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Research Article

The Influences of *Spirulina platensis* as an Eco-friendly Anticoccidial Agent on Growth Performance, Blood Biochemistry, Immune Response, Gut Microbiota in *Eimeria* Challenged Broiler Chickens

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Abstract

The escalating threat of antibiotic resistance drives the search for alternatives, leading this study to investigate *Spirulina platensis* extract (SPE) as a substitute in broiler chicken feed. The research evaluated the impact of SPE on growth, antioxidant levels, blood profiles, and gut bacteria. SPE was found to contain antimicrobial organic active compounds, such as heptadecane and geosmin. A 420 broilers were divided into seven groups for a 35-day study: a control group, an *Eimeria*-infected group, and five SPE-supplemented groups. Of the supplemented groups, four received 0.5, 1, 2, or 3 mg/kg SPE, respectively, while one *Eimeria*-infected group also received 3 mg/kg SPE. Supplementation with 3 mg/kg SPE improved body structure, feed efficiency, weight gain, carcass quality, and gut pH. Liver enzymes and kidney markers were reduced. SPE lowered oxidative stress while maintaining antioxidant enzymes and improving gut microbiota and immunity. *Eimeria* infection in broiler chickens led to reduced growth performance, increased oxidative stress, and compromised immunity compared to the control group. However, the dietary inclusion of *Spirulina platensis* extract (SPE) at 3 mg/kg demonstrated a significant mitigating effect on these negative impacts in *Eimeria*-infected birds. Specifically, broilers infected with *Eimeria* and treated with SPE exhibited notable improvements in growth performance, reaching levels comparable to or surpassing those of non-infected chickens receiving SPE. Furthermore, SPE supplementation in infected birds effectively improved their antioxidant status, evidenced by reduced oxidative stress markers, and bolstered their immune response. These findings suggest that SPE possesses properties capable of counteracting the detrimental effects of *Eimeria* infection in broiler chickens, highlighting its potential as a supportive agent in managing coccidiosis and maintaining overall health and productivity in poultry.

Keywords: Anticoccidial, *Eimeria*, *Spirulina platensis,* Gut microbiota, Immune response

Introduction

The emergence of antibiotic-resistant microbes has become a significant global health concern, prompting the search for reliable and sustainable alternatives to antibiotics in livestock production. In poultry farming, antibiotics have traditionally been used to promote growth, prevent diseases, and improve feed efficiency [1]. However, the overuse of antibiotics has led to the development of resistant bacterial strains, posing risks to animal and human health [2]. Consequently, there is a growing interest in natural alternatives, such as plant extracts, probiotics, and algal-based supplements, which can enhance poultry health and productivity without contributing to antibiotic resistance.

One of the most promising natural alternatives is *Spirulina platensis*, a cyanobacterium known for its high nutritional value and bioactive compounds [3]. *Spirulina* is rich in proteins, vitamins, minerals, and anti-oxidants, making it a valuable dietary supplement for livestock [4]. Previous studies have demonstrated that *Spirulina* supplementation can improve growth performance, enhance immune function, and reduce oxidative stress in broiler chickens. For instance, research by Abdelfatah et al.[5] showed that *Spirulina* supplementation increased body weight gain and improved feed conversion ratios in broilers. Similarly, Spínola et al.[6] reported that *Spirulina* enhanced anti-oxidant enzyme activity and reduced lipid peroxidation in poultry. More recently, Alghamdi et al.[7] found that dietary supplementation with *Spirulina* improved gut microbiota

composition and immune responses in broilers, further supporting its potential as a natural growth promoter.

Despite these benefits, poultry farming faces significant challenges from infectious diseases, particularly coccidiosis caused by *Eimeria* species. Coccidiosis is one of the most prevalent and economically devastating diseases in the poultry industry, leading to reduced growth performance, impaired feed efficiency, and increased mortality [8]. *Eimeria* infection damages the intestinal epithelium, resulting in malabsorption of nutrients, diarrhea, and secondary infections [9]. Traditional control methods rely heavily on anticoccidial drugs and vaccines, but the emergence of drug-resistant *Eimeria* strains has necessitated exploring alternative strategies. Recent studies have investigated using natural products, such as plant extracts and probiotics, to mitigate the effects of coccidiosis. For example, Elbaz et al.[10] found that dietary supplementation with essential oils reduced oocyst shedding and improved growth performance in *Eimeria*-infected chickens. Similarly, El-Ghareeb et al.[11] reported that herbal extracts enhanced immune responses and reduced oxidative stress in broilers challenged with *Eimeria*.

The impact of *Eimeria* infection on poultry extends beyond growth performance, affecting the histological integrity of the intestinal tract. The parasite invades and replicates within the intestinal epithelial cells, causing severe tissue damage, inflammation, and necrosis. Studies have shown that *Eimeria* infection leads to villus atrophy, crypt hyperplasia, and increased cellular infiltration in the intestinal mucosa. For instance, Hussein et al.[12] demonstrated that *Eimeria acervulina* infection resulted in shortened villi and deepened crypts in the duodenum of broiler chickens, impairing nutrient absorption and leading to weight loss. Similarly, Attia et al.[13] reported that *Eimeria tenella* infection caused extensive damage to the cecal mucosa, characterized by epithelial sloughing, hemorrhage, and inflammatory cell infiltration. These histological changes compromise intestinal function and predispose birds to secondary infections, further exacerbating the economic losses associated with coccidiosis.

In addition to histological damage, *Eimeria* infection has profound effects on the growth performance of poultry. Infected birds exhibit reduced feed intake, poor weight gain, and decreased feed efficiency due to the malabsorption of nutrients and the metabolic demands of the immune response. Freitas et al.[14] found that broilers infected with *Eimeria maxima* had significantly lower body weight gains and higher feed conversion ratios than uninfected controls. More recently, Choi et al.[15] reported that *Eimeria*-infected chickens showed a 20-30% reduction in body weight gain and a 15-25% increase in feed conversion ratio, highlighting the detrimental impact of coccidiosis on poultry productivity. These findings underscore the need for effective strategies to mitigate the effects of *Eimeria* infection on growth performance and intestinal health.

Recent studies have further highlighted the potential of natural products in mitigating the effects of *Eimeria* infection. For example, Abd El-Ghany [16] demonstrated that dietary supplementation with garlic extract reduced oocyst shedding and improved intestinal morphology in *Eimeria*-infected broilers. Similarly, Memon et al.[17] found that supplementation with Bacillus subtilis probiotics enhanced gut health and immune responses in chickens challenged with *Eimeria*. In 2024, a study by Zhang et al.[18] revealed that *Spirulina*-based diets significantly improved intestinal integrity and reduced oxidative stress in *Eimeria*-infected broilers, further supporting its potential as a natural remedy for coccidiosis.

Given the potential of *Spirulina platensis* to improve poultry health and productivity, as well as the need for effective alternatives to control coccidiosis, this study aimed to evaluate the effects of *Spirulina platensis* extract (SPE) on broiler chickens, including those infected with *Eimeria*. The specific objectives of this study were to assess SPE's impact on broiler chickens' growth performance, including body weight gain, feed conversion ratio, and growth rate, compared to control groups. Evaluate the effects of SPE on anti-oxidant status by measuring oxidative stress markers (e.g., malondialdehyde) and anti-oxidant enzyme activity (e.g., superoxide dismutase and glutathione peroxidase). Analyze blood parameters such as liver enzymes, kidney markers, and lipid profiles to determine the systemic effects of SPE supplementation. To investigate the influence of SPE on cecal microbiota composition, focusing on the abundance of beneficial and pathogenic microbes. Examine the histological changes in the intestinal tract of *Eimeria*-infected broilers and determine whether SPE supplementation can mitigate tissue damage and inflammation. Compare the effects of SPE in healthy broilers versus *Eimeria*-infected broilers, assessing its potential as a therapeutic agent for coccidiosis.

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/489/2024. Written informed consent was obtained from the owners for the participation of their animals in this study.

Table 1. Experimental layout design

Treatment	SPE (mg/kg)	*Eimeria tenella* (2.5×10^4)	Description
G1	–	–	Negative control (NC)
G2	–	+	Positive control (PC)
G3	0.5	–	SPE-treated
G4	1	–	SPE-treated
G5	2	–	SPE-treated
G6	3	–	SPE-treated
G7	3	+	Challenged and SPE-treated

Isolation, Cultivation, Extraction, and GC-MS Analysis of *Spirulina platensis*

Spirulina platensis was isolated and cultivated in Zarrouk's medium under controlled temperature and light conditions. A pure culture was established through streaking techniques and maintained on agar slants. Morphological identification was confirmed microscopically. The collected water sample was concentrated by the filtration method and examined under a compound microscope using low-power magnification. After repeated drop-by-drop microscopy, the cyanobacteria were fixed in the tube culture for multiplication. The isolated plankton community with cyanobacteria was cultured in the conical flasks with Zarrouk's media (Zarrouk C, 1966). Different pH levels were maintained in three culture containers, viz., 5.0, 7.0, and 9.0. Since the pH maintained was alkaline, there was a gradual eradication of other contaminant plankton as they could not thrive in higher pH. For extract preparation, *Spirulina platensis* powder underwent cold-water extraction involving freezing, thawing, and centrifugation. The supernatant was then freeze-dried to yield *Spirulina platensis* extract (SPE) [19]. Organic compounds within the SPE supernatant were identified using GC-MS spectroscopy [20]. 1 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 3 min. 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. Wiley 9 datasets were used to determine peaks.

Anticoccidial Activity

Eimeria tenella pure strain (1×10^7 parasites/ml) was cultured in 96-well plates. Each well was supplemented with SPE concentrations (50, 100, 200, and 300 µg/mL) and then kept for 24 h at 28°C. The resazurin was added to each well for a colorimetric test [21].

Experimental Design

The same *E. tenella* employed for the anticoccidial activity was used for the experimental infection. *E. tenella* oocysts were obtained from the supernatant using the flotation technique, then cleaned with tap water and centrifuged at 1500 rpm for 10 min. To induce sporulation, the oocysts were immersed in a solution containing 2.5% potassium dichromate and kept for 72 h at room temperature. The sporulated oocysts were rinsed with PBS at 1500 rpm for 10 min. Sporulated oocysts were vigorously mixed with 0.5 mm sterilized glass beads [22]. The infective dose was adjusted to 2.5×10^4 *E. tenella* sporulated oocysts via the McMaster counting technique [13].

Seven groups were carefully assigned to 420 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 (G1), and G2 was *Eimeria*-infected chickens. The other four groups (G3, G4, G5, and G6) 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; meanwhile, G7 was *Eimeria*-infected chickens and treated with 3 mg SPE/kg *(Table 1)*. 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 standard management conditions and water availability throughout the experiment. We tracked the weekly weights of each bird and recorded the daily feed intake for all groups. At the end of the 35-day experiment, blood was collected from the wing veins of 5 brids from each group, and the samples were collected and stored in EDTA vials for further analysis.

Growth Performance

Broiler chickens were assessed for their live body weights (LBW) and feed consumption. By deducting the beginning live body weight (7 days old) from the ending live body weight (35 days old), the body weight increase (BWG) is calculated by the feed conversion ratio (FCR) and divided by feed consumption following Saad et al.[23]. The performance index (PI) and the growth Rate (GR) were estimated [24].

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

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

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

Carcass Traits

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

Digestive Enzyme Activities

During the investigation, we carefully evaluated the concentrations of digestive enzymes in the intestine, namely amylase, protease, and lipase, with one measurement per duplicate. We dissected the chicken ileum. The contents of the intestine (ileum) were carefully gathered and put into sterile containers equipped with screw closures to avoid any contamination. The enzymes' activity in the ileum was evaluated using the methodology established by Najafi et al.[25].

Blood Parameter Determination

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), the plasma samples were obtained in labeled screw-top tubes.

Liver, 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, TC, TG, LDL, HDL, and VLDL, the assessment was conducted using a spectrophotometer and suitable kits following the instructions provided by the manufacturer.

Immunological Parameters

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

Anti-oxidant 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. Key biomarkers, including SOD, CAT, glutathione, malondialdehyde, anti-oxidant enzymes, and total anti-oxidant capacity, were assessed using top-quality commercial kits from a leading biodiagnostic company.

Histopathological Investigation

Intestinal tissues were collected, fixed in 10% formalin for 48 h, and then processed using an automated tissue processor. Following fixation, tissues were washed in distilled water for 30 min and then dried using different immersions in alcohol with different concentrations (70% for 120 min, 90% for 90 min). The dehydration was cleared by applying numerous cycles of xylene. Briefly, tissues were submerged in xylene (50%) for 60 min and alcohol (50%), then pure xylene for an additional 90 min. The tissues were put with melted paraffin wax, sealed, then paraffin cut sections for 4-5 µm, and then stained with Hematoxylin & Eosin [27].

Estimation of Caecal Bacterial Load

Three birds from each replicate (a total of 9 birds/group) were sacrificed at 35 days of age to collect caecal content. 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. The inoculums were carefully diluted using a 1 mL to 9 mL ratio of the suspension to normal saline in sterile ependymal tubes. This approach ensured the accuracy and reliability of our research process. The caecal samples underwent a process of repeated dilution, resulting in a dilution level of 10^{-6}. Then, 0.1 mL of the dilution was pipetted out and incubated for total bacterial count, coliforms, *E. coli*,

Salmonella, enterococcus, total fungal count, in Nutrient, McConkey's agar, Eosin-Methylene blue agar, XLD, and *Enterococcus* agar, sabaroud dextrose agar respectively. The samples were applied to the agar surface using a sterilized glass spreader while rotating the Petri dish beneath. For the total bacterial count, the nutrient broth is employed. The Petri dish was incubated at a temperature of 37°C for 24 h, and individual colonies were calculated with a colony counter and quantified as: CFU/mL = (No. of colonies x dilution factor)/volume of the culture plate.

Statistical Analysis

The statistical analysis was conducted using one-way ANOVA in 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: $n = (\frac{ZSD}{E})^2$.

Results

Table 2 highlighted the active components in SPE, where the GC-MS analysis table of *Spirulina platensis* extract highlights a diverse array of bioactive organic compounds, notably long-chain alkanes (such as octacosane, heneicosane, pentacosane, hexacosane), fatty acids (palmitic acid/n-hexadecanoic acid and cis-9-hexadecenoic acid), aromatic phenolic compounds, and organic esters. The most abundant constituents are octacosane and heneicosane, which together account for a substantial portion of the extract's composition. These compounds are well recognized for their membrane-disrupting, antioxidant, anti-inflammatory, and anti-parasitic properties, supporting the anticoccidial efficacy attributed to *Spirulina* extracts. The presence of both direct antiparasitic agents (alkanes, fatty acids) and supportive compounds (phenolics, esters) suggests a multimodal mechanism, contributing to gut protection and enhanced resistance against *Eimeria* infection. As mentioned in *Table 3*, the body weight demonstrated a substantial elevation in weeks three & five in all groups treated with SPE compared to control negative birds. From the first to third weeks of age, the body gain was considerably higher in all groups administered different dosages of SPE compared to the control group (T1), which fed a basic diet only. Compared to the birds in groups T1 and T5, there was a substantial elevation in body weight gain (BWG) for the birds in groups T2, T3, T4, and T5 throughout the third and fifth weeks of their lives. The cumulative body weight gain over the experimental time from week one to week five showed that all *Spirulina platensis* extract-supplied birds revealed a substantial elevation in BWG as opposed to T1, and the birds supplied with 3 mg SPE/kg diet revealed the best BWG (2405 g) at P-value <0.0001. The results in *Table 3* showed that feed intake declined substantially in all birds supplied with *Spirulina platensis* extract, contrasting with unsupplied birds (T1). Between the third and fifth weeks of age, it became apparent that all birds provided with SPE exhibited a substantial improvement in feed conversion ratio (FCR), indicating a reduction in FCR as opposed to the birds (T1). The cumulative FCR was the best (1.76 and 1.83) at (T4 and T5), respectively, (P-value (0.0235). *Eimeria*-infected chickens without SPE treatment showed reduced growth performance compared to the control group. However, *Eimeria*-infected chickens treated with SPE at 3 mg/kg demonstrate improved growth performance compared to untreated *Eimeria*-infected chickens, which is slightly lower than those treated with SPE at 3 mg/kg.

Table 4 shows no notable disparities in the liver's total weight and the percentage of the heart. The carcass and dressing exhibited a statistically significant increase in Group 5 (3 mg/kg diet) compared to Group 1 (birds), with values of 75.8 and 80.89, respectively, compared to 70 and 73 in the control birds. In addition, the examination of pH levels in the intestines revealed a substantial decrease

Table 2. *Organic active compounds in Spirulina platensis extract (SPE) detected by GC-MS*

Retention Time	Organic Active Compounds	% Area
9.60	Phenol, 2,5-bis(1,1-dimethyethyl)	1.01
15.16	Oxalic acid, isobutyl pentyl ester	0.52
27.02	Tricosane	2.63
29.13	Heneicosane	24.41
29.59	Pentacosane	10.75
30.86	Hexacosane	10.63
33.65	Octacosane	28.66
34.10	cis-9-Hexadecenoic acid	1.95
34.28	n-Hexadecanoic acid	2.12
36.49	Tetrapentacontane, 1,54-dibromo	1.86

a-f different lowercase letters at the same column indicate significant differences at P<0.05

Table 3. *The influence of dietary Spirulina platensis extract (SPE) at four concentrations at the growth performance parameters of broiler chickens*

SPE Treatments (mg/kg)	LBW (g)		BWG (g)	FI (g)	FCR	GR	PI
	1d	35d	1-35d	1-35d	1-35d	1-35d	1-35d
NC	44.9	2312e	2267e	3754d	1.59d	196b	131c
PC	44.8	2100f	2055f	3800e	1.85e	180d	120d
SPE 0.5	45.1	2339d	2294d	3768cd	1.65c	199a	134b
SPE 1	45.0	2370c	2325c	3778c	1.70bc	202a	136ab
SPE 2	45.7	2412b	2366b	3812b	1.76b	206a	139a
SPE 3	45.5	2450a	2405a	3878a	1.83a	210a	143a
PC+SPE 3 mg/kg	45.3	2380c	2335c	3820b	1.78b	204a	138a
P-value	0.89	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

a-e different lowercase letters at the same column indicate significant differences at P<0.05. SEM[1]: Pooled standard error, LBW: Live body weight, BWG: body weight gain, FCR: feed conversion ratio, PI: performance index, GR: growth rate, Control = basal diet + 0 mg/kg SPE; SPE 0.5 = basal diet + 0.5 mg/kg SPE, SPE 1 = basal diet + 1 mg/kg SPE, SPE 2 = basal diet + 2 mg/kg SPE; SPE 3 = basal diet + 3 mg/kg SPE

Table 4. *The impact of dietary Spirulina platensis on Carcass traits and intestinal pH of broiler chickens*

Traits (%)	SPE Treatments (mg/kg)							*P*-value
	NC	PC	0.5	1	2	3	PC+SPE	
Carcass	70.0c	68.5d	73.11b	74.36a	74.9a	75.8a	72.5b	0.001
Liver	2.20	2.18	2.23	2.22	2.17	2.20	2.19	0.9
Gizzard	1.9b	1.8c	2.2a	2.25a	2.26a	2.25a	2.1b	0.05
Heart	0.84b	0.80c	0.93ab	0.95a	0.96a	0.95a	0.90b	0.04
Dressing	73.23d	71.50e	75.11c	77.24b	78.88b	80.89a	76.50c	0.001
Intestinal pH	6.8b	7.0a	6.5b	6.7b	6.8b	7.2a	6.9a	0.05

a-e Means within the same raw with different superscripts differ significantly (P≤0.05). Control = basal diet + 0 mg/kg SPE; SPE 0.5 = basal diet + 0.5 mg/kg SPE, SPE 1 = basal diet + 1 mg/kg SPE, SPE 2 = basal diet + 2 mg/kg SPE; SPE 3 = basal diet + 3 mg/kg SPE

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

SPE Treatments (mg/kg)	Amylase	Lipase	Trypsin	*P*-value
0 (Control)	310d	12c	25d	<0.0001
0 (*Eimeria*-infected)	280e	10d	20e	<0.0001
0.5	420c	17d	30c	<0.0001
1	480b	22c	36b	<0.0001
2	500ab	26b	42ab	<0.0001
3	510a	29a	45a	<0.0001
3 (*Eimeria*-infected)	490b	24b	40b	<0.0001

a-e Means within the same column with different superscripts differ significantly (P≤0.05). Control = basal diet + 0 mg/kg SPE; SPE 0.5 = basal diet + 0.5 mg/kg SPE, SPE 1 = basal diet + 1 mg/kg SPE, SPE 2 = basal diet + 2 mg/kg SPE; SPE 3 = basal diet +3 mg/kg SPE

in G2 (6.5) and G5 (7.2) compared to the birds in G1 (6.8) (P-value of 0.05). The *Eimeria*-infected chickens without SPE treatment showed reduced carcass traits and altered intestinal pH compared to the control group. The treatment with SPE at 3 mg/kg demonstrated improved carcass traits and intestinal pH compared to untreated *Eimeria*-infected chickens.

Table 5 demonstrated that the digestive enzyme levels 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 substantially elevated T4 & T5 compared to T1, T2, and T3 birds. The *Eimeria*-infected chickens without SPE treatment reduced the activities of digestive enzymes compared to the control group. Meanwhile, the SPE (3 mg/kg) treatment improved digestive enzyme levels compared to untreated *Eimeria*-infected chickens.

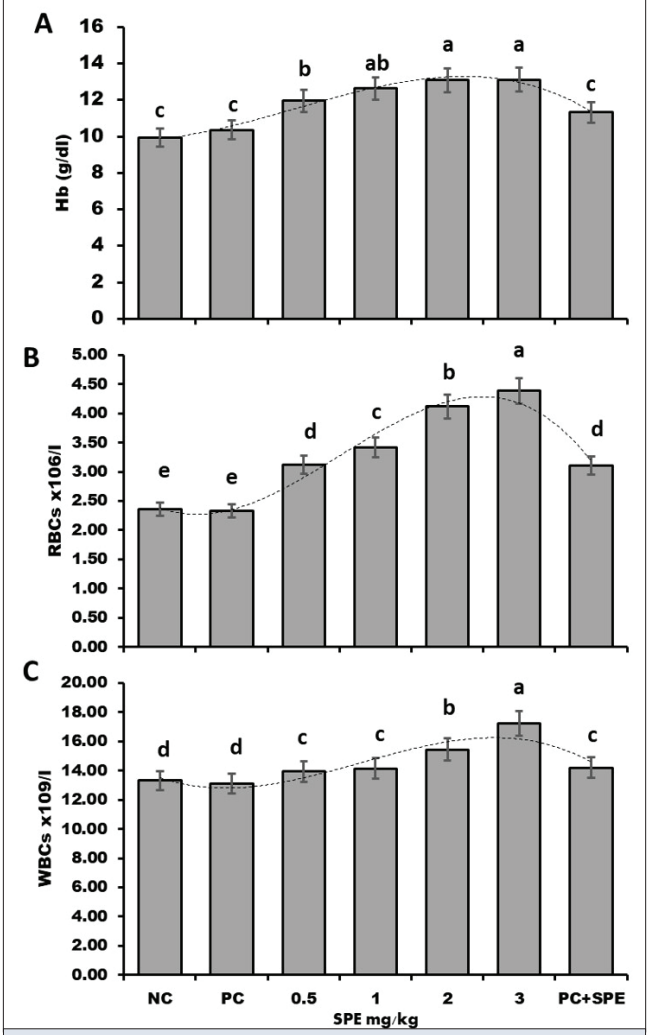

Fig 1. The effect of dietary *Spirulina platensis* extract (SPE) at four concentrations on the hematology of *Eimeria-infected* broiler chickens

Fig. 1 revealed that the blood parameters of birds supplied with dietary *Spirulina platensis* were significantly improved compared to those of birds in the control group (T1). The haemoglobin (Hb) level demonstrated a substantial rise in groups (T2, T3, T4, and T5), contrary to group (T1). When comparing G5 (4.5) to G1 (2.4) birds fed a 3 mg SPE/kg diet, the RBCs count revealed a considerable rise. The WBC count significantly increased T5 (17.33) compared to G1 (13.41) birds.

The *Eimeria* challenge (PC group) resulted in slightly increased liver enzyme levels (AST and ALT) compared to the healthy control (NC), although this increase was not always statistically significant. However, SPE treatment consistently and significantly reduced both AST and ALT compared to the challenged group, with the 3 mg/kg dose of SPE showing the most pronounced decrease (approximately a 34.4% reduction in AST and a 49.2% reduction in ALT relative to PC), bringing levels closest to those of the healthy control. Even in the presence of the parasite (PC+SPE group), SPE significantly lowered these enzymes (approximately 29.6% reduction in AST and 35.5% reduction in ALT relative to PC), demonstrating its ability to mitigate liver damage during infection. Notably, the AST and ALT values in the PC+SPE group approached those observed in the SPE 3 mg/kg group, highlighting its therapeutic potential *(Table 6)*.

Eimeria challenges slightly elevated uric acid, but didn't significantly impact creatinine. SPE treatment reduced uric acid considerably across all doses. It slightly decreased creatinine at higher doses, with the 3 mg/kg SPE group exhibiting the lowest uric acid levels (approximately 50% reduction relative to PC). The PC+SPE group also showed a considerable decrease in uric acid (approximately 35% relative to PC), suggesting that SPE benefits kidney function even during infection. The *Eimeria* challenge resulted in a reduction of total protein and albumin (approximately 10.7% and 5.9% reductions, respectively, relative to the NC). SPE treatment dose-dependently increased both, with the 3 mg/kg SPE group surpassing control levels (approximately 64.3% increase in total protein and 50% increase in albumin relative to PC). The PC+SPE group also showed a significant increase in total protein (approximately 60% increase relative to PC) and albumin (approximately 35.3% increase relative to PC), indicating SPE's role in recovery. Globulin levels also decreased with *Eimeria* (approximately an 8.3% reduction relative to NC), and SPE treatment increased them, with the 3 mg/kg dose showing the highest values (approximately a 90.9% increase relative to PC), similar to the PC+SPE group (approximately a 63.6% increase relative to PC). The albumin/globulin ratio was slightly higher in the challenged group (approximately a 3.3% increase relative to the NC group), and SPE treatment decreased it, with the 3 mg/kg SPE and PC+SPE groups showing comparable lower ratios (approximately a 17.7% and 17.4% reduction, respectively, relative to PC).

Eimeria challenges result in a slight increase in total cholesterol, triglycerides, and LDL (approximately 6.4%, 4.2%, and 4.2% increases relative to NC), while decreasing HDL (approximately 5.9% reduction relative to NC). SPE treatment significantly and dose-dependently reduced total cholesterol, triglycerides, LDL, and VLDL and increased beneficial HDL levels. The 3 mg/kg SPE group consistently demonstrated the most substantial improvements in all lipid parameters (approximately 55.6% reduction in total cholesterol, 62.5% reduction in triglycerides, 70% reduction in LDL, and 62.5% reduction in VLDL relative to PC, and a 37.5% increase in HDL relative to PC), reaching the lowest levels of harmful lipids and the highest HDL. The PC+SPE group also showed marked reductions in cholesterol (approximately 48% reduction), triglycerides (approximately 52.5% reduction), LDL (approximately 60% reduction), and

Table 6. *The influence of dietary Spirulina platensis extract (SPE) at four concentrations at the serum biochemical parameters of broiler chickens*

Serum Biochemistry		SPE Treatments (mg/kg)							P value
		NC	PC	0.5	1	2	3	PC+SPE	
Liver and kidney functions	AST (U/L)	255a	270a	220b	208c	189d	177e	190d	<0.0001
	ALT (U/L)	5.9a	6.2a	5.2b	4.7c	3.9d	3.1e	4.0d	<0.0001
	Uric acid (mg/dL)	5.5a	6.0a	4.8b	4.5c	3.8d	3.0e	3.9d	<0.0001
	Creatinine (mg/dL)	0.36a	0.38a	0.35a	0.32ab	0.28b	0.27b	0.29b	0.05
	Total protein (g/dL)	2.8c	2.5d	3.6bc	3.9b	4.2ab	4.6a	4.0b	0.00123
	Albumin (g/dL)	1.8d	1.7d	1.92c	2.1b	2.4ab	2.7a	2.3b	<0.0001
	Globulin (g/dL)	1.2c	1.1c	1.4bc	1.7b	1.9ab	2.1a	1.8b	0.0011
	Albumin/Globulin (%)	1.5a	1.55a	1.35b	1.23d	1.26c	1.28c	1.27c	0.0023
Lipid profile	Total cholesterol (mg/dL)	235a	250a	196b	141c	125d	111e	130d	<0.0001
	Triglycerides (mg/dL)	192a	200a	187b	168c	100d	74e	95d	<0.0001
	HDL (mg/dL)	85d	80d	92c	96c	100b	110a	98b	<0.0001
	LDL (mg/dL)	48a	50a	33b	25c	18d	15e	20d	<0.0001
	VLDL (mg/dL)	46a	48a	35b	28c	20d	18e	22d	<0.0001
	Abdominal fat	1.33a	1.48a	1.21b	0.91c	0.85d	0.69e	0.8d	<0.0001
Oxidative stress	GSH (ng/mL)	0.35c	30d	0.52bc	0.59b	0.67ab	0.69a	0.65b	<0.0001
	SOD (U/mL)	0.51e	0.45e	0.68d	0.72c	0.83b	1.01a	0.80b	<0.0001
	CAT (ng/mL)	0.33d	0.28d	0.51c	0.62bc	0.68b	0.77a	0.70b	<0.0001
	MDA (nmol/mL)	0.55a	0.60a	0.41b	0.35c	0.30c	0.21d	0.32c	<0.0001
	TAC (ng/mL)	0.35c	0.30c	0.42c	0.55c	0.68b	0.85a	0.70b	<0.0001
Immunity	IgG (mg/dL)	960e	900e	1050d	1071c	1099b	1120a	1100b	<0.0001
	IgA (mg/dL)	177.8e	170e	188.2d	191.3c	205.6b	211a	200b	<0.0001

a-e different lowercase letters at the same raw indicate significant differences at P<0.05. ALT Alanine aminotransferase: AST Aspartate aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, T_3: Triiodothyronine T_4: Thyroxine IgG, IgA Immunoglobulins Isotypes G, and A. Control = basal diet + 0 mg/kg SPE; SPE 0.5 = basal diet + 0.5 mg/kg SPE, SPE 1 = basal diet + 1 mg/kg SPE, SPE 2 = basal diet + 2 mg/kg SPE; SPE 3 = basal diet + 3 mg/kg SPE

VLDL (approximately 54.2% reduction) relative to PC, and an increase in HDL (approximately 22.5% increase relative to PC), though generally not as extreme as the highest SPE dose alone. Abdominal fat was also slightly higher in the challenged group (approximately 11.3% increase relative to NC). SPE treatment significantly reduced it, with the 3 mg/kg SPE group showing the greatest reduction (approximately 53.4% relative to PC). The PC+SPE group also exhibiting a significant decrease (approximately 45.9% reduction relative to PC).

Eimeria challenge significantly increased markers of oxidative stress (MDA) and decreased anti-oxidant defenses (GSH, SOD, CAT, TAC) (approximately 9.1% increase in MDA, 14.3% decrease in GSH, 11.8% decrease in SOD, 15.2% decrease in CAT, and 14.3% decrease in TAC relative to NC). SPE treatment significantly and dose-dependently reversed these effects, with the 3 mg/kg SPE group showing the most substantial increase in anti-oxidant enzyme activity and non-enzymatic anti-oxidant levels (approximately 130% increase in GSH, 124.4% increase in SOD, 175% increase in CAT, and 183.3% increase in TAC relative to PC), and the greatest reduction in MDA (approximately 65% reduction relative to PC). The PC+SPE group also demonstrated a significant recovery in all anti-oxidant markers (approximately 116.7% increase in GSH, 77.8% increase in SOD, 150% increase in CAT, and 133.3% increase in TAC relative to PC) and a reduction in MDA (approximately 46.7% reduction relative to PC), indicating SPE's protective effect against oxidative damage even during infection, with values often approaching those of the 3 mg/kg SPE group.

Eimeria challenge slightly decreased IgG and IgA levels (approximately 6.2% and 4.4% reduction, respectively, relative to NC). SPE treatment significantly and dose-dependently increased both immunoglobulins, with the 3 mg/kg SPE group exhibiting the highest levels (approximately 24.4% increase in IgG and 24.1% increase in IgA relative to PC). The PC+SPE group also showed a considerable increase in both IgG (approximately 22.2% increase relative to PC) and IgA (approximately 17.6% increase relative to PC), suggesting an enhanced immune response even in the presence of the parasite, with levels comparable to the higher SPE doses (*Table 6*).

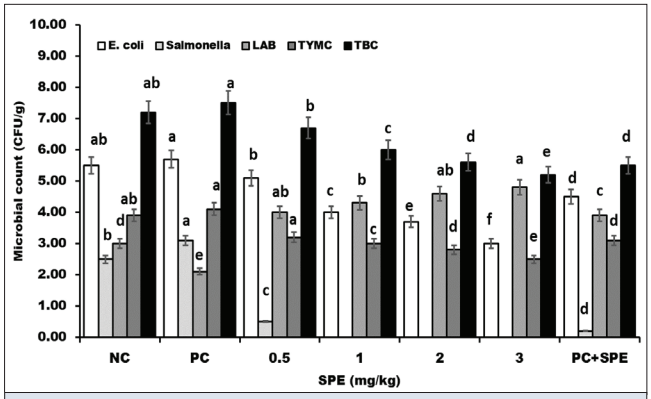

Fig 2. The influence of dietary *Spirulina platensis* extract (SPE) at four concentrations on the intestinal microbiota of *Eimeria-infected* broiler chickens

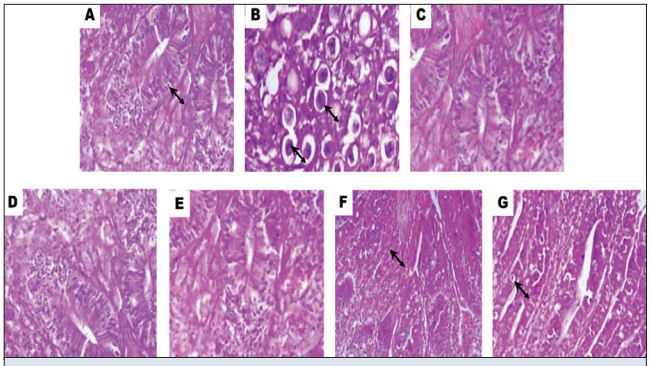

Fig 3. Photomicrographs, stained with Hematoxylin and Eosin (H&E), offer a histological evaluation of the impact of different concentrations of *Spirulina* extract on coccidiosis in broiler intestine. A, representing the negative control group, B, *Eimeria*-infected broiler chickens, C-F were SPE-treated broilers at different concentrations (0.5, 1, 2, 3 mg/kg), and G was *Eimeria-infected* broiler chickens and treated with SPE (3 mg/kg)

Spirulina extract, particularly at 3 mg/kg, generally mitigated the negative biochemical changes induced by *Eimeria* challenge to the greatest extent, often reversing the effects by significant percentages. SPE treatment consistently showed protective and restorative effects, and the PC+SPE group demonstrated that SPE could effectively counteract many of the detrimental effects of the parasite, often with results showing substantial percentage improvements compared to the challenged group alone and sometimes approaching the efficacy of the highest SPE dose.

Additionally, the total yeast and fungal count showed a substantial decrease in T2, T3, T4, & T5, contrasting with T1. Regarding the *E. coli* count, the birds fed a diet with the elevated SPE exhibited a substantial drop in contrast with their counts in control birds (G1). Furthermore, compared to control birds (T1), The SPE treatments showed a significant decline in TBC counts with a considerable rise in LAB count *(Fig. 2)*.

Fig. 3 shows that the photomicrographs, stained with Hematoxylin and Eosin (H&E), illustrate the effect of different *Spirulina* extract concentrations on broilers' intestinal histology. *Fig. 3-A*, representing the control group, exhibits normal intestinal villi architecture with intact villi, as indicated by the arrow. *Fig. 3-B* depicts a group under an unspecified condition, revealing significant alteration and parasitic presence within the intestinal tissue, marked by the arrow, as a reference for the treated groups. *Fig. 3-C* shows the intestinal tissue of broilers treated with 0.5 mg/kg *Spirulina* extract, displaying some preservation of villi structure with minimal pathological changes compared to *Fig. 3-B*. The group treated with 1 mg/kg *Spirulina* extract *(Fig. 3-D)* demonstrates improved intestinal villi structure and reduced pathological changes compared to the lower dose. Further improvement in villi integrity and minimal signs of damage are observed in Image E, representing the 2 mg/kg *Spirulina* extract treatment group. Image F showcases the intestinal tissue of broilers treated with the highest concentration of *Spirulina* extract, 3 mg/kg, exhibiting well-preserved intestinal villi architecture, closely resembling the control group (A). Finally, Image G illustrates the intestinal histology of an *Eimeria*-infected broiler treated with 3 mg/kg *Spirulina* extract, showing some preservation of villi structure and a reduction in the severity of damage typically associated with *Eimeria* infection, as indicated by the arrow, suggesting a protective effect of *Spirulina* extract in this context compared to a potentially untreated infected group.

From the microscopic images, control Group exhibits a healthy intestinal architecture, with a high VH:CD ratio (10.00), indicating optimal absorptive function and low crypt cell proliferation. Meanwhile *Spirulina*-treated group shows an increased villus height and a shallower crypt compared to control, resulting in a markedly higher VH:CD ratio (14.44). This suggests an enhancement in gut absorptive capacity and mucosal health-typically interpreted as a protective or promotive effect. However, *Eimeria*-challenged group demonstrates significant villus atrophy and deepened crypts, reflected in a much lower VH:CD ratio (4.67), indicative of intestinal damage and reduced absorption, commonly associated with coccidial infection.

Discussion

Coccidiosis is a widespread and economically significant parasitic disease in poultry caused by protozoan parasites of the genus *Eimeria*. These parasites infect the intestinal tract of chickens, leading to malabsorption of nutrients, diarrhea (often bloody), reduced growth rates, increased susceptibility to other diseases, and potentially high mortality rates, particularly in young birds. Control strategies have traditionally relied on anticoccidial drugs, but the emergence of drug-resistant *Eimeria* strains necessitates the exploration of alternative preventative and therapeutic approaches.

Discover the power of *Spirulina platensis*, a naturally occurring blue-green algae that grows in freshwater lakes [28]. Since ancient times, humans have consumed *Spirulina platensis*, and recent scientific findings confirm that *Spirulina platensis* is packed with essential fatty acids, protein, minerals, vitamins, amino acids, and volatile compounds, i.e., heptadecance that possess considerable activity [29]. These compounds showed anticoccidial properties through different mechanisms, where active compounds pass through membrane interference and disruption of parasite cellular processes, multiple alkanes and fatty acids present in the extract can reduce *Eimeria* oocyst viability and hinder parasite development. Also, phenolics and fatty acids found in *Spirulina* can modulate the inflammatory response, attenuate tissue damage, and foster mucosal recovery during infection episodes. Several identified compounds-particularly phenolics and organic esters-bolster the antioxidant capacity of the gut, mitigating the oxidative tissue injury caused by coccidial infection [29]. The array of bioactive organic compounds in *Spirulina platensis* extract-especially various alkanes, fatty acids, and phenolic agents-provides a multifaceted anticoccidial effect by disrupting parasite integrity, supporting host immune and antioxidative responses, and aiding recovery from intestinal pathology.

The main aim of this research was to evaluate the practicality of including SPE (selenium) in the diet of broiler chicks and its effects on their performance, lipid profiles, anti-oxidant activity, immune response, blood measurements, gut enzymes, and microbiota. *Table 2* and *Table 3* illustrates the impact of a food supplement containing SPE on the growth of Japanese broiler chicks from day 1 to day 35. The data indicates that broiler chicks were provided with *Spirulina platensis* supplementation for 35 days. Upon the completion of the experiment, the birds that were given a diet containing 3 mg of *Spirulina platensis* per kilogram of food had noticeably elevated body weight (BW), body weight gain (BWG), and lower feed conversion ratio (FCR) as opposed to the birds in the control group (G1) who fed a standard diet. This may be attributed to the fact that *Spirulina platensis* aids in preserving birds' natural microflora health, facilitating their ability to digest food and carry out efficient metabolic processes by absorbing essential vitamins and minerals. The findings align with Hanafy [30] study, which also saw comparable outcomes in broiler chickens given *Spirulina platensis* supplements. Hanafy noted that the enhanced absorption of minerals and vitamins caused the growth of the chicks' live body weight to rise. Also, *Spirulina platensis* might be applied as a substitute for antibiotics to enhance growth [31].

The study's findings, which evaluated the impact of several dietary doses of SPE (0.5, 1, 2, and 3 mg/kg) on several slaughter parameters, including the internal organs weight (Liver, heart, gizzard, and percentage of carcass body weight at 35 days), are shown in *Table 4*. When the subjects were 35 days old, the results indicated that adding *Spirulina platensis* to their diet significantly improved several slaughter criteria. The outcomes of this research are consistent with Hanafy [30] observations, which demonstrated enhanced carcass features and decreased abdominal fat in chickens that were given *Spirulina platensis*.

In contrast to the control group, the whole giblet data, which included the liver, heart, and gizzard, showed a substantial rise. The findings reported by Zahir et al.[31] are consistent with this observation. Moreover, the prior data align with the findings of Abou-Zeid et al.[32], which indicate that the collected data demonstrate that birds fed 2 mg SE/kg diet achieved higher average body weight. There were significant differences in the percentage of carcass and abdominal fat among the groups, but no significant differences were observed in the weight of the liver, heart, and gizzard.

Our research suggests that using the SPE raised the efficiency of digestive enzymes, potentially explaining the reason for the bird's increased output. Furthermore, these enzymes improved the bird's ability to digest feed and absorb nutrients. In addition, the groups that were provided with SE had a decreased microbial burden in the caecum as opposed to the control birds (G1). *Spirulina platensis* powder positively impacts the structure of the intestines, leading to longer villi and an increased number of goblet cells [33]. Additionally, it promotes a healthier population of microorganisms in the intestines, characterized by a higher abundance of *Lactobacillus* sp. and a decrease in *E. coli*. Phyto-additives positively impact nutritional digestibility primarily by increasing the production and enhancing the activity of digestive enzymes and improving gut morphology [34]. Enhanced absorption may cause improved protein digestibility, as seen in feed supplemented with *Spirulina platensis,* promoting broiler chickens' development [35].

In this study, groups supplied with SE significantly improved blood parameters, including WBCs, RBCs, and Hb levels. The present investigation assessed the clinical blood biochemicals, the state of the birds' health, the levels of circulating total protein, globulins, A/G ratio, and liver and kidney functions. These parameters showed improvement in the groups that were provided with SE. *Spirulina platensis* supplementation directly impacted blood hemoglobin levels because of its tendency and rich mineral content to alter the permeability and health of the intestines [36]. Furthermore, it was shown that increased hemoglobin levels were seen when 5 or 10 g of *Spirulina platensis* powder were added to the broiler diet [33].

The current study found that birds that got SE substantially improved their lipid profile compared to the control group (G1). Similar results were found by Abd El-HadyEl-Ghalid [37], who stated that compared to the control group, adding *Spirulina platensis* (3-6%) to broiler diets substantially decreased TC, TG, and LDL. The *Lactobacillus* population is increased by the dietary supplementation of *Spirulina platensis*, which results in the hypogastric-intestinal tract, which could be responsible for the cause of the broiler chickens' decreased blood lipid profile after consuming *Spirulina platensis* [38]. Serum high-density lipoprotein (HDL) concentration of broilers of 1, 2 gm *Spirulina platensis* significantly ($P \leq 0.05$) increased. The reduction in serum cholesterol was attributed to the impact of *Spirulina platensis* on lipoprotein metabolism and the elevation of lipoprotein enzyme activity levels. *Spirulina platensis* enhances the functions of hepatic triglyceride lipase and lipoprotein lipase, thereby reducing cholesterol levels in the liver and circulation. The lipases break down triglycerides and cholesterol. *Spirulina platensis* may increase the activity of these enzymes, which can reduce cholesterol levels while promoting overall heart health [39]. SA Majid et al.[40] noticed that oxidative stress increases the formation of ROS, which causes biomolecules, including nucleic acids, proteins, and enzymes, to become denaturized. Reduced protein consumption and a deficiency in vital amino acids may exacerbate oxidative damage brought on by heat stress, which may also be associated with the decline in blood protein levels [41].

Our results showed that the anti-oxidant and immunological status were improved in broiler chicks supplied with SPE instead of control birds (G1). Polyphenols are natural anti-oxidants with anti-oxidant and antimicrobial activity [42]. Anti-oxidants can inhibit lipid oxidation by performing the following actions: By scavenging free radicals, anti-oxidants can neutralize these harmful molecules before they can start the process of lipid oxidation. Interrupting sequential processes: Anti-oxidants can interrupt the consecutive events that transpire when lipids oxidize. Anti-oxidants may decrease the level of oxygen in a particular region by breaking down peroxides, which are substances formed during the oxidation of lipids. Anti-oxidants may reduce the oxygen supply for lipid oxidation.

Moreover, anti-oxidants such as metal ions and other catalysts can bind to chain-initiating catalysts, possibly initiating lipid oxidation [42]. The anti-oxidant qualities of these materials could potentially explain this. In their study, Park et al.[35] found that incorporating additives loaded with omega-3 fatty acids into the diet of broilers can improve the nutritional value and healthiness of their thigh meat. This is because essential fats like Omega-3 fatty acids can alleviate inflammation, strengthen the heart, and improve cognitive function, among other health advantages.

Chicken meat quality refers to physical, chemical, and biological attributes, including both the essential nature of the meat and its attributes. While the crucial quality of chicken meat relates to taste, color, muscle tissue texture, and meat nutritional quality, the specific characteristics of chicken meat include nutrient content, water-holding capacity, and pH value. Fatty acid content and flavor are also essential features. In addition, with the vast consumption of meat products, people are increasingly pursuing lower-fat, low-calorie, and high-nutrient content. Proper modification of the nutritional content of chicken meat can improve the quality of chicken meat and its market competitiveness, and it will be beneficial to the upgrade of the chicken industry [43]. Correlating with this study, microalgae, especially SPE, can be employed to improve the quality of meat and the performance of broiler poultry [44]. *Spirulina platensis* led to a varied meat color in their muscles. This effect was statistically significant ($P<0.01$) when *Spirulina platensis* was included in chicks' diets [45].

The cecum has a crucial role in preventing the establishment of infections, neutralizing harmful substances, reusing nitrogen, producing vitamins via microbial activity, breaking down certain carbohydrates, and absorbing additional minerals. The findings of our study demonstrated a substantial reduction in the overall levels of bacteria, coliforms, *E. coli*, *Salmonella* spp., *Enterococcus* spp., and fungi in the groups provided with SPE. The results of our investigation align with the findings of Ansari et al.[46], who stated the effects of feeding broiler chicks with *Spirulina platensis* on the microbes in their caecum; they found that supplementing the broiler meal with SE led to an elevated concentration of *Lactobacillus* in the caecum. Nevertheless, there was no fluctuation in the abundance of coliform bacteria. Numerous researches indicate that microalgae have a variety of antibacterial properties. *Candida albicans* growth is suppressed by 17.6% of different extracts of *Spirulina platensis*.

In contrast, the extract increased the proliferation of *Lactococcus* by 50% [47]. According to Kaushik and Chauhan [48] research, SE can eradicate or halt the growth of dangerous bacteria. The growth of *Lactobacillus acidophilus* was significantly enhanced by applying 10 mg/mL of dried *Spirulina platensis* to the LAB growth medium (de Man, Rogosa, and Sharpe medium), resulting in an 186% increase. This suggests that the microalgae may have prebiotic properties [49].

In addition, Abedin and Taha [50] research observed that the antibacterial activities of SE's lipopolysaccharides and alkaloids were practical against *E. coli*. Administering

chlorella microalgae to laying hens in a live experiment increased the cecum's count of lactic acid bacteria (LAB). Few studies have investigated the antibacterial characteristics of microalgae, mainly SE, in avian species. This research shows that adding a *Spirulina platensis* supplement would help sustain the LAB community and improve growth and digestibility.

In conclusion, including *Spirulina* extract (SPE) in the broiler diet at a 3 mg/kg ratio is advisable for improving overall production and health. This dietary supplementation has been shown to positively influence various aspects of broiler performance, including enhancements in body weight and weight gain, improved carcass characteristics, and a better feed conversion ratio. Furthermore, incorporating SPE at this level contributes to general health parameters by positively modulating blood and lipid profiles, enhancing digestive enzyme activity, and supporting healthy hepatic and renal functions. SPE at 3 mg/kg also boosts the immune system and elevates antioxidant levels, improving disease resistance. These beneficial effects ultimately reflect on the quality of the meat produced.

Spirulina platensis demonstrates promise as an eco-friendly anticoccidial agent in broiler chickens challenged with *Eimeria,* offering benefits for growth, immunity, and gut health. However, there are notable limitations and disadvantages. The active compounds in *Spirulina* can vary due to differences in cultivation and processing, leading to inconsistent efficacy. Determining optimal dosing is still challenging, and high inclusion rates may negatively affect feed palatability and intake. Most research is short-term and under controlled conditions, so results may not fully translate to commercial poultry settings. There are practical concerns related to production costs, supply scalability, and the risk of contamination with heavy metals or toxins if quality control is inadequate. *Spirulina* may also produce unintended changes in gut microbiota or, in rare cases, trigger hypersensitivity. Additionally, its anticoccidial effects may not match those of conventional drugs in severe infections and should not replace standard biosecurity and vaccination measures. Overall, while *Spirulina* has multiple advantages as a natural alternative, its application in poultry production should take these limitations into careful consideration.

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.

Competing Interests: The author declared that there is no conflict of interest.

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/489/2024. Written informed consent was obtained from the owners for the participation of their animals in this study.

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