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The effects of *Spirulina* sp. and vitamin E supplementation in laying hens diet on physical quality of eggs stored at different temperatures and times

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Abstract. This study evaluated the effects of *Spirulina* sp. supplementation combined with vitamin E in laying hen diets on egg physical quality and shell strength during storage under different durations and temperatures. Four dietary treatments were used: T0 (control diet with 100 IU/kg vitamin E), T1 (0.5% *Spirulina* sp. + 100 IU/kg vitamin E), T2 (1.0% *Spirulina* sp. + 100 IU/kg vitamin E), and T3 (2.0% *Spirulina* sp. + 100 IU/kg vitamin E). The experiment employed a completely randomized design with a 4 × 2 × 2 factorial arrangement and five replications. The factors consisted of diet, storage duration (7 and 14 days), and storage temperature (room and refrigerated). Storage duration and temperature significantly ($P<0.001$) reduced HU, while longer storage significantly decreased yolk and increased albumen percentages ($P<0.001$). Diet and storage duration significantly ($P<0.001$) influenced yolk color. Egg weight was not affected by the treatments. In conclusion, dietary treatment significantly affected yolk color and Haugh unit. The 0.5% *Spirulina* inclusion level resulted in the highest Haugh unit, indicating improved albumen quality, whereas 2.0% *Spirulina* enhanced yolk pigmentation. Storage temperature influenced internal egg quality, with refrigerated storage better maintaining albumen percentage and Haugh unit compared to room temperature.

1 Introduction

Eggs are an important source of high-quality protein and essential nutrients for human consumption. However, eggs physical quality tends to decrease during storage due to physical and chemical changes. During storage, moisture loss, carbon dioxide release through the shell, and albumen structure become thinner, leading to declines in egg weight, albumen height, and impact on Haugh unit [1]. These changes are strongly influenced by storage temperature and duration. Maintaining egg quality during storage is very important and crucial as it directly affects shelf life, consumer acceptance, and the economic value of eggs [2].

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Dietary manipulation of laying hens, especially through the supplementation of functional nutrients that enhance egg antioxidant capacity, represents an improve egg quality during storage. Dietary antioxidants have been reported to improve egg resistance to oxidative damage and maintain albumen quality during storage. Nadia *et al.*[3] stated that vitamin E supplementation in diets containing lemuru fish was effective in preserving the Haugh unit of eggs during storage, highlighting the role of antioxidants in maintaining internal egg quality. *Spirulina* sp. used as a natural feed ingredient because it contains many beneficial nutrients, such as unsaturated fatty acis, vitamins, minerals, pigments, and other bioactive compounds [4]. In addition to improving the nutritional value of eggs, *Spirulina* supplementation has the potential to enhance egg quality stability by reducing oxidative processes that occur during storage.

The high level of unsaturated fatty acids in *Spirulina* makes it highly susceptible to lipid oxidation, which can reduce egg quality during storage. Lipid oxidation may damage yolk quality and accelerate overall egg deterioration [5]. Therefore, the addition of antioxidants is required to protect these fatty acids from oxidative damage. Vitamin E is a lipid-soluble antioxidant that helps neutralize free radicals and prevent lipid oxidation [3]. The combined supplementation of *Spirulina* and vitamin E is expected to improve oxidative stability and help maintain the physical quality of eggs during storage at different temperatures and storage durations. However, research on the combined effects of *Spirulina* sp. and vitamin E supplementation on egg physical quality are still limited. Therefore, this study aimed to evaluate the effects *Spirulina* sp. and vitamin E supplementation in laying hen diets on egg physical quality and shell strength during storage at different times and temperatures.

2 Material and methods

2.1 Egg sampling and experimental design

Eggs were collected from 28-week-old ISA Brown laying hens. The hens were reared for six weeks under extensive management conditions. The basal diet consisted of yellow corn, rice bran, soybean meal, meat and bone meal, crude palm oil, calcium carbonate, dicalcium phosphate, sodium chloride, a commercial laying premix, L-lysine, DL-methionine, tryptophan, and vitamin E. In treatments T1 to T3, the diets were supplemented with *Spirulina* sp**.** Egg storage was conducted at the end of the experiment, with two eggs per replication used for analysis. A total of 160 eggs were included in this study. The eggs were stored either at room temperature or under refrigerated conditions for 7 and 14 days. The dietary treatments were as follows:

T0 = control + 100 IU/kg vitamin E
T1 = diet supplemented 0.5% *Spirulina* sp. + 100 IU/kg vitamin E
T2 = diet supplemented 1.0% *Spirulina* sp. + 100 IU/kg vitamin E
T3 = diet supplemented 2.0% *Spirulina* sp. + 100 IU/kg vitamin E

2.2 Egg physical quality measurements

Physical quality parameters observed were external quality, internal quality, Haugh unit, and shell strength. External quality observed was egg weight (g), shell percentage (%), and shell thickness (mm). Egg weight was determined by weighing the whole egg using an analytical scale. Shell percentage was determined by weighing the eggshell and calculating their proportion to the total egg weight. Eggshell thickness was measured using a digital micrometer.

Yolk percentage, albumen percentage, and yolk color are internal egg quality parameters. The percentages of yolk and albumen were calculated by weighing each part individually and dividing it by the total egg weight. The yolk color score was determined by comparing the yolk color to a Roche yolk color fan. Eggshell strength was measured using an Eggshell Strength Tester RH-DQ200 from Guangzhou Runhu Instruments Co., Ltd. Haugh unit was measured using the following equation (H is the height of albumen and W is the egg weight) [6]:

$$100 \times log_{10}(H - 1.7\, W^{0.37} + 7.73) \quad (1)$$

2.3 Statistical analysis

The experiment was conducted using a factorial completely randomized design with four levels of *Spirulina* (0, 0.5%, 1.0%, and 2.0%) and two storage durations (7 and 14 days), with five replications per treatment. Data were analyzed by ANOVA, with statistical significance set at $P<0.05$. When significant differences were detected, Duncan's Multiple Range Test was used. All statistical analyses were performed using SPSS Statistics 27.

3 Results and discussions

Result of diet, storage temperature, and storage time on egg external quality are presented in Table 1. Dietary treatments did not significantly affect egg weight, shell percentage, or shell thickness. Eggs from hens fed diets supplemented with Spirulina sp. at different inclusion levels exhibited comparable external quality characteristics to those of the control group (T0), indicating that Spirulina sp. supplementation up to 2.0% did not affect shell formation or overall egg mass. These findings suggest that the basal diet, which already met the nutritional requirements for laying hens, was sufficient to maintain egg external quality, and additional dietary treatments did not produce measurable improvements in these parameters.

In contrast, storage temperature had a greater effect on egg weight than dietary treatment and storage duration. Eggs stored at room temperature show lower egg weights compared to eggs under refrigerated conditions, regardless of diet or storage time. According to Sokolowicz *et al.* [7], reductions in egg weight during storage are mainly caused by water evaporation through the pores of the eggshell, which increases with higher storage temperatures. Refrigeration effectively slows down this evaporative process by reducing the vapor pressure gradient between the egg contents and the surrounding environment, thereby minimizing weight loss.

Storage time did not cause significant differences in egg weight, shell percentage, or shell thickness. This finding indicates that the storage periods used in this study were still sufficient to maintain overall egg external quality. Eggs show significant quality loss after 14 days [8]. Shell percentage and shell thickness remained relatively stable across dietary treatments, storage temperatures, and storage durations. This indicates that eggshell structural integrity was well maintained during storage, and that neither Spirulina sp. supplementation nor storage conditions had a notable effect on shell mineral composition.

The effects of diet, storage temperature, and storage time on egg internal quality are presented in Table 2. Dietary treatment had a significant ($P<0.001$) effect on yolk pigmentation, with increasing levels of Spirulina sp. supplementation leading to a higher yolk color score. This finding indicates that Spirulina sp. is an effective natural pigment source for enhancing yolk color. The improvement in yolk pigmentation can be attributed to the high content of bioactive pigments in spirulina, including carotenoids, phycocyanin, β-carotene, and xanthophylls, which are known to be efficiently deposited into the egg yolk. These results are consistent with the findings of Sujana *et al.* [9], who reported that dietary spirulina supplementation significantly enhances yolk color due to its rich pigment profile.

Table 1. Effects of diet, storage temperature, and storage time on egg external quality

Treatment[1]	Temperature[1]	Storage time[1] (days)	Egg weight (g)	Shell percentage (%)	Shell thickness (mm)
T0	Room temperature	7	62.80 ± 4.67	11.38 ± 0.75	0.38 ± 0.03
		14	62.80 ± 4.22	11.74 ± 0.96	0.47 ± 0.06
	Refrigerator	7	64.10 ± 4.94	12.05 ± 1.07	0.38 ± 0.01
		14	64.80 ± 3.05	11.98 ± 0.47	0.38 ± 0.01
T1	Room temperature	7	63.20 ± 1.92	11.71 ± 0.85	0.38 ± 0.02
		14	62.80 ± 2.93	11.30 ± 1.13	0.36 ± 0.05
	Refrigerator	7	64.20 ± 2.31	11.34 ± 0.25	0.38 ± 0.01
		14	64.60 ± 3.27	11.73 ± 0.28	0.39 ± 0.01
T2	Room temperature	7	61.90 ± 4.14	11.94 ± 0.64	0.39 ± 0.02
		14	61.70 ± 5.99	11.91 ± 0.93	0.37 ± 0.03
	Refrigerator	7	62.30 ± 4.10	12.37 ± 0.62	0.37 ± 0.01
		14	63.50 ± 3.24	11.87 ± 0.58	0.38 ± 0.03
T3	Room temperature	7	61.00 ± 3.10	12.56 ± 0.30	0.39 ± 0.02
		14	66.90 ± 3.15	11.31 ± 0.71	0.38 ± 0.02
	Refrigerator	7	63.80 ± 1.79	10.96 ± 1.23	0.37 ± 0.07
		14	61.90 ± 2.88	11.83 ± 0.37	0.40 ± 0.01
T0			63.63 ± 4.03	11.79 ± 0.82	0.40 ± 0.05
T1			63.70 ± 2.55	11.52 ± 0.70	0.38 ± 0.03
T2			62.35 ± 4.17	12.02 ± 0.68	0.38 ± 0.02
T3			63.40 ± 3.45	11.66 ± 0.92	0.39 ± 0.04
P-value[2]			0.625	0.208	0.063
	Room temperature		62.89 ± 3.95	11.73 ± 0.84	0.39 ± 0.04
	Refrigerator		63.65 ± 3.17	11.77 ± 0.75	0.38 ± 0.03
	P-value[3]		0.354	0.841	0.255
		7	62.91 ± 3.41	11.79 ± 0.88	0.38 ± 0.03
		14	63.63 ± 3.75	11.71 ± 0.71	0.39 ± 0.04
		P-value[4]	0.386	0.640	0.077

T0 = Control diet + 100 IU/kg vitamin E; T1= Diet with 0.5% *Spirulina* + 100 IU/kg vitamin E; T2= Diet with 1.0% *Spirulina* + 100 IU/kg vitamin E; T3= Diet with 2.0% *Spirulina* + 100 IU/kg vitamin E
[1]Significant interations: shell percentage = treatment*temperature*storage time; shell thickness = treatment*temperature, treatment*storage time, treatment*temperature*storage time
p-value[2] for diet treatment; *p*-value[3] for temperature, and *p*-value[4] for storage time

In addition to dietary effects, storage conditions also play an important role in maintaining yolk pigmentation. Vitamin E supplementation contributed to the preservation of yolk color during storage by protecting carotenoid pigments from oxidative degradation. Carotenoids are highly susceptible to oxidation, a process accelerated by exposure to light, elevated temperatures, and prolonged storage duration [10]. Eggs stored under refrigerated conditions exhibit greater yolk color stability than those stored at room temperature, indicating that lower temperatures effectively slows down oxidative reactions and pigment degradation, thereby preserving yolk color quality during storage.

Eggs stored at cooler temperatures have a higher albumen percentage than those stored at room temperature ($P<0.001$). Refrigeration markedly slows metabolic and physical changes within the egg, particularly water migration from the albumen to the yolk. At low temperatures, the vitelline membrane remains structurally stronger and less permeable, thereby reducing water transfer into the yolk and maintaining a higher proportion of albumen. Consequently, the yolk percentage under refrigerated storage in this study was lower ($P<0.001$). This preservation of albumen integrity under refrigerated storage conditions highlights the importance of post-laying handling practices in maintaining internal egg quality.

The effects of diet, storage temperature, and storage time on the Haugh unit and shell strength are presented in Table 3. Feed contains 0.5% *Spirulina* sp. significantly improved the Haugh unit during storage, whereas higher levels (1.0% and 2.0%) did not provide additional benefits. However, Omri [11] reported that the inclusion of *Spirulina* without vitamin E supplementation in the diet increased the Haugh unit. This finding suggests that 0.5% *Spirulina* sp. represents an optimal supplementation level when combined with 100 IU/kg vitamin E for maintaining albumen quality during storage. The improvement in Haugh unit may be attributed to the antioxidant properties of 0.5% *Spirulina* and 100 IU/kg vitamin E, which contains bioactive compounds such as phycocyanin, carotenoids, and phenolic compounds capable of scavenging free radical [12].

Table 2. Effects of diet, storage temperature, and storage time on egg internal quality

Treatment[1]	**Temperature**[1]	**Storage time**[1] **(days)**	**Yolk percentage (%)**	**Albumen percentage (%)**	**Yolk color**
T0	Room temperature	7	34.90 ± 7.56	53.72 ± 7.13	6.00 ± 0.61
		14	34.55 ± 2.55	53.72 ± 2.29	6.30 ± 1.48
	Refrigerator	7	30.72 ± 4.13	57.23 ± 4.96	7.40 ± 0.22
		14	27.71 ± 1.71	60.30 ± 1.36	5.80 ± 0.76
T1	Room temperature	7	33.74 ± 7.23	54.55 ± 7.27	8.00 ± 0.87
		14	33.49 ± 3.34	55.21 ± 3.80	8.20 ± 0.97
	Refrigerator	7	30.63 ± 1.74	58.03 ± 1.61	8.40 ± 0.42
		14	27.61 ± 3.45	60.67 ± 3.45	8.50 ± 0.35
T2	Room temperature	7	29.11 ± 1.82	58.96 ± 2.04	8.40 ± 0.22
		14	33.24 ± 4.34	54.85 ± 3.87	7.80 ± 0.27
	Refrigerator	7	32.76 ± 8.56	56.39 ± 5.71	9.30 ± 0.27
		14	27.05 ± 1.49	61.08 ± 1.24	9.40 ± 0.65
T3	Room temperature	7	35.33 ± 3.58	52.11 ± 3.61	9.40 ± 0.42
		14	31.96 ± 4.50	57.90 ± 3.55	10.40 ± 0.22
	Refrigerator	7	26.48 ± 4.43	63.56 ± 3.90	10.70 ± 1.10
		14	28.69 ± 4.01	60.65 ± 1.99	10.70 ± 0.67
T0			31.97 ± 5.18	56.24 ± 5.03	6.38 ± 1.04^{c}
T1			31.36 ± 4.80	57.12 ± 4.84	8.28 ± 0.68^{b}
T2			30.54 ± 5.25	57.82 ± 4.15	8.73 ± 0.77^{b}
T3			30.61 ± 5.13	58.55 ± 5.31	10.30 ± 0.83^{a}
P-value[2]			0.721	0.323	<0.001
	Room temperature		33.29 ± 4.72^{a}	55.13 ± 4.67^{b}	8.06 ± 1.54^{b}
	Refrigerator		28.96 ± 4.39^{b}	59.74 ± 3.83^{a}	8.78 ± 1.66^{a}
	P-value[3]		<0.001	<0.001	<0.001
		7	31.71 ± 5.72	56.82 ± 5.61	8.45 ± 1.44
		14	30.54 ± 4.21	58.05 ± 3.89	8.39 ± 1.82
		P-value[4]	0.252	0.179	0.688

T0 = Control diet + 100 IU/kg vitamin E; T1= Diet with 0.5% spirulina + 100 IU/kg vitamin E; T2= Diet with 1.0% spirulina + 100 IU/kg vitamin E; T3= Diet with 2.0% spirulina + 100 IU/kg vitamin E
Different superscript at the same column indicate significant differences (P<0.05)
[1]Significant interations: albumen percentage = treatment*temperature*storage time; yolk color = treatment*temperature*storage time
P-value[2] for diet treatment; *P*-value[3] for temperature, and *P*-value[4] for storage time

Storage temperature exerted a pronounced effect on albumen quality, with refrigerated storage resulting in significantly higher Haugh unit values (84.93) compared with room temperature storage (66.23). Lower storage temperatures effectively slow down biochemical and physical changes within the egg, including protein degradation and thinning of the thick albumen. Refrigeration reduces the rate of carbon dioxide loss and maintains albumen pH at a more stable level, thereby preserving the structural integrity of albumen proteins [13] and sustaining higher Haugh unit values during storage. No significant differences in eggshell strength were observed among dietary treatments, indicating that variations in diet composition, including *Spirulina* sp. supplementation, did not influence eggshell properties. This suggests that eggshell strength was primarily determined by mineral nutrition and shell formation processes rather than by dietary antioxidants.

Table 3. Effects of diet, storage temperature, and storage time on Haugh unit and shell strength

Treatment[1]	**Temperature[1]**	**Storage time[1] (days)**	**Haugh unit**	**Shell strength (N)**
T0	Room temperature	7	71.37 ± 6.46	28.46 ± 7.11
		14	61.43 ± 9.04	29.71 ± 4.55
	Refrigerator	7	72.72 ± 9.26	30.84 ± 4.08
		14	85.31 ± 5.14	31.89 ± 6.40
T1	Room temperature	7	75.81 ± 4.81	24.62 ± 3.87
		14	79.12 ± 5.86	26.47 ± 4.00
	Refrigerator	7	87.28 ± 1.73	29.98 ± 4.24
		14	87.07 ± 5.72	33.12 ± 3.14
T2	Room temperature	7	63.35 ± 6.95	31.22 ± 4.01
		14	60.17 ± 6.34	26.43 ± 3.28
	Refrigerator	7	97.34 ± 6.72	25.93 ± 2.64
		14	84.02 ± 8.23	27.77 ± 5.05
T3	Room temperature	7	54.68 ± 9.60	28.26 ± 3.22
		14	63.94 ± 6.18	37.69 ± 3.00
	Refrigerator	7	81.62 ± 11.24	28.33 ± 7.02
		14	84.04 ± 4.98	29.28 ± 1.81
T0			72.71 ± 11.20[b]	30.23 ± 5.37
T1			82.32 ± 6.78[a]	28.55 ± 4.86
T2			76.22 ± 16.94[b]	27.84 ± 4.12
T3			71.07 ± 14.73[b]	30.89 ± 5.62
P-value[2]			<0.001	0.118
	Room temperature		66.23 ± 10.20[b]	29.11 ± 5.46
	Refrigerator		84.93 ± 9.17[a]	29.64 ± 4.73
	P-value[3]		<0.001	0.592
		7	75.52 ± 14.46	28.46 ± 4.84
		14	75.64 ± 12.57	30.30 ± 5.21
		P-value[4]	0.942	0.070

T0 = Control diet + 100 IU/kg vitamin E; T1= Diet with 0.5% spirulina + 100 IU/kg vitamin E; T2= Diet with 1.0% spirulina + 100 IU/kg vitamin E; T3= Diet with 2.0% spirulina + 100 IU/kg vitamin E
Different superscript at the same column indicate significant differences ($P<0.05$)
[1]Significant interations: Haugh unit = treatment*temperature; treatment*storage time; treatment*temperature*storage time; Shell strength = treatment*temperature
P-value[2] for diet treatment; *P*-value[3] for temperature, and *P*-value[4] for storage time

4 Conclusion

Spirulina sp. supplementation combined with 100 IU/kg vitamin E had no significant effect on egg external quality traits. However, dietary treatment significantly affected yolk color and Haugh unit (*P*<0.001). The 0.5% Spirulina inclusion level resulted in the highest Haugh unit, indicating improved albumen quality, whereas 2.0% Spirulina enhanced yolk pigmentation. Storage temperature markedly influenced internal egg quality, with refrigerated storage better maintaining albumen percentage and Haugh unit compared to room temperature.

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