Effect of *Brevibacillus laterosporus* Probiotic on Hematology, Internal Organs, Meat Peroxidation and Ileal Microflora in Japanese Quails Fed Aflatoxin B_1

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ABSTRACT

In order to investigate the Aflatoxin B_1 (AFB_1) removal ability of *Brevibacillus laterosporus* (Bl), 125 male Japanese quails aged 21-day-old were divided into 5 experimental groups with 5 replicates of 5 birds each. The experimental groups were control (basal diet), *Bl* (basal diet+10^8 cfu *Bl* mL^{-1}), AFB_1 (basal diet+2.5 mg AFB_1 kg^{-1}), AFB_1+*Bl* (basal diet+2.5 mg AFB_1 kg^{-1} and 10^8 cfu *Bl* mL^{-1}), and AFB_1+Improved Milbond-TX® (basal diet+2.5 mg AFB_1 and 2.5 g Improved Milbond-TX® kg^{-1}). The AFB_1 decreased hematocrit (P= 0.003), red blood cells (P< 0.001), and white blood cells (P= 0.012) compared to the control while *Bl* probiotic improved those parameters. The highest relative liver and heart weight and lowest foam production and relative weight of bursa of Fabricius were observed in AFB_1 group (P< 0.05). However, the relative weight of bursa of Fabricius and cloacal gland of birds fed *Bl* were similar to those in the control group. The low oxidation stability of meat samples resulting from the use of AFB_1 improved due to use of *Bl* probiotic (P< 0.001). The ileal population of *Escherichia coli* increased in AFB_1 group while the lactic acid bacteria decreased. This condition was reversed due to administration of *Bl* probiotic (P< 0.001). This study clearly showed that indigenous *Bl* probiotic could be effectively used to lessen the negative effects of AFB_1 on meat quality and microbial ecosystem of growing quail chicks.

Keywords: Aflatoxicosis, Gut flora, Meat quality, Quail Spore former probioitcs.

INTRODUCTION

Mycotoxins are the most dangerous contaminants of poultry feeds around the world that result in economical losses (Wu, 2004). Many of disorders in human or animal species may be attributed to the feed-born mycotoxins, which interfere with nervous, cardiovascular, pulmonary, endocrine systems, and alimentary tract. Moreover, some mycotoxins are known as carcinogenic, mutagenic, teratogenic and immunosuppressive agents. Among aflatoxins, Aflatoxin B_1 (AFB_1) is the most potent poison that is primarily produced by *Aspergillus flavus* and *A. parasiticus* fungi (Diekman and Green, 1992). Common signs of aflatoxicosis in poultry are retarded growth, liver and kidney damage, impaired tissue integrity, delayed blood clotting and anemia, susceptibility to bruising and bacterial infections, resulting in disturbance of cellular and humoral immune systems (Pier, 1981). Likewise, feeding AFB_1 to Japanese quail decreased body weight gain and slaughter and carcass weights. Moreover, AFB_1 decreased serum albumin, total protein, and glucose and cholesterol levels, but increased serum uric

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acid, urea and creatinine (Bagherzadeh Kasmani *et al.,* 2012).

Several methods have been developed to reduce aflatoxin contamination of animal feed. Unfortunately, many of them are not of practical routine value (Diaz *et al.*, 2008), but, the biological methods are gaining attention due to their easy usage and inexpensive application (Teniola *et al.*, 2005). Probiotics administration as a biological way to remove aflatoxin (El-Nezami *et al.*, 1998; Peltonen *et al.*, 2000; Peltonen *et al.*, 2001; Lee *et al.*, 2003; Shahin, 2007; Hernandez-Mendoza *et al.*, 2009a; Hernandez-Mendoza *et al.*, 2009b) has been recently used in poultry nutrition (Bagherzadeh Kasmani *et al.*, 2012).

This research aimed to study the aflatoxin removal potential of an indigenous isolate of *Bacillus* probiotics, *Brevibacillus laterosporus* (Bl), in male Japanese quails and evaluate its effects on hematology, internal organs, meat peroxidation and intestinal microflora.

MATERIALS AND METHODS

Probiotic Isolation

Isolation and selection of bacilli is completely described in our previous study (Bagherzadeh Kasmani *et al.*, 2012). Identification of the selected isolate was performed using standard taxonomic descriptions from Sneath (1986) with commercially available strips (API 50CHB, API Laboratory Products Ltd.; Biomerieux-France). The results were analyzed using the API Web database (https://apiweb.biomerieux.com) for species-level identification. The selected isolate was identified as *Brevibacillus laterosporus* (Bl) with 92% identification (ID) which is interpreted as 'good identification'.

Birds and Experimental Diets

In total, 300 Japanese quails (*Coturnix japonica*), aged 21 days, were sexed and male ones were selected. The female of the

Japanese quail has brown speckled chest on a cream or pale brown base. The male has a plain chest with reddish brown tan. For the experiment, 125 male quails were randomly assigned to one of 5 treatment groups with 5 replicates of 5 birds each. The trial lasted for 28 days. The experimental groups were control (without any feed additive or AFB_1), *Bl* (containing 10^8 cfu *Bl* mL^{-1}), AFB_1 (containing 2.5 mg AFB_1 kg^{-1} of diet), AFB_1+*Bl* (containing 2.5 mg AFB_1 kg^{-1} of diet+ 10^8 cfu *Bl* mL^{-1} of drinking water), and AFB_1+Improved Milbond-TX$^®$ (containing 2.5 mg AFB_1 kg^{-1} of diet+2.5 g Improved Milbond-TX$^®$ kg^{-1} of diet), (Improved Milbond-TX$^®$ (IMTX) is an aluminosilicate with over 80% montmorillonite, specially manufactured in the United States to protect against the deleterious effects of aflatoxins and other mycotoxins in the animal industry (Milwhite, Inc.5487 Padre Island HighwayBrownsville, TX 78521). The composition of basal diet is shown in Table 1.

Aflatoxin Production

The AFB_1 was produced from *Aspergillus parasiticus* PTCC-526 culture grown on strile polished rice according to the method of Shotwell *et al.* (1966). The sterile rice was placed in Erlenmeyer flasks and inoculated with 2 mL of the mold suspension, which were allowed to grow for 7 days at 25°C. On day 7, the moldy rice was autoclaved to kill the fungus, dried at 56°C in a forced-air oven for 48 hours, and then ground to a fine powder. The contaminated rice powder was analyzed using an ELISA method (Ridascreen Aflatoxin B_1 Art. No. 1211, R-Biopharm, Darmstadt, Germany) to determine toxin concentration and, based on the analyzed value, the appropriate amount of rice was incorporated into the basal diet to provide the desired amount of 2.5 mg AFB_1 kg^{-1} of feed. Before contamination of feed with AFB_1, all feed ingredients were checked for any contamination with mycotoxins.

Table 1. Composition of the basal diet fed to Japanese quails.

Item	Alignment for Grower (21 to 49 days)
Ingredient (%)	
Yellow corn	42.32
Soybean meal (44% CP)	40.20
Vegetable oil	7.48
Fish meal (65% CP)	7.30
$CaCO_3$	1.21
Di-calcium phosphate	0.01
Sodium chloride	0.28
Mineral and vitamin premix[a]	0.50
DL-Methionine	0.03
Washed fine sand	0.67
Total	100.00
Calculated analysis (%)[b]	
ME (kcal kg^{-1})	3130
CP	25.90
Lys	1.40
Met	0.50
Arg	1.35
Thr	1.06
Met + Cys	0.81
Calcium	0.86
Available phosphorus	0.32

[a] Supplied the following per kilogram of diet: Retinyl acetate, 9,000 IU; cholecalciferol, 2,000 IU; DL-α-tocopheryl acetate, 12.5 IU; menadione sodium bisulfite, 1.76 mg; biotin, 0.12 mg; thiamine, 1.2 mg; riboflavin, 3.2 mg; calcium D-pantothenate, 6.4 mg; pyridoxine, 1.97 mg; nicotinic acid, 28 mg; cyanocobalamine , 0.01 mg; choline chloride, 320 mg; folic acid, 0.38 mg; $MnSO_4.H_2O$, 60 mg; $FeSO_4 .7H_2O$, 80 mg; ZnO, 51.74 mg; $CuSO_4 .5H_2O$, 8 mg; Iodized NaCl, 0.8 mg, Na_2SeO_3, 0.2 mg. [b] Calculated from NRC (1994).

Hematology

At 45 days of age, 2 birds of each replicate were randomly selected and 0.5 mL of blood was taken from the brachial vein using a syringe containing EDTA. Red Blood Cell (RBC) count was performed using a hemacytometer slide and light microscope on whole blood samples containing EDTA after 100-fold dilution with Natt and Herrick diluent (Natt and Herrick, 1952). Packed Cell Volume (PCV) was measured using micro-hematocrit tubes on whole blood samples by centrifugation at 12,000 rpm for 5 minutes (Alexander and Griffiths, 1993a). Hemoglobin (Hb) was determined using cyanomethemoglobin method (Alexander and Griffiths, 1993b).

Internal Organ Weights and Reproductive Organs

At 49 days of age, two birds of each replicate were weighed and then slaughtered for sampling the internal organs. Liver, spleen, bursa, heart, and testes were removed and immediately weighed. The length, width, and height of cloacal gland were measured to calculate the cloacal gland volume before sacrifice, and after squeezing the gland, foam production was measured.

Assessment of Lipid Peroxidation

At the end of experiment (49 days of age), two birds of each replicate were euthanized and deboned meats of breast and thigh portions were grounded with a blender and used for determination of oxidation stability on days 0 and 3 of refrigerated storage (at +4°C) and 30 days of storing in freezer (-20°C). In order to evaluate the extent of lipid peroxidation in meat samples, third-order derivative spectrophotometric method developed by Botsoglou *et al.* (1994) was adopted with minor modifications. In brief, one gram of grounded meat sample was weighed and homogenized (Polytron homogenizer, PCU, Switzerland) with 4 mL of 5% aqueous TriChloroacetic Acid (TCA) and 2.5 mL of 0.8% Butylated HydroxyToluene (BHT) in hexan, and then centrifuged at 3,000×*g* for 3 minutes. The top hexane layer was discarded and the bottom layer was filtered and made to 5 mL volume with 5% TCA, then placed into a screw-capped tube containing 3 mL of 0.8% aqueous 2-thiobarbituric acid. Finally, tubes were heated in 70°C water bath for 30 minutes and immediately cooled under tap water and submitted to spectrophotometry (UNIKON 933, Kontron Co. Ltd., Milan, Italy). The height of the third-order derivative peak that appeared at 521.5 nm was used for calculation of the MalonDiAldehyde (MDA) concentration, as secondary oxidation product. Tetraethoxypropane (1,1,3,3- Tetraethoxy propane, T9889, 97%, Sigma, USA.) was used as a MDA precursor in the standard curve. The concentration of MDA was expressed as mg/kg of meat samples (Botsoglou *et al.*, 1994).

Bacterial Populations of Ileal Content

The ileal contents of two birds in each replicate were individually collected into the sterile tubes for serial dilution. In brief, 1 g of ileal digesta was added into the test tube containing 9 mL of sterilized Phosphate Buffered Saline (PBS). Microbial populations were determined by serial dilution (10^{-4} to 10^{-6}) of ileal samples before inoculation onto Petri dishes. Plates for *Lactobacillus* bacteria (grown in deMan, Rogosa and Sharpe, MRS agar) and *Escherichia coli* bacteria (grown in Mac Conkey agar) were incubated at 37°C in anaerobic and aerobic media, respectively. Plate count agar was used for total count of bacteria. Finally, plates were counted between 24 and 48 hours after inoculation. All agar media were obtained from the Merck Company, Germany. Colony forming units were defined as distinct colonies measuring at least 1 mm in diameter (Rahimi *et al.*, 2011).

Statistical Analysis

The data were analyzed by GLM procedure for completely randomized experimental design with 5 treatments and 5 replicates by using the SAS software (SAS, 2002), and the means were compared by Tukey test at *P*< 0.05. Relative numbers were compared using Kruskal–Wallis test at *P< 0.05*.

RESULTS

The birds fed dietary AFB_1 showed a significant reduction in hematocrit (P= 0.003), red blood cells (P< 0.001), and white blood cells (P= 0.012) compared to the control and *Bl* groups (Table 2). However, the use of *Bl* in the contaminated diets with AFB_1 resulted in increase in the aforementioned parameters. The concentration of hemoglobin decreased with the use of AFB_1 in the diet and feed additives could not increase the hemoglobin concentration of the birds that received AFB_1. The birds fed *Bl* alone had the highest levels of these blood parameters when compared with those of AFB_1 and AFB_1+IMTX groups.

As shown in Table 3, dietary AFB_1 increased the relative weight of liver, gall

Table 2. Effect of Aflatoxin B_1 and feed additives on hematology of growing Japanese quail.

Groups	Aflatoxin B_1 (mg kg^{-1})	Additive [e]	Hematocrit (%)	Red blood cells (Number$\times 10^6$ µl^{-1})	White blood cells (Number$\times 10^6$ µl^{-1})	hemoglobin (g dl^{-1})
Control	0	-	45.6 [a]	47.6 [b]	4.8 [ab]	14.92 [ab]
Bl[a]	0	Bl	46.0 [a]	56.2 [a]	5.2 [a]	15.86 [a]
AFB$_1$[b]	2.5	-	40.8 [b]	33.8 [c]	3.2 [c]	13.80 [b]
AFB$_1$+Bl	2.5	Bl	45.2 [a]	44.6 [b]	4.6 [ab]	14.22 [b]
AFB$_1$+IMTX[c]	2.5	IMTX[f]	43.2 [ab]	43.6 [b]	3.6 [bc]	14.02 [b]
SEM [d]			0.538	1.649	0.227	0.219
P-value			0.003	<0.001	0.012	0.008

[a-c] Different letters in each column shows significant differences (P< 0.05). [a] *Brevibacillus laterosporus;* [b] Aflatoxin B$_1$; [c] Commercial toxin binder (Milwhite Inc.; Brownsville, Texas, USA); [d] Standard Error of Mean, [e] *Bl* (10^8 cfu ml^{-1} of drinking water); [f] (2.5 g kg^{-1} of feed).

Table 3. Effect of Aflatoxin B_1 and feed additives on relative internal organs weight of growing Japanese quail (g/100 g body weight).

Groups	Aflatoxin B$_1$ (mg kg^{-1})	Additive	Bursa of Fabricius	Liver	Gall bladder	Spleen	Heart
Control	0	-	0.041 [ab]	1.39 [b]	0.030 [e]	0.044	0.79 [c]
Bl[a]	0	Bl	0.049 [a]	1.41 [b]	0.047 [c]	0.044	0.88 [bc]
AFB$_1$	2.5	-	0.014 [c]	1.82 [a]	0.064 [a]	0.059	1.20 [a]
AFB$_1$+*Bl*	2.5	Bl	0.041 [ab]	1.53 [b]	0.036 [d]	0.050	0.94 [b]
AFB$_1$+IMTX	2.5	IMTX	0.039 [b]	1.87 [a]	0.056 [b]	0.050	1.12 [a]
SEM			0.002	0.049	0.002	0.002	0.033
P-value			< 0.001	< 0.001	< 0.001	0.192	< 0.001

[a-c] Different letters in each column shows significant differences (P< 0.05). [a] As defined under Table 2.

bladder, and heart when compared to the control and *Bl* groups (P< 0.001). However, the negative effects of AFB$_1$ on these internal organs were disappeared by the use of *Bl* in the diet. IMTX additive was not effective to alleviate the negative effect of AFB1 on the above mentioned tissues. The highest relative weight of bursa was observed in birds fed *Bl* alone, while dietary AFB$_1$ significantly decreased the size of bursa (P< 0.001). Both additives were sufficiently effective to remove the negative effects of AFB$_1$ on bursa, in which the relative weight of this immunity organ was increased up to the control level.

As shown in Table 4, dietary AFB$_1$ significantly decreased the size of testes (P< 0.001), cloacal gland (P= 0.003), and foam production (P< 0.001) in male Japanese quails. In all cases, the highest values were observed in birds fed the control and *Bl*

diets. Surprisingly, the use of *Bl* significantly improved the relative weight of testes and cloacal gland volume resulting in higher foam production than AFB$_1$ group. Although significant improvement in testes size was observed in birds fed AFB$_1$+IMTX, the IMTX additive was not as effective as *Bl* in improving the sexual parameters in male birds that received AFB$_1$.

The use of AFB$_1$ in the diet significantly increased the MDA concentration in stored meat samples (P< 0.001) for 3 or 30 days (Table 5). In fresh samples, the effect of *Bl* on reduction of MDA concentration in birds that received toxin diet was more profound than that of IMTX in which the use of *Bl* decreased MDA levels up to the control levels. The *Bl* was the most effective additive in stored meat samples regarding MDA index, while refrigerating or freezing the meat samples resulted in disclosure of

Table 4. Effect of Aflatoxin B_1 and feed additives on reproductive organs of male Japanese quail.

Groups	Aflatoxin B_1 (mg kg^{-1})	Additive	Testes (g)	Cloacal gland volume (cm^3)	Foam production (mg)
Control	0	-	7.28 [a]	5.54 [a]	44.04 [a]
Bl	0	Bl	7.27 [a]	4.96 [a]	46.50 [a]
AFB$_1$	2.5	-	5.02 [c]	2.92 [b]	15.34 [b]
AFB$_1$+Bl	2.5	Bl	7.22 [a]	4.77 [a]	40.04 [a]
AFB$_1$+IMTX	2.5	IMTX	6.11 [b]	3.32 [b]	20.08 [b]
SEM			0.221	0.279	2.853
P-value			<0.001	0.003	<0.001

[a-c] Different letters in each column shows significant differences (P< 0.05). [a] As defined under Table 2.

Table 5. Effect of Aflatoxin B_1 and feed additives on MalonDiAldehyde (MDA) concentration in growing quails' meat on days 0 and 3 of refrigerated storage (at +4°C) and 30 days of storing in freezer (at -18°C).

Groups	Aflatoxin B_1 (mg kg^{-1})	Additive	MDA (mg/kg of meat)		
			Days of meat storage		
			0	3	30
Control	0	-	0.107 [b]	0.148 [c]	0.204 [c]
Bl [a]	0	Bl	0.081 [c]	0.137 [c]	0.203 [c]
AFB1 [a]	2.5	-	0.121 [ab]	0.416 [a]	0.654 [a]
AFB$_1$+Bl	2.5	Bl	0.092 [c]	0.142 [c]	0.224 [c]
AFB$_1$+IMTX	2.5	IMTX	0.130 [a]	0.348 [b]	0.446 [b]
SEM			0.005	0.032	0.048
P-value			<0.001	<0.001	<0.001

[a-c] Different letters in each column shows significant differences (P< 0.05). [a] As defined under Table 2.

beneficial effects of IMTX. However, the positive effects of IMTX on MDA concentration of stored meat samples were not comparable with that of *Bl* additive.

In comparison with *Bl* group (Table 6), the use of dietary AFB$_1$ significantly decreased the total microbial counts, lactic acid bacteria, and spore-forming bacteria in the ileum of birds (P< 0.001). However, dietary AFB$_1$ increased the CFU of *Escherichia coli* and feed additives exerted the opposite effects. In term of *Escherichia coli* bacteria, the IMTX was more effective than *Bl* and resulted in sharp reduction in *Escherichia coli*. In contrast, the *Bl* probiotic was more effective than IMTX in increasing the lactic acid bacteria, and the lowest total microbial count was observed in AFB$_1$+IMTX group.

DISCUSSION

As shown in this research, blood variables such as hematocrit, red blood cells, and hemoglobin concentration decreased under aflatoxicosis. This finding was in line with Kececi *et al*. (1998), and Oguz and Kurtoglu (2000) who reported that the reduction in blood parameters might be related to the lower protein synthesis and higher hemolysis in affected birds than those in the control group. Indeed, increment in blood cells due to administration of *Bl* probiotic may offer an evidence to augment cell proliferation resulting in increasing hematocrit.

Table 6. Effect of Aflatoxin B_1 and feed additives on the ileal microbial populations of growing Japanese quail (Log CFU g^{-1}).

Groups	Aflatoxin B_1 (mg kg^{-1})	Additive	Total microbial counts	Lactic acid bacteria	*Escherichia coli*	Spore forming bacteria
Control	0	-	8.31 [b]	7.24 [c]	8.24 [c]	6.80 [c]
Bl	0	Bl	9.34 [a]	8.85 [a]	7.89 [d]	7.96 [a]
AFB_1	2.5	-	8.41 [b]	7.18 [c]	8.71 [a]	6.74 [cd]
AFB_1+Bl	2.5	Bl	9.24 [a]	8.07 [b]	8.59 [b]	7.62 [b]
AFB_1+IMTX	2.5	IMTX	7.71 [c]	6.41 [d]	0.00 [e]	6.63 [d]
SEM			0.127	0.172	0.685	0.111
P-value			<0.001	<0.001	<0.001	<0.001

[a-c] Different letters in each column shows significant differences (P< 0.05). As defined under Table 2.

The relative weights of splanchnic tissues increased in birds that received dietary AFB_1. Liver is the target tissue of aflatoxins in birds and increment in relative weight and fat content of liver are the most common signs of toxication. In addition, the relative weight of kidney, pancreas, and spleen of birds also increased under aflatoxicosis (Shi *et al.*, 2006). Denli and Okan (2002) showed that 80 μg kg^{-1} AFB_1 in the diet increased the relative weight of liver in broiler chickens but the addition of 2.5 g kg^{-1} *Saccharomyces cerevisiae* improved the health status of liver. Toxin binders covalently bind the aflatoxins in the gut decreasing toxin availability. It could be hypothesized that *Bl* probiotic may successfully bind AFB_1 in the alimentary tract of quail chicks and help the excretion of dietary toxin and, subsequently, improve the relative weight of splanchnic tissues.

In contrast to viscera tissues, testes size and foam production decreased in male birds fed AFB_1 and the use of *Bl* probiotic in the diet increased the relative weight of testes and foam production in male quails. Changes in genital organs and reduced reproductive performance due to mycotoxin consumption have been already reported (Clarke *et al.*, 1987). However, it has been postulated that negative effects of aflatoxins on reproduction might be indirect through other physiological systems (Diekman and Green, 1992).

The regular increase in MDA concentration in meat samples has been observed at high doses of AFs (Dalvi, 1986). In the present study, the highest concentration of MDA was observed in the birds that received AFB_1 without any additive, but the level of MDA in AFB_1+*Bl* group decreased up to the control level for stored samples (e.g. 3 and 30 days). Kabir (2009) reported that addition of probiotics to diet of broilers might improve the meat quality before and after freezing process. Mahajan *et al*. (2000) indicated that sensory specifications of meat such as appearance, texture, water-holding capacity, and overall desirability were significantly higher in birds that received probiotics than those in the control group.

In this study, lower MDA concentrations were observed in meat samples of birds fed probiotics when compared with those of AFB_1 group. This improvement in meat quality could be associated with higher hematocrit and hemoglobin concentration in birds fed probiotics because more oxygen and nutrients could be supplied to breast and thigh muscles (Hadad *et al*., 2014). An inhibitory effect of aflatoxins on protein synthesis may decrease the synthesis rate of seruloplasmin and transferrin in the liver, which may lead to increase in free copper and iron, respectively. The higher levels of these cations may impair the defense system against lipid peroxidation. Iron plays a particularly important role in the Fenton

reaction, which is one of the most important phases in lipid peroxidation (Gutteridge and Halliwell, 1990; Agil *et al.*, 1995; Kohen and Nyska, 2002). In addition, a decrease in vitamin absorption like vitamin A due to dietary AFB_1 may more weaken the antioxidant defense mechanism. It has been shown that vitamin A in the liver plays a major role in inhibition of lipid peroxidation and could be affected by aflatoxins (Decoudu *et al.*, 1992).

The use of *Bl* probiotic in the diet significantly decreased the *Escherichia coli* in the ileum. This bacterium could adhere to the mucosal surface of intestine and restrict *E-coli* colonization through the competitive exclusion. Dietary probiotics supplements could be used to manipulate the composition of gut microbiota. Theoretically, the mechanisms involved may include: (1) Reduction of lumen pH by lactic acid producing bacteria, (2) Direct antagonistic effects on pathogens, (3) Competition for binding and receptor sites that pathogens may occupy by probiotics, (4) Improved immune function and stimulation of appropriate immunomodulatory cells, and (5) Competition for available nutrients and other growth factors (Collins and Gibson, 1999). It is believed that transient balance between beneficial and harmful microbes may exist in the gut of non-stressed birds. If the microbial balance is prevalent, the birds may exhibit the best efficiency in performance, but under stress conditions the beneficial bacteria tend to be decreased and harmful microbes may increase resulting in either clinical (e.g. diarrhea) or subclinical (e.g. impaired performance and feed efficiency) disorders. Protective microbial population is naturally stable in the gut but it could be affected by some environmental and dietary factors (Kabir, 2009) and the use of probiotics in these conditions may help to re-settle the beneficial protective bacteria (Fuller, 1991).

This study clearly showed that *Bl* as a beneficial probiotic could be used to improve the health status of the birds and intestinal microbial ecosystem. Moreover, this probiotic has profound effects on meat quality and reproductive efficiency when birds have fed on contaminated diets with AFB_1.

ACKNOWLEDGEMENTS

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اثر پروبیوتیک بروباسیلوس لتروسپوروس بر هماتولوژی، اندام‌های داخلی، پراکسیداسیون گوشت و جمعیت میکروبی روده بلدرچین ژاپنی تغذیه شده با آفلاتوکسین

ف. باقرزاده کاسمانی، م. ا. کریمی ترشیزی و م. مهری

چکیده

به منظور بررسی توان یک پروبیوتیک بومی از جنس باسیلوس (بروباسیلوس لتروسپوروس) در حذف آفلاتوکسین B_1 آزمایشی با استفاده از 125 قطعه بلدرچین ژاپنی 21 روزه از جنس نر در 5 تیمار با پنج تکرار و 5 پرنده در هر تکرار انجام شد. تیمارها شامل شاهد (جیره پایه)، پروبیوتیک (جیره پایه + 10^8 واحد تشکیل دهندهٔ کلنی در میلی‌لیتر بروباسیلوس لتروسپوروس)، آفلاتوکسین B_1 (جیره پایه + 2/5 میلی‌گرم آفلاتوکسین B_1 در کیلوگرم)، آفلاتوکسین B_1 + پروبیوتیک (جیره پایه + 2/5 میلی‌گرم آفلاتوکسین B_1 در کیلوگرم و 10^8 واحد تشکیل دهندهٔ کلنی در میلی‌لیتر بروباسیلوس لتروسپوروس) و آفلاتوکسین B_1 + میلبوند TX (جیره پایه + 2/5 میلی‌گرم آفلاتوکسین B_1 در کیلوگرم و 2/5 گرم میلبوند TX در کیلوگرم). تیمار آفلاتوکسین B_1 در مقایسه با شاهد، هماتوکریت ($P = 0/003$)، گلبول‌های قرمز خون ($P < 0/001$) و گلبول‌های سفید خون ($P = 0/012$) را کاهش داد. بیشترین وزن نسبی کبد و قلب و کمترین مقدار تولید کف و کمترین وزن نسبی بورس فابریسیوس در تیمار آفلاتوکسین B_1 مشاهده شد ($P < 0/05$). اما، وزن نسبی بورس فابریسیوس و غدهٔ کلوآکی در پرندگان تغذیه شده با پروبیوتیک مشابه با شاهد بود. پایداری کم لیپید در اثر مصرف آفلاتوکسین، با تغذیهٔ پروبیوتیک بهبود یافت ($P < 0/001$). جمعیت اشریشیاکلی در تیمار آفلاتوکسین افزایش یافت در حالیکه جمعیت باکتری‌های اسید لاکتیک کاهش یافت. عکس این نتیجه در اثر مصرف پروبیوتیک مشاهده شد ($P < 0/001$). این مطالعه نشان داد که پروبیوتیک بومی بروباسلوس لتروسپوروس می‌تواند به‌طور کارآمدی برای حذف اثرات منفی آفلاتوکسین B_1 بر کیفیت گوشت و اکوسیستم میکروبی بلدرچین ژاپنی به کار رود.