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Intermittent supplementation of *Spirulina platensis*: effects on post-peak laying hen performance, protein digestibility, physiological responses, and egg quality

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ABSTRACT

This study evaluated the effects of different frequencies of *Spirulina platensis* (*S. platensis*) supplementation on blood parameters, protein digestibility, fecal characteristics, egg quality, and production performance in laying hens. A total of 224 ISA Brown hens (55 weeks old, BW ±1907 g) were assigned to four groups: T0 (control, basal feed), T1 (0.5% S. platensis daily), T2 (0.5% every other day), and T3 (0.5% twice a week). The trial lasted for eight weeks, with weekly measurements of feed intake, egg production, and egg quality. Blood was collected at the end of the experiment to assess hematological profiles, while fecal samples were analyzed for protein digestibility and nitrogen excretion. Results showed that T3 and T4 significantly increased red blood cell count, hemoglobin concentration, hematocrit value, and leukocyte count compared to the control ($P<0.05$). Compared to T0, nitrogen retention and protein digestibility coefficients (%) in T1, T2, and T3 were significantly different ($P<0.05$). Excretory nitrogen levels in the treatment groups (T1, T2, and T3) were substantially lower than those in T0 ($P<0.05$). Compared to T0, fecal ammonia levels in the treatment groups (T1, T2, and T3) were lower. The water content in T3 was significantly higher than that in T2 ($P<0.05$). Haugh Unit values in T1 and T2 were significantly higher than those in T0 and T3 ($P<0.05$). HDP at weeks 6 and 12 in T1, T2, and T3 were significantly higher than those in T0 ($P<0.05$). In conclusion, *S. platensis* supplementation enhanced hematological status, nutrient utilization, fecal characteristics, egg quality, and production performance. Twice-weekly supplementation (T3) was as effective as the more frequent regimens, suggesting a practical and cost-efficient strategy for laying hens.

Keywords: Egg quality, Laying hens, Laying performance, Protein digestion, Spirulina platensis.

INTRODUCTION

Poultry farming is essential to fulfill the worldwide demand for animal protein, with laying hens being the primary source of eggs (Laca *et al.*, 2021). The production efficiency of laying hen is affected by several factors, including nutrition, environmental conditions, and management practices (Gautron *et al.,* 2022). After the peak production period, laying hens undergo a gradual decrease in egg production, necessitating nutritional strategies to maintain egg quality and to prevent this decline in egg production (Gautron *et al.,* 2021).

S. platensis is a blue-green microalga that has garnered interest in poultry nutrition as a pro-

spective feed supplement due to its high protein content, essential fatty acids, vitamins, and bioactive substances (Usman *et al.,* 2024). The potential of *S. platensis* to boost immunity, promote intestinal health, and increase the overall poultry performance has been extensively researched (El-Shall *et al.,* 2023; Abbas *et al.,* 2024; Al-Otaibi *et al.,* 2022; Hassan *et al.,* 2022; Omri *et al.,* 2019). Nevertheless, research on its efficacy and optimal feeding regimen is still in progress, particularly in the context of post-peak laying hens production. The intermittent administration of *S. platensis* in poultry diets offers a cost-effective alternative to daily administration while still harnessing its nutritional and functional benefits. Previous studies have reported improvements in the growth performance, immune response, and feed efficiency of broilers and layers supplemented with *S. platensis* (Tufan and Kutlu, 2021; Khadanga *et al.,* 2023; Khalilnila *et al.,* 2023). However, no study has explored the effects of different feeding frequencies on the production performance and physiological responses of laying hens.

S. platensis contains a high amount of essential amino acids, which contribute greatly to protein synthesis in poultry (Bashir *et al.,* 2016). In addition, the Fe content of *S. platensis* has the potential to activate various enzymes involved in protein metabolism (Yiannikourides and Latunde-Dada, 2019). Another study showed that the use of *S. platensis* at a concentration of 0.3% improved egg quality and protein digestibility and reduced ammonia excretion in Arab laying hens during the final phase of production (Wahyuni *et al.,* 2023). Similarly, the reduction in serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) levels suggests that *S. platensis* can reverse liver damage in laying hens (Hasna *et al.*, 2024). In this respect, phycocyanin in *S. platensis* has demonstrated a hepatoprotective function to stabilize liver cell membranes and inhibit liver necrosis (Osman *et al.*, 2019; Hasna *et al.*, 2024). In addition to its use in poultry, *S. platensis* is widely used as a health supplement for humans. This gives *S. platensis* relatively high economic value and may become a financial hardship for farmers. In addition to its beneficial properties, several studies have demonstrated that excessive use of *S. platensis* can have negative effects on poultry (Alaqil and Abbas, 2023). Iwasa *et al.,* (2002) claimed that because *S. platensis* is known to possess hepatotoxins, using them at a certain level can be harmful to the liver. Roy-Lachapelle *et al.* (2017) reported that *S. platensis* contains cyanotoxin, which can interfere with liver function. Indeed, Shanmugapriya *et al.* (2015) found that broiler chickens treated with 1.5% *S. platensis* had lower ultimate body weights than those treated with 0.5% and 1% *S. platensis*. Thus, the dosage of *S. platensis* must be considered to ensure that it is not harmful and strains the organs of laying hens. Overuse of *S. platensis* may also result in metabolic problems and affect liver function (Sugiharto *et al.*, 2018). Taking all these into considerations, *S. platensis* will be administered intermittently to reduce costs and prevent toxicity from overuse while also preserving productivity or slowing the rate of production decline.

Egg quality is a key determinant of consumer preference and market value, and parameters such as shell strength, albumen quality, and yolk color are crucial indicators (Ulbad and Andre, 2023). Additionally, blood profiles, including erythrocyte and thrombocyte counts, reflect the overall health and physiological status of laying hens. Nutritional interventions, including the inclusion of *S. platensis*, may influence these parameters, thereby enhancing productivity and poultry welfare (Abbas *et al.,* 2022; Al-Otaibi *et al.,* 2022). The aim of this study was to investigate how the physiological state of laying hens, protein digestibility, egg quality, and egg-laying performance were affected by intermittent administration of *S. platensis* during post-peak production.

MATERIALS AND METHODS

Animals and Experimental Diets

A total of 224 Isa Brown laying hens (53 weeks old; body weight [BW] ± 1907 g) were used for the experiment. The study employed a completely randomized design with four treatments and seven replicates. The treatments included a control group receiving basal feed (T0)

Table 1. Composition and Nutrient Content of the Diet

Feed ingredients	Composition (%)
Maize	35.00
Corn glutten meal	5.00
Distillers dried grains with solubles	5.00
Sorghum	5.00
Soybean meal	8.00
Rice bran	20.00
Pollard	4.00
Meat bone meal	9.00
Crude palm oil	0.10
Limestone	6.90
Monocalcium phosphate	1.50
Premix*	0.50
Chemical Composition	
Metabolizable energy (Kcal/kg)**	2815
Crude protein	15.84
Crude fibre	3.38
Crude fat	2.89
Water	11.03
Ash	14.45
Calcium	3.31
Phosphorus	0.56

*Premix Composition (per 10 kg): vitamin A 12.000.000 IU, vitamin D_3 2.000.000 IU, vitamin E 8.000 IU, vitamin K_3 2.000 mg, vitamin B_1 2.000 mg, vitamin B_2 5.000 mg, vitamin B_6 500 mg, vitamin B_{12} 12.000 µg, vitamin C 25.000 mg, calcium-D-pantothenate 6.000 mg, niacin 40.000 mg, cholin cloride 10.000 mg, methionine 30.000 mg, lysine 30.000 mg, Mn 120.000 mg, Fe 20.00 mg, iodine 200 mg, Zn 100.000 mg, Co 200 mg, Cu 4.000 mg and santoquin (antioxidant) 10.000 mg.
**ME was calculated using the Bolton formula (1967) as cited by Sugiharto *et al.* (2018): 40.81 × (0.87 (crude protein + 2.25 crude fat + nitrogen-free extract) + 2.5)

and three other experimental groups receiving 0.5% *S. platensis* at different frequencies, that is, daily supplementation (T1), every two days (T2), and twice a week (T3).

The rearing period was carried out for 12 weeks, starting from 55 to 67 weeks (adaptation to experimental feed was conducted from 53 to 55 weeks of age). Feed was manually administered once a day at 11 a.m. Following feeding, the feed was inspected and leveled in the feeder five times at 3, 7, 11, 3, and 7 a.m. to ensure there was no clumping. The feed composition is listed in Table 1. Drinking water was provided *ad libitum by* a nipple drinker. Data on the physical quality of the eggs, blood profile, and protein digestibility were collected at the end of the experiment. The performance parameters were measured daily. The blood profile was determined at 66 weeks of age, whereas egg quality and protein digestibility were measured at 67 weeks.

Data Collection and Laboratory Analysis
Physiological status

Blood was collected from the brachial vein of randomly selected hens from each replicate. To measure the erythrocyte profile, 1 ml of blood was collected and placed in a vacutainer containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The samples were then placed in a cooler to prevent damage. While the remaining 2 ml of blood was used to assess liver function parameters, the blood was placed in a non-EDTA vacutainer tube and allowed to clot. The blood sample was then centrifuged for 15 min at 3000 rpm to extract blood serum. Blood serum

was collected for SGPT and SGOT tests.

Egg quality

For egg quality measurements, eggs were collected when the laying hens reached 67 weeks of age. To evaluate the physical quality of the eggs, one egg from each experimental unit was collected for three consecutive days prior to the end of the experiment. The albumen and yolk indices were calculated by measuring the heights and diameters, respectively, using a micrometer. After cleaning and drying, the eggshells were measured with a micrometer at three distinct locations: the narrow end, equator, and broad end. The shell thickness (mm) was calculated as the average of three measurements. The Haugh Unit (HU), an indicator of egg freshness and quality, was calculated using the following formula: HU=100 Ã–log(albumen height+7.57−1.7×egg weight$^{0.37}$).

Yolk color was assessed by visually matching the color of the egg yolk with the DSM Yolk Color Fan, which provides a standardized scale from 1 (light) to 15 (dark yellow/orange). To evaluate yolk cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels, yolks were separated, homogenized, and stored at − 20°C until analysis. Each yolk sample weighed approximately 0.5 grams, and was then diluted in isotonic saline solution and centrifuged for 10 min at 3000 rpm. After collecting the supernatant, enzymatic colorimetric techniques were used for the analysis. LDL and HDL levels were determined after the precipitation of other lipoprotein fractions. Absorbance was measured at 500 nm using a spectrophotometer and values were calculated against a standard curve and expressed in mg/dL.

Protein digestibility

Protein digestibility measurements were carried out when the chickens were 67 weeks old and the measurements were carried out *in vivo* using the total collection method. The hens selected for digestibility measurements were 58 hens, consisting of two chickens for each replication and two additional chickens to measure endogenous N. The total collection was carried out for three days. One day before the total collection of feces, the cage was prepared by installing a container to collect excreta. On the first day of the total collection, the chickens were fed according to the treatment feed mixed with 0.5% Fe_2O_3. At the end of the third day of total collection, the chickens were given treatment feed without an indicators, and the excreta was still collected until the color of the excreta returned to

Table 2. Complete Blood Counts and Biochemical Parameters in Laying Hens

Parameters	T0	T1	T2	T3	SEM	P value
Erythrocyte (g/dL)	2.11^{a}	1.81^{b}	1.99^{ab}	2.18^{a}	0.050	0.039
Hemoglobin (10^6/µL)	7.96	8.10	7.61	8.16	0.174	0.711
Hematocrit (%)	36.03	33.66	34.49	37.27	0.814	0.422
MCV (fL)	173.80	173.36	173.64	171.44	0.777	0.709
MCH (pg)	38.33	41.70	38.26	37.39	0.658	0.091
MCHC (g/dL)	21.59	23.59	21.59	21.34	0.343	0.062
Leucocytes (10^3/µL)	56.61	59.67	53.60	56.31	2.970	0.931
Lymphocytes (10^3/µL)	7.45	7.56	7.17	7.38	0.206	0.935
Heterophyl (10^3/µL)	1.11	1.19	1.05	1.11	0.030	0.490
H/L ration	0.15	0.16	0.15	0.15	0.004	0.816
Thrombocyte (10^3/µL)	42.57^{a}	35.86^{ab}	30.43^{b}	36.14^{ab}	1.441	0.020
SGOT (U/L)	212.39	225.00	211.61	228.97	6.075	0.689
SGPT (U/L)	22.5^{a}	24.74^{a}	13.72^{b}	17.85^{ab}	1.458	0.021

^{a,b}Means marked with superscript letters in the same row are significantly different (P<0.05). T0: control (basal feed); T1: basal feed + 0.5% *S. platensis* daily supplementation; T2: basal feed + 0.5% *S. platensis* every two days; T3: basal feed + 0.5% *S. platensis* twice a week; SEM: standard error mean.

Table 3. Protein Digestibility, Nitrogen and Ammonia Parameters of Laying Hens

Parameters	T0	T1	T2	T3	SEM	P value
Protein digestibility coefficient (%)	48.95[b]	57.24[a]	57.82[a]	58.25[a]	0.939	<0.001
Nitrogen retention	9.12[b]	10.68[a]	10.77[a]	10.84[a]	0.175	<0.001
Excreta nitrogen	10.83[a]	9.30[b]	9.18[b]	9.08[b]	0.175	<0.001
Fecal ammonia	12.38[a]	8.75[b]	8.79[b]	8.74[b]	0.365	<0.001

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05). T0: control (basal feed); T1: basal feed + 0.5% *S. platensis* daily supplementation; T2: basal feed + 0.5% *S. platensis* every two days; T3: basal feed + 0.5% *S. platensis* twice a week; SEM: standard error mean.

Table 4. Water Content, pH and Fecal Temperature of Laying Hens

Parameters	T0	T1	T2	T3	SEM	P value
Water content (%)	10.95[ab]	9.80[ab]	9.35[b]	11.67[a]	0.342	0.050
Fecal pH	7.57	7.43	7.86	7.00	0.215	0.582
Fecal temperature (°C)	29.61	30.55	29.40	29.30	0.194	0.081

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05). T0: control (basal feed); T1: basal feed + 0.5% *S. platensis* daily supplementation; T2: basal feed + 0.5% *S. platensis* every two days; T3: basal feed + 0.5% *S. platensis* twice a week; SEM: standard error mean.

its original state, and the time when the excreta came out was recorded.

The total nitrogen was calculated using the Kjeldahl method. N retention was measured by calculating the difference between the consumption of N entering the hen's body and the amount of N excreted after reduction by the amount of endogenous N (Tillman, 1998). Excreta ammonia levels were measured when the chickens were 67 weeks old.

Production performance

The eggs in each experimental unit were weighed using a digital scale. The number and weight of eggs were calculated and weighed daily. Data on feed consumption, feed conversion ratio (FCR), and hen day production (HDP) were obtained based on daily data taken during the research period. The remaining feed rations were weighed on a daily basis. The feed consumption was calculated by subtracting the feed from the remaining feed.

Statistical Analysis

The data were analyzed using SPSS software, version 16. One-way ANOVA was used to evaluate the collected data at a significance level of 5%. Duncan's multiple range test was used to assess differences between treatment groups. Non-parametric data (yolk color score) were analyzed using Kruskall-Wallis test.

RESULTS AND DISCUSION

Complete Blood Counts and Biochemical Parameters

The present study aimed to evaluate the impact of different frequencies of 0.5% *S. platensis* supplementation on the performance, blood parameters, protein digestibility, nitrogen and ammonia excretion, fecal characteristics, and egg quality of laying hens. These findings provide valuable insights into the potential benefits and limitations of *S. platensis* in poultry nutrition. The complete blood counts and biochemical parameters are shown in Table 2. Intermittent administration of *S. platensis* had a significant effect (P<0.05) on the erythrocytes of laying hens. Erythrocyte levels were higher (P<0.05) at T0 and T3 than at T1, but the difference was not significant (P>0.05) compared to T2. Thrombocyte levels were higher (P<0.05) in T0 than in T2, but not significantly different from those in T1 and T3. SGPT levels were lower (P<0.05) in T2 than in T0 and T1, but not significantly different (P>0.05) from those in T3. Other blood profiles were unaffected (P>0.05) by *S. platensis* administration, including hemoglo-

bin, hematocrit, MCV, MCH, MCHC, leukocytes, lymphocytes, heterophyl, H/L ratio, and SGOT.

Significant effects of *S. platensis* supplementation on erythrocyte counts and platelet levels were observed. Erythrocyte levels were significantly lower at T1 than at T0, T2, and T3, indicating that daily supplementation might influence red blood cell production. Because of the accumulation of harmful chemicals from *S. platensis*, the T1 chicken group that was exposed to *S. platensis* daily had the lowest erythrocyte values. In contrast, significantly higher erythrocyte counts were observed in the T0 and T3 groups, suggesting that less frequent *S. platensis* supplementation may improve oxygen-carrying ability.

The addition of *S. platensis* every day and every 2 days is suspected to have a toxic effect on laying hens. This is illustrated in the results of the study where the T1 that was given *S. platensis* every day had the lowest erythrocyte value, which is very likely caused by the accumulation of toxic substances of *S. platensis*. Roy-Lachapelle *et al.* (2017) reported, *S. platensis* is known to contain cyanotoxins, which are harmful chemicals that might lower the physiological status of animals. In this study, the intermittent administration of *S. platensis* can minimize the body's absorption of poisonous chemicals found in *S. platensis*, hence mitigating its harmful effects and optimizing its therapeutic effects.

Thrombocyte counts were highest in the T0 group and differed significantly from T2, indicating that the frequency of *S. platensis* supplementation may influence thrombocyte levels. This condition is likely related to ammonia exposure in the cage environment. As shown in Table 3, the hens that did not receive *S. platensis* supplementation (T0) had the highest ammonia concentration at 12.38 ppm. The study was conducted in an open housing system, where feces accumulated beneath the cages, allowing ammonia to evaporate and be inhaled by the birds. Prolonged inhalation of ammonia can irritate the respiratory tract, impair respiratory efficiency, and increase susceptibility to pathogen invasion (e.g., viruses and bacteria), thereby elevating the risk of respiratory diseases such as coccidiosis, aspergillosis, or salpingitis (Wang *et al.*, 2022).

In contrast, the groups supplemented with *S. platensis* had lower thrombocyte counts, which may be attributed to its role in enhancing immune function. *S. platensis* is rich in bioactive compounds such as phycocyanin, vitamin E, and carotenoids, which exhibit strong antioxidant and anti-inflammatory properties that can mitigate infection and inflammation (Mahmoud *et al.*, 2024). By improving immune resilience, *S. platensis* helps reduce oxidative stress and accelerate recovery from infections, processes that are closely associated with the regulation of thrombocyte counts (Maouia *et al.*, 2020).

Serum glutamic pyruvic transaminase (SGPT) levels illustrate damage to the liver condition of laying hens. SGPT levels were significantly lower in T2 and T3, indicating the potential hepatoprotective effects of *S. platensis*. This is consistent with previous studies that have reported the protective role of *S. platensis* against liver damage owing to its antioxidant content (Mohamed *et al.,* 2021; Fu *et al.,* 2018). *S. platensis* contains antioxidants, vitamins, β-carotene, ficocyanin and superoxide dismutase (SOD) which can maintain liver cell health from free radicals (Mahmoud *et al.*, 2024). The results in this study are also in line with research conducted by Tufarelli *et al.* (2021) which states that the addition of *S. platensis* by 1% and 2% can reduce SGPT levels in laying hens. Interesting finding was observed in this present study, in which SGPT levels in the T1 group (daily administration of *S. platensis*) showed the highest value among the treatment groups. This may confirm that the daily administration of *S. platensis* may be exaggerating, and that toxic substances in *S. platensis* in the form of cyanotoxins may cause damage to the liver. Indeed, supplementation of additives with high active compounds may increase the workload, and thereby jeopardize of the liver. Moreover, Roy-Lachapelle *et al.* (2017) stated that *S. platensis* is known to contain a toxic compound called cyanotoxin that can damage the liver.

The results presented in Table 3 demonstrate that the dietary treatments had significant effects ($P<0.001$) on all measured parameters, including protein digestibility, nitrogen retention,

excreta nitrogen, and fecal ammonia content. Protein digestibility coefficients and nitrogen retention were higher (P<0.001) in T1, T2, and T3 than in T0, but were not significantly different among T1, T2, and T3. Excreta nitrogen and fecal ammonia levels were lower (P<0.001) in T1, T2, and T3 than in T0, with no significant differences among the treatment groups.

Protein digestibility coefficients were significantly higher in T1, T2, and T3 compared to T0, with T3 showing the highest digestibility. This is consistent with other studies showing that *S. platensis* improves gut health, either directly affecting intestinal mucosa or by modifying the gut microbiota (Spinola *et al.*, 2022; Spinola *et al.,* 2025; Begum *et al.,* 2024). This is also in line with the research of Wahyuni *et al.* (2023) demonstrating that the addition of *S. platensis* at a dose of 0.3% can increase protein digestibility and nitrogen retention and reduce the amount of excreta nitrogen. Nitrogen retention was also significantly improved in T1, T2, and T3, while excreta nitrogen and fecal ammonia levels were significantly reduced, suggesting that *S. platensis* supplementation can improve nitrogen metabolism and reduce environment pollution. In addition, other previous studies reporting enhanced protein digestibility and nitrogen retention in poultry fed *S. platensis* diets (Lestingi *et al.,* 2024; Costa *et al.,* 2024; Prates, 2025). This indicates that the bioactive compounds of *S. platensis*, including peptides and enzymes, may enhance protein utilization in laying hens by increasing digestive enzyme activity and nutrient absorption, thereby improving protein digestibility and nitrogen retention, as evidenced by significantly higher nitrogen retention in all supplemented groups (T1, T2, and T3) along with reduced nitrogen excretion and fecal ammonia levels, which reflects more efficient nitrogen utilization and reduced nitrogen waste output, ultimately contributing to improved environmental sustainability (Anas *et al.,* 2020). These findings also suggest that *S. platensis* may enhance gut microbiota balance, which in turn may optimize nitrogen metabolism (Bondar *et al.,* 2023; Spinola *et al.,* 2024; Alaqil and Abbas, 2023; Wahyuni *et al.,* 2023).

Table 5. Egg Quality and Biochemical Traits of Laying Hens

Parameters	T0	T1	T2	T3	SEM	P value
Albumin index	0.07	0.08	0.08	0.08	0.002	0.179
Yolk index	0.43	0.42	0.45	0.42	0.005	0.196
Haugh unit	91.41[b]	95.73[a]	95.86[a]	95.17[b]	1.164	0.037
Yolk color	11.81	12.14	12.19	11.81	0.124	0.582
Shell thickness (mm)	0.58	0.66	0.64	0.63	0.014	0.296
Cholesterol (mg/dL)	885.37	872.80	855.15	841.26	11.110	0.687
Egg LDL (mg/dL)	531.46	576.72	637.95	675.75	8.566	0.902
Egg HDL (mg/dL)	645.43	642.02	733.06	715.41	0.613	0.448

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05). T0: control (basal feed); T1: basal feed + 0.5% *S. platensis* daily supplementation; T2: basal feed + 0.5% *S. platensis* every two days; T3: basal feed + 0.5% *S. platensis* twice a week; SEM: standard error mean.

Table 6. Feed Consumption, Hen Day Production, FCR and Egg Weight of Laying Hens

Parameters	T0	T1	T2	T3	SEM	P Value
Feed consumption (g/hens)	117.56	117.84	117.56	117.43	0.090	0.430
HDP 55 weeks (%)	97.45	96.68	96.68	97.45	0.321	0.723
HDP 60 weeks (%)	91.84[b]	95.15[a]	95.41[a]	95.92[a]	0.395	<0.001
HDP 67 weeks (%)	88.01[b]	93.11[a]	93.88[a]	94.39[a]	0.649	<0.001
FCR	2.04	2.01	1.94	2.00	0.023	0.540
Egg weight (g)	60.13	60.62	58.75	60.61	0.562	0.519

[a,b]Means marked with superscript letters in the same row are significantly different (P<0.05). T0: control (basal feed); T1: basal feed + 0.5% *S. platensis* daily supplementation; T2: basal feed + 0.5% *S. platensis* every two days; T3: basal feed + 0.5% *S. platensis* twice a week; SEM: standard error mean.

The decrease in fecal ammonia levels in this study was caused by an increase in protein digestibility and nitrogen retention in chickens due to the aministration of *S. platensis*. Low protein digestibility will lead to increased nitrogen levels in excreta (Sung *et al.*, 2022). The bioactive substances contained in *S. platensis*, including phycocyanin, peptides, and polysaccharides, may enhance the activity of digestive enzymes or alter the gut environment to promote effective nutrient absorption (Zhou *et al.,* 2022; Begum *et al.,* 2024). The decrease in fecal ammonia, in particular, raises the possibility of a change in gut microbial populations toward a more beneficial and balanced microbiota, away from ureolytic and proteolytic bacteria that produce ammonia. This theory is in line with earlier research that demonstrated the prebiotic and antibacterial properties of *S. platensis*, which can inhibit the growth of harmful bacteria and encourage the growth of beneficial species, such as *Lactobacillus* and *Bifidobacterium* (Zhou *et al.,* 2023; Kaoud, 2015). Additionally, the intermittent supplementation approach in T2 and T3 would enable microbial adaptability or resetting on a periodic basis, preserving microbial diversity and avoiding overstimulation or dysbiosis that could arise from a continuous dosage. These findings demonstrate that *S. platensis* is not only a nutritional supplement but also a possible modulator of intestinal ecology, which could lead to improved nitrogen consumption and lower environmental impact in the production of poultry (Spinola *et al.,* 2024; Alaqil and Abbas, 2023).

Water content, pH, and fecal temperature data are provided in Table 4. Water content of excreta was higher (P = 0.050) in T3 than in T2, whereas T0 and T1 showed intermediate values that were not significantly different (P > 0.05) between the two groups. No significant differences (P > 0.05) were observed between treatments for fecal pH and fecal temperature. Fecal water content in this study tended to decrease from group T0 to group T2, then increased in group T3. The decrease in fecal water content in groups T1 and T2 was caused by an increase in the value of protein digestibility as illustrated in Table 3. This is in accordance with research conducted by Wahyuni *et al.* (2023) which states that giving *S. platensis* as much as 0.3% can increase the value of protein digestibility while reducing excreta water content.

The increase in digestibility value illustrates that more feed nutrients are absorbed in the small intestine (Ravindran and Abdollahi, 2021). This reduces the amount of indigestible nutrients entering the cecum and colon, which means less water is required to remove unabsorbed nutrients and fiber. As a result, the water content in excreta is reduced (McDonald *et al.,* 2010). Similarly, fecal temperature was statistically constant across all groups, suggesting that *S. platensis* supplementation had little effect on thermoregulatory and intrinsic gut metabolic activities (Seidavi *et al.,* 2022; Seidavi *et al.,* 2023). However, small temperature changes could also be a sign of subtle fluctuations in the strength of microbial fermentation or in the amount of heat produced by metabolism.

The effects of dietary treatments on egg quality and selected biochemical parameters in laying hens were compiled in Table 5. The Haugh Unit was higher (P<0.05) in T1 and T2 than in T0 and T3, whereas no significant differences (P>0.05) were observed among treatments for albumin index, yolk index, yolk color, shell thickness, cholesterol, egg LDL, and egg HDL. Most egg quality parameters, including the albumin index, yolk index, yolk color, shell thickness, cholesterol, LDL, and HDL concentrations, were not significantly affected by *S. platensis* supplementation. However, Haugh Unit values, indicative of albumen quality, were significantly higher in T1 and T2 than in T0 and T3. This suggests that more frequent *S. platensis* supplementation can enhance internal egg quality, potentially because of the high protein and antioxidant contents of *S. platensis* (Panaite *et al.,* 2023).

The Haugh Unit is an indicator of albumen quality and freshness, and this increase may be associated with improved nutrient absorption and antioxidant status in hens (Salahuddin *et al.,* 2024). This suggests that regular *S. platensis* supplementation could enhance the internal egg quality. Lipid parameters (cholesterol, HDL, and LDL), yolk color, and shell thickness did not

differ significantly between the groups. Although the pigment content and lipid-lowering qualities of *S. platensis* are well known, the dosage and timeframe used in this study may not have been sufficient to produce noticeable changes in these characteristics. This suggests that *S. platensis* may have a dose-dependent effect on the biochemical makeup of eggs or that long-term supplementation may be necessary (Gadzama *et al.,* 2025). Deng and Chow (2009) stated that *S. platensis* contains alpha-linoleic acid that can bind cholesterol metabolites in bile and thus prevent their accumulation.

The effects of the dietary treatments on the performance indicators of laying hens were listed in Table 6. Hen-day production (HDP) at weeks 60 and 67 was higher ($P < 0.001$) in T1, T2, and T3 than in T0, while feed consumption, HDP at week 0, FCR, and egg weight showed no significant differences ($P > 0.05$) among treatments. Supplementation with *S. platensis* did not significantly affect feed consumption, feed conversion ratio (FCR), or egg weight across the treatment groups. These results are not in line with research conducted by Hasna *et al.* (2024) which explained that the addition of *S. platensis* by 1% can reduce feed consumption in Arabian laying hens because the color of the feed becomes darker due to the addition of *S. platensis* to the feed. Feed color is one of the factors that can affect poultry feed consumption because poultry are very sensitive to feed color in consuming rations (Radjulani *et al.*, 2022; Elahi *et al.*, 2020).

The decrease in feed consumption in this study did not occur probably because the dose of *S. platensis* given in this study was relatively low at 0.5% so that changes in feed color did not occur. Egg weight in this study was also not affected by the treatment given. These results are in line with research conducted by Salahuddin *et al.,* (2024) which stated that the addition of *S. platensis* by 1% in layer feed did not affect the egg weight of laying hens. However, hen day production (HDP) at weeks 60 and 67 was significantly higher at T1, T2, and T3 than at T0 ($P<0.05$), although there were no changes in feed intake or egg weight. This result is especially significant because it shows that laying performance improved over time, regardless of variations in feed intake or egg mass.

The higher HDP in the *S. platensis*-treated groups indicates that the chickens were able to sustain or improve their reproductive output without changing the properties of their eggs or increasing their nutritional intake. This improvement could be attributed to the rich nutritional profile and bioactive substances found in *S. platensis*, including vital amino acids, high-quality proteins, vitamins (including B_{12} and E), minerals (such as iron and calcium), antioxidants (such as β-carotene and phycocyanin), and polyunsaturated fatty acids (Seidavi *et al.,* 2023; Salahuddin *et al.,* 2024). These bioactive compounds are known to promote cellular metabolism, oxidative balance, and immunological functions, all of which are essential for long-term egg production (Al-Otaibi *et al.,* 2022; Wahyuni *et al.,* 2023). Antioxidants, for example, can improve ovarian function and follicular growth by reducing oxidative stress in the reproductive organs. Likewise, essential amino acids required for the production of yolk proteins and reproductive hormones can be found in the protein-rich matrix of *S. platensis* (Curabay *et al.,* 2021; Rey *et al.,* 2021). In addition to showing that regular, continuous dosage is not necessary to generate productive benefits, improvement in HDP, especially under intermittent supplementing regimens (T2 and T3), offers potential practical and financial benefits for poultry producers.

The long-term advantage of *S. platensis* in preserving reproductive performance was further supported by the consistent output improvement observed at weeks 60 and 67. Because of improved nutrition utilization, immunological modulation, and hormone regulation, the temporal stability of these benefits suggests a cumulative or supporting influence on the physiological systems of laying hens (Al-Otaibi *et al.,* 2022; Salahuddin *et al.,* 2024). Furthermore, it can be revealed that *S. platensis* functions more as a functional supplement than a feed stimulant, increasing productivity through internal physiological support rather than through increased feed intake, as no negative impacts were noted in terms of FCR or feed consumption (El-Shall *et al.,* 2023). In addition, feeding laying hens with *S. platensis* can slow down the rate of decline in productivity of laying hens after the peak

production phase. The decrease in the rate of productivity in laying hens after peak production is caused by degradation in organ function, resulting in a decrease in physiological status and metabolism in chickens (Kumalasari *et al.*, 2023). *S. platensis* contains complete amino acid compounds, vitamins and active compounds such as ficocyanins that can help improve physiological status in chickens so that it can slow down the rate of decline in productivity of laying hens after peak production (Mahmoud *et al.*, 2024). This is in accordance with research conducted by Wahyuni *et al.* (2023) that the group of laying hens added with *S. platensis* at a dose of 0.3% had better productivity compared to control group.

Based on the findings of this study, intermittent supplementation of *Spirulina platensis* twice weekly significantly enhances physiological health and productivity in laying hens. Erythrocyte and thrombocyte counts, liver function, protein digestibility, and HDP all showed notable improvements, especially in the T1, T2, and T3 groups. An increase in Haugh Unit scores suggested improved internal egg quality, even though the majority of egg quality metrics stayed constant. Crucially, these advantages were attained without changing egg weight, feed conversion ratio, or feed intake, suggesting that *S. platensis* can enhance performance without causing nutritional imbalance or extra feed expenses. A major highlight of this study is the environmental benefit of *S. platensis* supplementation. The notable decrease in nitrogen excretion and fecal ammonia levels shows more efficient nitrogen metabolism and less pollution. Lower ammonia emissions improve air quality in poultry housing, which supports environmental health, worker safety, and animal welfare. These findings show that *S. platensis* can be a sustainable option to reduce the environmental impact of egg production while also serving as a natural performance enhancer.

CONCLUSION

In conclusion, intermittent supplementation of *Spirulina platensis* twice weekly is strongly recommended as an effective natural strategy to improve physiological health, egg quality, and productivity in laying hens. This approach not only supports environmental sustainability but also offers economic efficiency by enhancing performance without increasing feed intake or production cost.

CONFLICT OF INTEREST

The authors declare that there are no conflict of interests.

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AUTHORS CONTRIBUTION

All the authors have the equal contribution in the writing of this manuscript.

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