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Full Length Research Paper

Efficacy of *Pediococcus acidlactici*-based probiotic on intestinal Coliforms and villus height, serum cholesterol level and performance of broiler chickens

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The objective of this research was to investigate the efficacy of *Pediococcus acidlactici*-based probiotic on broilers performance. One hundred and sixty male Ross broiler chicks were allocated in 2 experimental treatments for 6 weeks. The experimental birds received a corn-soybean meal basal diet that was supplemented as follows: "control," with no other additions; "PA," *Pediococcus acidlactici*. For broilers fed diet supplemented with PA, the results showed that body weight (BW) increased and feed conversion ratio (FCR) decreased ($P < 0.05$) when compared to those of the control. Treatment of PA increased ($P < 0.05$) villus height in duodenum and ileum when compared with control. Broilers fed diet supplemented with PA also had lower ($P < 0.05$) coliforms number of the ileum and serum cholesterol level than that of the control. This study showed the beneficial effects of *Pediococcus acidlactici*-based probiotic on broilers performance.

Key words: *Pediococcus acidlactici*, performance, probiotic, broiler chicken.

INTRODUCTION

Beneficial microflora promote gut development and health by influencing enterocyte turnover, competing with pathogenic bacteria for nutrients and binding sites, and producing bacteriostatic compounds that limit the growth of pathogenic bacteria (Farthing, 2004). Current research highlights the role of probiotic microorganisms as a sound alternative to antibiotic growth promoters. Probiotics have been defined as live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). So far, a variety of microbial species such as Lactobacillus, Bacillus, Bifidobacterium, Streptococcus, Enterococcus and Saccharomyces have been used as probiotics in poultry (Owings et al., 1990; Jin et al., 1998; Ghadban, 2002; Kalavathy et al., 2003; Patterson and Burkholder, 2003; Gil De Los Santos et al., 2005). Klaenhammer (1993) found that *Pediococcus acidlactici* can be a good source of probiotic, since it belongs to the homofermentative gram- positive bacteria, able to grow in a wide range of pH, temperatures and osmotic pressures, and thus able to colonize and inhabit the digestive tract. Guerra et al. (2006) suggested that the nonpathogenic and nontoxic bacterium P. acidilactici induces healthy intestinal conditions in pigs. Quarantelli et al. (2008) used the PA (*Pediococcus acidlactici*)-based probiotic to investigate laying hen performance.

Although Lee et al. (2007a,b) examined the influence of PA-based probiotic on coccidiosis in broiler chicken, there is no report about the using of Pediococus strains on broilers performance in a normal condition, because they

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Abbreviations: **PA**, *Pediococcus acidlactici*; **BW**, body weight; **FCR**, feed conversion ratio.

Table 1. Ingredient and chemical composition of the basal diet (% unless otherwise indicated).

Ingredient	1 to 10 days	11 to 28 days	29 to 42 days
Corn	61.00	61.00	62.10
Soybean meal	29.28	28.63	25.94
Corn gluten meal	5.00	4.00	4.50
Vegetable oil	0.00	2.00	3.50
Limestone	1.30	1.18	1.14
Dicalcium phosphate	1.85	1.63	1.48
Salt	0.30	0.30	0.25
Sodium bicarbonate	0.05	0.08	0.18
DL-Met	0.20	0.23	0.15
L-Lys HCl	0.52	0.45	0.26
Vitamin premix[1]	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25
Calculated analysis			
ME, kcal/kg	2900	3000	3100
CP	21.90	21.00	19.90
Met and Cys	0.92	0.92	0.82
Lys	1.38	1.30	1.10
Ca	1.00	0.90	0.85
Avail-P	0.50	0.45	0.42

[1] The vitamin premix supplied the following per kilogram of complete feed: vitamin A, 9,000 IU (retinyl acetate); cholecalciferol, 2,000 IU; vitamin E, 18 IU (dl-a-tocopheryl acetate); vitamin B_{12}, 0.015 mg; menadione, 2 mg; riboflavin, 6.6 mg; thiamine, 1.8 mg; pantothenic acid, 30 mg; niacin, 10 mg; choline, 500 mg; folic acid, 1 mg; biotin, 0.1 mg; pyridoxine, 3 mg.
[2] The mineral premix supplied the following per kilogram of complete feed: manganese ($MnSO_4 \cdot H_2O$), 60 mg; zinc (ZnO), 84 mg; iron ($FeSO_4 \cdot 7H_2O$), 50 mg; copper ($CuSO_4 \cdot 5H_2O$), 10.5 mg; selenium (Na_2SeO_3), 0.2 mg; iodine (Iodized NaCl), 0.8 mg; cobalt ($CoCl_2$), 0.4 mg.

only examined the weight gain of non-challenged and challenged birds just about 6 to 10 days after inoculation of Emeria (Lee et al., 2007a) and also did not use a non-infected supplemented group to show the practical and comparable results of broilers performance from 1 to 42 days of age (Lee et al., 2007b). This issue prompted us to investigate the efficacy of PA-based probiotic on growth performance (body weight and feed conversion ratio) of broiler chickens in a normal condition from 1 to 42 days of age and also to study some physiological parameters that have been usually used to examine the probiotic effects of new supplements in broiler nutrition such as villus height of intestine, serum cholesterol level and coliforms number of the ileum.

MATERIALS AND METHODS

Birds and dietary treatments

One hundred and sixty one (161) -day-old male Ross 308 broiler chicks were randomly divided into 8 groups. Each treatment had 4 replicates and each replicate was assigned to a pen with 20 broilers (2 × 1 m). Birds were raised on floored pen and in an environmentally controlled house with a 23:1 light: dark cycle. Birds were given ad libitum access to water and diet. All procedures were approved by the Animal Care and Welfare Committee of University of Tehran. The experimental treatments received a basal diet that was supplemented as follows: “control,” with no additives; “PA,” 1 × 10^6 *cfu* of *P. acidilactici*/g of the diet. Commercial product of P. acidilactici, Bactocell, was used in supplemented group. Before formulation, each ingredient was analyzed in triplicate for DM, CP, EE, CF, ash, calcium and phosphorus following AOAC (1995). The basal diet was a typical mash corn-soybean meal diet that was formulated to meet Ross 308 broiler nutrient requirements for starter (1 to 10 days), grower (11 to 28 days) and finisher (29 to 42 days) periods (Table 1).

Performance parameters measurement

Chickens were weighed at 1 and 42 days by pen basis to determine average body weight (BW). Feed Intake (FI) per pen was recorded at same age and feed conversion ratio (FCR) was calculated for whole period.

Coliforms count

Sixteen birds per treatment (8 birds on day 21 and 8 birds on day 42) were randomly selected and anesthetized with pentobabitoric

Table 2. Growth performance parameters and villus height of control and PA fed broilers.

Treatment	Growth performance parameters[2]			Villus height[3] (μm)		
	BW (g)	FI (g)	FCR	Duodenum	Jejunum	Ileum
Control[1]	2285[b]	4105	1.83[a]	1736[b]	845	777[b]
PA	2435[a]	4181	1.76[b]	1823[a]	853	826[a]
SEM	30.1	58.4	0.023	18.7	7.4	14.4

[1]Control, without supplementation; PA, 1 × 10^6 cfu of *P. acidilactici* /g of the diet; [2]Performance parameters are BW, body weight on day 42; FI, feed intake; and FCR , feed conversion ratio from 1 to 42 days of age. [3]Villus height was measured from the top of the villus to the top of the lamina propria. [a–b]Means within columns with no common superscript differ significantly (P < 0.05).

Table 3. Coliforms number of ileum and serum cholesterol level of control and PA fed broilers.

Treatment	Coliforms number of ileum (×10^3 cfu/g)	Serum cholesterol level (mg/dl)
Control[1]	9.67[a]	156[a]
PA	7.01[b]	126[b]
SEM	0.934	7.5

[1]Control, without supplementation; PA, 1 × 10^6 cfu of *P. acidilactici* /g of the diet.
[a–b]Means within columns with no common superscript differ significantly (P < 0.05).

acid and one gram of ileal digesta was sampled. Samples were serially diluted and subsequently plated on duplicate MacConkey agar media for the enumeration of coliforms. Plates were then incubated at 37°C for 24 to 72 h, aerobically.

Villus height measurement

Four birds per treatment on day 42 that were sampled for coliforms count were used for villus height measurement. A 1-cm segment of the midpoint of the duodenum, jejunum, and ileum were removed, washed in physiological saline solution, and fixed in 10% bufferedformalin. Each segment was then embedded in paraffin, and a 2-μm section of each sample was placed on a glass slide and stained with hematoxylin and eosin for examination (Sakamoto et al., 2000). Histological sections were examined with a Nikon phase contrast microscope (Nikon Eclipse 80i, Nikon Corp., Tokyo, Japan). Villus height was measured from the top of the villus to the top of the lamina propria. Fifteen measurements were taken per bird for this variable; for purposes of statistical analysis, the average of these values was used.

Analysis of serum cholesterol

Eight birds per treatment were randomly selected to measure the serum cholesterol level. The blood samples of these birds were taken on day 42. The concentration of total cholesterol in serum samples were analyzed by duplicate using an automatic biochemical analyzer (Clima, Ral. Co, Spain), following the instructions of the supplier of the kits (Pars Azmon Co. Tehran, Iran).

Statistical analysis

Data were analyzed in a completely randomized design using the General Linear Model procedures of SAS 9.1 (SAS Institute 2003), and means were compared using LSD test. The effect of time was considered for the analysis of the coliforms count data. However, only treatment effect was presented in the section of results, because time effect was not significant statistically.

RESULTS AND DISCUSSION

Feed intake was not affected by PA treatment (P > 0.05). However this group increased BW (P < 0.05) by 150 g more than control, and FCR decreased to 0.07 in this group (P < 0.05) when compared with control (Table 2). These beneficial effects might be related to general properties of probiotics such as lactic acid and enzyme production, competitive exclusion against pathogens and increase of villus height of intestine. The decreased number of coliforms of ileum (Table 3) and increased villus height of duodenum and ileum (Table 2) were observed by addition of PA in the diet.

Although there is no significant difference of villus height of jejunum, PA group had longer (P < 0.05) villus height in duodenum and ileum than that of the control (Table 2). The increased villus height observed in our study may be explained by the enhanced efficiency of digestion and absorption of the intestine due to a population of beneficial bacteria that supply nutrients and stimulate vascularization and enlargement of intestinal villus (Bedford, 2000; Gilmore and Ferretti, 2003). The PA treated birds had higher villus height than that of the control. This is not clear, although this effect might be related to the short chain fatty acids (especially lactic acid) production.

The coliforms number of the ileum decreased significantly (P < 0.05) in PA group when compared with control (Table 3). The antibacterial activities of LAB have been investigated *in vitro* (Taheri et al., 2009a, b) and in vivo (Jin et al., 1998). Pediococci exert antagonism against other microorganisms, including enteric pathogens, primarily through the production of lactic acid and secretion of bacteriocins known as pediocins (Daeschel and Klaenhammer, 1985). Our results showed the coliform reducing effect of PA-based probiotic.

Serum cholesterol was decreased (P < 0.05) by 19% in PA supplemented birds when compared with the control group (Table 3). A similar reduction of serum cholesterol levels has been found in broilers (Mohan et al., 1996; Jin et al., 1998) and layers (Abdulrahim et al., 1996) fed diets supplemented with Lactobacillus. The decrease in cholesterol level could be due to the coprecipitation of cholesterol with deconjugated bile salts (Klaver and Van der Meer, 1993), but there is no study to show this ability and also its mechanism in PA.

In conclusion, this study showed beneficial effects of dietary inclusion of PA-based probiotic. The treated birds had improved BW and FCR when compared with the control. Also the decrease of coilform number of ileum, increase villus height of duodenum and ileum, and reduction of serum cholesterol were observed by supplementation of PA in the diet of broilers. Hence *P. acidlactici*-based probiotic can be considered as a source of chicken nutritional supplement.

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