Mansoura Veterinary Medical Journal

Manuscript 1239

Influence of Spirulina Platensis Inclusion upon some biochemical markers besides quality of meat in broiler chickens

Enas Hassan

Gehad El-Sayed

Azza Hassan

Shimaa El-Sayed

ORIGINAL ARTICLE

Influence of *Spirulina platensis* Inclusion Upon Some Biochemical Markers Besides Quality of Meat in Broiler Chickens

Enas M. Hassan [a,*], Gehad R. El-sayed [b], Azza E-s. Hassan [a], Shimaa A.E. El-sayed [b]

[a] Biochemistry Department, Animal Health Research Institute, Mansoura, Egypt
[b] Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Mansoura University, P.O. 35516, Mansoura, Egypt

Abstract

OBJECTIVES: The current study aimed to evaluate the influence of *Spirulina platensis* inclusion on biochemical indices besides meat quality in broilers.
METHODS: One hundred newly hatched meat-yielding chicks purchased from a local hatchery in Gamasa City were randomly divided into five groups, with 20 birds per group.
RESULTS: *Spirulina platensis* acted as an excellent growth-promoting agent. *Spirulina platensis* inclusion at doses of 0.5, 1, and 2 g in the poultry diet enhanced growth performance indices, as body weight, body weight gain, feed consumption. Spirulina positive influence on meat quality was evident through decreased thawing and cooking losses, an increase in water holding capacity, and anti-oxidative potential as a significant increase in glutathione peroxidase and superoxide dismutase activities. A hypolipemic effect was clear by a decrease in total cholesterol, serum low-density lipoprotein cholesterol, and serum triacylglycerol levels, and rising in serum high-density lipoprotein levels. This study showed increased villus height and width.
CONCLUSION: From the preceding results, *Spirulina platensis* is recommended as a feed additive via enhancement of performance parameters, meat quality, anti-oxidative potential, hypolipidemic effect, and intestinal absorption. Increasing the dose to 4 g had the opposite effect on the previous parameters.

Keywords: *Spirulina platensis*, Broilers, Anti-oxidative, Hypolipidemic properties

1. Introduction

Spirulina platensis (SP), a filamentous cyanobacterium, possesses several health benefits, therapeutic aspects, and numerous additional biological actions. The pharmaceutical and medicinal activities of *SP* could be due to its nutritional significance, especially its high protein content, in addition to fatty acids [1]. A large proportion of current research has proven the anti-oxidative actions of Spirulina by improving disease resistance, efficiently eliminating free radicals, and suppressing lipid peroxidation, thus enhancing the production of poultry in addition to achieving a high level of profitability [2].

Spirulina contains high amounts of protein (55–65%) in addition to vital amino acids, vitamins, and minerals [3]. Moreover, it is considered an abundant supply of fatty acids and carotenoids, mainly (GLA) which supposes health profits [4] and is utilized all over the world as a feed constituent within meat yield, in addition to egg producer rations to improve the color of both yolk and flesh and the fertility of eggs [5]. The greater *SP* carotenoid component aids in retinol supplementation and offers anti-oxidative action in addition to boosting immunity [6]. Ref. [7] showed that SP at a dose of five grams per kilogram was able to improve the meat quality of meat-yielding chicks owing to

Received 20 February 2024; revised 12 May 2024; accepted 13 May 2024.
Available online 15 July 2024

* Corresponding author.
E-mail address: enashassan110@gmail.com (E.M. Hassan).

https://doi.org/10.35943/2682-2512.1239

increased eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA), in addition to docosahexaenoic acid (DHA) within the thigh muscles of meat-yielding chicks. In addition, SP can substitute for antibiotics in broiler diets. Ref. [8] reported that 2, 4, or 8 g supplementation of SP per kilogram ration elevated and reduced BW in addition to FCR(Feed conversion ratio) of meat-producing chicks. PUFA encountered within SP could aid in reducing the profiles of serum lipids [9]. A well-constructed small bowl results in enhanced nutritional consumption, in addition to additional growth pattern improvement [10]. The development of the bowl is assessed via morphometric measures of villi height in addition to crypt depth utilization, along with an extended villus that causes a rise within the surface of the mucosal area and enhances digestive potency. The objectives of the current study were to recognize the influence of *Spirulina platensis* inclusion within poultry diets on growth performance, antioxidant activities, meat quality parameters, lipid profile, and intestinal histopathological examination of meat-yielding chicks.

2. Material and method

2.1. Preparation of Spirulina platensis

SP source was the National Research Center. Spirulina from the Red Sea, dried it in an oven at 60 °C for 24 h, pounded it into powder, and then placed the powder in polyethylene bags to be stored until it was needed in formulations [11]. *SP* chemical composition was mentioned in Table 1.

2.2. Experimental animals

One hundred meat-producing chicks were subdivided randomly into five groups; each replicate was kept for forty-two days within floor cages containing litter. Conventional breeding, besides management procedures, was considered throughout the experiment, including applying GOVS (animals ethics committee Guidelines) and rulers for the treatment of animals used in research [12]. The foods, besides water, were presented to the birds for their satisfaction. Constitution of feed components based on [13] as shown in Table 2. Feed utilization and death rates were documented daily. The birds were weighed independently every week. Finally, the BWG and FCR were measured after the experiment was completed.

Table 1. Chemical composition of Spirulina platensis *algae (as air dry basis).*

Nutrient	value
Crude protein%	43.39
Moisture%	4.67
Crude fat%	16.46
Crude fiber%	0.75
Soluble carbohydrate%	1.97
Total ash%	32.67
Calcium%	2.44
Total Phosphorus%	6.27

2.3. Study design

The subsequent The experimental setup looked like this: one hundred meat-producing chicks were subdivided into five groups: a) (G1): chicks of the first group were given a control ration for 42 days (20 chicks); b) (G2): chicks fed a basal diet combined with a *SP* 0.5 g per kilogram diet for 42 days (20 chicks) [14]; c) (G3): chicks fed basal diet combined with SP 1 g per kilogram feed for 42 days (20 chicks); [14] d) (G4): Chicks were fed a basal diet combined with SP at 2 g per kilogram feed for 42 days (20 chicks) [15]; and E) (G5): Chicks fed a basal diet combined with SP 4 g/kg feed for 42 days (20 chicks) [16].

Table 2. Chemical compisition of experimental diet.

Ingredients (%)	Starter diet (0–21 days)	Finisher diet (23–42 days)
Corn ground	50	46
Rice broken	05	08
Soybean meal	21	18.7
Corn gluten meal 60%	2.5	2
Corn gluten meal 30%	05	05
Fish meal	06	05
Wheat bran	04	08
Vegetable oil	0.70	0.50
Lysine sulfate	0.50	0.50
Liquid methionine	0.30	0.30
Molasses	03	04
Marble chips	01	01
Di-calcium phosphate	0.50	0.50
[a]Vitamin/mineral premix	0.50	0.50
Total	100	100
Chemical composition (analyzed)		
Metabolizable energy, kcal/kg	3089	3007
Crude protein	21.87	20.02
Crude fat	3.26	3.08
Crude fiber	4.24	4.49
Total ash	6.38	6.14
Calcium	0.91	0.64
Total phosphorus	0.77	0.52
Available phosphorus	0.43	0.32

[a] Each kg of premix containing Vit. A (1,200,000 IU), Vit. D3 (350,000 IU), Vit. E (4000 mg), and Vit. B1 (250 mg), and Vit. B2 (800 mg), and Vit. B6 (600 mg), and Vit. B12 (3.2 mg), vit. K3 (450 mg), nicotinic acid (4.5 g), calcium pantothenate (1.5 g), folic acid (120 mg), biotin (5 mg), choline chloride (55 g), Fe (3 g), Cu (2 g), Mn (10 g), Zn (8 g), I (120 mg), and Co (40 mg).

2.4. Growth performance

Finally, when the experiment was over, performance indices like BW, BWG, and FI were evaluated to cover the whole rearing period. (FCR) were measured via dividing FI by BWG [17]. Body weights were recorded independently upon initial day age to determine the average first body weight, in addition to being weighed weekly for every bird, and were utilized for estimation of average body weight within every subgroup every week. Gains in body weight, at each period, was measured by subtracting BW between the initial weight and the final weight determined by BWG [18]. Each bird's BWG was added and divided by birds from every group to acquire the mean body weight gain for every week. Feed intake (FI/bird) = feed offered quantity minus feed residence/bird number within each replicate. Feed conversion ratio (FCR) g of experimental diets was utilized for the production of 1 g of body weight gain within every group, as stated by [19], like this:

$$(\mathrm{FCR}) = \frac{\text{feed intake/gram/bird/week consumption}}{\mathrm{BEG/g/bird/week}}$$

2.5. Dissection of chickens

Overall, birds from each group were euthanized in a humane manner. Blood samples were collected in non-heparinized test tubes and centrifuged at 3000/15 rpm to obtain a clear serum sample [20] for lipid profile estimation. A small piece of tissue (white muscle or right liver lobe) was carefully excised on ice to avoid squeezing the tissue, blotted dry with filter paper, and weighed to measure enzyme activity and oxidative stress parameters at 25 °C. Skinless breast samples (*M. pectoralis* superficialis) were prepared to determine meat quality parameters. Samples were vacuum-packaged and placed at −45 °C for further analysis. Duodenal tissues were excised and washed with normal saline for histopathological analysis. Euthanasia was achieved after administration of nembutal (20–30 mg/kg) [21]. Nembutal was introduced through the veins of the wings using germ-free needles.

2.6. Assessment of oxidant/antioxidant status in liver homogenates

Oxidant and antioxidant parameters were assayed using diagnostic kits that Bio-diagnostic offers in Egypt. The tissue was homogenized as previously described [22]. Hepatic tissue (1 g) was blended with 9 ml of (e-cold PBS a pH 7 PBS that was ice-cold. When the homogenization reactions were completed, supernatant aliquots were utilized for measuring GPx (Glutathione peroxidase) concentration estimation when the homogenate had been transported to a tube specialized centrifuge and rotated for 20 min at 10,000 rpm. The supernatant was kept at −80 °C until the test procedure was completed. Glutathione peroxidase activity (GPx) was assessed using colorimetric determination. According to Ref. [23], (MDA) besides (SOD) activities were evaluated using an enzymatic colorimetric technique according to Ref. [24].

2.7. Assessment of meat quality indices

Gathered muscles from the breast were utilized to examine the index value of potential hydrogen after 24 h (potential hydrogen 24). (WHC) was estimated as follows: % loss in muscle sample weight after centrifugation [25]. The percentage alteration among the primary and eventual weight was the thawing loss value [26]. Cooking loss was assessed using the method of Ref. [27]. The gathered breast muscles were utilized for quality index estimation, potential hydrogen after 24 h (potential hydrogen 24), and water-holding capacity [28]. Thawing loss and cooking loss [27].

2.8. Lipid profile measurement

Lipid profiles were assessed calorimetrically using serum samples provided by BioMerieux kits (Spinreact, Spain) using a typical test technique. Cholesterol amount was assessed by the CE method [29] and the cholesterol level was assayed by the CHOD/PAP method, using readymade commercial genesis reagent kits provided by Spectrum, Catalog no 230 002.

Test principle: Cholesterol esters + H_2O $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + Fatty acids.

Cholesterol + H_2O $\xrightarrow{\text{cholesterol oxidase}}$ Cholestenone + H_2O_2.

$2H_2O_2$ + 4-aminoantipyrine + Phenol $\xrightarrow{\text{peroxidase}}$ Quinoneimine (red complex).

The HDL-C assay is a precipitating method that uses readymade kits provided by spectrum catalog no 267 001 for the determination of HDL-C concentration in serum according to Zhao et al. [29]. VLDL-C and LDL precipitate with phosphotungstate in addition to magnesium ions. HDL) remained in the supernatant of the solution. HDL-

cholesterol was measured by the following reactions, explained below: Test principle:

Total cholesterol Specimen ⟶ VLDL, LDL depleted specimen

CE s carboxyl ester lipase ⟶ C + FA

C + H_2O Ch Oxs ⟶ Cholest-three -1+ hydrogen peroxide

2 hydrogen peroxide + four-p-HBA + four-AAP $\xrightarrow{\text{peroxidase}}$ quinone (colored dye) + water. The color concentration developed according to the HDL cholesterol concentration in the sample was measured at 600 min.

LDL-C. LDL besides CMs, LDL was precipitated using $H_3PW_{12}O_{40}$, determined according to the Friedewald equations [29] using readymade kits provided by the spectrum catalog no is 280 001 for the determination of LDL-C concentration in serum. Test principle: LDL-C = total cholesterol (HDL-C + triacylglycerol/5). Assessment of TGs was carried out using the lipase method using readymade Genesis Lab kits provided by Spectrum by the GPO/PAP method catalog no 314 002 explained in Zhao et al. [29]. Test principle by the hydrolysis of serum triacylglycerol into glycerol and free fatty acids. Glycerol was transformed into G3P in the presence of adenosine triphosphate and glycerol kinase. This was then oxidized by the GPO to create H_2O_2. A rose-colored dye is produced when the oxidative condensation of chlorophenol and 4-amino-antipyrine occurs with H_2O_2 in the presence of peroxidase (POD). Color saturation was calculated as 550.

Triacylglycerol + H_2O LIPA ⟶ glycerin + FFA

glycerin + Adenosine Tri Phosphate $\xrightarrow{\text{GYK}}$ G3P + Adenosine diphosphate

glycerol 3 phosphate + O_2 $\xrightarrow{\text{GPO}}$ di hydroxyacetone phosphate + H_2O_2

H_2O_2 + P-chlorophenol + MAA $\xrightarrow{\text{P-chlorophenol Dehydrogenase}}$ cyclohexadienedione + H_2O

2.9. Histopathological analysis

For analysis, 2 cm samples (3 from each group) were extracted from the small bowl (duodenum, jejunum, and ileum) and fixed in (NBF) (ten percent). Briefly, the specimens were dehydrated by exposing them to increasing concentrations of ethyl alcohol (75–100) %. Samples were placed in xylol I and II, fixed in paraffin oil, cut into 4 μm cross-slices and longitudinal slices using a microtome (Leica RM 2155, Wetzlar, Germany), and stained with (H&E) [30]. Twenty-five images per group) were taken via an AMScope 5.0 Megapixels digital inspection system (AMScope, Irvine, CA, United States of America) via a low-power field (40×magnification). (VH) was estimated (micrometer) from point to the villus's base, besides (CD) was estimated through villus-crypt conjugation to the crypt's distal boundary. Villi heights, VW beside CD, for areas from the epithelial layer were evaluated using Mitcam software (Motic Images plus 2.0, HK, China).

2.10. Statistical analysis

The acquired data were statistically analyzed. Via a statistical software program (SPSS version 20, United States of America). Data are expressed as the mean ± SE from the experimental study. Analysis of variance was used to determine alterations between the means of all groups via (MRT). One-way analysis of variance (ANOVA) was used with Duncan multiple comparison tests to determine the differences between the means of different groups. Variance outcomes were regarded as of considerable importance if the p value was less than or equal to 0.05, as stated by Ref. [31].

3. Results

3.1. Growth performance results

A significant ($p \leq 0.05\%$) increase in BW, BWG, and FI following SP administration in all groups, except for G5, showed a significant ($p \leq 0.05\%$) decrease in comparison with G1 (Table 3). Concerning FCR, it revealed a significant ($p \leq 0.05\%$) decrease in all groups except for G5, show a significant ($p \leq 0.05\%$) increase in comparison with G1 (Table 3).

3.2. SP influence upon some markers of oxidative damage besides a protective enzyme action presented at liver homogenate

A significant ($p \leq 0.05\%$) increase in GPx activity was observed, especially in (G3 and G4) compared with G1. G5 showed a significant ($p \leq 0.05\%$) decrease compared to the control group (Fig. 1A). A significant ($p \leq 0.05\%$) increase in SOD activity in all groups except G5 revealed a significant ($p \leq 0.05\%$) decrease in comparison with that of the control group (Fig. 1B). MDA levels exhibited a significant ($p \leq 0.05\%$) decrease in all groups except G5, which revealed a significant ($p \leq 0.05\%$) increase in comparison with that of the control group (Fig. 2A).

3.3. Meat quality results

PH24 residues constant (Table 4), the exudative loss percent decreased significantly ($p \leq 0.05\%$) within the overall groups compared to the control

Table 3. Influences of dietary Spirulina platensis *on growth performance parameters in broiler chickens after six weeks.*

Growth performance parameters	G1	G2	G3	G4	G5
Initial weight (g)	44.0 ± 0.58^{a}	44.33 ± 0.88^{a}	45.53 ± 0.09^{a}	45.33 ± 0.09^{a}	44.10 ± 0.87^{a}
Final weight (g)	2120.0 ± 5.77^{d}	2415.0 ± 7.64^{c}	2738.3 ± 4.41^{b}	2875.0 ± 2.89^{a}	1720.0 ± 11.5^{e}
BWG (g)	2076 ± 6.35^{d}	2370 ± 6.89^{c}	2692.77 ± 4.42^{b}	2829.7 ± 2.9^{a}	1676 ± 10.7^{e}
FI (g)	3977 ± 8.62^{c}	4471.3 ± 17.6^{c}	4649.3 ± 13.3 ^{b}	4779.0 ± 2.89^{a}	3652.7 ± 9.39^{d}
FCR (%)	1.91 ± 0.01^{b}	1.88 ± 0.01^{b}	1.72 ± 0.01^{C}	1.69 ± 0.00^{C}	2.19 ± 0.02^{a}

FCR = feed conversion ratio, BWG = weight gain, FI = feed intake. G1 Control basal diet, G2 basal diet plus 0.5 gm *S. platensis*, G3 basal diet plus 1 gm *S. platensis*; G4 Basal diet plus 2 gm *S. platensis*. G5 Basal diet plus 4 gm *S. platensis*. Data are presented as mean ± SE. Mean; mean values with different superscript letters in the same rows are significantly different at ($p \leq 0.05$).

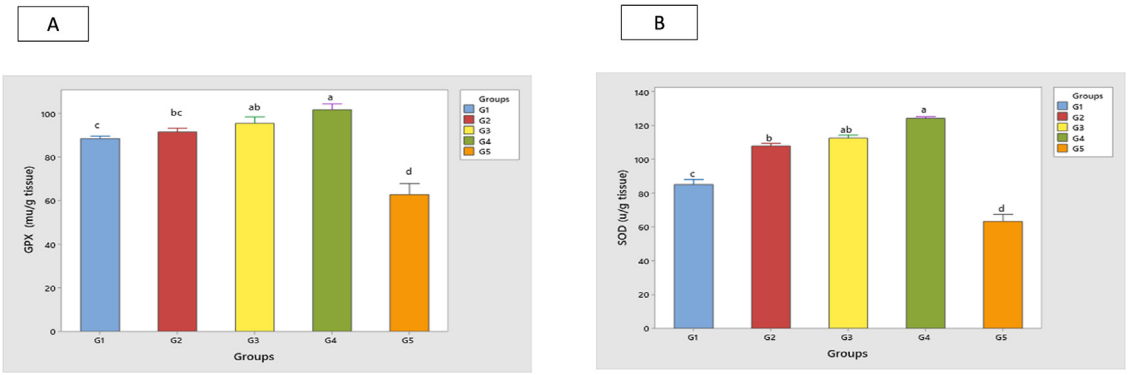

Fig. 1. (A) Influences of Spirulina platensis *dietary supplementation upon GPx activity in liver homogenate of different group birds (Mean ± S.E). G1 Control basal diet, G2 basal diet plus 0.5 gm* S. platensis*, G3 basal diet plus 1 gm* S. platensis*; G4 Basal diet plus 2 gm* S. platensis*. G5 Basal diet plus 4 gm* S. platensis*. Data represented as mean value ± standard error (S.E.). ^{a,b,c} Different superscripts indicate a significant difference ($p \leq 0.05$). (B) Influences of* Spirulina platensis *dietary supplementation upon SOD activity in liver homogenate of chickens of different groups (Mean ± S.E). G1 Control basal diet, G2 basal diet plus 0.5 gm* S. platensis*, G3 basal diet plus 1 gm* S. platensis*; G4 Basal diet plus 2 gm* S. platensis*. G5 Basal diet plus 4 gm* S. platensis*. Data represented as mean value ± standard error (S.E.). ^{a,b,c} Different superscripts indicate a significant difference ($p \leq 0.05$).*

group (Table 4). The cooking loss percent was significantly reduced ($p \leq 0.05\%$) within all groups compared with the normal group presented within Table 4. A considerable increase in water-holding capacity within chickens was observed in all groups compared to the control group (Table 4).

3.4. Lipid profile outcomes

The acquired outcomes revealed a significant ($p \leq 0.05\%$) decrease within the profile of lipids (cholesterol, LDL-C, and TG) after treatment of birds with *SP* dry leaves G2, G3, and G4,

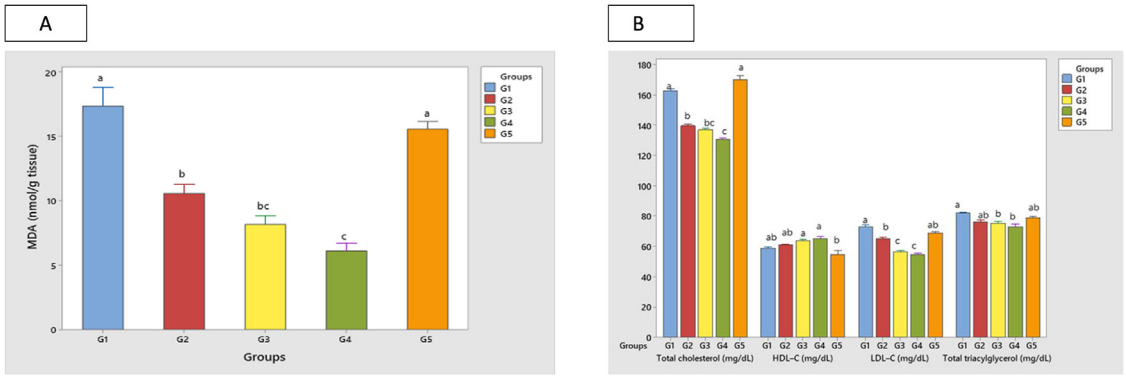

Fig. 2. (A) Influences of Spirulina platensis *dietary supplementation upon MDA level in liver homogenate of chickens of different groups (Mean ± S.E). G1 Control basal diet, G2 basal diet plus 0.5 gm* S. platensis*, G3 basal diet plus 1 gm* S. platensis*; G4 Basal diet plus 2 gm* S. platensis*. G5 Basal diet plus 4 gm* S. platensis*. Data represented as mean value ± standard error (S.E.). ^{a,b,c} Different superscripts indicate a significant difference ($p \leq 0.05$). (B) Influences of* Spirulina platensis *dietary supplementation upon lipid profile (mg/dL) in serum of chickens different groups (Mean ± S.E). G1 Control basal diet, G2 basal diet plus 0.5 gm* S. platensis*, G3 basal diet plus 1 gm* S. platensis*; G4 Basal diet plus 2 gm* S. platensis*. G5 Basal diet plus 4 gm* S. platensis*. Data represented as mean value ± standard error (S.E.). ^{a,b,c} Different superscripts indicate a significant difference ($p \leq 0.05$).*

Table 4. Influences of dietary Spirulina platensis *on meat quality parameters in breast muscles of chickens.*

Meat quality parameters	G1	G2	G3	G4	G5
PH24	5.80 ± 0.08^{ab}	5.70 ± 0.06^{ab}	5.77 ± 0.03^{a}	5.93 ± 0.08^{b}	5.50 ± 0.06^{ab}
Water holding capacity %	73.29 ± 0.79^{b}	78.02 ± 0.89^{a}	78.61 ± 0.47^{a}	78.61 ± 0.47^{a}	74.82 ± 0.99^{ab}
Thawing loss%	5.16 ± 0.08^{a}	4.50 ± 0.12^{b}	4.60 ± 0.12^{b}	4.62 ± 0.06^{b}	4.80 ± 0.06^{ab}
cooking loss%	18.57 ± 0.46^{a}	12.67 ± 0.49^{b}	10.76 ± 0.11^{c}	10.82 ± 0.08^{c}	10.79 ± 0.07^{c}

G1 Control basal diet, G2 basal diet plus 0.5 gm *S. platensis*, G3 basal diet plus 1 gm *S. platensis*; G4 Basal diet plus 2 gm *S. platensis*. G5 Basal diet plus 4 gm *S. platensis*. Data are presented as mean ± SE. Mean; mean values with different superscript letters in the same rows are significantly different at ($p \leq 0.05$).

respectively, although increasing doses at G5 significantly ($p \leq 0.05\%$) increased lipid indices compared to the control group (Table 5; Fig. 2B). HDL-c was significantly ($p \leq 0.05\%$) increased compared to that in the control group (Table 5; Fig. 2B).

3.5. Histopathological findings

Our results showed a significant ($p \leq 0.05\%$) elevation of VH in addition to VW in small bowl slices after *SPA* administration (Table 6; Fig. 3A–E, 4).

4. Discussion

Hatched broiler chicks are susceptible to various infectious diseases, oxidative stress, and high ambient temperatures, which lead to increased mortality and economic losses [32]. Spirulina acts as a formidable competitor. As asubstitute for antibiotics in chicken feeds. These substances showed possible therapeutic benefits, such as antimicrobial, anti-inflammatory, antitumor, immunostimulatory, and colorant activities [33]. The protein content of *SP* varies between 55 and 65%, with essential amino acid composition. Because of its superior nutritional profile and higher carotenoid content, the blue-green algae *Spirulina platensis (SP)* has been used for hundreds of years as a food source for both humans and animals [34]. In addition to cyanocobalamin, it contains l-ascorbic acid, vitamin B6, vitamin B2, and vitamin B1. Therefore, it is globally used to enhance meat quality. In addition to chlorophyll, *Spirulina platensis* is regarded as a great source of other

Table 6. Influences of dietary Spirulina platensis *on villi length and width in chickens.*

parameters	Villi length (μm)	Villi width (μm)
Control	1427.46 ± 5.16^{b}	294.08±9^{b}
0.5 gm group	1025.146±9^{d}	221.56 ± 2.8^{c}
1 gm group	1249.205±9^{c}	182.87 ± 3.7^{d}
2 gm group	2187.45 ± 6.7^{a}	324.56 ± 3.8^{a}
4 gm group	551.138 ± 6.2^{e}	97.66 ± 3.06^{e}

G1 Control basal diet, G2 basal diet plus 0.5 gm *S. platensis*, G3 basal diet plus 1 gm *S. platensis*; G4 Basal diet plus 2 gm *S. platensis*. G5 Basal diet plus 4 gm *S. platensis*. Data are presented as mean ± SE. Mean; mean values with different superscript letters in the same rows are significantly different at ($P \leq 0.05$).

nutrients, including β-carotenes, amino acids, phycocyanins, and tocopherols [35]. Our results revealed a significant increase in BW, BWG, and FI and better FCR within whole bird groups except group five exhibited considerable reduction as reported by Ref. [36]. Our results agree with those of Ref. [37] who similarly reported an analogous pattern within the growth performance of birds supplemented with SP supplements. WG was considerably influenced ($p \leq 0.05$) by *Spirulina platensis* addition. The improvement of WG within broilers could be due to supplementation of a greater component of Spirulina platensis, which has an outstanding nutritional profile besides its greater carotenoid and protein component, varying from 55 to 56%, encountered all of the indispensable amino acids essential for growth performance [38]. Our results are also in line with those of previous studies; the addition of SP to meals significantly increased BW, in addition to WG. The improvement of feed intake quality may be the primary mechanism by which dietary interventions, aside from

Table 5. Influences of dietary Spirulina platensis *on lipid profile (mg/dL) in chickens.*

Lipid profile parameters	G1	G2	G3	G4	G5
Total cholesterol (mg/dL)	162.67 ± 1.45^{a}	139.67 ± 0.88^{b}	137.00 ± 1.15^{bc}	130.67 ± 0.67^{c}	170.00 ± 2.83^{a}
HDL (mg/dL)	59.00 ± 0.57^{ab}	61.00 ± 0.57^{ab}	63.67 ± 0.88^{a}	65.33 ± 1.45^{a}	54.67 ± 2.60^{b}
LDL (mg/dL)	73.03 ± 1.52^{a}	65.00 ± 1.15^{b}	56.67 ± 0.88^{c}	54.67 ± 0.88^{c}	69.00 ± 0.58^{ab}
Totaltriacylglycerol (mg/dL)	82.20 ± 0.59^{a}	76.00 ± 1.73^{ab}	75.33 ± 1.45^{b}	73.00 ± 1.73^{b}	79.07 ± 0.58^{ab}

G1 Control basal diet, G2 basal diet plus 0.5 gm *S. platensis*, G3 basal diet plus 1 gm *S. platensis*; G4 Basal diet plus 2 gm *S. platensis*. G5 Basal diet plus 4 gm *S. platensis*. Data are presented as mean ± SE. Mean; mean values with different superscript letters in the same rows are significantly different at ($P \leq 0.05$).

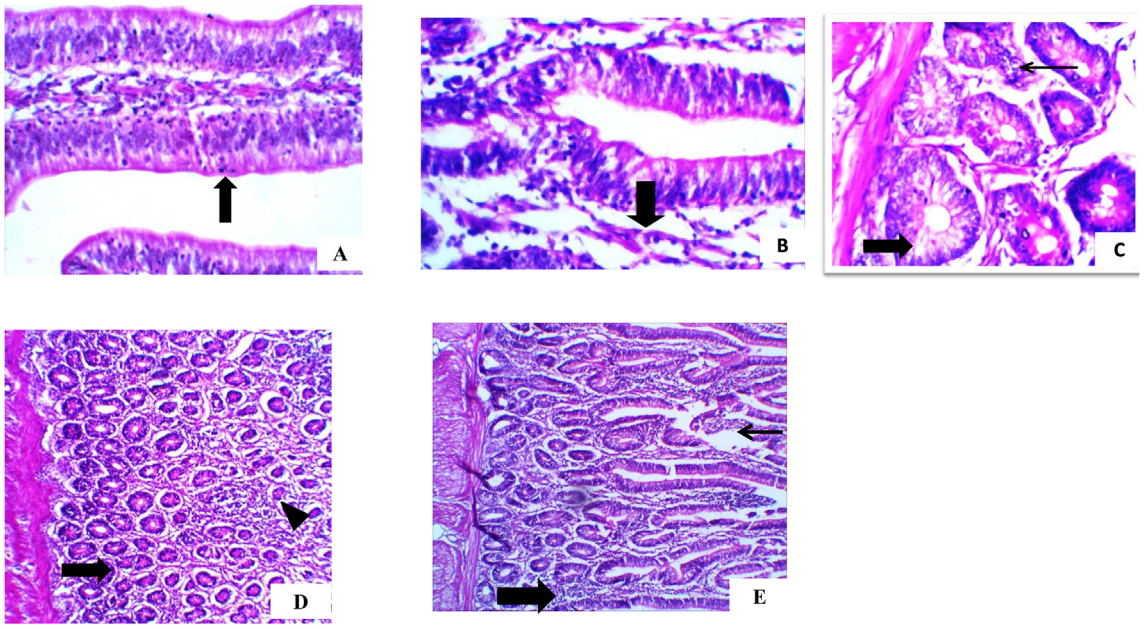

Fig. 3. (A) Control intestine: showing normal architecture of villi (arrowhead). (B) 0.5 gm group: showing minimal inflammatory cells in between villi. Image magnification = 400× and 100x (arrowhead). (C) 1 gm group: showing crypt proliferation with few interstitial inflammatory cells. (D) 2 gm group: showing marked crypt proliferation with focal aggregation of inflammation. (E) 4 gm group: showing mild villius desquamation (thin arrow) with the proliferation of crypts and moderate inflammatory cells separating the proliferated crypt (thick arrow).

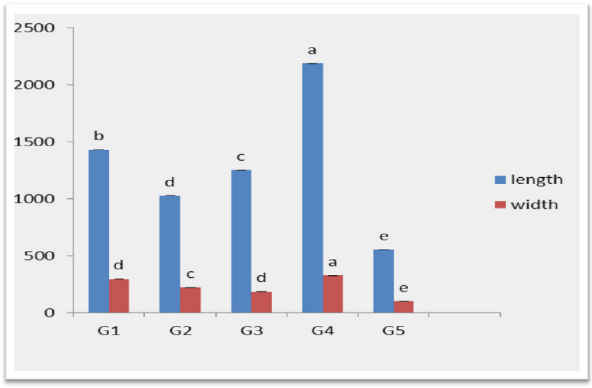

Fig. 4. Influences of Spirulina platensis *dietary supplementation upon villi length and width (μm) in chickens of different groups. G1 Control basal diet, G2 basal diet plus 0.5 gm* S. platensis*, G3 basal diet plus 1 gm* S. platensis*; G4 Basal diet plus 2 gm* S. platensis*. G5 Basal diet plus 4 gm* S. platensis*. Data represented as mean value ± standard error (S.E.).* ^{a,b,c} *Different superscripts indicate a significant difference (p ≤ 0.05).*

WG from broiler birds' SP diet, have a significant impact on BW [39]. The positive influence of SP might be a cause of improved food consumption in addition to microbial count balance within the gut as a result of antibacterial constituents such as laminarin and fucoidan [40]. Broilers fed algae meal gained weight rapidly, in addition to considerably ($p \leq 0.05$) consuming their meal. Moreover, elevated levels of highly digestible nutrients found in SP, such as carotenoids, proteins (lectins, for example), essential unsaturated fatty acids, pigments, and indispensable amino acids, in addition to PPhs, demonstrated increased antioxidant capacity and supported improved bird health and growth in environments with higher temperatures [41]. In our study, the SP-fed groups exhibited a significant ($p \leq 0.05$) increase in SOD and GPx levels and a decrease in MDA levels. Our results are in agreement with those in Ref. [36]. Superoxide dismutase, in addition to glutathione peroxidase, is considered a free radical scavenger enzyme within cells. Earlier research referred to the fact that Spirulina encountered antioxidants such as carotenoids, vitamin E, selenium polypeptide pigments, or phenolcarboxylic acids, some of which may aid in antioxidative activity along with or beside numerous other micronutrients [42]. In particular, Spirulina is thought to be a great source of phycocyanin, which functions as an antioxidant bili protein pigment and is connected to another potent (ARS) [43]. Another explanation may be that phenylic acid is linked to increased blood levels of glutathione peroxidase and superoxide dismutase in the *SP* groups. In addition to caffeic acids, several other phenolic compounds found in *SP*, including those containing salicylic, synaptic, chlorogenic, and quinic acids, may also be responsible for their antioxidant activities, either alone or in concert [44]. In addition to

lycopene, *S. platensis* contains additional carotenoids, such as lutein and polysaccharides, which are thought to have antioxidative properties [45]. Meat quality parameters exhibited significant improvements in the current research, including decreased exudative loss, cooking loss, and increasing WHC. The outcomes of our research were supported by an earlier study, which concluded that Spirulina might influence the quality of Japanese quail meat by reducing exudative loss [46]. Ref. [47] showed that including antioxidants in meals was a great way to promote development, somewhat restore the body's overall antioxidant fitness, and reduce exudative loss. Ref. [48] showed how antioxidants preserve the functioning of membranes, which increases their significance as semipermeable barriers, as opposed to drip loss. According to Ref. [49], dietary antioxidant inclusion also stops exudative loss from pale, soft, exudative muscles in stress-predisposed pigs. This activity appears to be a consequence of lessened (PLC) action through the greater antioxidant tissue membrane component, as a result of exudative loss that occurs when phospholipids are involved in the oxidation of the intracellular membrane, in addition to membrane weakness. In our study, the reduced exudative loss as a result of SP supplementation, probably due to cell membrane oxidation, was delayed. Development is considered a result of the optimistic influence of bioactive compounds, comprising *SP* antioxidants and muscle fibers, which stimulate their ability to maintain water [50]. The constructive effect of *SP* on carcass conformation, in addition to the quality of meat, could be explained by the enhancement of energy partitioning for the development of muscles. In addition, *SP* has an optimistic influence on a great component of nutrients, causing enhancements in the efficiency of feed in addition to nutrient transformation to lean meat. The hypolipidemic effects of *SP* found in this investigation have been verified previously [51]. Ref. [37] reported that HDL-C levels were elevated by *SP* administration. Spirulina platensis, in addition to lowering serum cholesterol and TG levels among groups, statistically, but not quantitatively [52]. Through the assessment of SP in local laying hens, found that cholesterol levels were significantly lowered in spirulina-administered chicks compared with the control. Decreasing serum lipid levels in *SP*-fed chicks has been reported as a result of the C-phycocyanin component of SP, which suppresses pancreatic lipase activity in a dose-dependent manner [53,54]. In addition to SP's ability to suppress cholesterol production and uptake within the intestine, the polyphenol component of SP may inhibit steapsin action while reducing hematological lipid levels [55]. In addition to the jejunum, the duodenum also plays a crucial role in the absorption and digestion of nutrients in chicks that produce meat. A fully formed small bowl results in increased nutritional intake and improved growth patterns [10]. In addition to growth, our study demonstrated increased villus breadth and height in small bowl slices treated with SPA, indicating a positive impact on intestinal health and nutritional absorption. Our results were similar to those of previous studies on broilers, which found that SPA administration had a positive impact on VW and VH from the small bowl, improving feed conversion ratio and nutrient absorption in addition to body mass [13]. Our findings reveal the optimistic influence of *Spirulina platensis* administration on gut morphology, digestibility, and, therefore, FI. A nutritional supplement of *Spirulina platensis* to poultry diets possesses a considerable ($p \leq 0.05$) increase in villi height and width. Rise within the VH could be a result of protein components, ranging from 55% to 65% in Spirulina platensis. Our study agrees with [56] who concluded that *SP* administration at a dose of ten grams per kilogram of diet significantly increases the length of the villous in addition to minimizing the count of harmful bacteria in meat-producing birds. SP administration enhanced the length of villi and increased the number of goblet cells within chicks given 1, 1.5, and 2 g SP/kg diet in comparison with control chicks [57]. Ref. [58] reported that *SP* inclusion by 15% within the basal diet considerably ($p \leq 0.05$) increased the size of intestinal segments compared with the control group. Elevated villous length and width and crypt could be the result of the pre- and probiotic effects of SP in broilers. As a result of elevated villi length and width, nutrient absorption was considerably elevated, resulting in better broiler performance.

5. Conclusion

It was concluded that (SPA) administration via 0.5, 1, 2 gm/kg dose was considered a growth enhancer substitute, improving birds production patterns characterized by increased final BW, BWG, and FI and decreased FCR. It acts as a hypolipidemic substance that decreases cholesterol, LDL-C, and triglyceride levels, while increasing HDL-C levels. It also enhanced antioxidant activity by increasing GPx and SOD activity and decreasing MDA levels. Improvements in meat quality decreased drip loss, whereas cooking loss increased in WHC. Improves intestinal histology and increases villus length and width. Increasing the dose to 4 g/kg had the

opposite effect on the previous parameters. Given its cost-effectiveness and ability to support broiler growth, *SP* could be a natural feed supplement for the enhancement of broiler production.

Ethics approval statement

Managing birds throughout the current research, in addition to their performance based on the Guidelines for the conduct of AECs, Mansoura University.

Funding

This study has received no funding.

Authors' contributions

All authors contributed equally to the research conception. Preparation of materials, data collection, and investigation.

Availability of data and materials

All relevant data are within the manuscript and its supporting information files.

Conflict of interest

The authors declare that they have no conflicts of interest with any financial organization regarding the material discussed in the manuscript.

Acknowledgments

All authors are grateful to the editor and reviewers. The technical support of Dr. Amr Mohammed is gratefully appreciated.

References

[1] Mendiola JA, Jaime L, Santoyo S, Reglero G, Cifuentes A, Ibañez E, et al. Screening of functional compounds in supercritical fluid extracts from Spirulina platensis. Food Chem 2007;102(4):1357−67.
[2] Farag MR, Alagawany M, Abd El-Hac ME, Dhama K. Nutritional and healthical aspects of spirulina (Arthrospira) for poultry, animals and human. Int J Pharmacol 2015;12(1): 36−51.
[3] Sahil S, Bodh S, Verma P: Spirulina platensis: a comprehensive review of its nutritional value, antioxidant activity and functional food potential. J Cell Biotechnol:1-14.
[4] Güroy B, Şahin İ, Mantoğlu S, Kayalõ S. Spirulina as a natural carotenoid source on growth, pigmentation and reproductive performance of yellow tail cichlid Pseudotropheus acei. Aquacult Int 2012;20(5):869−78.
[5] Curubay B, Sevim B, Cufadar Y, Ayasan T. Effects of adding spirulina platensis to laying hen rations on performance, egg quality, and some blood parameters. 2021.
[6] Januszewski J, Forma A, Zembala J, Flieger M, Tyczyńska M, Dring JC, et al. Nutritional supplements for skin health—a review of what should Be chosen and why. Medicina 2023; 60(1):68.
[7] Bonos E, Kasapidou E, Kargopoulos A, Karampampas A, Christaki E, Florou-Paneri P, et al. Spirulina as a functional ingredient in broiler chicken diets. S Afr J Anim Sci 2016; 46(1):94.
[8] Jamil ABM, Akanda M, Rahman M, Hossain M, Islam M. Prebiotic competence of spirulina on the production performance of broiler chickens. J Adv Vet Anim Res 2015;2(3): 304.
[9] Hajati H, Zaghari M. Effects of *Spirulina platensis* on growth performance, carcass characteristics, egg traits and immunity response of Japanese quails. Iran J Appl Anim Sci 2019;9(2): 347−57.
[10] Amer SA, Mohamed WA, Gharib HS, Al-Gabri NA, Gouda A, Elabbasy MT, et al. Changes in the growth, ileal digestibility, intestinal histology, behavior, fatty acid composition of the breast muscles, and blood biochemical parameters of broiler chickens by dietary inclusion of safflower oil and vitamin C. BMC Vet Res 2021;17:1−18.
[11] Raji AA, Junaid QO, Oke MA, Taufek N-HM, Muin H, Bakar N-HA, et al. Dietary *Spirulina platensis* and Chlorella vulgaris effects on survival and haemato-immunological responses of Clarias gariepinus juveniles to Aeromonas hydrophila infection. Aquac Aquar Conserv Legis 2019;12(5): 1559−77.
[12] Alderman TS, Carpenter CB, McGirr R. Animal research biosafety. Appl Biosaf 2018;23(3):130−42.
[13] Khan S, Mobashar M, Mahsood FK, Javaid S, Abdel-Wareth A, Ammanullah H, et al. Spirulina inclusion levels in a broiler ration: evaluation of growth performance, gut integrity, and immunity. Trop Anim Health Prod 2020;52: 3233−40.
[14] Omar AE, Al-Khalaifah HS, Osman A, Gouda A, Shalaby SI, Roushdy EM, et al. Modulating the growth, antioxidant activity, and immunoexpression of proinflammatory cytokines and apoptotic proteins in broiler chickens by adding dietary spirulina platensis phycocyanin. Antioxidants 2022;11(5):991.
[15] Abed HA, Ahmed A, Abdel-Azeem F, Shourrap M. Effect of spirulina platensis algae supplementation on growth performance, physiological status of broilers during summer season. Egypt J Nutr Feeds 2023;26(1):101−7.
[16] Jamil AR, Akanda MR, Rahman MM, Hossain MA, Islam MS. Prebiotic competence of Spirulina on the production performance of broiler chickens. 2015.
[17] Yi Z, Li X, Luo W, Xu Z, Ji C, Zhang Y, et al. Feed conversion ratio, residual feed intake and cholecystokinin type A receptor gene polymorphisms are associated with feed intake and average daily gain in a Chinese local chicken population. J Anim Sci Biotechnol 2018;9(1):1−9.
[18] Rashad A, Soliman AA. Comparative analysis of growth performance and profitability of four broiler commercial strains raised in Egypt. Egypt Poult Sci J 2023;43(3):505−21.
[19] Ogbuewu I, Modisaojang-Mojanaga M, Mokolopi B, Mbajiorgu C. A meta-analysis of responses of broiler chickens to dietary zinc supplementation: feed intake, feed conversion ratio and average daily gain. Biol Trace Elem Res 2023;201(5):2491−502.
[20] Onyango DM, Ramkat RC. Phenotypic and genotypic methods for the identification and quantification of cyanobacteria in lake water. In: Plant functional genomics: methods and protocols. vol. 2. Springer; 2024. p. 397−410.
[21] Overmyer KA, Thonusin C, Qi NR, Burant CF, Evans CR. Impact of anesthesia and euthanasia on metabolomics of mammalian tissues: studies in a C57BL/6J mouse model. PLoS One 2015;10(2):e0117232.
[22] Kasem N, Hamza DS. Antioxidant effect of sepia pharaonis ink extract and ellagic acid on oxidative stress induced by cyclophosphamide in male albino rats. Egypt Acad J Biol Sci C Physiol Mol Biol 2020;12(2):213−27.
[23] Andrade IGA, Suano-Souza FI, Fonseca FLA, Lago CSA, Sarni ROS. Selenium levels and glutathione peroxidase activity in patients with ataxia-telangiectasia: association with oxidative stress and lipid status biomarkers. Orphanet J Rare Dis 2021;16:1−10.

[24] Hamad SK, Elomda AM, Sun Y, Li Y, Zong Y, Chen J, et al. The in vitro evaluation of rooster semen pellets frozen with dimethylacetamide. Animals 2023;13(10):1603.
[25] Geng X, Zhao Y, Zhao N, Zhu Q, Zhang M. Quality characteristics and gastrointestinal fate of low fat emulsified sausage formulated with konjac glucomannan/oat β-glucan composite hydrogel. Int J Biol Macromol 2023;239:124251.
[26] Corrette GE, Jeneske HJ, Rimmer LA, Koulicoff L, Hene S, Zumbaugh MD, et al. Effect of electrostatic field assisted thawing on the quality of previously frozen beef striploins. Meat Muscle Biol 2024;8(1).
[27] Muhammad A, Dalia A, Samsudin A. Effect of selenium-enriched bacteria and other selenium sources on meat quality parameters of mid-producing lohman Brown laying hens. Mal J Anim Sci 2023;26(2).
[28] Zhang C, Li Y, Xia X, Liu Q, Chen Q, Kong B. Changes in muscle quality and physicochemical characteristics of chicken breast subjected to ultrasound-assisted immersion freezing during long-term frozen storage. Int J Refrig 2022; 142:10−8.
[29] Zhao L, Zhao X, Tian P, Liang L, Huang B, Huang L, et al. Predictive value of remnant cholesterol level for all-cause mortality in heart failure patients. Front Cardiovasc Med 2023;10:1063562.
[30] Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. Elsevier health sciences; 2018.
[31] Orlhac F, Lecler A, Savatovski J, Goya-Outi J, Nioche C, Charbonneau F, et al. How can we combat multicenter variability in MR radiomics? Validation of a correction procedure. Eur Radiol 2021;31:2272−80.
[32] Stelios V, Ilias G, Ioannis P, Christos A, Elias P, Paschalis F. Effect of three different insect larvae on growth performance and antioxidant activity of thigh, breast, and liver tissues of chickens reared under mild heat stress. Trop Anim Health Prod 2024;56(2):80.
[33] El-Shall NA, Jiang S, Farag MR, Azzam M, Al-Abdullatif AA, Alhotan R, et al. Potential of *Spirulina platensis* as a feed supplement for poultry to enhance growth performance and immune modulation. Front Immunol 2023;14:1072787.
[34] Wehr J, van Vuuren SJ. Algae and cyanobacteria communities. In: Wetzel's limnology. Elsevier; 2024. p. 463−510.
[35] Mourelle ML, Gómez CP, Legido JL. The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. Cosmetics 2017;4(4):46.
[36] Abdelfatah SH, Yassin AM, Khattab MS, Abdel-Razek AS, Saad AH. Spirulina platensis as a growth booster for broiler; Insights into their nutritional, molecular, immunohistopathological, and microbiota modulating effects. BMC Vet Res 2024;20(1):11.
[37] Mirzaie S, Zirak-Khattab F, Hosseini SA, Donyaei-Darian H. Effects of dietary Spirulina on antioxidant status, lipid profile, immune response and performance characteristics of broiler chickens reared under high ambient temperature. Asian-Australas J Anim Sci 2018;31(4):556.
[38] Velten S, Neumann C, Bleyer M, Gruber-Dujardin E, Hanuszewska M, Przybylska-Gornowicz B, et al. Effects of 50 percent substitution of soybean meal by alternative proteins from Hermetia illucens or *Spirulina platensis* in meat-type chicken diets with graded amino acid supply. Open J Anim Sci 2018;8(2):119.
[39] Attia YA, Hassan RA, Addeo NF, Bovera F, Alhotan RA, Al-Qurashi AD, et al. Effects of spirulina platensis and/or allium sativum on antioxidant status, immune response, gut morphology, and intestinal lactobacilli and coliforms of heat-stressed broiler chicken. Vet Sci 2023;10(12):678.
[40] Hossain MS, Small BC, Kumar V, Hardy R. Utilization of functional feed additives to produce cost-effective, eco-friendly aquafeeds high in plant-based ingredients. Rev Aquacult 2024;16(1):121−53.
[41] Abdel-Moneim A-ME, Shehata AM, Khidr RE, Paswan VK, Ibrahim NS, El-Ghoul AA, et al. Nutritional manipulation to combat heat stress in poultry−A comprehensive review. J Therm Biol 2021;98:102915.
[42] Mousa NAE-SM. The efficacy of biotechnology applications in rabbit production. Damietta University; 2023.
[43] Rahim A, Salhi S, El Khelfaoui N, Badaoui B, Essamadi A, El Amiri B. Effect of C-phycocyanin purified from *Spirulina platensis* on cooled ram semen quality and in vivo fertility. Theriogenology 2024;215:234−40.
[44] Seghiri R, Kharbach M, Essamri A. Functional composition, nutritional properties, and biological activities of Moroccan Spirulina microalga. J Food Qual 2019;2019.
[45] Assuncao LS, Bezerra PQM, Poletto VSH, de Oliveira Rios A, Ramos IG, Ribeiro CDF, et al. Combination of carotenoids from Spirulina and PLA/PLGA or PHB: new options to obtain bioactive nanoparticles. Food Chem 2021;346:128742.
[46] Cheong DSW, Kasim A, Sazili AQ, Hishamuddin O, Teoh JY. Effect of supplementing Spirulina on live performance, carcass composition and meat quality of Japanese quail. Walailak J Sci Technol 2016;13(2):77−84.
[47] Lu T, Harper A, Zhao J, Dalloul R. Effects of a dietary antioxidant blend and vitamin E on growth performance, oxidative status, and meat quality in broiler chickens fed a diet high in oxidants. Poultry Sci 2014;93(7):1649−57.
[48] Perna A, Simonetti A, Grassi G, Gambacorta E. Effect of a cauliflower (Brassica oleraceae var. Botrytis) leaf powder-enriched diet on performance, carcass and meat characteristics of growing rabbit. Meat Sci 2019;149:134−40.
[49] Wang C, Wang J, Qi S, Xu C, Wang Y, Wang H. Effect of dietary inclusion of antioxidants and organic trace minerals on growth performance, carcass characteristics, and meat quality of finishing pigs with pre-slaughter transportation. Can J Anim Sci 2020;100(3):577−85.
[50] Dal Bosco A, Gerencsér Z, Szendrő Z, Mugnai C, Cullere M, Kovàcs M, et al. Effect of dietary supplementation of Spirulina (Arthrospira platensis) and Thyme (Thymus vulgaris) on rabbit meat appearance, oxidative stability and fatty acid profile during retail display. Meat Sci 2014;96(1): 114−9.
[51] Aziz AS, KH Tawfeeq F, S Ahmed M. Therapeutic role of vitamin D3, K2, garlic extract, calcium and spirulina algae powder on induced hyperlipidemia in rats. Egypt J Vet Sci 2024;55(3):651−9.
[52] Dewi Y, Yuniza A, Sayuti K, Mahata M. Effects of different dietary concentration of fermented brown algae Sargassum binderi on plasma lipid profiles, yolk lipid, and cholesterol total of laying hens. J Anim Plant Sci 2023;33(1).
[53] Prete V, Abate AC, Di Pietro P, De Lucia M, Vecchione C, Carrizzo A. Beneficial effects of spirulina supplementation in the management of cardiovascular diseases. Nutrients 2024; 16(5):642.
[54] Deng R, Chow TJ. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. Cardiovasc Ther 2010;28(4):e33−45.
[55] Abdel-Moneim A-ME, Elbaz AM, Khidr RE-S, Badri FB. Effect of in ovo inoculation of Bifidobacterium spp. on growth performance, thyroid activity, ileum histomorphometry, and microbial enumeration of broilers. Probiotics Antimicrob Proteins 2020;12:873−82.
[56] Alwaleed EA, El-Sheekh M, Abdel-Daim MM, Saber H. Effects of *Spirulina platensis* and Amphora coffeaeformis as dietary supplements on blood biochemical parameters, intestinal microbial population, and productive performance in broiler chickens. Environ Sci Pollut Control Ser 2021;28: 1801−11.
[57] Khan F, Shuvo A, Khan M, Islam K. Effects of dietary inclusion of *Spirulina platensis* on egg yolk pigmentation. Development 2021;33:8−15.
[58] Pestana J, Puerta B, Santos H, Madeira M, Alfaia C, Lopes P, et al. Impact of dietary incorporation of Spirulina (Arthrospira platensis) and exogenous enzymes on broiler performance, carcass traits, and meat quality. Poultry Sci 2020; 99(5):2519−32.