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Effect of Probiotic Supplements on Egg Quality and Laying Hen's Performance

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Abstract: The effect of probiotic supplements (0, 400, 1000 and 2000 gr Bioplus 2B ton^{-1} feed providing 0, 1.28×10^6, 3.2×10^6 and 4.6×10^6 cfu gr^{-1} feed concentration) on egg quality and laying hen's performance was investigated on eighty white leghorn Hy-Line, W-36 strain. Evaluated traits were egg production, egg weight, egg mass, feed consumption, feed conversion ratio, shell thickness, shell hardness, Haugh unit, egg cholesterol, plasma cholesterol, plasma triglyceride and histological changes of duodenum. Although, using the different levels of probiotic caused highly significant increase ($P<0.01$) in goblet cell numbers, significant increase ($P<0.05$) in destroying apical cells of villus and significant decrease ($P<0.05$) in plasma cholesterol, plasma triglyceride and egg cholesterol (mg gr^{-1} of yolk), but it had no significant effects on other traits.

Key words: Probiotic, duodenum, egg quality, performance, laying hens

Introduction

Probiotics (meaning ‘‘for life’’) are defined as microbial cell preparations that have a beneficial effect on the health and wellbeing of the host (Fuller, 1989). Direct fed microbials benefit the host animal by stimulating appetite (Nahashon *et al*., 1992; Nahashon *et al*., 1993), improve intestinal microbial balance (Fuller, 1989), synthesize vitamins (Coates and Fuller, 1977), stimulate the immune system (Toms and Powrie, 2001), produce the digestive enzyme (Gilliland and Kim, 1984; Saarela *et al*., 2000), utilize undigestible carbohydrate (Prins, 1977), stimulate lactic acid and volatile fatty acids production (Bailey, 1987), produce toxic compounds such as volatile fatty acids, decrease pH and release bacteriocins (Rolfe, 2000) that compete with other microbes for adhesive site (Dunham *et al*., 1993). Regarding the controversial results about using biological additives, the strain, concentration and form of them (viability, dryness or their products) shoud be considered.

Feeding viable *Lactobacillus* at 1100 mg kg^{-1} (4.4×10^7 colony forming units [(cfu) mg^{-1}] increased daily feed consumption, egg size, nitrogen and calcium retentions and decreased intestinal length from 7 to 59 weeks of age (Nahashon *et al*., 1996). Haddadin *et al*. (1996) reported that egg production, egg size and egg quality were improved by the addition of a liquid culture of *Lactobacillus acidophilus* to the basal diet. Goodling *et al*. (1987) observed no improvement in hen day egg production, feed efficiency, livability and egg size when laying pullets were fed a drived non-viable *Lactobacillus* product. The addition of *Lactobacillus acidophilus* plus *Lactobacillus casei* mixed culture to maize-barley (50/50) diet improved hen day egg production, feed conversion ratio, egg weight and albumen quality (Tortuero and Fernandez, 1995). Although, in barley based diets, addition of Lactobacillus acidophilus plus Lactobacillus casei mixed culture and Bacillus cereus, increased hen day egg production, egg weight and albumen quality, but there were no differences in feed intake, feed conversion ratio and egg specific gravity (Tortuero and Fernandez, 1995). It is also reported that some body and product factors are influenced by biological additives, for instance probiotic supplementation can depress cholesterol concentrations in blood and egg yolk (Abdulrahman *et al*., 1996; Haddadin *et al*., 1996; Mohan *et al*., 1995).

The purpose of this study was to investigate the effects of probiotic inclusion supplements on laying hen's performance, egg quality, blood factors and histological changes in duodenum.

Materials and Methods

Eighty white leghorn hens Hy-Line, W-36 strain were randomly allocated in a completely randomized design considering 4 treatments with 4 replicates and 5 samples in each. Supposed treatments included four probiotic concentration (0, 400, 1000 and 2000 gr ton^{-1} feed providing 0, 1.28×10^6, 3.2×10^6 and 4.6×10^6 cfu gr^{-1} feed concentration). Bioplus 2B, a commercial probiotic preparation, was used in this study. The product contained 2 strains of bacilli. *Bacillus subtilis* (CH201) and *Bacillus licheniformis* (CH200) with a minimum of 3.2×10^9 cfu gr^{-1} of the product.

During the 12 weeks of the experiment (28-39 weeks-old) hens had free access to feed and water. The basal diets are shown in Table 1. The photoperiod was 14 h light d^{-1}. Feed consumption were recorded at the end of each four weeks of the experimental period. Egg weight, shell thickness, shell hardness and albumen quality (Hough unit score) were measured for 3 consecutive days at the end of each four weeks period and egg

Table 1: Composition of experimental basal diets

Ingredients (%)	
Yellow corn	64
Soybean meal	20
Fish meal	3
Soybean oil	0.5
Oyster shell	8.1
Dicalcium phosphate	0.8
Vitamin premix[1]	0.25
Mineral premix[2]	0.25
Salt	0.3
DL-methionine	0.1
Vitamin D_3	0.03
Sand	2.67
Calculated analysis	
Metabolizable energy (kcal/kg)	2717.3
Crude protein (%)	16
Crude fiber (%)	3.06
Methionine (%)	0.4
Methionine+Cysteine (%)	0.65
L-Lysine (%)	0.84
Calcium (%)	3.48
Available phosphate (%)	0.35

[1]Vitamin premix provided per kilogram of diet: vitamin A, 10000 IU; vitamin D3, 2500 IU; vitamin E, 10 IU; 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}, .015 mg; folic acid, 0.5 mg; biotin, 0.15 mg; cholin 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.

production were recorded daily. Yolk cholesterol, plasma cholesterol and triglyceride were determined during the last week of the trial. Yolk cholesterol was extracted by the method of Folch *et al*. (1956) as modified by Washburn and Nix (1974) from two eggs of each replicate.

Blood samples from the brachial vein of two hens in each replicate, were drawn and centrifuged (3000×g for 15 min) immediately and plasma collected. Plasma and yolk cholesterol was estimated by the colorimetric Libermann-Burchard method.

At the end of the trial, 2 hens were randomly sacrificed from each treatment for studying histological changes in duodenum. A 2-cm length of descending duodenal segment was excised for light microscopic observations. They were immediately fixed at 10% formaldehyde solution, and the fixed samples were embedded in paraffin. Transverse and longitudinal sections were prepared with microtome with 5-µm thickness, then stained with hematoxyline - eosin (HE) and examined under the light microscope.

Statistical analysis: Data were analyzed using the General Linear Models (GLM) procedure of Statistical Analyses Systems (SAS, 1999). The following model was assumed in the analysis of all traits. $Y_{ij} = \mu + A_i + e_{ij}$ where Y_{ij}= observed value for a particular character, μ = overal mean, A_i= effect of the i^{th} treatment and e_{ij}= random error associated with the ij^{th} recording .

Results and Discussion

Production characteristics: Analysis of the egg weight, egg production, egg mass, feed consumption and feed conversion ratio data are shown in Table 2. Inclusion of probiotic caused no significant decrease in feed consumption, egg production and egg weight (P>0.05). Because using the third and fourth levels of probiotic caused serious damages to absorptive area of digestive system (Fig. 1). Another reason to variable effect of biological additives may be confounded by variations in gut flora and environmental conditions. In research conducted with laying hens under different climatic and geographical locations, Miles *et al.* (1981) showed that feeding live *Lactobacillus acidophilus* culture resulted significant increase in egg production at one location, a numerical improvement at the second and no difference at the third location.

Probiotic inclusion did not influence the egg weight significantly, which has already been reported by Cerniglia *et al.* (1983), Mohan *et al*. (1995), Haddadin *et al*. (1996) and Chen and Chen (2003). But there are also some reports which have different opinions (Nahashon *et al*., 1992; Tortuero and Fernandez, 1995), that might be related to the strain of bacteriae, concentration and the form of bacteria used (viability, dryness or their products). Nahashon *et al*. (1992) and Tortuero and Fernandez, (1995) showed that using vital biomass of probiotic supplements affects the egg weight significantly (P<0.05). Complementary reports by the Nahashon *et al*. (1996) and Haddadin *et al.* (1996) suggested that addition of biological additives did not influence the egg weight significantly (P>0.05). These controversial results might be related to the dosages of probiotic and concentration of bacteria used in the diet. In Nahashon *et al*. (1992) and Tortuero and Fernandez, (1995) diets there are more bacteria per gr of feed comparing with Nahashon *et al*. (1996) and Haddadin *et al*. (1996), 2200 mg kg^{-1} vs 1100mg kg^{-1}; 10^9cfu gr^{-1} feed vs 10^6-10^7 cfu gr^{-1} feed respectively). Thus, increase of egg weight might be related to the vital form with higher doses up to 10^9 cfu gr^{-1} feed of probiotic.

Blood cholesterol and triglyceride: The probiotic reduced the plasma cholesterol and triglyceride significantly (P<0.05). This findings is in agreement with relevant reports (Abdulrahman *et al*., 1996; Haddadin *et al*., 1996; Mohan *et al*., 1995), confirming the important roles of gastrointestinal tract (GIT) microorganisms in recycling of lipids. Primary bile salts in the presence of specific microorganisms such as *Bacillus subtilis* and *Bacillus licheniformis* are prevented from the

Table 2: Effects of probiotics on production characteristics in laying hens

Source of variation	Egg weight (gr)	egg production (%)	feed consumption (gr/hen/d)	feed conversion ratio(gr/gr)	egg mass (gr/hen/d)
Probiotic levels (cfu gr^{-1} feed)					
0	57.88	86.43	97.86	1.96	50.00
1.28×10^6	57.18	84.60	97.73	2.02	48.47
3.2×10^6	58.57	82.50	96.82	2.00	48.40
4.6×10^6	56.58	85.36	97.23	2.01	48.29
SE	0.856	2.16	2.41	0.031	1.84

Table 3: Effects of probiotics on egg quality treats

Source of variation	Shell thickness (mm)	Shell hardness (kg cm^{-1})	Haugh unit	Egg cholesterol (mg gr^{-1} yolk)
Probiotic levels (cfu gr^{-1} feed)				
0	34.75	3.38	82.33	10.73^a
1.28×10^6	34.75	3.77	81.77	10.67^a
3.2×10^6	34.58	3.78	85.17	10.27^{ab}
4.6×10^6	33.83	3.32	84.93	0.23

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

Table 4: Effects of probiotics on plasma cholesterol and triglyceride

Source of variation	Plasma cholesterol (mg dl^{-1})	Plasma triglyceride (mg dl^{-1})
Probiotic levels (cfu gr^{-1} feed)		
0	237.5^a	1586.1^a
1.28×10^6	207.5^b	1573.6^{ab}
3.2×10^6	201.0^b	1462.4^b
4.6×10^6	213.0^{ab}	1475.0^{ab}
SE	8.01	35.17

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

reabsorption and have more chance to be converted to second type and this inhibits their absorption. On the other hand these organisms are able to synthesize estrase enzymes alongside with lipase enzymes, which the former converts free fatty acids to esterified form different from triglyceride in intestinal content and finally less chance for triglyceride absorption into the plasma.

Egg quality traits: Addition of probiotic had no significant effect (P>0.05) on shell hardness and shell thickness and these were expected which have already been reported (Cerniglia *et al*., 1983; Chen and Chen, 2003; Haddadin *et al*., 1996; Mohan *et al*., 1995). Although, the increase of albumen quality was not significant (P>0.05), no reasonable explanation can be offered for the improvement in albumen quality in the microbial additive groups. Damron *et al*. (1976) and Jensen *et al*. (1978) found significant improvements in interior egg quality as measured by Hough units in hens fed distillers feeds and corn fermentation solubles. Subsequent studies indicated that trace elements may have been involved (Jensen and Maurice, 1978). But Tortuero and Fernandez, (1995) described that the variations in plasma mineral concentration were not sufficient to implicate supporting the hypothesis that trace elements improve albumen quality with microbial supplementation.

Addition of probiotic had significant effect on egg yolk cholesterol (mg gr^{-1} of yolk). Haddadin *et al.* (1996) observed a similar response. They reported that inclusion of *lactobacillus acidophilus* in three ages (40, 44 and 48 week) affects egg cholesterol in 40 week of production not 44 and 48. The correlation coefficient among plasma cholesterol and yolk cholesterol was not significant in this trial. These results have already been confirmed by Sutton *et al*. (1984) and Marks and Washburn (1991) reports.

Histological changes: Results of histological changes of duodenum are shown in Table 5 and sample Fig. 1. Probiotic supplementation had almost damaged the apical cells significantly (P<0.05) and increased the goblet cells more seriously (P<0.01) without any effect on folding of villus. Since the gastrointestinal mucosa is the surface of contact with probiotics, it seems evident that the first effects of probiotics relate to digestive function. A brief review of the literature indicates that probiotics have very few effects on the main physiological functions of the gastrointestinal tract, which are digestion, absorption and propulsion. The main action of probiotics can be summarized as a reinforcement of the intestinal mucosal barrier against deleterious agents. Experimental data indicate that some probiotics reduce pathological alterations in paracellular permeability to large molecules or bacteria,

Table 5: Effects of probiotic on histological changes of duodenum

Probiotic levels (cfu gr^{-1} feed)	Goblet cell numbers	Villus epithelium surface folds	Villus destroyed apical cells
0	-	-	-
1.28×10^6	++	-	++
3.2×10^6	+++	++	+++
4.6×10^6	++++	++	++++
Sources of variation			
Probiotic levels	0.0001**	0.0001**	0.0229**

Significant at P<0.05, **Significant at P<0.01, (-) No effect, (+) Least effect, (++) Less effect, (+++) Moderate effect, (++++) Serious effect.

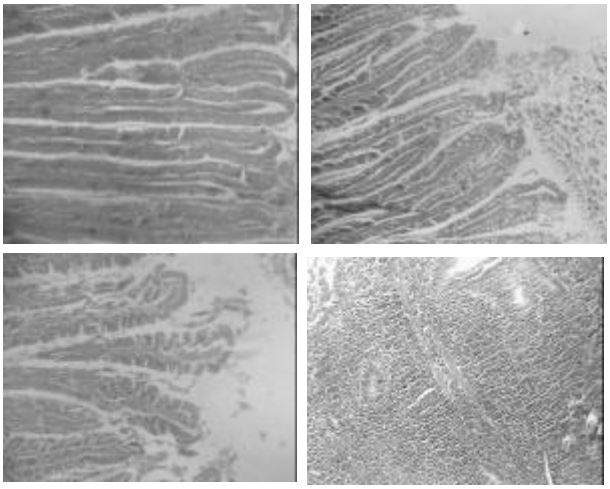

Fig. 1: Effects of probiotics on histological changes of duodenum. Scale bar are (40×) for P1, P2, P4a and (100×) for P4b

stimulate mucosal immunity, display a trophic action on the mucosa, reduce mucus degradation and interact with mediators of inflammation (Fioramonti *et al*., 2003). The action of probiotics on the immune response is relatively well documented. It is clearly established that intestinal microorganisms are necessary for the development of the gut immune system (Blum *et al*., 2002). Using of third and fourth levels of probiotic caused the lymphatic system in the lamina propria layer be significantly proliferated with hyperplasia conditions, which is more likely similar to defensive reaction against antigens. These reactions might be also related to response of animal cells to microbial enzymes such as phospholipase A_2, because increasing the level of probiotic at third and fourth levels damaged the tissue more seriously.

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