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Effect of probiotic supplementation on eggs production performance in laying hens

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Abstract
The present investigations on "Effect of Probiotic Supplementation on Eggs Production Performance in Laying Hens" were conducted at the Poultry Unit, Division of Animal Husbandry and Dairy Science, College of Agriculture, Pune. A total number of 160 White leghorn laying hen, of 32 weeks of age at the beginning of the study were used for the present study. The laying hens were weighed and distributed randomly into four treatment groups viz., T_0 (Diet without Probiotic (*Bacillus subtilis*) (Control), T_1 (Diet + 500 gm Probiotic (*Bacillus subtilis*)/ton of feed.), T_2 (Diet + 1000 gm Probiotic (*Bacillus subtilis*)/ton of feed) and T_3 (Diet + 1500 gm Probiotic (*Bacillus subtilis*)/ton of feed.) with 40 laying hen in each treatment as replicates on equal weight basis. The laying hens were fed the same experimental diets with different levels of probiotic supplementation in laying hen trial during the experimental period from 32 to 41 weeks of age. The objectives of this study to evaluate the egg production performance of White Leghorn laying hens supplemented with *Bacillus subtilis* and to assess the feed conversion ratio to determine the efficiency of feed utilization. Key performance indicators, including feed intake, hen day egg production (HDEP), egg mass and feed conversion ratio (FCR) were assessed over the experimental period. Results indicated that hens receiving *Bacillus subtilis* supplementation (especially T_2) exhibited significantly higher feed intake and enhanced egg production, with HDEP values of 75.96, 79.25, 82.42 and 77.58 percent for T_0, T_1, T_2 and T_3, respectively. The overall egg mass also increased notably in the T_2 group, reaching an average of 45.31 g compared to 40.62 g in the control group. Furthermore, the lowest FCR was recorded in the T_2 group at 1.46, demonstrating improved feed efficiency in converting feed to egg mass. In conclusion, the incorporation of *Bacillus subtilis* in poultry diets may provide a viable approach to improve the overall productivity and specifically, the supplementation resulted in an improvement in egg weight an increase in egg mass was observed in the T_2 treatment group.

Keywords: Poultry, probiotic supplementation, eggs production

Introduction
Poultry industry is a fast growing segment of Indian economy, contributes about eight percent of the gross national income. India's poultry sector represents one of the biggest success stories of the country over the past decade. India ranks 3rd in egg production and 7th in chicken meat production in the world (BAHS 2023) [2]. About 3.4 million tons (74 billion) of eggs are produced from 260 million layers and 3.8 million tons of poultry meat is produced from 3000 million broilers per annum in India. Nearly 20 million farmers are employed in poultry industry with around 1,000 hatcheries operating across India. In India, broiler production has assumed the dimensions of poultry in which quantity and quality of meat production is of paramount importance. As feed comprises of about 70-80 percent of the total cost of poultry enterprises, the increased production of quality meat at lower cost is need of the day.

There is a vast scope for growth of poultry industry in India. Global economic pressures have driven a tendency of the poultry production to produce more products per unit of feed intake with minimum stress to the poultry and environment. Economical poultry production largely depends on optimum utilization of feed, improved growth performance, absence of diseases, minimum morbidity and mortality. Feed plays an important role in the poultry production system and represents about 70-75 percent of total cost of production. The feeding cost of poultry production therefore can be minimized by precise nutrients supply and efficient nutrients utilization.

Various feed additives are being included in poultry diet to improve growth rate, feed efficiency and product quality and to reduce the production cost.

The word 'probiotic' was used by Elie Metchnikoff in 1906, a Russian scientist and he was awarded Noble prize in medicine. He showed the beneficial effects of microbes replacing those which were harmful for treating intestinal illness. He is known as the father of probiotics (Rautray *et al.*, 2011) [11]. Application of biotechnological tools to boost up the production efficiency of poultry is a dynamic feed technology. Such technology needs to be well justified so that it does not create any harm to poultry as well as to the ultimate consumers of poultry products. With the advancement of biotechnology in poultry feeds and nutrition and banning of harmful growth promoters, antibiotics in particular, the use of probiotics is gaining momentum globally in feed formulation strategy. Antimicrobial resistance is now a worldwide anxiety (WHO, 2018) [16] and alteration of immune response due to feeding of antibiotic growth promoters, has lead poultry nutritionists to find out suitable alternatives to antibiotics. Probiotics of various preparations are considered to be important tools in this regard (Kabir, 2009) [7]. It is now well established that newly hatched chicks 'gut is sterile and establishment of micro flora in the gut of chicken starts just after hatch from hatching tray and Hatcher which is called "cloacal drinking", thereafter gradually with the introduction of feed and water. Microorganisms which colonize in the gut may be either beneficial or harmful based on their response to the host animal. Attachment of beneficial micro flora is always desired but negative to it may also occur. The beneficial organisms maintain gut equilibrium, improve health of birds and enhance, or at least maintain production. On the contrary, harmful bacteria like *E. coli*, *Salmonella*, *Coliform* and others may alter the gut equilibrium to negative direction. This problem can be elucidated by the supplementation of probiotics as they are believed to have a positive impact on poultry. Until recently, a number of beneficial effects have been reported from research works with probiotics although there are some controversies. Supplementation of commercial preparations of probiotics through diet comprising either single or multi-strain positively affected laying performance and egg quality characteristics specifically shell thickness and Haugh unit of commercial layers (Ray, 2018) [12].

Materials and Methods

A total number of 160 White leghorn laying hen, of 32 weeks of age at the beginning of the study were used for the present study. The laying hens were weighed and distributed randomly into four treatment groups viz., T_0, T_1, T_2 and T_3 with 40 laying hen in each treatment as replicates, on equal weight basis. The laying hens were fed the same experimental diets with different levels of probiotic supplementation in laying hen trial during the experimental period from 32 to 41 weeks of age.

Treatment details

1. T_0: Basal Diet without Probiotic (*Bacillus subtilis*) (Control)
2. T_1: Basal Diet + 500 gm Probiotic (*Bacillus subtilis*)/ton of feed.
3. T_2: Basal Diet + 1000 gm Probiotic (*Bacillus subtilis*)/ton of feed.
4. T_3: Basal Diet + 1500 gm Probiotic (*Bacillus subtilis*)/ton of feed.

Data collection

1. Feed consumption

Daily feed consumption of each group was estimated as difference between the total quantity of feed offered and quantity of left over during 24 hours period. Feed consumption so recorded was added together for seven days of the week and will be considered as weekly feed consumption.

2. Egg production

2.1 Hen day egg production

First, computed the number of hen-days in the period by totaling the number of hens alive on each day of the period and calculated the number of eggs laid during the same period. Then the following formula was used to calculate hen day percent production as described by Singh and Kumar (1994).

$$\text{HDEP (\%)} = \frac{\text{Number of eggs laid during the period}}{\text{Number of hen-days in period}} \times 100$$

Daily egg production was recorded for each treatment group in all experiments. The eggs were collected at 12:00 noon each day.

2.2 Eggs mass

Daily eggs mass was calculated by multiplying the eggs weight with percent hen-day eggs production (HDEP) by 100.

$$\text{Eggs Mass} = \frac{\text{Average eggs weight in grams x \% hen day eggs production}}{100}$$

3. Feed conversion ratio

Feed conversion ratio was calculated on the basis of per dozen of eggs as well as per Kg mass of eggs on weekly basis for each experimental unit, by using the following formulae as described by Singh and Kumar (1994).

3.1 Feed conversion ratio/dozen of eggs

The amount of feed consumed to produce per dozen of eggs will be calculated.

$$\text{FCR/dozen eggs} = \frac{\text{Total feed consumed (kg) during the period}}{\text{Total number of eggs produced during the period}} \times 12$$

3.2 Feed conversion ratio/kg of eggs

The amount of feed consumed to produce per kg of eggs will be calculated.

$$\text{FCR/Kg egg mass} = \frac{\text{Total feed consumed during the period (kg)}}{\text{Total egg mass produced during the period (kg)}}$$

Statistical analysis

The data were analyzed using General Linear Model procedure of statistical package for social sciences (SPSS) 15th version and comparison of means tested using Duncan's multiple range test (1955) and significance was considered at (p<0.05).

Results and Discussion

1. Feed Intake (g/hen/day)

Data related to feed intake presented in the table 1. It was observed that overall feed intake by the birds of various treatment groups were 99.59, 99.66, 99.75 and 99.70 g/hen/day in T_0, T_1, T_2 and T_3 treatment groups, respectively. The average feed intake was significantly (p<0.05) higher in T_2 group. However, treatment T_1, T_3 and T_2 and T_3 were at par to each other. The outcomes were consistent with previous studies by Falaki *et al.* (2010) [5] and Sheoran *et al.* (2018) [14] which discovered a significant (p<0.05) increase in feed intake in White Leg horn layers.

When compared to the control group, feed consumption was increased in the treated groups. The probiotic-supplemented group's feed consumption is higher than the control group because naturally occurring beneficial bacteria are present in the birds' intestinal tracts. In contrast to the previously findings, studies by Yoruk *et al.* (2004) [18] and Fathi *et al.* (2018) [6] reported that there was not significant in feed intake among commercial White Leghorn birds supplemented with probiotic and yeast culture.

Table 1: Effect of probiotic supplementation on feed intake (g/hen/day) of White Leghorn layers in different treatment groups and periods

	T_0	T_1	T_2	T_3	S.Em. (±)	CD @ 5 %
Phase I	99.55^b	99.61^{ab}	99.70^a	99.67^a	0.0373	0.1150
Phase II	99.59^c	99.66^b	99.75^a	99.69^b	0.0176	0.0543
Phase III	99.64^b	99.72^a	99.79^a	99.73^a	0.0241	0.0741
Average	99.59^c	99.66^b	99.75^a	99.70^{ab}	0.0168	0.054

The mean values in same row with different superscripts differ significantly (p<0.05)

2. Egg Production

2.1 Hen-day egg production (HDEP %)

It was observed that the overall hen-day egg production under different treatment groups in T_0, T_1, T_2 and T_3 was 75.96, 79.25, 82.42 and 77.58 percent, respectively. The hen day egg production usually differed between groups and statistically significant in I, II and III tri-week period. Overall average HDEP was highest found in treatment T_2 (82.42 %) whereas, the lowest production was found in treatment T_0 (75.96 %). However, treatment T_0 and T_3 at par with each other. Also, treatment T_1 and T_3 were at par with each other. Surprisingly the hen-day egg production in this study was found improved with probiotics powder addition in layers diet.

The findings agree with previous studies on the enhancement and increase in percent hen day egg production in commercial layer birds Abdelqader *et al.*, (2013) [1]. Similar results match with findings conducted by Berrin (2011) [4] in Japanese quail and Shaoxing ducks. Moorthy *et al.* (2010) [10] on the other hand, held differing views and found that probiotic treatment in White Leghorn layers significantly (p<0.05) decreased the percentage of hen day egg production.

Table 2: Effect of probiotic supplementation on average hen day egg production of laying hens (per period/treatment)

Phase	T_0	T_1	T_2	T_3	S.Em. (±)	CD @ 5%
Phase I	75.25^b	78.50^{ab}	80.75^a	78.00^{ab}	1.20	3.72
Phase II	76^b	79.13^{ab}	82.38^a	77.75^b	1.22	3.77
Phase III	76.63^b	80.13^{ab}	84.13^a	77.00^b	1.81	5.58
Average	75.96^c	79.25^b	82.42^a	77.58^{bc}	0.93	2.87

The mean values in same row with different superscripts differ significantly (p<0.05)

2.2 Egg Mass (gm/hen/day)

It was revealed from Table 3 that the overall average egg mass under different treatment groups T_0, T_1, T_2 and T_3 was 40.62, 42.31, 45.70 and 43.15, respectively. The egg mass differed between groups and statistically significant (p<0.05) in tri-week period. In overall average egg mass was highest found in treatment T_2 (45.70 g) which is significantly superior rest on the group whereas, the lowest production was found in treatment T_0 (40.62 g). Whereas treatment T_1 and T_3 were at par with each other. The egg mass in this study was found improved with probiotics powder supplementation in layers diet.

Mikulski *et al.* (2012) [9] found that Hisex Brown layers showed a significant (p<0.05) increase in egg mass (9.21 kg of egg/hen in the control group vs. 9.67 kg of egg/hen in the probiotic supplemented group). In Lohmann white laying hens, Abdelqader *et al.* (2013) [1] observed a non-significant increase in egg mass between 64 and 75 weeks of age (42.1 g/h/d in the control group vs. 48.0 g/h/d in birds fed with *Bacillus subtilis* @ 1 g/Kg baseline diet).

Table 3: Effect of probiotic supplementation on average egg mass (g/hen/day) of laying hens (per period/treatment)

Phase	T_0	T_1	T_2	T_3	S.Em. (±)	CD @ 5%
Phase I	40.30^c	41.39^c	44.73^a	43.24^b	1.05	3.24
Phase II	40.91^b	42.16^b	45.07^a	44.44^a	0.57	1.47
Phase III	40.68^b	43.37^b	47.31^a	41.76^b	1.05	3.27
Average	40.62^c	42.31^b	45.70^a	43.15^b	0.31	1.55

The mean values in same row with different superscripts differ significantly (p<0.05)

3. Feed conversion ratio

3.1 Feed conversion ratio/dozens of eggs

The tri-weekly average feed conversion ratio of various treatment groups in study period were found to be 1.55, 1.53, 1.46 and 1.53 in T_0, T_1, T_2 and T_3, treatment groups, respectively. The comparison of feed conversion ratio at different tri-weeks revealed that all treatments were non-significant during entire period.

The results of study revealed that feed intake per dozen egg production were not significantly affected by the supplementation of different levels of probiotic in diet of layers. As per the findings of this investigation, Mahdavi *et al.* (2005) [8] and Yousefi and Karkoodi (2007) [19] there was no discernible change in the feed conversion ratio per dozen eggs in White Leghorn layers fed with probiotics. The results of the present study are in contrary to those of previous studies by Shalaei *et al.* (2014) [13] and Sheoran *et al.* (2018) [14] which reported a significant (p<0.05) increase in feed conversion ratio in commercial layer birds fed probiotic supplementation at various levels.

Table 4: Effect of probiotic supplementation on feed conversion/dozens of eggs of laying hens (per period/treatment)

Phase	T_0	T_1	T_2	T_3	S.Em. (±)	CD @ 5%
Phase I	1.48	1.55	1.45	1.48	0.43	NS
Phase II	1.60	1.56	1.53	1.55	0.45	NS
Phase III	1.56	1.50	1.41	1.56	0.44	NS
Average	1.55	1.53	1.46	1.53	0.44	NS

3.2 Feed conversion ratio/kg of Eggs

The tri-weekly average feed conversion ratio of various treatment groups in study period were found to be 2.37, 2.32, 2.19 and 2.26 in T_0, T_1, T_2 and T_3 treatment groups, respectively. Better feed efficiency/kg of eggs was observed in T_2 treatment followed by T_3, T_1 and T_0 groups. The average feed conversion ratio was unaffected by the dietary treatments phase I and phase III though slight numerical difference occurred among the treatment groups. The statistical analysis revealed that there was significant difference in feed efficiency per kg egg mass during first and third phase of experimental period in different treatment group.

This was in agreement with the results of the following studies Yalcin *et al.* (2008) [17] Lohmann Brown laying hens (2.03 in control group vs 2.01 in probiotic supplemented birds), Mahdavi *et al.* (2005) [8] in Hy-line 36 strain (1.96 in control group vs 2.02 in probiotic supplemented group), Wei Fen Li *et al.* (2011) in Shaoxing Ducks (3.135 in control group vs 3.020 *Bacillus subtilis* supplemented birds). However, the probiotic-supplemented group showed significantly higher feed conversion efficiency (kg feed/kg eggs) as reported by Yalcin *et al.* (2008) [17] and Mikulski *et al.* (2012) [9].

Table 5: Effect of probiotic supplementation on feed conversion ratio/kg of egg of laying hens (per period/treatment)

Phase	T_0	T_1	T_2	T_3	S.Em. (±)	CD @ 5%
Phase I	2.47	2.41	2.24	2.31	0.057	NS
Phase II	2.30^{ab}	2.37^{a}	2.21^{b}	2.24^{b}	0.030	0.095
Phase III	2.33	2.20	2.11	2.22	0.048	NS
Average	2.37^{a}	2.32^{ab}	2.19^{bc}	2.26^{c}	0.03	0.093

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

Conclusion

It may be concluded that supplementation of probiotic *Bacillus subtilis* powder improve the performance of White Leghorn layers in term of egg production, eggs laid/day/treatment and hen day eggs production (HDEP). The inclusion of probiotic *Bacillus subtilis* powder in white leghorn layers diet is beneficial in improving the feed consumption and feed conversion ratio.

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