http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 8 (11): 1521-1528, 2005
ISSN 1028-8880

Effect of Probiotic Inclusion in Different Levels of Barley Substitution for Corn Diets on Egg Quality and Laying Hen's Performance

A.H. Mahdavi, H.R. Rahmani, J. Pourreza and M.A. Edriss
Department of Animal Sciences, Isfahan University of Technology, Isfahan, 84156, Iran

Abstract: The purpose of this study was to investigate the effect of probiotic (0, 400, 1000 and 2000 g Bioplus 2B ton^{-1} feed providing 0, 1.28×10^{6}, 3.2×10^{6} and 4.6×10^{6} cfu g^{-1} feed concentration) in different levels of barley substitution for corn diets (0, 50 and 100%) on laying hen's performance, egg quality, blood factors and histological changes in duodenum. 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. Using different levels of substitution of barley for corn showed highly significant decrease ($p<0.01$) in feed consumption and feed conversion ratio, highly significant increase ($p<0.01$) in goblet cell numbers and epithelium surface folds of villus, significant increase ($p<0.05$) in egg weight and damaged apical cells of villus, without any effect on other traits. Although, using the different levels of probiotic caused highly significant increase ($p<0.01$) in goblet cell numbers, significant increase ($p<0.05$) in feed consumption, feed conversion ratio and destroying apical cells of villus and significant decrease ($p<0.05$) in plasma cholesterol and triglyceride, but it had no effects on other traits. Consumption of probiotic alone had no effect on egg cholesterol (mg g^{-1} of yolk), but caused significant decrease ($p<0.05$) in egg cholesterol (mg g^{-1} of yolk) among fourth level of probiotic and another levels, in corn based diets. Interaction of different percentages of substitution of barley for corn and probiotic levels had highly significant effect ($p<0.01$) on feed consumption, feed conversion ratio and egg cholesterol (mg g^{-1} of yolk) and significant effect ($p<0.05$) on Hough unit.

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

INTRODUCTION

In the last few years using of barley has increased in layer feed formulations for cost considerations. The feeding value of barley, which is generally considered to be lower than of maize for poultry, is thought to be affected by starch type, acid detergent fiber and total mixed linked β-glucans. Although, β-glucanase supplementation of barley based diets for laying hens has little or no effect on nutritive value[1], but it has been proved that manipulation of the microbial flora in the intestine may affect the digestive enzyme activity[2]. Therefore we can expect comparable results due to inclusion of probiotics in commercial barley based diets on laying hens performance with corn based diets.

Direct fed microbials benefit the host animal by stimulating appetite[3,4], improve intestinal microbial balance[5], synthesize vitamins[6], stimulate the immune system[7], produce the digestive enzyme[8,9], utilize undigestible carbohydrate[10], produce toxic compounds such as volatile fatty acids, decrease pH and release bacteriocins[11] that compete with other microbes for adhesive site[12].

Regarding the controversial results about using biological additives, the strain, concentration and form (viability, dryness or their products) of them should 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[13]. Haddadin *et al.*[14] 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.*[15] 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[16]. Although, in barley based diets,

Corresponding Author: H.R. Rahmani, Department of Animal Sciences, Isfahan University of Technology, Isfahan, 84156, Iran
Tel: +98 311 391 3511 Fax: +98 311 391 3500

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[16]. It was 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[14,17,18].

The purpose of this study was to investigate the effects of probiotic inclusion in different levels of substitution of barley for corn diets on laying hen's performance, egg quality, blood factors and histological changes in duodenum.

MATERIALS AND METHODS

Two hundred and forty white leghorn hens Hy-Line, W-36 strain were randomly allocated in a factorial arrangement (3×4) based on a completely randomized design considering 12 treatments with 4 replicates and 5 samples in each. Both supposed factors included 4 levels of probiotic (0, 400, 1000 and 2000 g ton^{-1} feed providing 0, 1.28×10^6, 3.2×10^6 and 4.6×10^6 cfu g^{-1} feed concentration) and 3 levels of barley substitution for corn (0, 50 and 100%).

Bioplus 2B, a commercial probiotic preparation, was used in this study. The product contained two strains of bacilli. *Bacillus subtilis* (CH201) and *Bacillus licheniformis* (CH200) with a minimum of 3.2×10^9 cfu g^{-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 three consecutive days at the end of each four weeks period and egg 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.*[19] as modified by Washburn and Nix[20] in 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, two 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.

Table 1: Composition of experimental basal diets

	Percentage substitution of barley for corn		
	0	50	100
Ingredients (%)			
Yellow corn	64.00	32.000	-
Barley	-	32.000	64
Soybean meal	20.00	18.000	15.5
Fish meal	3.00	3.000	3.0
Soybean oil	0.5	4.000	7.6
Oyster shell	8.10	8.100	8.1
Dicalcium phosphate	0.8	0.800	0.8
Vitamin premix[1]	0.25	0.250	0.25
Mineral premix[2]	0.25	0.250	0.25
Salt	0.3	0.300	0.30
DL-methionine	0.10	0.120	0.14
Vitamin D3	0.03	0.030	0.03
Safizym GP5003	-	0.036	0.06
Sand	2.67	1.110	-
Calculated analysis			
Metabolizable energy (kcal kg^{-1})	2717.3	2719.200	2717.80
Crude protein (%)	16.00	16	16
Crude fiber (%)	3.06	4.68	6.27
Methionine (%)	0.40	0.40	0.4
Methionine+Cysteine(%)	0.65	0.66	0.67
L-Lysine (%)	0.84	0.83	0.81
Calcium (%)	3.48	3.48	3.48
Available phosphate (%)	0.35	0.37	0.38

[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, [3]β-glucanase activity of 5600 IU g^{-1}

Statistical analysis: Data were analyzed using the General Linear Models (GLM) procedure of Statistical Analyses Systems (SAS)[21]. The following model was assumed in the analysis of all traits. $Yijk = \mu + Ai + Bj + ABij + eijk$ where, Yijk = observed value for a particular character, μ = overall mean, Ai= effect of the ith levels of A factor (different levels of probiotic), Bj = effect at the jth levels of B factor (different levels of barley substitution for corn), Abij = Interaction effects between A and B factors, eijk = random error associated with the ijkth recording .

RESULTS

Analysis of the egg weight, egg production, egg mass, feed consumption and feed conversion ratio data are

Table 2: Analysis of variance for the production characteristics

Source of variation	df	Egg weight (g)	Egg production (%)	Feed consumption (g/hen/d)	Feed conversion ratio (g g^{-1})	Egg mass (g/hen/d)
Barley substitution for corn levels (B)	2	12.31*	1.73	267.07**	0.177**	5.51
Probiotic levels (P)	3	2.64	3.52	51.63*	0.017*	1.35
B×P	6	1.52	5.83	60.64**	0.018**	3.02
Random error	36	3.22	14.13	17.11	0.005	9.79
CV		3.08	4.44	4.4	3.690	6.34

*Significant at p<0.05, **Significant at p<0.01

Table 3: Effects of different levels of barley substitution for corn and different levels of probiotic on production characteristics in laying hens

Source of variation	Egg weight (g)	Egg production (%)	Feed consumption (g/hen/d)	Feed conversion ratio (g g^{-1})	Egg mass (g/hen/d)
0	57.55^{b}	84.72	97.41^{a}	2.00^{a}	48.79
50	57.89^{b}	84.97	95.05^{a}	1.93^{b}	49.20
100	59.21^{a}	84.32	89.46^{b}	1.79^{c}	49.95
SE	0.449	0.94	1.034	0.017	0.782
Probiotic levels (cfu g^{-1} feed)					
0	57.91	85.00	91.24^{b}	1.85^{b}	49.23
1.28×10^{6}	58.25	85.19	96.17^{a}	1.94^{a}	49.66
3.2×10^{6}	58.88	83.98	94.74^{ab}	1.92^{a}	49.47
4.6×10^{6}	57.87	84.52	93.74^{ab}	1.92^{a}	48.89
SE	0.518	1.085	1.193	0.020	0.903

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

Table 4: Analysis of variance for the egg quality treats

Source of variation	df	Shell thickness (mm)	Shell hardness (kg cm^{-1})	Haugh unit	Egg cholesterol (mg g^{-1} yolk)
Barley substitution for corn levels (B)	2	308×10^{-7}	0.196	1.73	0.8
Probiotic levels(P)	3	821×10^{-7}	0.039	6.27	0.525
B×P	6	527×10^{-7}	0.141	42.85*	1.0**
Random error	36	1628×10^{-7}	0.081	16.87	0.324
CV		3.71	7.75	4.91	5.43

*Significant at p<0.05, **Significant at p<0.01

Table 5: Effects of different levels of barley substitution for corn and different levels of probiotics on egg quality treats

Source of variation	Shell thickness (mm)	Shell hardness (kg cm^{-1})	Haugh unit	Egg cholesterol (mg g^{-1} yolk)
Barley substitution for corn levels (%)				
0	0.345	3.56	83.55	10.25
50	0.344	3.65	83.24	10.63
100	0.342	3.78	83.90	10.63
SE	0.00319	0.071	1.03	0.143
Probiotic levels (cfu g^{-1} feed)				
0	0.345	3.60	83.28	10.79
1.28×10^{6}	0.346	3.70	82.70	10.46
3.2×10^{6}	0.343	3.72	83.97	10.48
4.6×10^{6}	0.340	3.64	84.32	10.29
SE	0.00368	0.082	1.19	0.165

Table 6: Analysis of variance for the plasma cholesterol and triglyceride

Source of variation	df	Plasma cholesterol (mg dL^{-1})	Plasma triglyceride (mg dL^{-1})
Barley substitution			
For corn levels (B)	2	36.88	1785.98
Probiotic levels (P)	3	959.81*	16280.70*
B×P	6	152.52	1030.08
Random error	36	345.55	6165.88
CV		8.72	5.16

*Significant at p<0.05

shown in Table 2 and 3. Using different levels of substitution of barley for corn showed highly significant decrease (p<0.01) in feed consumption and feed conversion ratio, significant increase (p<0.05) in egg weight without any effect on the other performance traits. Inclusion of different levels of probiotic caused significant increase (p<0.05) in feed consumption and feed conversion ratio, without any effect on other performance traits. Interaction of different percentages of substitution of barley for corn and probiotic levels had highly significant effect (p<0.01) on feed consumption and feed conversion ratio.

Although feeding different levels of substitution of barley for corn and different levels of probiotics motivated no significant effects on egg quality traits (Table 4,5), but in corn based diets, there was a significant decrease in egg cholesterol (mg g^{-1} of yolk) among fourth level of probiotic and another levels (data not shown). Intraction of these factors had highly significant (p<0.01) effect on egg cholesterol (mg g^{-1} yolk) and significant effect (p<0.05) on Hough unit. Although using different levels of substitution of barley for corn had no significant effects on plasma cholesterol and triglyceride, but addition of different levels of probiotic decreased plasma cholesterol and triglyceride significantly (p<0.05, Table 6 and 7).

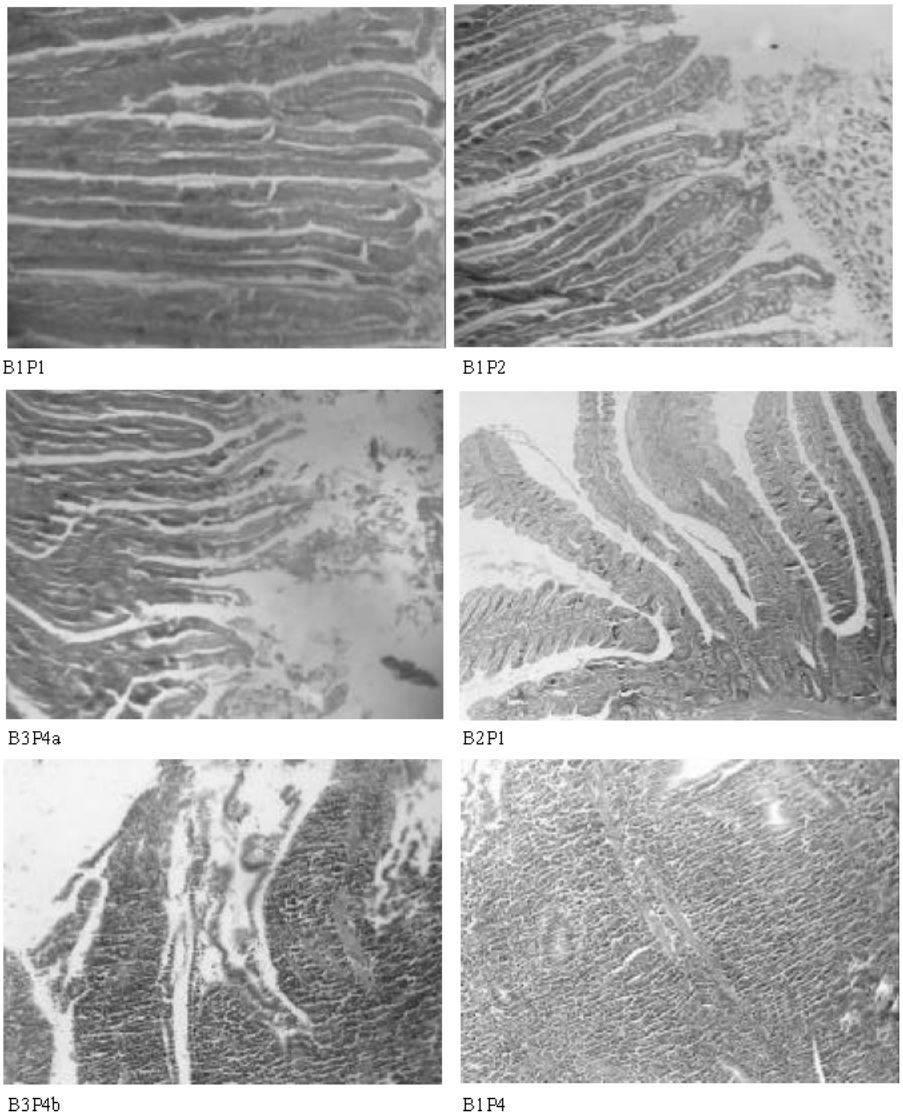

Fig. 1: Effects of different levels of barley substitution for corn (B) and different levels of probiotics (P) on histological changes of duodenum. Scale bar are (40x) for B1P1, B1P2, B2P1, B3P4a and (100x) for B1P4 and B3P4b

Table 7: Effects of different levels of barley substitution for corn and different levels of probiotics on plasma cholesterol and triglyceride

Source of variation	Plasma cholesterol (mg dL^{-1})	Plasma triglyceride (mg dL^{-1})
Barley substitution for corn levels(%)		
0	211.62	1521.59
50	214.62	1512.12
100	213.53	1533.22
SE	4.65	19.63
Probiotic levels (cfu g^{-1} feed)		
0	224.50^{a}	1529.92^{ab}
1.28×10^{6}	213.71^{ab}	1559.37^{a}
3.2×10^{6}	202.67^{b}	1471.33^{b}
4.6×10^{6}	212.17^{ab}	1528.62^{ab}
SE	5.37	22.67

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

Results of histological changes of duodenum are shown in Table 8 and Fig. 1. Utilization of different levels of substitution of barley for corn motivated highly significant increase ($p<0.01$) in goblet cell numbers and epithelium surface folds of villus, significant increase ($p<0.05$) in damaged apical cells of villus, without any effect on other histological changes. Although the inclusion of probiotic caused highly significant increase ($p<0.01$) in the number of goblet cells per microscopic field and significant increase ($p<0.05$) in destroyed apical cells of villus, but it had no effects on other histological changes of duodenum.

Table 8: Interaction of different percentages of substitution of barley for corn and probiotic levels on histological changes of duodenum

Barley substitution for corn levels (%)	Probiotic levels (cfu g^{-1} feed)	Goblet cell numbers	Villus epithelium surface folds	Villus destroyed apical cells
0	0	-	-	-
0	1.28×10^6	++	-	++
0	3.2×10^6	+++	++	+++
0	4.6×10^6	++++	++	+++
50	0	-	++	+++
50	1.28×10^6	++	++	++
50	3.2×10^6	+++	++	++++
50	4.6×10^6	+++	++	++++
100	0	+	++++	+++
100	1.28×10^6	+++	++++	++
100	3.2×10^6	++++	++++	+++++
100	4.6×10^6	+++++	++++	+++++
Sources of variation				
Barley substitution for corn levels(B)		0.0012**	0.002**	0.0429*
Probiotic levels(P)		0.0001**	ns	0.0229*

*Significant at $p<0.05$, **Significant at $p<0.01$, (-) No effect, (+) Least effect, (++) Less effect, (+++) Moderate effect, (++++) Serious effect, (+++++) Very serious effect

DISCUSSION

Production characteristics: Barley substitution affected significantly some production factors including, feed consumption, feed conversion ratio and egg weight, without any significant effect on egg production and egg mass. These results are consistent with Jeroch and Danike[22] report. As the barley level was increased in the ration, the feed consumption was decreased significantly ($p<0.05$), considering the isocaloric level of rations the reduced feed intake might be related to fiber content of barley based diets. Despite the reducing food intake, egg production was not significantly affected and consequently the Feed Conversion Ratio (FCR) was decreased significantly ($p<0.05$). In this relation the histological changes (Fig. 1) in the cellular structure of duodenum showed that the absorptive area is highly ($p<0.01$) elongated and folded with enormous proliferated goblet cells in order to make more active absorption in 2nd and 3rd levels of substitution of barley for corn.

The other interesting point is significant increase ($p<0.05$) in egg weight particulary at the 3rd level of barley substitution for corn, which might be related to the presence of soybean oil for making the isocaloric diets.

Although, inclusion of different levels of probiotic caused significant increase in feed consumption and feed conversion ratio ($p<0.05$), but it had no positive effects on egg production and egg weight. 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.*[23] 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.*[24], Mohan *et al.*[18], Haddadin *et al.*[14] and Chen and Chen[25]. But there are also some reports which have different opinions[3,16], that might be related to the strain of bacteria, concentration and the form of bacteria used (viability, dryness or their products). Nahashon *et al.*[3] and Tortuero and Fernandez[16] showed that using vital biomass of probiotic supplements affects the egg weight significantly ($p<0.05$). Complementery reports by the Nahashon *et al.*[13] and Haddadin *et al.*[14] suggested the 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.*[3] and Tortuero and Fernandez[16] studies there are more bacteria g^{-1} of feed comparing with Nahashon *et al.*[13] and Haddadin *et al.*[14] (2200 mg kg^{-1} vs 1100 mg kg^{-1}; 109 cfu g^{-1} feed vs 106-107 cfu g^{-1} feed respectively). Thus, increase of egg weight might be related to the vital form with higher doses up to 109 cfu g^{-1} feed of probiotic.

Blood cholesterol and triglyceride: Biliary excretion of bile acids, including cholesterol, is increased by dietary fiber[26], resulting in reduced availability of cholesterol for incorporation into lipoproteins. Oat and barley fibers have been shown to be effective dietary ingredients for lowering plasma cholesterol in humans and laboratory animals[27-29]. Barley contains other factors that affect blood plasma cholesterol, as Qureshi *et al.*[30] have reported that barley contains compounds that inhibit 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA)

reductase in chickens, the rate-limiting enzyme in the cholesterol biosynthesis pathway. In spite of these considerable reports, results of the present study is not parallel and is more close to the results of the research done by Scott Beyer and Jensen[31] who showed high protein barley flour had no significant effect on plasma and egg cholesterol. They suggested that inclusion of barley in the hen diets required additional oil in order to maintain the energy content, which may be a factor leading to the apparent increase of cholesterol absorption by the cholesterol-fed hens.

In contrast to the results of barley, the probiotic reduced the plasma cholesterol and triglyceride significantly ($p<0.05$). This findings is in agreement with relevant reports[14,17,18], 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 reabsorption and have more chance to be converted to second type and this inhibits their reabsorption. 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 triglyceride absorption in to the plasma.

Egg quality traits: Using different levels of substitution of barley for corn had relatively little or no effect on egg quality traits. Addition of probiotic had no effect on shell hardness and shell thickness and these were expected which have already been reported[14,18,24,25]. Although, the increase of albumen quality was not significant no reasonable explanation can be offered for the partial improvement in albumen quality with microbial additive groups. Damron *et al.*[32] and Jensen *et al.*[33] 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[34]. But Tortuero and Fernandez[16] 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 no significant effect on egg yolk cholesterol. Haddadin *et al.*[14] 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 but not 44 and 48 weeks. If dietary supplementation with probiotic makes any significant effect on egg cholesterol, then it might be anticipated that the initial impact will be on the laying hen. It seems meaningful that the reduction in egg cholesterol content will likely occur only through the genetic manipulation than the diet[35]. The inclusion of probiotic decreased plasma cholesterol and triglyceride, whereas no significant effect on yolk cholesterol. Really, the correlation coefficient among plasma cholesterol and yolk cholesterol was not significant in this trial. These results confirm the Sutton *et al.*[36] and Marks and Washburn[37] reports.

One of the factors that influence egg cholesterol content is the intake of energy and fat. Dietary fat *per se* does not seem to be a factor, although in most instances high fat diets imply that high energy diets are used. The influence of dietary energy on egg cholesterol is mediated through their effect on egg size[35]. In this trial the estimated correlation coefficient among egg weight and yolk cholesterol was highly significant ($p<0.01$) and $r = + 0.36$.

Histological changes: The more interesting results in this study was related to the gut performance at cellular stage. Compatibility of apical cells with different rations were definitely obvious with different levels of barley substitution particularly at second and third levels. The apical cells were refurnished by increasing the folding the villus. This manner was accompanied by increasing the goblet cell proliferation, which together protected the gut wall by unstirred water layer. The effect of DF on epithelial morphology and cell turnover is variable and depends on the physico-chemical characteristics of the DF, their level of incorporation in the diet, the duration of ingestion, the animal species and age and the site in the intestinal tract. Montagne *et al.*[38] suggested that effect of DF on intestinal epithelial anatomy and structural development seems to be dependant on the ability of particular DF to increase digesta viscosities. Although some authors suggested that treatments decreasing the digesta viscosity through the use of exogenous enzymes have reduced the deleterious effect of fiber on the small intestine mucosa[39], but addition of β-glucanase enzyme in production period of laying hens in this trial had no positive effect on duodenum health. Blottieres *et al.*[40] suggested that the increase of crypt-cell proliferation induced by DF can also be explained by the trophic effect of SCFA and especially butyrate, acting through mechanisms that are still incompletely understood.

The probiotic affected the cellular changes but in another from. Probiotic supplementation caused highly significant increase ($p<0.01$) in the number of goblet cells and significant increase ($p<0.05$) in destroyed apical cells of villus 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 summarised 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, stimulate mucosal immunity, display a trophic action on the mucosa, reduce mucus degradation and interact with mediators of inflammation[41]. 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[42].

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.

REFERENCES

1. Albustany, Z. and K. Elwinger, 1998. Whole grains, unprocessed rapeseed and ß-glucanase in diets for laying hens. Swed. J. Agric. Res., 18: 31-40.
2. Collington, G.K., D.S. Parker and D.G. Armsterong, 1990. The influence of either an antibiotic or a probiotic in the diet on the development of digestive enzyme activity in the pig. Br. J. Nutr., 64: 59-70.
3. Nahashon, S.N., H.S. Nakaue and L.W. Mirosh, 1992. Effect of direct-fed microbials on nutrient retention and production parameters of laying pullets. Poult. Sci., 71: 111.
4. Nahashon, S.N., H.S. Nakaue and L.W. Mirosh, 1993. Effect of direct fed microbials on nutrient retention and productive parameters of Single Comb White Leghorn pullets. Poult. Sci., 72: 87.
5. Fuller, R., 1989. Probiotics in man and animals. A review. J. Applied Bacteriol., 66: 365-378.
6. Coates, M.E. and R. Fuller, 1977. The Genotobiotic Animal in the Study of Gut Microbiology. In: Clarke, R.T.J. and T. Bauchop (Eds.). Microbial Ecology of the Gut. Academic Press. London, pp: 311-346.
7. Toms, C. and F. Powrie, 2001. Control of intestinal inflammation by regulatory T cells. Microbes Infect., 3: 929-935.
8. Gilliland, S.E. and S.H. Kim, 1984. Effect of viable starter culture bacteria in yogurt on lactose utilization in humans. J. Dairy Sci., 97: 1-6.
9. Saarela, M., G. Mogensen, R. Fonden, J. Matto and T. Mattila-Sandholm, 2000. Probiotic bacteria: Safety, functional and technological properties. J. Biotechnol., 84: 197-215.
10. Prins, R.A., 1977. Biochemical Activities of Gut Microorganisms. In: Microbial Ecology of the Gut. Clarke, R.T.J. and T. Hauchop (Eds.). Academic Press, London, pp: 73-183.
11. Rolf, R.D., 2000. The role of probiotic cultures in the control of gastroinjtestinal health. J. Nutr., 130: 396S-402S.
12. Dunham, H.J., C. William, F.W. Edens, I.A. Casas and W.J. Dobrogosz, 1993. *Lactobacillus reuteri* immunomodulation of stressor associated disease in newly hatched chickens and turkeys. Poult. Sci., 72: 103.
13. Nahashon, S.N., H.S. Nakaue and I.W. Mirosh, 1996. Performance of single comb white leghorn fed a diet supplemented with a live microbial during the growth and egg laying phases. Anim. Feed. Sci. Technol., 57: 25-38.
14. Haddadin, M.S.Y., S.M. Abdulrahim, E.A.R. Hashlamoun and R.K. Robinson, 1996. The effects of *Lactobacillus acidophilus* on the production and chemical composition of hen's eggs. Poult. Sci., 75: 491-494.
15. Goodling, A.C., 1987. Production performance of white leghorn layers fed *Lactobacillus* fermentation products. Poult. Sci., 66: 480-486.
16. Tortuero, F. and E. Fernandez, 1995. Effect of inclusion of microbial culture in barley-based diets fed to laying hens. Anim. Feed. Sci. Technol., 53: 255-265.
17. Abdulrahim, S.M., M.S.Y. Haddadin and A.R. Hashlamoun, 1996. The influence of *Lactobacillus acidophilus* and bacitracin on layer performance and cholesterol of plasma and egg yolk. Br. Poult. Sci., 37: 341-346.
18. Mohan, B., R. Kadirvel, M. Bhaskaran and A. Notarajan, 1995. Effect of probiotic supplementation on serum/yolk cholesterol and on egg shell thickness in layers. Br. Poult. Sci., 36: 799-803.
19. Folch, J., M. Less and G.H. Solane Stanley, 1956. A simple method for the isolation and purification of total lipid from animal tissues. J. Biol. Chem., 226: 497-509.

20. Washburn, K.W. and D.F. Nix, 1974. A rapid technique for extraction of yolk cholesterol. Poult. Sci., 53: 1118-1122.
21. Anonymous, 1999. SAS User's Guide. Release 8.2 Edn. SAS Institute Inc. Cary. NC.
22. Jeroch, H. and S. Danicke, 1995. Barley in poultry feeding: A review. World. Poult. Sci., 51: 271-291.
23. Miles, R.D., A.S. Arofa, R.H. Harms, C.W. Carlson, B.L. Reid and J.S. Crawford, 1981. Effects of a living non freeze-dried *Lactobacillus acidophilus* culture on performance, egg quality and gut microflora in commercial layers. Poult. Sci., 60: 993-1004.
24. Cerniglia, G.J., A.C. Goodling and J.A. Hebert, 1983. The response of layers to feeding *Lactobacillus* fermentation product. Poult. Sci., 62: 1399.
25. Chen, Y.C. and T.C. Chen, 2003. Effects of commercial probiotic or prebiotic supplementation on production, size and quality of hen's egg. Poult. Sci., 82: 330.
26. Story, J.A. and E.J. Furumoto, 1990. Dietary Fiber and Bile Acid Metabolism. In: Health Effects (Kritchevsky, D., D. Richevsky, C. Bonfield and J.W. Anderson Eds.). Plenum Press, New York, pp: 364-365.
27. Klopfenestein, C.F. and R.C. Hoseney, 1987. Cholesterol-lowering effect of β-glucan enriched bread. Nutr. Rep. Int, 36: 1091-1098.
28. Davidson, M.H., L.D. Dugan, J.H. Burns, J. Bova, K. Story and K.B. Drennan, 1991. The hypocholesterolemic effects of ß-glucan in oat meal and oat brane. A dose-controlled study. J. Am. Med. Assoc., 265: 1833-1839.
29. McIntosh, G.H., J. Whyte, R. McArthur and P.J. Nestel, 1991. Barley and wheat foods: Influence on plasma cholesterol concentrations in hypercholesterolemic men. Am. J. Clin. Nutr., 53: 1205-1209.
30. Qureshi, A.A., W.C. Burger, N. Prentice, H.R. Bird and M.L. Sunde, 1980. Suppression of cholesterol and stimulation of fatty acid biosynthesis in chicken livers by dietary cereals supplemented with culture filtrate of *Trichoderia viride*. J. Nutr., 110: 1014-1022.
31. Scott Beyer, R. and L.S. Jensen, 1993. Tissue and egg cholesterol concentration of laying hen fed high-protein barley flour, α-tocotrienol and cholesterol. Poult. Sci., 72: 1339-1348.
32. Damron, B.L., A.R. Eldered and R.H. Harms, 1976. An improvement in interior egg quality by the feeding of brewers dried grains. Poult. Sci., 55: 1365-1366.
33. Jensen, L.S., C.H. Chang and S.P. Wilson, 1978. Iinterior egg quality: Improvement by distillers feeds and trace elements. Poult. Sci., 57: 448-454.
34. Jensen, L.S. and D.V. Maurice, 1978. Effect of chromium and corn fermentation solubles on interior egg quality. Poult. Sci., 57: 1147-1151.
35. Leeson, S. and J.D. Summers, 1997. Commercial Poultry Nutrition. 2nd Edn. University books. Guelph. Ontario. Canada.
36. Sutton, C.D., W.M. Nuir and G.E.J. Mitchell, 1984. Cholesterol metabolism in laying hen as influenced by dietary cholesterol, caloric intake and genotype. Poult. Sci., 63: 972-980.
37. Marks, H.L. and H.W. Washburn, 1991. Plasma and yolk cholesterol levels in Japanese quail divergently selected for plasma cholesterol response to adrenocoticotropin. Poult. Sci., 70: 429-433.
38. Montagne, L., J.R. Pluske and D.J. Hampson, 2003. A review of interactions between dietary fibre and the intestinal mucosa and their consequences on digestive health in young non-ruminant animals. Anim. Feed. Sci. Technol., 108: 95-117.
39. Simon, O., 1998. The mode of action of NSP hydrolyzing enzymes in the gastrointestinal tract. J. Anim. Feed Sci., 7: 115-123.
40. Blottieres, H.M., M. Champ, C. Hoebler, C. Michel and C. Cherbut, 1999. Production and digestive effects of short chain fatty acids. Sci. Aliment., 19: 269-290.
41. Fioramonti, J., V. Theodorou and L. Bueno, 2003. Probiotics: what are they? What are their effects on gut physiology? Best Pract. Res. Clin. Gastroenterol., 17: 711-724.
42. Blum, S., D. Haller, A. Pfeifer and E.J. Schiffrin, 2002. Probiotics and immune response. Clin. Rev. All Immunol., 22: 287-309.