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Effect of dietary inclusion of probiotics and prebiotics on external egg quality traits in White Leghorn layers

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Abstract
A 16 week feeding trial was carried out to study the influence of dietary inclusion of probiotics and prebiotics on livability, egg shape index and egg shell quality parameters. A total of 140 White Leghorn layers in the age group of 22 to 38 weeks were divided randomly into seven experimental groups containing 4 replications of 5 birds each. In (T_1) control group layers were fed a basal diet formulated as per BIS (2007) standards. In treatment groups T_2, T_3 and T_4 hens were fed control diet supplemented with probiotics (containing 5×10^8 cfu/g of *Lactobacillus fermentum*, 1×10^9 cfu/g of *Bacillus spp*. and 1×10^9 cfu/g of *Saccharomyces cerevisiae*) @ 0.5g, 1.0g and 2.0g Kg^{-1} feed, respectively, and layers in experimental groups T_5, T_6 and T_7 were fed basal diet added with prebiotics (mannan oligosaccharide of *Saccharomyces* cell wall-47gm, formic acid-32gm, HSCAS-upto 1Kg) @ 0.5g, 1.0 g and 2.0 g Kg^{-1} feed, respectively. Study results revealed that there was ($P<0.05$) increase in egg shell thickness, egg shell weight and egg shell percent under different levels of probiotic and prebiotic regimes (T_3, T_4, T_5, T_6 and T_7) as compared to the control (T_1) group. Shell thickness increased from 0.348 to 0.374 mm indicating an advantage of 0.026mm in favour of probiotics and prebiotics. Egg shape index was not affected, however, egg width and egg length were ($P<0.05$) increased under various levels of probiotics and prebiotics supplementation in comparison to control. The mean body weight gain in hens were not affected significantly and no mortality was observed under different dietary regimes. Thus, result findings clearly indicate that supplementation of probiotics and prebiotics has beneficial effects by significantly improving the egg shell thickness, shell weight, shell weight percentage and egg shape parameters, without affecting health and livability of the layers.

Keywords: Probiotics, prebiotics, egg shell thickness, egg shape index, laying hens

Introduction
Eggshell quality is one of the main concerns in the poultry industry, influencing the economic profitability of egg production and hatchability. Today in this competitive world hen eggs are forced to keep up with the latest trends in nutrition and management of laying hens. The effort of the breeders used to focus particularly on increasing egg yield, weight of eggs and increasing industrialization of their production while an insufficient attention was paid to the quality of eggs and eggshells. Cracked and broken eggs account to about 10% of all the eggs produced between oviposition and their sale in the retail [24]. Egg producers realize the problem of egg breakage as it seriously affects the total profit. Therefore, enhancing the eggshell quality increases the retail value of the eggs in the market. One of the major causes of decrease in eggshell quality is due to increase in egg weight without increase in the amount of calcium carbonate deposited in their shells. When trying to improve the quality of eggs and their shells, one should also consider the rate of calcium absorption. Thus, one of the approach to improve the egg shell quality is by increasing the intestinal Ca^{2+} absorption rate, phenomenon facilitated by the presence within feed of some additives like probiotics and prebiotics. This beneficial effect on eggshell quality due to probiotic feeding may be attributed to a favorable environment in the intestinal tract by feeding of *L. sporogenes*, which might have helped to assimilate more calcium, which was evident by increased concentration of Ca in serum [13]. Thus, in recent years, the use of probiotic and prebiotic in poultry diet has become popular as an alternative substances to antibiotics [7]. In case of probiotics, lactic acid bacteria such as *Lactobacilli*, *Streptococci* and *Bifidobacteria* are the most common organisms used. The US national food ingredient association defined probiotic (direct fed microbial) as a source of live naturally occurring microorganisms, and this includes bacteria, fungi, and yeast [3]. Prebiotics are defined as non-digestible food ingredients which beneficially affect the host by selectively stimulating the growth of and/or activating metabolism of one or limited number of health promoting bacteria in intestinal tract, thus improving host microbial balance [5].

Probiotics and prebiotics influence on the metabolic activity of the beneficial bacteria colony within the layers' intestine, which positively influence mineral absorption rate, especially those of Ca^{2+} and Mg^{2+} [14]. Therefore, this study was thus designed to evaluate the effect of feeding laying hens with probiotic and prebiotic supplemented diets and their effect on egg shell quality parameters and to assess whether they cause any diminishing effects on hens' body weight and livability

Materials and Methods

A total of one hundred and forty single comb White Leghorn hens of commercial strain of the age group of twenty two weeks were randomly distributed under seven treatment groups, every treatment has four replications with five birds in each replication. All the feed ingredients used for ration formulation were analysed for proximate principles as per A.O.A.C [1]. are presented in Table 1. The feed ingredients composition and feed additives added in basal diet in laying phase of the hens are shown in Table 2. The laying hens of control group (T_1) were fed a basal diet formulated as per BIS [4] standards consisting of 18.04% crude protein, 4.34% crude fibre, 3.61 The feed grade commercially available probiotic (containing 5 × 10^8cfu/g of *Lactobacillus fermentum*, 1× 10^9cfu/g of *Bacillus spp*. and 1× 10^9cfu/g of *Saccharomyces cerevisiae*) and prebiotic (containing mannonoligosacchride of *Saccharomyces* cell wall-47gm, formic acid-32gm, HSCAS-upto 1Kg) were mixed along with the ration as premix. The seven dietary treatment groups are as follows:-

T_1 (maize based control diet), T_2 (0.5g probiotic/Kg of feed), T_3 (1.0g probiotic/Kg of feed), T_4 (2.0g probiotic/Kg of feed), T_5 (0.5g prebiotic/Kg of feed) T_6, (1.0g prebiotic/Kg of feed) T_6 and T_7 (2.0g prebiotic/Kg of feed). A 16 week feeding trial was run where the hens were fed the formulated diets beginning at 22 weeks of age till they attain age of 38 weeks. All the laying hens were housed individually in the cage system. Feed and water were offered *ad libtum* through linear feeders and waterers. All the treatment birds were weighed initially before the start of the experiment and any change in the body weight gain was noticed by difference in weight at the end of the trial.

Four eggs (1 egg per replication) were collected randomly from each treatment group, thus a total of 28 eggs at weekly intervals were analyzed for the measurement of egg shell thickness, egg shell weight, egg shell weight percentage using Screw Guage and electronic balance, respectively. While, egg length, egg width and egg shape index were measured using Vernier Calliper. To determine egg shell weight, the eggs were broken at its equator and the internal contents were discarded. Then, eggshells were cleaned of any adhering albumen, the membrane was removed; eggshells were then dried at room temperature. Eggshell weight is described as the ratio of eggshell weight to total egg weight, expressed as a percentage. After removing shell membranes manually, egg shell thickness (without inner and outer shell membranes)was measured at 3 different locations viz. at the large end, the equatorial region, and the small end (middle, broad, and narrow ends)without membrane by a micrometer to the nearest 0.01 millimeter. Shell thickness was recorded as the average of the three measurements. Mortality if any was recorded daily for knowing the livability percent. Data were analyzed by one-way ANOVA as a completely randomized design using the GLM procedure of SAS Institute [15]. Significant differences between treatment means were separated using Duncan's multiple range test, with a 5% probability (P<0.05). Each experimental unit was a replicate consisting of four adjacent cages in laying hen trial.

Results and Discussion

Body weight changes and liveability

The mean body weights of the experimental laying hens at the beginning of the experiment and at the end of the experiment are presented in Table 3. The results of the study depicted that all the experimental birds under different dietary treatments were in positive weights. However, the statistical analysis of the data revealed that no significant effect on the body weight gain of hens was observed due to dietary supplementation of basal ration with different levels of probiotics and prebiotics as compared to control. No mortality was observed in any of the dietary treatment groups of laying hens in the first phase of their production cycle during the whole experimental period. Thus, it indicated that addition of probiotics and prebiotics to the basal ration of hens do not have any ill effects on the health and liveability of layers.

In agreement with the results of the present trial, Nahashon *et al.* [11] found no significant effect on hen body weight in response to dietary probiotic supplementation, compared to control. The results of several investigations have also reported good livability and improved performance goals when brown layer hens were fed on diets with probiotic preparations (Protexin) [2, 20]. Inconsistent to our findings, Nahashon *et al.* [10] reported that the laying hens given probiotic and prebiotic added diets, containing mainly *Lactobacillus* microorganisms, gained more body weight and did not experience decreased egg production and feed efficiency, as compared to the control. The growth-promoting effects of dietary probiotic (*Lactobacillus*) and prebiotic (mannonoligosaccharide) supplementation were attributed to their proven effects on nitrogen, fat, calcium and phosphorus retention. No study has yet shown that probiotic and prebiotic feeding has any detrimental effects on health status and productivity of layer hens. Thus, earlier research, as well as this study, have shown that feeding layer hens with probiotic-supplemented diets do not have detrimental effects on hens' body weight, rather it can benefit laying performance.

Egg shell quality parameters

The findings show that there was significant (P<0.05) increase in egg shell thickness over the different experimental periods, however, the mean values of shell thickness show variable significance under different dietary levels of probiotics and prebiotics during progressive weeks (Table 4). The mean values of shell thickness show significant (P<0.05) increase with the use of highest (2g/Kg of feed) level of probiotic in treatment T_4, however, it was at par with treatment group T_7 (2g of prebiotic Kg^{-1} feed) during the periods from 22nd to 26th week; and 34th to 38th weeks, respectively. While, during 28th, 32nd and 34th weeks of age the mean values of shell thickness were found to be non-significant (P>0.05). With respect to the whole period, it was observed that there was significant (P<0.05) increase in egg shell thickness under various probiotic and prebiotic regimes (T_3, T_4, T_5, T_6 and T_7) as compared to the control (T_1) group and lowest (0.5 g Kg^{-1} feed) level of probiotic in T_2. While, the differences in egg shell thickness over the various supplemental levels of their use did not differ significantly amongst themselves, highest value being observed in T_7. The values of the shell thickness increased from 0.348 to 0.374 mm indicating an advantage of 0.026mm in favour of

probiotics and prebiotics as compared to control group. The beneficial effect on eggshell quality due to probiotic feeding may be attributed to a favorable environment in the intestinal tract by feeding of *Lactobacillus*, which might have helped birds to assimilate more calcium, which was evident by increased concentration of Ca in serum [13].
Similar significant improvements in egg weight and eggshell quality were also obtained in hens fed diets with a mixture of *Lactobacillus* cultures [8, 25]. The increase in egg shell thickness in hens fed probiotic supplemented diets, noted in our study, seems to confirm the hypothesis that an acidic environment created in the digestive tract by lactic acid bacteria facilitates the ionization of minerals and improves their absorption [6]. Also, an improvement of the physiological conditions of digestion and intestinal absorption (or "gut health") of fructooligosacchride (FOS) treated hens could be assumed. A strong correlation was established between increase absorption of minerals and fermentation of nondigestible oligosaccharides in the large intestine [16]. Thus, it can be suggested that the improvement in egg shell quality in this study might be due to the increased mineral absorption.
Similarly, the results depicted that there was significant (P<0.05) increase in egg shell weight and weight percent over the different experimental periods (Table 4), however, the mean values show variable significance under different dietary levels of probiotics and prebiotics during progressive weeks. The mean values of shell weight during 26th to 30th weeks and shell weight percent in 24th, 30th, 32nd, 36th and 38th weeks of age were found to be non-significant respectively. With respect to the whole period, it was observed that there is significant (P<0.05) increase in both egg shell weight and shell % under various probiotic and prebiotic regimes (T_2, T_3, T_4, T_5, T_6 and T_7) as compared to the control (T_1) group. While, the mean values of egg shell weight under different supplemental levels did not differ significantly amongst themselves. Thus, the result findings clearly indicate that there is a notable increase in egg shell weight and shell percent with the supplementation of probiotics and prebiotics in the ration of layer hens. However, it should be noted that an increase in the eggshell proportion was accompanied by a considerable increase in eggshell thickness leading to a decrease in the rate of damaged eggs. The improvement in egg shell percentage and egg shell thickness may be attributed to the enhancement of calcium absorption and retention associated with adding yeast into the diet [21].
Several reports are in agreement with the research findings of our present study with laying hens which have also shown that prebiotic fructans such as inulin or oligofructose may positively affect mineral utilization and in this way, improve eggshell and bone quality [18, 19]. The mechanism of the positive effect of prebiotics on mineral utilization can be attributed to the high solubility of minerals because of the increased production of short chain fatty acids which resulted from colonic fermentation of non-digestible carbohydrates [16].

Egg shape parameters

The results revealed that there was significant (P<0.05) increase in egg width and egg length over the different experimental periods and maximum values were observed in the treatment (T_7) group of hens fed with highest level of prebiotic (2g/Kg feed) during progressive weeks of age of birds (Table 5). The mean values of egg width during 28th, 32nd weeks and egg length in 32nd weeks of age were found to be non-significant, respectively. With respect to the whole period, it was observed that there was significant (P<0.05) increase in both egg width and egg length under various probiotic and prebiotic regimes (T_2, T_3, T_4, T_5, T_6 and T_7) as compared to the control (T_1) group. While, the maximum increase was observed with the highest level (2g/Kg feed) of prebiotic in T_7 treatment group, followed by the hens in treatment group T_4 fed with highest level (2g/Kg feed) of probiotic. Thus, the result findings clearly indicate that there is a notable increase in egg width and egg length with the supplementation of probiotics and prebiotics in the ration of layer hens.
While, the study results illustrated in Table 5 shows that there were no significant differences in Egg shape Index among different dietary treatments during progressive weeks of age of hens as well as with respect to the whole period. Thus, it can be concluded that feeding of different levels of probiotics and prebiotics in the diet of laying hens did not affect egg shape index. In confirmation to our findings several researchers also observed no significant difference in shape index and yolk index due to supplementation of either probiotics or prebiotics in diet of layers [23, 21]. In contrast to our results, Swain *et al*. [17] reported that shape index was increased (P<0.05) due to probiotic and yeast supplementation @ 0.5 or 1.5 or 2.0g/Kg of diet.

Table 1: Chemical composition (%DM basis) and metabolizable energy (ME, Kcal/Kg) of feed ingredients used in 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.,* [12] 2002

Table 2: Ingredients (%) and chemical composition (% DM basis) of basal diet

Feed ingredients	Percentage (%)
Maize	50.0
Groundnut cake	7.0
Soybean meal	13.0
Deoilled rice polish	12.0
Rice Polish	5.0
Fish Meal	6.0
Mineral Mixture	3.0
Salt	0.5
Shell Grit	3.5
Feed additive	*
Chemical composition	(%) DM basis
CP	18.04
CF	4.34
EE	3.61
Ash	7.80
Metabolizable energy (Kcal/Kg)**	2697.17

***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)
** Calculated value as per Panda *et al*., [12] 2002

Table 3: Body Weight changes (Kg) of layers during the experimental period under different dietary treatments

Treatments	Initial Body Weight	Final Body Weight	Body Weight gain(g)
T_1	1.78 ±0.05	1.98 ±0.05	0.20 ±0.01
T_2	1.78 ±0.06	1.98 ±0.06	0.20 ±0.02
T_3	1.80 ±0.06	2.01 ±0.05	0.20 ±0.03
T_4	1.75 ±0.05	1.95 ±0.05	0.20 ±0.02
T_5	1.77 ±0.04	1.95 ±0.05	0.19 ±0.02
T_6	1.67 ±0.05	1.89 ±0.04	0.22 ±0.02
T_7	1.68 ±0.06	1.90 ±0.05	0.22 ±0.05
CD	NS	NS	NS

Table 4: Average values of egg shell thickness (mm), egg shell weight (g) and egg shell weight percentage (%) during progressive weeks of age under different dietary treatments.

	Treatments						
Parameter	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**	**T_7**
Weeks Egg shell thickness (mm)							
22 – 24	$0.362^{a}\pm0.002$	$0.370^{a}\pm0.002$	$0.404^{bc}\pm0.003$	$0.408^{c}\pm0.003$	$0.383^{d}\pm0.006$	$0.392^{bd}\pm0.005$	$0.394^{bd}\pm0.006$
24 – 26	$0.352^{a}\pm0.005$	$0.366^{ab}\pm0.002$	$0.382^{bc}\pm0.007$	$0.387^{c}\pm0.005$	$0.366^{ab}\pm0.010$	$0.387^{c}\pm0.002$	$0.375^{bc}\pm0.003$
26 – 28	0.327±0.003	0.325±0.014	0.333±0.005	0.340±0.012	0.332±0.007	0.339±0.010	0.355±0.014
28 – 30	$0.319^{a}\pm0.005$	$0.322^{ab}\pm0.010$	$0.332^{ab}\pm0.008$	$0.346^{ab}\pm0.009$	$0.330^{ab}\pm0.020$	$0.347^{ab}\pm0.014$	$0.359^{b}\pm0.016$
30 – 32	0.370±0.010	0.376±0.006	0.392±0.012	0.394±0.020	0.391±0.009	0.389±0.018	0.381±0.017
32 – 34	0.359±0.005	0.361±0.007	0.365±0.012	0.372±0.009	0.369±0.011	0.363±0.004	0.379±0.003
34 – 36	$0.349^{a}\pm0.011$	$0.350^{ab}\pm0.010$	$0.358^{ab}\pm0.004$	$0.364^{ab}\pm0.005$	$0.367^{ab}\pm0.003$	$0.372^{b}\pm0.010$	$0.370^{ab}\pm0.005$
36 – 38	$0.351^{ab}\pm0.007$	$0.334^{b}\pm0.010$	$0.353^{ab}\pm0.009$	$0.379^{c}\pm0.007$	$0.372^{ac}\pm0.004$	$0.379^{c}\pm0.008$	$0.384^{c}\pm0.004$
Mean	$0.348^{a}\pm0.003$	$0.350^{a}\pm0.004$	$0.364^{b}\pm0.004$	$0.373^{bc}\pm0.004$	$0.363^{bc}\pm0.004$	$0.371^{bc}\pm0.004$	$0.374^{c}\pm0.004$
Weeks Egg shell weight (g)							
22 – 24	$4.80^{a}\pm0.22$	$4.99^{ab}\pm0.07$	$5.14^{ab}\pm0.16$	$5.05^{ab}\pm0.03$	$4.89^{ab}\pm0.18$	$4.97^{ab}\pm0.07$	$5.21^{b}\pm0.06$
24 – 26	$4.86^{a}\pm0.05$	$5.63^{bc}\pm0.05$	$5.75^{bc}\pm0.12$	$5.86^{b}\pm0.12$	$5.46^{c}\pm0.11$	$5.91^{b}\pm0.15$	$5.92^{b}\pm0.24$
26 – 28	5.59±0.14	5.78±0.16	5.78±0.05	5.60±0.05	5.68±0.09	5.73±0.04	5.81±0.03
28 – 30	5.35±0.14	5.49±0.09	5.56±0.10	5.50±0.07	5.49±0.06	5.50±0.12	5.58±0.11
30 – 32	$5.40^{a}\pm0.07$	$5.48^{ab}\pm0.05$	$5.55^{ab}\pm0.07$	$5.56^{ab}\pm0.09$	$5.53^{ab}\pm0.04$	$5.59^{b}\pm0.05$	$5.60^{b}\pm0.02$
32 – 34	$5.23^{a}\pm0.01$	$5.52^{b}\pm0.06$	$5.46^{bc}\pm0.07$	$5.41^{bc}\pm0.07$	$5.36^{abc}\pm0.07$	$5.34^{ac}\pm0.04$	$5.34^{ac}\pm0.07$
34 – 36	$5.30^{a}\pm0.04$	$5.34^{ab}\pm0.03$	$5.46^{b}\pm0.05$	$5.40^{ab}\pm0.07$	$5.34^{ab}\pm0.02$	$5.44^{b}\pm0.06$	$5.43^{ab}\pm0.06$
36 – 38	$5.43^{ab}\pm0.03$	$5.38^{a}\pm0.09$	$5.40^{ab}\pm0.02$	$5.50^{ab}\pm0.04$	$5.51^{b}\pm0.05$	$5.49^{ab}\pm0.01$	$5.41^{ab}\pm0.04$
Mean	$5.24^{a}\pm0.06$	$5.45^{b}\pm0.05$	$5.51^{b}\pm0.04$	$5.49^{b}\pm0.04$	$5.41^{b}\pm0.04$	$5.50^{b}\pm0.05$	$5.54^{b}\pm0.05$
Weeks Egg shell weight percentage (%)							
22 – 24	9.09±0.38	9.45±0.22	9.53±0.35	9.40±0.23	9.86±0.49	9.66 ±0.24	10.08 ±0.36
24 – 26	$9.02^{a}\pm0.12$	$10.24^{bc}\pm0.10$	$10.39^{bc}\pm0.22$	$10.62^{bc}\pm0.17$	$10.06^{b}\pm0.25$	$10.89^{c}\pm0.25$	$10.70^{bc}\pm0.35$
26 – 28	$10.63^{ab}\pm0.29$	$11.04^{a}\pm0.33$	$10.79^{ab}\pm0.14$	$10.30^{b}\pm0.09$	$10.79^{ab}\pm0.20$	$10.87^{ab}\pm0.17$	$10.81^{ab}\pm0.04$
28 – 30	10.04±0.30	10.34±0.20	10.43±0.22	10.11±0.09	10.44±0.25	10.32±0.24	10.27±0.21
30 – 32	9.96±0.20	10.16±0.11	10.27±0.13	10.24±0.16	10.19±0.07	10.22±0.10	10.32±0.05
32 – 34	$9.70^{a}\pm0.10$	$10.10^{b}\pm0.12$	$10.2^{b}\pm0.13$	$9.95^{ab}\pm0.16$	$10.04^{ab}\pm0.14$	$9.92^{ab}\pm0.09$	$9.85^{ab}\pm0.18$
34 – 36	9.75±0.10	9.99±0.18	10.12±0.16	9.81±0.11	9.90±0.13	9.92±0.13	9.85±0.14
36 – 38	9.99±0.04	10.06±0.23	10.00±0.07	9.99±0.13	10.21±0.12	10.01±0.12	9.83±0.12
Mean	$9.77^{a}\pm0.11$	$10.17^{b}\pm0.09$	$10.21^{b}\pm0.08$	$10.05^{b}\pm0.07$	$10.18^{b}\pm0.09$	$10.22^{b}\pm0.09$	$10.21^{b}\pm0.09$

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

Table 5: Mean values of egg width (cm), egg length (cm) and egg shape index under various dietary treatments of probiotic and prebiotic supplementation during progressive weeks of age.

	Treatments						
Parameter	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Weeks Egg width (cm)							
22 – 24	3.8^{a}±0.06	3.89^{ab}±0.04	3.95^{bc}±0.03	3.96^{bc}±0.02	3.91^{ab}±0.04	3.94^{abc}±0.02	4.05^{c}±0.07
24 – 26	4.05^{a}±0.02	4.06^{ab}±0.02	4.14^{abc}±0.03	4.20^{bc}±0.08	4.08^{ab}±0.03	4.13^{abc}±0.05	4.23^{c}±0.07
26 – 28	4.09±0.03	4.14±0.01	4.23±0.05	4.28±0.07	4.11±0.03	4.24±0.13	4.33±0.16
28 – 30	4.01^{a}±0.05	4.13^{ab}±0.03	4.15^{ab}±0.03	4.26^{b}±0.04	4.11^{ab}±0.05	4.18^{ab}±0.03	4.24^{b}±0.14
30 – 32	4.13±0.03	4.18±0.05	4.20±0.05	4.24±0.02	4.16±0.02	4.19±0.05	4.23±0.03
32 – 34	4.11^{a}±0.04	4.15^{a}±0.02	4.23^{ab}±0.05	4.28^{ab}±0.08	4.18^{ab}±0.09	4.23^{ab}±0.03	4.34^{b}±0.05
34 – 36	4.0^{a}±0.05	4.11^{abc}±0.07	4.23^{bc}±0.01	4.29^{bc}±0.14	4.04^{ab}±0.02	4.15^{abc}±0.02	4.26^{bc}±0.05
36 – 38	4.03^{a}±0.03	4.05^{a}±0.04	4.10^{ab}±0.03	4.23^{ab}±0.14	4.18^{ab}±0.05	4.21^{ab}±0.04	4.26^{b}±0.10
Mean	4.03^{a}±0.02	4.09^{ab}±0.02	4.15^{bc}±0.02	4.22^{cd}±0.03	4.10^{a}±0.02	4.16^{bc}±0.02	4.24^{d}±0.03
Weeks Egg length (cm)							
22 – 24	5.39^{abc}±0.03	5.30^{bc}±0.04	5.48^{ab}±0.01	5.6^{a}±0.17	5.23^{c}±0.06	5.46^{ab}±0.07	5.50^{ab}±0.04
24 – 26	5.55^{a}±0.12	5.75^{ab}±0.09	5.78^{ab}±0.08	5.85^{b}±0.09	5.65^{ab}±0.05	5.74^{ab}±0.08	5.90^{b}±0.10
26 – 28	5.55^{a}±0.07	5.65^{ab}±0.07	5.76^{ab}±0.07	5.85^{b}±0.04	5.71^{ab}±0.12	5.78^{ab}±0.07	5.83^{b}±0.09
28 – 30	5.56^{a}±0.07	5.69^{ab}±0.05	5.78^{abc}±0.05	5.91^{bc}±0.04	5.78^{abc}±0.12	5.88^{bc}±0.09	5.95^{c}±0.13
30 – 32	5.66±0.05	5.71±0.15	5.74±0.13	5.40±0.04	5.76±0.09	5.86±0.04	5.93±0.06
32 – 34	5.63^{a}±0.08	5.74^{ab}±0.05	5.88^{bc}±0.09	5.96^{c}±0.08	5.68^{ab}±0.10	5.83^{abc}±0.05	5.96^{c}±0.06
34 – 36	5.61^{a}±0.08	5.68^{ab}±0.03	5.79^{abc}±0.10	5.95^{bc}±0.07	5.80^{abc}±0.19	5.84^{abc}±0.11	6.00^{c}±0.02
36 – 38	5.35^{a}±0.02	5.56^{b}±0.05	5.66^{bc}±0.03	5.86^{de}±0.10	5.70^{bcd}±0.10	5.79^{cde}±0.04	5.91^{e}±0.03
Mean	5.54^{a}±0.03	5.63^{ab}±0.03	5.73^{bc}±0.03	5.80^{cd}±0.04	5.66^{b}±0.05	5.77^{c}±0.03	5.89^{d}±0.04

Conclusion

Thus from the result findings of the present study, it can be concluded that dietary supplementation of different levels of probiotics and prebiotics in laying hens' diet resulted in significant increase in the egg shell thickness, egg shell weight, shell weight percent, egg width, egg length which attributed to the better egg handling and reduced breakage during transport. Thus, probiotic and prebiotic supplements can promote laying hen performance without diminishing body weight and liveability.

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