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Full Length Research Paper

Effects of dietary probiotic supplementation on promoting performance and serum cholesterol and triglyceride levels in broiler chicks

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The aim of this study was to investigate the effect of two commercially-available probiotics, based on *Enterococcus faecium* and *Bifidobacterium* genera on performance and cholesterol and triglyceride amounts of broiler chicks' sera. One hundred and fifty-six (156), day-old, Ross chicks were randomly divided into groups A, B, and C (52 each). The birds in group A received control diet during the experiment but those in groups B and C were, respectively, given control diet supplemented with *Enterococcus faecium* and *Bifidobacterium* genera probiotics based on their instruction. Each treatment had 4 replicates of 13 broilers. Twenty chicks were bled from each group on days 21, and 42 of age. The sera were assayed for cholesterol and triglyceride levels using commercial biochemical kits. Treatment effects on broiler body weight, feed intake, and feed conversion ratio were determined on a weekly basis. The results showed that in group A, the cholesterol and triglyceride amounts increased with no significant changes until 42 days of age. In groups B and C, these amounts had a non significant decline. At first and second bleeding, the cholesterol and triglyceride amounts of B and C groups were significantly lower than group A, but there was no difference between groups B and C. The administration of these two probiotics affected positively on parameters of broiler performance compared to the control group but there was no significant difference between probiotic-treated groups. It is concluded that inclusion of probiotics based on *Enterococcus faecium* or *Bifidobacterium* genera displayed a growth-promoting effect that was comparable to control diet and also decreased the cholesterol and triglyceride components of broiler chicks' sera.

Key words: Probiotic, *enterococcus faecium* and *bifidobacterium* genera, cholesterol, triglyceride, performance, broiler chicks.

INTRODUCTION

Probiotics or direct-fed microbials are live microbial supplements that, when administered in adequate amounts, confer a beneficial effect on the health of the host by improving its intestinal microbial balance (FAO/WHO, 2001; Fuller, 1989). Among the numerous intestinal microbes, those that are expected to beneficially affect the host by improving the intestinal microbial balance, and hence are selected as probiotics, include species of the genera *Lactobacillus, Bifidobacterium*, and *Enterococcus* (Fuller, 1991; Gordin and Gorbach, 1992). Cholesterol is essential for many functions in body because it acts as a precursor to certain hormones and vitamins; also, it is a component of cell membranes and nerve cells. It is known that elevated levels of total blood cholesterol or other blood lipids (such as triglycerides as the main components) are considered risk factors for development of human coronary heart diseases (Lim et al., 2004).

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Table 1. Composition of control diet[1].

Item	Control diet		
	1 to14days	15 to 28 days	29 to42 days
Ingredients (%)			
Corn	60.12	63.27	66.82
Soybean meal (45%)	26.76	24.81	23.81
Fish meal	7.11	5.00	2.21
Vegetable fat	3.00	4.00	4.00
Limestone	1.59	1.28	1.23
Dicalcium phosphate	0.68	0.90	1.18
Mineral and vitamin premix[2]	0.40	0.40	0.40
Salt	0.24	0.25	0.27
DL-Met	0.10	0.07	0.07
L-Lys	0.00	0.014	0.006
Calculated analysis (per kg of diet)			
ME (MJ)	12.4	12.7	12.7
CP (g)	220.0	200.0	180.0
Fat (g)	61.7	70.30	68.7
Met and Cys (g)	9.5	8.5	7.7
L-Lys (g)	13.7	12.0	10.0

[1]Basal diets contained salinomycin Na as a coccidiosat at 60 mg/kg of feed.
[2]The mineral and vitamin premix provided the following per kilogram of diet: vitamin A 12000 IU; vitamin D3 4000 IU; vitamin E 75 mg; Menadione (vitamin K3) 9 mg; Thiamine 3 mg; Riboflavin 7 mg; Pyridoxine 6 mg; Cyanocobalamin 35 µg; Nicotinic acid 40 mg; Pantothenic acid 15 mg; Folic acid 1.5 mg; Biotin 135 µg; Ascorbic acid 100 mg; Choline chloride 400 mg; Cobalt 250 µg; Iodine 1.5 mg; Selenium 200 µg; Iron 50 mg; Magnesium 150 mg; Copper 15 mg; and Zinc 70 mg.

One of the main roles of probiotics bacteria, clearly identified in human and mice, is control of serum cholesterol and triglyceride levels (Lin et al., 1989; Taranto et al., 1998). Kurtoglu et al. (2004) showed the decrease of serum cholesterol levels with the use of probiotic product containing *Lactobacillus* cultures as an additive to diet for layers. Reduction of serum cholesterol and triglyceridelevels in broiler chicks were also reported by Ignatova et al. (2009) who used a probiotic based on *Lactobacillus* and *Bifidobacterium* strains. There are some evidences proposing that *Lactobacillus* feed supplementation reduces the cholesterol and fatty acid composition of broiler chick's body (Kalavathy et al., 2006).

Numerous studies show that probiotics have positive effects on chicken performance-growth and feed efficiency (Ahmad, 2006; Mountzouris et al., 2007; Abazza et al., 2008; Midilli et al., 2008).

The main aim of this experiment was to study the effects of two commercially-available probiotics (based on *Enterococcus faecium* and *Bifidobacterium* genera, respectively) supplementation to diets on cholesterol and triglyceride levels of broiler chicks' sera. Effect of these two probiotics on broiler performance was also examined.

MATERIALS AND METHODS

Chickens and diets

A total of one hundred and fifty-six (156) day-old broiler chicks (Ross 308) which consists of male sex were obtained from a commercial hatchery with a good reputation of producing disease-free chicks and randomly divided into 3 groups (A, B, and C) of 52 birds each. The birds in group A received control mash diet during the experiment, but those in groups B and C were fed control diet supplemented with commercial probiotics based on *E. faecium* and *Bifidobacterium* genera according to their instructions, respectively. The control diet was a typical corn-soybean diet that was formulated to meet broiler nutrient requirements for starter (1 to 14 days), grower (15 to 28 days), and finisher (29 to 42 days) growth periods (Table 1) (National Research Council, 1994). The basal diet was prepared every 2 wk and was stored in sacks in a cool place.

Each dietary treatment had four replicate pens with 13 chicks per pen and the pens were randomized with respect to the dietary treatments. All chicks were reared under sanitary conditions and feed and water were provided *ad libitum*.

Sampling procedure

On 21st and 42nd days of age, twenty chicks from each group were bled via brachial vein puncture using sterilized needle. It must be

Table 2. Effects of dietary probiotics on serum cholesterol and triglyceride amounts[1] (mg/100 ml) in broiler chicks.

Treatment groups	Cholesterol (mg/100 ml)		Triglyceride (mg/100 ml)	
	21	42	21	42 days
No probiotics (A)	169±0.47[Aa]	175±0.13[Aa]	172±0.33[Aa]	186±0.51[Aa]
Enterococcus faecium (B)	139±0.22[Bb]	126±0.25[Bb]	135±0.28[Bb]	119±0.23[Bb]
Bifidobacterium genera (C)	131±0.32[Cb]	121±0.15[Cb]	132±0.19[Cb]	124±0.27[Cb]

[1]Values represent means ± SE for each treatment; n = 20.
[A-C] For each factor, values in lines with no common capital alphabetic superscripts differ significantly ($p < 0.05$). [a-b] For each factor, values in columns followed by different lower-case superscripts are significantly different ($p< 0.05$).

Table 3. Effects of dietary probiotics on evolution of broiler weekly BW in control, *Enterococcus faecium*, and *Bifidobacterium* genera treatments during the experiment.

Treatment groups	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6
No probiotics (A)	230±8[a]	494±7[a]	826±6[a]	1305±5[a]	1758±8[a]	2260±12[a]
Enterococcus faecium (B)	225±5[a]	489±7[a]	823±8[a]	1323±6[b]	1783±9[b]	2301±6[b]
Bifidobacterium genera (C)	227±11[a]	487±5[a]	822±10[a]	1326±7[b]	1786±6[b]	2307±9[b]

[a-b] The mean values and their SD within a column showing different letters are significant ($p < 0.05$).

explained that, on each sampling, the mean weights of these triple groups were similar. After collecting blood from chicks, they were marked with leg bands, so that they were not reused for blood collection. The separated sera by centrifugation (1000 rpm, 5 min) were stored at - 40°C until the end of the experiment.

Chemical analysis

The assessment of cholesterol and triglyceride levels of prepared sera was made by using commercially-available biochemical kits (ZiestChem, Iran). All sera were analyzed in duplicate.

Performance parameters

Chickens in each pen were weighed on a weekly basis (that is, wk 1 to 6) to determine average body weight (BW) and weight gain (WG). Feed intake (FI) per pen was recorded weekly, and FCR was also calculated weekly (FI/WG). Feed intake was calculated from the difference between supplied feed and feed left in each pen. Feed weighing was performed on the same dates the birds were weighed. Feed conversion was calculated from the ratio between total feed intake and weight gain in the period in each pen and was adjusted for mortality (weight gain and feed intake of birds that died were included).

Statistical analysis

The data were compared by analysis of variance (ANOVA) and Duncan multiple range test (Snedecor and Cochran, 1980) using SPSS software (SPSS, 2002). The level of p-*value* <0.05 were considered as significant.

RESULTS

The results of Table 2 show that in control chicks (group A), the cholesterol and triglyceride amounts increased without any significant changes ($p > 0.05$) until 42 days of age. In group B and C, the cholesterol and triglyceride amounts had a non-significant decline with aging ($p > 0.05$). At first and second bleeding, the cholesterol and triglyceride amounts of B and C groups were significantly lower than group A ($p < 0.05$), whereas there was no difference ($p > 0.05$) among group B and C.

The weekly progress of broiler BW and FCR values, in each group, during the experiment are shown in Table 3 and 4, respectively. Average BW of newly hatched broilers was 40.9 ± 0.61 g and did not differ among treatments.

DISCUSSION

The broilers from groups receiving probiotic had higher live weight compared with those from the control group as well at 4, 5, and 6th weeks of age as at the end of the experimental period (Table 3). These results are in agreement with other research studies with chickens fed probiotics (Mountzouris et al., 2007; Samli et al., 2007; Abaza et al., 2008).

Total feed intake was higher for broilers fed with probiotic supplemented diets, than for those fed with the control diet without additive, but there was no significant

Table 4. Effects of dietary probiotics on feed convertion ratio in control, *Enterococcus faecium*, and *Bifidobacterium* genera treatments during the experiment.

Treatment groups	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6
No probiotics	1.68^a	1.71^a	1.80^a	1.88^a	1.89^a	2.02^a
Enterococcus faecium (B)	1.65^a	1.7^a	1.72^b	1.80^b	1.86^a	1.92^b
Bifidobacterium genera (C)	1.66^a	1.68^a	1.73^b	1.79^b	1.86^a	1.94^b

The mean values and their SD within a column showing different letters are significant ($p < 0.05$).

difference between two probiotic groups. Similar results for feed intake and feed utilization in chickens fed probiotics were reported by other authors (Mountzouris et al., 2007; Chafai et al., 2007).

The probiotic supplementations reduced the serum cholesterol and triglyceride significantly. The results obtained in this study are in accordance with those reported by Chafai et al. (2007) and Ignatova et al. (2009) who in experiment with broiler chickens fed probiotic, have found a significant difference between treatments for serum lipids.

Nowadays, cardiovascular diseases are the main causes of death in most countries and it is strongly associated with hypercholesterolemia and hyperlipidemia (Lee et al., 1992). Because serum cholesterol and triglyceride levels are strongly associated to the lipid component of diet, decreasing the cholesterol and lipid composition of human food could, therefore, be very important to decrease the rate of cardiovascular disorders (Hu and Willett, 2002; Wang et al., 2003; Rivellese, 2005).

With renewal of interest in the use of probiotics in broiler nutrition, development of more effective probiotic preparations complying with various probiotic characteristics has been found to improve the lipid component of blood serum and thus the lipid composition of carcass. Cholesterol levels in meat have received an increased interest considering their implications for human health and product quality (Wood et al., 2004; Muchenje et al., 2009). Kalavathy et al. (2006) proposed that Lactobacillus probiotic supplementation can reduce the cholesterol in the carcass and liver and fat contents in the carcass, muscle and liver.

With due attention to the results obtained from the current study, it is concluded that, like *Lactobacillus* probiotics, diet supplementation with *E. faecium* or *Bifidobacterium* genera probiotics decreases the cholesterol and triglyceride components of broiler chicks' sera. However, an overall judgment about their effects on lipid composition of carcass needs further study for evaluating the cholesterol and triglyceride levels of muscles and liver.

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1