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The Effect of Probiotic Supplementation on Production Performance, Egg Quality and Serum and Egg Chemical Composition of Lying Hens

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Abstract: A study was conducted to evaluate the effects of addition different levels of probiotic to practical-type diet on the performance, egg quality and blood parameters of laying hens. The levels of probiotc was (0, 1000 and 2000 g Bioplus 2B ton^{-1} DM which provide 0, 3.2×10^6 and 6.4×10^6 cfu g^{-1} bacteria in feed). Twenty-seven Hy-Line W-36 strains of white Leghorn laying hens were assigned by a completely randomized design to 3 treatments with 3 replicates for each. Addition probiotic to the diets caused to significant increase ($p<0.05$) of egg production and egg mass and significant decrease ($p<0.05$) of feed conversion ratio. Probiobic improved egg weight, shell hardness and Haugh unit but not significant ($p>0.05$). Probiotic supplementation significantly decrease ($p<0.05$) the cholesterol and triglyceride of plasma also yolk cholesterol. Generally, the results showed that using 1000 g ton^{-1} probiotic had the best results than level of 2000 g ton^{-1}.

Key words: Hens, egg, probiotic, performance, cholesterol, Iran

INTRODUCTION

Antibiotics have been used to stimulate growth in poultry and to avoid of disease. But by long term use, side effects of them occur like residues in meat (Cavazzoni *et al*., 1998). So, the uses of probiotic in poultry diets have become popular as an alternative to antibiotic for poultry production in recent years.

Microbial flora of the gastro intestinal tract plays an important role in the health and performance of the poultry (Yu *et al*., 1999). Pathogenic microbial flora competes with the host for nutrients in gastro intestinal tract (Alp *et al*., 1999). Probiotics are defined as live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance which production acids (e.g., acetic acid and lactic acid) and other compounds that prevent from the growth of pathogenic bacteria, help the growth, multiplication and establishment of beneficial in the intestinal environment (Fuller, 1989). Probiotic is a generic term and products can contain yeast cells, bacterial cultures or both that stimulate micro organisms capable modifying the gastrointestinal environment to favor health status and improve feed efficiency (Dierck, 1989). For example bacteria such as (Bacilli, Bifidobacteria, Enterococcus, Lactobacillus and Streptococcus), Yeasts (*Saccharomyces cerevisiae*), Fungi (*Aspergillus oryzae* and *Aspergillus niger*) and indefinite mixed cultures have been used as probiotics (Simon *et al*., 2001). Using of probiotics for broiler chickens caused to improvement in growth (Yeo and Kim, 1997), reduction mortality and improvement feed conversion efficiency (Yeo and Kim, 1997). In laying hens Yoruk *et al*. (2004) showed that addition of probiotics which contains bacterial cultures (Lactobacillus, Bifidobacterium and streptococcus) in the second period of laying, egg production was positively affected and linearly increased with increasing dose of probiotics, feed consumption was better but different between egg weights not significant.

Addition probiotic improved egg size, egg production and egg quality (Haddadin *et al*., 1996) and supplementation of a lactobacillus culture improved feed efficiency, egg weight, egg production and albumen quality (Tortuero and Fernandoz, 1995). Although, other experiment showed no improvement in hen day egg production, feed efficiency and egg size when laying hens were fed probiotic in their diets (Mahdavi *et al*., 2005). It is also reported probiotic supplementation can depress cholesterol concentration in blood and egg yolk (Haddadin *et al*., 1996; Mohan *et al*., 1995). Numerous studies have been conducted to determine the effect of probiotic on performance during the growing period in broiler therefore, the objective of this study were to investigate the effects of supplementation of probiotic on production, quality parameters and blood parameters of hen's egg.

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MATERIALS AND METHODS

Twenty-seven Hy-Line W-36 strains of white Leghorn laying hens was used in a completely randomized design considering 3 treatments with 3 replicates and 3 samples in each. At the beginning of the experiment, hens of all treatments had similar body weight, egg production and egg weight. During 8 weeks experiment periods all hens in treatments received the same basal diet (Table 1) which was formulated according to nutrient requirement of laying hens (National Research Council, 1994). Treatments included three probiotic concentrations (0, 1000 and 2000 g ton^{-1} of feed). Commercial probiotic used in this study was Bioplus 2B. The product contained 2 strains of bacilli, *Bacillus subtilis* (CH201) and *Bacillus licheniformis* (CH 200) with a minimum of 3.2×10^9 cfu g^{-1} of the product.

Data and sample collection: During the 8 weeks of the experiment (41-49 weeks old), conventional management procedures were employed, artificial light was provided for 16 h day^{-1}, ambient temperature was controlled and hens had free access to feed and water. Feed consumption was recorded at the end of 4 weeks of the experiment period. Egg weight, shell thickness, shell hardness, yolk quality and albumen quality were measured for consecutive day at the end of 4 weeks period and egg production was recorded daily.

Table 1: Composition of laying hen basal diet

Ingredient (%)	Basal diet
Corn	57.10
Soybean meal	25.30
Fish meal	2.50
Soybean oil	2.85
Oyster shell	5.50
Limestone	3.80
Dicalcium phosphate	1.30
Salt	0.17
Bicarbonate sodium	0.15
DL-Methionine	0.15
Vitamin complex[1]	0.50
Mineral complex[2]	0.50
Vitamin D	0.10
Vitamin E	0.10
Calculated chemical composition	
ME (Kcal kg^{-1})	2800.00
Crud protein (%)	17.50
Calcium (%)	4.03
Available phosphate	0.42
Methionine (%)	0.42
Methionine+cystein (%)	0.70
Lysine (%)	0.94

[1]vitamin complex provided per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 2500 IU; vitamin E, 10IU; vitamin B_1, 2.2 mg; vitamin B_2, 4 mg; pantothenic acid, 8 mg; vitamin B_6, 2 mg; niacin, 30 mg; vitamin B_{12}, 0.15; Folic acid, 0.5 mg; biotin, 0.15 mg; Colin chloride, 200 mg; [2]Mineral premix provided per kilogram of diet: manganese, 80 mg; Copper, 10 mg; iodine, 0.8 mg; cobalt, 0.25 mg; selenium, 0.3 mg; zinc, 80 mg; Iron, 80 mg

Chemical composition: At the 8th week of the experimental period 4 mL of blood was collected from wing vein from 6 birds in each treatment (2 from each subgroup). Blood samples were centrifuged at 4000 rpm for 15 min to obtain blood serum. Serum was stored at -20°C for analysis. For measurement cholesterol and triglyceride blood using appropriate laboratory kits (Gowenlock *et al*., 1988). Yolk cholesterol was determined during last week of the trial. Yolk cholesterol was extracted by the method of Folch *et al*. (1957) as modified by Washburn and Nix (1974) from three eggs of each replicate.

Statistical analysis: All data were analyzed using General Linear Model (GLM) producer of SAS Institute (1999) and when the means were significant ($p<0.05$), Duncan multiple range test was used to determine treatments difference.

RESULTS AND DISCUSSION

Production characteristics: Effects of dietary probiotics on egg production, egg weight, feed consumption, egg mass and feed conversion ratio shown in Table 2. Probiotic supplementation significantly increased egg production and egg mass ($p<0.05$) also decreased feed conversion ratio compared to control treatment but inclusion of probiotic had no significant affect ($p>0.05$) on feed consumption and egg weight. The performance of lying hens for diets contain 1000 g porobiotic ton^{-1} was better than 2000 g ton^{-1} in all traits.

Schneitz *et al*. (1998) reported that there were no changes in intestinal pH and volatile fatty acid concentration but there was linear increase in digestibility of nutrients in hens supplemented with increasing levels of probiotic also Mohan *et al*. (1995) reported a quadratic increase in egg production in chickens supplemental with 0, 100 and 150 mg probiotic (*Lactobacillus*, *Bifidobacterium*, *Aspergillus* and *Torulopsis* sp. at 27×10^9 cfu log^{-1}) per kilogram of diet during the peak period which was agree with the results of present experiment because use probiotic at level of 1000 g ton^{-1} had best performance.

Results this experiment are agreement with Balevi *et al*. (2001) that found feed efficiency was better ($p<0.05$) in the group of hens that consume probiotic but contract with Nahashon *et al*. (1994) that reported feed efficiency for hens fed a control diet significantly was better than the hens fed diets supplemented with lactobacillus. Also, Mahdavi *et al*. (2005) did not find any significant difference on production characteristics in laying hens which fed by diets contain probiotic. Nahashon *et al*. (1996) and Haddadin *et al*. (1996)

Table 2: Effects of probiotic on production characteristic s in laying hen's

Probiotic concentration ($g\ ton^{-1}$ feed)	Egg production (%)	Egg weight (g)	Feed consumption (g/hen/d)	Egg mass (g/hen/d)	Feed conversion ratio (g/g)
0	79.69[b]	57.43	103.80	45.90[b]	2.263[a]
1000	84.17[a]	60.66	103.70	51.03[a]	2.032[b]
2000	82.03[ab]	59.33	102.20	49.19[a]	2.078[ab]
SE	0.78	0.79	1.32	0.79	0.042

Means followed by the same superscript letters in each column are not significant ($p<0.05$)

Table 3: Effects of probitic on egg quality traits

Probiotic concentration ($g\ ton^{-1}$ Feed)	Shell thickness (mm)	Shell hardness ($kg\ cm^{-1}$)	Haugh unit	Yolk index
0	0.373	3.100	55.70	0.402[b]
1000	0.370	3.460	65.34	0.420[a]
2000	0.376	3.206	67.39	0.407[ab]
SE	0.002	0.078	2.69	0.003

Means followed by the same superscript letters in each column are not significant ($p<0.05$)

suggested that addition of biological additives had no significant influence on the egg weight ($p>0.05$) that was agree with the results of present experiment.

Egg quality trait: Analysis of the shell thickness, shell hard, Haugh unit and yolk index are shown in Table 3. Addition of probiotic had no significant effect ($p>0.05$) on shell thickness, shell hardness and Haugh unit, although adding probiotic trend to improved these traits. Probiotc supplementation significantly increased ($p<0.05$) yolk index. Addition of probiotic had no significant effect on shell hardness and thickness (Haddadin *et al*., 1996; Mohan *et al*., 1995) which was agree with the present experiments. Nahashon *et al*. (1994) showed the decrease of pH in gastrointestinal tract of laying hen due to probiotic dietary supplementation caused in an increase in absorption of calcium and phosphorus from the digestive tract. Mohan *et al*. (1995) reported slight improvement in egg shell thickness in hens supplemented with probiotic during the peak period but Tortuero and Fernandoz (1995) showed that egg shell calcium was not affected by the addition of *Streptococcus faecium* to the diet of laying hen.

Blood parameters: The effect of probiotic on plasma cholesterol and triglycerides and yolk cholesterol are shown in Table 4. Results showed that using probiotic had significant effect ($p<0.05$) on plasma cholesterol and triglyceride and yolk cholesterol as addition probiotic decreased these trait. Haddadin *et al*. (1996) reported that probiotics reduce the plasma cholesterol and triglyceride which the results of present experiment confirmed them. The use of probiotc may active the lactic acid producing bacteria, production of enzymes disintegrating bile salts and de-conjugating them

Table 4: Effects of probiotic on plasma cholesterol, triglyceride and yolk cholesterol

Probiotic concentration ($g\ ton^{-1}$ Feed)	Plasma cholesterol ($mg\ dL^{-1}$)	Plasma triglyceride ($mg\ dL^{-1}$)	Yolk cholesterol $mg\ g^{-1}$ yolk
0	167.60[a]	1694.6[a]	11.730[a]
1000	141.60[b]	1379.0[b]	10.100[b]
2000	145.60[b]	1461.0[b]	10.800[b]
SEM	4.72	51.9	0.269

Means followed by the same superscript letters in each column are not significant ($p<0.05$)

as well as reduction of the pH in the intestinal tract these changes can be effective in reducing the cholesterol. In low pH solvability of non-conjugated bile acids reduced, thus they absorbed decrease from the intestine (Klaver and Van der Meer, 1993). Probiotic such as lactobacilli can assimilate cholesterol and de-conjugated bile acids and this leads to a reduction in serum cholesterol levels (Gilliland and Walker, 1990; Gilliland and Speck, 1977). Elkin and Yan (1999) reported that cholesterol content in the eggs is influenced by genetic factors, diet composition, lay intensity and layer age. Haddadin *et al*. (1996) and Hargis (1988) suggested a link between serum and egg lipid. So reduce serum cholesterol can lead to a reduction yolk cholesterol levels.

CONCLUSION

Therefore, addition probiotic improved the egg production, egg weight, egg mass, feed conversion ratio, shell hardness, cholesterol and triglyceride of plasma and yolk cholesterol and diet contain 1000 g probiotic ton^{-1} had the best results.

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