The Impact of Adding Trehalose to the Diet on Egg Quality and Tibia Strength in Light Laying Hens

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Article

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Simple Summary: The egg industry is always looking for ways to improve the quality of eggs, and one approach is to use additives that can enhance the antioxidant properties of eggs. This study tested different amounts of a sugar called trehalose in the diet of hens to see how it affects egg quality. We used 384 hens and added trehalose at various levels to their food. We measured how much they ate, the quality of their eggs, and other factors like bone strength. We found that adding 0.60% trehalose to their diet improved egg quality and reduced the spoilage of fats in the eggs without affecting bone health. This suggests that using trehalose in hen feed could be a useful way to produce better-quality eggs.

Abstract: Trehalose, a disaccharide made of two D-glucose molecules, is found in various organisms like bacteria, yeast, fungi, insects, and plants. It serves as an energy and carbon source in plants and has signaling functions in yeast and plants, affecting metabolic pathways and growth regulation. It also plays a role in protecting proteins and cell membranes from damage caused by stress. This study aims to determine the best level of trehalose supplementation in layer diets between 34 to 49 weeks of age, due to limited literature on its impact on laying hen productivity. Experimental diets were based on nutritional recommendations and included six trehalose levels. The study was conducted over five periods of 21 days each, evaluating various parameters such as feed intake, egg production, egg quality, and bone characteristics. The results showed that trehalose supplementation influenced the performance of laying hens, as well as some aspects of egg and bone quality.

Keywords: bone strength; disaccharide; egg production; lipid peroxidation; performance

1. Introduction

Trehalose, a disaccharide composed of two D-glucose molecules, is distributed among various organisms including bacteria, yeast, fungi, insects, invertebrates, and plants. Its role within plants revolves around serving as a potential energy and carbon reservoir. Additionally, in yeast and plants, trehalose can act as a signaling molecule, exerting influence on metabolic pathways and growth regulation. Moreover, it is acknowledged for its ability to protect proteins and cell membranes from inactivation or denaturation under a variety of stressors such as desiccation, dehydration, heat, cold, and oxidation.

The antioxidant properties of trehalose have attracted attention in animal husbandry, particularly in mitigating lipid peroxidation. Research indicates that supplementing diets with trehalose leads to reduced mRNA levels of antigen receptor, inflammatory cytokine, and pro-oxidant enzyme genes. Noteworthy findings demonstrate that trehalose supplementation can alleviate oxidative stress in dairy cows, consequently improving the quality of milk production [1] Aoki et al. (2010).

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An investigation evaluating the impact of 0.5% trehalose supplementation in broiler diets unveiled potential growth advantages for young chicks due to enhanced intestinal innate immune system post-hatching supplementation [2] Kikusato et al. (2016). Additionally, certain disaccharides, trehalose among them, are believed to aid in calcium absorption in the gastrointestinal tract. Furthermore, disaccharides have exhibited potential in inhibiting bone resorption, particularly in ovariectomized rats within an osteoporosis setting.

In conclusion, trehalose shows promise for integration into laying hen diets to counteract oxidative stress, boost immune response, facilitate calcium absorption, and enhance bone health, thereby contributing to the overall well-being of birds during the laying period. This study seeks to evaluate the optimal level of trehalose supplementation in layer diets, with a focus on performance, egg quality, and bone characteristics between 34 to 49 weeks of age, considering the limited available literature on the effects of this component on laying hen productivity parameters.

2. Materials and Methods

The study was carried out at Campus II of the Federal University of Paraiba, situated in Areia city, positioned at a latitude of 06° 57' 48'' S, a longitude of 35° 41' 30"W, and an altitude of 618 m in Paraiba, Brazil. All protocols and tests adhered to the animal welfare guidelines and received approval from the local Ethics Committee at the Federal University of Paraiba (Areia, Paraiba, Brazil).

A total of 384 34-week-old Hy-line W-80® layers were utilized in the research. These birds were procured at one day old and managed in accordance with the instructions outlined in the breed manual until the commencement of the experimental phase. Their diet followed the nutritional guidelines [3] Rostagno et al. (2017). The hens were accommodated in a traditional laying structure, roofed with clay tiles, equipped with gutter feeders and nipple drinkers, and housed in galvanized wire cages sized at 100 x 45 x 45 cm.

Egg productivity was recorded and tallied between the 32nd and 34th weeks of the hens' life cycle. Upon reaching 34 weeks, the hens were weighed and allocated randomly to six treatments, each with eight replications and eight birds per experimental unit. Feeding was conducted twice daily, at 8:00 a.m. and 4:00 p.m., with the birds having ad libitum access to feed and water throughout the study period.

The lighting schedule implemented encompassed 17 hours of illumination daily, regulated by an electronic clock timer to manage the light-dark cycles. Temperature readings within the facility were taken once per day, at 4:00 p.m., using maximum and minimum thermometers strategically positioned at various spots within the building at the birds' level. The mean maximum and minimum temperatures recorded inside the experimental premises during the trial period were 27.75°C and 21.25°C, respectively.

The experimental diets were formulated according to the nutritional recommendations [3] Rostagno et al. (2017) (Table 1). Treatments were of six trehalose levels (0.0; 0.05; 0.10; 0.30; 0.60 and 1.00%).

The experiment was divided into five periods of 21 days each. The variables evaluated were: feed intake (FI), egg production (EP), egg per hen housed (EHH), egg weight (EW), egg mass (EM), feed conversion per egg mass (FCREm) and feed conversion per dozen of eggs (FCRDz), livability (LIVA), yields of egg components like shell (RWS), albumen (RWA) and yolk (RWY), along with egg quality parameters like shell thickness (Tshell), specific gravity (SG) and yolk color (YC), lipid oxidation of yolk and tibiotarsus weight (TW), length (L), bone strength (BS), calcium (Ca) and phosphorus (P) content. The chemical analyses were according to AOAC.

Feed Intake: Leftover feed was weighed and discounted from the amount of feed provided for the entire period. At the end of each 21-day period, the amount of feed consumed was divided by the number of hens in each treatment and the number of days to determine grams of feed consumed/hen/day. In case of mortality during the experimental period, the average intake was discounted and corrected to obtain the true average intake for the experimental unit in question.

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Egg Production: The average egg production was obtained by calculating the daily number of eggs produced, including broken, cracked, and abnormal eggs (soft shelled eggs), and was expressed as the average produced by poultry of the period (egg produced/hen/day).

Average egg weight: Eggs were weighed twice each week throughout the experimental period. All intact eggs collected in the last three days of each experimental unit were used in all 21-day sub periods. The average egg weight was obtained by dividing the total weight of eggs collected by the number of eggs collected per experimental unit and was expressed in grams.

Egg mass: The average egg weight was multiplied by the total number of eggs produced during the experimental period, thus obtaining the total egg mass. This value was subsequently divided by the total number of birds per day of the period, expressed in grams of egg/bird/day.

Table 1. Composition of experimental diets and calculated nutritional content.

Items	*Trehalose levels (%)*					
	0.00	**0.05**	**0.10**	**0.30**	**0.60**	**1.00**
Corn, 7,88%	55.950	55.950	55.950	55.950	55.950	55.950
Soybean meal, 45,22%	24.620	24.620	24.620	24.620	24.620	24.620
Soybean oil	4.940	4.940	4.940	4.940	4.940	4.940
Limestone, 37%	10.780	10.780	10.780	10.780	10.780	10.780
Dicalcium phosphate, 18%	1.717	1.717	1.717	1.717	1.717	1.717
Salt	0.488	0.488	0.488	0.488	0.488	0.488
DL-Methionine	0.333	0.333	0.333	0.333	0.333	0.333
L-Lysine HCl	0.066	0.066	0.066	0.066	0.066	0.066
Choline chloride	0.070	0.070	0.070	0.070	0.070	0.070
Vitamins	0.050	0.050	0.050	0.050	0.050	0.050
Minerals	0.025	0.025	0.025	0.025	0.025	0.025
Inert	1.000	0.950	0.900	0.700	0.400	-
Trehalose	-	0.050	0.100	0.300	0.600	1.000
Chemical Composition						
Linoleic Acid, %	3.88	3.88	3.88	3.88	3.88	3.88
ME, kcal.kg^{-1}	2900	2900	2900	2900	2900	2900
CP, g.kg^{-1}	15.80	15.80	15.80	15.80	15.80	15.80
SID Methionine, %	0.54	0.54	0.54	0.54	0.54	0.54
SID Methionine + cysteine, %	0.77	0.77	0.77	0.77	0.77	0.77
SID Lysine, %	0.79	0.79	0.79	0.79	0.79	0.79
SID Threonine, %	0.54	0.54	0.54	0.54	0.54	0.54
SID Tryptophan, %	0.17	0.17	0.17	0.17	0.17	0.17
SID Valine, %	0.67	0.67	0.67	0.67	0.67	0.67
SID Arginine, %	0.97	0.97	0.97	0.97	0.97	0.97
SID Isoleucine, %	0.60	0.60	0.60	0.60	0.60	0.60
SID Leucine, %	1.28	1.28	1.28	1.28	1.28	1.28
Calcium, %	4.56	4.56	4.56	4.56	4.56	4.56
Non-phytate P, %	0.38	0.38	0.38	0.38	0.38	0.38
Sodium, %	0.21	0.21	0.21	0.21	0.21	0.21
Chloride, %	0.33	0.33	0.33	0.33	0.33	0.33
Potassium, %	0.61	0.61	0.61	0.61	0.61	0.61
Fibre	2.27	2.27	2.27	2.27	2.27	2.27
Dry matter	90.00	90.00	90.00	90.00	90.00	90.00
Electrolytic balance mEq.kg^{-1}	153.00	153.00	153.00	153.00	153.00	153.00

Vitamin Supplementation: vit. A - 8,000,000 IU; vit. D3 - 2,400,000 IU; vit. E - 22,500 mg; vit. B1 - 2,800 mg; vit. B27,700 mg; vit. B12 - 18,000 mcg; vit. B6 - 4,500 mg; pantothenic acid - 13,000,000 mg; vit. K3 - 1,800.00 mg; folic acid - 1,300.00 mg; nicotinic acid - 31,500 mg; selenium-400 mg; antioxidant 0.25 g; and excipient q. s.p. - 1,000g. 2- 2- Mineral supplementation: manganese 80.0 g;

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iron - 80.0 g; zinc 50.0 g; copper - 10.0 g; cobalt 2.0 g iodine 1.0 g; and excipient q. s. mp 500 g. 3- Antioxidant - BHT.

Feed conversion: The feed conversion per dozen eggs was expressed as the total feed intake in kilograms divided by the dozen eggs produced (kg/dz), and the feed conversion per egg mass was obtained by dividing the total feed intake by egg mass produced in kilograms (kg/kg).

Livability: The total number of dead hens was recorded daily, and the cumulative number of dead hens was subtracted from the total number of live birds, and the values obtained were converted as a percentage at the end of the experiment.

Final weight: The hens of each experimental unit were weighed at the end of the experiment, to determine the average final weight of the birds, expressed in kg.

Egg Components: The egg yolk and albumen of each egg were weighed separately on a three-digit digital scale (0.001g). Yolk and albumen percentages were determined by the ratio between the average yolk and albumen weight and the average egg weight. Egg shells were identified, oven-dried at 55-60°C for 24 hours, and weighed on a digital scale, accurate to 0.01 g, to obtain the average shell weight. The percentage of the shell was obtained by the ratio of the average shell weight to the average egg weight multiplied by 100 and the shell thickness was obtained by using a Mitutoyo 0-25 mm digital micrometer with an accuracy of 0.001 mm.

To evaluate albumen quality, eggs were first weighed individually on a precision scale, and then broken on a special glass table and albumen height measured by using a special AMES altimeter. The Haugh unit was calculated [4] Card & Nesheim (1968) through the equation $HU = 100 \log (H + 7.57 - 1.7W^{0.37})$, where: HU= Haugh Unit; H=albumen height (mm); W = egg weight (g).

The shell strength was determined by TA.X T2 (Texture Analyzer). A 4mm diameter stainless steel P4 DIA Cylinder probe was used with 6mm and a pre, during, and post-test velocity of 3.0; 0.5; and 5.0mm/s, respectively. Specific gravity was determined by the saline flotation method according to the methodology [5] Hamilton (1982). Eggs were immersed in sodium chloride (NaCl) solutions with densities ranging from 1.0700 to 1.0975 g / cm^3, with a gradient of 0.0025 between them. The density of the solutions was routinely measured by means of an oil densimeter.

Lipid Peroxidation: Seven eggs were collected per replicate to determine lipid peroxidation. The thiobarbituric acid reactive substances (TBARS) method was used, by means of the aqueous acid extraction method [6] Cherian et al. 2007.

Tibia resistance: At the end of the experiment and period of production, one bird from each experimental unit was slaughtered (sensitized by electronarcosis) and the left tibia was collected. Bone strength was determined on the TA-XT Plus Stable Micro Systems universal tester (Surrey, UK) with a 50 kg load cell at a speed of 50 mm/min. Point Bend Rig (HDP/3PB) fracture fixture, Stable Micro Systems, was adjusted to allow the shaft clearance to be 3.0 cm [7] Park et al. 2003.

Tibial mineral, phosphorus, and calcium content: The right tibiotarsus were stripped, weighed, pressed and pre-degreased for 4 hours, then taken to the forced ventilation oven where they remained for 16 hours. Afterwards, the tibiotarsus was weighed again, and then ground in a ball mill. For the quantification of dry matter, the tibiotarsus was oven dried at 105ºC for 16 hours. For the quantification of mineral matter, they were burned at 600ºC for 4 hours. Mineral matter was expressed in grams and as a percentage of the pre-degreased tibia dry matter. The mineral solution was prepared according to the methodology [8] AOAC (1998), by applying the wet procedure. The phosphorus content of the mineral solution was quantified by the colorimetric method, and the calcium content was calculated by the atomic absorption method. Calcium and phosphorus contents in the tibia were expressed in grams and as a percentage of pre-defatted tibia dry matter.

Statistical analyses were performed by software R, 3.4.1. Data were analyzed by one-way ANOVA with subsequent Dunnet and Tukey test at the level of 5%. Data were also submitted to polynomial regression.

3. Results

3.1. Performance

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The different levels of trehalose supplementation influenced the performance of light laying hens in the laying phase from 34 to 49 weeks of age, except for EP, EHH and LIVA (P> 0.05) (Table 2).

Table 2. Effect of Trehalose levels in Feed intake (FI), egg production (EP), eggs per hen housed (EHH), egg weight (EW), egg mass (EM), feed conversion per egg mass (FCREm), feed conversion per dozen of eggs (FCRDz) and livability (LIVA) of light hens.

	Parameters							
Levels (%)	**FI (g/hen)**	**EP (egg produced/ hen)**	**EHH (%)**	**EW (g)**	**EM (g)**	**FCREm (g/g)**	**FCRDz (kg/dz)**	**LIVA (%)**
0.00	101.40 ab	0.9593	98.81	62.72 b	60.16 b	1.687 a	1.270 ab	100.00
0.05	103.57 a	0.9638	99.27	*64.37 a	*62.03 a	1.671 a	1.291 a	97.38
0.10	103.08 a	0.9609	98.97	*64.96 a	*62.41 a	1.652 ab	1.287 a	100.00
0.30	102.40 a	0.9583	98.70	*64.61 a	*61.91 a	1.658 ab	1.286 ab	100.00
0.60	102.45 ab	0.9483	97.68	*64.83 a	61.46 ab	1.668 a	1.297 a	99.88
1.00	99.81b	0.9621	98.76	*64.78 a	*62.32 a	*1.603 b	1.246 b	98.29
P value	0.002	0.548	0.604	0.0001	0.002	0.003	0.008	0.227
L	0.0026	ns	ns	0.0007	ns	0.0006	0.0411	ns
Q	0.0182	ns	ns	0.0022	ns	0.1817	0.0025	ns
CV (%)	1.71	1.79	1.81	1.09	1.82	2.29	2.10	2.68

* Means differ from control (0.0) by Dunnet's test (P <0.05). Different letters indicate statistical difference by Tukey test (P <0.05). L-linear effect; Q-quadratic effect; ns-not significant. FI: $y = -6.231x^2 + 3.6512x + 102.39$. $R^2 = 0.7304$. Max= 0.29; EW: $y = -3.5195x^2 + 4.4877x + 63.703$. R^2=0.4305. Max=0.69; FCREm: $y = -0.0602x + 1.6771$. $R^2 = 0.66$; FCRDz: $y = -0.1338x2 + 0.1048x + 1.2763$. $R^2 = 0.8459$. Max=0.39. .

The supplementation levels of 0.05, 0.10, and 0.30% trehalose exhibited increased FI in comparison to the highest trehalose concentration of 1.0% (p <0.05). An observed statistical disparity in FCREm indicated that the elevated trehalose content in the diet led to decreased conversion by egg mass relative to the control group and other supplementation levels. Concerning FCRDz, the control group's performance resembled that of the other concentrations. The proportions of 0.05%, 0.10%, and 0. surpassed the highest assessed trehalose concentration. These findings suggest that enhanced dietary trehalose supplementation enabled the birds to achieve superior outcomes for FCREm and FCRDz (Refer to Table 2).

3.2. Egg Quality

Regarding EW, the birds that received trehalose supplementation had higher values compared to the control diet. For the RWS, the 1.00% trehalose treatment was superior to the others when submitted to the Tukey test. The variable EM presented significant difference between the treatments. The treatments of 0.05%, 0.10%, 0.30%, and 1.00% presented higher EM when compared to the control treatment (Tables 2 and 3).

Table 3. Effect of trehalose levels in relative weight of shell (RWS, %), yolk (RWY, %), and albumen (RWA, %), yolk color (YC), Haught Unit (HU), specific gravity (SG, g/cm³), thickness of shell (Tshell, μm), and shell strength (SS) of light hens eggs.

	Parameters							
Levels (%)	**RWS (%)**	**RWY (%)**	**RWA (%)**	**YC**	**HU**	**SG (g/cm³)**	**Tshell (μm)**	**SS (kgf)**
0.00	9.94	27.08	62.78	5.61 b	83.01	1.087	0.442	3.761
0.05	9.95	27.55	62.49	5.75 ab	82.36	1.087	0.449	3.518
0.10	9.94	26.99	63.07	*5.93 a	82.23	1.088	0.448	3.514
0.30	9.91	27.02	63.09	*5.89 a	81.26	1.088	0.448	3.519

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0.60	9.82	26.91	63.27	5.60 b	82.35	1.087	0.442	3.559
1.00	9.91	27.14	62.97	5.60 b	81.83	1.088	0.445	3.711
P value	0.575	0.365	0.122	<.0001	0.088	0.542	0.114	0.725
L	ns	ns	ns	0.00345	ns	Ns	ns	ns
Q	ns	ns	ns	0.00883	ns	Ns	ns	ns
CV (%)	1.55	2.25	0.90	2.60	1.36	0.11	1.56	9.14

* Means differ from control (0.0) by Dunnet's test (P <0.05). Different letters indicate statistical difference by Tukey test (P <0.05). L-linear effect; Q-quadratic effect; ns-not significant. YC: y = -0.5921x2 + 0.3803x + 5.7444. R^2 = 0.4215. Max=0.50.

Feeding light laying hens from 34 to 49 weeks of age with varying concentrations of trehalose supplementation exhibited no significant effect (P>0.05) on parameters such as RWS, RWY, RWA, HU, SG, TShell, and SS, as indicated in Table 3. Notably, a notable impact of diet was observed on YC. Specifically, levels of 0.10% and 0.30% demonstrated superiority compared to the control, as well as levels of - 0.60% and 1.00% of supplementation (Table 3). The antioxidant status was most favorable with trehalose supplementation at levels 0.30% and 0.60% solely during the final evaluation period, as depicted in Table 4.

Table 4. Effect of trehalose levels on MDA levels (nmol/mg protein) of eggs of light hens.

Levels	**Subperiods**				
(%)	**1**	**2**	**3**	**4**	**5**
0	0.122	0.123	0.124	0.131	0.135b
0.05	0.124	0.121	0.132	0.134	0.145b
0.1	0.131	0.134	0.142	0.144	0.143b
0.3	0.114	0.122	0.132	0.154	0.211a*
0.6	0.124	0.131	0.136	0.156	0.262a*
1	0.122	0.132	0.145	0.161	0.153b
P value	0.7359	0.7208	0.8047	0.2146	0.0063
L	ns	ns	ns	ns	0.0047
Q	ns	ns	ns	ns	0.0124
CV (%)	6.87	4.36	5.43	7.62	5.62

* Means differ from control (0.0) by Dunnet's test (P <0.05). Different letters indicate statistical difference by Tukey test (P <0.05). L-linear effect; Q-quadratic effect; ns-not significant. 5: y= 0.0104 x^2 + 0.0859 x +0.0401. R^2=0.83. Max=0.236.

3.3. Bone quality

The increase in trehalose levels caused a quadratic effect on TW with the maximum point at 0.187, and with the highest weight at 0.30 in relation to BS at the levels of 0.10 and 1.00 compared to the other levels of trehalose. Other parameters, such as L, DM, Ca and P, did not present significant difference (Table 5).

Table 5. Effect of trehalose levels in weight (TW), length (L), bone strength (BS), dry matter (DM), phosphorus (P) and calcium (Ca) of tibiotarsus of light hens.

	Parameters					
Levels	**TW**	**L**	**BS**	**DM**	**P**	**Ca**
(%)	**(g)**	**(mm)**	**(kgf)**	**(g/kg)**	**(g/kg)**	**(g/kg)**
0.00	8.50 b	115.83	20.57 c	462.3	101.1	170.2
0.05	9.00 ab	117.19	21.88 bc	459.2	103.4	167.2
0.10	8.64 ab	116.77	*24.79 a	451.2	99.3	168.4
0.30	*9.14 a	117.97	21.08 c	454.7	100.1	169.1
0.60	8.62 ab	116.78	19.89 c	461.4	102.2	162.2

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1.00	8.77 ab	116.21	*23.96 ab	458.9	103.1	161.3
P value	0.0241	0.2841	0.0001	0.6402	0.8903	0.596
L	0.62	0.73	0.61	0.82	0.65	0.10
Q	0.10	0.04	0.75	0.54	0.56	0.76
CV (%)	11.27	5.04	1.58	0.009	0.016	0.022

* Means differ from control (0.0) by Dunnet's test (P <0.05). Different letters indicate statistical difference by Tukey test (P <0.05). L-linear effect; Q-quadratic effect; ns-not significant. L: y= -0.2279 x2 + 1.6484x +114.48. R2= 0.97. Max = 0.19.

4. Discussion

Supplementation of trehalose in the diet of laying hens resulted in a decrease in feed intake, an increase in egg weight and egg mass, as well as a reduction in feed conversion ratio for both egg mass and egg dozens at the 0.6% level of inclusion. However, upon examination of the relative weights of egg components such as shell, albumen, and yolk, no statistically significant variances were detected among the different dietary treatments. This suggests that with the incorporation of trehalose into the diets, all egg components experienced a proportional increase in weight.

Upon ingestion, trehalose does not undergo direct absorption as a disaccharide into the bloodstream. Its sweetness potency is lower compared to sucrose, and it undergoes enzymatic cleavage into two glucose molecules by the action of the enzyme trehalose. Interestingly, this enzyme is present in humans and is situated on the brush border of the intestinal endothelial cells as discussed [9]. Similar physiological mechanisms are involved in the breakdown of other common disaccharides like maltose, sucrose, and lactose by their respective specific enzymes according to studies [10, 11, and 12].

Sugars play a crucial role in the generation of ATP and serve as carbon sources in metabolic processes as highlighted [13]. The utilization of trehalose has been linked to enhanced glucose metabolism and the regulation of postprandial blood glucose levels in rabbits [14]. However, investigations have shown that trehalose activity remains low from the 18th day of incubation up to 7 days after hatching in broilers, particularly when assessed in the jejunum and ileum segments, with a subsequent decline in activity beyond this developmental stage [15]. Therefore, it is hypothesized that for trehalose to exert its physiological effects in this species, the molecule may potentially contribute to improved gut health through the modulation of gut microbiota composition, with the bacteria utilizing trehalose as part of their metabolic processes.

In an evaluation of the impact of trehalose supplementation in the diets of broiler chickens, [16] noted enhancements in growth performance among females. Additionally, in a separate study involving both male and female birds, improvements in intestinal morphology were observed, further underscoring the potential benefits of incorporating trehalose into the diets of poultry.

In the current experiment conducted, it was observed that at the 0.60% level of inclusion of trehalose in the diet, there was a notable decrease in feed intake while maintaining a consistent rate of egg production. This phenomenon suggests an enhancement in the efficiency of energy utilization of the dietary regimen upon the incorporation of this additive. The authors of the study suggest that this specific molecule possesses the capability to regulate glucose homeostasis through a minimum of 7 distinct molecular pathways in various other species [17].

Into the exploration of trehalose as a probiotic in broilers that were subjected to a challenge involving *Salmonella Typhimurium* [18]. The findings of this investigation revealed significant outcomes associated with the utilization of this supplement in modulating the composition of the cecal microbiota. Notably, there was an elevation in the levels of lactobacilli, leading to the inhibition of the proliferation of Salmonella. Moreover, this modulation brought about advantageous effects on the daily feed intake and the rate of feed conversion in the avian subjects under study.

Various scientific studies have reported that certain disaccharides have the capacity to facilitate the absorption of calcium within the human body, [19, 20]. Trehalose falls within this category of disaccharides, and its supplementation could potentially result in an increased deposition of this essential mineral within eggshells. Furthermore, research explored the interaction between trehalose

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and calcium through the utilization of 13C nuclear magnetic resonance (NMR) spectroscopy [21]. The study findings revealed that upon the addition of trehalose at a rate of 10%, there was an augmentation in the soluble calcium content within a mixture of CaCl2 in 50 mM K-NaPO4 to approximately 24 ppm [21].

Commercially raised laying hens have been found to transfer roughly 10% of their total body calcium content daily towards the production of eggshells within their oviducts. Interestingly, only about half of this calcium contribution originates from their dietary intake, as highlighted in the studies conducted by [22 and 23]. Throughout the process of eggshell formation, which predominantly comprises calcium carbonate, a substantial proportion of the required calcium (ranging between 20% to 40%) must be mobilized from the skeletal reserves, particularly the medullary bone. This specialized bone tissue is situated within the medullary cavity of the midsection of long bones, notably those found in the limbs [24].

During this scientific investigation, the analysis of the calcium content present in the eggshell was not conducted. However, since the calcium content in the tibiotarsus remained constant throughout the study, it can be deduced that the increment in eggshell weight might have been induced by trehalose. This disaccharide likely facilitated enhanced absorption of calcium, subsequently channeling it towards the development and composition of the eggshell structure.

Conversely, considering the role of trehalose as an energy reservoir within the biological system of animals, its supplementation probably induced alterations in feed efficiency. Previous research has indicated that laying hens exhibiting superior Feed Conversion Ratio (FCR) also displayed increased albumen weight and height, along with a higher Haught Unit [25 and 26].

Insights from molecular modeling investigations propose that when trehalose is present in a solution, it may serve as a protective agent against water loss during processes of dehydration or freezing. This protective mechanism is achieved by substituting the hydration water molecules typically associated with biological entities [27]. Trehalose, in comparison to other disaccharides, demonstrates the highest capacity for hydration, indicating its potential to stabilize lipid bilayers by organizing the neighboring water molecules or through direct engagement with hydrophilic biomolecules as water is extracted [28 and 12].

A significant factor contributing to the loss of activity in labile biologics, such as proteins, during freezing or desiccation processes, is the alteration in their molecular conformation resulting from water removal. Dehydration disrupts the various intermolecular forces responsible for upholding the protein's native structure, leading to denaturation. Trehalose and other polyhydroxy compounds possess the capability to mitigate these transformations by forming hydrogen bonds with the protein surface, thereby preserving its conformation, supporting the water replacement theory [29].

When trehalose was included at levels of 0.10% and 0.30% in the experimental treatments, a notable increase in the intensity of stained yolks was observed compared to the other conditions. Investigations have confirmed that trehalose can enhance the stability of protein-based food items when subjected to extended heat processing, thus averting undesired discoloration. Consequently, the incorporation of trehalose into food formulations not only extends their shelf life but also safeguards against quality deterioration linked to discoloration issues [29].

The vibrant coloration of the yolk is closely linked to the accumulation of xanthophylls, a type of fat-soluble pigment. Diets characterized by a higher lipid content have been shown to stimulate increased absorption of these essential nutrients within the avian gastrointestinal tract. Upon supplementation of trehalose in rodent diets and subsequent examination of mesenteric and intestinal tissues, [30] noted a suppression in adipocyte proliferation in response to the reduced migration of chylomicrons from the intestine to the epithelium induced by this disaccharide.

In the laying hens, enhancing the quality of eggs stands out as a paramount aspect that requires enhancement and advancement. A limited number of research endeavors have delved into exploring the impacts of trehalose on avian metabolism. Nevertheless, its role as an antioxidant has already been firmly established and confirmed through a multitude of studies showcasing its efficacy in ameliorating lipid peroxidation [31, 32, 33, and 1].

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The current study unveiled that trehalose exhibited no significant impact on reducing lipid peroxidation during the initial 4 production cycles spanning 112 days. However, as the experiment progressed, the levels of 0.3% and 0.6% showcased superior outcomes in comparison to other concentrations of trehalose. Conversely, the 1.0% level of trehalose yielded results akin to the lower concentrations, potentially indicating a pro-oxidant role at this level. Variations in lipid peroxidation levels when assessing plasma, muscle, and liver samples of broilers aged from 0 to 18 days [2]. Noteworthy findings highlighted that trehalose supplementation in dairy cows led to a decrease in milk's lipid peroxide content while elevating its antioxidant properties [34].

Overall, it is evident that trehalose might not exert a significant influence on the antioxidant mechanisms when integrated into the diet for a brief duration. Nonetheless, it is postulated that administering this disaccharide from the early stages of laying hens' development could potentially play a pivotal role in mitigating lipid peroxidation and enhancing egg quality. Given the scarcity of research on trehalose utilization in avian diets, the appropriate levels of inclusion of this compound in the diet necessitate meticulous evaluation in alignment with the specific role intended in poultry metabolism.

The amassed data puts forth the notion that supplementing the diet of laying hens with trehalose could yield favorable outcomes in terms of performance, egg quality, and lipid peroxidation metrics. Further exploration is imperative to unravel the biological and physiological attributes of trehalose, particularly concerning the optimal inclusion levels for optimal outcomes.

5. Conclusions

Supplementation of laying hens' diets with 0.60% trehalose improves feed efficiency, increases egg weight and mass, and enhances egg quality without altering egg component weight proportions. Short-term use of trehalose does not significantly affect lipid peroxidation, but it has the potential to enhance gut health and egg quality.

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