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Research Article

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Reducing Egg and Serum Cholesterol by Dietary Inclusion of Probiotics in White Leghorn Layers

Nancy Sheoran[1*], Jyoti Shunthwal[1], Vinus[1], Suman Bishnoi[2] and N. S. Maan[1]

[1]Department of Animal Nutrition, College of Veterinary Sciences,

[2]Department of Livestock product technology, College of Veterinary Sciences,

Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

*Corresponding Author E-mail: sheorannancy@gmail.com

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ABSTRACT

The aim of this study was to evaluate the effect of supplementation of probiotics on egg yolk cholesterol, serum lipid profile, internal egg quality and economics of feeding in layers. A total of eighty White Leghorn layers of 22 to 38 weeks of age were randomly allotted to four dietary treatments containing 4 replications of 5 birds each. In (T_1) control group layers were fed basal diet formulated as per BIS (2007) standards, while basal diet was supplemented with probiotics (containing 5×10^8cfu/g of Lactobacillus fermentum, 1×10^9cfu/g of Bacillus spp. and 1×10^9cfu/g of Saccharomyces cerevisiae) @ 0.5g, 1.0g and 2.0g Kg^{-1} feed in T_2, T_3 and T_4 treatment groups, respectively. Study results indicated that there was ($P<0.05$) improvement in the egg quality parameters viz. egg weight, albumen index and haugh unit with increasing levels of probiotic supplementation in T_2, T_3, and T_4 as compared to the control (T_1). Also, significant reduction was observed in the serum as well as egg yolk cholesterol and LDL concentrations while egg yolk HDL concentrations were not affected. Supplementation of probiotic @ 2g/Kg feed in T_4 was most effective as it resulted in maximum reduction i.e. 11.13g/dl in serum cholesterol and 0.14mg/g decrease in LDL concentration as compared to control. Also, in T_4 there was significant reduction in feed cost per dozen of egg production and per kg of egg mass production, respectively. Thus, probiotics in addition to improvement in egg quality traits possess marked hypocholesterolemic effects on egg yolk and serum cholesterol levels in layers.

Key words: *Probiotics, egg cholesterol, serum cholesterol, internal egg quality, economics, layers.*

INTRODUCTION

Chicken eggs are excellent source of proteins, vitamins, minerals and lipids. However, these days there is growing concern among the consumers to limit the consumption of eggs because of their high cholesterol content which is considered as major cause of increase in cardiovascular diseases[9]. Yolk is one of the richest sources of cholesterol in chicken egg, cholesterol vary considerably from a range of about 200-250 mg in egg and around 150mg% in chicken blood.

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So, it has encouraged various researchers to provide people a designer egg with reduced cholesterol content and enriched internal egg quality like improved albumen index and haugh unit, which would be consumers' health friendly. The level of yolk cholesterol in hen eggs may be decreased by targeted nutrition of hens and selection of genetically suitable breeds[4]. So, one way to regulate the internal egg quality naturally and to supply buyers the food with enhanced nutrient composition is by supplementing the laying hens with probiotics added in their ration. Thus, probiotics were introduced as natural feed additives in poultry diet as an alternative to antibiotics. Probiotic are biopreparations which contain living cells or metabolites of stabilized micro-organisms that optimize colonization and composition of gut microflora in both animals and humans and have a stimulative effect on digestive processes and immunity of the macroorganism[6]. Several relevant reports have shown that supplementation of probiotics in layers could reduce the cholesterol content of egg yolk[13,15] as well as serum[22] by altering the lipid metabolism of chickens. Thus our current study is focused on evaluating the hypocholesterolemic effects of probiotics in reducing the cholesterol concentration in serum and egg-yolk of laying hens and, at the same time, on potential improvement in the quality of hen eggs at lower costs.

MATERIAL AND METHOD

The animal experiment was conducted in accordance with guidelines approved by the Institutional Animal Ethics Committee (IAEC), 235/CPCSEA dated 1-8-2000 in the Department of Animal Genetics and Breeding (AGB), LUVAS.

Experiment Design, Egg quality traits and Cost analysis

A 16 week feeding trial was conducted with eighty 22 weeks old White Leghorn layers which were randomly assigned to 4 treatment groups having 4 replicates with 5 birds in each. The other standard and uniform managemental practices were followed during the trial. Proximate analysis was done as per AOAC, (2007)[1] for all the feed ingredients used during ration formulation (Table 1).

Table 1: Chemical composition (%DM basis) and metabolizable energy (ME, Kcal/Kg) of feed ingredients used during formulation of the experimental diets

Ingredients	CP	CF	EE	Ash	OM	NFE	ME*
Maize	9.10	2.65	3.39	2.50	97.50	82.36	3309
Groundnut cake	40.90	8.90	7.94	4.52	95.48	37.74	2596
Soybean Meal	43.15	3.78	3.43	6.93	93.07	42.71	2230
DORP	13.70	12.88	1.10	11.25	88.75	61.07	2235
Rice Polish	10.20	4.69	14.78	12.83	87.17	57.50	2937
Fish Meal	48.15	2.05	5.30	22.43	77.57	22.07	2600

**Panda et al.* [18]

Composition of the feed ingredients used in basal ration and the feed additives added to basal diet during the laying phase in hens are presented in Table 2. In (T_1) control group layers were fed basal diet formulated as per BIS (2007) standards[5], while basal diet was supplemented with probiotics (containing 5×10^8cfu/g of *Lactobacillus fermentum*, 1×10^9cfu/g of *Bacillus spp*. and 1×10^9cfu/g of *Saccharomyces cerevisiae*) @ 0.5g, 1.0g and 2.0g Kg^{-1} feed in T_2, T_3 and T_4 treatment groups, respectively. All diets were similar in energy, protein and other nutrients contents (CP% = 18.04%, ME = 2697.17 kcal/ kg). Effects of supplementation of probiotics was judged on the basis of egg quality traits viz. egg weight, albumen index, haugh unit, yolk index; serum and egg yolk cholesterol parameters were measured using reagent kits (ERBA Kit) in ERBA-EM-200 Automatic Analyzer and economics of feeding.

Table 2: Ingredient composition (%) and quantities of feed additives used in the laying phase of the hens under different dietary treatments of probiotic supplementation

Ingredient composition (%) under different dietary treatments during laying phase				
Feed ingredients (%)	**T_1**	**T_2**	**T_3**	**T_4**
Maize	50.0	50.0	50.0	50.0
Groundnut Cake	7.00	7.00	7.00	7.00
Soyabean Meal	13.0	13.0	13.0	13.0
Deoiled rice polish	12.0	12.0	12.0	12.0
Rice polish	5.00	5.00	5.00	5.00
Fish Meal	6.00	6.00	6.00	6.00
Mineral Mixture	3.00	3.00	3.00	3.00
Salt	0.50	0.50	0.50	0.50
Shell grit	3.50	3.50	3.50	3.50
Total	100	100	100	100
Feed additives* (g/100g of feed)	340	340	340	340
Probiotic (g/Kg of the feed)[1]	-	0.5	1.0	2.0

***Spectromix**-10g/ quintal , (Each g contained vitamin A- 82,500 IU, vitamin D_3-12,000 IU, vitamin B2- 50mg, and vitamin K- 10m) **Spectrimix-BE**-10g/ quintal, (Each g contained vitamin B1- 80mg, vitamin B_6 -16mg, Niacin-120mg, vitamin B_{12}- 80mg, Calcium Pantothenate- 80mg, vitamin E -160mg, L-lysine HCl- 10mg, DL-Methionine -10mg, and Calcium- 260mg)

[1] Probiotic composition (w/w): *Bacillus* spp. -1billion cfu/g, *Lactobacillus* spp.-0.5 billion cfu/g, Yeast (*Sacchromyces cerivisiae*) -1 billion cfu/g, excipients-Q.S. Mixing rate: 50g, 100g, 200g per quintal in 2nd, 3rd and 4th treatment groups, respectively.

Statistical analyses

All data were subjected to one-way ANOVA according to General Linear Models procedures procedure for SAS institute software[19]. Significant differences between treatment means were separated using Duncan's multiple range test, with a 5% probability (P<0.05).

RESULT AND DISCUSSION

Egg weight and egg quality parameters

Study results (Table 3) indicated that probiotic supplementation @ 2g/Kg of feed in treatment group T_4 led to significant (P<0.05) increase in egg weight, in comparison to control, while in case of treatment group T_3 (1g probiotic/Kg of feed) only a numerical improvement was observed (Table 3). Egg weight was measured to be highest (54.58 g) in hens fed highest level (2g/Kg of feed) of probiotic in T_4, whereas the egg weight of hens given the control diet were (53.62g), indicating a 0.96 g advantage in favor of probiotic fed hens. Similarly, Balevi *et al.*[3] also documented 1.22 g increases in egg weight when brown layers' diet was supplemented with probiotic. Supplementation of layers' diet with probiotic might have resulted in enhanced retention of nutrients, including nitrogen, fat, calcium and phosphorus which led to significant increases in both egg weight and egg mass.

Table 3: Average values of egg weight and internal egg quality parameters during progressive weeks of age under different levels of probiotic supplementation.

Parameter	T_1	T_2	T_3	T_4
Weeks	**Egg Weight (g)**			
22 – 24	$52.73^{ab}\pm0.33$	$52.92^{ab}\pm0.78$	$53.98^{b}\pm1.38$	$53.82^{b}\pm1.16$
24 – 26	53.92±0.70	54.95±0.72	55.36±0.40	55.18±0.67
26 – 28	$52.57^{ab}\pm0.17$	$52.32^{b}\pm0.34$	$53.49^{ac}\pm0.21$	$54.36^{c}\pm0.36$
28 – 30	$53.24^{ab}\pm0.59$	$53.10^{ab}\pm0.56$	$53.34^{ab}\pm0.32$	$54.40^{b}\pm0.30$
30 – 32	54.21±0.49	53.88±1.01	54.07±0.74	54.29±0.47
32 – 34	53.88±0.61	54.66±1.15	53.49±0.30	54.44±0.49
34 – 36	$54.24^{ab}\pm0.23$	$53.48^{b}\pm0.68$	$53.99^{ab}\pm0.39$	$55.09^{a}\pm0.75$
36 – 38	$54.23^{ab}\pm0.24$	$53.47^{b}\pm0.67$	$53.98^{ab}\pm0.38$	$55.08^{a}\pm0.75$
Mean	$53.63^{a}\pm0.18$	$53.60^{a}\pm0.28$	$53.95^{ab}\pm0.22$	$54.58^{b}\pm0.22$
Weeks	**Albumen index percent (%)**			
22 – 24	8.15±0.10	8.34±0.04	8.45±0.18	8.59±0.12
24 – 26	8.30±0.07	8.30±0.02	8.48±0.11	8.56±0.07
26 – 28	8.74±0.09	8.67±0.15	8.95±0.00	8.80±0.04
28 – 30	9.21±0.01	9.22±0.06	9.29±0.06	9.38±0.07
30 – 32	9.82±0.04	9.86±0.06	10.05±0.06	9.90±0.01
32 – 34	9.94±0.05	9.98±0.03	10.13±0.04	10.15±0.07
34 – 36	10.11±0.05	10.15±0.01	10.22±0.03	10.18±0.02
36 – 38	10.70±0.06	10.77±0.08	10.96±0.01	10.93±0.03
Mean	$9.36^{a}\pm0.16$	$9.41^{a}\pm0.16$	$9.57^{b}\pm0.16$	$9.56^{b}\pm0.15$
Weeks	**Yolk index percent (%)**			
22 – 24	42.62±0.50	42.76±0.28	42.62±0.17	42.37±0.21
24 – 26	$43.71^{a}\pm0.37$	$44.43^{ab}\pm0.13$	$44.61^{b}\pm0.21$	$44.51^{ab}\pm0.14$
26 – 28	$46.63^{ab}\pm0.19$	$46.98^{ab}\pm0.24$	$46.90^{ab}\pm0.19$	$47.09^{a}\pm0.17$
28 – 30	$45.32^{ab}\pm0.14$	$45.49^{ab}\pm0.48$	$45.35^{ab}\pm0.36$	$46.21^{a}\pm0.28$
30 – 32	44.41±0.61	45.04±0.71	45.04±0.36	45.16±0.15
32 – 34	$46.45^{ab}\pm0.18$	$47.13^{bc}\pm0.23$	$47.38^{c}\pm0.10$	$47.23^{bc}\pm0.26$
34 – 36	$47.39^{a}\pm0.21$	$47.46^{a}\pm0.18$	$47.51^{a}\pm0.17$	$48.05^{b}\pm0.08$
36 – 38	$47.26^{ab}\pm0.32$	$46.84^{a}\pm0.55$	$47.50^{ab}\pm0.23$	$48.63^{c}\pm0.22$
Mean	45.47±0.31	45.76±0.30	45.86±0.31	46.15±0.35
Weeks	**Haugh unit**			
22 – 24	$75.14^{a}\pm0.99$	$76.94^{ab}\pm0.57$	$77.81^{ab}\pm1.78$	$79.47^{b}\pm0.98$
24 – 26	$76.26^{a}\pm0.75$	$77.64^{ab}\pm0.31$	$79.29^{bc}\pm0.91$	$79.59^{c}\pm0.53$
26 – 28	$81.73^{ab}\pm0.71$	$81.17^{b}\pm0.93$	$83.62^{a}\pm0.06$	$82.46^{ab}\pm0.54$
28 – 30	$85.81^{a}\pm0.09$	$85.84^{ab}\pm0.38$	$86.83^{abc}\pm0.32$	$87.62^{bc}\pm0.69$
30 – 32	$91.59^{a}\pm0.44$	$92.00^{ab}\pm0.53$	$93.94^{b}\pm0.30$	$92.76^{ab}\pm0.22$
32 – 34	$92.89^{a}\pm0.37$	$93.33^{a}\pm0.36$	$94.90^{b}\pm0.42$	$94.53^{bc}\pm0.19$
34 – 36	$94.02^{a}\pm0.35$	$94.56^{ab}\pm0.22$	$95.25^{b}\pm0.12$	$94.99^{ab}\pm0.32$
36 – 38	$96.11^{a}\pm0.68$	$96.67^{ab}\pm0.63$	$98.47^{c}\pm0.23$	$98.10^{bc}\pm0.30$
Mean	$86.63^{a}\pm0.84$	$87.27^{ab}\pm0.93$	$88.76^{b}\pm0.73$	$88.69^{b}\pm0.82$

The mean values in same row with different superscripts differ significantly ($P < 0.05$).

The perusal of the data presented in Table 3 revealed that, the mean values of albumen index percent increased (P<0.05) by 22.4% and 21.36% in hens of treatment groups T_3, T_4, respectively. Similarly, the haugh unit values also differ significantly (P<0.05) between different dietary treatment groups and show similar trend as that of albumen index (Table 3). The internal quality of an egg can be assessed by Haugh unit, which is further related to its weight and albumin height[13]. In full agreement with our findings, Gallazzi *et al*.[7] reported higher egg albumen (Haugh Units) mean viscosity values for probiotic treated eggs (96.29±.33 HU) when compared to control eggs (95.29±0.35 HU) (P<0.05). Probiotics produce weak organic acids can lower the pH of the gut environment below that essential for the survival of such pathogenic bacteria as *E. coli* and *Salmonell*[14]. Probably the acidification is also responsible for the improvement of the albumen quality and haugh unit.

In resemblance to our results, Mohammadian *et al*.[16] observed that yolk color and haugh unit were affected significantly (P<0.05) by dietary inclusions of probiotics at the levels of 750 g/t feed. The result findings depicted in Table 3 shows that overall with respect to the whole period there was no significant increase in the yolk index percent by dietary inclusion of probiotics in hens. Thus, probiotic supplementation in laying hens' diet resulted in the significant increase in the egg weight and improved egg albumen quality in terms of albumen index and haugh unit.

Egg and serum cholesterol, HDL, LDL

The data pertaining to the cholesterol levels in the egg yolk and serum revealed that there was a prominent decrease (P<0.05) in the cholesterol content due to dietary inclusion of probiotics at higher levels i.e. 1g and 2g/Kg of the feed in the diet of laying hens. Table 4 represents mean values of cholesterol, HDL and LDL in the egg yolk.

Table 4: Mean values of cholesterol, HDL and LDL in egg yolk of layers under different treatments of probiotic supplementation

Treatments	Cholesterol (mg/g egg yolk)	HDL (mg/g egg yolk)	LDL (mg/g egg yolk)	Cholesterol (mg/dl in blood)
T_1	$13.55^a \pm 0.23$	5.58 ± 0.05	$7.67^a \pm 0.01$	$166.66^a \pm 0.84$
T_2	$13.46^a \pm 0.08$	5.54 ± 0.03	$7.65^a \pm 0.01$	$163.64^{ab} \pm 1.47$
T_3	$12.13^b \pm 0.11$	5.51 ± 0.03	$7.61^b \pm 0.01$	$158.09^c \pm 0.57$
T_4	$11.97^b \pm 0.11$	5.54 ± 0.05	$7.53^c \pm 0.02$	$155.53^c \pm 1.68$
CD	0.42	NS	0.03	3.13

The mean values in same column with different superscripts differ significantly (P< 0.05).

It was observed that there was a significant (P<0.05) decrease in the mean values of cholesterol, from 13.55 mg/g in control group to 12.13 and 11.97 mg/g in treatment groups T_3 and T_4 in hens fed @ of 1g/Kg and 2g/Kg feed of probiotic, respectively. However, T_3 and T_4 treatment groups had no significant differences among themselves. The mean values of total serum cholesterol in Table 4 indicated that cholesterol in serum was reduced to maximum by 11.13 g/dl of blood in T_4 than control. Consistent to our result findings, Kurtoglu *et al*.[11] identified that the application of *Bacillus spp*. can significantly (P<0.05) reduce the serum cholesterol levels in layers, as well as reducing egg yolk cholesterol.

The reduction of cholesterol could be attributed to reduced absorption and synthesis of cholesterol in the gastrointestinal tract[17]. Lee *et al*.[12] clarified that higher population of lactic acid bacteria could inhibits synthesis of cholesterol enzymes in the host, assists the elimination of cholesterol in the faeces, utilizes circulating cholesterol for the synthesis of bacterial cell wall and thus reducing the cholesterol level. There are reports which support our findings of reduction in cholesterol concentrations in egg yolk[17] as well as in serum of laying hens[22]. One of the possible reasons for reduction in yolk cholesterol in present study is that probiotic supplementation resulted in lowered levels of circulating cholesterol making it less available to be deposited into the egg yolk.

In current study (Table 4) there was a significant ($P<0.05$) decrease in the LDL content from 7.67 mg/g in control group to 7.61 and 7.53 in treatment groups T_3 and T_4, respectively. These findings indicate that LDL values for laying hens fed probiotic added diet was 0.14g lower than that of hens given a control diet. Consistent with our results Zarei *et al*.[21] also reported that hens fed the probiotic diets did have decreased blood levels of LDL compared to control. Thus, egg LDL cholesterol decreased ($P<0.05$) more dramatically in layers fed the probiotics @ 1g and 2g of the feed compared lower levels.

Results findings (Table 4) shows that the High Density Lipoprotein value ranged from 5.58 (T_1) to 5.47 (T_4) mg per g egg yolk. However, effect of probiotic supplementation on the egg yolk HDL concentrations was non-significant and was at par with control. However, Khan *et al*.[10] found that serum HDL cholesterol was increased by probiotic supplementation to a layer diet compared with the control. Thus, dietary inclusion of probiotics to the basal ration of laying hens did not affect the HDL levels in egg yolk significantly.

Feed Cost per dozen of egg production and per kg egg mass production

In present study there was notable ($P<0.05$) decrease in feed cost value for dozen egg production and per kg of egg mass production in hens supplemented with higher levels of probiotics @ 1g and 2g/ Kg of the feed in comparison control.

Table 5: Average feed cost (Rs) per dozen egg production during progressive age (Weeks) under different probiotic treatments

Weeks/ Treatment	T_1	T_2	T_3	T_4
22 – 24	40.98	43.49	41.67	42.02
24 – 26	43.19	47.57	43.29	42.99
26 – 28	41.86	45.75	43.06	43.47
28 – 30	43.64	44.39	44.91	43.95
30 – 32	46.29	44.85	45.61	45.40
32 – 34	46.29	44.85	45.61	45.40
34 – 36	48.95	48.47	48.62	47.82
36 – 38	48.51	48.92	47.46	47.58
Mean	44.96	46.04	45.03	44.83
Difference	0.00	1.08	0.07	-0.13

There was a reduction in cost of Rs. 0.13 per kg of egg production (Table 5) and Rs. 1.13 per kg of egg mass production (Table 6) in T_4 in comparison to T_1. Thus, highest net profit was obtained treatment group T_4. In full agreement with our findings Swain *et al*.[20] and Ayassan *et al*.[2] reported that net profit was increased in laying hens fed probiotics and yeast compared to that of control diet which might be due to lower feed consumption and better egg size. Although, dietary inclusion of probiotics increased the cost per kg feed, but the beneficial effects of supplementation such as increased egg production, less feed intake and improved nutrient availability has curtailed the increase in feed cost.

Table 6: Average feed cost per kg egg mass production (Rs.) under different probiotic regimes during overall experimental period (16 weeks)

Weeks/ Treatment	T_1	T_2	T_3	T_4
22 – 24	64.90	69.08	64.82	65.45
24 – 26	67.11	72.48	65.28	65.21
26 – 28	66.67	72.93	67.14	66.65
28 – 30	68.44	69.76	70.61	67.62
30 – 32	71.54	69.76	70.61	69.79
32 – 34	76.20	74.07	75.93	73.66
34 – 36	74.65	76.33	73.62	72.21
36 – 38	65.56	72.25	71.07	65.45
Mean	69.38	72.08	69.88	68.25
Difference	0.00	2.70	0.50	-1.13

CONCLUSION

Probiotics are the natural feed additives which lead to production of large sized eggs with improved internal egg quality in the form of increased albumen index and haugh unit, without affecting the yolk index. Also, in addition to lowering the feed cost, there is significant reduction in the egg as well as serum cholesterol levels. Thus, from current study it can be concluded that the dietary supplementation of probiotics may lead to the development of low-cholesterol chicken eggs with enriched albumen quality as demanded by health-conscious consumers.

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