Research Article

The Influence of *Spirulina* Algae Supplementation in Drinking Water on Egg Quality and Immune Response to Newcastle Disease in Laying Hens

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Abstract | The quality of chicken eggs is largely influenced by the nutritional composition of the feed and water provided to laying hens. Proper nutrition is essential for maintaining hen health, optimizing egg production, and enhancing the nutritional value of eggs. This study examined the effects of supplementing *Spirulina* algae in the drinking water of laying hens on egg quality and immune responses to the Newcastle disease virus. A completely randomized design was implemented, with five treatment groups receiving different concentrations of *Spirulina*. The immune response to Newcastle disease was evaluated using the hemagglutination inhibition assay, while egg nutritional composition, including lipids, proteins, vitamins, and minerals, was analysed using specialized methods. The results showed that *Spirulina* supplementation in drinking water did not significantly affect the lipid, protein, or the tested mineral content (calcium, potassium, magnesium, and iron) of the eggs. However, hens receiving 1.5 g and 2 g of *Spirulina* per bird per day exhibited a significant improvement in immune response to Newcastle disease ($P<0.05$). Moreover, the inclusion of *Spirulina* in the diet significantly enhanced the vitamin A content of egg yolks, with a clear dose-dependent effect observed. Notably, supplementation at a level of 2 g/day resulted in a 2.6-fold increase in vitamin A concentration compared to the control group ($P<0.05$). In conclusion, adding *Spirulina* to the drinking water of laying hens enhances immune function and increases egg yolk vitamin A levels without altering other nutritional components. These findings highlight the potential of *Spirulina* as a natural dietary supplement in poultry production, offering a practical approach to improving both animal health and egg quality.

Keywords | *Spirulina* algae, Egg quality, Immune response, Laying hens, Vitamin A, Newcastle disease

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INTRODUCTION

Eggs are a staple food providing essential nutrition. With rising demand for quality, farmers focus on enhancing egg composition. Studies show dietary supplements can improve egg quality; for example fish oil boosts Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (Fraeye *et al.*, 2012), plant seed oils increase unsaturated fatty acids (Gładkowski *et al.*, 2011), iodine-rich fungi raise iodine content by 80% (Opaliński *et al.*, 2012), and broccoli stems reduce cholesterol while improving yolk colour (Hu *et al.*, 2011). Thus, egg nutrition varies based on hens diets.

Newcastle disease virus (NDV) is one of the most dangerous pathogens affecting the poultry industry. It primarily infects the respiratory and digestive systems, leading to reduced egg production, significant weight loss, and high mortality in laying hens (OIE, 2024). Consequently, Newcastle disease has been included in vaccination programs for poultry. However, the overuse of vaccines, veterinary drugs, and high stocking densities have negatively impacted chickens' immune systems. As a result, vaccination effectiveness remains low due to poor immune responses or insufficient antibody production, failing to protect chickens against the virus (Fesseha, 2020). Therefore, supplementing laying hens' diets with immune-boosting additives to enhance resistance to Newcastle disease has gained attention from poultry farmers.

Spirulina algae is highly regarded for its exceptional nutritional value, containing a rich composition of 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). Due to its impressive nutrient profile, *Spirulina* has been widely recognized as a valuable food source for both humans and animals (Parikh *et al*., 2001; Rasool *et al*., 2006). In particular, research by Ramadan *et al.* (2008) has demonstrated that the lipid content in *Spirulina* serves as an alternative source for natural antioxidant compounds, which play a crucial role in reducing oxidative stress and protecting cellular structures from damage. Furthermore, studies have indicated that *Spirulina* supplementation can have positive effects on poultry health and productivity. Specifically, *Spirulina* has been shown to reduce physiological stress, enhance antioxidant capacity, and strengthen the immune system in broiler chickens, ultimately leading to improved overall health and performance (Fathi *et al*., 2018; Mirzaie et al., 2018; Khan *et al*., 2020; Kolluri *et al*., 2021). Additionally, research has explored the potential of *Spirulina* in enhancing disease resistance in poultry. For example, supplementing diets with *Spirulina* has been found to improve immune responses to the Newcastle disease virus in both quails (Ibrahim *et al*., 2018) and laying hens (Samia *et al*., 2018; Nia *et al*., 2021). These findings suggest that *Spirulina* supplementation not only improves the general health of poultry but may also play a role in preventing infectious diseases. Therefore, incorporating *Spirulina* into poultry diets holds promise for improving egg quality and strengthening immune responses against viral infections such as Newcastle disease.

Despite these promising benefits, most previous studies have primarily focused on adding *Spirulina* to poultry feed, while little attention has been given to its incorporation into drinking water (Park *et al*., 2015; Dogan *et al*., 2016; Selim *et al*., 2018). Introducing *Spirulina* into drinking water could offer a more practical and cost-effective supplementation strategy, particularly for small-scale poultry farms. This method avoids the potential degradation of nutrients that can occur during the high-temperature pelleting process used in commercial feed production (Kolluri *et al*., 2021; Wang *et al*., 2022). Furthermore, research has demonstrated that adding *Spirulina* to broilers' drinking water enhances nutrient absorption in the small intestine, especially under heat stress conditions. This improvement in nutrient uptake contributes to better antioxidant activity, reduced physiological stress, and overall enhanced health in broiler chickens (Kolluri *et al*., 2021). However, there remains a significant gap in research regarding the effects of *Spirulina* supplementation in the drinking water of laying hens, particularly in terms of its impact on egg quality and immune function. Therefore, the objective of this study is to evaluate the effects of *Spirulina* supplementation in the drinking water of laying hens, with a specific focus on its influence on egg quality and immune response. By addressing this research gap, the study aims to provide new insights into alternative dietary strategies that can enhance poultry health and productivity in a sustainable and practical manner.

MATERIALS AND METHODS

Animals

A total of 120 commercial Isa Brown laying hens, each 38 weeks old and with consistent body weights ranging from 1.84 to 1.92 kg, were used in this study to maintain experimental uniformity. Prior to the study, all hens had been properly vaccinated against common infectious diseases to minimize potential confounding effects. Specifically, the Newcastle disease vaccine (LaSota strain) was re-administered every 5 weeks. All experimental procedures were carefully designed and conducted following ethical guidelines. The study received official approval from the Animal Care and Use Committee of Tra Vinh University, Vietnam, under license number 219/2022/HD-HDKH&DT-DHTV.

Throughout the study, the welfare of the hens was prioritized. Birds were housed in standard commercial layer cages, providing approximately 600 cm^2 of floor space per hen, consistent with accepted poultry welfare guidelines. The housing environment was maintained under optimal bio-climatic conditions, including controlled temperature, ventilation, and lighting, to reduce environmental stress. Special attention was given to minimize handling stress, particularly during procedures such as vaccination and blood sampling. Hens were gently restrained by trained personnel to reduce discomfort and avoid injury. To further support their well-being, all birds were supplemented with vitamin C immediately after handling to help alleviate stress and promote recovery. These comprehensive welfare measures were implemented to ensure that all hens remained healthy and comfortable throughout the experiment, thereby maintaining the integrity of the study and aligning with current standards for ethical animal research.

Experimental design

The experimental design followed a completely randomized layout consisting of five distinct treatment groups, each receiving different levels of *Spirulina* algae supplementation through drinking water. The treatment groups were designated as T1, T2, T3, T4, and T5, corresponding to *Spirulina* supplementation levels of 0, 0.5, 1.0, 1.5, and 2.0 grams of fresh *Spirulina* per hen per day, respectively. To ensure statistical reliability, each treatment was replicated four times, with each replicate containing six hens, resulting in a total of 24 hens per treatment group. The experiment was conducted over a 10-week period, beginning when the hens were 38 weeks old and continuing until they reached 47 weeks of age. This duration allowed for a comprehensive assessment of the effects of *Spirulina* supplementation on egg quality and the immune response against Newcastle disease in laying hens.

Animal management

The hens were housed in battery cages equipped with nipple drinkers and trough feeders. They were exposed to 16 hours of continuous light daily (04:00–20:00). All hens received the same commercial layer feed, with ingredient and nutrient composition detailed in Table 1. Diets were provided ad libitum, allowing hens to consume feed freely according to their needs. Importantly, no probiotics or antibiotics were included in the diets, ensuring that the study focused solely on the effects of the experimental treatments without external influences from growth-promoting additives.

Table 1: Nutritional composition of compound feed for laying hens.

Composition	Concentration
Metabolizable energy (min)	2.850 Kcal/kg
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%

Fresh *Spirulina* algae were obtained from an aquaculture farm at Tra Vinh University, Vietnam. To prepare *Spirulina*-infused drinking water, a manual stirrer was used to maintain constant circulation, ensuring a uniform suspension at room temperature. Treatments were prepared by adding increasing amounts of fresh *Spirulina* algae (0, 0.5, 1.0, 1.5, and 2.0 g per hen per day), corresponding to 0, 12, 24, 36, and 48 g per 4 liters of water, respectively, in 25 liters containers per treatment. Birds had voluntary access to *Spirulina* water from 08:00 to 12:00, after which any remaining algae water was replaced with fresh water, provided ad libitum until the next day. The nutritional composition of *Spirulina* algae is shown in Table 2.

Table 2: Nutritional composition of the experimental *Spirulina*.

Parameters	Concentration
β-caroten (mg/100g)	150±2.00
Protein (µg/100g)	49.2±0.60
Vitamin A (µg/100g)	-
Calcium (mg/kg)	3050±30.00
Potassium (mg/kg)	12500±200.00
Magnesium (mg/kg)	2990±90.00
Iron (mg/kg)	598±8.00
Saturated fatty acid (%)	2.09±0.04
Unsaturated fatty acid (%)	2.77±0.12
Polyunsaturated fatty acid (%)	1.89±0.09
Monounsaturated fatty acid (%)	0.89±0.09
Omega 3 (%)	0.06±0.01
Omega 6 (%)	1.87±0.07
Omega 9 (%)	0.79±0.07

Blood sampling method for evaluating immune response

During the experimental period (from 38 to 47 weeks of age), the hens were vaccinated against Newcastle disease at 38, 43, and 48 weeks of age. Blood samples were collected 2 and 5 weeks after vaccination to assess antibody levels against Newcastle disease. The blood sampling schedule and the number of samples required after Newcastle disease vaccination are summarized in Table 3.

Table 3: Blood sampling schedule and the number of samples required after Newcastle disease vaccination.

Treatments	Number of blood samples					Total number of blood samples
	Week 38	Week 40	Week 43	Week 45	Week 48	
T1	8	8	8	8	8	40
T2	8	8	8	8	8	40
T3	8	8	8	8	8	40
T4	8	8	8	8	8	40
T5	8	8	8	8	8	40

T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5, and 2.0 g of *Spirulina* per bird per day.

Method for determining antibody titer

To evaluate the immune response of the hens, serum samples were analysed for antibody titers using the hemagglutination inhibition (HI) assay. This method was conducted in accordance with the standardized guidelines outlined in TCVN 8400-4:2010, ensuring accuracy and consistency in the assessment of antibody levels against Newcastle disease. The HI assay is widely recognized as a reliable technique for detecting and quantifying specific antibodies that inhibit the agglutination of red blood cells by the Newcastle disease virus. During the assay, serial dilutions of each serum sample were prepared and mixed with a fixed amount of the Newcastle disease virus antigen. After incubation, a suspension of red blood cells was added to the mixture to observe whether hemagglutination was inhibited. The highest dilution at which hemagglutination was completely prevented was recorded as the HI antibody titer. Samples that exhibited an HI antibody titer of ≥4 log^2 were considered to have reached the protective threshold, indicating a sufficient immune response to protect the birds against Newcastle disease infection. This threshold value reflects an adequate level of circulating antibodies capable of neutralizing the virus and reducing the risk of disease outbreak within the flock.

Evaluation of nutrient composition in egg yolk

To assess the nutritional profile of egg yolk, eggs were collected at the end of the experimental period, specifically at 47 weeks of age. The analysis focused on key nutrients, including lipid, protein, vitamins, and minerals, to provide a comprehensive understanding of the egg yolk's composition. The lipid content in the yolk was evaluated using gas chromatography with a flame ionization detector (GC-FID), a highly sensitive technique that allows precise quantification of lipid components. This analysis was conducted following the AOAC standard 996.06, ensuring accuracy and compliance with internationally recognized protocols. The protein content in the egg yolk was determined using the Kjeldahl method, a widely used procedure for assessing total nitrogen content, which is then converted into protein concentration. This method provides reliable results and is commonly employed in food composition studies. To measure the concentrations of β-carotene and vitamin A, high-performance liquid chromatography with ultraviolet detection (HPLC-UV) was utilized. This technique enables the separation and quantification of these essential micronutrients, ensuring precise measurement of their levels in the egg yolk. The analysis followed the AOAC standard 992.06, ensuring standardized and accurate results. The mineral composition of the egg yolk was assessed using inductively coupled plasma mass spectrometry (ICP-MS), a highly advanced technique capable of detecting trace elements with exceptional sensitivity. This method provided detailed insights into the mineral profile of the yolk, including essential elements necessary for human nutrition.

Statistical analysis

The data were analyzed using SPSS software version 22. Before conducting the analysis of variance (ANOVA), the data were tested for normality using the Shapiro-Wilk test. If the data met the assumption of normality, ANOVA followed by Tukey's test was used to compare the mean values of the treatments. Mean values were considered statistically significant when $P \leq 0.05$.

RESULTS

Immune response against Newcastle disease in laying hens

Throughout the 10-week experimental period, laying hens were systematically vaccinated against Newcastle disease at three specific time points: 38, 43, and 48 weeks of age. In order to monitor the immune response of the hens following vaccination and to assess the impact of *Spirulina* supplementation on antibody production, blood samples were collected on five different occasions. These blood samples were obtained at 38, 40, 43, 45, and 48 weeks of age and were subsequently analysed to determine the antibody levels against the Newcastle disease virus. The antibody concentrations measured in hens subjected to different dietary treatments, including those supplemented with *Spirulina*, are systematically presented in Table 4.

Table 4: The effect of *Spirulina* on antibody levels against Newcastle disease.

Periods	Antibody levels ($\log_2$)					P-value
	T1	T2	T3	T4	T5	
Week 38	9.00±0.01	9.00±0.01^{B}	8.88±0.35^{B}	9.00±0.01^{B}	8.88±0.35^{B}	0.565
Week 40	8.63±0.92	8.75±0.46^{B}	9.00±0.01^{B}	9.00±0.53^{B}	9.13±0.83^{B}	0.516
Week 43	8.63±0.92	8.63±0.52^{B}	8.88±0.64^{B}	8.88±0.83^{B}	9.13±0.83^{B}	0.664
Week 45	9.38±0.52^{b}	9.75±0.46^{abA}	9.88±0.35^{abA}	10.00±0.01^{aA}	10.00±0.01^{aA}	0.005
Week 48	9.50±0.53^{b}	9.88±0.35^{abA}	10.00±0.01^{aA}	10.00±0.01^{aA}	10.00±0.01^{aA}	0.005

Mean ± standard deviation. Lowercase superscript letters (a, b, or c) in the same row indicate a significant difference among treatments ($P<0.05$), and uppercase superscript letters (A or B) in the same column indicate a significant difference within treatments over different time periods ($P<0.05$). T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5, and 2.0 g of *Spirulina* per bird per day.

The results of the study indicated that during the initial five weeks of the experiment, there were no statistically significant differences in antibody levels among the different treatment groups (P>0.05). This suggests that, in the early phase of the study, *Spirulina* supplementation did not have a noticeable effect on the hens' immune response compared to the control group. However, in the later stages of the experiment, particularly during the last four weeks, a marked difference in antibody levels was observed. Specifically, at weeks 7 and 10 of the study, the treatment groups that received *Spirulina* in their drinking water exhibited a noticeable increase in antibody concentrations. More importantly, the hens that were supplemented with 1.5 g and 2 g of *Spirulina* per bird per day demonstrated significantly higher antibody levels compared to those in the control group, with statistical significance (P<0.05). This finding strongly suggests that the inclusion of *Spirulina* at these dosages played a crucial role in enhancing the immune response against Newcastle disease. The results indicate that supplementing *Spirulina* at levels between 1.5 g and 2 g per bird per day provided a beneficial boost to the hens' antibody production, particularly between weeks 7 and 10 of the experiment (P=0.005). Furthermore, in the control group, where no *Spirulina* supplementation was provided, antibody levels remained stable and exhibited no significant fluctuations throughout the entire 10-week experimental period. In contrast, in the groups that received *Spirulina* supplementation, antibody concentrations showed a noticeable upward trend from week 7 onward. Additionally, these elevated antibody levels in the *Spirulina*-supplemented groups were significantly higher than those recorded in the earlier weeks of the study (P<0.05). These findings further reinforce the idea that *Spirulina* supplementation has the potential to enhance immune function in laying hens by increasing their antibody response to Newcastle disease, particularly in the later stages of the experiment.

Contents of β-carotene, protein, vitamin A, and minerals in egg yolk

At the final stage of the experiment, eggs collected from each treatment group were utilized to conduct a detailed analysis of the nutritional composition of the egg yolk. This analysis aimed to determine the impact of supplementing *Spirulina* in the drinking water of laying hens on the levels of various nutrients present in the yolk. The comprehensive results obtained from this study are systematically presented in Table 5, providing insights into how *Spirulina* supplementation influenced the nutritional profile of egg yolks. Based on the findings, it was observed that the concentrations of β-carotene, protein, calcium, potassium, magnesium, and iron in the egg yolk did not exhibit any statistically significant differences between the groups that received *Spirulina* supplementation and the control group (P>0.05). This suggests that the inclusion of *Spirulina* in the hens' diet did not notably alter these specific nutrient levels in the egg yolk. However, when examining the vitamin A content, a statistically significant difference was detected among the various treatment groups (P<0.05), indicating that *Spirulina* supplementation had a measurable impact on the vitamin A concentration in the egg yolk. A clear trend was observed regarding the vitamin A levels in the egg yolk, which progressively increased in response to higher amounts of *Spirulina* supplementation. Specifically, in the control group, where no *Spirulina* was added, the vitamin A content in the egg yolk was measured at 173.67±19.76 µg/100g. However, when 1 g of *Spirulina* per bird per day was supplemented, the vitamin A concentration rose significantly to 331.00±18.52 µg/100g. Furthermore, in the group that received the highest level of *Spirulina* supplementation, at 2 g per bird per day, the vitamin A content in the egg yolk reached 448.00±8.19 µg/100g. This substantial increase in vitamin A concentration highlights the potential of *Spirulina* as a dietary supplement for enhancing the nutritional quality of eggs, particularly in terms of their vitamin A content.

Table 5: The effect of *Spirulina* on β-carotene, protein, vitamin A, and mineral composition in egg yolk.

Parameters	Treatments					P-value
	T1	T2	T3	T4	T5	
β-carotene (mg/100g)	0.03 ±0.01	0.03 ±0.01	0.03 ±0.01	0.04 ±0.01	0.04 ±0.01	0.499
Protein (%)	15.30±0.10	15.27±0.06	15.50±0.30	15.53±0.12	15.50±0.10	0.190
Calcium (mg/kg)	1228.33±7.64	1230.00±5.00	1233.33±5.77	1230.00±10.00	1231.67±7.64	0.934
Potassium (mg/kg)	1030.00±10.00	1040.00±10.00	1031.67±10.41	1043.33±11.55	1040.00±10.00	0.480
Magnesium (mg/kg)	104.67±1.53	105.67±2.08	107.33±1.53	104.67±1.53	106.00±1.00	0.278
Iron (mg/kg)	63.47±0.83	63.37±0.76	64.07±0.76	65.27±1.12	65.40±0.62	0.064
Vitamin A (µg/100g)	173.67±19.7^{6}e	276.67±15.28^{d}	331.00±18.5^{2}c	390.00±10.00^{b}	448.00±8.1^{9}a	<0.001

Mean ± standard deviation. Different letters (a, b, c, d, or e) in the same row indicate a statistically significant difference among treatments (P<0.05). T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5, and 2.0 g of *Spirulina* per bird per day.

Table 6: The effect of *Spirulina* on the lipid profile of egg yolk.

Parameters	Treatments					P-value
	T1	T2	T3	T4	T5	
Saturated fatty acid (%)	9.44±0.13	9.43±0.10	9.45±0.12	9.47±0.100	9.46±0.09	0.992
Unsaturated fatty acid (%)	19.44±0.06	19.47±0.21	19.57±0.24	19.56±0.07	19.57±0.15	0.798
Polyunsaturated fatty acid (%)	5.60±0.10	5.57±0.10	5.62±0.02	5.63±0.08	5.65±0.05	0.740
Monounsaturated fatty acid (%)	13.94±0.14	13.97±0.21	13.95±0.13	13.80±0.10	13.92±0.08	0.620
Docosahexaenoic acid (DHA) (%)	0.097±0.002	0.099±0.001	0.095±0.005	0.096±0.004	0.094±0.002	0.365
Omega 3 (%)	0.231±0.011	0.231±0.001	0.229±0.006	0.232±0.010	0.233±0.010	0.990
Omega 6 (%)	7.45±0.11	7.42±0.23	7.46±0.08	7.41±0.04	7.45±0.13	0.992
Omega 9 (%)	12.50±0.10	12.47±0.13	12.63±0.10	12.62±0.13	12.65±0.05	0.184

Mean ± standard deviation. T1: control; T2, T3, T4, and T5: 0.5, 1.0, 1.5, and 2.0 g of *Spirulina* per bird per day.

The composition of lipid in egg yolk

The composition of lipid present in the egg yolk was carefully evaluated at the final stage of the experiment to determine whether the supplementation of *Spirulina* in the drinking water of laying hens had any impact on the lipid profile of the eggs they produced. This analysis aimed to assess key lipid components, including saturated fatty acid, unsaturated fatty acid, polyunsaturated fatty acid, monounsaturated fatty acid, docosahexaenoic acid (DHA), and essential fatty acids such as Omega-3 (alpha-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid), Omega-6 (linoleic acid), and Omega-9 (oleic acid and erucic acid). The detailed results obtained from this assessment are systematically presented in Table 6, providing valuable insights into how different dietary treatments influenced the lipid composition of the egg yolk. Based on the findings of the study, it was observed that the concentrations of saturated fatty acid, unsaturated fatty acid, polyunsaturated fatty acid, monounsaturated fatty acid, DHA, Omega-3, Omega-6, and Omega-9 in the egg yolk remained consistent across all experimental groups. In other words, there were no noticeable variations in the fat composition between the groups that received *Spirulina* supplementation and the control group. Statistical analysis confirmed that the differences in fat content among the treatment groups were not significant ($P>0.05$), indicating that the inclusion of *Spirulina* in the hens' diet did not have a measurable effect on the lipid profile of the egg yolk.

DISCUSSION

Spirulina algae have attracted growing interest as a natural dietary supplement in both aquaculture and poultry farming, due to their exceptional nutritional composition rich in proteins, essential fatty acids, vitamins, and antioxidant compounds. Rather than simply cataloguing its benefits, recent research has begun to explore how *Spirulina*'s bioactive components actively influence animal physiology. In poultry, these effects are particularly relevant to immune modulation, oxidative stress reduction, and productivity enhancement. While previous studies have shown improvements in parameters such as immune response and egg quality, the outcomes often depend on how *Spirulina* is administered and at what dosage. Building on this foundation, our study focused on a more practical application for small-scale farms incorporating *Spirulina* into the drinking water of laying hens. This approach allowed us to assess not only the influence on egg quality but also its potential role in enhancing the birds' immune response to Newcastle disease.

In this study, the supplementation of *Spirulina* in the drinking water of laying hens resulted in a significant improvement in the immune response to Newcastle disease. The enhancement of the immune system in laying hens can be attributed to the rich nutritional composition of *Spirulina*, which contains high levels of protein, essential vitamins, and important minerals. These nutrients play a crucial role in supporting the development, activation, and overall function of immune cells, thereby strengthening the birds' ability to fight infections and maintain overall health. Furthermore, *Spirulina* is a rich source of bioactive compounds such as phycocyanin, β-carotene, and vitamin E - all of which possess strong antioxidant properties. These antioxidants play a vital role in neutralizing harmful free radicals, thereby reducing oxidative stress and preventing cellular damage. By protecting immune cells from oxidative damage, *Spirulina* helps to sustain a more effective and resilient immune system in laying hens, ultimately enhancing their overall health and resistance to disease. Among these compounds, phycocyanin plays a particularly important role in immune enhancement. It has been shown to stimulate the activity of key innate immune cells such as macrophages and natural killer cells, which serve as the first line of defence against invading pathogens. Phycocyanin also promotes the production of cytokines, which are essential signalling molecules for coordinating immune responses (Hirahashi *et al*., 2002). In addition, phycocyanin contributes to adaptive immunity by enhancing lymphocyte proliferation and antibody production, especially following vaccination. This supports a more robust immune response to specific pathogens such as the Newcastle disease virus (Qureshi *et al*., 1996). Moreover, phycocyanin helps regulate immune balance by reducing the production of pro-inflammatory cytokines (e.g., TNF-α, IL-6), thereby preventing excessive inflammation that can negatively impact poultry health and performance (Liu *et al*., 2022). These findings are in agreement with previous research, which has consistently demonstrated the immune-enhancing effects of *Spirulina*. The use of *Spirulina* as a dietary supplement has been widely recognized for its ability to stimulate various immune processes, contributing to overall disease resistance in poultry. Studies have specifically highlighted its effects on monocytes and natural killer cells, both of which play crucial roles in the innate immune system. The administration of *Spirulina* has been shown to enhance the macrophage phagocytic response and increase the activity of natural killer cells in both chickens and humans, thereby improving the overall immune defence mechanisms (Al-Batshan *et al*., 2001; Hirahashi *et al*., 2002). Kaoud (2012) reported that chickens fed diets containing *Spirulina* exhibited increased relative and absolute thymus and bursa weights compared to control groups. Similarly, dietary supplementation with *Spirulina* platensis at levels of 0.7 and 0.9 g/kg in broiler diets significantly increased the weights of immune organs such as the bursa, thymus, and spleen (Fathi *et al*., 2018). Further supporting this, (Qureshi *et al*., 1996) found that all *Spirulina*-supplemented groups with K-strain chicks had elevated total against Sheep Red Blood Cells (SRBC) titers during the secondary response, with the 10,000-ppm group displaying the highest response (6.8 $\log^2$) compared to the control group (5.5 $\log^2$).

In laying Japanese quails, different concentrations of *Spirulina* (1, 3, or 5 g/kg of feed) resulted in significantly higher levels of total antibody against SRBC and IgG titers (Hajati, 2019). Additionally, Khan *et al*. (2020) showed that *Spirulina* supplementation significantly improved growth performance, gut integrity, and immunity in broiler production while also enhancing the antibody titer response to Newcastle disease vaccination. Ibrahim *et al*. (2018) observed that adding *Spirulina* at concentrations of 0.5, 1, and 2 g/L in the drinking water of growing Japanese quail chicks significantly increased serum antibody levels against Newcastle virus and enhanced plasma total protein profiles. Moreover, Golden Montazah laying hens and cocks supplemented with 3 g *Spirulina*/kg diet during the laying period exhibited significantly higher antibody titers against Newcastle virus, Avian Influenza, anti-SRBC, and Interferon-gamma concentrations (Samia *et al*., 2018). Similarly, Nia *et al*. (2021) demonstrated that dietary *Spirulina* supplementation at levels of 0.3%, 0.6%, or 0.9% in Lohmann Selected Leghorn laying hens significantly increased antibody titers following Newcastle vaccination. However, certain immune parameters, such as the heterophil-to-lymphocyte ratio, humoral immunity against SRBC, cell-mediated immunity response to phytohemagglutinin injection, and the relative weight of the bursa and spleen, were not significantly affected by *Spirulina* supplementation.

Other microalgae, such as *Chlorella*, have also been shown to enhance immune responses in laying hens. Nasroallah *et al*. (2015) reported that supplementing *Chlorella* microalgae at 400 ppm in drinking water improved immune responses in laying hens. Yehia *et al*. (2024) highlighted the effectiveness of using 400 mg/kg of *Spirulina* extract as a natural immunostimulant in conjunction with the H9N2 vaccine to achieve optimal protection against H9N2 infection. Furthermore, Abotaleb *et al*. (2020) found that dietary *Spirulina* at a minimum concentration of 1 g/kg was sufficient to optimize immune response, enhance protection against heterologous strains, and reduce viral shedding in SPF chickens. Additionally, Salah *et al*. (2025) proposed that incorporating *Spirulina* platensis into broiler diets could be a novel strategy to mitigate the harmful effects of aflatoxin B1 while enhancing immunity through improved phagocytic activity and phagocytic index. Al-Khalaifah *et al*. (2022) further supported the role of algal supplementation in enhancing both cellular and humoral immune responses in broiler chickens. Collectively, these findings highlight the potential of *Spirulina* as a valuable dietary supplement for improving immune function and disease resistance in poultry production.

The assessment of the nutritional composition of egg yolks demonstrated that the inclusion of *Spirulina* in the diet of laying hens did not lead to significant improvements in the protein content, β-carotene levels, or mineral composition of the egg yolk. However, a notable effect was observed on the vitamin A content, which increased progressively with higher levels of *Spirulina* supplementation across the experimental groups. This suggests that while *Spirulina* does not enhance all nutritional aspects of the egg yolk, it plays a crucial role in influencing the levels of certain essential nutrients, particularly vitamin A. The observed increase in vitamin A concentration can be attributed to the naturally high β-carotene content found in *Spirulina*. As laying hens consume *Spirulina*, the β-carotene undergoes metabolic conversion into vitamin A, which is subsequently stored in the egg yolk. This process highlights the ability of *Spirulina* to act as a natural source of provitamin A, contributing to enhanced vitamin A deposition in eggs. The presence of β-carotene, a powerful precursor to vitamin A, plays a fundamental role in this conversion, supporting the bioavailability and accumulation of this vital nutrient in the yolk. These findings align with the research conducted by Rey *et al.* (2021), which demonstrated that the dietary inclusion of *Spirulina* in laying hens feed significantly increased the retinol content in egg yolks. Retinol, a biologically active form of vitamin A, is essential for various physiological functions, including vision, immune response, and cellular growth. The increase in vitamin A concentration observed in the present study can likely be attributed to the conversion of β-carotene derived from *Spirulina*. Furthermore, previous studies have consistently reported similar outcomes, showing that diets enriched with β-carotene or direct sources of vitamin A contribute to elevated levels of vitamin A in eggs (Jiang *et al.*, 1994; Surai *et al.*, 1998). These findings reinforce the idea that dietary modifications, particularly those involving natural carotenoid-rich sources like *Spirulina*, can be an effective strategy for enhancing the nutritional profile of eggs. Consequently, the use of *Spirulina* as a feed additive may offer potential benefits in improving the vitamin A content of eggs, which could be of significant nutritional value to consumers.

The results of this study indicated that the concentrations of saturated fatty acid, unsaturated fatty acid, DHA, Omega-3, Omega-6, and Omega-9 in egg yolks remained consistent across all experimental groups. There were no statistically significant differences observed between the groups supplemented with *Spirulina* and the control group. This suggests that *Spirulina* supplementation did not alter the overall fatty acid composition of the egg yolk. These findings align with those reported by Rey *et al.* (2021), who concluded that the short-term inclusion of *Spirulina platensis* in the diet of laying hens had no noticeable impact on the fatty acid profile of egg yolks. However, their study also highlighted that different chicken breeds may influence variations in fatty acid levels, fat-soluble vitamin concentrations, and the structural composition of fatty acids in the yolk. This implies that factors beyond dietary supplementation, such as genetic variations among poultry breeds, may contribute to differences in yolk composition. Contrary to these findings, research by Boiago *et al.* (2019) demonstrated that the dietary inclusion of *Spirulina platensis* in quail feed led to a reduction in saturated and polyunsaturated fatty acids while simultaneously increasing the levels of monounsaturated fatty acids in egg yolks. This suggests that *Spirulina* may have species-specific effects, impacting fatty acid metabolism differently in quails compared to laying hens. Similarly, a study by Park *et al.* (2015) investigated the effects of marine microalgae *Schizochytrium* in the diets of laying hens and reported a significant enhancement in DHA content within egg yolks. This indicates that while certain algal species, such as *Schizochytrium*, may influence the DHA concentration in eggs, the effects of *Spirulina platensis* on fatty acid composition may be less pronounced or dependent on additional factors such as diet formulation and feeding duration. In addition to fatty acid composition, several studies have explored the effects of *Spirulina* supplementation on egg yolk cholesterol levels. Dogan *et al.* (2016) and Selim *et al.* (2018) reported that incorporating *Spirulina* into the diets of both laying hens and quails resulted in a significant reduction in yolk cholesterol levels. This finding suggests that *Spirulina* may possess cholesterol-lowering properties, potentially benefiting egg consumers concerned with dietary cholesterol intake. However, conflicting results have been reported by Zahroojian *et al.* (2013), who found that *Spirulina* supplementation did not influence yolk cholesterol levels. Similarly, Omri *et al.* (2019) observed no significant changes in total cholesterol content in the egg yolks of laying hens following *Spirulina* supplementation. These inconsistencies across studies suggest that the effects of *Spirulina* on cholesterol metabolism in poultry may be influenced by multiple factors, including the duration of supplementation, the concentration of *Spirulina* used, and the overall nutritional composition of the diet. The variations in findings between this study and previous research could be attributed to several factors, including differences in poultry breeds, the specific strain and composition of *Spirulina* used, the dosage and duration of supplementation, and variations in feeding strategies. Additionally, environmental conditions, management practices, and interactions with other dietary components may also play a role in determining the extent to which *Spirulina* influences egg yolk composition. Further research is needed to clarify these discrepancies and establish standardized guidelines for optimizing the use of *Spirulina* in poultry nutrition.

CONCLUSIONS

Supplementing *Spirulina* in the drinking water of laying hens at a dosage of 1.5–2 g/bird/day contributed to improving the immune response against the Newcastle disease virus and increased the vitamin A content in egg yolks. The findings of this study highlight the beneficial role of *Spirulina* in enhancing poultry health and egg quality while also contributing to improved nutrition for consumers. Further research is recommended to better understand the effects of *Spirulina* on poultry nutrition. Future studies should include longer trial periods to assess long-term impacts on poultry health and productivity. Additionally, research should explore different poultry breeds to determine if the observed effects vary across genetic lines. Other factors such as varying *Spirulina* doses, the form of administration (e.g., feed vs. drinking water), and the influence of environmental conditions should also be considered to establish more comprehensive guidelines for the optimal use of *Spirulina* in poultry diets.

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NOVELTY STATEMENT

This study is the first to investigate the effects of supplementing *Spirulina* algae in the drinking water of laying hens on egg quality and immune response to Newcastle disease. While earlier studies have primarily examined *Spirulina* supplementation through feed, this study demonstrates that delivering *Spirulina* via drinking water enhances immune function and significantly increases vitamin A content in egg yolks without affecting other nutritional components. The findings highlight a novel and practical approach to improving poultry health and egg quality, particularly in small-scale farming systems where feed-based supplementation may be less feasible.

AUTHOR'S CONSTRIBUTION

NVV: Conceived and designed the experiments.
NVV, NTKQ, and DHO: Performed the experiments.
NVV and NTKQ: Analysed the data.
DHO: Contributed materials.
NVV, NTKQ, and DHO: Wrote the paper.
All authors reviewed and approved the final manuscript.

Generative AI and AI-assisted technology statement

The authors state that ChatGPT was employed to enhance the English grammar of the manuscript. The entire content was carefully reviewed, revised, and validated by the authors to guarantee both accuracy and originality.

Conflict of interest

The authors have declared no conflict of interest.

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