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Effects of a multi-strain probiotics against aflatoxicosis in growing Japanese quails

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ABSTRACT

An experiment was conducted to determine the effects of a multi-strain probiotics (PRO) on growth performance and immune status of growing Japanese quails fed aflatoxin B_1 (AFB_1). A total of 320 quail chicks were assigned to 4 treatments, control (basal diet), AFB_1 (basal diet + 2.5 mg AFB_1/kg), PRO (basal diet + 150 mg PRO/kg), and AFB_1 + PRO (basal diet + 2.5 mg AFB_1 and 150 mg PRO/kg) with 4 replicate pens and 20 quails per pen. The quail chicks had free access to water and experimental diets from 7 to 35 d of age, and feed intake (FI) and body weight gain (BWG) were measured weekly. Overall feed intake and BWG were reduced in AFB_1 treatment ($P < 0.05$), but feeding probiotics improved those criteria in quails received dietary AFB_1 ($P < 0.05$). During 7–35 d of age, AFB_1 increased FCR while the PRO supplementation decreased FCR in quails fed AFB_1 diet ($P < 0.05$). Moreover, FCR in AFB_1 + PRO treatment was better than PRO treatment ($P < 0.05$). The most hypertrophy in internal organs (*e.g.*, liver and spleen) and suppressed growth in testes and bursa of Fabricius were observed in AFB_1 group but feeding PRO improved the status of these organs ($P < 0.05$). The humoral (*e.g.*, antibody response against sheep red blood cell antigen) and cellular responses (*e.g.*, skin thickness against 2,4-Dinitro 1-chlorobenzene) were suppressed in quails fed AFB_1 but feeding PRO boosted those immune systems ($P < 0.05$). The use of AFB_1 in diet invariably increased the levels of aspartate transaminase, alanine aminotransferase, and alkaline phosphatase in the sera of Japanese quails ($P < 0.05$). The opposite effect was observed for aforementioned enzymes because of PRO in the diets. This study showed that under aflatoxicosis, the use of dietary multi-strain probiotics could improve the growth performance and immunocompetence in growing Japanese quails.

1. Introduction

Aflatoxins are produced by *Aspergillus* fungi and considered as the main natural contaminants of feedstuffs in the sub-tropical and tropical regions of the world (Sehu et al., 2005). Among different aflatoxins, aflatoxin B_1 (AFB_1) is the most potent aflatoxin, which is produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus* (El-Desouky et al., 2012). In poultry industry, finding an effective way to alleviate the negative effects of AFB_1 on poultry performance and product safety has gained increasing attention in recent years (Gabal and Azzam, 1998; Kececi et al., 1998; Kubena et al., 1998; Sur and Celik, 2003). The main target organ for aflatoxins is the liver and their effects may be attributed to its destructive effects on DNA and RNA strands in the cells. Therefore, not only hepatotoxic consequences of aflatoxins in poultry may result in low growth performance and impaired immunity but also in some changes in relative weight of other organs,

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including spleen, heart, and bursa of Fabricius (Chami et al., 2005; Diaz and Murcia, 2011; Hussain and Chandrasekhara, 1994; Ledoux et al., 1999).

Probiotics (PRO) are live microorganisms, which, when consumed in adequate amounts, can confer a health benefit to the host (Guarner and Schaafsma, 1998). In poultry, probiotics have been shown to promote growth performance, improve the efficiency of feed utilization, modulate the gastrointestinal microbial ecosystem, stimulate the immune system and protect the host from gastrointestinal tract diseases (Gaggìa et al., 2010). Some anti-AFB_1 properties of PRO in avian species have been reported in several *in vitro*, *ex vivo,* and *in vivo* studies (Bagherzadeh Kasmani et al., 2012; Gratz et al., 2004; Hernandez-Mendoza et al., 2009). Possibly, polysaccharides in the cell wall of PRO, such as peptidoglycan and mannans, are the main components playing major role in AFB_1 binding (Decoudu et al., 1992; El-Desouky et al., 2012; Hadiani et al., 2009; Kramer and Hoffmann, 1997). Because of limited information on the possible effects of PRO on the productivity and health status of Japanese quails challenged with AFB_1, this study was conducted to determine the effect of a multi-strain PRO on growth performance and immune status of quail chicks fed AFB_1.

2. Materials and methods

2.1. Japanese quails and experimental diets

Management and handling of the Japanese quails were performed according to the Animal Care and Welfare Committee of Research Institute (University of Zabol, Zabol, Iran). A total of 320 seven-day-old Japanese quail chicks were assigned to 4 teatments, basal control diet (without AFB_1 and PRO), AFB_1 diet (containing 2.5 mg AFB_1/kg), PRO diet (containing 150 mg PRO/kg), and AFB_1+PRO diet (2.5 mg AFB_1 plus 150 mg PRO/kg) in a completely randomized design with 4 replicate pens and 20 quails per pen. The basal diet consisting of corn, soybean meal, corn gluten meal, and rice was formulated to meet or exceed all nutritional needs of growing Japanese quails according to the NRC (1994) recommendations (Table 1). The PRO product used in the present study, which composed of spray-dried bacteria containing *Lactobacillus plantarum* (1.89×10^{10} cfu), *Lactobacillus delbrueckii subsp. Bulgaricus* (3.09×10^{10} cfu), *Lactobacillus acidophilus* (3.09×10^{10} cfu), *Lactobacillus rhamnosus* (3.09×10^{10} cfu), *Bifidobacterium bifidum* (3.00×10^{10} cfu), *Streptococcus salivarius subsp. Thermophilus* (6.15×10^{10} cfu), *Enterococcus faecium* (8.85×10^{10} cfu), *Aspergillus oryza* (7.98×10^{9} cfu), and *Candida pintolopesii* (7.98×10^{9} cfu), was manufactured by a commercial company (Probiotics International Ltd, Somerset, United Kingdom).

2.2. Preparation of aflatoxin B_1

Aflatoxin B_1 was produced by PTCC-5286 strain of *Aspergillus parasiticus* through growing on rice grain and fermentation reactions under constant stirring and controlled temperature (Dashkevicz and Feighner, 1989). Thenafter, the concentration of AFB_1 in contaminated rice samples was determined using an ELISA method (Ridascreen Aflatoxin B_1 Art. No. 1211; R-Biopharm, Darmstadt, Germany). Contaminated rice was incorporated into the basal diet to provide the desired amounts of 2.5 mg AFB_1/kg of feed.

Table 1
Composition of basal diet [a].

Item	Content
Ingredient (%)	
Corn, grain	47.97
Soybean meal (44% CP)	35.00
Corn gluten meal	9.04
Rice[b]	2.18
Sunflower oil	1.60
Dicalcium phosphate	1.44
Limestone	1.26
Sodium bicarbonate	0.30
L-Lys ≅ HCl	0.34
DL-Met	0.14
NaCl	0.15
L-Thr	0.08
Trace mineral premix[c]	0.25
Vitamin premix[d]	0.25
Calculated chemical composition	
ME (kcal/kg)	2950
CP (%)	25.00
Lys (%)	1.40
Met (%)	0.60
TSAA (%)	1.05
Thr (%)	1.00
Trp (%)	0.26
Ca (%)	0.85
Available P (%)	0.35
DEB (mEq/kg)	240

[a] CP=crude protein, ME=metabolizable energy, TSAA=total sulfur amino acids, and DEB=dietary electrolyte balance (Na+K−Cl).

[b] Rice was contaminated with aflatoxin B_1 (AFB_1; 114.68 ppm/kg) for AFB_1 treatments.

[c] Mineral premix provided per kilogram of diet: Mn ($MnSO_4 \cdot H_2O$), 65 mg; Zn (ZnO), 55 mg; Fe ($FeSO_4 \cdot 7H_2O$), 50 mg; Cu ($CuSO_4 \cdot 5H_2O$), 8 mg; I [$Ca(IO_3)2 \cdot H_2O$], 1.8 mg; Se, 0.30 mg; Co (Co_2O_3), 0.20 mg; and Mo, 0.16 mg.

[d] Vitamin premix provided per kilogram of diet: vitamin A (vitamin A acetate), 11,500 IU; cholecalciferol, 2100 IU; vitamin E (dl-α-tocopheryl acetate), 22 IU; vitamin B12, 0.60 mg; riboflavin, 4.4 mg; nicotinamide, 40 mg; calcium pantothenate, 35 mg; menadione (menadione dimethylpyrimidinol), 1.50 mg; folic acid, 0.80 mg; thiamine, 3 mg; pyridoxine, 10 mg; biotin, 1 mg; choline chloride, 560 mg; and ethoxyquin, 125 mg.

2.3. Body growth performance

Feed intake (FI) and body weight gain (BWG) were recorded weekly on a pen basis and mortality was recorded as it occurred. Feed conversion ratio (FCR) was calculated from the FI and BWG data. Two Japanese quails per replicate were killed at the end of experiment by cervical dislocation and internal organs including small intestine, testes, liver, spleen, heart, and bursa of Fabricius were removed and weighed immediately.

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