Original Article

Journal of Animal Feed Science and Technology
Volume 7 Number 1, January - June 2019
DOI: http://dx.doi.org/10.21088/jafst.2321.1628.7119.4

Effect of Dietary Inclusion of Probiotics on Performance and Serum Parameters of Layers in Post Peak Production

Nare Ravi Kumar[1], J.V. Ramana[2], B. Devasena[3], S. Shakila[4]

[1]MVSc student [2]Professor, Dept. of Animal Nutrition [3]Professor, ILFC [4]Professor and Head, Dept. of Poultry Science, College of Veterinary Science, Tirupati Andhra Pradesh 517502, India.

How to cite this article:

Nare Ravi Kumar, J.V. Ramana, B. Devasena *et al*. Effect of Dietary Inclusion of Probiotics on Performance and Serum Parameters of Layers in Post Peak Production. Journal of Animal Feed Science and Technology. 2019;7(1):17-24.

Abstract

The present study was carried out with a view to investigate the production performance of layers fed with probiotic in diets at different concentration 0 (T1), 50 (T2), 100 (T3) and 150 g (T4) per ton of basal diets during post peak production (47 to 58 weeks of age). The basal diet was formulated with commonly available feed ingredients like maize, soybean meal, sunflower meal, de-oiled rice bran, fish meal as per the specifications of BIS, (1992) suitable for Indian conditions. The chemical composition (%) of the basal diet was 90.0 (DM), 18.09 (CP), 2.82 (EE), 7.65 (CF), 55.22 (NFE), 17.22 (TA) and 3.81 (AIA). The calculated ME content of the basal diet was 2654 Kcal/Kg. One hundred and eighty Single Comb White Leghorn layers were used in the experiment and divided into four dietary treatments with three replicates of fifteen birds each. During the experimental period feed intake, feed efficiency and body weights were recorded in three periods of 28 days each (47 to 50, 51 to 54 and 55 to 58 weeks). Serum parameters like total serum proteins and serum cholesterol levels were recorded at the start and at the end of the experiment. There was no significant difference observed in feed intake (g) during overall experimental laying period (47-58 weeks) and feed intake ranged from 118.53 (T4) to 120.18 g (T1). There was no significant difference in feed efficiency (kg feed consumed for dozen eggs) during 47-58 weeks of age. Better feed efficiency (kg feed consumed for dozen eggs) was observed in T2, T3 and T4 groups (1.68 Kg) over T1 (1.69 Kg). Similarly non-significant differences were observed in birds fed with experimental diets with regard to feed efficiency expressed as Kg feed consumed for Kg egg produced. The total protein was reduced by 7.33% in control (T1) but increased in T2, T3 and T4 by 2.60, 1.19 and 2.58% respectively. There was no significant difference observed among the treatment groups. The serum cholesterol increased by 0.56% in control (T1) but reduced in T2, T3 and T4 by 2.39, 6.22 and 8.82% respectively. During 47-58 weeks of age significantly ($p \leq 0.05$) higher body weights (g) were observed in T1 (1296) and lower body weight was observed in T4 (1234) group. There was no significant difference in feed consumption and income over feed cost per 12 eggs produced among different treatments. Income over feed cost/12 eggs in T1, T2, T3 and T4 were 4.91, 5.13, 5.09 and 5.06 respectively. Percent improvement in income over the control (T1) was T2 (4.51), T3 (3.83) and T4 (3.14). There was no mortality observed in all the groups during the experimental period and birds did not show any illness on probiotic addition. Better performance of the birds, reduced cholesterol contents and improved relative economics over control suggest that 50g of probiotic addition to one ton of layer diet is beneficial without any adverse effects during post peak production. The inclusion of probiotics can replace the usage of antibiotics in poultry feeds.

Keywords: Probiotic; Layers; Post peak production; Feed efficiency; Serum cholesterol; Serum protein.

Corresponding Author: J.V. Ramana, Professor, Department of Animal Nutrition, College of Veterinary Science, Tirupati, Andhra Pradesh 517502, India.

E-mail: jvenkataramana@rediffmail.com

Received on 22.05.2019; **Accepted on** 20.06.2019

Introduction

Due to continuous demand from consumers for low cost chicken meat and egg resulted in the intensification of poultry production system and extensive administration of antimicrobials as growth promoters (NRC, 1980; Fuller, 1992). The antibiotics as growth promoters appear to act by reducing the pathogenic bacteria and modifying the microflora in the gut of the animals (Radostits *et al.*, 1994). Continuous use of sub-therapeutic level of antibiotics in animal feeds may result in not only bacterial resistance (Adams, 2004) but also accumulation of antibiotic residues in various tissues of the birds resulting in development of drug resistant microorganisms in humans upon consumption of such poultry products (Jin *et al.*, 1996). The adverse effect of antibiotic feeding has encouraged a shift in favor of feeding probiotics to boost up productive performance of chicken.

Probiotics were introduced as an alternative to antibiotics. Probiotics are defined as live microbial feed supplements, which beneficially affect the host animal by improving the intestinal microbial balance (Fuller, 1989). The mechanism by which probiotics improve feed conversion efficiency include alteration in intestinal flora, inhibition of growth of pathogenic microorganisms and enhancement of growth of non pathogenic, facultative anaerobic and gram positive bacteria. The probiotics will enhance digestion, nutrient utilization, improve feed conversion efficiency, improved growth rate, reduction in mortality and maintain health status of birds.

Mohan *et al*., (1995) reported that probiotic supplementation can depress serum/yolk cholesterol and improve egg production by 5% in layers. Nahashon *et al*., (1996) observed increased egg size upon supplementation of *Lactobacillus* @110 mg/kg diet. Panda *et al*., (2008) reported that addition of probiotics significantly increased the egg production, shell weight, shell thickness and serum calcium and reduced the cholesterol content in serum and yolk. Yoruk *et al*., (2004) reported that supplementation of humate and probiotics increased egg production, reduced mortality, improved feed conversion efficiency in Hisex brown layers during late laying periods (54 weeks of age). The egg production starts declining after reaching the peak egg production. The ever increasing cost of production may marginally be compensated by improving the production and feed efficiency during this declining phase of production by using probiotic supplements. Therefore the present research work was taken up to study the effect of probiotic supplementation on feed intake, feed efficiency, serum parameters and cost economics in layers during post peak production.

Materials and Methods

Maize (ground), soybean meal, fish meal, sunflower meal, de-oiled rice bran (DORB), mineral mixture and shell grit were obtained from local market. Commercial probiotic supplement (Lactoplus) comprising of mixed cultures of *Lactobacillus acidophilus*. *Lactobacillus bulgaricus, Lactobacillus casei*, *Lactobacillus returi* , *Lactobacillus lactis, Streptococcus faecium, Asperigillus oryzae* and *Saccharomyces cervisiae* at the concentration of 32 billion CFU/100 g was procured from local market. Representative samples of feed ingredients and layer diets were analysed for proximate composition as per AOAC (2005). Representative samples of feed ingredients and layer diets were analysed for calcium and phosphorus as per method of Talapatra *et al*., (1940).

One hundred and eighty Single Comb White Leghorn layers of same hatch were used in the experiment. A 12-week feeding trial (March 2013 to May 2013) was conducted from 47th week of age and continued for 3 laying periods of 28 days each. The birds were weighed individually and randomly divided into 4 treatments of 3 replicates of 15 birds each (with uniform average body weight per replicate) housed in individual cages of California two tier system in completely randomized design. In the biological trial probiotic was included at 0, 50, 100 and 150 g/ ton in T_1, T_2, T_3 and T_4 experimental diets respectively. These iso-caloric and iso-nitrogenous layer diets were formulated according to BIS (1992) specifications.

All the birds were housed in cages with optimum floor space per bird and adequate ventilation throughout the experiment. The hens had free access to feed and water throughout the experiment. All birds were dewormed one week before commencement of experiment and the other managemental practices like vaccination were adopted uniformly for all the treatments. The birds were provided with 16 hours of light/day. Feed intake was recorded for every 28 days period replicate wise and based on this feed efficiency was calculated as the feed required in kilos to produce one dozen eggs/ one kg eggs.

Serum samples were collected from 2 birds in each replicate twice during the experiment i.e. at the beginning and at the end of the experiment. Serum total proteins were estimated by using diagnostic kit (M/s Span Diagnostics Limited) by Biuret method (Varley *et al*., 1980). Serum cholesterol was estimated by using diagnostic kit (M/s Excel Diagnostics Private Limited) by enzymatic method of Allian (1974) for *in vitro* estimation. Individual body weights of all birds were recorded in grams at the beginning and also at the end of the every 28 day period during the experiment. Income over feed cost per dozen eggs in different treatments and relative economy of each dietary regimen were worked out based on the prevailing prices of various inputs and outputs. All the data obtained in the experiment were subjected to standard statistical analysis (Snedecor and Cochran 1994).

Results and Discussion

The results obtained with regard to chemical composition of feed ingredients and experimental diets, performance of layers during post peak production fed on diets with different levels of probiotics in terms of feed intake, feed efficiency, serum parameters and economics are discussed in conjunction with the available literature.

The chemical composition of the feed ingredients used in the experiment is given in the Table 1. The percent DM, CP, EE, CF, TA, NFE and AIA of maize, soybean meal, sunflower cake, fish meal, and de-oiled rice bran were 88.9, 10.59, 4.12, 2.74, 6.87, 75.68 and 0.72; 90.89, 42.9, 0.97, 6.13, 10.01, 39.99 and 1.32; 91.13, 27.18, 0.51, 24.49, 10.4, 37.42 and 0.94; 91.76, 42.23, 6.2, 3.12, 21.9, 26.55 and 8.61; 88.86, 15.0, 1.51, 15.79, 15.94, 51.76 and 8.42, respectively.

The CP content of the maize in the present study was 10.59% which is as per the values reported by Arora and Harjit kaur (2010). The CP content of soybean meal, sunflower cake, fish meal, and de-oiled rice bran used in the present study were lower than the values given by Arora and Harjit kaur (2010), which were 51.0, 35.3, 71.4 and 16.1, respectively. This may be due to variations in soil texture, season of harvesting and other variable factors.

The experimental diets were formulated based on the recommendations of BIS, (1992) suitable for Indian conditions (Table 2). The feed ingredients like maize, soybean meal, sunflower meal, de-oiled rice bran, fish meal were used in formulating the diets, as these are the most commonly used and available in this region. The chemical composition of layer diets along with probiotic supplementation at different levels used in the experiment is given in Table 3. The protein : energy ratio of T_1, T_2, T_3 and T_4 diets were 1:147, 1:147, 1:147 and 1:148, respectively which were nearer to the protein : energy ratio of 1:150 as recommended by BIS, (1992).

The feed intake during experimental period was not significantly affected in layers fed on experimental diets compared to control diet (Table 4). The feed intake (g/d) in T_1, T_2, T_3 and T_4 were 120.18, 119.36, 119.10 and 118.53, respectively.

Table 1: Chemical composition[+] (%) of feed ingredients used in experimental diets

Constituent	Ingredient				
	Maize	Soybean meal	Sunflower cake	Fish meal	De-oiled rice bran
Dry matter	88.9	90.89	91.13	91.76	88.86
Crude protein	10.59	42.9	27.18	42.23	15.0
Ether extract	4.12	0.97	0.51	6.2	1.51
Crude fibre	2.74	6.13	24.49	3.12	15.79
Total ash	6.87	10.01	10.4	21.9	15.94
Nitrogen free extract	75.68	39.99	37.42	26.55	51.76
Acid insoluble ash	0.72	1.32	0.94	8.61	8.42
Calcium	0.24	0.34	0.4	5.39	0.33
Phosphorus	0.4	0.87	0.3	1.67	1.79
Lysine*	0.18	2.57	1.95	4.17	0.44
Methionine*	0.15	0.76	1.56	1.42	0.29
ME (Kcal/kg)	3309**	2300**	2230*	2500**	2235*

+On dry matter basis except for DM; * Values from Ramasubba Reddy and Bhosale (2001)

**Values from Narahari (1996)

Table 2: The ingredient composition (%) of experimental diets

Ingredient	T_1	T_2	T_3	T_4
Maize	49	49	49	49
Deoiled rice bran	16	16	16	16
Soybean meal	9	9	9	9
Sunflower cake	12	12	12	12
Fish meal	8	8	8	8
Shell grit [+]	5	5	5	5
Mineral mixture *	1	1	1	1
Additives				
Probiotic (g)	0	5	10	15
Other feed additives **	+	+	+	+

+Contained *Ca*, 38%

*Contained *Ca*,32%; *P*,6%; *NaCl*; 2.5%; *Cu*, 100 ppm; *Mn*, 200 ppm, *Co*,50 ppm; *Zn*, 50 ppm and *I*, 100 ppm

**Meriplex DS @ 10g/100 kg: *Vit* B_1 8 mg, B_6 16 mg, B_{12} 80 mcg, *Niacin* 120 mg and *Ca* 88 mg

**Hyblend – AB_2D_3K @10g/100 kg: *Vit A* 82,500 IU, *Vit* B_2*-50 mg*, *Vit* D_3 12,000 IU, Vit K 10 mg.

Table 3: Chemical composition * (%) of experimental diets

Constituent	Layer diet			
	T_1	T_2	T_3	T_4
Dry matter	90.0	88.91	91.85	90.42
Crude protein	18.09	18.0	17.94	17.96
Ether extract	2.82	2.91	2.79	2.96
Crude fibre	7.65	7.37	7.51	7.27
Nitrogen free extract	54.22	54.62	54.03	54.44
Total ash	17.22	17.10	17.73	17.37
Acid insoluble ash	3.81	3.95	3.89	3.72
Calcium	3.78	3.54	3.81	3.69
Phosphorus	1.12	1.17	1.08	1.21
Lysine**	0.96	0.96	0.96	0.96
Methionine**	0.49	0.49	0.49	0.49
ME (Kcal/Kg)**	2654	2654	2654	2654

* On dry matter basis except for DM

** Calculated values.

Table 4: Effect of feeding probiotic supplemented diets on feed intake (g/hen/day)

Treatment	Age (weeks)			Mean for treatments
	47-50	51-54	55-58	
T_1	120.93 ± 0.92	121.03 ± 2.31	118.56 ± 0.97	120.18 ± 0.72
T_2	120.84 ± 0.82	118.71 ± 0.79	118.54 ± 1.05	119.36 ± 0.32
T_3	119.91 ± 0.82	117.79 ± 1.01	119.60 ± 1.26	119.10 ± 0.54
T_4	119.30 ± 1.19	117.99 ± 1.00	118.30 ± 1.91	118.53 ± 0.63
Mean for periods	120.25 ± 0.45	118.88 ± 0.72	118.75 ± 0.59	

Table 5: Effect of feeding probiotic supplemented diets on feed efficiency (kg feed consumed for dozen eggs)

Treatment	Age (weeks)			Mean for treatments
	47-50	51-54	55-58	
T_1	1.65 ± 0.009	1.70 ± 0.038	1.74 ± 0.021	1.69 ± 0.012
T_2	1.64 ± 0.009	1.64 ± 0.003	1.77 ± 0.049	1.68 ± 0.015
T_3	1.65 ± 0.019	1.66 ± 0.007	1.74 ± 0.024	1.68 ± 0.012
T_4	1.63 ± 0.010	1.67 ± 0.012	1.75 ± 0.045	1.68 ± 0.013
Mean for periods	1.64 ± 0.45	1.67 ± 0.011	1.75 ± 0.016	

Table 6: Effect of feeding probiotic supplemented diets feed efficiency (kg feed consumed for kg eggs)

Treatment	Age (weeks)			Mean for treatments
	47-50	51-54	55-58	
T_1	2.57 ± 0.03	2.62^{a} ± 0.02	2.70 ± 0.07	2.64 ± 0.01
T_2	2.57 ± 0.01	2.54^{c} ± 0.01	2.72 ± 0.07	2.61 ± 0.02
T_3	2.60 ± 0.01	2.56^{bc} ± 0.01	2.66 ± 0.04	2.61 ± 0.01
T_4	2.56 ± 0.01	2.61^{ab} ± 0.03	2.66 ± 0.04	2.61 ± 0.01
Mean for periods	2.58 ± 0.01	2.58 ± 0.01	2.69 ± 0.03	

Table 7: Effect of feeding probiotic supplemented diets on total serum protein (g/dl)

Treatment	At the start of experiment	At the end of experiment
T_1	6.73 ± 0.13	6.27 ± 0.31
T_2	6.73 ± 0.22	6.91 ± 0.16
T_3	6.61 ± 0.14	6.69 ± 0.10
T_4	6.78 ± 0.13	6.96 ± 0.12
Mean for periods	6.71 ± 0.08	6.71 ± 0.11

Table 8: Effect of feeding probiotic supplemented diets on serum cholesterol (mg/dl)

Treatment	At the start of experiment ^{NS}	At the end of experiment*
T_1	137.98 ± 4.5	138.76^{a} ± 2.3
T_2	132.43 ± 2.0	129.26^{b} ± 1.6
T_3	130.81 ± 1.7	122.67^{b} ± 2.6
T_4	136.67 ± 1.3	124.61^{b} ± 3.1
Mean for periods	134.47 ± 1.4	128.83 ± 1.7

Means with similar superscripts in a column do not differ significantly

NS: Non significant

* Significant at $P \leq 0.01$

Table 9: Effect of feeding probiotic supplemented diets on body weight (g)

Treatment	Age (weeks)			Mean for treatments*
	47-50*	51-54*	55-58^{NS}	
T_1	1,353^{a} ± 11.28	1,297^{a} ± 19.90	1,238 ± 18.77	1,296^{a} ± 14.87
T_2	1,290^{b} ± 28.17	1,264^{ab} ± 18.81	1,249 ± 11.31	1,268^{ab} ± 14.39
T_3	1,286^{b} ± 12.25	1,247^{ab} ± 11.47	1,215 ± 24.53	1,249^{b} ± 10.11
T_4	1,268^{b} ± 14.48	1,234^{b} ± 7.23	1,200 ± 15.95	1,234^{b} ± 2.81
Mean for periods	1,299 ± 12.33	1,261 ± 9.69	1,226 ± 9.74	

Means bearing similar superscripts in a column do not differ significantly.

NS: Not significant

*Significant at $p \leq 0.05$

Table 10: Effect of feeding probiotic supplemented diets on relative economics

S. No	Criterion	T_1	T_2	T_3	T_4
1	Cost of feed per kg (Rs)	25.50	25.52	25.54	25.56
2	Feed consumption/12 eggs (kg)	1.69	1.68	1.68	1.68
3	Feed cost/12 eggs (Rs)	43.10	42.87	42.91	42.94
4	Selling price of 12 eggs (Rs)	48	48	48	48
5	Income over feed cost/12 eggs (Rs)	4.91	5.13	5.09	5.06
6	Percent improvement in income over the control	-	4.51	3.83	3.14

Similar findings were also reported by Yoruk *et al.,* (2004) in Hisex brown layers (123.9g in control group vs 122.6 g in probiotic group); Mahdavi *et al.,* (2005) in Hyline 36 layers (97.86g in control group vs 96.82g in probiotic group); Yousefi and Karkoodi (2007) in Hyline 36 layers (106.25 g in control group vs 108.11 g in probiotic group); Panda *et al.,* (2006) in Single Comb White leghorn layers (102.42g in control group vs 104.29g in probiotic group).

Further non-significant difference with regard to feed intake also observed by Ashayerizadeh *et al.,* (2009) in broilers (106.13 g in control group vs 105.46g in probiotic group); Balevi *et al.,* (2009) in Hyline brown layers (113.23 g in control group vs 112.88 g in probiotic group), Yalcin *et al.,* (2010) in Hyline brown layers (103.4 g in control group vs 104.3 g in probiotic group); Kalavathy Ramasamy *et al.,*(2009) in Lohmann Brown pullets (101.23 g in control group vs, 104.17 g in probiotic group); Sattar Bagheri Dizaji and Rasoul Pirmohammadi (2009) in Hyline 36 (103.25 g in control group vs 105.8 g in probiotic group); Berrin (2011) in quails (36.88 g in control group vs 36.99 g in probiotic group); Mikulski *et al.,* (2012) in Hisex brown layers (124.0 g in control group vs 123.7 g in probiotic group); Abdelqader *et al*., (2013) in Single Comb White leghorn layers (110.8 g in control group vs 110.2g in probiotic group). The addition of probiotic supplementation did not show any impact on feed intake. This might be due to probiotics which do not impart any palatability to the diets and also might be due to the feeding of iso-caloric and iso-nitrogenous diets throughout the experimental period.

On contrary significantly ($p < 0.05$) high feed intake was observed by Nahashon *et al*., (1996) in DeKalb XL Single Comb White Leg horn pullets (118g in control group vs 121g in probiotic group). On the other hand Swain *et al.,* (2011) reported significantly ($p < 0.05$) decreased feed intake in Vanaraja layers per bird in kg during 14 weeks of experimental period fed on probiotic supplemented group (10.87 kg) over control (11.18 kg).

Feed efficiency (kg feed/dozen eggs) was numerically better in layers fed on probiotic supplemented diets as compared to control diets during the experimental period (Table 5). The feed efficiency of T_1, T_2, T_3 and T_4 diets was 1.69, 1.68, 1.68 and 1.68, respectively. This effect might be due to the establishment of normal gut flora, prevention of pathogenic microbes and better availability of nutrients by the action of probiotics added in the diet. The result in the present investigation for probiotics were in conformity with the findings of Panda *et al.,* (2006) in Single Comb White Leghorn breeders during the late laying period (1.42 in control group vs 1.42 in probiotic supplementation @ 150 mg /kg basal diet group); Yalcin *et al*., (2008) in Lohmann Brown laying hens (1.42 in control group vs 1.42 in probiotic group). On the contrary, Moorthy *et al*., (2010) reported that feed efficiency was not improved in layers fed with probiotic supplemented diets.

There was no significant difference observed in feed efficiency (kg feed / kg eggs) in layers fed on probiotic supplemented diets among the treatment groups during the experimental period (Table 6). The results in T_1, T_2, T_3 and T_4 groups were 2.64, 2.61, 2.61 and 2.61, respectively (Fig. 1). This was in conformity with the findings of Mahdavi *et al.,* (2005) in Hy-line 36 strain (1.96 in control group vs 2.02 in probiotic supplemented group); Yousefi and Karkoodi (2007) in Hyline 36 layers during 63 to 75 weeks of age (2.4 in control group vs 2.57 in *Saccharomyces cerevisiae* supplemented group); Yalcin *et al*., (2008) in Lohmann Brown laying hens (2.03 in control group vs 2.01 in probiotic supplemented birds), Wei Fen Li *et al.,* (2011) in Shaoxing Ducks (3.135 in control group vs 3.020 *Bacillus subtilis* supplemented birds).

On contrary, significantly better feed conversion efficiency (kg feed / kg eggs) observed in probiotic supplemented group by Balevi *et al.,* (2009) in Hysex Brown layers (2.59 in control vs 2.49 in probiotic supplemented birds); Yalcin *et al.,* (2010) in Hyline Brown layers (2.8 in control vs 2.3 in probiotic supplemented birds); Mikulski *et al.,* (2012) in Hisex Brown layers from 22 to 37 weeks of age (2.27 in control group vs 2.21 in probiotic supplemented group). The reason of variable effect of biological additives may be confounded by variations in gut flora and environmental conditions (Mahdavi *et al*., 2005). The contradictory results reported by various researchers might be related to the dosages of probiotic and concentration of bacteria used in the diet (Shivani Katoch *et al*., 2011).

Total serum protein in layers fed with different levels of probiotics was not affected during experimental period (Table 7). At the end of the experiment the total serum protein (g/dl) in T_1, T_2, T_3 and T_4 was 6.71, 6.27, 6.91 and 6.69, respectively. The total protein was reduced by 7.33% in control (T_1) but increased in T_2, T_3 and T_4 by 2.60, 1.19 and 2.58% respectively. Similar findings were also observed by Panda *et al.,* (2006) in White Leghorn layers (4.62 in control group vs 4.39g/100 ml in probiotic supplemented group) during 65 to 76 weeks of age. Yalcin *et al.,* (2008) in Lohmann Brown layers (4.46 in control group vs

4.35g/100 ml in probiotic supplemented group); Ashayerizadeh *et al.*, (2009) in Ross 308 broilers (3.89 in control group vs 3.80g/100 ml in probiotic supplemented group).

In the present study, serum cholesterol (mg/dl) was estimated at the start and at the end of experimental period. The twelve weeks of probiotic supplementation to the layers resulted in significantly ($p < 0.05$) low serum cholesterol levels compared to control group (Table 8). High serum cholesterol (mg/dl) was observed in T1 (138.76) and low levels (122.67) in T4 group (Fig 2). The serum cholesterol was increased by 0.56% in control (T1) but reduced in T2, T3 and T4 by 2.39, 6.22 and 8.82% respectively. These findings are in accordance to findings of Panda *et al.*, (2006) in White Leghorn layers (133.81 in control group vs 116.17 mg/100 ml in probiotic supplemented group) during 65 to 76 weeks of age; Al-Kassie et al., (2008) in Arbor-Acres broilers (196.83 in control group vs 186.50 mg/100 ml in probiotic supplemented birds); Wei Fen Li *et al.*, (2011) in Shaoxing Ducks (126.96 in control group vs 97.09 mmol/L in probiotic supplemented birds).

This reduction in serum cholesterol could be attributable to reduced absorption and/or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan *et al.*, 1995). Utilization of cholesterol by microbes for their own metabolism (Nelson and Gilliland, 1984) and deconjugation of bile salts in the intestine, thereby preventing them from acting as precursors in cholesterol synthesis (Abdulrahim *et al.*, 1996) and also observed that the hypocholesteraemic effect on the host due to probiotic feeding was also dependent on duration of feeding.

The results (Table 9) obtained on body weights during the experimental period in layers were significantly ($p \leq 0.05$) higher in T_1 (1296 g), than the probiotic supplemented groups T_2 (1268 g) followed by T_3 (1249 g) and lowest body weight was recorded in T_4 (1234) group (Fig. 3). This might be due to the fact that as egg production increases depletion of nutrients from body increases which leads to lower body weights and also FCR value in T_4 was lowest (2.61) over T_1 (2.64) indicating that the birds consumed less feed per unit gain in live weight. The results of the present study corroborating with the reports of Pedrosso *et al.*, (1999) who concluded that addition of probiotics (*Bacillus subtilis*) increased feed utilization without significantly affecting the live weight gain in pullets. On contrary non-significant results were observed by Sattar Bageri Dizaji and Rasoul Pirmohammadi (2009) in Hy-line W-36 strain laying hens during 46 to 55 weeks of age (1462 in control group vs 1450.6 g in probiotic supplemented group). Panda *et al.*, (2006) observed non-significant difference in body weights in White Leghorn layers (1657 in control vs 1644 g in probiotic group) with probiotic supplementation during 65-76 weeks of age.

Similar findings were also observed by Yalcin *et al.*, (2010) in Hyline Brown layers (1745 in control vs 1811g in probiotic supplemented birds); Wei Fen Li *et al.*, (2011) in Shaoxing Ducks (1710 in control group vs 1699 g/d/bird in probiotic supplemented birds); Abdelqader *et al.*, (2013) in Lohmann white laying hens (1714 in control group vs 1715g in probiotic supplemented group) during 64 to 75 weeks of age.

The results of the present study revealed that probiotic supplementation to the laying hens during post peak period (Table 10), improved percent income from the egg production by 4.51 in T_2, 3.83 in T_3 and 3.14 in T_4 over the control (T_1). These findings are in accordance with the earlier observations of Moorthy et *al.*, (2010). Non-significant improvement in returns in the present study can be attributed to non-significant results obtained with egg production and feed efficiency. On contrary Davis and Anderson (2002) reported net income ($/hen) of 9.74 in control group and 10.33 in probiotic supplemented group in Single Comb White Leghorn layers. Chaurasia *et al.*, (2008) found significant gain in net profits (Rs. 19.67 vs 28.03) in Vanaraja birds; this might be due to significant improvement in FCR. Swain *et al.*, (2011) reported that net profit increased (9.2%) due to supplementation of probiotics and yeast @ 2.0g/kg diet compared to control diet (8.1%).

Conclusion

It can be concluded that supplementation of probiotics at 0, 5, 10 and 15% level in layer diets during post peak production has no significant impact on feed intake, feed efficiency, and serum protein. Serum cholesterol levels were significantly ($p < 0.05$) reduced in treatment groups over control group during the experimental period. The percent improvement in income over the control was higher in treatment group with 5% level of probiotic supplementation in layer diets.

Based on the performance, relative economics and serum cholesterol levels it may be concluded that probiotics can be supplemented @ 50g / ton of layer diets during post peak production without any adverse effects. The use of probiotics in the present study is an alternative source for the use of antibiotic feed supplements in the poultry diets without causing any deleterious effects on the health of the birds. Further studies are required to ascertain the level of incorporation of probiotics with different strains at different periods of egg production in laying hens.

References

1. Abdelqader Anas, Abdur-Rahman Al-Fataftah, Gurbuz das. Effects of dietary *Bacillus subtilis* and inulin supplementation on performance, eggshell quality, intestinal morphology and microflora composition of laying hens in the late phase of production. Animal Feed Science and Technology. 2013;179:103-111.
2. Abdulrahim S.M, Haddadin M.S.Y, Hashlamoun E.A.R and Robinson R.K. The influence of *Lactobacillus acidophilus* and Bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. British Poultry Science 1996;37:341-46.
3. Adams CA. Nutricines in poultry production: focus on bioactive feed ingredients. Nutr. Abstr. Rev. 2004;74(6B):1-12.
4. Al-Kassie G.A.M, Al-Jumaa Y.M.F, and Jameel Y.J. Effect of probiotic (*Asperigillus niger*) and prebiotic (*Taraxacum officinale*) on Blood picture and Biochemical properties of broiler chicks. International Journal of Poultry Science. 2008;7 (12):1182-84.
5. Allian. In vitro enzymataic method of cholesterol estimation. Clinical Chemistry. 1974;26(13):1775.
6. AOAC. Official methods of analysis. 18th Edn. Association of Official Analytical Chemists, Washington DC. 2005.
7. Arora SP and Harjit kaur. Principles of Animal Nutrition and Nutrient Dynamics. ICAR, 2010.
8. Ashayerizadeh. A, Dabiri. N, Ashayerizadeh. O, Mirzadeh. K.H. Effect of Dietary Antibiotic, Probiotic, and Prebiotic as Growth Promoters, on Growth Performance, Carcass Characteristics and hematological Indices of Broiler Chickens. Pakistan Journal of Biological Sciences. 2009;12(1):52-57.
9. Balevi T, Ucan U S, Coskun B, Kurtoglu and Cetingul S. Effect of dietary probiotic on performance and humoral immune response in layer hens. *Archiva zootechnica*. 2009;12(2):14-23.
10. Berrin. Effects of probiotic and prebiotic (mannon oligosaccharide) supplementation on performance, egg quality and hatchability in quail breeders. Ankara univ vet Fak Derg. 2011;58:27-32.
11. BIS. Bureau of Indian Standards. Requirements for chicken feeds. IS 1374-1992. Manak Bhavan, 9, Bhadursha Zafar marg, New Delhi. 1992.
12. Chaurasia RK, Vidyarthi VK, Rai DC, Zuyie and Sharma VB. Effect of dietary probiotic on the performance and economy of rearing vanaraja birds. Indian Journal of Animal Production Management. 2008;24(1-2):18-21.
13. Davis GS and Anderson KE. The effects of feeding of direct-fed microbial, primalac, on growth parameters and egg production in Single Comb White Leghorn hens. Poultry Science. 2002;81:755-759.
14. Fuller R. History and Development of Probiotics. *J. Appl. Bacteriol.*, 1989;66:365-78.
15. Fuller R. History and development of probiotics. In: *Probiotics- the Scienctific Basis*. (Ed.) R. Fuller, Chapman and Hall, London, 1992.pp.1-8.
16. Jin LZ, Ho YW. Ali MA, Abdullah N and Jalaludin S. Adhesion of *Lactobacillus* isolates to the intestinal epithelial cells of chicken. Letters in Applied Microbiology. 1996;22:229-32.
17. Kalavathy Ramasamy, Abdullah Norhani, Jalaludin S, Wong M and Ho Y.W. Effects of *Lactobacillus* cultures on performance of laying hens, and total cholesterol, lipid and fatty acid composition of egg yolk. Jsci Food Agric. 2009;89:482-86.
18. Mahdavi A.H, Rahmani H.R and Pourreza J. Effect of probiotic supplements on egg quality and laying hens performance. International Journal of Poultry Science. 2005;4(7):488-92.
19. Mikulski D, Jankowski J, Naczmanski J, Mikulska. M and Demey V. Effect of dietary probiotic (*Pediococcus acidilactici*) supplementation on performance, nutrient digestibility, egg traits, egg yolk cholesterol and fatty acid profile in laying hens. Poultry Science. 2012;91:2691-2700.
20. Mohan B, Kadirvel R, Bhaskaran M and Natarajan A. Effect of probiotic supplementation on serum/ yolk cholesterol and on egg shell thickness in layers. British Poultry Science. 1995;36:799-803.
21. Moorthy M, Ravi.S and Ravi Kumar. M 2010 Effect of feeding probiotics in combinations with Aloe vera and Curcuma longa on the performance of White Leghorn Layers. Indian Journal of Animal Nutrition. 2010;27(4):409-10.
22. Nahashon SN, Nakaue HS, Mirosh LW. Performance of Single Comb White Leghorn fed a diet supplemented with a live microbial during the growth and egg laying phases. Animal Feed Sciences Technology. 1996;57:25-38.
23. Narahari, D. Commercial broiler production, Emkay Publications, Delhi, 1st Ed., 1996.pp.122.
24. Nelson C R and Gilliland S E 1984 Cholesterol uptake by *Lactobacillus acidophilus*. Journal of Dairy Science. 1984;67(Suppl. 1):50. Paper presented at the American Dairy Science Association, 79th Annual Meeting, 24-27 June.
25. Panda A.K, Rama Rao S.V and Raju M.V.L.N. Dietary supplementation of probiotics during the late laying period on production performance of

white Leghorn layer breeders and their progeny. Indian Journal of Poultry Science. 2006;41(3):304-308.

26. Panda A.K, Rama Rao S, Raju M.V.L.N. Effect of Probiotic (*Lactobacillus sporogenes*) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. Jsci Food Agric. 2008;88:43-47.
27. Pedrosso AA, Moraes VMB and Ariki J. Effect of protein and probiotic (*Bacillus subtilis*) levels in the diets of pullets and laying hens. *Revista Brasileira – de-ciencia-avicola*, 1999;1(1):49-54.
28. Radostits OM, Leslie KE and Fetrow J. Planned animal health and production in swine herds. In: *Herd Health*. Food Animal Production Medicine. 2nd edn., W.B. Saunders Co, London, 1994.pp.435-26.
29. Ramasubba Reddy, V. and Bhosale, D.T. Hand book of poultry nutrition. American Soybean Association 168, Jor bagh, New Delhi, India. 2001.p.110.
30. Satbir Singh, Sharma VP, Panwar VS and Singh S. Influence of levels of probiotic and energy on mortality and economics of broilers in summer. Indian Journal of Animal Sciences. 1999;69:830-31.
31. Sattar Bagheri Dizaji and Rasoul Pirmohammadi. Effect of *Saccharomyces cervisiae* and Bioplus 2B on Performance of Laying Hens. International Journal of Agriculture & Biology. 2009;11:495-97.
32. Shivani Katoch, Mukul Kaistha, Sharma K.S, Sharma V.K and Katoch B.S. Effect of dietary supplementation of direct fed microbes on growth and production performance of egg type chicken. Indian Journal of Poultry Science. 2011;46(1):61-66.
33. Snedecor GM and Cochran WG. Statistical methods (8th ed) Oxford and IBH publishing Company, Calcuta. 1994.
34. Span Diagnostics limited, Plot No. 336 Sachin (Surat), Gujarat.
35. Swain B.K, Naik P.K, Chakurkar E.B and Singh N.P. Effect of probiotic and yeast supplementation on performance, egg quality characteristics and economics of production in vanaraja layers. Indian Journal of Poultry Science. 2011;46(3):313-15.
36. Talapatra S K, Roy SC and Sen KC. Estimation of Phosphorus, Chlorine, Calcium, Magnesium, Sodium and Potassium in food stuffs. Indian Journal of Veterinary Science and Animal Husbandry 1940;10:243-58.
37. Talebi A, Amirzadeh B, Mokhtari B and Gahri H. Effects of a multi-strain probiotic (Primilac) on performance and antibody responsed to Newcastle disease virus and Infectious bursal disease virus vaccination in broiler chickens. Avian Pathology 2008;37(5):509-12.
38. Varley H, Gowenlock AH and Bell M. Practical Clinical Biochemistry, 5th Edition, William Hienemann Books Ltd., London: 1980.pp.550.
39. Wei Fen Li, Imran Rashid Rajput, Xin Xu, Ya Li Li, Jian Lei, Qin Huang and Min Qi Wang. Effects of probiotic (Bacillus subtilis) on laying performance, Blood Biochemical Properties and intestinal Microflora of Shaozing Duck. International Journal of Poultry Science. 2011;10(8):583-89.
40. Yalcin. S, Ozsoy. B, Erol. H, and Yalcin. S. Yeast culture supplementation to laying hen diets containing soybean meal or sunflower seed meal and its effect on performance, egg quality traits, and blood chemistry. J.Appl.Poult.Res. 2008;17:229-36.
41. Yalcin S, Yalcin S, Cakin K, Eltan O and Dagasan L. Effect of dietary yeast autolysate (*Saccharomyces cerevisiae*) on performance, egg traits, egg cholesterol content, egg yolk fatty acid composition and humoral immune response of laying hens. Jsci Food Agric. 2010;90:1695-1701.
42. Yoruk M.A, Gul.M , Hayirli A and Macit M. The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. Poultry Science Association Inc. 2004;83:84-88.
43. Yousefi M and Karkoodi. Effect of probiotic Thepax® and *Saccharomyce cerevisiae* supplementation on performance and Egg quality of Laying Hens. International Journal of Poultry Science. 2007;6(1):52-54.