290.25	4.33	8527.2	2797.6
B	171.75	3.50	7684.3	1910.8
G	219.75	3.55	7767.8	2406.7
SEM	11.679	0.182	337.498	109.536
Treat				
C	226.50^{bc}	3.33^{b}	7304.0^{b}	1991.7^{b}
AF	354.00^{a}	5.33^{a}	9750.3^{a}	3603.5^{a}
B	172.17^{c}	3.42^{b}	7643.5^{b}	1908.3^{b}
G	167.50^{c}	3.64^{b}	7561.7^{b}	1780.0^{b}
AF+B	171.33^{c}	3.59^{b}	7725.0^{ab}	1913.3^{b}
AF+ G	272.00^{b}	3.45^{b}	7973.8^{ab}	3033.3^{b}
SEM	16.516	0.257	477.295	154.907
P-value				
Pro	<0.0001	0.0045	0.1681	<0.0001
AF	<0.0001	0.0039	0.0175	<0.0001
AF× Pro	<0.011	0.0004	0.0397	<0.0001

Means with common superscripts in same column are not significantly different ($P < 0.05$). *SEM*: Standard error means; AST: Aspartate amino transferase; ALT: Alanine amino transferase; ALP: Alkaline phosphatase; LHD: Lactate dehydrogenase. AF: Aflatoxin B1; 500 ug/kg; Pro: Probiotic; B: *Bacillus sp*. MABI.A2.40; G: Gallipro

Table 4. Effects of *Bacillus sp*. MBIA2.40 and Gallipro on antibody titers against Newcastle disease, Sheep red blood cell and Phytohemagglutinin of broilers fed AFB1

Groups	ND14	ND32	SRBC32	SRBC37	PHA-P (mm)
AF (ppb)					
0	2.85	2.28^{a}	2.46^{a}	4.67	0.61^{a}
500	1.78	1.32^{b}	1.54^{b}	3.53	0.37^{b}
SEM	0.085	0.049	0.099	0.143	0.030
Pro					
0	2.00	1.50^{b}	1.64^{b}	3.56	0.35^{b}
B	2.79	2.29^{a}	2.39^{a}	4.50	0.59^{a}
G	2.14	1.60^{b}	1.95^{b}	4.23	0.53^{a}
SEM	0.104	0.061	0.122	0.175	0.037
Treat					
C	2.67^{a}	2.00	2.20	4.46^{ab}	0.51
AF	1.33^{b}	1.00	1.08	2.67^{c}	0.19
B	3.00^{a}	2.71	2.62	4.75^{a}	0.65
G	2.87^{a}	2.12	2.54	4.79^{a}	0.68
AF+B	2.59^{a}	1.87	2.17	4.25^{ab}	0.54
AF+ G	1.42^{b}	1.08	1.37	3.67^{bc}	0.38
SEM	0.147	0.086	0.172	0.247	0.053
P-value					
Pro	<0.0001	<0.0001	0.0006	0.0021	<0.0001
AF	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AF× Pro	0.0021	0.4472	0.0860	0.0464	0.1084

Means with common superscripts in same column are not significantly different ($P < 0.05$). *SEM*: Standard error means; ND: Newcastle disease; SRBC: Sheep red blood cell. PHA-P: Phytohemagglutinin; AF: Aflatoxin B1; 500 ug/kg; Pro: Probiotic; B: *Bacillus sp.* MABI.A2.40; G: Gallipro

Humolar and cellular Immune Response

Antibody titers against ND and SRBC on d 14 and 37 respectively were significantly lower in birds fed contaminated diet with AFB1 compared with others ($P < 0.05$) (Table 5). In aflatoxin- treated groups, AFB1 and AFB1 + Gallipro, antibody production

against ND and SRBC on d 14 were considerably decreased compared to control diet and diet containing *Bacillus sp.* MBIA2.40 ($P < 0.05$). In reviewing immune response to Phytohemagglutinin injection, the lowest skin thickness was observed in AFB1 group ($P < 0.05$). Birds fed *Bacillus sp.* MBIA2.40 and Gallipro could improve swelling of skin rather than control diet ($P < 0.05$).

Gut morphology

There is a decrease in the villus height of birds fed contaminated diet with AFB1 compared to other treatments ($P < 0.05$) (Table 6). Inclusion of *Bacillus sp*. MBIA2.40 and Gallipro in contaminated diets could not well decrease the villus height in compared with inclusion of *Bacillus sp*. MBIA2.40 and Gallipro in diets without AFB1 ($P < 0.05$). Villus with was increased and decreased in birds feeding by probiotics and AFB1 respectively in comparison with control group ($P < 0.05$). Birds feeding by 500 ppb diet had the most crypt depth ($P < 0.05$). All probiotic diets with and without AFB1 had the similar effect on crypt depth ($P > 0.05$). The number of goblet cells in birds fed 500 ppb AFB1 was significantly decreased in compared with control diet ($P < 0.05$). The ratio of villus height to crypt depth was considerably decreased in AFB1 group rather than other groups ($P < 0.05$). Diets containing probiotics without AFB1 had higher villus height/crypt depth rather than those diets with AFB1 and control diet ($P < 0.05$).

Table 5. Effects of *Bacillus sp.* MBIA2. 40 and Gallipro on gut morphology of broilers feeding by 500 ppb AFB1

Groups	Villus Height	Villus With	Crypt depth	Villus Height/crypt depth	Goblet cell
		(um)			
AF (ppb)					
0	1556.50	277.76^{a}	98.56	3.62	7.78^{a}
500	1414.85	212.86^{b}	184.59	2.02	8.78^{b}
SEM	9.171	5.596	4.464	0.097	0.256
Pro					
0	1252.54	144.46^{b}	263.54	0.78	8.92
B	1607.65	302.89^{a}	81.50	3.85	8.08
G	1596.84	288.58^{a}	79.69	3.84	7.83
SEM	11.232	6.853	5.467	0.119	0.313
Treat					
C	1346.79^{c}	190.15	160.35^{b}	1.28^{c}	8.17
AF	1158.28^{d}	98.78	366.72^{a}	0.27^{d}	9.67
B	1657.66^{a}	330.05	70.56^{c}	7.72^{a}	7.67
G	1665.04^{a}	313.10	64.78^{c}	4.86^{a}	7.5
AF+B	1557.63^{b}	275.74	92.45^{c}	2.99^{b}	8.5
AF+ G	1528.63^{b}	264.07	94.61^{c}	2.18^{b}	8.17
SEM	15.884	9.962	7.732	0.168	0.443
P-value					
Pro	<0.0001	<0.0001	<0.0001	<0.0001	0.0516
AF	<0.0001	<0.0001	<0.0001	<0.0001	0.0097
AF× Pro	0.0307	0.0745	<0.0001	0.0141	0.6143

Means with common superscripts in same column are not significantly different ($P < 0.05$). *SEM*: Standard error means; AF: Aflatoxin B1; 500 ug/kg; Pro: Probiotic; B: *Bacillus sp*. MABI.A2.40; G: Gallipro

Discussion

Growth performance

In poultry and livestock, aflatoxicosis symptoms are usually appeared in diminished growth rate and reduced performance. In this study broilers fed diets contaminated with 500 ppb AFB1 showed a significantly declined in FI and BW gain compared with other treatments. This suggesting a toxic effect of lower level of AFB1 on the growth performance which is consistent with previous researches (Zuo *et al.,* 2013; Chibanga *et al.,* 2014) The effects of aflatoxins on BW gain, FI and FCR are likely due to anorexia, reluctance and inhibition of protein synthesis and lipogenesis (Kana *et al.,* 2014; Dhanapal *et al.,* 2014). In current study adding of *Bacillus sp.* MBIA2.40 and Gallipro in contaminated diets were ameliorated the negative effects of aflatoxin B1. In consistent with this finding, Zhang *et al.* (2016) demonstrated that the adding of B. subtilis ANSB060 into moldy diets was significantly recovered the growth performance of ducks Also, Bagherzadeh Kasmani *et al.* (2012) reported that supplementation of *Berevibacillus laterosporus* increased BW gain in contaminated diets of quails as similar as control diet. These results indicate that probiotics have a certain effect on BW gain loss so

that, the inclusion of probiotics in feed have improved the intestinal microflora balanced and eliminated the harmful effects of toxins and foreign agents. It was documented that spores of bacillus probiotics can survive and colonize in broiler intestinal and degrade aflatoxins by theirs polysaccharides cell walls, so that the adsorption of aflatoxins were declined (Fan *et al.,* 2013).

Serum biochemistry and enzymes activities

In this study, AFB1-contaminated diet caused eliminate in serum urea and uric acid concentrations and decline in the levels of serum total protein, albumin, glucose, triglyceride, cholesterol, calcium and phosphorous. It was found that feeding of aflatoxin was significantly decreased serum total protein, albumin, globulin, and phosphorous (chen *et al.,* 2014). Results indicate that inclusion of especially B. sp. MBIA2.40 and Gallipro into AFB1-contaminated feed improve the adverse effects of AFB1 on serum biological parameters. Japanese quails feeding by *Brevibacillus laterosporus* have been shown improves in serum biochemistry in diets contaminated with AFB1 (Bagherzadeh Kasmani *et al.,* 2012). A negative effect of aflatoxin on serum glucose concentration was ameliorated by aluminosilicates, cell wall yeast and probiotic bacteria such as *Bacilli* (Bagherzadeh Kasmani *et al.*, 2012; Bovo *et al.,* 2015). Serum activity of AST, ALT and ALP are known as sensitive serological indicators of the depletion of hepatic tissues and biliary system. In current study, broilers fed 500 ppb AFB1 had significantly increased serum enzymes concentrations in compared with control birds, illustrating aflatoxin toxigenic on liver function that in accordance with previous researches (El-Afifi *et al.,* 2013). Increased in serum AST, ALT, ALP, and LDH were due to hepatocytes damage, consistent with what occurs when the liver is damaged by viral hepatitis. It was found that feeding of aflatoxin significantly decreased serum total protein, albumin, globulin, and phosphorous (Chen *et al.,* 2014). The mechanism of reducing aflatoxins by microorganisms is probably due to their incorporation into cell wall peptidoglycans and polysaccharides (Li *et al.,* 2010). These results showed the degradation of AFB1 by *Bacillus* probiotic bacteria which in agreement with previous researches conducted by Bagherzadeh Kasmani *et al.* (2012) and Zhang *et al.* (2016).

Humolar and cellular Immune Response

Aflatoxins have significant effects on immunosuppression which can be due to prevented RNA polymerase and disturbed the synthesis of albumin, globulins and immunoglobulins (Makinia, 2014). A decrease in the relative weight of bursa of fabricius can be created by necrosis or cell depletion of its, therefore, this phenomenon may be caused immunosuppressive (Sur and Celik, 2003). In this study, broilers fed 500 ppb AFB1 had lower relative weight of bursa of Fabricius rather than others. Several reports have shown that aflatoxins reduce humoral immune by depletion of bursa of Fabricius (Verma *et al.,* 2004). These findings suggest that inclusion of *Bacillus sp*. MBIA2.40 and Gallipro in diets contaminated with AFB1 have a great ability to ameliorate the toxic effects of aflatoxin on immune response. Hashmi *et al.* (2006) reported that inclusion of 100, 200 and 300 ppb aflatoxin with and without yeast cell wall did not significant effect on antibody titer produced against ND. One report demonstrated that broilers fed 0.5, 1 and 2 ppm aflatoxin B1 have shown significant decreased antibody production against SRBC at 32, 37, 42, and 47 in compared with ones in the control group (Verma *et al*, 2004). The lowest skin thickness after challenge with PHA-P was observed in AFB1 group which confirmed the previous results (Barati *et al.,* 2017; Reed *et al.,* 2018). Valchev *et al.,* 2017 have found that the antibody titers against Newcastle disease were decreased by adding of 0.2 or 0.4 ppm AFB1 into turkey broilers diets.

Gut morphology

Gastro intestinal tract is the first organ which it contacts with chemicals, natural toxins and foods and such should be affected with greater potency compared to other organs (Bouhet *et al.,* 2004). The structure and integrity of the gut are important agents in intestinal health and absorption capacity. The small intestine is the main place of nutrient absorption (Zuo *et al.,* 2003). In this work, AFB1- contaminated diet have adverse effect on the height, width, crypt depth, ratio of villus height/crypt depth and goblet cell number in ileum. The results of present experiment is in agreement with Girish and Smith (2008) statement that there was a decreased significantly in the height, width, and surface of villus in the duodenum and jejunum when broilers were fed grains naturally contaminated with deoxynivalenol. Our finding showed that height and crypt depth of villus were improved in birds feeding by probiotics + AFB1. However, the efficacy of probiotics have been proven on improve intestinal morphology. The current study also suggested that probiotics as binder of AFB1 by decreasing in the crypt depth were able to improve intestinal morphology and nutrient absorption. This result is demonstrate that an increased in the villus width of treatments were clearly improved BW gain and FCR. The crypt depth of gut was linearly increased with aflatoxin concentrations (Applegate *et al.,* 2009).

Conclusion

In conclusion, our experiment demonstrates that the presence of low level of aflatoxin B1 in diets could

decrease growth performance, reduce immune response, gut injury, and serum biochemical changes in broilers. *Bacillus sp.* MBIA2.40 specially and Gallipro significantly ameliorated the adverse effects of AFB1. Hence, B. MBIA2.40 specially and Gallipro, as a feed additive for biodegradation of aflatoxins, and B. MBIA2.40 may have promising potential in feed industrial applications.

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