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RESEARCH ARTICLE

The Potential of *Spirulina platensis* to Substitute Antibiotics in Broiler Chickens Diets: Influences on Growth Performance, Serum Biochemical Profiles, Meat Quality, and Gut Microbiota

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Abstract

The rise of antibiotic-resistant microbes has prompted a search for effective alternatives to antibiotics. This study evaluated the effects of *Spirulina platensis* extract (SPE) as a dietary supplement and a potential alternative to antibiotics for broiler chickens, focusing on growth performance, antioxidant activity, blood parameters, and cecal microbiota. SPE contained antimicrobial active compounds, including heptadecane and geosmin. A total of 300 broilers were divided into five groups (T1-T5). T1 received a basal diet (control), while T2-T5 were supplemented with 0.5, 1, 2, and 3 mg SPE/kg of diet, respectively, for 35 days. Results showed that including SPE (3 mg/kg) in the broiler diet significantly enhanced growth, decreased the feed conversion ratio (FCR), and improved body weight gain (BWG, 9%), while maintaining optimal carcass quality and intestinal pH at 6.8. Liver enzymes remained stable, with a 13-45% reduction in kidney markers based on the SPE concentration. SPE (3 mg/kg) also reduced oxidative stress by decreasing malondialdehyde (MDA) levels while sustaining antioxidant enzyme levels. The cecal microbiota showed an increase in lactic acid bacteria and exhibited enhanced immunity compared to the control. Additionally, SPE improved meat quality by boosting protein and moisture content, enhancing juiciness and tenderness. In conclusion, supplementing broiler diets with SPE (3 mg/kg) enhances growth performance, productivity, overall health, and disease resistance, making it a potential viable alternative to antibiotics.

Keywords: Eco-friendly antibiotic, Broiler chickens, *Spirulina platensis,* Immune response, Gut microbiota

Introduction

Different pathogens can significantly impact the health and productivity of poultry, which in turn affects their welfare and production efficiency. This can lead to the formation of antimicrobial or multidrug-resistant strains of pathogens [1], which increases the risk of poultry products being contaminated with pathogens that can be transmitted to humans. Additionally, consumers are increasingly demanding organic poultry [2]. Some pathogens, such as *Salmonella* and *Campylobacter* species, exacerbate the situation by forming biofilms, which contribute to the severity of poultry diseases and promote resistance to antimicrobial drugs. These biofilms are complex structures of bacterial cells and the substances they produce, creating a protective barrier that makes eradication efforts more challenging [3].

The poultry industry relies heavily on the use of synthetic antimicrobial agents, which are commonly administered through feed or drinking water. While this practice has contributed to the industry's success, a significant risk is associated with the prolonged use of antibiotics at low levels. This can lead to the emergence of drug-resistant pathogens, which can have negative consequences for human, animal, and environmental health [4].

Additionally, the overreliance on antimicrobials for controlling diseases in the poultry industry poses financial sustainability risks, as it promotes the growth of bacterial reservoirs that are resistant to treatment [1]. Thus, it is essential to practice proper antimicrobial stewardship that reduces the use of antimicrobials in animal feeds, particularly for preventive purposes, to mitigate the impact of antimicrobial resistance on human health [5].

The rise of antibiotic-resistant bacteria in food, including *Campylobacter jejuni, Bacillus cereus, Escherichia coli,* and *Staphylococcus aureus,* necessitates a deeper understanding of their pathogenesis [6]. It is imperative to investigate alternatives to the use of preventive antibiotics to prioritize public health and ensure revenue in livestock production. One potential solution is the use of phytogenic additives, which are plant-derived and classified as non-antibiotic antimicrobials [7,8]. These additives show promise as feed supplements for promoting growth and preventing diseases in poultry. Plant-derived natural antimicrobial compounds can control both susceptible and resistant pathogens, thereby minimizing their presence in the food chain and enhancing microbial food safety [9].

Spirulina is a nutritious and widely used ingredient in broiler feed worldwide [10]. *Spirulina* is a single-celled cyanobacterium, often referred to as "blue-green algae." *Spirulina* can thrive and proliferate in saline and freshwater environments [11]. *Spirulina* is commonly used as a dietary supplement and growth promoter for animals due to its high concentrations of Fe, protein, P, and all essential and non-essential amino acids [12]. Antibiotics are used in poultry production due to ongoing efforts to decrease illnesses and improve the quality of meat and eggs [13]. The widespread use of antibiotic alternatives in the diet has led to the disappearance of drug-resistant microbes, antibiotic residues, and the development of natural microflora [14,15].

Spirulina is a safe, non-toxic, and nutritious organism that promotes growth, reproduction, and immune function in poultry and animals [16]. Broilers supplemented with Spirulina as a growth enhancer exhibit improved performance, as evidenced by a higher feed conversion ratio (FCR) and enhanced live body weight gain (BWG) [17]. Feeding chicken diets containing *Spirulina platensis* resulted in a substantial rise in meat production compared to the control group [18]. *Spirulina platensis* improves nutrient digestion and mineral absorption and reduces diarrhea [19].

Spirulina platensis is a powerful natural supplement that enhances reproductive function and strengthens the immune system [20]. *Spirulina platensis* in the diet may significantly improve the immune system's ability to process antigens (T-cells) and reduce the presence of harmful microbes [21]. Despite the promising preliminary findings on Spirulina's benefits in poultry, a comprehensive study is still needed that integrates various aspects of broiler health and performance. Specifically, research is limited on the combined impact of Spirulina supplementation on immune responses, oxidative stress markers, detailed lipid profiles, and the modulation of the caecal microbiome in broilers, particularly as a complete replacement for conventional antibiotics. Further investigation into these interconnected physiological parameters will provide a more holistic understanding of Spirulina's potential as a sustainable and effective alternative to antibiotic growth promoters in poultry production. Therefore, this research aims to examine the effects of replacing antibiotics with *Spirulina platensis* extract (SPE) in broiler chickens' diets, specifically investigating the potential benefits of this supplementation on the birds' immunity, oxidation status, lipid profile, caecal bacterial content, blood parameters, liver and kidney functions, productivity, and carcass and meat quality.

Material and Methods

Ethical Approval

The animal study has been reviewed and approved by ZU-IACUC committee. was performed in accordance with the guidelines of the Egyptian Research Ethics Committee and the guidelines specified in the Guide for the Care and Use of Laboratory Animals (2024). Ethical code number ZU-IACUC/2/F/394/2024. Written informed consent was obtained from the owners for the participation of their animals in this study.

Spirulina platensis Extract Preparation

A pure culture of *Spirulina platensis* was recovered and grown using the Zarrouk medium, which was created by Zarrouk in 1966. The composition of the Zarrouk medium included the following consistunants dissolved in 1L of water: 1 g of NaCl, 16.8 g of $NaHCO_3$, 2.5 g of $NaNO_3$, 0.5 g of K_2HPO_4, 1 g of K_2SO_4, 0.2 g of $MgSO_4.7H_2O$, 0.04 g of $CaCl_2.2H_2O$, 0.01 g of $FeSO^4.7H_2O$, and 0.08 g of EDTA. Using a 1 M KOH solution, the medium's pH was adjusted to 9.5. To initiate a fresh *Spirulina platensis* culture, 10 mL of a 5-day-old culture was added to a 250 mL amount of Zarrouk's media in 500 mL screw bottles. The bottles were placed in an environment with a constant temperature of 25±2°C and exposed to continuous light from a 36W white fluorescent lamp with an intensity of 600-800 lux for ten days. The *Spirulina platensis* pure culture was successfully obtained through the effective streaking method on Zarrouk's media. We isolated a single culture from this strain using the streaking technique on Zarrouk's medium to produce a pure culture of *Spirulina platensis* [22]. The plates were carefully stored in an environment with a temperature of 25°C and a constant light exposure of 600 lux. Once the colonies were obtained, they were carefully collected and inspected under a microscope. Zarrouk's medium was then used to preserve the *Spirulina platensis* cells on slants.

Identification of *Spirulina platensis* Isolates

The morphological identification, as determined by microscopic examination of Spirulina platensis isolates, was applied at various phases of development in Zarrouk's

medium. In the process of cold-water extraction of *Spirulina platensis*, as follows: 10 g of *Spirulina platensis* powder was homogenized in 90 mL of distilled water (1:9, *w/v)* for two hours. To produce a solid mass, the resultant supernatant was quickly frozen at a temperature of -20°C. This mass was then thawed at 4°C and subjected to centrifugation (10,000 rpm for 10 min) to separate the supernatant, which was subsequently freeze-dried (Heto PowerDry lyophilizer) until it reached a powdered state.

Identification of Volatile Compounds in SE by GC-MS Spectroscopy

One g of SPE was dissolved in 10 mL of Hexane (1:10, w/v), then sonicated for 10-30 min at room temperature. The obtained extract was filtered through centrifugation, and the supernatant was obtained. The solvent was removed under reduced pressure (rotary evaporator) to obtain the crude extract. An extract volume of 1 µL was injected into the GC-MS system (Agilent 6890, Foster City, CA), which had an HP-5 MS column and an Agilent mass spectrometer detector. The carrier gas was helium, with a flow rate of 1.0 mL/min. After adding 1 µL of volume to the sample, the solvent was left in place for three minutes. The rate of temperature increase was 8°C/min, starting at 40°C and reaching 260°C. The detector temperature was set to 280°C, while the injector temperature was maintained at 250°C. Following Saad et al.[23] Wiley 9 datasets were used to determine peaks.

Experimental Design

Five groups were carefully assigned to a total of 300 broiler chicks, ensuring a fair and balanced distribution for the study, with each group consisting of 3 replicates of 20 chicks. The standard basal diet was given to the control negative group (T1). For 35 consecutive days, the other four groups (T2, T3, T4, and T5) received a basic diet supplemented with 0.5 mg SPE/kg, 1 mg SPE/kg, 2 mg SPE/kg, and 3 mg SPE/kg, respectively. Using a randomized methodology, every chick in the study was grown on a litter model. In a shed with adequate ventilation, rice husk was employed as litter. All broiler chicks were provided with standard management conditions and access to water throughout the experiment. We tracked the weekly weights of each bird and recorded the daily feed intake for all groups. Once the experiment was over, we extracted blood from the veins in the wings and stored it in EDTA vials for further analysis.

Growth Performance

Broiler chickens were assessed for their live body weights (LBW) and feed consumption. Body weight gain (BWG) was calculated by deducting the initial live body weight (7 days old) from the final live body weight (35 days old). The feed conversion ratio (FCR) was calculated by dividing feed consumption by body weight gain, as explained in Saad et al. [24]. The performance index (PI) and the growth Rate (GR) were estimated.

$$\text{Body weight gain (BWG)} = \text{FBW} - \text{IBW} \quad (1)$$

$$\text{GR} = (\text{LBW35} - \text{LBW7})/0.5 \times (\text{LBW7} + \text{LBW35}) \quad (2)$$

$$\text{PI} = \text{BWG/FCR} \quad (3)$$

Carcass Traits

After the experiment, three birds were randomly selected from each replication, and their weights were measured. After collecting body measures, the birds were butchered to evaluate carcass features, including carcass weight, dressing percentage, and the weight of the visceral organs, which included giblets, heart, liver, and gizzard. Intestinal pH was also estimated.

Digestive Enzyme Activities

The concentrations of digestive enzymes, including amylase, protease, and lipase, in the intestine were evaluated during the investigation. The chicken ileum was dissected, and then the contents of the intestine (ileum) were carefully gathered and placed into sterile containers equipped with screw closures to prevent contamination. The activity of the enzymes in the ileum was evaluated using the methodology established by Najafi et al.[25].

Hematology

Plasma samples were obtained using gauge needles from the broiler chickens' wing veins (3 birds per replicate). The samples included 200 µL of EDTA, which was applied as an anticoagulant. To provide a comprehensive analysis of essential blood parameters, including red blood cells (RBCs), packed cell volume (PCV), haemoglobin, and white blood cells (WBCs), plasma samples were obtained in labeled screw-top tubes.

Liver and Kidney Function

Blood samples were meticulously extracted from slaughtered chicks that were 35 days old and promptly preserved in an anticoagulant-containing tube for efficient plasma extraction using high-speed centrifugation at 4000 rpm for 10 min. After the plasma was collected, it was securely sealed in a sterile tube and stored at a temperature of -20°C till it was needed. Spectrophotometers (Apel 310 Spectrophotometer, Japan) were used to measure photometric biological processes. Calorimetric analysis was conducted using specific commercial kits to evaluate the biochemical characteristics of blood components. The biochemical profiles including alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, total protein (TP), total globulin (TG), albumin/globulin (A/G) ratio,

Lipid Profile

The lipid profile, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and very low-density lipoprotein (VLDL), was assessed using a spectrophotometer and commercial kits according to the manufacturer's instructions

Immunological Parameters

The colorimetric estimation of IgA and IgG immunoglobulin isotypes was tested using a spectrophotometer with respective kits [26].

Antioxidant Status

At slaughter, nine birds from each group were blood sampled and centrifuged for twenty minutes at 4500 rpm. Subsequently, the plasma was stored at -20°C to preserve its integrity. The antioxidant key biomarkers, including SOD, CAT, glutathione, malondialdehyde, and antioxidant enzymes, were measured using top-quality commercial kits provided by Biodiagnostic company.

Estimation of Caecal Bacterial Load

For the collection of caecal content, three birds from each replicate (a total of 5 birds/group) were sacrificed at 35 days of age. The careful dissection of the caeca and collection of contents in sterile cups was performed using aseptic techniques to ensure the integrity of the samples. 1 mL of cecal content was homogenized in 9 mL of sterilized saline solution to obtain 10-1 diluation, serial diluations were conducted up to 10^{-6}. Then, 0.1 mL of each dilution was spread on specific media for counting total bacterial *Salmonella*, coliforms, *E. coli*, *Salmonella, Enterococcus,* total fungal on Nutrient, McConkey's agar, Eosin-Methylene blue agar, XLD, and *Enterococcus* agar, sabaroud dextrose agar media, respectively. The samples were applied to the agar surface using a sterilized glass spreader while the Petri dish was rotated beneath. For the total bacterial count, the nutrient broth is employed. The Petri plates were incubated at a temperature of 37°C for 24 h, and individual colonies were counted with a colony counter and quantified as:

CFU/mL = (No. of colonies ×dilution factor)/volume of the culture plate

Meat Quality

The color of the chicken breasts (2 cm in diameter) was measured using a Hunter spectrophotometer. Lipid and protein oxidation were determined according to Sayed-Ahmed et al.[27]. The chemical composition of the chicken breast was determined according to the AOAC [28]. Sensory evaluations were measured using a 9-hedonic scale [29].

Statistical Analysis

The statistical analysis was conducted using one-way ANOVA in the SPSS program (SPSS, 2021). The LSD test was used to compare all tested means (treatments) at a significance level of $P<0.05$. The sample size was calculated using the following equation: $n = (\frac{ZSD}{E})^2$.

PCA was performed using R (FactoMineR and factoextra packages) or equivalent statistical software, following standard procedures for multivariate analysis.

Results

Table 1 highlights the active components in SPE, where the main compounds in the SPE profile, detected by GC/MS, were identified as the active compounds of SPE, including heptadecane (63.21%), pentadecane (6.32%), and β-ionone (5.21%), which were found to be the primary components. These components are followed by β-cyclocitral and 2-methylisoborneol (0.77%). The SPE contains geosmin (0.06%), a critical component of cyanobacterial odor.

As mentioned in *Table 2*, supplementating the broiler diets with Spirulina platensis extract (SPE) produced clear, dose-dependent improvements in growth performance parameters. As the concentration of SPE increased from T1 (control) to T5 (highest dose), both live body weight (LBW) and body weight gain (BWG) showed progressive

Table 1. *Active compounds in Spirulina platensis extract (SPE) detected by GC-MS*

Retention Time	Volatile Compounds	% Area
10.21	2-Pentylfuran	1.55±0.2d
19.99	Geosmin	0.06±0.001f
20.53	β-cyclocitral	0.45±0.05e
27.05	2-Methylisoborneol	0.32±0.08e
31.51	β-ionone	5.21±0.3bc
32.10	Pentadecane	6.32±0.8b
34.74	Hexadecane	4.94±0.4c
37.79	Heptadecane	63.21±1.9a

Data are presented mean ±SE, n=3

Table 2. *The influence of dietary Spirulina platensis extract (SPE) at four concentrations on the growth performance parameters of broiler chickens*

Growth performance		SPE Treatments (mg/kg)					P value
		T1	T2	T3	T4	T5	
LBW (g)	1d	44.9±0.1	45.1±0.0	45±0.0	45.7±0.0	45.5±0.0	0.89
	35d	2312±11.2e	2339±10.9d	2370±12.3c	2412±11.1b	2450±10.2a	<0.0001
BWG (g)	1-35d	2267±9.8e	2294±8.7d	2325±11.2c	2366±11.3b	2405±8.9a	<0.0001
FI (g)	1-35d	3878±12.7a	3812±11.2b	3778±10.8c	3768±9.9cd	3754±8.3d	<0.0001
FCR	1-35d	1.71±0.5a	1.65±0.2b	1.62±0.1bc	1.59±0.1c	1.56±0.0c	<0.0001
GR	1-35d	196±3.2b	199±3.8a	202±2.9a	206±4.2a	210±5.2a	<0.0001
PI	1-35d	226±6.8c	231±7.5b	233±6.9b	237±5.3ab	240±6.5a	<0.0001

n=5, data are presented as mean ±SD. Different lowercase letters in the same raw indicate significant variation at P<0.05. ***T1*** *= basal diet + 0 mg/kg SPE;* ***T2****, SPE 0.5 = basal diet + 0.5 mg/kg SPE,* ***T3****, SPE 1 = basal diet + 1 mg/kg SPE,* ***T4****, SPE 2 = basal diet + 2 mg/kg SPE;* ***T5****, SPE 3 = basal diet + 3 mg/kg SPE*

Table 3. *The impact of dietary Spirulina platensis on Carcass traits and intestinal pH of broiler chickens*

Traits (%)	SPE Treatments, mg/kg					P-value
	T1	T2	T3	T4	T5	
Carcass	70.0±2.5c	73.11±3.6b	74.36±2.6a	74.9±4.1a	75.8±3.9a	0.001
Liver	2.20±0.1	2.23±0.2	2.22±0.0	2.17±0.0	2.20±0.2	0.9
Gizzard	1.9±0.2b	2.2±0.3a	2.25±0.5a	2.26±0.5a	2.25±0.6a	0.05
Heart	0.84±0.01b	0.93±0.02ab	0.95±0.01a	0.96±0.03a	0.95±0.04a	0.04
Dressing	73.23±4.5d	75.11±5.1c	77.24±5.5b	78.88±4.9b	80.89±4.5a	0.001
Intestinal pH	6.8±0.6b	6.5±0.7b	6.7±0.4b	6.8±0.6b	7.2±0.7a	0.05

n = 5; data are presented as mean ± SD. Different lowercase letters in the same row indicate significant variation at P<0.05. ***T1*** *= basal diet + 0 mg/kg SPE;* ***T2****, SPE 0.5 = basal diet + 0.5 mg/kg SPE,* ***T3****, SPE 1 = basal diet + 1 mg/kg SPE,* ***T4****, SPE 2 = basal diet + 2 mg/kg SPE;* ***T5****, SPE 3 = basal diet + 3 mg/kg SPE*

Table 4. *The influence of dietary Spirulina platensis extract (SPE) at four concentrations on the serum digestive enzymes of broiler chickens*

Digestive Enzymes Activity	SPE Treatments, mg/kg					P-value
	T1	T1	T1	T1	T1	
Amylase	310±12.3d	420±11.3c	480±13.1b	500±13.6ab	510±14.6a	<0.0001
Lipase	12±1.1e	17±0.9d	22±0.9c	26±0.6b	29±1.1a	<0.0001
Trypsin	25±0.9d	30±1.2c	36±1.3b	42±1.0ab	45±1.1a	<0.0001

n = 5; data are presented as mean ± SD. Different lowercase letters in the same row indicate significant variation at P<0.05. ***T1*** *= basal diet + 0 mg/kg SPE;* ***T2****, SPE 0.5 = basal diet + 0.5 mg/kg SPE,* ***T3****, SPE 1 = basal diet + 1 mg/kg SPE,* ***T4****, SPE 2 = basal diet + 2 mg/kg SPE;* ***T5****, SPE 3 = basal diet + 3 mg/kg SPE*

increases, with the highest dose (T5) resulting in a 5.97% increase in LBW and a 6.09% increase in BWG compared to the control. This indicates that higher levels of SPE can effectively enhance the growth rate of broiler chickens.

Feed intake (FI) demonstrated a gradual reduction as SPE concentration increased, with T5 showing a 3.20% decrease relative to T1. This reduction in feed intake, coupled with increased weight gain, led to a marked improvement in feed conversion ratio (FCR), which decreased by 8.77% at the highest SPE level. A lower FCR reflects more efficient conversion of feed into body mass, highlighting the positive impact of SPE on feed efficiency.

Additionally, both growth rate (GR) and performance index (PI) improved with higher SPE supplementation. The greatest enhancements were observed at T5, where GR increased by 7.14% and PI by 6.19% over the control. These findings collectively demonstrate that dietary inclusion of Spirulina platensis extract not only boosts growth and performance metrics but also optimizes feed utilization, making it a valuable additive for improving broiler production outcomes.

As presented in *Table 3*, dietary supplementation with Spirulina platensis extract (SPE) led to notable improvements in carcass traits and intestinal pH in broiler chickens. As the level of SPE increased from the control (T1) to the highest dose (T5), both carcass yield and dressing percentage showed significant enhancements, with the highest values observed at the greatest SPE

inclusion. This indicates that SPE can effectively boost meat yield in broilers. Additionally, there were modest but statistically significant increases in gizzard and heart percentages at higher SPE levels, suggesting a potential influence on organ development. Liver weight, however, remained unaffected across all treatments, indicating that SPE does not notably impact liver size. Intestinal pH exhibited a slight but significant increase at the highest SPE dose, which may have implications for gut health and digestive processes. Overall, the results demonstrate that increasing dietary SPE improves key carcass characteristics and dressing percentage, with minimal effect on liver weight and a modest rise in intestinal pH at higher supplementation levels.

Table 4 demonstrated that the digestive enzyme levels, such as the lipase enzyme level, showed that lipase level exhibited a substantial elevation in T5 as opposed to T1 (P-value <0.0001) while the level of protease exhibited a significant elevation of all groups supplied with *Spirulina platensis* in contrast with unsupplied birds (G1). The amylase level was substantially elevated in T4 and T5 compared to T1, T2, and T3 birds.

Fig. 1 revealed that the blood parameters of birds supplied with dietary Spirulina platensis were significantly improved compared to those of the birds (T1). The haemoglobin (Hb) level demonstrated a substantial rise in groups (T2, T3, T4, and T5), contrary to group (T1). When comparing T5 (4.5) to T1 (2.4) birds fed a 3 mg SPE/kg diet, the RBC count revealed a considerable rise. The WBC count significantly increased at T5 (17.33) compared to T1 (13.41) birds.

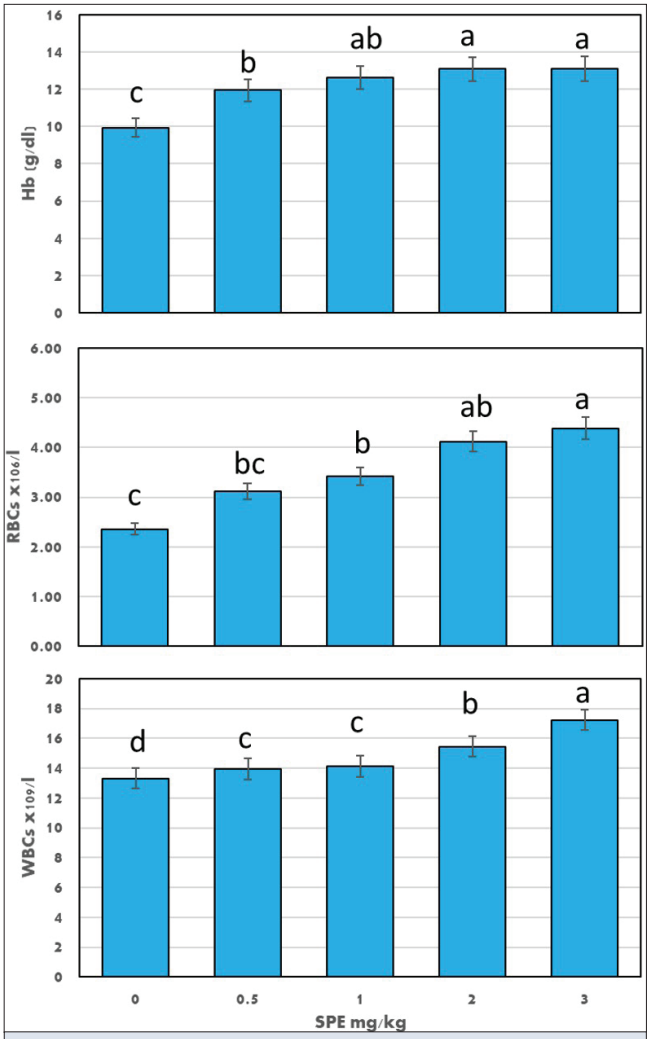

Fig 1. The effect of dietary *Spirulina platensis* extract (SPE) at four concentrations on the hematology of broiler chickens

The results in *Table 5* showed that liver and kidney functions indicated a significant increase in total protein (TP) in birds fed a diet of 3 mg/kg in T5, compared to those in T1. Albumin (ALB) levels appear to have a significant elevation in T3 birds as opposed to birds in T1, T2, and T4. Globulin (GLOB) levels showed a substantial increase in birds in T5 compared to T1, T2, T3, and T4. A notable decrease in the A/G ratio in T5 in contrast with T1. Regarding liver functions, ALT and AST levels revealed a substantial reduction in T5 compared to birds in T1. Regarding renal function, urea and creatinine levels exhibited a considerable decrease in birds supplied with different Spirulina platensis extracts from T2 to T5, as opposed to control birds (T1).

In *Table 5*, the birds in treatments (T2, T3, T4, and T5), treated with varied quantities of SE, showed a substantial drop in total cholesterol (TC) compared to those in group G1 that were not supplied with SE. Birds exhibiting the most significant stress-induced sympathetic excitation (SE) showed a notable reduction in total cholesterol (TC) levels, measured at 74 mg/dL. This drop was shown to be statistically significant compared to other groups (P-value <0.0001). Triglyceride levels (TG) showed a substantial reduction in all experimental groups supplied with SE, as opposed to birds in T1 (P-value <0.0001). The birds in T5 showed a significant increase in high-density lipoprotein (HDL), demonstrating that the highest quantity of SPE enhanced the lipid profile with a P-value of <0.0001. Low-density lipoprotein (LDL) exhibited a substantial drop in the birds supplied with different SE, while birds in T5 revealed a significant drop in LDL level (15 mg/dL) in contrast with its level in T1 (48 mg/dL) (P-value <0.0001). Very low-density lipoprotein (VLDL) appeared to have a significant decline in birds in T5 (18 mg/dL) when compared with birds in T1 (P-value <0.0001). The findings suggest that adding SPE to the birds' food improved their lipid profile compared to birds that did not receive the supplement.

The data in *Table 5* demonstrate that the birds in T5 exhibited a noteworthy increase in IgG and IgM levels (17-19%) compared to the control birds (T1). Additionally, the

Table 5. *The influence of dietary Spirulina platensis extract (SPE) at four concentrations at the serum biochemical parameters of broiler chickens*

Parameter	Serum Biochemistry	SPE Treatments (mg/kg)					P-value
		T1	T2	T3	T4	T5	
Liver and kidney functions	AST (U/L)	255±12.3a	220±11.3b	208±10.5c	189±9.5d	177±8.8e	<0.0001
	ALT (U/L)	5.9±0.5a	5.2±0.3b	4.7±0.6c	3.9±0.2d	3.1±0.2e	<0.0001
	Uric acid (mg/dL)	5.5±0.6a	4.8±0.2b	4.5±0.1c	3.8±0.5d	3.0±0.5e	<0.0001
	Creatinine (mg/dL)	0.36±0.01a	0.35±0.02a	0.32±0.05ab	0.28±0.02b	0.27±0.03b	0.05
	Total protein (g/dL)	2.8±0.6c	3.6±0.3bc	3.9±0.3b	4.2±0.4ab	4.6±0.1a	0.00123
	Albumin (g/dL)	1.8±0.2d	1.92±0.1c	2.1±0.4b	2.4±0.2ab	2.7±0.2a	<0.0001
	Globulin (g/dL)	1.2±0.3c	1.4±0.2bc	1.7±0.6b	1.9±0.3ab	2.1±0.3a	0.0011
	Albumin/Globulin (%)	1.5±0.5a	1.35±0.3b	1.23±0.2d	1.26±0.1c	1.28±0.5c	0.0023
Lipid profile	Total cholesterol (mg/dL)	235±9.2a	196±6.7b	141±7.1c	125±3.2d	111±4.5e	<0.0001
	Triglycerides (mg/dL)	192±6.8a	187±7.1b	168±6.6c	100±6.1d	74±4.3e	<0.0001
	HDL (mg/dL)	85±2.3d	92±1.9c	96±3.5c	100±3.3b	110±4.0a	<0.0001
	LDL (mg/dL)	48±0.8a	33±1.1b	25±0.7c	18±0.5d	15±0.7e	<0.0001
	VLDL (mg/dL)	46±0.9a	35±0.5b	28±0.6c	20±0.4d	18±0.2e	<0.0001
	Abdominal fat	1.33±0.2a	1.21±0.2b	0.91±0.1c	0.85±0.1d	0.69±0.01e	<0.0001
Oxidative status	GSH (ng/mL)	0.35±0.02c	0.52±0.01bc	0.59±0.03b	0.67±0.01ab	0.69±0.02a	<0.0001
	SOD (U/mL)	0.51±0.03e	0.68±0.03d	0.72±0.03c	0.83±0.01b	1.01±0.7a	<0.0001
	CAT (ng/mL)	0.33±0.02d	0.51±0.02c	0.62±0.01bc	0.68±0.02b	0.77±0.01a	<0.0001
	MDA (nmol/mL)	0.55±0.01a	0.41±0.01b	0.35±0.02c	0.30±0.03c	0.21±0.02d	<0.0001
	TAC (ng/mL)	0.35±0.02c	0.42±0.02c	0.55±0.04c	0.68±0.01b	0.85±0.03a	<0.0001
Immunity	IgG (mg/dL)	960±13.2e	1050±14.3d	1071±14.8c	1099±15.0b	1120±13.8a	<0.0001
	IgA (mg/dL)	177.8±3.5e	188.2±3.6d	191.3±4.1c	205.6±4.5b	211±4.3a	<0.0001

n = 5; data are presented as mean ± SD. Different lowercase letters in the same row indicate significant variation at P<0.05. ***T1*** *= basal diet + 0 mg/kg SPE;* ***T2****, SPE 0.5 = basal diet + 0.5 mg/kg SPE,* ***T3****, SPE 1 = basal diet + 1 mg/kg SPE,* ***T4****, SPE 2 = basal diet + 2 mg/kg SPE;* ***T5****, SPE 3 = basal diet + 3 mg/kg SPE*

Table 6. *Effect of Spirulina platensis dietary treatments on meat quality of broiler chickens*

Parameters		SPE Treatments (mg/kg)					P-value
		T1	T2	T3	T4	T5	
Chemical composition	Moisture	60.3±3.5d	62.3±4.1c	63.4±3.6b	65.9±4.2ab	66.7±5.2a	<0.0001
	Protein	20.9±1.1c	21.5±0.9bc	22.0±1.0b	23.2±1.1ab	24.0±1.3a	<0.0001
	Fat	14.1±0.4a	11.2±0.5b	10.6±0.7c	8.7±0.5d	7.1±0.3e	<0.0001
	Ash	0.88±0.01a	0.74±0.02b	0.68±0.02c	0.61±0.01c	0.60±0.02c	<0.0001
	pH	5.9±0.5c	6.1±0.3b	6.3±0.2b	6.6±0.5ab	6.7±0.3a	<0.0001
	TVBN	7.1±0.4a	5.5±0.2b	4.8±0.2c	4.4±0.1c	4.2±0.0c	<0.0001
	TBA	0.79±0.02a	0.59±0.01b	0.41±0.03c	0.28±0.01d	0.25±0.01d	<0.0001
Sensory properties	Juiciness	9±0.0a	8.8±0.1b	8.5±0.3b	8.6±0.3b	9.0±0.0a	0.025
	Tenderness	8.7±0.2ab	8.6±0.1b	8.7±0.2ab	8.7±0.2ab	8.8±0.1a	0.031
	Aroma	8.5±0.1a	8.3±0.2b	8.1±0.5b	8.2±0.4b	8.5±0.3a	0.032
	Taste	8.8±0.1a	8.5±0.2b	8.5±0.2b	8.4±0.3b	8.8±0.1a	0.034
Color properties	*L**	60±2.3d	61.2±2.5c	62.1±2.6b	62.9±2.9ab	63.1±2.1a	0.031
	*a**	6.1±0.2	5.9±0.5	6.0±0.5	6.1±0.5	6.2±0.2	0.6
	*b**	14.9±0.9	14.8±1.1	15.2±0.6	15.3±0.8	15.1±0.9	0.7

n = 5; data are presented as mean ± SD. Different lowercase letters in the same row indicate significant variation at P<0.05. ***T1*** *= basal diet + 0 mg/kg SPE;* ***T2****, SPE 0.5 = basal diet + 0.5 mg/kg SPE,* ***T3****, SPE 1 = basal diet + 1 mg/kg SPE,* ***T4****, SPE 2 = basal diet + 2 mg/kg SPE;* ***T5****, SPE 3 = basal diet + 3 mg/kg SPE*

antioxidant status in birds fed a diet containing 3 mg/kg of T5 revealed a significant improvement in MDA, SOD, CAT, GSH, and TAC levels compared to control birds (T1). The birds fed with varying levels of SE significantly improved immunity and antioxidant status compared to control birds (T1).

Table 6 illustrates that the chemical composition of chicken breast meat varied in response to different levels of SPE supplementation in the broiler diet. Notably, the group receiving 3 mg/kg of SPE exhibited the most favorable results compared to the other treatments. In this group, the moisture content increased by 25% and the protein content rose by 12% compared to the control group. These improvements in moisture and protein were reflected in the sensory evaluation, with panelists awarding the highest scores for juiciness (9) and tenderness (8.8) to the 3 mg/kg SPE group. Both the control and the 3 mg/kg SPE groups were noted for their superior taste.

Additionally, increasing SPE levels led to reductions in both fat and ash content in the breast meat. Regarding meat quality parameters, the pH value rose to 6.7, while nitrogen compounds and TBA values decreased to 4.4 and 0.25, respectively. The addition of BP significantly influenced the color characteristics of the meat, enhancing its lightness, although the a* (redness) and b* (yellowness) values remained unchanged.

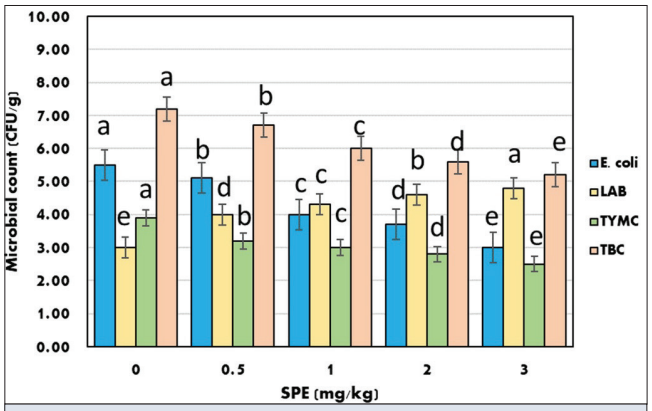

Fig 2. The influence of dietary *Spirulina platensis* extract (SPE) at four concentrations on the intestinal microbiota of broiler chickens

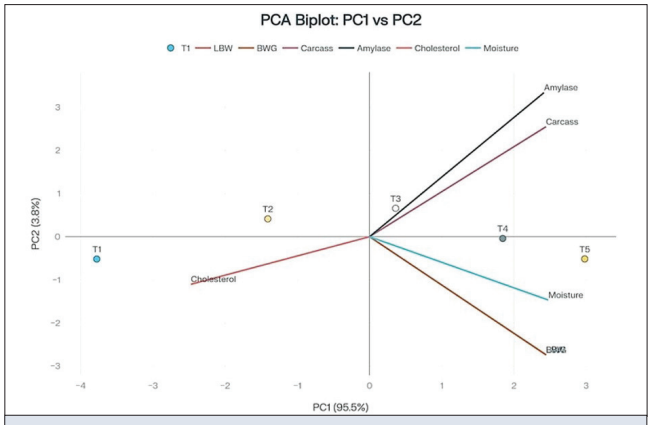

Fig 3. PCA biplot showing parameter vectors for PC1 and PC2. PC1 (95.5% variance) and PC2 (3.8% variance). Treatments (T1-T5) and measured properties (growth, blood biochemistry, enzymes, carcass, meat quality)

Additionally, the total yeast and fungal count showed a substantial decrease in T2, T3, T4, and T5, in contrast to T1. Regarding the *E. coli* count, the birds fed a diet with elevated SPE exhibited a substantial drop compared to their counts in control birds (T1). Furthermore, compared to control birds (T1), the SPE treatments showed a significant decline in TBC counts, accompanied by a considerable rise in LAB counts *(Fig. 2)*.

The PCA biplot visualizes the relationships between five treatments (T1-T5) and six measured properties: live body weight (LBW), body weight gain (BWG), carcass percentage, amylase activity, cholesterol, and meat moisture (*Fig. 3*). The first two principal components (PC1 and PC2) collectively account for over 99% of the total variance, with PC1 contributing 95.5% and PC2 contributing 3.8%. This means the biplot provides an accurate and comprehensive summary of the multivariate data structure. The results showed that T1 and T2 are positioned on the left side of the biplot, indicating lower values for most performance and quality traits, but higher cholesterol. However, T3, T4, and T5 are distributed to the right, aligning with higher values for LBW, BWG, carcass percentage, amylase activity, and moisture, and lower cholesterol levels.

LBW, BWG, carcass, amylase, and Moisture vectors all point in a similar direction, indicating these properties are positively correlated. Treatments in this direction (T3-T5) exhibit higher values for these traits. While cholesterol vector points in the opposite direction, showing a strong negative correlation with the other properties. Treatments closer to this vector (T1, T2) have higher cholesterol levels.

Fig. 3 shows that T5 is closest to the vectors for LBW, BWG, carcass, amylase, and moisture, suggesting it yields the best overall performance and meat quality, with the lowest cholesterol levels. T1 is positioned near the cholesterol vector, indicating the least favorable profile in terms of growth, carcass, and enzyme activity, but the highest cholesterol. meanwhile T3 and T4 show intermediate profiles, with improvements over T1 and T2 but not as pronounced as T5.

Discussion

The global poultry industry faces a critical challenge: how to maintain high productivity and animal health in broiler chickens while reducing or eliminating the use of antibiotics, which have been linked to the rise of antimicrobial resistance in both animals and humans. In

this context, *Spirulina platensis*, a blue-green microalga renowned for its rich nutritional and bioactive profile, has emerged as a promising natural alternative to antibiotic growth promoters (AGPs).

Multiple recent studies have consistently demonstrated that dietary supplementation with *Spirulina platensis* can significantly improve growth performance in broiler chickens. Supplementation levels ranging from 0.1% to 1% in feed have been shown to increase body weight gain, enhance feed intake, and improve feed conversion ratios (FCR) [11,30-32]. For example, Abdelfatah et al.[31] found that broilers fed 0.3% and 0.5% *Spirulina* had higher body weight gains and better FCR compared to controls. Similarly, Khan et al. [33] reported that a 1% inclusion of *Spirulina* resulted in significantly higher body weight gain and improved FCR, with optimal results at this level, while higher inclusion (2%) did not yield further benefits.

The mechanisms behind these improvements are multifaceted. *Spirulina* is rich in high-quality protein, essential amino acids, vitamins (such as B-complex and vitamin E), minerals (including iron and selenium), and bioactive compounds like phycocyanin and beta-carotene [34]. These nutrients support efficient nutrient utilization, promote muscle growth, and enhance overall metabolic activity in broilers [11,35]. Additionally, the presence of polyunsaturated fatty acids and antioxidants in *Spirulina* may help reduce oxidative stress, further supporting growth under intensive rearing conditions [36,37].

The inclusion of *Spirulina platensis* in broiler diets has been shown to beneficially modulate serum biochemical parameters, which are key indicators of animal health and metabolic status [38,39]. Several studies have reported that *Spirulina* supplementation can lowered total cholesterol, triglycerides, and LDL-cholesterol levels, while increasing HDL-cholesterol, contributing to better cardiovascular health in broilers [40,41]. Additionally, increased the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), as well as total antioxidant capacity (TAC) in serum, thereby reducing oxidative damage [42,43].

Furthermore *Spirulina* can mitigate the elevation of liver enzymes (ALT, AST) and renal markers (uric acid, creatinine) that are often associated with dietary or environmental stressors, such as ochratoxin A exposure [36]. They found that broilers supplemented with *Spirulina* exhibited significant decreases in abdominal fat percentage and improved liver and kidney function test results, even under toxin-induced stress. These effects are attributed to the bioactive compounds in *Spirulina*, including phycocyanin and phenolic acids, which possess hepatoprotective and nephroprotective properties [44,45].

Meat quality is a critical determinant of consumer acceptance and market value. Recent research indicates that *Spirulina platensis* supplementation can positively influence several aspects of broiler meat quality, where broilers fed *Spirulina* exhibit increased redness (a*) and yellowness (b*) in their meat, likely due to the deposition of natural pigments, such as beta-carotene and phycocyanin, from the microalga [30,32]. This can improve the visual appeal of poultry products. Additionally, the improved water-holding capacity results in reduced cooking and thawing losses, leading to juicier and more tender meat [32]. Meat from *Spirulina*-fed broilers tends to have higher protein levels, reflecting improved muscle accretion [30,32].

On the other hand, the antioxidant properties of *Spirulina* help maintain meat freshness and extend shelf life by reducing oxidative rancidity [31,35]. Sensory evaluations have also shown that meat from *Spirulina*-supplemented broilers is more tender, juicy, and generally preferred by panelists [30]. These improvements are particularly valuable in markets where meat quality attributes drive consumer choice.

A healthy gut microbiota is essential for nutrient absorption, immune function, and disease resistance in broilers. Antibiotics, while effective in controlling pathogenic bacteria, can disrupt the balance of gut microbiota and contribute to the development of resistance issues. In contrast, *Spirulina platensis* acts as a prebiotic and immunomodulatory agent, promoting beneficial bacteria. Studies have shown that *Spirulina* supplementation increases the population of *Lactobacillus* species in the cecum, which are associated with improved gut health and pathogen exclusion [11,46]. On the other hand, there is a concurrent decrease in *Escherichia coli* and other potential pathogens, reducing the risk of enteric infections [11].

Additionally, supplementation improves villus height, the villus-to-crypt ratio, and the villus surface area, thereby facilitating better nutrient absorption and gut barrier function [46]. It is found that when combined with native probiotics, *Spirulina* further enhances gut health and immune response, providing a robust alternative to antibiotics [37]. These effects are attributed to the oligosaccharide-rich content and bioactive molecules in *Spirulina*, which serve as substrates for beneficial microbes and modulate the gut environment [30].

One of the most compelling arguments for using *Spirulina platensis* as an alternative to antibiotics is its ability to support the immune system. *Spirulina* supplementation leads to larger bursa, thymus, and spleen, indicating enhanced development of these immune organs [47]. Also, increased WBC counts and improved phagocytic activity suggest a more robust innate immune response [11], which enhances both humoral (antibody-mediated) and cellular immune responses, making broilers more resilient to

infections [35]. Under stress conditions, such as heat or mycotoxin exposure, *Spirulina* helps maintain immune function and reduces pathological changes in lymphoid tissues [36]. These immune-enhancing effects are crucial for maintaining flock health in the absence of antibiotic protection, especially in intensive production systems.

Several studies have directly compared *Spirulina platensis* with conventional antibiotics such as enrofloxacin and zinc bacitracin. The results indicate that *Spirulina* can match or even surpass antibiotics in promoting growth, optimizing feed conversion, supporting egg and meat quality, and maintaining liver health [48,49]. For instance, a 2024 study on laying hens found that 1% *Spirulina* was as effective as zinc bacitracin in improving performance and protecting liver health, without the risk of antibiotic residues or resistance [49].

The safety profile of *Spirulina platensis* is well-established, with no reports of adverse effects at recommended inclusion levels (typically up to 1% of the diet for broilers) [31,32]. Higher inclusion rates (e.g., 15%) may impair growth, suggesting that moderation is key [30]. The optimal dosage appears to be between 0.3% and 1% of the diet, balancing efficacy and cost-effectiveness [50].

While the evidence supporting *Spirulina platensis* as an antibiotic substitute is robust, some limitations remain: Large-scale production and consistent supply of high-quality *Spirulina* are necessary for widespread adoption in the poultry industry [36]. The nutrient and bioactive content of *Spirulina* can vary depending on cultivation conditions, affecting its efficacy [30]. Long-term effects, where most studies are short-term; long-term impacts on productivity, health, and resistance development warrant further investigation.

Declarations

Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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