



■Author(s)

Abideen ZU¹  <https://orcid.org/0000-0002-9751-6382>
Farooq M¹  <https://orcid.org/0000-0001-5932-1982>
Ruby T¹  <https://orcid.org/0000-0003-2033-1627>
Khan AA¹  <https://orcid.org/0000-0002-3453-5818>

¹ Bahauddin Zakariya University, Institute of Zoology, Multan, Punjab, Pakistan.

² Ghazi University, Department of Zoology, Dera Ghazi Khan, Punjab, Pakistan.

■Mail Address

Corresponding author e-mail address

Zain Ul Abideen

Bahauddin Zakariya University, Institute of Zoology, Multan, Punjab, 60000, Pakistan.

Phone: +92 331 738 0678

Email: zaenulabidin71@gmail.com

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Physiology and Growth Performance of *Gallus gallus domesticus* Supplemented with Algal Extracts

ABSTRACT

The present study aimed to determine how dietary *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris* extract (CVE) supplementation affects growth performance, hematological parameters, serum biochemistry, and blood hormones in *Gallus gallus domesticus*. The experiment included 70 chickens grouped across seven treatment groups consisting of a control group along with Spirulina doses from 0.25g/L to 5g/L, and CVE doses from 0.25g/L to 5g/L. The seven-week trial aimed to monitor how dietary interventions affect physiology in *Gallus domesticus*. Study findings showed that the supplementation treatment showed substantial impacts on both body development and biological functioning. Group 3 (2.5g/L CVE) had the greatest body weight gain of 35.50 ± 0.58 g ($p < 0.001$), and Group 1 (2.5g/L SP) had the lowest cholesterol measurement of 125.00 ± 0.58 mg/dL ($p < 0.001$). Group 6 (5g/L SP + 5g/L CVE) achieved the highest platelet count at $161.00 \pm 0.58 \times 10^3/\mu\text{L}$ ($p < 0.001$) along with a moderate cholesterol increase to 155.00 ± 0.58 mg/dL ($p < 0.001$). The blood triglyceride concentrations decreased similarly in Group 1 (127.00 ± 0.58 mg/dL) compared with Group 6 (122.00 ± 0.58 mg/dL). Group 3 (2.5g/L CVE) demonstrated elevated hemoglobin together with RBC counts, whereas Group 6 (5g/L SP + 5g/L CVE) showed optimal platelet counts. Thyroid hormone levels remained unaffected. The combined use of Spirulina and CVE at the same level of 2.5g/L effectively reduced cholesterol together with triglycerides and enhanced both hemoglobin production and RBC counts. The combined treatment using 5g/L SP with 5g/L CVE delivered the most comprehensive results by improving body weight, platelet count, and biochemical assessments. The combination of 5g/L SP and 5g/L CVE improved body weight, platelet count, and biochemical markers. Future research should determine the most effective dosages and long-term benefits regarding poultry productivity improvement.

INTRODUCTION

Poultry production has become a cornerstone of the global livestock economy in the past decades, significantly contributing to food security and economic development (Acheampong, 2024). The poultry sector in Pakistan is playing an important role in the livelihoods of rural and urban households and is an accessible source of income and means to fight protein malnutrition (Afzal *et al.*, 2024). Despite its rapid annual growth rate of 20–25%, driven predominantly by Punjab's contribution of 61%, challenges such as nutritional deficiencies, rising feed costs, and environmental stressors persist (Qiu *et al.*, 2024). Two-thirds of Pakistan's population is suffering from protein malnutrition, so the



improvement of poultry production to reduce protein deficiencies is an issue that remains paramount, which is true in various other developing countries where poultry products represent a good source of economically-accessible protein source (Erdaw, 2023, Gulati & Juneja, 2023).

Gallus gallus domesticus (Linnaeus, 1758) belongs to the Phasianidae family and Galliformes order (Haider *et al.*, 2022), and is a major source of quality protein through its eggs and meat (Abed *et al.*, 2021). Due to its ecological adaptability, efficient growth, and suitability to small-scale farming systems, especially in rural and peri-urban areas, this terrestrial species is strongly valued (Shaikh *et al.*, 2024). Poultry in South Asia, including Pakistan, also plays a big role in meeting the nutritional needs of a region that is home to around 22% of the world's population (Ghose, 2024). Traditional management practices, for instance scavenging systems based on household waste and foraging, are often unable to meet increasing poultry products as well as quality feed demand, hence leading to the need for innovative practices that help promote productivity sustainability (Fatima *et al.*, 2015).

Recently, microalgae have been presented as suitable alternatives to conventional protein sources in bird feeds, due to their rapid growth rate, easy cultivation, and rich bio-nutritional profiles (Shields & Lupatsch, 2012). Of these, *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris* are highlighted their great nutritional and bioactive potential. Spirulina, a filamentous cyanobacterium, is rich in protein (60–70% dry weight), essential amino acids (leucine, isoleucine), vitamins (B12, β -carotene), minerals (iron, calcium), and bioactive compounds like phycocyanin and γ -linolenic acid (Kulshreshtha *et al.*, 2008, Lestingi *et al.*, 2024). Its antioxidant, immunomodulatory, and hepatoprotective properties have been demonstrated in poultry, improving nutrient utilization, disease resistance, and meat quality (El-Shall *et al.*, 2023, Spinola *et al.*, 2024). However, the specific impact of these algal supplements on the physiology and productivity of broiler chickens remains underexplored.

Similarly, *Chlorella vulgaris*, a unicellular green microalga, is known for its protein-dense composition and high chlorophyll content, as well as its variety of micronutrients. Dietary inclusion of 5–10% *C. vulgaris* could, according to studies, effectively replace traditional protein sources such as fishmeal without having an effect on growth or feed efficiency in broilers. Being a functional feed additive, its ability to

increase intestinal microbial diversity, promote immune responses, and facilitate tissue repair is also valuable (Gadzama, 2024). Additionally, *Chlorella vulgaris*-derived products have shown success at improving growth rates and egg quality when added to poultry feeds (Panaite *et al.*, 2023).

Microalgae have bioactive compounds that contain polyunsaturated fatty acids (PUFAs), antioxidants and prebiotics which make poultry healthier and promote their growth, while also improving product quality (Kalia & Lei, 2022). The consumption of microalgae promotes nutritional substance intake, and strengthens immune response and gut health, leading to better feed efficiency and lower mortality rates (Hassan *et al.*, 2022). Food enriched with microalgae gains increased nutritional value due to their omega-3 fatty acids and carotenoids contents, which improve the appeal of eggs and meat (Bruneel *et al.*, 2013). Due to their sustainability in farming and their capacity to substitute traditional animal feed components, these products are positioned as environmentally friendly solution for poultry feeding (Kusmayadi *et al.*, 2021). Multiple studies have confirmed that microalgae effectively enhance poultry growth performance and boost both immunity and feed efficiency. A study conducted by Hanafy (2022) verified that feeding broiler chickens *Spirulina platensis* at 0.07% led to better weight development and improved feed conversion ratio (FCR) due to improved nutrient processing capabilities and digestive system health. *Spirulina platensis* supplements (0.1–0.2%) are beneficial for body weight gain, FCR improvement, and intestinal villi height enhancement, all of which enhance nutrient absorption. A research by Hassan *et al.* (2022) demonstrated that *Spirulina platensis* supplementation elevated globulin and lymphocyte percentage levels, thus demonstrating immune-stimulating activity. Several studies have demonstrated that microalgae are effective at improving poultry growth rates, feed efficiency, and immune system functioning.

Hormonal regulation plays a crucial role in growth performance, metabolism, and overall physiological health in poultry. Thyroid hormones (T3, T4, TSH) are key regulators of metabolic rate and energy balance. Previous research suggests that certain bioactive compounds in Spirulina and *Chlorella vulgaris* may influence endocrine function, particularly thyroid activity and lipid metabolism (Abbas *et al.*, 2022). Thyroid hormones regulate energy metabolism, growth, and feed efficiency in poultry, and algal bioactive compounds (e.g., iodine, polyphenols) may



modulate their activity (El-Shallet *et al.*, 2023, Lestingiet *et al.*, 2024).

Researchers have previously examined the individual effects of *Spirulina platensis* and *Chlorella vulgaris* on poultry growth and immunity, but they have not extensively explored their combined impact. The majority of the studies carried out on the subject are on isolated impacts without an understanding of synergistic impacts on physiological and hemato-biochemistry. However, little is known about how simultaneous supplementation will augment comprehensive physiological parameters in *G. domesticus*. The primary objective of this study was to evaluate the effects of *Spirulina platensis* and *Chlorella vulgaris* supplementation on the growth performance of *Gallus gallus domesticus* and to assess their impact on hematological parameters, serum biochemistry, and hormonal regulation. We hypothesized that dietary supplementation with these algal extracts would improve growth performance, enhance immune function, and optimize physiological health in poultry, thereby supporting sustainable poultry production systems and nutritional security in resource-constrained regions. This study aims to address the underexplored synergistic effects of combined algal supplementation on comprehensive physiological parameters in poultry.

MATERIALS AND METHODS

Experimental Design

This study was conducted to examine the physiological effects of the algal extracts of *Arthrospira platensis* (Spirulina) and *Chlorella vulgaris* (CVE) in *Gallus gallus domesticus*. This study was conducted in an poultry house at the Botanical Garden BZU, Multan, for seven weeks. A total of 70 chicks (350g) were purchased from the local market (Poultry Hatchery Multan). All birds were kept in an animal care facility with free access to water and feed. All birds were provided with a commercially available standard broiler diet formulated to meet the nutritional requirements of *Gallus gallus domesticus*, following NRC (1994) guidelines. The diet contained approximately 21-23% crude protein, 3,000-3,200 kcal/kg metabolizable energy, 1.0% calcium, 0.45% available phosphorus, 0.9% lysine, and 0.45% methionine. Feed was provided *ad libitum* throughout the experimental period. At the end of the seven-week experimental period, all birds were humanely euthanized using cervical dislocation, following institutional ethical guidelines.

Blood collection and organ sampling were performed immediately after euthanasia. This procedure ensured minimal stress and was conducted under the supervision of trained personnel in accordance with the animal welfare protocols approved by the Institutional Research Ethical Committee (Letter No. Zool 868/24, Bahauddin Zakariya University, Multan, Pakistan).

We randomly assigned the birds to 7 experimental groups after they had acclimatized for two weeks with free access to water and standard poultry feed. There were 10 chicks in each group. Six groups received varying doses of Spirulina (SP) and *Chlorella vulgaris* extract (CVE), while the control group received no supplementation. These six treatment groups were designated as Group 1 (2.5 g/L Spirulina), Group 2 (5 g/L Spirulina), Group 3 (2.5 g/L Chlorella), Group 4 (5 g/L Chlorella), Group 5 (2.5 g/L Spirulina + 2.5 g/L Chlorella), and Group 6 (5 g/L Spirulina + 5 g/L Chlorella). Extracts were fed daily through drinking water. The doses of 2.5 g/L and 5 g/L for Spirulina (SP) and *Chlorella vulgaris* extract (CVE) were selected based on previous poultry studies demonstrating efficacy at these concentrations without adverse effects. Park *et al.* (2018) used 1–5 g/kg SP, while Abdelfatah *et al.* (2024) reported optimal growth at 2.5 g/L CVE). Additionally, preliminary trials conducted in our laboratory (unpublished) also supported this decision, as doses <1 g/L showed negligible effects, while >7.5 g/L reduced palatability. Therefore, these concentrations were chosen as optimal for evaluating the synergistic effects of Spirulina and Chlorella supplementation in broiler diets.

Birds were observed daily for general health, behavior and feed intake. To monitor growth and physiological changes, body weight and body temperature were recorded weekly using a digital thermometer and an electronic weighing scale. Temperature and humidity were controlled and documented throughout this study.

Measurement of Growth Parameters

In this study, the growth performance of *G. domesticus* was evaluated by measuring several key parameters, including initial body weight, final body weight, body weight gain, percentage weight gain (PWG%), total feed intake, and feed conversion ratio (FCR). Body weight was recorded weekly using an electronic weighing scale (precision ± 0.01 g), and body temperature was measured using a digital thermometer (precision $\pm 0.1^\circ\text{C}$). Feed intake was monitored daily



by measuring the amount of feed provided and the remaining feed after 24 hours. The feed conversion ratio (FCR) was calculated as the ratio of total feed consumed to body weight gain. The percentage weight gain was calculated as the difference between final and initial body weight, expressed as a percentage of the initial weight. These parameters were analyzed across seven treatment groups, including a control group and groups supplemented with varying doses of *Spirulina platensis* (0.25–5 g/L) and *Chlorella vulgaris* (0.25–5 g/L), both individually and in combination.

Organ Weight Measurements

Organ weights were measured to evaluate the effects of SP and CVE supplementation on the physiological development of *G. domesticus*. After the seven-week experimental period, birds were humanely euthanized, and the following organs were excised and weighed using a precision balance (accuracy ± 0.01 g): lungs, spleen, heart, thymus, kidneys, muscle, gizzard, bones, liver, brain, intestine, bursa of Fabricius, and stomach. The weights of these organs were recorded and compared across the seven treatment groups, including the control group and groups supplemented with varying doses of SP (0.25–5 g/L) and CVE (0.25–5 g/L), both individually and in combination.

Body Measurements and Slaughter Characteristics

After seven weeks of trial, birds were fasted for 4 hours, individually weighed, and transported to a commercial poultry processing plant. Following standard commercial slaughtering procedures, carcass weights were recorded after 2 hours of refrigeration at 2 °C to determine carcass yield. Representative carcasses from each treatment group, selected based on average live weight and variability, were examined for gross morphological traits, including live body weight, body length, wings, beak length, culmen length, head length, foot length, feather length, tail length, and femur length. Post-slaughter measurements included post-slaughter weight, carcass weight, and dressing weight. The carcasses were then transported to the laboratory, stored at 4 °C, and further processed 24 hours post-mortem to separate major parts, including the breast, wings, and drumsticks with thighs. These measurements were performed to evaluate the effects of algal supplementation on growth performance and meat yield.

Blood Collection

Using sterile syringes, we collected blood samples from each bird's wing vein on the final day of the seven-week experiment. Blood was directly transferred to EDTA-coated tubes for hematological tests. We centrifuged blood samples at 3,000 rpm for 10 min to separate serum for serological analysis, then stored them at -20°C until the analyses.

Hormonal Assays

TSH, T3, and T4 levels were determined using ELISA kits to evaluate the effects of Spirulina and Chlorella supplementation on thyroid hormone levels. We followed the manufacturing instructions to perform the assays and quantified the hormone levels using an automated microplate reader at a wavelength of 450 nm.

Serum Biochemical and Hematological Analysis

Serum biochemical parameters, including cholesterol, triglycerides, total bilirubin, aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase, blood urea, and serum creatinine were analyzed using an automatic analyzer (Micro-lab 200, Diasys). Hematological parameters such as total red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), platelets, and packed cell volume (PCV) were also evaluated. The PCV was determined by centrifuging whole blood in micro-hematocrit capillary tubes at 10,000 rpm for 10 minutes, and the hematocrit was calculated based on the height of the packed cells. Erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were derived from the hematocrit and RBC counts. Differential leukocyte counts were performed to determine the percentages of neutrophils and lymphocytes, reflecting the immune response. Hemoglobin concentration was measured using a colorimetric kit based on the cyanomethemoglobin method. All analyses were conducted following standard clinical procedures to ensure accuracy and reproducibility.

Statistical Analysis

The data obtained from the study were analyzed using one-way ANOVA to determine significant differences among the treatment groups. *Post hoc*



comparisons were conducted using Tukey's test to identify specific group differences, with multiple comparisons between treatments and the control group denoted by alphabetical superscripts. All statistical analyses were performed using SPSS software (version 26), and differences were considered statistically significant at $p < 0.05$.

RESULTS

Growth Performance

The growth performance of *Gallus domesticus* fed different amounts of *Arthrospira platensis* (Spirulina, SP) and *Chlorella vulgaris* extract (CVE) was significantly improved across all treatment groups in terms of body weight gain, feed consumption, and feed conversion ratio (FCR) (Table 1). The groups supplemented with *Chlorella vulgaris* extract (G3 and G4) had slightly higher weight gains. G3, which received 2.5 g/L of Chlorella, showed a final weight of 847.00 g and a Percentage Weight Gain (PWG%) of 172.50%, while G4, with 5 g/L of Chlorella, achieved a final weight of 875.50 g and a PWG% of 168.97%. The combination of 2.5 g/L Spirulina and 2.5 g/L Chlorella (G5) led to the highest weight gain among the experimental groups, with a final weight of 953.17 g, a weight gain of 605.67 g, and a PWG% of 174.29%. Finally, the group supplemented with the highest concentrations of both Spirulina and Chlorella (G6, 5 g/L SP + CVE) exhibited the greatest PWG% of 237.40%, with a final weight of 899.17 g and a weight gain of 632.67g.

Table 1 – The growth performance of *Gallus domesticus* with various amounts of *Arthrospira platensis* (Spirulina, SP) and *Chlorella vulgaris* extract (CVE).

Parameters	Control	G1 (2.5g/L SP)	G2 (5g/L SP)	G3 (2.5g/L CVE)	G4 (5g/L CVE)	G5 (2.5g/L SP+CVE)	G6 (5g/L SP+CVE)
Initial Weight (g)	214.33	272.33	294.50	310.83	325.50	347.50	266.50
Final Weight (g)	608.67	808.17	836.33	847.00	875.50	953.17	899.17
Weight Gain (g)	394.34	535.84	541.83	536.17	550.00	605.67	632.67
PWG%	183.99 ^a	196.76 ^b	184.00 ^a	172.50 ^a	168.97 ^a	174.29 ^a	237.40 ^c
Total Feed Intake (g)	757.72 ^a	976.48 ^b	1008.73 ^{bc}	1015.24 ^{bc}	1075.37 ^c	1193.39 ^d	971.06 ^b
FCR	1.92 ^c	1.82 ^b	1.86 ^{bc}	1.89 ^{bc}	1.96 ^c	1.97 ^c	1.54 ^a

Feed consumption varied significantly across the experimental groups, showing a consistent increase over the study period of seven weeks. The supplementation groups demonstrated enhanced feed consumption, with the combined Spirulina and Chlorella groups showing the most pronounced

effects. Groups G1 (2.5 g/L Spirulina) and G2 (5 g/L Spirulina) exhibited comparable feed consumption trends, with total intakes of 976.48 g and 1008.73 g, respectively. While these values were higher than the control group, they were significantly lower than those of the combined Spirulina and Chlorella groups. Groups supplemented with Chlorella alone, such as G3 (2.5 g/L Chlorella) and G4, showed slightly better feed consumption compared to Spirulina-only groups, particularly in later weeks. Group G4 (5 g/L Chlorella) also showed high feed intake, with a total consumption of 1075.37 g, beginning at 68.36 g in Week 1 and increasing to 280.16 g in Week 7. Group G5 (2.5 g/L SP + CVE) recorded the highest feed intake across all weeks, starting at 72.98 g in Week 1 and peaking at 305.01 g in Week 7. This was significantly higher than all other groups ($p < 0.05$), indicating the synergistic effect of both microalgae on feed palatability or appetite stimulation. Interestingly, Group G6 (5 g/L Spirulina + 5 g/L Chlorella) exhibited inconsistent feed consumption, starting with relatively low values (55.97 g in Week 1) but peaking at 287.73 g in Week 7, with a total intake of 971.06 g. This group's feed intake, while lower than G5, remained significantly higher than the control ($p < 0.05$).

The feed conversion ratio (FCR), a key indicator of feed efficiency, varied significantly among the experimental groups. The control group had an FCR of 1.92, indicating suboptimal feed utilization, consistent with its low weight gain and total feed consumption. The Spirulina-supplemented groups (G1 and G2) demonstrated improved feed efficiency compared to the control, with FCR values of 1.82 and 1.86, respectively. This improvement indicates Spirulina's role in enhancing nutrient assimilation and promoting growth. Similarly, Chlorella-supplemented groups (G3 and G4) showed intermediate FCR values (1.89 and 1.96), suggesting that while Chlorella supports growth, it may not be as efficient as Spirulina in feed utilization. Group G4 (5 g/L Chlorella) exhibited the highest FCR of 1.96, reflecting relatively inefficient feed conversion despite its high total feed consumption of 1075.37 g. Group G6 (5 g/L Spirulina + 5 g/L Chlorella) exhibited the lowest FCR of 1.54, indicating optimal feed utilization for growth. Despite its slightly lower total feed consumption (971.06 g), this group achieved the highest weight gain (632.67 g), highlighting the synergistic effect of Spirulina and Chlorella when combined at higher concentrations. Conversely, Group G5 (2.5 g/L Spirulina + 2.5 g/L Chlorella) had a slightly higher FCR (1.97). Overall, these results highlight the



potential of Spirulina and Chlorella supplementation, particularly in combination, to improve feed efficiency and growth performance in *Gallus domesticus*.

Weekly body weight development

Table 2 shows the development of weekly body weight among the experimental groups after feeding with Spirulina (SP) and *C. vulgaris* extract (CVE). Statistical analyses revealed that adding higher concentrations of algal extract to the treatments, especially in combination, improved the growth performance over all the weeks.

Table 2 – Weekly body weight development with Spirulina and *C. vulgaris* extract supplementation.

Week	Control	G1 (2.5g/L SP)	G2 (5g/L SP)	G3 (2.5g/L CVE)	G4 (5g/L CVE)	G5 (2.5g/L SP+CVE)	G6 (5g/L SP+CVE)
Week 1	214.33 ± 3.87 ^e	272.33 ± 1.31 ^d	294.50 ± 0.96 ^c	310.83 ± 2.85 ^{bc}	325.50 ± 3.69 ^b	347.50 ± 1.67 ^a	266.50 ± 9.43 ^d
Week 2	258.17 ± 11.72 ^e	345.00 ± 9.24 ^{cd}	371.67 ± 5.14 ^{bc}	375.17 ± 7.86 ^{bc}	394.67 ± 4.71 ^b	445.50 ± 5.13 ^a	312.00 ± 8.37 ^d
Week 3	309.83 ± 15.16 ^d	376.83 ± 11.71 ^c	402.17 ± 5.00 ^{bc}	405.67 ± 10.52 ^{bc}	426.17 ± 6.32 ^b	476.83 ± 10.19 ^a	327.33 ± 14.64 ^d
Week 4	406.50 ± 19.29 ^d	475.33 ± 12.61 ^{bc}	473.00 ± 5.77 ^{bc}	462.67 ± 8.42 ^{bcd}	515.33 ± 6.87 ^{ab}	570.83 ± 10.43 ^a	421.50 ± 19.59 ^{cd}
Week 5	431.33 ± 23.47 ^d	572.83 ± 11.63 ^{bc}	580.83 ± 7.93 ^{bc}	576.67 ± 9.68 ^{bc}	624.33 ± 8.06 ^b	701.83 ± 12.50 ^a	548.83 ± 16.41 ^c
Week 6	520.67 ± 33.42 ^c	684.17 ± 7.88 ^b	699.83 ± 10.73 ^b	706.17 ± 9.44 ^b	744.00 ± 8.12 ^b	838.67 ± 11.38 ^a	707.67 ± 16.93 ^b
Week 7	608.67 ± 33.88 ^d	808.17 ± 8.77 ^c	836.33 ± 10.78 ^{bc}	847.00 ± 9.22 ^{bc}	875.50 ± 16.63 ^{abc}	953.17 ± 30.66 ^a	899.17 ± 16.86 ^{ab}

During the initial week, the control group showed the lowest weight gain (214.33 ± 3.87 g), considerably less than all other treatment groups ($p < 0.001$). Group G5 (2.5g/L SP + CVE) exhibited the greatest weight gain at 347.50 ± 1.67 g, followed by Group 4 (5g/L CVE) at 325.50 ± 3.69 g and Group 6 (5g/L SP + CVE) at 266.50 ± 9.43 g. These changes revealed the enhanced efficacy of combined supplementation relative to singular algal extracts. By the second week, the highest weight gain was observed in Group 5 (2.5g/L SP + CVE) at 445.50 ± 5.13 g, followed by the Group 4 (5g/L CVE group) at 394.67 ± 4.71 g, and Group 2 (5g/L SP) at 371.67 ± 5.14 g. By the third week, the trend of weight gain significantly changed between the treatment groups ($p < 0.001$). The combined supplementation of Group 5 (2.5 g/L SP + CVE) resulted in the highest weight gain at 476.83 ± 10.19 g, followed by the Group 4 (5g/L CVE) at 426.17 ± 6.32 g. In the fourth week, the highest weight gain was recorded for Group 5 (2.5 g/L SP + CVE) at 570.83 ± 10.43 g, which was significantly higher than all other groups ($p < 0.001$). Notably, Group 4 (5g/L CVE) recorded 515.33 ± 6.87 g. By week 5, the

weight gain in Group 5 (2.5 g/L SP + CVE) achieved the highest weight gain at 701.83 ± 12.50 g. Group 4 (5 g/L CVE group) at 624.33 ± 8.06 g also showed remarkable growth, reinforcing the beneficial effects of higher concentrations of algal extracts. In the sixth week, the combined supplementation of Group 5 (2.5 g/L SP + CVE) resulted in the maximum weight gain at 838.67 ± 11.38 g, which was significantly higher than all other groups ($p < 0.001$). By the final week, Group 5 (5 g/L SP + CVE) maintained its superior performance with the highest weight gain of 953.17 ± 30.66 g (Figure 1).

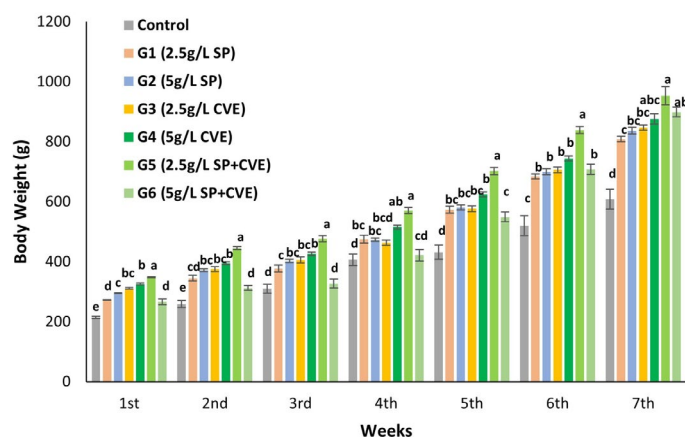


Figure 1 – Effects of Spirulina and *C. vulgaris* extract on the weekly body weight gain of the experimental groups.

Organ Weights

We found different responses in the organ weights of chickens subjected to varying concentrations of Spirulina (SP) and Chlorella (CVE) (Table 3). The heart showed the greatest divergence, followed by the gizzard and spleen, while other organs like the kidney, liver, and brain did not significantly change.

A general increase of lung weights was observed in the treated groups, with group G1 (2.5 g/L SP) having a slightly increased weight (4.30 ± 0.10 g) compared to the control (3.90 ± 0.40 g), and G3 (2.5 g/L CVE) showing a high increase (5.65 ± 0.35 g). Groups G5 (2.5 g/L SP + CVE) and G4 (5 g/L CVE) also had increased lung weights (5.60 ± 0.10 g and 5.05 ± 0.85 g, respectively). This group showed a marginally significant difference ($p = 0.062$) compared to the control group. SP and CVE administered at the highest doses showed an increase in spleen weights. In the control group, the spleen weighed 0.95 ± 0.25 g, and splenomegaly occurred at 1.75 ± 0.15 g in G4 (5 g/L SP) and 2.65 ± 0.35 g in G3 (2.5 g/L CVE). Group G5 (2.5g/L SP + CVE) had the highest spleen weight, 4.15 ± 1.45g, but no differences were found by ANOVA ($p = 0.126$).

**Table 3** – Organ weights of domestic chickens fed with varying concentrations of Spirulina and chlorella.

Organ	Control	G1 (2.5g/L SP)	G2 (5g/L SP)	G3 (2.5g/L CVE)	G4 (5g/L CVE)	G5 (2.5g/L SP+CVE)	G6 (5g/L SP+CVE)
Lungs	3.90 ± 0.40	4.30 ± 0.10	4.05 ± 0.05	5.65 ± 0.35	5.05 ± 0.85	5.60 ± 0.10	4.45 ± 0.05
Spleen	0.95 ± 0.25	1.35 ± 0.05	1.75 ± 0.15	2.65 ± 0.35	1.90 ± 0.00	4.15 ± 1.45	2.05 ± 0.85
Heart	2.65 ± 0.45	3.90 ± 0.10	3.05 ± 0.05	4.05 ± 0.05	3.35 ± 0.15	3.20 ± 0.40	2.50 ± 0.30
Thymus	2.35 ± 0.45	1.55 ± 0.05	1.95 ± 0.25	2.25 ± 0.05	2.00 ± 0.00	2.10 ± 0.30	2.50 ± 0.50
Kidney	3.55 ± 1.35	3.95 ± 0.05	4.25 ± 0.35	4.60 ± 0.70	4.55 ± 1.25	5.35 ± 0.85	4.40 ± 0.40
Muscle	3.55 ± 1.75	1.95 ± 0.05	5.00 ± 0.00	3.65 ± 1.25	3.65 ± 0.15	1.89 ± 0.91	4.30 ± 2.10
Gizzard	12.75 ± 1.15	7.35 ± 0.15	18.40 ± 0.60	18.55 ± 3.75	16.25 ± 2.55	100.00 ± 80.00	13.85 ± 2.15
Bones	7.90 ± 4.40	6.90 ± 0.10	8.50 ± 0.50	1.55 ± 0.35	7.40 ± 1.70	7.14 ± 2.04	4.90 ± 0.10
Liver	14.75 ± 2.25	9.80 ± 0.20	14.55 ± 2.05	22.15 ± 1.55	23.15 ± 6.25	14.75 ± 2.25	14.75 ± 1.75
Brain	1.95 ± 0.05	2.45 ± 0.05	2.15 ± 0.45	2.00 ± 0.20	2.00 ± 1.00	2.15 ± 0.45	2.00 ± 0.10
Intestine	11.45 ± 10.05	1.80 ± 0.10	39.25 ± 5.75	21.60 ± 19.40	39.50 ± 16.50	33.50 ± 0.50	22.40 ± 0.60
Bursa of Fabricius	1.45 ± 0.35	2.45 ± 0.05	3.70 ± 0.50	3.15 ± 0.35	2.65 ± 0.85	3.05 ± 0.95	1.80 ± 0.00
Stomach	3.05 ± 0.85	3.95 ± 0.05	4.25 ± 0.25	4.15 ± 0.05	4.05 ± 0.15	4.50 ± 0.10	4.10 ± 0.10

Furthermore, there was a difference in heart weights, with group G1 (2.5g/L SP) having an increase (3.90 ± 0.10 g) compared to control (2.65 ± 0.45). G3 (2.5 g/L CVE) resulted in slightly higher (4.05 ± 0.05 g) weight than other treatments (G2, G4 and G6), whereas weight was lower (3.0 ± 0.40 g) in birds from G5 (2.5 g/L SP + CVE). There was a difference in heart weights, with group G1 (2.5g/L SP) having an increase (3.90 ± 0.10 g) compared to control (2.65 ± 0.45). G3 (2.5 g/L CVE) resulted in slightly higher (4.05 ± 0.05 g) weight than other treatments (G2, G4 and G6), whereas weight was lower (3.0 ± 0.40 g) in birds treated with G5 (2.5 g/L SP + CVE). An ANOVA was carried out to determine if treatments made a significant difference in heart weights, showing that there was a significant group difference ($p=0.029$). There were no effects of treatments on thymus weight. The control thymus weight was 2.35 ± 0.45 g and was only slightly reduced (1.55 ± 0.05 g) in the G1 (2.5 g/L SP) group. We observed no significant effects ($p=0.441$). Kidney weights were generally elevated with treatment, and G2 (5 g/L SP; 4.55 ± 1.25 g) had the highest kidney weight in comparison to the control (3.55 ± 1.35 g). Kidney weight did not differ between the groups ($p=0.821$). The muscle weights differed

significantly among the groups, with the highest muscle weight for the G2 (5g/L SP; 5.00 ± 0.00 g) and the lowest muscle weight for the control group (3.55 ± 1.75 g). However, there was no significant difference ($p=0.526$), implying that Spirulina and Chlorella had no significant effect on muscle weight, despite there being a significant group difference ($p=0.029$). There were no effects of treatments on thymus weight. The control thymus weight was 2.35 ± 0.45 g and was only slightly reduced (1.55 ± 0.05 g) in the G1 (2.5 g/L SP) group. No significant effects ($p=0.441$) were measured. Kidney weights were generally elevated with treatment, and G2 (5 g/L SP; 4.55 ± 1.25 g) had the highest kidney weight in comparison to the control (3.55 ± 1.35 g). Kidney weight did not differ between the groups ($p=0.821$), while muscle weights differed significantly, with the highest muscle weight observed in G2 (5g/L SP; 5.00 ± 0.00 g) and the lowest muscle weight for the control group (3.55 ± 1.75 g). However, there was no significant difference ($p=0.526$), suggesting that Spirulina and Chlorella had no significant effect on muscle weight.

The response of gizzard weights was most striking in G1 (2.5g/L SP), where there was downregulation (7.35 ± 0.15 g v. 12.75 ± 1.15 g (control)). Conversely, this value was significantly higher in the G2 (5 g/L SP) and G3 (2.5 g/L CVE) groups, amounting to respectively 18.40 ± 0.60 g and 18.55 ± 3.75 g. The G5 (2.5 g/L SP + CVE) group recorded the highest gizzard weight (100.00 ± 80.00 g), and the G6 group (5 g/L SP + CVE) recorded moderate gizzard weight (13.85 ± 2.15 g). The values showed no statistically significant differences ($p=0.423$) due to their extreme variation. The G2 (5g/L SP) group had slightly higher (8.50 ± 0.50 g) bone weight than control (7.90 ± 4.40 g), although only slightly. The results showed no statistically significant variations ($p=0.306$), suggesting that treatments did not significantly affect weight. Liver weight was similarly measured and reported to be 14.75 ± 2.25 g in the control group, and was significantly increased in the G3 (2.5g/L CVE) (22.15 ± 1.55 g) and G4 (5g/L CVE) groups (23.15 ± 6.25 g), but there were no significant differences when comparing treatment groups to control ($p=0.114$).

Brain weights did not vary, with the control group weighing 1.95 ± 0.05 g. We found no difference between treatment groups ($p=0.985$), so Spirulina and Chlorella had no effect on brain weight. We also observed significant variation of intestinal weights across treatment groups. An intestinal weight of 11.45 ± 10.05 g was observed in the control group, while



intestinal weight rose in the G2 (5g/L SP) (39.25 ± 5.75 g) and G4 (5g/L CVE) groups (39.50 ± 16.50 g). Such treatment was also quite effective in the G5 (2.5g/L SP + CVE) group, leading to an increase of 33.50 ± 0.50 g. However, the ANOVA showed no significance ($p=0.226$). Several treatment groups showed increases in the weight of the bursa of Fabricius. When compared with the control ($1.45 \text{ g} \pm 0.35$ g), the G2 (5 g/L SP) had the highest average weight (3.70 ± 0.50 g), but this result not reach significance ($p=0.188$). Mean stomach weight for the 2.5g/L SP group was high (3.95 ± 0.05 g) in comparison to control (3.05 ± 0.85 g) but no significant variation was observed ($p=0.679$).

Body Measurements

Table 4 shows the effects of varying levels of Spirulina (SP) and Chlorella (CVE), individually and in combination, on the growth performance, body measurements, and slaughter characteristics of domestic chickens. There were significant differences ($p<0.05$) in the live body weight, body measurements, and slaughter parameters between the treatment groups. Supplementation significantly influenced the studied traits.

Table 4 – Effects of Spirulina and Chlorella supplementation on body measurements and slaughter characteristics of domestic chickens.

Parameter	Control	G1 (2.5g/L SP)	G2 (5g/L SP)	G3 (2.5g/L CVE)	G4 (5g/L CVE)	G5 (2.5g/L SP+CVE)	G6 (5g/L SP+CVE)
Live Body Weight (g)	709.0 ± 41.0^a	820.0 ± 10.0^a	800.0 ± 5.0^a	860.0 ± 95.0^a	874.0 ± 116.0^a	847.0 ± 56.0^a	612.5 ± 77.5^a
Body Length (cm)	16.5 ± 6.5^{ab}	14.5 ± 0.5^{ab}	9.75 ± 0.25^b	26.5 ± 0.5^a	11.25 ± 0.25^b	12.0 ± 2.0^{ab}	11.5 ± 1.5^b
Wings (cm)	14.2 ± 11.8^a	10.5 ± 0.5^a	17.0 ± 6.0^a	30.5 ± 1.5^a	11.5 ± 1.5^a	11.5 ± 1.0^a	9.25 ± 0.75^a
Beak (cm)	4.25 ± 0.75^a	1.55 ± 0.05^a	3.75 ± 0.75^a	4.5 ± 0.5^a	4.75 ± 0.25^a	3.8 ± 0.1^a	3.2 ± 1.8^a
Culmen (cm)	1.9 ± 0.0^a	2.05 ± 0.05^a	2.0 ± 0.0^a	2.5 ± 0.5^a	2.15 ± 0.35^a	1.95 ± 0.05^a	1.35 ± 0.05^a
Head (cm)	6.5 ± 0.5^{ab}	6.5 ± 0.5^{ab}	4.75 ± 1.25^b	8.25 ± 0.25^a	7.5 ± 0.5^{ab}	6.5 ± 0.5^{ab}	6.2 ± 0.3^{ab}
Foot (cm)	13.75 ± 1.25^a	6.25 ± 0.25^a	9.5 ± 4.5^a	15.0 ± 1.0^a	15.5 ± 0.5^a	13.75 ± 0.25^a	8.75 ± 3.25^a
Feathers (cm)	8.75 ± 1.25^{ab}	3.75 ± 0.25^b	9.25 ± 0.25^a	9.25 ± 1.75^a	10.75 ± 0.75^a	8.75 ± 0.25^{ab}	7.6 ± 0.6^{ab}
Tail (cm)	14.0 ± 1.0^a	4.65 ± 0.15^b	15.0 ± 0.0^a	17.5 ± 2.5^a	14.75 ± 1.75^a	16.0 ± 2.0^a	13.5 ± 0.5^a
Femur (cm)	9.0 ± 0.0^{ab}	5.25 ± 0.25^b	10.0 ± 0.0^{ab}	13.0 ± 1.0^a	10.75 ± 0.75^{ab}	7.5 ± 3.0^{ab}	8.6 ± 0.4^{ab}
Post-Slaughtering Weight (g)	405.5 ± 2.5^a	402.5 ± 7.5^a	538.5 ± 11.5^a	568.5 ± 44.5^a	641.0 ± 50.0^a	572.0 ± 20.0^a	296.0 ± 161.0^a
Carcass Weight (g)	524.0 ± 4.0^{ab}	534.0 ± 6.0^a	202.0 ± 12.0^{ab}	142.25 ± 18.25^b	507.5 ± 17.5^{ab}	470.0 ± 60.0^{ab}	302.5 ± 167.5^{ab}
Dressing Weight (g)	121.5 ± 6.5^{abc}	66.0 ± 1.0^{bc}	37.5 ± 2.5^c	36.5 ± 8.5^c	159.0 ± 21.0^{abc}	231.0 ± 79.0^{ab}	257.5 ± 2.50^a

Weight gain/body weight increased in most of the treated groups when compared to the control. Group G4 (5 g/L CVE) had the highest mean live body weight (874.0 ± 116.0 g), followed by group G3 (2.5 g/L CVE), with a mean weight of 860.0 ± 95.0 g; however, the mean weight of group G6 (5 g/L SP + 5 g/L CVE) (612.5 ± 77.5 g) was lower than that of the control group (709.0 ± 41.0 g). There were no meaningful differences between the groups with regard to live body weight ($p=0.211$). Group length had a significant ($p=0.032$) effect on body length; group G3 (2.5 g/L CVE) had the longest mean length (26.5 ± 0.5 cm), while group G2 (5 g/L SP) recorded the shortest (9.75 ± 0.25 cm). Statistical analysis indicated that there was a significant effect of treatments on body length ($p=0.032$). There was no statistical significance for relationships between these groups with regard to other parameters (width length, beak length, culmen length). Significantly longer tail length in group G3 (17.5 ± 2.5 cm) than in control (14.0 ± 1.0 cm) was found overall ($p=0.007$), as well as a significant interaction ($p=0.048$). The femur length was longest in the Group G3 (2.5 g/L SP; 13.0 ± 1.0). As in BWG, feathers analyzed for treatment effects were noteworthy; with Group G4 being found to have the longest feathers (10.75 ± 0.75 cm) ($p=0.017$).

Serum Biochemical Analysis

Table 5 shows the effects of Spirulina and Chlorella supplementation on physiological markers in domestic chickens. Mean cholesterol concentrations among the experimental groups were significantly different from the control group, which had a mean cholesterol concentration of 135.00 ± 1.15 mg/dL ($p<0.001$). Cholesterol levels were reduced significantly in Group 1 (2.5 g/L Spirulina; 125.00 ± 0.58 mg/dL, $p<0.001$), and were increased significantly in Group 2 (5 g/L Spirulina; 152.00 ± 1.15 mg/dL, $p<0.001$). The cholesterol levels (118.00 ± 0.58 mg/dL) in Group 3 (2.5 g/L Chlorella) were the lowest and significantly lower than that of the control ($p<0.001$). On the other hand, Group 6 (5 g/L Spirulina + 5 g/L Chlorella) had highest cholesterol (155.00 ± 0.58 mg/dL) among all groups ($p<0.001$). The triglyceride levels for the control group were 151.00 ± 0.58 mg/dL, in comparison to which Group 1 had a significant decrease (127.00 ± 0.58 mg/dL, $p<0.001$) and Group 2 had a significant increase (176.00 ± 0.58 mg/dL, $p<0.001$). Group 6 presented the lowest levels for this variable (122.00 ± 0.58 mg/dL), significantly lower than the control ones ($p<0.001$). In regards to the overall triglyceride levels, the administration of Spirulina and Chlorella



combinations had dose-dependent effects. There was no big difference between the groups' bilirubin levels; the control group had 0.50 ± 0.06 mg/dL and the experimental groups varied between 0.30 and 0.40 mg/dL ($p=0.377$).

Table 5 – Physiological markers of domestic chickens fed with Spirulina and Chlorella supplementation.

Parameter	Control	2.5g/L SP	5g/L SP	2.5g/L CVE	5g/L CVE	2.5g/L SP+CVE	5g/L SP+CVE
Serum Biochemical Parameters							
Cholesterol	135.00 ± 1.15^c	125.00 ± 0.58^d	152.00 ± 1.15^b	118.00 ± 0.58^e	123.00 ± 0.58^d	180.00 ± 0.58^a	155.00 ± 0.58^b
Triglycerides	151.00 ± 0.58^c	127.00 ± 0.58^e	176.00 ± 0.58^a	129.00 ± 0.58^e	141.00 ± 0.58^d	160.00 ± 0.58^b	122.00 ± 0.58^f
Total Bilirubin	0.50 ± 0.06^a	0.30 ± 0.06^a	0.40 ± 0.06^a	0.40 ± 0.06^a	0.40 ± 0.06^a	0.40 ± 0.06^a	0.40 ± 0.06^a
AST	15.00 ± 0.58^{bc}	18.00 ± 0.58^a	17.00 ± 0.58^{ab}	18.00 ± 0.58^a	17.00 ± 0.58^{ab}	14.00 ± 0.58^c	11.00 ± 0.58^d
ALT	12.00 ± 0.58^c	15.00 ± 0.58^b	14.00 ± 0.58^{bc}	12.00 ± 0.58^c	14.00 ± 0.58^{bc}	19.00 ± 0.58^a	13.00 ± 0.58^{bc}
Alkaline Phosphatase	856.00 ± 0.58^f	892.00 ± 0.58^e	892.00 ± 0.58^e	954.00 ± 0.58^d	1124.00 ± 0.58^a	1004.00 ± 0.58^b	969.00 ± 0.58^c
Blood Urea	20.00 ± 0.58^a	12.00 ± 0.58^d	16.00 ± 0.58^b	15.00 ± 0.58^{bc}	11.00 ± 0.58^d	13.00 ± 0.58^{cd}	15.00 ± 0.58^{bc}
Serum Creatinine	0.30 ± 0.06^a	0.20 ± 0.06^a	0.30 ± 0.06^a	0.40 ± 0.06^a	0.20 ± 0.06^a	0.20 ± 0.06^a	0.30 ± 0.06^a
Hematological Parameters							
Hemoglobin	12.90 ± 0.06^f	16.20 ± 0.06^d	17.00 ± 0.06^b	18.60 ± 0.06^a	16.60 ± 0.06^c	12.80 ± 0.06^f	14.90 ± 0.06^e
RBC	1.60 ± 0.06^d	2.30 ± 0.06^b	2.50 ± 0.06^{ab}	2.70 ± 0.06^a	2.40 ± 0.06^b	2.00 ± 0.06^c	1.70 ± 0.06^d
WBC	22.40 ± 0.06^f	49.20 ± 0.06^c	58.10 ± 0.06^a	54.50 ± 0.06^b	49.10 ± 0.06^c	30.10 ± 0.06^e	39.40 ± 0.06^d
Platelet	18.00 ± 0.58^a	16.00 ± 0.58^d	15.00 ± 0.58^d	7.00 ± 0.58^e	14.00 ± 0.58^d	21.00 ± 0.58^c	32.00 ± 0.58^b
PCV	30.00 ± 0.58^c	35.00 ± 0.58^b	36.00 ± 0.58^{ab}	38.00 ± 0.58^a	35.00 ± 0.58^b	27.00 ± 0.58^d	32.00 ± 0.58^c
MCV	151.00 ± 0.58^b	148.00 ± 0.58^c	144.00 ± 0.58^d	138.00 ± 0.58^e	142.00 ± 0.58^d	134.00 ± 0.58^f	181.00 ± 0.58^a
MCH	76.00 ± 0.58^b	68.00 ± 0.58^c	67.00 ± 0.58^c	67.00 ± 0.58^c	67.00 ± 0.58^c	64.00 ± 0.58^d	84.00 ± 0.58^a
MCHC	50.00 ± 0.58^a	45.00 ± 0.58^c	47.00 ± 0.58^{bc}	48.00 ± 0.58^{ab}	47.00 ± 0.58^{bc}	47.00 ± 0.58^{bc}	46.00 ± 0.58^{bc}
Neutrophils	2.00 ± 0.58^c	4.00 ± 0.58^{bc}	3.00 ± 0.58^{bc}	4.00 ± 0.58^{bc}	5.00 ± 0.58^b	8.00 ± 0.58^a	2.00 ± 0.58^c
Lymphocytes	98.00 ± 0.58^a	96.00 ± 0.58^{ab}	97.00 ± 0.58^{ab}	96.00 ± 0.58^{ab}	95.00 ± 0.58^b	92.00 ± 0.58^c	98.00 ± 0.58^a

AST levels varied only mildly, being observed to range from 11.00 ± 0.58 U/L in Group 6 to 18.00 ± 0.58 U/L in Group 1, and this variation was statistically significant ($p<0.001$). ALT levels were shown to vary considerably across groups, ranging from the highest (19.00 ± 0.58 U/L, $p<0.001$; Group 5 (5 g Spirulina + 5 g Chlorella)) to the lowest (12.00 ± 0.58 U/L, $p<0.001$; Group 1 (control)) levels. The control group level of alkaline phosphatase was 856.00 ± 0.58 U/L. In all experimental groups, the levels increased, with

the highest levels (1124.00 ± 0.58 U/L, $p<0.001$) observed in Group 4 (5 g/L Chlorella), demonstrating that higher Chlorella doses had a significant effect on alkaline phosphatase activity. The control group shows a blood urea of 20.00 ± 0.58 mg/dL. Significant reductions were seen in all the experimental groups, and Group 4 had the lowest level (11.00 ± 0.58 mg/dL; $p<0.001$). There were no significant differences in serum creatinine levels between groups; their variation between 0.20–0.40 mg/dL ($p=0.190$) reflected no adverse effect of treatments on kidney function.

Hematological Parameters

There were significant changes in hemoglobin levels between groups ($p<0.001$) (Table 5). The control group had hemoglobin levels of 12.90 ± 0.06 g/dL, while the highest levels were found in group 3 (2.5 g/L Chlorella), at 18.60 ± 0.06 . This shows that Chlorella supplementation greatly increased hemoglobin levels. RBC count significantly differed among groups ($p<0.001$). Chlorella supplementation resulted in a dose-dependent increase, with the highest count observed for Group 3 (2.70 ± 0.06) and the lowest for the control group (1.60 ± 0.06). Significantly ($p<0.001$), these also affected white blood cell counts. Both the control group and Group 2 (5 g/L Spirulina) had the maximum and minimum counts, respectively, of 22.40 ± 0.06 and 58.10 ± 0.06 . These findings indicate that Spirulina stimulated immune function in a dose-dependent manner. There was a marked variation in the platelet count across the groups ($p=0.001$). Group 6 had a platelet count of significantly fewer platelets (64.00 ± 0.58) than the control group (161.00 ± 0.58).

The packed cell volume (PCV) and the mean corpuscular volume (MCV) were significantly different ($p<0.05$) from the control (PCV = $30.00 \pm 0.58\%$ and MCV = 151.00 ± 0.58 fL). Group 3 had the statistically significant ($p<0.001$) highest PCV ($38.00 \pm 0.58\%$), and Group 6 had the statistically significant ($p<0.001$) highest MCV (181.00 ± 0.58 fL). Group 6 also significantly altered MCH and MCHC, with the highest MCH (84.00 ± 0.58 pg, $p<0.001$) and the control group having the highest MCHC (50.00 ± 0.58 g/dl, $p<0.001$). A significant effect of the treatments was shown on neutrophil percentages ($p<0.001$). Group 5 ($8.00 \pm 0.58\%$) had the highest count in neutrophils, and the control group had $2.00 \pm 0.58\%$. Lymphocyte percentages decreased in general across groups, but the lowest lymphocyte percentage was observed in Group 5 ($92.00 \pm 0.58\%$, $p<0.001$).



Endocrine Hormonal Parameters

There were no significant changes in TSH, T3, or T4 levels across groups. Also, all thyroid hormone levels (TSH < 0.005 μ IU/mL, T3 < 0.10 ng/dL, and T4 < 10.23 μ g/dL) were below what could be detected in both the control and supplemented groups (Table 5). This shows that adding algae did not change the levels of thyroid hormones. Lipid profiles, hematological indices, and enzyme activities primarily reflected the overall effect of spirulina and chlorella supplementation on *Gallus Gallus domesticus* physiology. These results suggest that these algal extracts may serve as nutritional supplements to enhance physiological health in poultry.

DISCUSSION

This study demonstrated that Spirulina and Chlorella supplementation significantly improved growth performance, feed efficiency, and selected physiological parameters in broiler chickens. The increase in body weight gain and percentage weight gain (PWG%) suggests that these microalgae play a role in enhancing metabolism and nutrient absorption efficiency, in agreement with previous studies (El-Bahr *et al.*, 2020). The relationship between feed intake and weight gain remains crucial. While some groups exhibited higher feed consumption, others showed improved feed conversion ratio (FCR), indicating that the supplements not only stimulated appetite but may have also enhanced nutrient utilization and metabolic efficiency. Spirulina's phycocyanin and essential fatty acids, along with Chlorella's polysaccharides and bioactive compounds, are known to influence lipid metabolism, gut microbiota, and immune responses, potentially contributing to better growth outcomes (Alfaia *et al.*, 2021, Sugiharto *et al.*, 2018). Previous studies confirm the potential use of microalgae as growth boosters in poultry diets based on these results. Khan *et al.* (2023) reviewed the therapeutic effects of medicinal plants on immunology and growth, emphasizing the potential of bioactive compounds to improve nutrient utilization and growth performance in poultry. Similarly, Saleem *et al.* (2023) discussed the importance of optimizing poultry health through dietary interventions, which supports the observed improvements in growth parameters in this study.

In this study, the combined supplementation of Spirulina and Chlorella (Group 6) resulted in the highest weight gain despite marginally lower total feed intake compared to Group 5, suggesting an improved feed efficiency rather than mere increased consumption.

This implies that algal extracts may enhance nutrient utilization through bioactive compounds (e.g., phycocyanin in Spirulina, chlorophyll in Chlorella) that promote protein synthesis, gut health, and metabolic efficiency (El-Shallet *et al.*, 2023, Spínola *et al.*, 2024). Future studies should investigate the specific metabolic and digestive mechanisms underlying these effects, particularly their impact on gut microbiota and nutrient assimilation.

In the present study, liver, lung, spleen, heart, and gizzard organ weights showed significant effects of SP and CVE supplementation. Lung weight was increased ($p=0.01$) in the treatment groups, more so in the group receiving 2.5 g/L CVE (5.65 ± 0.35 g), although only marginally significantly. The observed increases in organ weights, particularly the spleen and lungs, suggest that algal supplementation may enhance immune organ development. This is consistent with the findings of Mustafa *et al.* (2023), who reviewed the global prevalence of parasitic infections in poultry and emphasized the role of dietary interventions in improving organ health and disease resistance. Additionally, Siddiqui *et al.* (2024) highlighted the importance of managing heat stress in broiler chickens, which can influence organ development and meat quality, further supporting the relevance of this study's findings.

There was a significant effect of the treatment on the heart weight ($p=0.029$), and CVE supplementation induced higher values. Our findings support previous research, such as the one conducted by An *et al.* (2016), who found a role of *C. vulgaris* in improving cardiovascular health through enhancing lipid metabolism and antioxidant activity. Gizzard weight also showed variability extremes that were most pronounced in the 2.5 g/L SP + CVE (100.00 ± 80.00 g). Despite these variations, differences in digestive organ weights were not statistically significant ($p=0.423$), disagreeing with Cabrol *et al.* (2022), who showed consistent increases in weights of the given digestive organs when supplemented daily with *C. vulgaris*. The difference may arise from lower inclusion rates or dietary formulations in our study.

The liver and brain had minimal or non-significant changes. The increase in the weight of liver with CVE (23.15 ± 6.25 g at 5 g/L) was studied by Kulkarni & Nikolov (2018), who reported better liver functions in microalgae-fed chickens. Nevertheless, a lack of significant differences in our study indicates a dose-dependent effect or poor bioavailability of bioactive compounds. This led to the conclusion that 2.5 g/L



CVE supplementation led to the best performance (860.0 ± 95.0 g live body weight, 26.5 ± 0.5 cm body length, 17.5 ± 2.5 cm tail length) in group G4. However, the treatments that combined SP and CVE (Group G6) showed lower performance compared to those that did not, indicating antagonistic effects at higher doses, as noted by Cabrol *et al.* (2022) in cases of over-supplementation. CVE significantly improved body length and femur length ($p=0.032$ $p=0.048$, respectively) and showed potential in skeletal development.

Carcass weight in group G6 (combined treatment) was lower than in G3, which may be a sign of metabolic stress or poor utilisation of nutrients at higher doses. That is exactly what An *et al.* (2016) found: diminishing returns at very high inclusion rates. Group G6 dressing weight was significantly higher, which is consistent with the findings of Cabrol *et al.* (2024), who reported that increasing microalgae inclusion could help increase meat dressing percentages. While dose combination seems to offer other advantages, variability in these other parameters suggests the need for optimised dose combinations. This study indicates that *Spirulina* and *Chlorella vulgaris* may be useful as dietary supplements for increasing selected organ weights, growth, and slaughter characteristics in domestic chickens. However, although moderate doses of CVE (2.5 g/L) were the most effective, combined treatments at higher doses showed decreased effectiveness, indicating the need for dose optimisation.

Cholesterol levels were dose-dependent in this study. A concentration of 2.5 g/L of spirulina and chlorella decreased cholesterol levels, while a concentration of 5 g/L of spirulina increased it. The cholesterol reductions with the lower concentration of *Spirulina* are similar to the ones observed in the work of Hassan *et al.* (2022), which also demonstrated a cholesterol reduction in Fayoumi broiler chickens with the bioactive components of *Spirulina* (10 g/kg). Hassan *et al.* (2022) also reported that phenolic compounds and C-phycoerythrin previously increased lactobacilli counts and reduced cholesterol digestion and synthesis (Torres-Duran *et al.*, 2007; Mariey *et al.*, 2012). Early research has found that carotenoids and chlorophylls in supplements helped lower lipid levels. The lower dose of *Chlorella vulgaris* also lowered cholesterol (Hynstova *et al.*, 2018). However, a high dose (5g/L SP and 5g/L SP+CVE) led to an increase in the level of cholesterol in *Gallus gallus domesticus*. We found opposite results to earlier studies by Fathi (2018) and Omar *et al.* (2022), who reported a decrease in

cholesterol in broiler chickens fed *Spirulina platensis* as dietary supplementation. Methodological parameters, the dosage and source of microalgae, and the basal diet can explain the observed differences. Spínola *et al.* (2024) found that an excessive amount of *Spirulina* could lead to an increase in cholesterol, likely due to its high fat digestibility when supplemented at high levels.

The current study's showed an increase in triglyceride levels at higher doses of *Spirulina*. While some studies report triglyceride reductions with *Spirulina* supplementation (Sugiharto *et al.*, 2018), our higher-dose group (5 g/L SP) showed increased levels—a divergence potentially explained by dose-dependent effects or basal diet composition. Notably, Hassan *et al.* (2022) observed neutral effects on triglycerides at lower *Spirulina* inclusions (1–3 g/kg), suggesting threshold-specific outcomes.

The AST levels in all the groups tested were significantly different ($p<0.001$), and group 1 showed the highest. Similarly, a highly significant increase in the level of ALT in the same parameter was found in Group 5 (SP+ CVE), $p<0.001$. While no significant changes were observed in total bilirubin levels, the increase in ALP activity suggests improved bone formation and metabolic activity. These findings are supported by Khan *et al.* (2024), who demonstrated the mitigating effects of dietary supplements against oxidative stress and liver damage in broiler birds. Furthermore, Akram *et al.* (2023) discussed the public health implications of chemical residues in poultry products, underscoring the importance of natural feed additives like *Spirulina* and *Chlorella* in reducing oxidative stress and improving overall health.

Experimental groups were confirmed to have a considerable reduction in blood urea level to a highly significant level ($p<0.001$), while no considerable changes were observed in serum creatinine levels ($p=0.190$). These results are in agreement with Spínola *et al.* (2024), who recorded lower urea levels in poultry fed *Spirulina*, possibly because of elevated nitrogen absorption or reduced protein degradation. The rats' creatinine levels remained unchanged, indicating that the algal supplementation had no effect on their kidney function. This observation aligns with the findings by Yadav *et al.* (2014), who showed that *Spirulina* and *Chlorella* had no effect on kidney function. Abbas *et al.* (2022) observed that the lower protein digestibility of algal supplements leads to low urea levels.

The current study's results demonstrated an increase in both hemoglobin and RBC count in Group 3, with *Chlorella* being the most effective treatment. The WBC



count was higher in Group 2 (5 g/L Spirulina), which may also suggest that the immunocompetent effect of Spirulina is likely to have a dose-associated effect. These results are consistent with findings reporting that oxidative stress and toxicological impacts on erythrocytes could be mitigated through dietary interventions (Hussein & Saeed, 2025, Wang *et al.*, 2025). Additionally, Shafqat *et al.* (2023) highlighted the role of bioactive compounds in improving hematological profiles and immune responses, which aligns with the enhanced immune function observed in this study.

The present study showed an increase in packed cell volume (PCV), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCH), with the highest PCV of $38.00 \pm 0.58\%$ in Group 3 and the highest MCV of 181.00 ± 0.58 fL in Group 6. These are aligned with the studies of Abbas *et al.* (2022), who argued that broilers and fish fed Spirulina showed better hematological features. Spirulina supplements were associated with a higher MCV and a higher MCH, indicating an increased erythrocyte size and, more specifically, a higher hemoglobin concentration, likely due to the strong antioxidant effect of spirulina protecting erythrocytes from oxidative stress and improving their quality (Elmalawany *et al.*, 2014). However, Emami & Olfati (2017) found that microalgae increased MCV levels and decreased PCV levels in diabetic animals. This suggests that microalgae can help slow down the effects of stress on the hematological profile. The present work showed an increased level of neutrophils and a decrease in the percentage of lymphocytes in poultry from Group 5. This aligns with the findings of Sneha *et al.* (2022) and (Rezzani *et al.*, 2014), who discovered that Spirulina enhances neutrophil maturation and innate immunity efficacy. Other research on Spirulina's immune system modulation (Abbas *et al.*, 2022) shows that these changes may be caused by the way it controls the percentage of lymphocytes, which speeds up the body's neutrophil response to pathogens.

The results of this study highlight the potential of *Spirulina platensis* and *Chlorella vulgaris* as sustainable feed additives for improving poultry health and productivity. However, further research is needed to optimize dosage levels and evaluate long-term effects. This aligns with the recommendations of Khan *et al.* (2023), who emphasized the need for dose optimization in dietary interventions to maximize benefits while minimizing potential adverse effects. Additionally, Shafqat *et al.* (2023) suggested that

future studies should explore the synergistic effects of combining multiple bioactive compounds to enhance growth performance and immune function in poultry.

CONCLUSION

In conclusion, the dietary supplementation of Spirulina (SP) and *Chlorella vulgaris* extract (CVE) in *Gallus gallus domesticus* significantly enhanced growth performance, serum biochemical markers, and hematological parameters, with distinct effects depending on the type and dosage of supplementation. Spirulina at a dose of 2.5g/L (Group 1) notably improved cholesterol and triglyceride levels, while *Chlorella vulgaris* supplementation at 2.5g/L (Group 3) significantly boosted hemoglobin concentration and red blood cell counts. The combined supplementation of Spirulina and *Chlorella vulgaris* (5g/L SP + 5g/L CVE, Group 6) showed the most comprehensive benefits, with the highest body weight gain, increased platelet count, and improved overall biochemical markers, although cholesterol levels were slightly elevated. This combined formulation proved to be the most effective, showing the best overall performance in terms of growth and physiological health parameters, highlighting its potential for enhancing poultry health and productivity.

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Conceptualization, Z.U.A., M.F., T.R., and A.A.K.; methodology, Z.U.A.; software, Z.U.A.; validation, Z.U.A., M.F., T.R., and A.A.K.; formal analysis, Z.U.A.; investigation, Z.U.A.; resources, Z.U.A.; data curation, Z.U.A.; writing—original draft preparation, Z.U.A.; writing—review and editing, Z.U.A., M.F., T.R., and A.A.K.; visualization, Z.U.A.; supervision, M.F., T.R., and A.A.K.; project administration, Z.U.A. No funding



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DATA AVAILABILITY STATEMENT

Data will be made available upon request.

CONFLICT OF INTEREST

All authors declare no potential conflict and no competing interests.

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