Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.053

RESEARCH ARTICLE

Spirulina platensis as a Sustainable Protein Source: Impacts on Broiler Growth Performance, Immunity, Physiology, and Profitability

Abdulaziz A. Alaqil[1], Hanan Al-Khalaifah[2], Noura K. Al-Suwailem[1], Nancy N. Kamel[3], and Ahmed O. Abbas[1*]

[1]Department of Animal and Fish Production, College of Agricultural and Food Sciences, King Faisal University, P.O. Box 420, Al-Ahsa 31982, Saudi Arabia; [2]Environment and Life Sciences Research Center, Kuwait Institute for Scientific Research (KISR), P.O. Box 24885, Safat, Kuwait City 13109, Kuwait; [3]Department of Animal Production, National Research Centre, El Buhouth St., Dokki, P.O. Box 12622, Giza 12622, Egypt
*Corresponding author: aabbas@kfu.edu.sa; abbasosman591@gmail.com

ARTICLE HISTORY (26-032)

Received: January 08, 2026
Revised: February 27, 2026
Accepted: March 03, 2026
Published online: March 10, 2026

Key words:
Broiler chickens
Economic efficiency
Histological traits
Immune response
Physiological status
Spirulina platensis

A B S T R A C T

The present study investigated the effects of partially replacing conventional protein sources (CPS) with *Spirulina platensis* powder (SPP) on growth performance, immune response, physiological traits, histomorphology, and economic efficiency of broiler chickens. A total of 240 one-day-old male Cobb500 chicks were allotted to 24 cages and fed a starter diet from days 1 to 13. From days 14 to 35, birds were randomly assigned to four dietary treatments (six replicates each): a CPS-based finisher diet replaced with 0%, 5%, 10%, or 15% SPP. Growth performance parameters exhibited significant quadratic responses to SPP inclusion ($P<0.05$), with 10% SPP producing the highest BW, weight gain, feed efficiency, and production index. Regarding the immune response, leukocyte counts and lymphocyte proliferation increased linearly, while leukocyte viability, anti-SRBC antibody titers, phytohemagglutinin response, and heterophil-to-lymphocyte ratio improved quadratically, reaching a peak at 10% SPP. A linear increase in total antioxidant capacity and reduced glutathione, along with a corresponding linear decrease in malondialdehyde and pro-inflammatory cytokines, was observed following SPP treatment. Plasma T3 displayed a quadratic increase, with the highest value at 10% SPP. ALT, creatinine, and urea were linearly declined, while triglycerides and cholesterol were linearly increased by the SPP treatment. Histological evaluation revealed normal liver and intestinal architecture, with only mild alterations at 15% SPP. Economically, SPP reduced feed and protein costs linearly, while net profit was maximized at 10% SPP. In conclusion, SPP is a functional and sustainable protein source for broilers, with a 10% inclusion level providing optimal biological and economic outcomes.

To Cite This Article: Alaqil AA, Al-Khalaifah H, Al-Suwailem NK, Kamel NN and Abbas AO, 2026. *Spirulina platensis* as a sustainable protein source: Impacts on broiler growth performance, immunity, physiology, and profitability. Pak Vet J, 46(3): 645-652. http://dx.doi.org/10.29261/pakvetj/2026.053

INTRODUCTION

Poultry production supplies over 35% of global meat demand and depends largely on conventional protein sources (CPS), particularly soybean meal and corn gluten, which substantially increase feed costs, especially in developing countries due to import dependence and competition with human food markets (Voora *et al.*, 2024). Consequently, identifying alternative, cost-effective protein sources has become essential for sustaining poultry productivity (Mallick *et al.*, 2020).

Spirulina platensis (SP), a nutrient-rich cyanobacterium, has gained significant attention as a functional ingredient in poultry nutrition (Al-Khalaifah and Al-Nasser, 2024). SP contains 50–70% high-quality protein along with considerable amounts of carbohydrates, fats, minerals, vitamins, essential amino acids, fatty acids, carotenoids, and various phytopigments (Mohan *et al.*, 2014). In addition to its nutritive properties, SP exhibits several bioactive effects, including antioxidant, anti-inflammatory, and immunomodulatory activities (Anvar and Nowruzi, 2021). These attributes have encouraged its use in broiler diets as a potential substitute for traditional plant proteins (Lestingi *et al.*, 2024).

Previous studies have shown promising outcomes: up to 42% of soybean meal can be replaced by approximately

9% SP in layer diets under diverse environmental conditions (Al-Otaibi *et al.*, 2022); in broilers, SP inclusion up to 15% has been reported to maintain growth performance and meat quality (Tavernari *et al.*, 2018; Altmann *et al.*, 2020). Supplementation with 20 g/kg SP has also been linked to improved growth and enhanced antioxidant defense (Coudert *et al*., 2020). Recent reviews further indicate that SP levels of 3–15% generally produce growth comparable to that of CPS-based diets, while maintaining hematological stability and normal hepatic and renal function (Spínola *et al.*, 2024). Despite these advantages, the economic feasibility of SP remains debated (Al-Khalaifah and Uddin, 2022). Although some researchers cite its higher cost relative to conventional ingredients, others report that its health, productivity, and meat-quality benefits can make it a cost-effective supplement (Coudert *et al*., 2020).

Although numerous studies have investigated SP as a feed additive in poultry diets, most have evaluated it at low supplementation levels or focused on isolated physiological traits such as antioxidant status. Limited information is available regarding its role as a graded replacement for CPS within nutritionally balanced diets. Moreover, growing interest in SP as a CPS replacer underscores the need to clarify optimal inclusion rates and to understand the physiological and immunological mechanisms underlying broiler responses to SP. Accordingly, the present study was designed to address these gaps by comprehensively evaluating the biological and economic responses of broiler chickens to graded replacement levels of CPS with SP powder (SPP). Therefore, the present study aimed to evaluate the effects of gradually replacing CPS (soybean and gluten meals) with graded levels of SPP on broiler performance, immune response, physiological parameters, liver and intestinal histomorphology, and economic efficiency, thereby identifying practical, cost-effective substitution levels.

MATERIALS AND METHODS

Ethical statement*:* Birds were monitored daily to ensure humane handling and minimal discomfort. All procedures complied with King Faisal University research ethical guidelines (Approval No. KFU-REC-2025-MAY-ETHICS3317).

Spirulina platensis source and analysis: Organic Spirulina platensis powder (SPP) was obtained from Naturya (Hoofddorp, Netherlands). Proximate composition was analyzed in triplicate according to AOAC methods (Latimer Jr., 2023). Total polyphenols and flavonoids were determined using the Folin–Ciocalteu and aluminum chloride assays, respectively, and expressed as gallic acid and quercetin equivalents, respectively (Seghiri *et al*., 2019). Antioxidant activity was assessed using the DPPH assay and expressed as IC_{50} values of ascorbic acid equivalents (Yang *et al.*, 2007). The proximate composition, total polyphenols, total flavonoids, and radical-scavenging activity of SPP are presented in Table 1.

Birds and experimental design: A total of 240 one-day-old male Cobb500 chicks (50±2.7g) were obtained from Al-Wataniya Poultry Co. (Riyadh, Saudi Arabia). Birds were randomly allocated to 24 raised-floor cages of 125×90×60cm^3 cm³ (10 birds/cage) under controlled environmental conditions. The birds were provided with a constant daily light schedule of 23h light and 1h darkness during the first week, after which the birds were provided with 18h light and 6h darkness till the end of trial. The brooder temperature was maintained at 32°C for the first 3 days, then gradually decreased by 2°C per week until reaching a final temperature of 22-24°C. Cages were equipped with nipple drinkers and feeders according to Cobb500 management guidelines, and randomly positioned within the facility to minimize potential environmental bias. Feed and water were provided ad libitum. Birds received a starter diet from days 1-13. From days 14-35, birds were randomly assigned to four dietary treatments using a completely randomized design (six replicates each): a control diet and three diets in which soybean meal and corn gluten meal were partially replaced by SPP at 5%, 10%, or 15% (Table 2). The inclusion levels of SPP were chosen to represent graded replacement levels of CPS within the range reported in broiler studies (Altmann *et al.*, 2020; Spínola *et al*., 2024), while allowing identification of potential dose-dependent linear and quadratic responses. The finisher phase (14-35 days) was specifically targeted due to its high metabolic demand, rapid muscle accretion, and economic importance in commercial broiler production (Hossain and Islam, 2025). Personnel responsible for daily husbandry were aware of dietary treatments due to practical feeding requirements. However, laboratory analyses, immune assays, biochemical measurements, and histological evaluations were conducted by investigators blinded to treatment allocation to reduce measurement bias.

Table 1: The chemical composition, total polyphenols, flavonoids, and radical scavenging activity of Spirulina platensis powder (SPP) used in the broiler diet.[1]

Item	Values (DM basis) [1]
DM (%)	94.4±1.71
Protein (g)[2]	67.0±0.58
Fat (g)[2]	0.9±0.03
Carbohydrate (g)[2]	15.0±0.72
Fiber (g)[2]	3.5±0.04
Total ash (g)[2]	7.5±0.60
Energy (kcal)[2]	345.2±6.22
Calcium (mg)[2]	333.3±8.84
Phosphorus (mg)[2]	124.5±1.66
Sodium chloride (mg)[2]	3.7±0.01
Magnesium (mg)[2]	300.8±10.04
Manganese (mg)[2]	2.8±0.05
Iodine (µg)[2]	168.0±3.37
Total polyphenols (mg GAE)[3]	21.1±0.96
Total flavonoids (mg QE)[4]	8.1±0.47
Radical scavenging activity (mg AAE)[5]	14.8±0.51

[1]Values express means±SD of three SPP samples. [2]Nutrients determined per 100g DM. [3]GAE: gallic acid equivalent per g DM. [4]QE: quercetin equivalent per g DM. [5]AAE: ascorbic acid equivalent per g DM.

Growth performance: BW was recorded on days 14 (BW_{14}) and 35 (BW_{35}). BW gain (BWG_{14-35}), feed intake (FI), and gain-to-feed (G: F) ratio were calculated per cage. Mortality was recorded daily, and performance data were adjusted accordingly. Production efficiency factor (PEF) was calculated using standard equations (average daily BWG × survival rate) / (Feed Conversion × 10).

Immunological assessments: At the end of the experiment, birds were randomly selected for immune evaluation, as described in a previous study (AL-Kahtani *et al*., 2022). Briefly, blood samples were collected from the brachial vein of two birds per cage using heparinized syringes and put into heparinized tubes to prevent coagulation. A part of the whole blood was stained with Brilliant Cresyl Blue solution (1:50 v/v) to determine total leukocyte count (TLC) using a haemocytometer slide (American Optical Bright-Line, NY, USA) under a light microscope at 200× magnification. Meanwhile, a smear of whole blood was stained with HEMA-3 solutions (Fisher Scientific, Pittsburgh, PA, USA) and examined under a microscope (1000× magnification with oil immersion) to differentiate heterophil (H) and lymphocyte (L) cells among 200 leukocytes and to calculate the H/L ratio. The remaining blood sample was layered on a separation medium (Histopaque-1077, Sigma Chemical Co., St. Louis, MO, USA) and centrifuged at 1030×g for 20min at 4°C to obtain the peripheral blood mononuclear cells (PBMCs). PBMCs were washed and resuspended in phosphate-buffered saline (pH 7.2) and then used in an MTT assay kit (Serva, Heidelberg, Germany). Finally, the absorbance of generated formazan color was measured at 570nm using an automated ELISA reader (Bio-Rad Laboratories Inc., USA) to evaluate leukocyte cell viability (LCV%). In addition, viable lymphocytes in the isolated PBMCs were detected with trypan blue stain and adjusted to a concentration of 10^6 cells/mL. Next, B- and T-cells in each sample were stimulated by incubating the samples with Lipopolysaccharide or Concanavalin-A mitogen at 42ºC for 48h. Lymphocyte proliferation was determined by calculating B- and T-cell stimulation indices (BSI and TSI) using the MTT protocol. Moreover, two birds per replicate were intradermally injected at a designated wattle site with 0.1 mL of PBS supplemented with 0.5 mg of phytohemagglutinin (PHA) (Thermo Fisher Scientific). One day later, the PHA-reaction response was assessed by measuring the thickness of wattle swelling. Furthermore, antibody titer against sheep red blood cells (SRBCs-AB) was assessed as an indicator of the humoral immune response. On day 28 of age, two birds from each replicate were intravenously injected with SRBCs suspended at 5%. On day 35, blood samples were collected from injected birds and sera were separated by centrifugation at 200×g for 20min at room temperature. Serial dilutions of the sera samples were incubated overnight with 2% suspension of SRBCs at room temperature. After that, the log2 value of the highest dilution at which visible agglutination occurred was used to express the antibody titer.

Physiological traits: After the trial termination, plasma was collected from heparinized blood samples (2 birds per replication) by centrifugation (1030×g, 4ºC, 20min) and stored at −20°C for further assay. Physiological traits, including free-T3 hormone, metabolites, antioxidants and pro-inflammatory cytokines, were analyzed as described in a previous study (Al-Otaibi *et al.*, 2022). Free T3 hormone (fT3) was measured in plasma according to the protocols provided with the chicken ELISA kits (MBS8807417; MyBioSource, San Diego, CA, USA). Optical density was measured at 450nm using the Bio-Rad ELISA microplate reader. ntra- and inter-assay CVs were ≤8 and 10%, respectively, with a detection range of 0.13-8ng/mL. In addition, plasma metabolites, such as triglycerides (TG), cholesterol (CH), alanine and aspartate aminotransferase (ALT and AST), creatinine (CR) and urea (UR), were determined following the manufacturer's instructions of colorimetric test kits (Abcam, MA, USA) with a spectrophotometer (CE1010, Cecil Instruments Limited, Cambridge, UK). Moreover, systemic antioxidant biomarkers, such as total antioxidant capacity (TAOC), reduced glutathione (rGSH) and malondialdehyde (MDA), were measured according to the manufacturer's protocols of available colorimetric test kits (Elabscience Biotechnology Inc., Houston, TX, USA). Intra- and inter-

Table 2: Ingredients and nutritional analysis of the feed rations introduced to the Cobb500 broiler chickens.

Period (Days of age)	Phase 1 (1-13d)	Phase 2 (14-35d)			
Groups	All broilers	SPP (0%)	SPP (5%)	SPP (10%)	SPP (15%)
Ingredients per kg as fed					
SPP[1], 67% CP (g)	-	0	50	100	150
Gluten meal, 60% CP (g)	75	100	75	50	25
Soybean meal, 44% CP (g)	300	200	175	150	125
Corn meal (g)	576	650.5	650.5	650.5	650.5
Vegetable oil (g)	0	8.5	8.5	8.5	8.5
Di-calcium phosphate (g)	22	17	17	17	17
Limestone (g)	14	11	11	11	11
Salt (g)	4.5	4.5	4.5	4.5	4.5
Premix[2] (g)	5.5	5.5	5.5	5.5	5.5
L-Lysine (g)	1.5	1.5	1.5	1.5	1.5
L-Threonine (g)	1.5	1.5	1.5	1.5	1.5
Calculated composition					
ME (MJ)	12.2	13.0	13.1	13.2	13.3
Calcium (g)	11.0	8.5	8.6	8.7	8.8
Available phosphorus (g)	5.5	4.5	4.5	4.4	4.4
Met + Cyst (total)	9.4	8.7	8.8	8.9	9.1
Analyzed composition					
Moisture (g)	116.6	116.4	116.8	116.4	116.6
Ash (g)	51.3	46.5	44.6	43.8	43.0
CP (g)	220.2	196.8	204.3	211.8	219.3
Total lipids (g)	23.2	34.2	34.0	33.8	33.7
CF (g)	33.8	28.9	28.2	27.6	27.0

[1]SPP, Spirulina platensis powder. [2]Contents per kg of diet: 10000IU retinyl acetate, 5000IU cholecalciferol, 65IU DL-α tocopherol acetate, 3mg menadione sodium bisulfite, 3mg thiamine, 9mg riboflavin, 4mg pyridoxine, 0.02mg vitamin B_{12}, 0.20mg biotin, 20mg niacin, 15mg pantothenic acid, 2mg folic acid, 500mg choline chloride, 100mg Mn, 40mg Fe, 100mg Zn, 15mg Cu, 0.35mg Se, and 1mg Iodine.

assay CVs were 2.7 and 8.2% for TAOC, 1.9 and 3.2% for rGSH and 4.9 and 8% for MDA, respectively, with detection ranges of 0.62–145.2U/mL, 2–100µmol/mL and 0.38–133.33nM/mL, respectively. Further, pro-inflammatory cytokines, including interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), were measured in the plasma samples using protocols outlined by ELISA kits specific for chickens (MBS2024496, MBS2021018, and MBS2031870, respectively, from MyBioSource Inc.). All assays had intra- and inter-assay CVs ≤10 and 12%, respectively, with detection ranges of 15.6- 1000pg/mL for IL-1β, 7.8-500pg/mL for IL-6 and 7.8- 500pg/mL for TNF-α.

Histomorphology: One bird per replicate was euthanized via cervical dislocation for tissue collection (Abbas *et al.*, 2022). Jejunum midpoint (from the pre-Meckel's diverticulum area) and liver specimens were fixed in 10% formalin and subjected to standard histological processing, including dehydration in graded ethanol, clearing in xylene and embedding in paraffin, using a rotatory microtome (RM2125 RTS, Leica Biosystems, Illinois, USA). After that, samples were sectioned at ≈5µm and stained with hematoxylin and eosin. Jejunal villus height (VH), crypt depth (CD), and VH/CD ratio were measured using digital image analysis (Leica Microsystems, Germany), while liver sections were examined for histological alterations (structural integrity and degenerative or inflammatory changes).

Economic efficiency evaluation: Only the feed cost was considered as input for production costs in the current study, since all other costs, such as labor, medicine, water, power, housing, etc., were assumed to be the same for each treatment. The total feed cost (TPC) and protein cost in each experimental diet were estimated based on the prices of the feed consumed per bird and the SPP, soybean meal, and gluten meal consumed as the main protein sources in the diet. The process of selling the birds at marketing age (35 d) was regarded as the source of revenue and profit margins. The broiler's economic efficiency was assessed based on data derived from the cost-benefit ratio (CBR) and the return on investment (RoI), which were calculated according to prior research (Nassar *et al.*, 2023).

Statistical analysis: Data were analyzed using SPSS v22. Normality and homogeneity were verified prior to one-way ANOVA. Means were compared using Tukey's test, with differences considered significant at $P<0.05$. Linear and quadratic regression models were used to assess dose–response relationships associated with SPP inclusion. The number of birds per treatment and replicates was determined according to commonly adopted standards in poultry nutrition trials and was sufficient to detect biologically meaningful differences in growth performance and physiological parameters at $P<0.05$, as supported by previous comparable studies (Costa *et al.*, 2024; Zampiga *et al.*, 2024).

RESULTS

Growth performance: The effect of partially replacing CPS with SPP on broiler growth performance is shown in Table 3. Data revealed a quadratic relationship with SPP inclusion levels ($P<0.05$), indicating that a 10% SPP diet maximized BW_{35}, $BWG_{14–35}$, G:F ratio, and PEF (R^2=0.510, 0.505, 0.248 and 0.415, respectively). In contrast, TFI showed no differences among the SPP treatment groups ($P>0.05$).

Immunological markers: The effects of SPP inclusion at partial replacement levels of CPS on broiler immunological parameters are shown in Table 4. Replacing CPS with higher levels of SPP resulted in a linear ($P<0.05$) increase in TLC, BSI, and TSI (R^2=0.196, 0.888 and 0.922, respectively). The H/L ratio decreased by 5-10% SPP compared to the control but significantly increased again at 15% SPP, indicating a quadratic pattern (R^2=0.316, $P<0.05$). A strong quadratic relationship was observed in LCV, with a peak at 10% SPP compared to the control (R^2=0.680, $P<0.05$). Compared to the control, SPP significantly increased the SRBCs-Ab titer and PHA responses, with the highest values at 10% SPP (R^2=0.831 and 0.807, respectively).

Antioxidant and pro-inflammatory indices: The effect of CPS replacement on antioxidant and proinflammatory indices with increasing SPP levels is shown in Table 5. TAOC and rGSH showed a linear increase with increasing SPP inclusion levels (R^2=0.181 and 0.728, respectively; $P<0.05$). In contrast, MDA and pro-inflammatory cytokines decreased linearly with increasing SPP inclusion (R^2=0.642, 0.312, 0.354, and 0.679 for MDA, IL-1β, IL-6, and TNF-α, respectively; $P<0.05$).

Table 3: Growth performance in broiler chickens fed on different levels of *Spirulina platensis* powder (SPP) as a replacer of the conventional protein sources (CPS) in the diet

SPP inclusion level	BW_{14} (g)	BW_{35} (g)	BWG_{14-35} (g)	TFI_{14-35} (g)	G:F ratio	PEF
0%	571	2597^{b}	2026^{b}	3293	0.62^{b}	445.0^{b}
5%	572	2615^{b}	2044^{b}	3307	0.62^{b}	450.1^{b}
10%	572	2673^{a}	2102^{a}	3307	0.64^{a}	473.2^{a}
15%	570	2617^{b}	2046^{b}	3301	0.62^{b}	451.8^{b}
SEM	3.8	9.1	8.9	9.6	0.005	4.34
P-value						
Overall	0.973	<0.001	<0.001	0.468	0.002	<0.001
Linear regression	0.989	0.046	0.041	0.433	0.273	0.066
Quadratic regression	0.638	0.001	0.001	0.170	0.030	0.005

Data represent the means of 6 replicates per treatment group (superscript letters rank the differences between the means within the same column at P-value<0.05). Parameters: BW_{14}, initial body weight at the start of the trial (14 d of age); BW_{35}, final body weight at the end of the trial (35d of age); BWG_{14-35}, body weight gain during the trial (14–35d of age); TFI_{14-35}, total feed intake during the trial (14–35d of age); G:F ratio, gain-to-feed ratio; PEF, production efficiency factor. Treatment groups: CPS in the broiler diet was replaced by 0, 5, 10 and 15% SPP.

Table 4: Immunological parameters in broiler chickens fed on different levels of *Spirulina platensis* powder (SPP) as a replacer of the conventional protein sources (CPS) in the diet.

SPP inclusion level	TLC (10^3/mL)	H/L ratio	LCV (%)	SRBCs-Ab (log2)	PHA test (mm)	BSI	TSI
0%	53.59^{b}	0.43^{ab}	100.0^{b}	6.53^{b}	0.46^{d}	2.17^{d}	3.92^{d}
5%	57.04^{a}	0.41^{b}	109.9^{a}	7.54^{a}	0.55^{c}	2.61^{c}	4.51^{c}
10%	56.78^{a}	0.42^{b}	112.3^{a}	7.61^{a}	0.69^{a}	3.86^{b}	5.63^{b}
15%	57.43^{a}	0.45^{a}	97.4^{b}	7.43^{a}	0.57^{b}	4.44^{a}	6.56^{a}
SEM	1.025	0.008	1.765	0.076	0.005	0.116	0.115
P-value							
Overall	0.002	0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Linear regression	0.002	0.071	0.585	<0.001	<0.001	<0.001	<0.001
Quadratic regression	0.063	<0.001	<0.001	<0.001	<0.001	0.483	0.053

Data represent the means of 12 replicates per treatment group (superscript letters rank the differences between the means within the same column at P-value <0.05). Parameters: TLC, total leukocyte cell count; H/L ratio, heterophil to lymphocyte ratio; LCV, leukocyte cell viability; SRBCs-Ab, sheep red blood cells antibody titer; PHA, phytohemagglutinin; BSI, B-cell stimulation index; and TSI, T-cell stimulation index. Treatment groups: CPS in the broiler diet was replaced by 0, 5, 10 and 15% SPP.

Table 5: Antioxidant and pro-inflammatory indices in broiler chickens fed on different levels of *Spirulina platensis* powder (SPP) as a replacer of the conventional protein sources (CPS) in the diet.

SPP inclusion level	TAOC (U/mL)	rGSH (nM/mL)	MDA (nM/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
0%	7.15^{b}	27.68^{c}	2.58^{a}	264.78^{a}	3.75^{a}	103.51^{a}
5%	8.59^{ab}	29.06^{c}	2.25^{a}	252.90^{ab}	2.62^{b}	96.67^{b}
10%	10.66^{a}	31.29^{b}	1.89^{b}	226.84^{bc}	2.47^{b}	93.31^{c}
15%	9.81^{a}	37.21^{a}	1.47^{c}	214.84^{c}	2.32^{b}	92.18^{c}
SEM	0.975	0.745	0.13	12.392	0.261	1.06
P-value						
Overall	0.005	<0.001	<0.001	0.001	<0.001	<0.001
Linear regression	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic regression	0.107	<0.001	0.673	0.994	0.011	<0.001

Data represent the means of 12 replicates per treatment group (superscript letters rank the differences between the means within the same column at P-value<0.05). Parameters: TAOC, total antioxidant capacity; rGSH, reduced glutathione; MDA, malondialdehyde; IL-1β, interleukin-1 beta; IL-6, interleukin-6; and TNF-α, tumor necrosis factor-alpha. Treatment groups: CPS in the broiler diet was replaced by 0, 5, 10 and 15% SPP.

Table 6: Metabolites assay in broiler chickens fed on different levels of *Spirulina platensis* powder (SPP) as a replacer of the conventional protein sources (CPS) in the diet.

SPP inclusion level	fT_3 (ng/mL)	ALT (U/mL)	AST (U/mL)	CR (mg/dL)	UR (mg/dL)	TG (mg/dL)	CH (mg/dL)
0%	6.16^{b}	11.69	29.68^{a}	0.28^{a}	5.54^{a}	175.89^{b}	123.66^{b}
5%	7.25^{ab}	11.75	23.79^{b}	0.25^{ab}	5.10^{b}	180.37^{ab}	126.65^{ab}
10%	8.13^{a}	11.7	23.37^{b}	0.25^{ab}	5.05^{b}	182.03^{a}	129.60^{a}
15%	6.91^{ab}	11.76	19.94^{c}	0.24^{b}	4.94^{b}	182.47^{a}	129.16^{a}
SEM	0.473	0.491	1.234	0.009	0.101	1.791	1.542
P-value							
Overall	0.002	0.999	<0.001	0.005	<0.001	0.002	0.001
Linear regression	0.069	0.919	<0.001	0.001	<0.001	<0.001	<0.001
Quadratic regression	0.001	0.997	0.184	0.354	0.027	0.114	0.121

Data represent the means of 12 replicates per treatment group (superscript letters rank the differences between the means within the same column at P-value<0.05). Parameters: fT_3, free T3; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CR, creatinine; UR, urea; TG, triglycerides; and CH, cholesterols. Treatment groups: CPS in the broiler diet was replaced by 0, 5, 10 and 15% SPP.

Metabolite assay: The effect of partial CPS replacement with increasing SPP inclusion levels on broiler metabolite assays is shown in Table 6. Compared with the control (0% SPP), plasma fT3 levels increased in a quadratic pattern with SPP treatments, peaking in the 10% SPP group (R^2=0.262, P<0.05). In contrast, AST, CR, and UR decreased linearly as SPP levels increased (R2=0.532, 0.222, and 0.400, respectively; P<0.05). Conversely, TG and CH levels increased linearly with higher SPP levels (R^2=0.234 and 0.254, respectively; P<0.05).

Histomorphology traits: The histological measurements of the jejunum are summarized in Table 7. The VH increased linearly with higher SPP inclusion (R2=0.372, P<0.05). The CD significantly decreased in the SPP groups compared to the control, following a quadratic pattern, with the lowest CD at 5-10% SPP (R^2=0.934, P<0.05). Meanwhile, SPP treatment increased the VH/CD ratio at all levels compared to the control, with a strong quadratic relationship, showing the highest ratio in the 10% SPP group (R^2=0.805, P<0.05). The effect of replacing CPS with SPP on liver and jejunal histomorphology is shown in Fig. 1 and Fig. 2, respectively. Overall, liver sections showed common hepatocellular architecture across the studied groups. However, there was slight cytoplasmic vacuolation and mild fatty infiltration in the hepatocytes of both the 10% and 15% SPP groups. In contrast, the jejunum's histological appearance improved gradually with increasing SPP levels up to 10%, especially in the villus structure. Higher levels (15% SPP) tended to cause more extensive but less dense villi, with some widening of the intestinal lumen.

Economic efficiency: The economic efficiency of using SPP with graded levels as a partial replacement for CPS in broiler diets is summarized in Table 8. Results showed a linear reduction in protein cost and TFC (R^2 = 0.999 and 0.990, respectively) as the SPP inclusion level increased (P<0.05). In contrast, total revenue and profit margin exhibited quadratic responses, with peak values at 10% SPP (R^2 = 0.510 and 0.762, respectively; P<0.05). Nonetheless, a linear trend was observed in the CBI and RoI responses to increased SPP levels in broiler diets (R^2 = 0.953 for both CBR and RoI, P<0.05).

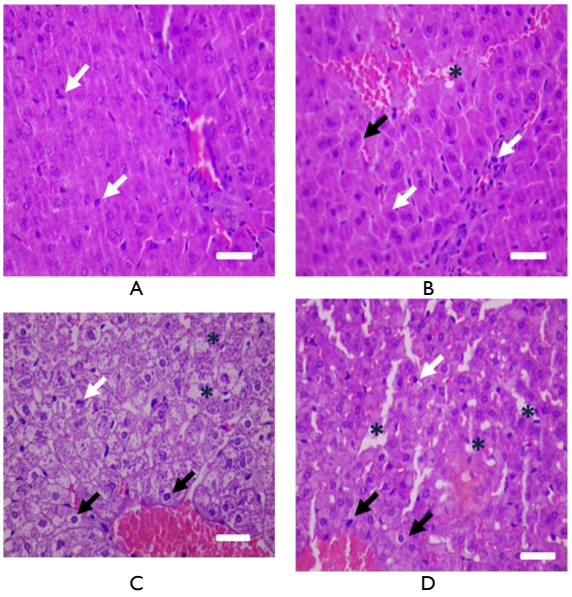

Fig. 1: Microscopic examination of liver histological sections obtained from broiler chickens fed diets in which conventional protein sources (CPS) were partially replaced with *Spirulina platensis* powder (SPP) at inclusion levels of 0% (A), 5% (B), 10% (C), and 15% (D). (Hematoxylin and eosin stain, 40× magnification; scale bar=50µm). White arrows indicate normal hepatocellular architecture, black arrows indicate cytoplasmic vacuolation, and asterisks indicate lipid (fatty) infiltration. Results show common hepatocellular architecture across the studied groups; however, there was slight cytoplasmic vacuolation and mild fatty infiltration in the hepatocytes of both the 10% and 15% SPP groups.

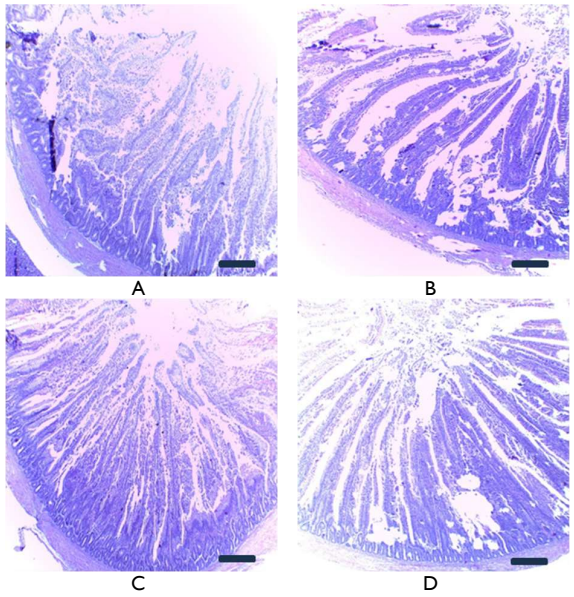

Fig. 2: Microscopic examination of jejunal histological sections obtained from broiler chickens fed diets in which conventional protein sources (CPS) were partially replaced with Spirulina platensis powder (SPP) at inclusion levels of 0% (A), 5% (B), 10% (C) and 15% (D). (Hematoxylin and eosin stain, 40× magnification, scale bar=50µm). An apparent improvement in villus morphology was observed at inclusion levels up to 10%. In the 15% SPP group, the villi appeared less compact, with a relatively wider intestinal lumen.

Table 7: Jejunal morphology parameters in broiler chickens fed on different levels of *Spirulina platensis* powder (SPP) as a replacer of the conventional protein sources (CPS) in the diet

SPP inclusion level	VH (µm)	CD (µm)	VH/CD ratio
0%	1952.7^{c}	491.8^{a}	3.97^{c}
5%	1960.0^{bc}	388.3^{c}	5.05^{b}
10%	2167.0^{ab}	373.0^{c}	5.81^{a}
15%	2171.2^{a}	435.3^{b}	4.99^{b}
SEM	74.01	7.75	0.177
P-value			
Overall	0.006	<0.001	<0.001
Linear regression	0.002	0.035	0.002
Quadratic regression	0.977	<0.001	<0.001

Data represent the means of 6 replicates per treatment group (superscript letters rank the differences between the means within the same column at P-value<0.05). Parameters: VH, villi height; and CD, crypt depth. Treatment groups: CPS in the broiler diet was replaced by 0, 5, 10 and 15% SPP.

DISCUSSION

The CP content of the SPP used in the present study reached 67%, exceeding that of the CPS in the control diet (44% and 60% in the soybean and corn gluten meals, respectively). Although SPP provides all essential amino acids required for broiler nutrition (Mullenix *et al.*, 2022), approximately 10% of its protein fraction consists of nucleic acids and membrane-bound proteins, which are poorly bioavailable to poultry (Spínola *et al.*, 2023). This limitation underscores the importance of evaluating SPP as a partial, rather than complete, replacement for CPS, particularly at graded inclusion levels (Lestingi *et al.*, 2024). Furthermore, this experiment focused on the finisher phase (14-35d), a critical period characterized by maximal muscle accretion, feed efficiency, and economic relevance (Hossain and Islam, 2025). Dietary treatments replaced 16.7%, 33.3%, and 50% of CPS via SPP inclusion at 5%, 10%, and 15%, respectively, corresponding to approximately 12.5-37.5% replacement of soybean meal and 25-75% replacement of corn gluten meal. All diets met Cobb broiler nutritional recommendations for the finisher phase, even though there was a slight increase in the ME (13.0-13.3MJ/kg) and CP (197-219g/kg) due to increasing SPP in the broiler diet.

Growth performance, feed conversion, and production efficiency improved in SPP-fed birds, with a clear quadratic response. The 10% SPP diet yielded the most favorable performance indicators under the present experimental conditions, suggesting that replacing one-third of CPS falls within a biologically suitable range. Earlier studies using lower SP levels (≤6%), replacing less than 20% of soybean meal, often reported negligible growth benefits (Zampiga *et al.*, 2024), whereas higher replacement rates (>50-55%) were associated with SP intake may increase digesta viscosity due to the gel-forming properties of its proteins, thereby reducing nutrient digestibility and enzyme efficiency. This proposed mechanism may be related to the slight performance decline observed at 15% SPP compared with 10% inclusion and warrants further investigation.

Immune-related parameters improved with SPP inclusion in the present study, indicating enhanced leukocyte activity, humoral and cellular immune responses, and lymphocyte proliferation. SPP's immunomodulatory effects are attributed to its bioactive compounds such as phycocyanin and polysaccharides, and other bioactive constituents (Lestingi *et al.*, 2024). Interestingly, the observed

Table 8: Economic efficiency in broiler chickens fed on different levels of *Spirulina platensis* powder (SPP) as a replacer of the conventional protein sources (CPS) in the diet.

SPP inclusion level	CPS replacement %	TFC ($US/bird)	Protein cost %	Total revenue (US$/bird)	Profit margin (US$/bird)	CBR	RoI
0%	0	2.88[a]	0.91[a]	13.84[b]	10.96[d]	4.80[d]	380.3[d]
5%	16.7	2.77[b]	0.79[b]	13.94[b]	11.16[c]	5.02[c]	402.4[c]
10%	33.3	2.66[c]	0.67[c]	14.25[a]	11.59[a]	5.37[b]	436.6[b]
15%	50	2.53[d]	0.55[d]	13.95[b]	11.41[b]	5.51[a]	450.8[a]
SEM	-	0.008	0.002	0.048	0.052	0.029	2.87
P-value							
Overall	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Linear regression	-	<0.001	<0.001	0.046	<0.001	<0.001	<0.001
Quadratic regression	-	0.179	0.197	0.001	0.002	0.133	0.133

Data represent the means of 6 replicates per treatment group (superscript letters rank the differences between the means within the same column at P-value<0.05). Parameters: TFC, total feed cost; CBR, cost-benefit ratio (total revenue/TFC); and RoI, return on investment (profit margin/TFC × 100). Treatment groups: CPS in the broiler diet was replaced by 0, 5, 10 and 15% SPP. Prices were US$0.60 per kg of the diet, US$0.13 per g of the SPP, US$1.05 per g of the soybean meal, US$0.65 per g of the gluten meal, and US$5.33 per kg of fresh broilers (US$1.0=SAR3.75 during this study).

dose-dependent responses align with previous reports suggesting that excessive inclusion of SP may attenuate immunological gains, depending on the broiler strain, feeding phase, or SP processing method (Spínola, Costa, and Prates, 2024).

In agreement with other studies (Salah *et al.*, 2025), antioxidant status was significantly improved by SPP inclusion, as evidenced by increased TAOC and rGSH and decreased MDA. These effects are consistent with SP's high levels of phenolics, carotenoids, and antioxidant vitamins, which enhance endogenous defense systems and mitigate oxidative stress (Kumar *et al.*, 2022). In parallel, pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) declined linearly with increasing SPP, indicating a reduction in systemic inflammation. These results are consistent with other findings that SP reverses inflammation induced by nutritional stressors such as low-protein diets (Mullenix *et al.*, 2021). The combined antioxidant and anti-inflammatory effects may have contributed to the enhanced growth and immune responses observed at moderate SPP levels.

Biochemical parameters further supported the physiological benefits of SPP. Plasma fT3, a hormone closely linked to growth and metabolic activity (Abd El-Hack *et al.*, 2019), increased in SPP-fed birds, potentially explaining improvements in performance and immunity. In contrast, plasma AST, CR, and UR declined linearly with increasing SPP, suggesting preserved hepatic and renal function. These findings are generally consistent with previous reports that highlight the hepatoprotective and nephroprotective effects of SP in poultry (Spínola *et al.*, 2024).

Unlike the commonly reported hypolipidemic effects of SP (Abed *et al.*, 2023), the current study revealed a linear increase in plasma CH and TG with higher SPP inclusion. This response may reflect enhanced intestinal lipid absorption, which could elevate circulating lipid levels (Alwaleed *et al.*, 2021). Consistent with this interpretation, mild hepatic fatty infiltration was observed histologically at the highest inclusion level (Fig. 1). Intestinal morphology was improved at moderate SPP inclusion (≤10%), as indicated by increased VH and VH/CD ratio, suggesting enhanced absorptive capacity (Wlaźlak and Biesek, 2025). However, these benefits appeared to plateau or decline at 15% SPP, which may have contributed to the slight reduction in growth performance (Ogbuewu and Mbajiorgu, 2025).

From an economic perspective, replacing CPS with SPP reduced protein and total feed costs despite the relatively high market value of SP (Bature *et al.*, 2022), as only small inclusion rates (5–15%) were required to achieve substantial CPS replacement. Although the 15% SPP diet achieved the highest CBR and RoI, the most balanced combination between biological performance and economic efficiency was observed at 10% SPP. This outcome reflects inherent limitations in SP digestibility at higher inclusion levels (Costa *et al.*, 2024) and supports recommendations for processing strategies, such as extrusion or enzymatic treatment, to improve its utilization at higher inclusion levels (Bature *et al.*, 2022).

The present study extends current knowledge by evaluating SPP as a graded replacer of conventional protein sources rather than merely as a functional feed additive in broiler diets. Unlike previous reports that primarily assessed isolated performance or antioxidant outcomes at relatively low supplementation levels, this study integrated growth performance, immune competence, oxidative and inflammatory biomarkers, endocrine responses, tissue histomorphology, and economic indicators within a single experimental framework. The convergence of biological optima at approximately 10% inclusion—corresponding to one-third replacement of conventional protein sources—provides a clearer definition of a practical substitution threshold under nutritionally balanced finisher diets. Furthermore, the identification of mild physiological and histological constraints at higher inclusion levels contributes mechanistic insight into the limits of SP utilization in broiler nutrition. Collectively, these findings refine evidence-based recommendations and support the strategic use of SP as a partial, biologically optimized, and economically viable protein alternative in commercial broiler production systems.

Conclusions: The findings of this study indicate that SPP may represent a sustainable and functional alternative to CPS in broiler nutrition. Moderate inclusion levels (e.g., 10%) appeared to support growth performance, immune responses, and histomorphology of the liver and intestines. Meanwhile, 15% SPP provided the greatest economic return and boosted key physiological indicators, including lymphocyte proliferation, antioxidant capacity, and anti-inflammatory activity. However, replacing more than one-third of CPS with SPP negatively affected growth, underscoring the importance of staying within the practical range of 10–15%. These results suggest that SPP can be a

cost-effective ingredient when carefully incorporated into broiler diets. Future research should explore processing methods, such as enzymatic hydrolysis or extrusion, to enhance nutrient availability at higher inclusion levels. Additionally, it should assess responses across different broiler genotypes, production environments, and feeding phases to improve recommendations for commercial use.

Acknowledgments: The authors thank the Deanship of Scientific Research and the Vice Presidency for Graduate Studies and Scientific Research at King Faisal University in Saudi Arabia for their financial support (Project: KFU252098), which enabled the execution and publication of this work.

Author contribution: AOA, AAA, HA, and NNK conceived and designed the study. AOA, AAA, HA, and NKA executed the experiment and analyzed the sera and tissue samples. NNK analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

REFERENCES

Abbas AO, Alaqil AA, Mehaisen GMK, *et al*., 2022. Effect of organic selenium-enriched yeast on relieving the deterioration of layer performance, immune function, and physiological indicators induced by heat stress. Frontiers in Veterinary Science 9:880790.

Abd El-Hack ME, Alagawany M, Mahrose KM, *et al*., 2019. Productive performance, egg quality, hematological parameters and serum chemistry of laying hens fed diets supplemented with certain fat-soluble vitamins, individually or combined, during summer season. Animal Nutrition 5:49–55.

Abed A, Ahmed A, Abdel-Azeem F, *et al*., 2023. Effect of spirulina platensis algae supplementation on growth performance, physiological status of broilers during summer season. Nutrition and Feeds 26:101–107.

AL-Kahtani SN, Alaqil AA and Abbas AO, 2022. Modulation of Antioxidant Defense, Immune Response, and Growth Performance by Inclusion of Propolis and Bee Pollen into Broiler Diets. Animals 12:1658.

Al-Khalaifah H and Uddin S, 2022. Assessment of Sargassum sp., Spirulina sp., and Gracilaria sp. as poultry feed supplements: feasibility and environmental implications. Sustainability 14:8968–8968.

Al-Khalaifah HS and Al-Nasser AY, 2024. Evaluating the potential of Marine Algae as sustainable ingredients in poultry feed. Agriculture 14:1889.

Al-Otaibi MIM, Abdellatif HAM, Al-Huwail AKA, *et al*., 2022. Hypocholesterolemic, Antioxidative, and Anti-Inflammatory Effects of Dietary Spirulina platensisis Supplementation on Laying Hens Exposed to Cyclic Heat Stress. Animals 12:2759.

Altmann BA, Wigger R, Ciulu M, *et al*., 2020. The effect of insect or microalga alternative protein feeds on broiler meat quality. Journal of the Science of Food and Agriculture 100:4292–4302.

Alwaleed EA, El-Sheekh M, Abdel-Daim MM, *et al*., 2021. Effects of Spirulina platensis and Amphora coffeaeformis as dietary supplements on blood biochemical parameters, intestinal microbial population, and productive performance in broiler chickens. Environmental Science and Pollution Research 28:1801–1811.

Anvar AA and Nowruzi B, 2021. Bioactive properties of Spirulina: A Review. Microb Bioact 4:134–142.

Bature A, Melville L, Rahman KM, *et al*., 2022. Microalgae as feed ingredients and a potential source of competitive advantage in livestock production: A review. Livestock Science 259:104907.

Costa MM, Spínola MP, Tavares B, *et al*., 2024. Effects of high dietary inclusion of Arthrospira platensis, either extruded or supplemented with a super-dosing multi-enzyme mixture, on broiler growth performance and major meat quality parameters. BMC Veterinary Research 20.

Coudert E, Baéza E and Berri C, 2020. Use of algae in poultry production: a review. Worlds Poultry Science Journal 76:767–786.

Hossain E and Islam S, 2025. Phase-specific nutrient requirements in broilers: implications for behavior, gut health, performance, meat quality, welfare, and economics. Research and Reviews : A Journal of Life Sciences 515.

Kumar A, Ramamoorthy D, Verma DK, *et al*., 2022. Antioxidant and phytonutrient activities of Spirulina platensis. Energy Nexus 6:100070.

Latimer Jr. GW, 2023. Official Methods of Analysis of AOAC INTERNATIONAL, 22. Oxford University Press: New York, USA.

Lestingi A, Alagawany M, Di Cerbo A, *et al*., 2024. Spirulina (Arthrospira platensis) used as functional feed supplement or alternative protein source: a review of the effects of different dietary inclusion levels on production performance, health status, and meat quality of broiler chickens. Life 14:1537.

Mallick P, Muduli K, Biswal JN, *et al*., 2020. Broiler poultry feed cost optimization using linear programming technique. Journal of Operations and Strategic Planning 3:31–57.

Mohan A, Misra N, Srivastav D, *et al*., 2014. Spirulina-The Nature's Wonder: A Review. Scholars Journal of Applied Medical Sciences (SJAMS 2:1334–1339.

Mullenix GJ, Greene ES, Emami NK, *et al*., 2021. Spirulina platensis inclusion reverses circulating pro-inflammatory (chemo)cytokine profiles in broilers fed low-protein diets. Frontiers in Veterinary Science 8:640968.

Mullenix GJ, Maynard CJ, Owens CM, *et al*., 2022. Spirulina platensis meal inclusion effects on broilers fed a reduced protein diet. Journal of Applied Poultry Research 31:100199.

Nassar FS, Alsahlawi AM, Al-Mahaish MA, *et al*., 2023. Effect of dietary mealworm meal inclusion as a replacement for soybean meal on growth, physiological, and economic efficiency of broiler chickens. Advances in Animal and Veterinary Sciences 11:310–319.

Ogbuewu IP and Mbajiorgu CA, 2025. Unlocking the feed supplement potentials of blue-green alga (spirulina) in broiler nutrition: a comprehensive review. Tropical Animal Health and Production 2025 57:7 57:1–15.

Salah AS, Lestingi A, El-Tarabany MS, *et al*., 2025. Effect of spirulina supplementation on growth, immunity, antioxidant status and pathomorphological perspectives in broilers exposed to dietary aflatoxin B1. Journal of Applied Poultry Research 34:100519.

Seghiri R, Kharbach M and Essamri A, 2019. Functional composition, nutritional properties, and biological activities of moroccan spirulina microalga. Journal of Food Quality 2019: 3707219.

Spínola MP, Costa MM and Prates JAM, 2023. Studies on the impact of selected pretreatments on protein solubility of Arthrospira platensis Microalga. Agriculture 13:221.

Spínola MP, Costa MM and Prates JAM, 2024. Analysing the impact of spirulina intake levels on performance parameters, blood health markers and carcass traits of broiler chickens. Animals 14:1964.

Spínola MP, Alfaia CM, Costa MM, *et al*., 2024. Impact of high Spirulina diet, extruded or supplemented with enzymes, on blood cells, systemic metabolites, and hepatic lipid and mineral profiles of broiler chickens. Frontiers in Veterinary Science 11: 1342310.

Voora V, Bermudez S, Le H, *et al*., 2024. Global market report: Soybean prices and sustainability. Sustainable commodities marketplace series. Manitoba, Canada.

Wlaźlak S and Biesek J, 2025. Spirulina platensis and Chlorella vulgaris in poultry nutrition - a review of current research and potential opportunities. Poultry Science 104:105456.

Yang J, Paulino R, Janke-Stedronsky S, *et al*., 2007. Free-radical-scavenging activity and total phenols of noni (Morinda citrifolia L.) juice and powder in processing and storage. Food Chemistry 102:302–308.

Zampiga M, Laghi L, Soglia F, *et al*., 2024. Partial substitution of soybean meal with microalgae meal (Arthrospira spp. – Spirulina) in grower and finisher diets for broiler chickens: implications on performance parameters, footpad dermatitis occurrence, breast meat quality traits, amino acid digestibility and plasma metabolomics profile. Poultry Science 103:103856.