



The Impact of Adding *Spirulina* Algae to Drinking Water on the Productivity, Egg Quality, Yolk Lipid Oxidation, and Blood Biochemistry of Laying Hens

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Abstract | The quality of chicken eggs is affected by the nutritional composition of the feed and water provided to the laying hens. This research aimed to assess the impact of supplementing laying hens' drinking water with *Spirulina* algae on their productive performance, egg quality, yolk lipid peroxidation, and blood biochemical parameters. The experiment followed a completely randomized design, featuring five treatments with varying concentrations of *Spirulina*, monitored over a 10-week period. Reproductive performance was assessed daily, while egg quality was evaluated on a weekly basis. The antioxidant capacity of the egg yolk was measured at the start, midpoint, and end of the experiment using the TBARS assay. Blood biochemical parameters were analysed at the end of the experiment using an automated Cobas biochemical system. The results indicated that supplementing *Spirulina* in the drinking water of laying hens did not affect reproductive performance indicators. Nevertheless, there was a statistically significant difference in egg yolk color core between the *Spirulina* supplemented groups and the control group. Additionally, differences were observed between treatments in serum HDL and LDL levels. HDL levels increased and LDL levels decreased in the *Spirulina* supplemented groups as the concentration increased. Notably, the antioxidant capacity of the egg yolk increased with higher *Spirulina* concentrations ($P < 0.05$). It was concluded that the addition of *Spirulina* to the drinking water of laying hens enhanced egg yolk color, increased the yolk's antioxidant capacity, and improved serum lipoprotein levels, creating potential benefits for poultry farming and human nutrition.

Keywords | *Spirulina* algae, Performance, Egg quality, Lipid peroxidation, Serum parameters, Laying hens

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INTRODUCTION

Eggs are one of the most common and essential foods meeting daily nutritional needs for humans. Now-

adays, as human lifestyles improve and demand not only for quantity but also for quality food rises, the production of high-quality eggs to meet competitive quality demands is receiving increasing attention from farmers. Particular-

ly, the quality of eggs linked to functional foods has been a focus of scientific research. Many previous studies have added various types of supplements to the feed for laying hens, which has contributed to improving the quality of eggs corresponding to the nutrients in the supplements (Hu *et al.*, 2011; Fraeye *et al.*, 2012; Opaliński *et al.*, 2012). Thus, the nutritional value of eggs may vary depending on the nutritional components in the diet of laying hens.

Spirulina algae is a type of freshwater algae found in ponds and lakes. *Spirulina* is extremely nutritious, packed with proteins, essential amino acids, vitamins, essential fatty acids, minerals, and antioxidant pigments (such as carotenoids and xanthophylls) (Holman and Malau-Aduli, 2013; Asghari *et al.*, 2016; Hynstova *et al.*, 2018). *Spirulina* has been recognized as a valuable nutritional source for both animals and humans (Parikh *et al.*, 2001; Rasool *et al.*, 2006). Ramadan *et al.* (2008) demonstrated that the lipid content in *Spirulina* serves as an alternative source for antioxidant agents. *Spirulina* has the ability to lower blood cholesterol concentration and raise high-density lipoprotein levels, contributing to the prevention of atherosclerosis in humans (Colla *et al.*, 2008). Moreover, *Spirulina* can reduce stress, increase antioxidant capacity, and boost the immune system of broiler chickens (Mirzaie *et al.*, 2018; Kolluri *et al.*, 2021). Additionally, several studies have supplemented *Spirulina* in the diet of laying hens, which have shown improvements in the color of egg yolks, and a reduction in serum and egg yolk cholesterol (Park *et al.*, 2015; Dogan *et al.*, 2016; Selim *et al.*, 2018). Therefore, the addition of *Spirulina* to the diet of laying hens has the potential to improve productivity, egg quality, yolk color, reduce serum cholesterol, and enhance the antioxidant capacity of egg yolk.

Table 1: The nutritional components of commercial mixed feed for laying hens.

Components	Percentage (%)
Crude protein (min)	17.5
Crude fiber (max)	7.0
Calcium (min – max)	3 – 6
Total phosphorus (min – max)	0.4 – 1.8
Lysine (min)	0.5
Methionine + Cystine (min)	0.5

Furthermore, previous studies have focused exclusively on incorporating *Spirulina* into the feed of laying hens, without exploring its addition to their drinking water (Park *et al.*, 2015; Dogan *et al.*, 2016; Selim *et al.*, 2018). Introducing *Spirulina* into the drinking water for laying hens is more feasible for small-scale farms and avoids nutrient degradation caused by high-temperature pelleting. It has been shown that adding *Spirulina* to the drinking water of

broilers enhances the rapid absorption of nutrients in the small intestine during heat stress, and improves antioxidant and anti-stress capabilities, thereby promoting better health in broilers (Kolluri *et al.*, 2021). Yet, there is presently a lack of research on administering *Spirulina* in the laying hens’ drinking water and examining the antioxidant potential of eggs. The objective of this research is to examine how adding *Spirulina* to the drinking water of laying hens influences egg production, egg quality, serum composition, and the antioxidant capacity of egg yolks.

MATERIALS AND METHODS

ANIMALS

This study involved 120 commercial Isa Brown laying hens aged 38 weeks with consistent body weights. All hens were vaccinated against infectious diseases. The procedures conducted in this study were approved by the Animal Care and Use Committee of Tra Vinh University, Vietnam, under license number 219/2022/HD-HDKH&DT-DHTV. The laying hens were maintained in welfare conditions and optimal bio-climatic. The chickens were carefully restrained when administering vaccines or drawing blood samples. After vaccination or blood sampling, all chickens were provided with additional vitamin C to ensure stress reduction.

EXPERIMENTAL DESIGN

The experimental setup followed a completely randomized design comprising five treatment groups with varying levels of *Spirulina* algae supplement in drinking water. Treatment groups received 0, 0.5, 1.0, 1.5, and 2.0 grams of fresh *Spirulina* per hen per day, denoted as T1, T2, T3, T4, and T5, respectively. Four replicates were conducted, each with 6 hens (totaling 24 hens per treatment). The experiment spanned from 38 to 47 weeks of age, covering a 10-week experimental period.

ANIMAL MANAGEMENT

The hens were accommodated in battery cages featuring nipple drinkers and trough feeders. Lighting was set to 16 hours of continuous illumination daily, from 04:00 to 20:00. All hens across treatments received identical commercial layer feed, detailed in Table 1 for ingredient and nutrient composition. Diets were provided *ad libitum* without probiotics or antibiotics.

Fresh *Spirulina* algae was sourced from an aquaculture farm at Tra Vinh University, Vietnam. To prepare the drinking water with the designated amounts of fresh *Spirulina* algae, constant circulation was ensured using a manual stirrer, achieving a consistent suspension and even distribution at room temperature. The required treatments were created by adding increasing quantities of fresh *Spirulina* algae (0, 0.5, 1.0, 1.5, and 2.0 grams per hen per day) equivalent to 0, 12,

Table 2: Effects of *Spirulina* algae supplementation in drinking water on productive performance of laying hens.

Parameters	Periods	Treatments					P-value
		T1	T2	T3	T4	T5	
Body Weight (g)	Initial BW	1.89±0.11	1.87±0.05	1.84±0.03	1.92±0.03	1.86±0.06	0.433
	Final BW	1.93±0.10	1.92±0.03	1.87±0.03	1.96±0.01	1.89±0.06	0.215
	Gain Weight	0.04±0.01	0.05±0.02	0.03±0.02	0.04±0.02	0.03±0.01	0.581
Feed intake (g/hen/day)	Week 38-39	119.50±3.27	121.54±2.06	117.43±2.82	118.14±2.78	120.95±3.30	0.255
	Week 40-41	108.59±3.03	106.04±11.03	105.98±3.79	106.21±6.41	105.75±4.73	0.969
	Week 42-43	104.03±0.70	106.46±3.07	105.50±3.58	104.19±13.57	103.67±8.55	0.982
	Week 44-45	103.66±1.35	105.06±1.42	105.86±3.82	105.85±2.86	105.10±2.04	0.720
	Week 46-47	110.33±1.37	110.78±3.20	110.06±2.73	110.61±5.98	111.46±7.36	0.995
Egg weight (g)	Week 38-39	55.76±0.96	55.99±0.38	55.90±1.02	55.53±0.58	55.95±1.28	0.948
	Week 40-41	54.92±0.19	55.01±0.90	54.77±0.24	54.89±1.14	54.97±0.65	0.991
	Week 42-43	50.64±1.44	50.62±0.94	50.99±1.45	50.84±0.82	50.73±0.81	0.990
	Week 44-45	50.77±1.16	50.50±1.28	50.71±1.39	50.52±0.60	50.61±1.64	0.997
	Week 46-47	49.96±0.57	49.75±1.26	49.63±2.77	49.58±0.56	49.79±0.89	0.996
Egg number (egg/hen/week)	Week 38-39	6.78±0.05	6.59±0.35	6.52±0.11	6.77±0.14	6.65±0.17	0.302
	Week 40-41	6.59±0.29	6.54±0.05	6.52±0.14	6.42±0.20	6.50±0.12	0.766
	Week 42-43	6.29±0.26	6.29±0.05	6.34±0.14	6.29±0.14	6.36±0.12	0.958
	Week 44-45	6.27±0.17	6.19±0.28	6.29±0.16	6.19±0.24	6.23±0.27	0.947
	Week 46-47	6.35±0.11	6.33±0.24	6.31±0.16	6.27±0.11	6.29±0.09	0.948
Egg production (%)	Week 38-39	96.78±0.69	94.05±5.05	93.16±1.50	96.73±2.03	94.94±2.45	0.298
	Week 40-41	94.05±4.12	93.46±0.69	93.16±2.03	91.67±2.91	92.86±1.68	0.755
	Week 42-43	89.88±3.70	89.89±0.69	90.59±2.05	89.89±2.06	90.79±1.80	0.959
	Week 44-45	89.59±2.46	88.40±3.94	89.89±2.28	88.39±3.42	88.99±3.82	0.948
	Week 46-47	90.78±1.50	90.48±3.51	90.18±2.26	89.59±1.50	89.89±1.19	0.940
Egg mass (g)	Week 38-39	53.97±1.19	52.67±3.07	52.06±0.76	53.72±1.58	53.13±2.27	0.650
	Week 40-41	51.66±2.39	51.42±1.09	51.02±1.13	50.34±2.60	51.05±1.33	0.873
	Week 42-43	45.56±3.08	45.50±1.05	46.16±0.47	45.71±1.62	46.07±1.59	0.976
	Week 44-45	45.50±2.18	44.67±3.02	45.60±2.42	44.64±1.39	45.03±2.27	0.960
	Week 46-47	45.35±0.41	45.02±2.37	44.80±3.50	44.42±1.08	44.75±0.77	0.974
FCR (g feed/g egg)	Week 38-39	2.21±0.08	2.31±0.10	2.26±0.04	2.20±0.09	2.28±0.10	0.366
	Week 40-41	2.11±0.15	2.07±0.23	2.08±0.09	2.12±0.21	2.07±0.15	0.991
	Week 42-43	2.29±0.15	2.34±0.09	2.28±0.07	2.28±0.29	2.26±0.20	0.970
	Week 44-45	2.28±0.08	2.36±0.16	2.33±0.09	2.37±0.08	2.34±0.08	0.752
	Week 46-47	2.44±0.03	2.46±0.11	2.47±0.22	2.50±0.18	2.49±0.12	0.980

Values are mean ± standard deviation. T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5 and 2.0 g *Spirulina*/hen/day, respectively.

24, 36, and 48 grams of fresh *Spirulina* per 4 liters of water, respectively, in water containers with a capacity of 25 liters for each treatment. Birds were given access to the algae-containing water voluntarily from 08:00 to 12:00 in the morning. Once the chickens finished drinking the algae water, any remaining water was replaced with fresh normal water until the next day, provided *ad libitum*

EVALUATION OF PRODUCTIVE PERFORMANCE

Each hen was individually weighed at the start (38th week of age) and conclusion (47th week of age) of the trial. Dai-

ly measurements were taken for feed intake (in grams per hen), egg weight (in grams), egg number (eggs per hen), and egg production percentage. Egg mass was computed by multiplying egg weight by egg production. The feed conversion ratio (FCR) was calculated as the grams of feed consumed per gram of egg produced. All production parameters were assessed based on replicates and represented the bi-weekly averages.

EVALUATION OF EGG QUALITY

Each week, a total of 24 eggs (6 eggs per replicate) were

Table 3: Effects of *Spirulina* algae supplementation in drinking water on egg quality of laying hens.

Parameters	Periods	Treatments					P-value
		T1	T2	T3	T4	T5	
Albumin weight (%)	Week 38-39	61.57±0.80	61.55±0.72	61.23±0.53	61.40±0.57	61.83±0.73	0.781
	Week 40-41	60.44±0.68	60.25±0.75	60.53±0.32	60.26±0.91	60.57±1.39	0.977
	Week 42-43	60.65±0.49	60.61±1.11	60.58±0.55	61.63±0.18	61.71±0.63	0.050
	Week 44-45	61.40±0.70	61.79±1.18	61.30±0.64	61.72±0.73	61.58±1.42	0.946
	Week 46-47	61.73±1.31	61.58±0.86	61.32±1.15	61.68±1.11	61.99±0.57	0.923
Yolk weight (%)	Week 38-39	25.10±0.74	25.24±0.18	25.86±0.72	25.88±0.48	25.27±0.83	0.301
	Week 40-41	26.92±0.63	26.96±0.65	27.05±0.54	27.15±0.67	27.19±1.04	0.979
	Week 42-43	26.30±0.51	26.23±0.58	26.61±0.77	26.24±0.18	26.27±0.33	0.824
	Week 44-45	25.56±0.89	25.55±0.97	26.02±0.80	25.66±0.32	26.01±1.35	0.900
	Week 46-47	25.37±1.27	25.46±0.41	25.48±0.90	25.41±1.05	25.68±0.96	0.993
Shell weight (%)	Week 38-39	12.21±0.47	12.34±0.59	12.79±0.30	12.48±0.33	12.27±0.47	0.393
	Week 40-41	12.65±0.28	12.91±0.26	12.54±0.49	12.58±0.40	12.49±0.36	0.562
	Week 42-43	12.92±0.44	13.04±0.60	12.82±0.50	12.72±0.15	12.85±0.13	0.852
	Week 44-45	13.04±0.43	13.16±0.61	13.18±0.47	12.88±0.18	13.04±0.41	0.868
	Week 46-47	13.03±0.28	13.22±0.30	13.20±0.44	13.16±0.25	13.21±0.23	0.905
Egg shape index	Week 38-39	0.793±0.013	0.795±0.031	0.793±0.010	0.808±0.010	0.815±0.006	0.237
	Week 40-41	0.773±0.015	0.773±0.010	0.775±0.017	0.783±0.010	0.790±0.008	0.252
	Week 42-43	0.783±0.013	0.783±0.005	0.788±0.010	0.778±0.010	0.780±0.008	0.646
	Week 44-45	0.783±0.013	0.785±0.010	0.783±0.010	0.790±0.018	0.783±0.019	0.932
	Week 46-47	0.793±0.013	0.803±0.013	0.793±0.010	0.798±0.005	0.800±0.014	0.648
Albumin Index	Week 38-39	0.024±0.001	0.023±0.002	0.023±0.002	0.022±0.002	0.023±0.001	0.904
	Week 40-41	0.024±0.001	0.025±0.001	0.024±0.002	0.025±0.001	0.025±0.001	0.336
	Week 42-43	0.026±0.001	0.026±0.001	0.026±0.002	0.026±0.001	0.026±0.002	0.958
	Week 44-45	0.021±0.002	0.022±0.001	0.021±0.003	0.021±0.002	0.023±0.001	0.498
	Week 46-47	0.022±0.001	0.022±0.003	0.021±0.003	0.022±0.002	0.022±0.002	0.964
Yolk index	Week 38-39	0.365±0.006	0.355±0.006	0.363±0.013	0.363±0.022	0.360±0.014	0.864
	Week 40-41	0.363±0.015	0.360±0.012	0.358±0.010	0.355±0.010	0.355±0.017	0.892
	Week 42-43	0.310±0.018	0.310±0.023	0.310±0.008	0.315±0.013	0.310±0.024	0.993
	Week 44-45	0.348±0.017	0.338±0.005	0.338±0.015	0.355±0.013	0.348±0.013	0.315
	Week 46-47	0.345±0.017	0.355±0.017	0.348±0.013	0.358±0.015	0.353±0.026	0.860
Haugh units	Week 38-39	78.92±0.43	78.06±2.17	78.30±2.12	77.73±1.90	78.04±2.77	0.939
	Week 40-41	83.03±2.01	84.55±1.56	84.16±2.38	84.77±1.85	85.33±1.64	0.537
	Week 42-43	84.95±2.21	83.19±2.24	84.63±2.50	85.41±1.14	84.47±1.59	0.609
	Week 44-45	75.83±3.69	75.96±3.32	76.08±0.68	76.12±1.65	77.97±2.12	0.774
	Week 46-47	78.71±2.83	77.28±2.43	77.03±2.37	78.36±1.69	79.97±2.46	0.445
Shell thickness (mm)	Week 38-39	0.113±0.010	0.109±0.006	0.111±0.009	0.116±0.014	0.113±0.006	0.872
	Week 40-41	0.108±0.006	0.113±0.001	0.119±0.016	0.119±0.007	0.110±0.012	0.409
	Week 42-43	0.110±0.010	0.112±0.009	0.107±0.008	0.108±0.004	0.108±0.011	0.917
	Week 44-45	0.107±0.007	0.110±0.012	0.106±0.007	0.107±0.008	0.110±0.005	0.936
	Week 46-47	0.110±0.007	0.110±0.012	0.107±0.008	0.103±0.007	0.103±0.007	0.654
Yolk color core	Week 38-39	12.63±0.10 ^b	13.03±0.21 ^a	13.07±0.08 ^a	13.16±0.12 ^a	13.19±0.07 ^a	<0.001
	Week 40-41	12.66±0.06 ^c	13.07±0.26 ^{bc}	13.13±0.18 ^{ab}	13.19±0.16 ^a	13.41±0.24 ^a	0.001
	Week 42-43	12.60±0.29 ^b	13.10±0.21 ^a	13.16±0.12 ^a	13.22±0.12 ^a	13.50±0.20 ^a	<0.001
	Week 44-45	12.72±0.06 ^c	13.13±0.18 ^b	13.29±0.19 ^{ab}	13.35±0.12 ^{ab}	13.66±0.26 ^a	<0.001
	Week 46-47	12.63±0.34 ^c	13.16±0.12 ^b	13.35±0.16 ^{ab}	13.44±0.13 ^{ab}	13.72±0.06 ^a	<0.001

Values are mean ± standard deviation. Superscript letters (a, b or c) in the same row indicates significant difference among treatments (P<0.05). T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5 and 2.0 g *Spirulina*/hen/day, respectively.

randomly selected from each treatment over a span of 10 weeks to evaluate various egg quality parameters. These parameters, such as yolk weight, albumin weight, shell weight, albumin index, yolk index, shape index, shell thickness, Haugh units, and yolk color core, were measured according to the methodology outlined by (Kirubakaran *et al.*, 2011).

EVALUATION OF EGG YOLK LIPID PEROXIDATION

Egg yolk lipid peroxidation was assessed via the thiobarbituric acid reactive substance (TBARS) assay to quantify malondialdehyde (MDA) production, following the method described by Siu and Draper (1978). Eight eggs per treatment (2 eggs per replicate) were collected at the beginning (38th week of age), middle (42nd week of age), and end of the trial (47th week of age) for evaluating egg yolk lipid peroxidation. All eggs were stored at room temperature for 10 days before analysis.

Prior to MDA measurement, egg yolks were homogenized with distilled water (at a ratio of 1:5). The sample was then subjected to lipid peroxidation by adding 0.24 mM ferric sulphate and incubated for 15 minutes at 38°C. Subsequently, 0.5 ml of the egg yolk sample was mixed with 1 ml of TBA reagent (containing 0.375% thiobarbituric acid (w/v), 15% trichloroacetic acid (w/v), and 0.25N hydrochloric acid). The mixture was boiled for 20 minutes, cooled, and then centrifuged to separate the supernatant. The absorbance of the supernatant was measured at 535 nm using a microplate spectrophotometer. MDA levels were determined by comparing the absorbance of each sample at 535 nm with a standard curve of MDA. The results were expressed in nmol MDA per 0.5 g of egg yolk.

EVALUATION OF SERUM CHEMICAL PARAMETERS

At the conclusion of the experiment (at 47 weeks of age), blood samples were collected from the wing vein of 12 hens per treatment group. The serum was separated and transported to the laboratory under refrigerated conditions to maintain its integrity. Each sample was then analysed individually for serum protein, calcium, low-density lipoprotein (LDL), high-density lipoprotein (HDL), cholesterol, and uric acid using an automated Cobas biochemical system (C702).

STATISTICAL ANALYSIS

A one-way ANOVA was conducted using IBM SPSS Statistics, version 22. To compare means across treatments, the Tukey method was employed. Statistical significance was determined at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

SPIRULINA ALGAE SUPPLEMENTATION AND LAYING PERFORMANCE

Table 2 illustrates the impact of supplementing *Spirulina* algae in drinking water on the productive performance of laying hens. In general, supplementing algae in drinking water for laying hens did not affect laying performance indicators such as body weight, feed intake, egg number, egg weight, egg production, egg mass, and FCR during 10 weeks ($P > 0.05$).

SPIRULINA ALGAE SUPPLEMENTATION AND EGG QUALITY

Table 3 displays the impact of supplementing *Spirulina* algae in drinking water on egg quality over a period of 10 weeks. The analysis revealed no notable variances in egg quality parameters, including yolk weight, albumin weight, shell weight, yolk index, albumin index, egg shape index, shell thickness, and Haugh units ($P > 0.05$). Nevertheless, the addition of algae to the drinking water of laying hens did influence the color of egg yolks. The experiments showed that supplementation of algae at higher concentrations resulted in darker yellow egg yolks, and vice versa. Specifically, the experiments with higher scores of yolk color significance were statistically meaningful compared to the control experiments ($P < 0.05$).

EGG YOLK LIPID PEROXIDATION

Table 4 illustrates the levels of malondialdehyde (MDA) in egg yolks following a 10-day storage period at room temperature. The findings suggest that during the initial week of the experiment, there were no significant variations in antioxidant capacity across the treatments. However, in the middle and final phases of the experiment, the concentration of MDA decreased compared to the initial stage, and notable differences were observed between the groups supplemented with algae in their drinking water

Table 4: Effects of *Spirulina* algae supplementation in drinking water on the concentration of malondialdehyde (MDA) of laying hens egg yolk (nmol MDA/0.5 g egg yolk).

Periods	Treatments					P-value
	T1	T2	T3	T4	T5	
Beginning of experiment (Week 38)	34.03±1.35	34.25±5.09	34.03±2.59	34.08±1.57	34.16±2.94	1
Middle of experiment (Week 42)	33.14±2.00 ^a	27.01±1.42 ^b	25.37±0.77 ^{bc}	23.92±0.70 ^{bc}	22.48±1.20 ^c	<0.001
End of experiment (Week 47)	34.03±1.54 ^a	26.14±0.34 ^b	24.70±0.38 ^{bc}	23.36±0.39 ^{cd}	22.03±1.26 ^d	<0.001

Values are mean ± standard deviation. Superscript letters (a, b, c or d) in the same row indicates significant difference among treatments ($P < 0.05$). T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5 and 2.0 g *Spirulina*/hen/day, respectively.

and those without supplementation ($P < 0.05$). Particularly, in the algae-supplemented treatments, higher algae concentrations corresponded to lower MDA values, whereas in the control treatment, the MDA concentration was highest, and there were statistically significant differences when comparing with the algae-supplemented treatments in the middle and final stages of the experiment ($P < 0.05$).

SERUM CHEMICAL PARAMETERS

Table 5 summarises the effects of *Spirulina* algae supplementation in drinking water on serum biochemical parameters of laying hens. The results indicate that supplementing *Spirulina* in laying hens' drinking water affects the HDL and LDL indicators but does not affect protein, uric acid, triglyceride, cholesterol, and calcium indicators in the serum of laying hens ($P > 0.05$). In treatments with higher concentrations of *Spirulina* supplementation, the HDL levels in the blood are higher, while the LDL levels are lower. In terms of the HDL parameter, the group receiving the highest *Spirulina* supplementation (2g per bird per day) exhibited the highest HDL levels, demonstrating statistically significant variances compared to the control group ($P < 0.05$). Conversely, for the LDL indicator, the control group demonstrated the highest LDL levels, with statistically significant differences observed compared to all other treatments ($P < 0.05$).

Spirulina algae has been explored as a natural feed supplement in aquaculture and poultry farming. Recent studies, such as El-Ghany (2020), have reported on the algae's effects on poultry's immune response, carcass traits, microbial resistance, and productivity. One particular study focused on the effects of adding *Spirulina* algae to the laying hens' drinking water, examining its impact on their laying performance, serum chemical parameters, egg quality, and lipid peroxidation in egg yolks. The findings indicate that while algae supplementation did not significantly affect reproductive productivity or egg quality parameters, it did enhance egg yolk color, oxidative stability of the yolk, and HDL and LDL levels in the hens' serum.

LAYING PERFORMANCE

The study's data reveal that adding *Spirulina* to the laying hens' drinking water did not influence egg productivity metrics, including egg count, laying rate, feed consumption, and feed conversion efficiency. These results align with those of earlier research. Zahroojian *et al.* (2013) found that adding 1.5%, 2.0%, and 2.5% *Spirulina platensis* to the Hy-line White hens' diet did not impact the rate of laying or the weight of the egg. Similarly, Nasroallah Moradikor *et al.*, (2015) reported that varying concentrations of *Chlorella* algae (0, 100, 200, 300, and 400 ppm) in the laying hens' drinking water over a 9-week period did not have a significant impact on egg mass or egg production. Dogan *et al.* (2016) observed that incorporating 0.5%, 1%, and 2% *Spirulina* into the diet of laying quails did not influence feed consumption or egg production. Additionally, Abouelezz (2017) found that adding 1% *Spirulina* powder to the feed and 0.25% to the drinking water of Japanese quails did not significantly impact the egg weight, egg laying rate or daily egg mass. The comparable reproductive performance observed across the treatments in this study may be attributed to the insufficient quantity of *Spirulina* added to the drinking water, which was likely too small to impact feed intake or nutrient absorption in the chickens. As a result, the varying concentrations of *Spirulina* supplementation did not significantly affect the reproductive performance metrics in the laying hens.

EGG QUALITY

In this study, adding *Spirulina* algae to the drinking water did not result in significant differences in the percentages of eggshell, albumen, yolk, egg shape index, or Haugh unit. These results are consistent with previous research. Zahroojian *et al.* (2013) showed that adding *Spirulina* to the diet did not impact the physical qualities of eggs. Dogan *et al.* (2016) also found that supplementing 0.5%, 1.0%, and 2.0% *Spirulina* in the quail diet for 8 weeks did not significantly improve egg quality parameters. Likewise, Selim *et al.* (2018) observed that including 0.1%, 0.2%, and 0.3% *Spirulina* in the diet had no impact on egg physical

Table 5: Effects of *Spirulina* algae supplementation in drinking water on serum biochemical parameters of laying hens.

Parameters	Treatments					P-value
	T1	T2	T3	T4	T5	
Protein (g/L)	55.00±1.30	56.80±1.59	57.55±1.98	59.25±6.97	59.48±3.23	0.430
Uric acid (µmol/L)	290.87±32.91	314.76±40.30	320.36±31.57	321.02±40.80	333.14±66.35	0.738
Triglyceride (mmol/L)	17.13±0.80	14.48±2.63	14.17±1.67	14.00±4.19	13.39±3.79	0.439
Cholesterol (mmol/L)	3.25±0.60	2.92±0.48	2.67±0.24	2.78±0.80	2.60±0.34	0.465
HDL (mmol/L)	0.21±0.07 ^b	0.19±0.05 ^b	0.25±0.08 ^{ab}	0.55±0.30 ^{ab}	0.66±0.29 ^a	0.009
LDL (mmol/L)	2.90±0.12 ^a	2.55±0.11 ^b	2.37±0.08 ^b	2.15±0.08 ^c	1.84±0.09 ^d	<0.001
Calcium (mmol/L)	6.41±0.55	6.43±0.29	6.53±0.86	6.50±0.42	6.44±0.42	0.998

Values are mean ± standard deviation. Superscript letters (a, b, c or d) in the same row indicates significant difference among treatments ($P < 0.05$). T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5 and 2.0 g *Spirulina*/hen/day, respectively.

characteristics by the conclusion of the fourth week of the study. Moreover, [Omri et al. \(2019\)](#) found no significant differences in egg yolk weight, height, diameter, and index in hens fed a basal diet with 1.5%, and 2.5% *Spirulina* over a period of 6 weeks. However, [Dogan et al. \(2016\)](#) reported that adding 2% *Spirulina* to the diet of laying quails improved the shell weight, egg yolk index, and shell thickness. Similarly, [Selim et al. \(2018\)](#) showed that the shell thicknesses of hens fed with *Spirulina* were thicker than that of hens fed with the control diet. The variations in outcomes between our study and earlier research could stem from diverse factors such as differing levels of supplementation, varied administration techniques, various *Spirulina* strains, care practices, housing setups, feed compositions, and the breed or age of the laying hens. Notably, the mineral composition in different *Spirulina* varieties might influence eggshell thickness, although there is currently no scientific proof elucidating the underlying mechanism of this impact ([Selim et al., 2018](#)).

EGG YOLK COLOR

Consumer preferences for eggs now consider yolk color in addition to cholesterol and fatty acid profile content in egg yolk ([Englmaierová et al., 2013](#)). The preferred level of yolk pigmentation differs both between and within countries, with shades ranging from golden to yellow generally being more appealing ([Baiaon et al., 1999](#)). The color of egg yolk can be improved by adding carotenoids to the hens' diet, as laying hens are unable to produce these pigments themselves and must obtain them through their food. Carotenoids are generated by fungi, certain bacteria, plants, and algae ([El-Ghany, 2020](#)). This study investigated the enhancement of egg yolk color through the dietary supplementation of *Spirulina* algae in the laying hens' drinking water. The results indicated a dose-dependent enhancement in egg yolk color score, likely caused by the deposition of carotenoids and xanthophylls in the yolk, resulting in more vivid pigmentation. The outcomes closely resemble the conclusions drawn by earlier investigators ([Zahroojian et al., 2013](#); [Park et al., 2015](#); [Selim et al., 2018](#); [Omri et al., 2019](#); [Curabay et al., 2021](#)).

SERUM CHEMICAL PARAMETERS

The findings suggest that adding *Spirulina* to the drinking water of laying hens impacts HDL and LDL levels in their serum but has no effect on protein, uric acid, cholesterol, and calcium indicators. The study shows that supplementing algae in the drinking water increases HDL levels and decreases LDL levels in egg-laying hens' serum, which is consistent with the findings of ([Dogan et al., 2016](#)). Similarly, ([Gao et al., 2020](#)) noted a rise in HDL and a decline in LDL with the inclusion of *Haematococcus pluvialis* algae in the laying hens' diet. The rise in HDL levels and the reduction in LDL levels in the serum of laying hens

may be attributed to the high concentration of essential fatty acids in spirulina, particularly gamma-linolenic acid, which is known to improve lipid metabolism by enhancing the activity of lecithin-cholesterol acyltransferase (LCAT). LCAT converts free cholesterol into cholesteryl ester, which is then incorporated into HDL, thereby increasing HDL levels ([Moor et al., 2017](#)). *Spirulina* is also rich in antioxidants such as phycocyanin, beta-carotene, and vitamin E, which can reduce oxidative stress and inflammation, potentially improving lipid profiles ([Hynstova et al., 2018](#)). By decreasing oxidative stress, *Spirulina* can lower LDL oxidation, a key factor in the development of atherosclerosis, thus reducing LDL levels. Additionally, compounds in *Spirulina*, like phycocyanin, can inhibit HMG-CoA reductase, an enzyme crucial for cholesterol biosynthesis. Inhibiting this enzyme can lower overall cholesterol levels, including LDL ([Sheu et al., 2013](#)). Chronic inflammation is linked to higher LDL and lower HDL levels, and the anti-inflammatory properties of *Spirulina* can help reduce systemic inflammation, positively affecting the lipid profile by lowering LDL and increasing HDL ([Abu-Taweel et al., 2019](#)). Furthermore, *Spirulina* is high in dietary fiber, which can bind to cholesterol in the digestive system, reducing its absorption and lowering LDL cholesterol levels. *Spirulina* can also positively influence the gut microbiota, promoting the growth of beneficial bacteria that enhance lipid metabolism ([van den Driessche et al., 2020](#)). A healthier gut microbiota can increase bile acid excretion, which is derived from cholesterol, thereby reducing overall cholesterol levels.

Furthermore, the study results indicate that protein, uric acid, cholesterol, and calcium levels remain unchanged with *Spirulina* supplementation in the drinking water, aligning with ([Curabay et al., 2021](#)) research. However, this contradicts the findings of ([Dogan et al., 2016](#)), who presented a decrease in plasma total cholesterol level with *Spirulina platensis* supplementation in quail diets. [Park et al. \(2015\)](#) similarly found a reduction in serum cholesterol with the incorporation of marine microalgae in laying hens' diets. Several studies have also noted a significant decrease in egg yolk cholesterol levels with *Spirulina* supplementation in laying hens' and quails' diets ([Dogan et al., 2016](#); [Selim et al., 2018](#)). However, [Zahroojian et al. \(2013\)](#) reported no effect on egg yolk cholesterol concentration with dietary addition of *Spirulina*. Likewise, [Omri et al. \(2019\)](#) found no change in total cholesterol level of egg yolk with *Spirulina* supplementation in hens' diets. The differences in research results compared to previous studies may be due to the use of different sources of algae, varying concentra

EGG YOLK LIPID PEROXIDATION

Moreover, during the egg preservation process, malondialdehyde (MDA) may be produced as a result of lipid

NOVELTY STATEMENT

The novelty of this study lies in the use of *Spirulina* supplementation in the drinking water of laying hens to evaluate the antioxidant capacity of egg yolk as well as to improve egg yolk color and serum lipoproteins. The results of this study have a positive impact on egg quality and hold potential for application in poultry farming.

AUTHOR'S CONTRIBUTIONS

Nguyen Van Vui: Conceived and designed the experiments.

Nguyen Van Vui, Nguyen Thi Kim Quyen, Kim Nang and Nhan Hoai Phong: Performed the experiments.

Nguyen Van Vui and Nguyen Thuy Linh: analysed the data.

Duong Hoang Oanh: Contributed materials.

Nguyen Van Vui, Nguyen Thi Kim Quyen and Nguyen Thuy Linh: Wrote the paper.

All authors reviewed and approved the final manuscript.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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oxidation (Buege and Aust, 1978). The presence of malondialdehyde serves as an indicator of chicken eggs' susceptibility to spoilage, as evidenced in previous research studies (Galobart *et al.*, 2001; Faitarone *et al.*, 2016). The results of this research endorse the notion that adding *Spirulina* algae to the water intake of laying hens leads to decreased MDA levels in egg yolks when compared to the control. These findings align with studies carried out by (Abou-Elkhair *et al.*, 2018; Gao *et al.*, 2020), both of which have observed an inverse relationship between levels of lipid peroxidation and the quality of eggs. Incorporating *Spirulina* algae into the drinking water decreased MDA levels in egg yolks, indicating the efficacy of its antioxidant properties in diminishing lipid peroxidation and the liberation of free radicals. The beneficial effects of *Spirulina* algae on MDA concentrations in egg yolks may be attributed to its primary active components. *Spirulina* harbours both enzymatic and non-enzymatic antioxidants, boasting abundant quantities of superoxide dismutase, glutathione peroxidase, and catalase enzymes, alongside vitamins E, C, K, and various B group vitamins (Asghari *et al.*, 2016). Furthermore, it includes β -carotene, chlorophyll, provitamin A, and phycocyanin (Romy *et al.*, 2003; Hynstova *et al.*, 2018), all of which have exhibited antioxidant properties and the capacity to neutralize free radicals (Gad *et al.*, 2011; Kumar *et al.*, 2022). Moreover, *Spirulina* furnishes 18 amino acids, encompassing all essential ones, along with minerals like calcium, potassium, magnesium, zinc, iron, copper, and selenium, all of which may demonstrate antioxidant characteristics (Khan *et al.*, 2005; Holman and Malau-Adu-li, 2013). Nevertheless, in clinical investigations, the blue pigment biliprotein C-Phycocyanin is regarded as the primary antioxidant within *Spirulina* (Finamore *et al.*, 2017).

CONCLUSIONS AND RECOMMENDATIONS

To sum up, introducing *Spirulina* algae into the drinking water of laying hens at a dosage of 2 g/hen/day proves to be the ideal approach for enhancing egg yolk color, bolstering antioxidant capability, and maintaining serum lipoproteins levels, all while ensuring no adverse impact on the reproductive productivity of hens. The study results emphasized the role of *Spirulina* in enhancing hen health and egg quality, contributing to human nutrition. Further research is recommended to explore the impact of *Spirulina* on the nutritional composition of eggs and the antioxidant capacity of hen serum.

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