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Dietary supplementation of sodium bicarbonate, probiotic and L-ascorbic acid effects laying performance and eggshell quality in older hens

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Abstract

Eggshell quality issues are a substantial hidden cost for egg producers and a matter of concern for older hens. To address the issue, a trial was conducted to determine the effects of various dietary supplements on eggshell quality in older hens. A total of 48 Hy-line brown commercial laying hens aged 76 weeks were considered for the experiment. There were four dietary treatments with three replications per treatment, maintaining four birds per replication. All the diets had similar energy and protein contents except for the doses of test ingredients. The diets contained T1 (control: layer mash feed), T2 (control + 3g $NaHCO_3$/kg feed), T3 (control + 2 g/kg multi-strain probiotics) and T4 (control + 0.4 g/kg L-ascorbic acid). Laying performances and eggshell qualities were measured three times during the whole experimental period. Dietary inclusions of all the test materials reflected a significant increase in egg production ($p<0.0001$). Similar to egg production, a rise in egg mass output ($p=0.0006$) was depicted in all the treated groups. An improvement ($p=0.0001$) in feed conversion ratio (feed:egg mass) was obtained due to the feeding of the additives as well. The addition of $NaHCO_3$, a multi-strain probiotic, and L-ascorbic acid to the diet increased shell percent ($p=0.0611$) and shell thickness ($p=0.0293$). The inclusion of three test ingredients in the layer diet showed the better shell thickness ($p=0.0293$) than the control group. Taken together, it may, therefore, be opined that the dietary supplementation of sodium bicarbonate, multi-strain probiotics, and L-ascorbic acid significantly improves the quality of eggshell and overall laying performance of older hens.

Keywords: Eggshell, sodium bicarbonate, probiotic, L-ascorbic acid, older hen

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INTRODUCTION

Eggshell resistance decreases naturally as layers' age increase due to older hens' lower calcium absorption from the regimen. The decreased calcium uptake along with increased egg weight results in a rise in eggshell quality concerns. Cracked eggs can reach 20% in elderly chickens, and for obvious reasons, these eggs reflect poorly on the egg industry. Eggs produced in the initial stages of egg production are smaller in size than those obtained from mature birds. As the size of the eggs increases, the thickness begins to diminish. The eggshell structure is complex and serves critical biological functions such as protecting the inner content of the egg during deposition and assisting in the development of chicken embryos (Sirri *et al*., 2018). Additionally, there is a significant risk of eggshell damage and bacterial contamination during the collecting, classification, packaging, transportation, storage, and processing processes (Li *et al.*, 2019). Between 10% and 15% of laying eggs are lost before and during the collection process due to shell quality issues (Dunn *et al.*, 2009). The cracking of eggshells is an expensive problem in the egg industry, and it grows worse as shell quality deteriorates with age (Zhang *et al.*, 2017). Thus, eggshell quality is critical for poultry producers. The decline in eggshell feature as the hen ages is due to the rising size of the egg, for which the hen cannot deposit more than 2,000 mg calcium, and also to the decreased supply of feed-derived calcium. Finally, aged chickens tend to deplete their bone stores, resulting in mobility difficulties and decreased egg production. Increased dietary calcium intake is not an option because it will aggravate an already strained calcium absorption mechanism. Trace elements may affect the quality of eggshells either through their catalytic characteristics as important enzymes participating in the shell membrane and eggshell development processes or by their direct interaction with the calcite crystals in the forming eggshell (Mabe *et al.*, 2003). Among the many nutritional supplements, sodium bi-carbonate,

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probiotics, and L-ascorbic acid (Vitamin C) have demonstrated significant impacts on eggshell quality, gut quality, and calcium absorption in older chickens. Numerous studies have been conducted on those substances for enhancing eggshell quality. Of these additives, sodium bicarbonate ($NaHCO_3$) is associated with the acid-base balance and has a favorable link with the shell matrix structure (Fu *et al.*, 2021). The adding of 1% sodium hydroxide to the diet increased shell-breaking strength (Balnave and Muheereza, 1997). The other additive, vitamin C, though birds can synthesize it, the production is insufficient under stressful conditions. In such conditions, Vitamin C supplementation is crucial to combat stress (Rajabi and Torki, 2021). Numerous researchers have established that ascorbic acid supplementation has a beneficial effect on egg production, growth rate, thickness, and eggshell strength in stressed birds (Thornton, 1962; Bains, 1996). Supplementing the poultry diet with vitamin C improves performance under stressful conditions (Pardue and Thaxton 1986). Probiotic, the other one, aids in the improvement of intestinal health and facilitates better nutrient absorption. The supplementation of probiotics in the diet is effective in improving hen performance, egg quality, and gastrointestinal health (Xiang *et al.*, 2019). Though these additives are widely used, there are no specific recommendations and the results are also inconsistent. As a result, the proposed experiment was taken to evaluate the use of NaHCO3, multi-strain probiotics, and L-ascorbic acid (Vitamin C) as feed supplements in the diets of older layers to increase eggshell thickness as well as to evaluate laying performance.

MATERIALS AND METHODS

Experimental layout, birds, and dietary treatment

The research was carried out at the Bangladesh Agricultural University Poultry Farm, Mymensingh, for eight weeks in the experimental shed of an open-sided house with Hy-line Brown commercial layers. For this experiment, 48 Hy-line-Brown layer chickens aged 76 weeks were chosen from BAU Poultry Farm. The experimental birds were randomly categorized into 4 dietary treatment groups, each with three replications of four birds. The initial body weight of birds in each treatment group was nearly identical. All feed materials were procured from Mymensingh's local market and blended properly by hand according to the layer ration's performance. Following mixing, the diet was split into four components. T1 was fed a control diet; T2 was provided a control diet plus 3g/kg sodium bicarbonate salt; T3 was fed a control diet plus 2g/kg multi-strain probiotics, and; T4 was fed a control diet plus 0.4g/kg L-ascorbic acid. Every morning, waterers were cleaned with tap water, while feeders were cleaned once a week. Additionally, waterers were cleaned and rinsed completely weekly with detergent. Regular feces removal was performed. During the study period, birds in all treatment groups received identical care and management. Throughout the experimental period, strict sanitary controls and sanitation initiatives were implemented. Throughout the trial period, daily temperature and humidity readings were taken using a thermometer and hygrometer.

Record Keeping

At the start of the feeding trial, all birds were weighed according to protocol. The birds' weights were then recorded weekly until the trial concluded. The early ten days were regarded as an adjustment period. Each replication was recorded daily for the number of eggs and average egg weight of each treatment. Temperature and humidity readings were taken twice daily, in the morning and afternoon. Every day, immediately after collection, the fresh eggs were weighed using a digital egg weighing balance. Weekly records of bird feed intake and mortality were kept throughout the trial.

Egg Quality Characteristics

Throughout the study period, eggs laid by birds fed various diets were moved to BAU's Poultry Science Laboratory to assess the effect of dietary additives on eggshell quality attributes. During the experimental period, one egg per replication was collected in the morning at three-week intervals. The eggs were weighed using a digital egg weighing balance. A slide caliper was used to determine the egg's length. The egg's breadth was also determined using a slide caliper. For each variable, the mean of three recordings was used to determine the length and width of an egg. To calculate eggshell thickness, the measurements were obtained from the small, large, and waist regions. The mean of three measurements was used to determine the thickness of shells. Thereafter, the shape index (egg width/egg length, shell percentage (shell weight/test egg weight), and shell thickness (average of three measurements) of the eggshells were determined.

Table 1: Ingredients and nutrient composition of control diet.

Ingredients	**Quantity (Kg)**
Yellow corn	56.625
Rice Polish	4.00
Soybean meal (44% CP)	17.00
Protein Concentrate	5.00
Limestone	13.00
DCP	1.25
Salt	0.25
Lysine	0.25
Methionine	0.125
Layer premix	0.375
Choline chloride	0.125
Soybean oil	2.00
Total	100.00
ME (Kcal/Kg)	2715
CP %	16
Ca %	4.6
Linoleic acid %	2.4
Lysine %	1.1
Methionine %	0.4
P %	0.5
CF %	2.6

Statistical Analysis

The data were organized methodically in preparation for analysis using the Statistical Computer Package Software (SAS, 2009). In a Completely Randomized Design, data were subjected to one-way ANOVA (CRD). Duncan's Multiple Range Test (DMRT) was used to compare means, and differences with p-values <0.05 or greater were considered significant.

Results and Discussion

Laying Performances

Data recorded on laying performances have been depicted in Table 2 and Figure 1. Supplementing the diet with all of the additives studied in the experiment resulted in a significant increase (p<0.0001) in egg production. Although, in the case of multi-strain probiotics, the weight of the egg was a slightly less (P=0.0311), other additives ($NaHCO_3$ and L-ascorbic acid) did not influence the egg weight. Similar to egg production, a rise in egg mass output (P=0.0006) was found in all the treated groups. An improvement (P=0.0001) in feed conversion ratio (feed: egg mass) was obtained due to the feeding of the additives as well. Although probiotic-fed birds had a lower egg weight, greater egg production resulted in increased egg mass, which contributed to improved feed conversion.

In a study by Abbas (2017), it was found that supplementing commercial layers' diets with sodium bicarbonate boosted feed efficiency, egg output, and egg weight significantly (p<0.05). Similarly, increased egg production was noticed in $NaHCO_3$ treated commercial laying hens by Wang *et al.*, (2020). A study conducted by Ray (2018) revealed that single and multi-strain probiotics significantly improved egg production, egg weight, egg mass output, and feed conversion ratio. Zhang *et al.*, (2014) experimented and reported that supplementing layer hen diets with a probiotic containing heat-inactivated *Lactobacillus salivarius* CB and *Bacillus subtilis* resulted in highly significant (p<0.01) increases in egg production and daily egg yield. Likewise, Yuruk *et al.*, (2004) stated improved egg production in laying hens. The suppressing effects of probiotics on undesirable bacteria and stimulating effects on the growth and/or activity of beneficial bacteria in the intestines probably increased the absorptive capacity to enhance egg production (Ray, 2018). The results indicate that the addition of these additives is beneficial for improving the performance of birds. Vitamin C supplementation in birds in comparison with control birds improved the production of hen housed eggs, feed conversion efficiency, egg weight, and egg mass (p <0.01) (Ahmed *et al.* 2008). Skrivan *et al.*, (2013) discovered that ascorbic acid boosted the productive performance of laying hens. Consistent with our findings, Sahin *et al.*, (2002) found that adding 250 mg L-ascorbic acid increased feed efficiency and egg weight in laying hens. Wang *et al.*, (2015) observed that egg weight was significantly greater in laying hens kept under heat stress when provided diets containing 200 and 300 mg ascorbic acid in comparison to those fed diets containing 0 and 100 mg ascorbic acid.

External Quality of Egg

Egg quality was assessed three times during the whole experimental period and the results have been displayed in Table 2. In the first assessment of egg quality at 80-week, none of the external egg quality attributes showed any significant differences (p>0.05) due to either $NaHCO_3$ or multi-strain probiotic or L-ascorbic acid supplementations. However, with the progress of time during the evaluation at 82-week, dietary inclusion of both probiotic and L-ascorbic acid feeding reflected an increase in egg weight (p=0.0041) and shell percent (p=0.0611). In addition, along with these two, $NaHCO_3$ addition in the diet revealed greater shell thicknesses, of which MSP was the highest this week. An 84-week, shell percent was improved (p=0.0517) by both $NaHCO_3$ and multi-strain probiotic supplementation, whereas all the additives showed greater (p=0.0293) shell thicknesses. At the moment, consumer demand for eggs has turned away from quantity toward quality (Xiang *et al.*, 2019). In support of our study, Fu *et al.*, (2021) opined that the inclusion of $NaHCO_3$ in

Table 2: Overall laying performance of hens (76-84 wks).

Parameter	T1	T2	T3	T4	Level of significance
Hen Day Egg production (%)	69.39[b] ±0.491	81.55[a] ±1.717	83.61[a] ±0.940	82.14[a] ±1.236	**
Egg Weight (g)	63.41[a] ±0.345	63.82[a] ±0.353	61.68[b] ±0.284	64.23[a] ±0.829	*
Egg Mass Output (g/b/d)	44.00[b] ±0.260	52.03[a] ±0.902	51.57[a] ±0.737	52.78[a] ±1.459	**
FCR	2.41[a] ±0.014	2.02[b] ±0.0353	2.04[b] ±0.028	2.01[b] ±0.054	**

*Where, T1 = Hand-made layer mash (control diet); T2 = Control diet + NaHCO3; T3 = Control diet + multi-strain probiotics; T4 = Control diet + L-ascorbic acid; The means having alphabets not alike in the same raw differ significantly at the stated level of probability; * p<0.05 and ** p<0.01; The value indicates mean± standard error.*

feed resulted in the quadratic increase of eggshell breaking strength ($p< 0.05$). A decrease in the bicarbonate content within the shell gland's lumen has a detrimental effect on the quality of the eggshell (Balnave and Muheereza, 1997). As a result, hens require bicarbonate at elevated temperatures to maintain the shell's quality. The use of sodium bicarbonate may aid in improving eggshell calcification by adjusting the acid-base balance, particularly under conditions of heat stress (Ghorbani and Fayazi, 2009). Abdelqader *et al.*, (2013) investigated the effect of Bacillus subtilis ($2.3x10^8$ cfu/g of probiotic) supplementation on the efficiency and eggshell consistency of aged-laying chickens at 64

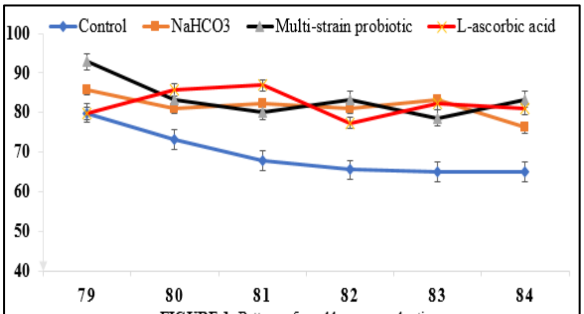

FIGURE 1: Pattern of weekly egg production

weeks of age and discovered enhanced eggshell quality. Additionally, Fathi *et al.*, (2018) observed an improvement in eggshell quality and breaking strength in hens fed a probiotic-containing diet. In commercial layer hens, feeding *Lactobacillus sporogenes* at 100 mg ($6x10^8$ spore per kg diet) dramatically increased eggshell breaking strength, shell weight, and shell thickness (Panda *et al.*, 2008). In commercial layer hens, feeding *L. sporogenes* at 100 mg ($6x10^8$ spores) kg^1 diet dramatically increased eggshell breaking strength,

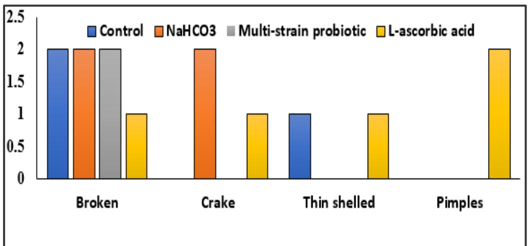

Figure 2: Eggshell Defects

shell weight, and shell thickness (Panda *et al.*, 2008). The probiotic bacteria cause greater fermentation and produce short-chain fatty acids to lower the luminal pH (Scholz-Ahrens *et al.*, 2007). The lower pH facilitates Ca solubility and causes greater absorption. In addition, the lower pH also improves the villi health to increase absorption efficiency. As more calcium is internalized, it may be deduced that the shell glands will utilize more calcium, which will eventually be deposited in the eggshell (Ray, 2018). The uterus or shell gland plays a critical role in the production of the eggshell. The presence of more Ascorbic acid in the eggshell glands of young layers compared to older layers increases their antioxidant status, which benefits eggshell consistency (Gan *et al.*, 2018). Additionally, vitamin C has a favorable effect on shell thickness in chickens reared in heat stress circumstances by stimulating 1.25 dihydroxy-cholecalciferol and increasing calcium mobilization from bone, both of which are necessary for eggshell development and thus egg quality (Demir *et al.*, 1995). However, Wang *et al.*, (2015) found no variation in eggshell quality between commercial layers at 31 weeks of age upon Vitamin C supplementation.

Eggshell Defects

In the whole experiment, a few abnormal eggs were found in different treatments that are shown in Figure 2. A lower number (only 1) of the broken eggs were found in L-ascorbic acid treatment. Crake was found in only $NaHCO_3$ and L-ascorbic acid. No thin shell egg was found in $NaHCO_3$ and Probiotics. Pimple was only found in L-ascorbic acid treatment. In this experiment, the main focus was to improve the eggshell thickness. In total feeding trials, data were analysed three times. In the first analysis (80th week) insignificant (P>0.05) results were found. But in the next two analyses (82 and 84th weeks) significant results (P<0.03 and P<0.0293) were found. That means in 84th week, better results were found than 82th week. Diets with different supplementation provided significant effects in this experiment.

Table 3: External egg quality parameters of laying hens (76-84 wks).

Parameter	T1	T2	T3	T4	Level of significance
			80th week		
Test egg weight (g)	62.0±1.9	63.0±1.5	60.3±2.6	60.7±1.4	NS
Shape index (%)	73.0±0.9	75.7±2.7	77.5±1.7	72.4±1.2	NS
Shell weight (g)	6.0 ±0.0	6.5±0.3	6.3±0.1	6.4±0.2	NS
Shell percent	9.7 ±0.2	10.3±0.3	10.6±0.2	10.5±0.2	NS
Shell thickness (mm)	0.3±0.0	0.3±0.0	0.4±0.0	0.4±0.0	NS
			82th week		
Test egg weight (g)	57.8±1.2	62.2±1.1	59.1±1.6	63.1±1.1	NS
Shape index (%)	77.6±1.8	73.3±0.1	74.4±1.1	75.4±2.9	NS
Shell weight (g)	5.5^{c}±0.1	6.2^{ab}±0.2	6.1^{b}±0.0	6.6^{a}±0.0	**
Shell percent	9.7±0.1	10.0±0.1	10.4±0.3	10.5±0.1	NS
Shell thickness (mm)	0.3^{b}±0.0	0.3^{a}±0.0	0.4^{a}±0.0	0.4^{a}±0.0	*
			84th week		
Test egg weight (g)	59.1±2.4	59.0±0.8	59.3±0.6	60.8±0.8	NS
Shape index (%)	71.3±1.7	71.4±1.5	74.3±1.1	74.3±0.3	NS
Shell weight (g)	5.5±0.2	6.0±0.3	5.8±0.1	6.1±0.0	NS
Shell percent	9.3^{b}±0.0	10.2^{a}±0.2	9.8^{ab}±0.1	10.1^{a}±0.2	*
Shell thickness (mm)	0.3^{b}±0.0	0.3^{a}±0.0	0.3^{a}±0.0	0.3^{a}±0.0	*

*Where, T1 = Hand-made layer mash (control diet); T2 = Control diet + NaHCO3; T3 = Control diet + multi-strain probiotics; T4 = Control diet + L-ascorbic acid; NS = non-significant, *p<0.05, **p<0.0; Common superscripts within a column indicate significantly (p<0.05) different values.*

Conclusion

The results indicate that dietary supplementation with sodium bicarbonate, a multi-strain probiotic, and L-ascorbic acid improves the eggshell quality of older laying hens significantly. As a result, these additives may be considered to enhance the shell quality and laying performance of older commercial laying hens.

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