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## Effect of probiotic supplementation on eggs quality performance in laying hens

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### Abstract

The present investigations on “Effect of Probiotic Supplementation on Eggs quality 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<sub>0</sub> (Diet without Probiotic (*Bacillus subtilis*) (Control), T<sub>1</sub> (Diet + 500 gm Probiotic (*Bacillus subtilis*) /ton of feed.), T<sub>2</sub> (Diet + 1000 gm Probiotic (*Bacillus subtilis*) /ton of feed) and T<sub>3</sub> (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 quality of White Leghorn laying hens supplemented with *Bacillus subtilis*. Egg quality analysis revealed that probiotic supplementation positively influenced egg weight, with T<sub>2</sub> yielding an average of 54.15 g, while no significant changes were observed in specific gravity, shell thickness, or internal quality parameters such as yolk and albumen weight. These findings underscore the potential of *Bacillus subtilis* as a beneficial feed additive, enhancing both the quantity and quality of eggs produced by laying hens. In conclusion, the incorporation of *Bacillus subtilis* in poultry diets may provide a viable approach to positive effects were achieved without any adverse impact on egg shell thickness, shell weight, egg yolk weight and egg albumen weight. The internal qualities of eggs were not affected due to treatment variation.

**Keywords:** Poutry, probiotics, eggs quality, treatment variation

### Introduction

Poultry sector in India is an integral part of the agricultural industry with valued at about Rs. 1,93,000 crore (2022). This sector is largely subdivided into two; one with a highly organized commercial sector and another one is unorganized or backyard poultry sector, having about 80 and 20 per cent of the total market share, respectively. India's egg production soared to 138.38 billion during the 2022-23 periods, marking a notable 6.77 per cent increase from the previous year. This surge solidifies India's position as the world's third-largest egg producer, according to data from the FAO. With a per-capita availability of 101 eggs annually, the country showcases a significant contribution to global egg production and consumption trends (BAHS, 2023) [2].

The “Poultry production strategy and action plan 2022” set a target of 6.0 million tones of poultry meat production with per capita meat availability of 5.00 kg per annum in 2022 and the “egg production strategy and action plan 2022” set some targets such as, increasing the egg productions up to 133 billion with per capita egg availability of 93 eggs per annum, doubling the farmer's income, fulfill the protein enriched food requirement of the growing population and achieve 2 per cent of world egg market trade through exports in 2022.

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) [16]. The probiotics are considered as “direct-feed microbial” and they affect the host positively by balancing the intestinal microbial populations (Fuller, 1989) [8].

Microorganisms that are commonly used as probiotics are *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Clostridium*, *Bifido bacterium* species and *E. coli* while yeast and fungus used as probiotics include *Saccharomyces cerevisiae* and *Aspergillus oryzae*

(Fuller, 1989) [8]. Bacteria and yeasts are used as spores or as living microorganisms. Noncolonizing species are *Saccharomyces cerevisiae* and *Bacillus* spp. (spores) while colonizing species include *Lactobacillus* and *Enterococcus* spp. Among probiotic organisms, *Saccharomyces* are known to offer a source of good quality protein and B complex vitamins. Probiotics help in the digestion of food more efficiently, exert better metabolism, maintain the health status of the birds and cause faster weight gain in chicken (Park *et al.*, 2016) [15]. Probiotics are supplied through powder, in encapsulated form or liquid form either in feed or water. Their use seems to be very much strain specific and no explicit recommendation has yet been developed for particular strains and thus has deviations in their feeding results. They deliberate beneficial effects by producing favorable conditions inside the gut of poultry to enhance performances.

## 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<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> 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<sub>0</sub>:** Basal Diet without Probiotic (*Bacillus subtilis*) (Control)
2. **T<sub>1</sub>:** Basal Diet + 500 gm Probiotic (*Bacillus subtilis*) /ton of feed.
3. **T<sub>2</sub>:** Basal Diet + 1000 gm Probiotic (*Bacillus subtilis*) /ton of feed.
4. **T<sub>3</sub>:** Basal Diet + 1500 gm Probiotic (*Bacillus subtilis*) /ton of feed.

### Data collection

#### A. External Egg quality parameters

- **Egg weight:** All eggs laid were numbered and weighed for each replicate at the 7<sup>th</sup> day of each ending week and average egg weight
- **Specific gravity:** Specific gravity is the ratio of the weight of an object to the weight of an equal volume of water. In other words, weigh the egg and then divide the weight by the volume of the egg. Egg specific gravity usually declines as the hen ages. This is partly due to the size of the egg increasing more rapidly than shell weight. Therefore, differences in specific gravity among eggs of similar weights are mainly due to variations in the amount of shell.
- **Egg shell thickness:** Shell thickness was recorded in millimeters (mm) using a Vernier Caliper. Shell membrane was removed manually and two readings were recorded from two different places of the shell. Average of the two was taken as the final reading.
- **Eggs Shell weight:** The weight of shell along with shell membrane was recorded in grams. Each egg was broken on flat glass surface. The egg shell was cleaned with a tissue paper and then weighed on an electronic balance.

## B. Internal Egg quality parameters

- **Yolk weight:** Yolk will be separated from albumen with a separating spoon and weighed with an electronic balance.
- **Albumen weight:** Albumen will be separated from yolk and weighed with an electronic balance.
- **Statistical analysis:** The data were analyzed using General Linear Model procedure of statistical package for social sciences (SPSS) 15<sup>th</sup> version and comparison of means tested using Duncan's multiple range test (1955) and significance was considered at ( $p < 0.05$ ).

## Results and Discussion

### External egg quality parameter

#### Egg weight

The cumulative average egg weight during experimental period was 52.37, 53.24, 54.15 and 54.30 g, in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, group respectively. The average egg weight was differed significantly during entire period of experiment under different treatment groups. During first tri-weekly periods statistically highest production was found in treatment T<sub>2</sub> (54.46 g) followed by T<sub>3</sub> (53.32), T<sub>1</sub> (52.64) and T<sub>0</sub> (51.73) treatment groups. Second and third tri-weekly significantly ( $p < 0.05$ ) higher egg weight recorded in T<sub>2</sub> treatment (supplemented 1000 gm Probiotic (*Bacillus subtilis*) /ton of feed) group. However, treatment in T<sub>0</sub>, T<sub>1</sub> and T<sub>3</sub> were at par to each other.

As was previously documented by Tortuero and Fernandez (1995) [19], the addition of probiotics has a substantial impact on egg weight. This effect may be connected to the strain, concentration and kind of bacteria utilized (viability, dryness, or their products). According to findings by Haddadin *et al.* (1996) [9] and Nahashon *et al.* (1996) [14], the inclusion of biological additives did not substantially ( $p > 0.05$ ) affect the egg weight. These findings may be connected to the probiotic dosages and bacterial concentrations utilized in the diet.

**Table 1:** Effect of probiotic supplementation on average egg weight (g) of laying hens (per period/treatment)

Phase	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	S.E.M. (±)	CD @ 5%
Phase I	51.73 <sup>c</sup>	52.64 <sup>bc</sup>	54.46 <sup>a</sup>	53.32 <sup>b</sup>	0.31	0.95
Phase II	52.67 <sup>b</sup>	53.56 <sup>b</sup>	55.34 <sup>a</sup>	53.34 <sup>b</sup>	0.38	1.18
Phase III	53.09 <sup>b</sup>	54.13 <sup>b</sup>	56.23 <sup>a</sup>	54.25 <sup>b</sup>	0.51	1.56
Average	52.37 <sup>c</sup>	53.24 <sup>b</sup>	54.15 <sup>a</sup>	54.30 <sup>a</sup>	0.22	0.679

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

#### Egg Specific Gravity (g/ml)

Egg specific gravity is an indirect indicator of the amount of shell present in relation to the size of the egg. From table, it was indicated that egg specific gravity not influenced by the of probiotic powder treatment being the differences were statistically non-significant in all phases. The specific gravity ranged from 1.078 to 1.083. The overall average specific gravity of eggs during experimental period were 1.082, 1.081, 1.079 and 1.078 in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> groups, respectively. The statistical analysis revealed that feeding of probiotic powder had no significant effect on egg specific gravity during entire experimental period in different treatment.

The egg specific gravity is an index of shell thickness and porosity. There was non-significant difference among all treatments in the present study. Similarly, Balevi *et al.* (2001

& 2009) [4, 5] and Khan *et al.* (2011) [10] also found no effects of probiotics on egg specific gravity.

**Table 2:** Effect of probiotic supplementation on average egg specific gravity (g/ml) of laying hens (per period/treatment)

Phase	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	S.E.M. (±)	CD @ 5%
Phase I	1.081	1.080	1.078	1.079	0.0024	NS
Phase II	1.083	1.081	1.078	1.079	0.0020	NS
Phase III	1.082	1.081	1.080	1.078	0.0021	NS
Average	1.082	1.081	1.079	1.078	0.44	NS

### Egg shell thickness (mm)

From Table 3, it was found that the overall average egg shell thickness under different treatment groups T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 0.363, 0.358, 0.355 and 0.352 mm, respectively. The statistical analysis revealed that there was significant difference in eggs shell thickness during experimental period in different treatment group. Statistically highest average egg shell thickness was reported in T<sub>0</sub> (0.363). However treatment T<sub>1</sub> and T<sub>2</sub> was at par to each other.

The introduction of probiotics to birds may have produced a favorable environment in their gastrointestinal tract, which may have contributed to this positive effect. During the research, a significant effect on egg shell thickness was noted. These findings concur with those of Sobczak *et al.* (2015) [17] and Fathi *et al.* (2018) [6]. Whereas Mahdavi *et al.* (2005) [12] and Yalcin *et al.* (2008) [20] found that shell thickness was not significantly different across the treatment groups in layers birds, which runs contradictory to the results of the current study.

**Table 3:** Effect of probiotic supplementation on egg shell thickness (mm) of laying hens (per period/treatment)

Phase	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	S.E.M. (±)	CD @ 5%
Phase I	0.365 <sup>a</sup>	0.360 <sup>b</sup>	0.354 <sup>c</sup>	0.350 <sup>d</sup>	0.001	0.004
Phase II	0.364 <sup>a</sup>	0.359 <sup>ab</sup>	0.356 <sup>b</sup>	0.353 <sup>b</sup>	0.002	0.006
Phase III	0.361 <sup>a</sup>	0.355 <sup>b</sup>	0.354 <sup>bc</sup>	0.351 <sup>c</sup>	0.001	0.003
Average	0.363 <sup>a</sup>	0.358 <sup>b</sup>	0.355 <sup>b</sup>	0.352 <sup>c</sup>	0.001	0.003

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

### Egg shell weight (gm)

The overall average egg shell weight under different treatment groups T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> was 6.75, 6.78, 6.85 and 6.80 g respectively. The statistical analysis revealed that there was no significant difference in eggs shell weight during experimental period in different treatment group. Thus, it can be concluded that supplementation of probiotic at different levels in the diet of layers did not affect on average egg shell weight. A non-significant difference was seen in the egg shell weight of White leghorn layers between the ages of 32 and 41 weeks when probiotic supplements were added to their diet at varying amounts.

Forte *et al.* (2016) [7] reported similar results. Yousefi and Karkoodi (2007) [21] and Fathi *et al.* (2018) [6] observed a significant ( $p < 0.05$ ) difference in shell weight among the treatment groups, which is in contrast to the above results.

**Table 4:** Effect of probiotic supplementation on eggs average egg shell weight (gm) of laying hens (per period/treatment)

Phase	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	S.E.M. (±)	CD @ 5%
Phase I	6.75	6.81	6.88	6.63	0.41	NS
Phase II	6.69	6.75	6.81	6.88	0.43	NS
Phase III	6.81	6.78	6.88	6.91	0.49	NS
Average	6.75	6.78	6.85	6.80	0.60	NS

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

### Internal egg quality parameter

#### Yolk weight

It was found that the overall average eggs yolk weight under treatment T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> was 14.70, 14.73, 14.78 and 14.71 g. The eggs yolk weight ranges from 14.69 (T<sub>0</sub>) to 14.84 (T<sub>2</sub>) during experiment period. Statistically feeding the probiotic powder had no significant effect on egg yolk weight during entire experimental period. The trend in the current study indicated that probiotic powder was used no changes in yolk weight compared with eggs from the hen fed the control diet showed. Similar finding were reported by Yalcin *et al.* (2008) [20], Abou El-Ella *et al.* (1996) [11] and Loh *et al.* (2014) [11].

**Table 5:** Effect of probiotic supplementation on average yolk weight (gm) of laying hens (per period/treatment)

Phase	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	S.E.M. (±)	CD @ 5%
Phase I	14.69	14.71	14.73	14.68	0.62	NS
Phase II	14.71	14.74	14.84	14.73	0.48	NS
Phase III	14.72	14.75	14.78	14.71	0.54	NS
Average	14.70	14.73	14.78	14.71	0.59	NS

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

#### Albumen weight

The tri-weekly average egg albumen weight of various treatment groups in study period were found to be 33.22, 34.19, 34.22 and 34.27 g, in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> treatment groups, respectively.

The statistical analysis showed that the feeding of different levels of probiotic powder had no significant effect on albumen weight during entire experimental period. Above finding are in agreement with Yalcin *et al.* (2008) [20], Abou El-Ella *et al.* (1996) [11] and Tang *et al.* (2015) [18] reported similar results.

**Table 6:** Effect of probiotic supplementation on average albumen weight (gm) of laying hens (per period/treatment)

Phase	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	S.E.M. (±)	CD @ 5%
Phase I	33.21	34.16	34.19	34.22	0.62	NS
Phase II	33.22	34.19	34.20	34.25	0.54	NS
Phase III	33.23	34.22	34.28	34.34	0.40	NS
Average	33.22	34.19	34.22	34.27	0.59	NS

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

### Conclusion

The present results indicate that dietary supplementation of probiotic *Bacillus subtilis* powder have shown positive effects on various parameters related to egg quality. Specifically, the supplementation resulted in an improvement in egg weight and these positive effects were achieved without any adverse impact on egg shell thickness, shell weight, egg yolk weight and egg albumen weight. The internal qualities of eggs were not affected due to treatment variation.

Overall, the results indicate the potential benefits of incorporating probiotic *Bacillus subtilis* powder into the diet of laying hens, presenting opportunities for farmers and the poultry industry to improve egg quality and meet consumer demands for healthier and more nutritious eggs.

### Conflict of Interest

Not available

**Financial Support**

Not available

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