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Evaluation of the Role of *Spirulina platensis* and *Chlorella vulgaris* on Growth Performance, Meat Quality and Blood Parameters of Broiler Chickens

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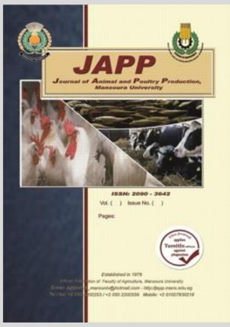

ABSTRACT

The current study focused on the effects of dietary supplementation of two types of microalgae on broiler chickens; namely *Spirulina plantesis* (SP) and *Chlorella vulgaris* (CV), separately. This study was to determine their effects on productive performance, carcass traits, blood parameters, immune functions and meat quality. 210 One-day-old Ross 308 broiler chickens were divided into seven treatment groups, each of which included three replicates. The diet treatments were (0.0, 2.0, 4.0, 6.0 g/kg dried of SP group and 2.0, 4.0, 6.0 g/kg of dried CV group for 42 d. The results showed significant improvements in FCR and weight gain in groups supplementation microalgae compared with control group. Significant increase has been determined at RBCS and hemoglobin in most treated groups and the best results occurred at 6.0 g/Kg SP and 4.0 g/kg CV. While the levels of bursal weight ratio increased significantly in all groups of CV and the best result occurred at the group level of 2.0 g/kg CV. Feeding diets with different types and levels of algae had no significant effect on ALT, total protein, albumin, cholesterol, HDL, IgA, and SOD among all groups. However significantly lower serum levels of AST and triglycerides for broilers supplementation CV at level 6g/kg compared with other groups. Accordingly, it is concluded that dietary supplementation of SP or CV has positive impacts on productive performance, hematological parameters, and meat quality of broilers. Generally, the maximal benefits can be obtained at inclusion levels of 4.0 g/kg of SP or CV.

***Keywords*: ** Broilers, Spirulina platensis, Chlorella vulgaris, lipids profile, complete blood picture, immune response, antioxidant status, meat quality.

INTRODUCTION

The global trend tends to restrict the use of antibiotics and replace it with natural and effective alternatives. That’s because antibiotic resistance has developed threatening public health and food safety. Probiotics, prebiotics, symbiotics, natural essential oils, and herbal pharmaceuticals are all examples of these alternatives. Aquatic substances have been introduced to such a field, especially algae which become promising players with satisfactory actions as dietary supplementation to diets of human, animals, and poultry.

Microalgae are tiny, photosynthetic algae with one or more cells. They may grow in fresh, brackish, and marine waters, among other aquatic conditions. They are regarded as naturally occurring nutritional sources enhanced by vitamins, minerals, vital fatty acids, proteins, carbs, pigments, and antioxidants. They can therefore achieve good growth and feed efficiency once added to the rations (El-Hady *et al.*, 2018).

There are many types of algae existing in wide geographical areas all over the world. This paper gives particular interest to *Spirulina platensis* (SP) and *Chlorella vulgaris* (CV). Such species have paid attention to their preferable supplementation to poultry, owing to their nutraceutical and pharmaceutical advantages. (Toyomizu *et al.*, 2001)

Spirulina platensis (also called blue green algae) is a microscopic single-cell alga and it is rich in complex nutrients and phytopigments (Farag *et al.*, 2016). Since it contains all essential amino acids, it has been introduced to poultry feeds to partially replace the common protein feedstuffs as fishmeal and others (Altmann *et al.*, 2018, Jubie S. *et al.*, 2012). Spirulina is rich in highly valuable constituents such as flavonoids, tannins, phenolics, saponins, and steroids (Anbarasan *et al.*, 2011). Furthermore, C-phycocyanin; a photosynthetic pigmented protein present in Spirulina, provides additional advantages, since it has antioxidant and anti-inflammatory properties (Hemalatha *et al.*, 2012). Other actions have also been studied including antibacterial, antiviral, immunomodulatory, and anticancer effects (Hoseini *et al.*, 2013).

Chlorella vulgaris is a unicellular freshwater microalga rich in protein (60.6 %) and other nutrients. It was considered a candidate to be added as a growth promoter in poultry rations even at a very low inclusion rate (0.5-1.0% of the diet) Abdelnour *et al.*, 2019. It also contains several micronutrients, fibers, polyunsaturated fatty acids, and a lot of natural pigment. The potential nutritive values of Chlorella have been shown to affect some biochemical and physiological functions, such as enhancing immune function and the growth rate of animals Singh *et al.*, 1998.

This study aimed to investigate the effects of dietary supplementation of *Spirulina platensis* (SP) (at inclusion rates of 2 ,4 and 6 g/kg) and *Chlorella vulgaris* (CV) (at inclusion rates of 2 ,4 and 6 g/kg) on growth performance, blood profiles, carcass characteristics, immune system and meat quality in broiler chickens.

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MATERIALS AND METHODS

The current study's experimental work was conducted from August to September 2022 at the Centre of Agricultural Research and Experiments, Poultry Production Farm, Faculty of Agriculture, Mansoura University, Egypt. The study's goal was to assess how marine microalgae affected broiler chicken growth performance, carcass production, immunity, lipid peroxidation, and meat quality. The following subtitles are included in this section.

The algal used in treatments:

The dried microalgae *Spirulina platensis* (SP) and *Chlorella vulgaris* (CV) were acquired from the National Research Institute's Algae Production Unit in Cairo, Egypt. The AOAC (2000) standard procedures were used in determining the chemical analyses of SP and CV.

Birds, Management and Experimental Design:

308 Ross broiler chicks (n = 210), at one day of age, had been divided up into seven treatment groups, with three replicates (pens) in each group. The diets used for the treatments consisted of 30 birds per treatment for 42 days: 0.0 control group, 2.0 g/kg dried SP group, 0.4 g/kg dry SP group, 6.0 g/kg dried SP group, 2.0 g/kg dried CV group, 4.0 g/kg dried CV group, and 6.0 g/kg dried CV group. Battery cages measuring 70, 60 and 40 centimeters in length, width and height were used to raise the birds. During the first week of the experiment, the farm's daily temperature was 32°C. After that, it dropped progressively to a range of 30 to 28°C in the second week, and it was kept at 18 to 24°C in the third week until the experiment's conclusion. The experiment was conducted with a photoperiod of 23L: 1 D.

The chickens were raised to 42 days of age, during which time they were fed a starter ration (3200 kcal of ME/kg of diet and 23% CP) and a grower ration (3041.36 Kcal of ME/kg of diet and 20% CP) from 22 to 42 days of age. According to NRC (1994), diets were designed to satisfy or above the required needs of broiler chicks. Water and mash feed were given out without restriction. Table 1 displays the experimental diets' composition and chemical analysis.

Table 1. Broiler chicken basal diet composition and calculated analysis.

Ingredients (%)	Starter	Grower
Yellow corn	63	67.8
Soybean meal 44	12.4	20.2
Corn Gluten Meal 60.2	18.9	8.0
Di calcium Phosphate	1.85	1.3
Limestone	1.5	1.45
DL-methionine	0.05	0.05
L-Lysine	0.45	0.35
Sodium chloride	0.3	0.3
Vit+Min Premix[1]	0.3	0.3
Soybean oil	1.25	0.25
Total	100	100
Calculated Analysis		
ME, kcal/Kg	3200	3041
CP, %	23.03	20.01
Crude Fiber, %	2.499	3.00
Ether extract %	2.965	2.938
Calcium %	1.03	0.91
Av-Phosphorus, %	0.45	0.363
Lysine, %	1.14	1.15
Methionine, %	0.52	0.41
Meth. +Cys. (TSAA, %)	0.925	0.76

“[1]Premix provided the following per kilogram of diet: VA (retinyl acetate), 2654 µg; VD3 (cholecalciferol), 125 µg; VE (dl-α-tocopheryl acetate), 9.9 mg; VK3 (menadionedimethylpyrimidinol), 1.7 mg; VB1 (thiamin mononitrate), 1.6 mg; VB12 (cyanocobalamin), 16.7 µg; riboflavin, 5.3 mg; niacin (niacinamide), 36 mg; calcium pantothenate, 13 mg; folic acid, 0.8 mg; d-biotin, 0.1 mg; choline chloride, 270; BHT, 5.8; Fe (iron sulphate monohydrate), 50 mg; Cu (copper sulphatepentahydrate), 12 mg; I (calcium iodate), 0.9 mg; Zn (zinc oxide), 50 mg; Mn (manganous oxide), 60 mg; Se (sodium selenite), 0.2 mg; Co (cobalt sulphate), 0.2 mg.

Performance of broiler chickens.

Throughout the duration of the experiment, weekly measurements of live body weight (LBW), feed intake (FI), and body weight gain (BWG) were taken. The feed conversion ratio (FCR) was then determined. During the trial period, birds were individually weighed to the closest gramme in the morning before being given anything to consume. This process was repeated every week. Broiler live weights were measured at the start of the experiment and then once a week after that. On a replication group basis, weekly records on broiler FI and BWG were also kept. As a result, feed consumption per BWG unit was used to compute the feed conversion ratio (FCR).

Carcass Characteristics.

Three chickens in each group, whose LBW was roughly the average weight of their respective group at the end of the study (42 days of age), were selected for a slaughter test. After the chicken had completely bled, each one was weighed separately, sacrificed, and then weighed again. After they were skinned, their corpses were disemboweled. We kept weight records for carcasses and giblets, which included the liver, kidney, gizzard, and heart.

Blood sampling and biochemical analysis.

Three birds were selected from each treatment group, slaughtered, and blood samples were taken in heparinized tubes. The blood samples were then centrifuged for 15 minutes at 4000 rpm, and the resulting plasma was kept at -20°C until analysis. To determine glucose (Trinder, 1969), total protein (Doumas *et al.,* 1981), albumin (Doumas *et al.,* 1971), cholesterol (Allain *et al.,* 1974), triglycerides (Fossati and Prencipe, 1982), high-density lipoprotein (HDL) (Myers *et al.,* 1994), and low-density lipoprotein (LDL) (Friedewald *et al.,* 1972), plasma samples were tested colorimetrically using commercial kits by the manufacturer's instructions. Nishikimi *et al.* (1972) reported that superoxide dismutase (SOD) was measured using an enzymatic colorimetric approach with ready diagnostic kits from Bio-diagnostic, Egypt. Also, based on the available kits, malondialdehyde (MDA) according to Miharaand Uchiyama (1978). Enzymatic Randox kits UK were used to determine ALT by Reitman and Frankel (1957). Reitman and Frankel (1957) state that ready-made kits from Randox kits UK were used to measure AST. According to Engvall and Perlman's 1972 publication, the ELISA technique was used to measure the levels of immunoglobulins (IgG, IgA, and IgM).

Estimation of Meat Quality Parameters.

The gathered breast muscles were examined using the following quality parameters pH, water-holding capacity (WHC), and cooking loss.

a. pH value:

The pH value was determined using pH meter (model pH 211, HANNA instruments, Inc, USA) according to Egan et al. (1981). 5 g breast sample was homogenized with 50 ml distilled water at 25°c for 30 min. Then, the mixture was filtrated to record the pH value.

b. Water holding capacity (WHC) and plasticity:

As per Egan *et al.* (1981), the WHC of breast samples that were chilled was assessed. A 0.5 g sample of the breast was placed above filter paper No. 4 and compressed with a 1 kg weight for 10 minutes. On the filter paper, two zones developed, and the surface areas of each were measured. The bound water was identified by the outer zone that formed as the water disengaged from the pushed breast tissue. The indicator of tenderness, plasticity, was computed as follows:

Plasticity (cm^2/g) = Cm^2 internal zone/ Sample weight.

Bound water (%) = $\{[(a \times m) - 8.4 \times (b - c)] / m\} \times 100$

Where

a: Moisture content /100.
m: weight of sample (mg).
b: the outer zone area (Cm^2).
c: the internal zone area (Cm^2).
1 Cm^2 of squeezed juice absorbed on filter paper was found to be about 8.4 mg water.

Water holding capacity (WHC) and plasticity (Cm^2): were determined as described by Huff and Lonergan (2005).

c. Cooking characteristic of strips fried chicken:

Cooking loss after frying: Cooking loss was calculated using the equation described by Dreeling *et al.* (2000) as follows:

Cooking loss (%) = un-cooked sample weight (g) – cooked sample weigh ×100 un-cooked sample weight (g)

7. Statistical analysis.

Using the least squares analysis of the covariance approach, a one-way investigation of variance was used to do a measurable evaluation of the collected data (SAS, 2006). Tukey (1977) employed Duncan's multiple range test to distinguish significant differences between means.

RESULTS AND DISCUSSION

Growth performance of broiler chicks:

Live body weight:

The effects of dietary supplementation of *Spirulina plantesis* (SP) and *Chlorella vulgaris (CV)* of algae on the live body weight of broiler chickens from 0 to 42 days of age are given in Table 2. In the present study, supplementation of different levels and types of algae in the diet influenced the LBW of broiler chicks at 7, 14, 21, 28, 35, or 42 days of age. In addition, supplementation of *Chlorella vulgaris(CV)* at different levels had a positive effect (P≤0.05) on LBW of chicks during the different ages and whole experiment period compared to *Spirulina plantesis* and control groups. However, the supplementation of CV at 6 g/kg had the best LBW compared with other groups but the lower body weight was obtained in the control group. This result agreed with Alfaia *et al.*, 2021 found that dietary incorporation of 10% C.vulgaris microalga, whether supplemented or not with exogenous enzymes, in broiler diets was not detrimental to the growth performance of broilers. In addition, Kotrbácek *et al.*, 1994 concluded that the live weight of broilers was unaffected by the addition of Chlorella together with other biological feed additives. In contrast, Roques *et al.*, 2022 discovered that throughout the 35-day trial, broiler meals containing Chlorella vulgaris biomass tended to increase the birds' final BW. An *et al.* (2016) and Abdelnour *et al.* (2019) have shown that the growth performance of broilers was positively influenced by C. vulgaris supplementation at very low amounts (0.15–1.0% of the diet). In addition, Kang *et al.*, 2013 discovered that broiler chickens' BW gain was greatly increased by dietary chlorella supplementation. The high quantity and high quality of protein in chlorella, which promotes broiler weight gain, may be responsible for the improved growth performance of broiler chickens Kay, 1991

Table 2. Effect of SP and CV supplemented- diet on performance of broiler chickens at different ages in terms of live body weight (LBW):

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV (4g/kg)	CV (6g/kg)	Pooled SEM	Sig.	P value
LBW 0-old (kg)	0.0418	0.0418	0.0418	0.0418	0.0418	0.0418	0.0418	0.0001	NS	1.0
LBW 7-old (kg)	0.1130^{b}	0.1253^{ab}	0.1306^{ab}	0.1316^{ab}	0.1331^{a}	0.1343^{a}	0.1345^{a}	0.0039	*	0.0196
LBW 14-old (kg)	0.2665^{b}	0.3173^{a}	0.3105^{ab}	0.3198^{a}	0.2978^{ab}	0.3283^{a}	0.3198^{a}	0.0094	*	0.0070
LBW 21-old (kg)	0.4880^{b}	0.5895^{a}	0.5880^{a}	0.5705^{a}	0.6005^{ab}	0.6181^{a}	0.5970^{a}	0.0176	*	0.0032
LBW 28-old (kg)	0.8653^{b}	1.032^{a}	1.056^{a}	1.008^{a}	1.056^{a}	1.094^{a}	1.035a	0.0287	*	0.0018
LBW 35-old (kg)	1.408^{b}	1.576^{ab}	1.681^{a}	1.589^{ab}	1.645^{a}	1.641^{a}	1.610^{a}	0.0386	*	0.0048
LBW 42-old (kg)	1.791^{c}	2.001^{b}	2.083^{ab}	2.052^{ab}	2.001^{b}	2.077^{ab}	2.166^{a}	0.0329	*	0.0001

"a-b Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Body weight gain:

Table 3 displays the effects of dietary supplementation of *Spirulina plantesis* and *Chlorella vulgaris* of algae on body weight gain of broiler chickens. It was observed that dietary supplementation of CV at a level of 6 g/kg did significantly influence (P≤ 0.05) BWG of broiler chicks for the whole experimental period (0-42 days of age) compared to other supplementation algae (0.0 control, 2.0 g/kg dried SP, 0.4 g/kg dried SP, 6.0 g/kg dried SP, 2.0 g/kg dried CV, 4.0 g/kg dried CV).

Table 3. Effect of SP and CV supplemented- diet on performance of broiler chickens at different ages in terms of body weight gain (BWG):

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV(4g/kg)	CV(6g/kg)	Pooled SEM	Sig.	P value
BWg 0-7 old (kg)	0.071^{b}	0.083^{ab}	0.088^{ab}	0.089^{ab}	0.091^{a}	0.092^{a}	0.092^{a}	0.0039	*	0.019
BWg 7-14 old (kg)	0.1535^{b}	0.1920^{a}	0.1798^{ab}	0.1881^{ab}	0.1646^{ab}	0.1940^{a}	0.1853^{ab}	0.0075	*	0.0150
BWg 14-21 old (kg)	0.2215^{b}	0.2721^{ab}	0.2775^{ab}	0.2506^{ab}	0.3026^{a}	0.2898^{ab}	0.2771^{ab}	0.0145	*	0.027
BWg 21-28 old (kg)	0.3773^{b}	0.4430^{ab}	0.4683^{a}	0.4383^{ab}	0.4555^{a}	0.4758^{a}	0.4380^{ab}	0.0159	*	0.014
BWg 28-35 old (kg)	0.5430	0.5441	0.6253	0.5809	0.5890	0.5476	0.5750	0.0275	NS	0.364
BWg 35-42 old (kg)	0.3827	0.4252	0.4016	0.4626	0.3566	0.4361	0.5566	0.0500	NS	0.193
TBWg (kg)	1.749^{c}	1.960^{b}	2.041^{ab}	2.010^{ab}	1.959^{b}	2.035^{ab}	2.124^{a}	0.0329	*	0.0001

"a-b Means in the same row with different superscripts differ significantly (P≤ 0.05)".

But the lower body weight gain was obtained in the control group compared to algae supplementation groups. On the other hand, adding had no effect on FI of chicks during the periods of 0-7, 14-21, 21-28, 35-42, or 0-42 days of age while adding red cranberry powder significantly increased FI of birds during the period of 7-14 days of age. El-Abd and Hamouda, 2017 indicated that the growth rates of the various categories had not changed significantly. However, the chicks treated with freezing and thawing algae had the best performance index.

Feed intake:

Table 4 shows the effects of dietary supplementation of *Spirulina plantesis* and *Chlorella vulgaris* of algae on the feed intake of broiler chicks aged 0 to 42 days. The effect of supplementation of different levels and types of algae had a significant effect (P≤0.05) on the FI of chicks during the periods of 14-21 or 21-28 days of age, the control group to a significant reduction in the FI of chicks compared to microalgae types groups. On the other hand, the addition of *Spirulina plantesis* and *Chlorella vulgaris* of algae had no effect (P>0.05) on the FI of chicks during the whole experimental period compared to the control group. Mariey *et al.,* 2014 discovered that when commercial broiler chickens were fed varying amounts of dietary Spirulina (0.1, 0.2, and 0.3g Spirulina/kg) and their feed consumption was monitored during the starting period, the chicks consumed noticeably less feed. Following that, nutritional interventions had no discernible impact on the birds' feed intake during the finisher period. Moustafa *et al.,* 2021 discovered that while supplementing with *spirulina* did not raise feed intake, it did improve feed conversion, which in turn raised the heat-stressed chickens' final body weight.

Table 4. Effect of SP and CV supplemented- diet on performance of broilers chickens at different ages in terms of feed intake (FI)

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV (4g/kg)	CV (6g/kg)	Pooled SEM	Sig.	P value
FI 0-7 old (kg)	0.0895	0.0975	0.1040	0.1073	0.0995	0.1133	0.1076	0.0091	NS	0.6290
FI7-14 old (kg)	0.2571	0.2648	0.2848	0.2888	0.2658	0.2815	0.2818	0.0114	NS	0.3945
FI 14-21old (kg)	0.3405^{b}	0.4090^{ab}	0.4328^{a}	0.3998^{ab}	0.4220^{ab}	0.4301^{a}	0.4120^{ab}	0.0185	*	0.0481
FI 21-28 old (kg)	0.5870^{b}	0.6846^{ab}	0.7013^{a}	0.6740^{ab}	0.7041^{a}	0.7170^{a}	0.6976^{a}	0.0217	*	0.0148
Fi 28-35 old (kg)	0.8916	0.8933	0.9303	0.9020	0.9041	0.8986	0.9175	0.0327	NS	0.9776
FI 35-42 old (kg)	0.9540	0.8860	0.8723	0.8941	0.9595	0.8198	0.9200	0.0481	NS	0.4493
TFI (kg)	3.120	3.235	3.326	3.266	3.355	3.260	3.336	0.0612	NS	0.1906

"a-b Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Feed conversion ratio:

Table 5 displays the effects of dietary supplementation of *Spirulina plantesis* and *Chlorella vulgaris* of algae on the feed conversion ratio of broiler chicks aged 0 to 42 days. The effect of the addition of *Spirulina plantesis* and *Chlorella vulgaris* of algae had no effect (P>0.05) on the feed conversion ratio of chicks during the whole experimental period compared to the control group. This result agrees with El-Bahr *et al.* 2020 recommended supplementation of S. *platensis* to broilers chickens' diet for improvement of performance parameters. These results agree with previous studies conducted by Kharde *et al.,* 2012 and Shanmugapriya and SaravanaBabu, 2014. reported that dietary *Spirulina* significantly improved the feed efficiency of broiler chickens compared with the control groups. These results contradict the findings of previous researchers Toyomizu *et al.,* 2001 and Gongnet *et al.,* 2001 discovered that adding dietary spirulina to measurements of performance had no discernible effects. However, Nikodémusz *et al.,* 2010 found that the feeding of dietary spirulina to birds improved their productivity. Shanmugapriya *et al.,* 2015 found that adding 1% of Spirulina platensis to the feed significantly reduced the broilers' FCR when compared to the control group. Furthermore, in broilers fed an enclosed diet containing 5 or 10 g/kg of feed, there was a noteworthy improvement in feed conversion ratio and growth rate Alwaleed *et al.,* 2020.

Table 5. Effect of SP and CV supplemented- diet on performance of broiler chickens at different ages in terms of feed conversion rate (FCR):

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV(4g/kg)	CV (6g/kg)	Pooled SEM	Sig.	P value
FCR 7 old (kg)	1.261	1.176	1.173	1.193	1.085	1.225	1.1550	0.0732	NS	0.7436
FCR 14 old (kg)	1.674	1.379	1.611	1.535	1.620	1.453	1.5190	0.085	NS	0.2657
FCR 21old (kg)	1.535	1.5043	1.563	1.641	1.414	1.485	1.488	0.1215	NS	0.9028
FCR 28 old (kg)	1.562	1.544	1.498	1.538	1.546	1.508	1.594	0.0385	NS	0.6575
FCR 35 old (kg)	1.642	1.647	1.509	1.572	1.534	1.643	1.599	0.0996	NS	0.9172
FCR 42 old (kg)	2.535	2.165	2.224	1.942	2.704	2.033	1.681	0.2726	NS	0.2094
TFCR (kg)	1.784^{a}	1.652^{ab}	1.629^{ab}	1.626^{ab}	1.713^{ab}	1.602^{ab}	1.571^{b}	0.0410	*	0.0377

"a-b Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Broiler chick carcass characteristics and lymphoid organs:

Table 6 shows the effects of dietary supplementation of *Spirulina plantesis* and *Chlorella vulgaris* of algae on the carcass characteristics and percentage of lymphoid organs in broiler chicks (42 days old). It revealed no significant difference at the levels of carcass %, heart %, and spleen %. However, a significant increase has been detected in the bursa of Fabricius % in group of inclusion of 4.0 g/Kg SP and 2.0 g/kg CV. This observation indicates improvement of the immune functions since the bursa of Fabricius is known to be the most important immune organ in birds. Such a result is in harmony with a previous study that observed a reduction of the immunopathy caused by gumboro disease in the group supplemented with 1% Spirulina (Pravesh Kumari *et al.,* 2019). In addition, a similar increase has been detected according to the current study in the liver and gizzard especially at levels of 2.0 g/kg CV. This result agrees with El-Abd and Hamouda, 2017 showed that the liver, heart, gizzard, and spleen did not significantly differ between. In the meantime, the weight of the thymus, the proportion of dressings breast meat, abdomen fat, and the bursa of Fabricius were all significantly impacted by algae (Chlorella vulgaris). Significant effects of algae were observed on lymphoid organs, which are primarily responsible for the immune response in chicks. Kaoud, 2012 revealed that as compared to the control group, all groups given dietary spirulina had higher absolute and relative weights of the thymus and bursa. That is congruent with Bennett and Stephens, 2006 stated that a bird's bursa serves as half of its immune system and that the size of the bursa indicates the general health of the bird.

Table 6. Effect of SP and CV supplemented-diet on carcass and lymphoid organs weights of 42-day-old broiler chicks.

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV (4g/kg)	CV (6g/kg)	Pooled SEM	Sig.	P value
LBW (Kg)	1.843	1.766	1.923	1.900	1.980	1.995	1.966	0.066	NS	0.2383
Carcass (%)	70.96	70.47	70.19	71.19	70.78	69.79	71.69	1.227	NS	0.2821
Heart (%)	1.123	1.016	1.143	1.096	1.180	1.040	1.223	0.082	NS	0.1287
Gizzard (%)	3.720	3.813	3.610	3.560	3.666	3.033	3.403	0.279	NS	0.7698
Liver (%)	5.230	4.976	5.266	5.840	5.623	5.060	4.263	0.506	NS	0.1853
Spleen (%)	0.300	0.303	0.276	0.243	0.303	0.296	0.270	0.050	NS	0.9565
Giblets (%)	11.19	10.83	11.16	11.58	11.65	10.17	10.11	0.668	NS	0.0644
Bursa (%)	0.286[b]	0.263[b]	0.563[a]	0.283[b]	0.476[a]	0.336[ab]	0.336[ab]	0.070	*	0.0357

"[a-b]Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Blood Profile.

Results of serum parameters (ALT, AST, total protein, albumin, glucose, cholesterol, triglycerides, HDL, LDL, antioxidant status, and immunoglobin G) for the different experimental groups of broiler chickens fed diets supplemented with different types and levels of algae are illustrated in Table 7 and 8. Feeding diets with different types and levels of algae had no significant effect on ALT, total protein, albumin, cholesterol, HDL, IgA, and SOD among all groups. However significantly lower serum levels of AST and triglycerides for broilers supplementation CV at level 6 g/kg compared with other groups. On the other hand, CV at level 6 g/kg supplement group had significantly higher levels of glucose than the other groups. While SP at level 4 g/kg group had a significantly lower level of LDL compared with other groups. According to El-Abd and Hamouda, 2017 water treatment the serum concentrations of HDL, LDL, triglycerides, and cholesterol were all reduced by chlorella alga. Moreover, total protein, globulin, and albumin did not significantly change from the control, but ALT, AST, and creatinine were significantly higher. Moradi Kor *et al.* (2016) demonstrated that high doses of Chlorella microalgae had favorable effects on blood triglyceride, cholesterol, LDL, and HDL concentrations. This is consistent with previous findings by Canogullari Dogan *et al.,* 2016 found that although not statistically, supplementing with Spirulina platensis also reduced the levels of plasma triglycerides between groups. Furthermore, broilers exposed to heat stress showed decreased blood cholesterol, LDL, total lipids, and triglycerides when supplemented with spirulina at 0.5 to 2% Abdel-Moneim *et al.,* 2021 and Mirzaie *et al.,* 2018.

Rashid *et al.* (2021) reported a noteworthy elevation in the white blood cell count of broiler hens given diets enriched with spirulina. Additionally, broiler blood-fed diets supplemented with 2-4 g/kg of Spirulina showed a substantial rise in hemoglobin and RBC counts Jamil *et al.,* 2015.

Table 7. Effect of SP and CV supplemented- diet on some blood parameters of 42-day-old broiler chicks.

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV (4g/kg)	CV (6g/kg)	Pooled SEM	Sig.	P value
ALT (U/L)	11.00	7.000	8.333	7.000	8.333	7.000	8.333	1.309	NS	0.3686
AST (U/L)	139.0[ab]	177.0[a]	183.3[a]	129.0[ab]	132.6[ab]	97.00[bc]	71.00[c]	11.52	***	0.0001
Tp (g/dl)	5.133	4.500	4.700	5.666	5.050	5.600	4.300	0.438	NS	0.2707
Alb (g/dl)	3.000	2.533	2.700	3.200	2.900	3.300	2.500	0.251	NS	0.2334
Glucose (mg/dl)	173.6[bc]	182.6[abc]	166.6[c]	204.0[abc]	220.0[ab]	208.3[abc]	231.6[a]	10.85	**	0.0060
Chol (mg/dl)	128.3	172.3	118.0	132.3	161.0	143.3	168.6	13.09	NS	0.0628
Tri g (mg/dl)	93.66[ab]	71.00[b]	151.0[a]	79.00[b]	79.66[b]	85.00[ab]	74.00[b]	14.22	*	0.0187
HDL (mg/dl)	36.33	28.66	36.33	33.66	31.66	36.33	28.33	3.534	NS	0.4544
LDL (mg/dl)	73.26[ab]	129.4[a]	51.73[b]	82.86[ab]	113.4[ab]	90.00[ab]	125.5[ab]	15.65	*	0.0282

"[a-c]Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Table 8. Effect of SP and CV supplemented-diet on immunoglobin G and antioxidant status of 42-day-old broiler chicks.

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV(4g/kg)	CV (6g/kg)	Pooled SEM	Sig.	P value
IgG (mg/dl)	706.8[a]	564.2[ab]	432.7[ab]	396.9[b]	406.5[b]	460.0[ab]	561.3[ab]	59.71	*	0.0237
IgM (mg/dl)	65.93[a]	50.63[ab]	41.19[ab]	31.71[b]	43.10[ab]	32.83[b]	45.400[ab]	5.273	**	0.0070
IgA (mg/dl)	29.64	30.53	22.99	29.00	29.03	28.06	28.23	2.855	NS	0.6294
MDA (nmol/ml)	46.33[b]	66.46[ab]	74.86[a]	56.30[ab]	59.26[ab]	43.66[b]	42.66[b]	5.142	**	0.0036
SOD (U/ml)	448.5	355.0	370.1	386.3	381.7	434.5	475.6	28.65	NS	0.0739

"[a-b]Means in the same row with different superscripts differ significantly (P≤ 0.05)".

Immune response and antioxidant status

There are previous reports of immune system improvement by the addition of microalgae, especially SP. However, in this study, there were no significant differences between the control group and groups supplementation microalgae, but with the high levels of supplementation SP and CV showed decreased concentrations of the antibodies including IgG, IgM, and IgA. This result agrees with, Kang *et al.,* 2013 and An *et al.,* 2016 In comparison to control chicks' plasma IgM concentrations of 155 µg/ml, chicks fed a diet containing 0.05% or 0.15% dried Chlorella vulgaris powder or 0.15% Chlorella vulgaris growth factor (CGF) displayed significantly higher concentrations (480 µg/ml, 501 µg/ml, and 472 µg/ml), representing increases of 32.3%, 30.9%, and 30.3%, respectively. These outcomes can be primarily attributed to Chlorella vulgaris high antioxidant and omega-3 PUFA concentrations, which regulate host immunological responses. On the contrary, Kang *et al.,* 2013 discovered that the chicks in the Chlorella supplemental group had a considerably greater plasma IgA concentration than the chicks in the antibiotic growth promoter group and that the level of total plasma immunoglobulin was significantly influenced by fresh liquid Chlorella.

Malondialdehyde (MDA) has decreased in the group of CV, especially at levels 4 and 6 g/kg but the group of SP at level 4g/kg higher concentration of MDH compared to other groups. Since increasing MDA is indicative of oxidative stress and mutagenesis, CV seems to be preferable in this aspect to SP according to our results. Superoxide dismutase (SOD) has not been significant between all groups, but has also group had supplementation with 6g/kg CV higher concentration when compared to other groups.

Hematological evaluation of blood:-

Table 9 shows the effects of dietary supplementation of *Spirulina plantesis* and *Chlorella vulgaris* of algae on hematological evaluation of blood that has been investigated. Feeding diets with different types and levels of algae had significant improvement in most parameters detected of particular interest, HB, RBCs count, Platelets and lymphocytes have all increased significantly. It is worth noting that most improvement occurs more significantly at inclusion levels of 6.0 g/kg SP and 4.0 g/kg CV. This observation was in line with a previous study that concluded that Spirulina has exhibited a preventive effect against anemia due to its content of vitamins and iron (Hemalatha K. *et al.,* 2012). This result agrees with Kang *et al.,* 2013 who found that the number of lymphocytes was significantly higher (P < 0.05) in 1.0% fresh liquid *Chlorella* compared with antibiotic growth promoters and 1.0% dried *Chlorella* powder; however, supplemental *Chlorella* did not affect other blood leucocytes of broiler chickens. In addition, Swati *et al.,* 2022 discovered that there were similarities between the groups' RBC counts, haemoglobin concentrations, and HCT levels. Little variations were seen in MCV, MCH, and MCHC, with the exception that MCV values were lowered when Spirulina platensis (5%) and Chlorella vulgaris (5%) were supplemented at a higher level. Additionally, compared to the control group, the group supplemented with 1.25% of both Chlorella vulgaris and Spirulina platensis had a higher MCV value. It has been observed that feeding broiler chicks dietary supplements containing S. platensis increases their erythrocyte and haemoglobin counts Jamil *et al.,* 2015. In contrast, Sugiharto *et al.,* 2018 feeding broilers such algae over the entire growing period caused the animals' erythrocyte, haemoglobin, and hemocrit levels to drop. McCarty (2007) In chickens, the activity of phagocytic cells—monocyt/macrophages, heterophils,and thrombocytes—was enhanced by supplementing with spirulina.

Table 9. Effect of SP and CV supplemented-diet hematological evaluation of 42-day-old broiler chicks.

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV(2g/kg)	CV(4g/kg)	CV(6g/kg)	Pooled SEM	P value	Sig.
Hb (g/dl)	10.16^{b}	11.83^{ab}	12.56^{ab}	13.93^{ab}	13.53^{ab}	15.40^{a}	13.93^{ab}	0.956	0.0358	*
RBCs (×10^{6}/µL)	3.510^{b}	4.053^{ab}	4.433^{ab}	4.666^{ab}	4.066^{ab}	5.400^{a}	4.933^{ab}	0.299	0.0104	*
PCV (%)	28.83	32.50	35.36	40.00	38.36	44.53	40.46	3.986	0.1766	NS
MCV (fl)	83.13^{b}	85.10^{ab}	85.33^{ab}	84.76^{ab}	86.53^{ab}	85.76^{ab}	87.50^{a}	0.857	0.0664	*
MCH (pg)	26.76^{b}	27.50^{ab}	28.60^{a}	28.66^{a}	28.20^{ab}	28.93^{a}	28.56^{a}	0.335	0.0048	**
MCHC (%)	33.93	34.23	32.63	33.60	34.20	35.06	32.73	1.048	0.6663	NS
Platelete (×10^{3}/µL)	411.0	462.3	453.6	474.3	429.3	403.3	413.0	38.38	0.7726	NS
WBC (×10^{3}/µL)	16.66	18.66	18.00	21.00	19.33	22.66	20.66	1.685	0.2669	NS
Heterocyte (×10^{3}/µL)	21.33	14.00	19.33	18.00	19.33	14.66	16.66	2.167	0.2457	NS
Lymphocyte (×10^{3}/µL)	66.33^{b}	77.00^{ab}	72.33^{ab}	73.66^{ab}	74.66^{ab}	83.33^{a}	79.00^{ab}	3.005	0.0342	*
Esinophil (×10^{3}/µL)	2.666^{a}	2.333^{ab}	0.666^{ab}	0.333^{ab}	0.333^{ab}	0.000^{b}	1.666^{ab}	0.487	0.0074	*
Monocyte (×10^{3}/µL)	9.666	6.666	7.666	8.000	5.666	2.000	2.666	1.808	0.0793	NS

"a-bMeans in the same row with different superscripts differ significantly (P≤ 0.05)".

Meat quality parameters of broiler chicken.

Different parameters of meat quality have been examined in (Table 10) in terms of cooking loss, pH%, bound water, water holding capacity and plasticity. It is observed that significant improvement in meat quality can be detected especially after the addition of 4.0 g/kg CV. An *et al.* (2016) found that low levels (0.05, 0.15, and 0.5%) of incorporation of Chlorella in broiler diets had no significant effect on the color of breast meat. Contrary, it improvement may be referred to the fact that high content of xanthophyll, carotenoids, and polyunsaturated fatty acids (PUFA) are present in CV (Alfaia *et a*l., 2021). Otherwise, inclusion levels of 2.0 and 6.0 g/kg SP have also shown decreased plasticity and cooking loss, respectively.

Table 10. Effect of SP and CV supplemented-diet on meat quality parameters of broiler chickens at 42-day-old broiler chicks.

Main effects	Control	SP (2g/kg)	SP (4g/kg)	SP (6g/kg)	CV (2g/kg)	CV (4g/kg)	CV (6g/kg)	Pooled SEM	Sig.	P value
Cooking loss%	32.33^{a}	25.66^{b}	24.33^{bc}	22.66^{bc}	21.00^{bc}	20.33^{c}	21.66^{bc}	0.975	***	0.0001
Ph %	6.570	6.463	6.650	6.573	6.480	6.610	6.603	0.045	NS	0.0983
WHC (cm) ^{2}	6.466	6.893	8.170	6.780	9.963	7.846	6.946	1.316	NS	0.5525
Bound water %	64.13	63.41	61.27	63.60	58.25	61.81	63.32	2.212	NS	0.5530
Plasticity (cm)^{2}	3.853	2.913	3.203	3.016	2.690	2.656	3.210	0.418	NS	0.4933

"a-cMeans in the same row with different superscripts differ significantly (P≤ 0.05)".

CONCLUSION

According to the aforementioned results, it can be concluded that dietary supplementation of SP or CV have positive impacts on productive performance, hematological parameters, and meat quality of broiler chicken. The maximal benefits can be generally obtained at inclusion levels of 4.0 g/kg of each of SP or CV. Future research have to be directed to minimize the cost of production to maximize the economic benefit of microalgae supplementation in poultry rations.

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تقييم دور سبيرولينا بلاتنسيس وكلوريلا فولجارس في أداء النمو وجودة اللحوم ومؤشرات الدم لدجاج التسمين

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قسم إنتاج الدواجن، كلية الزراعة، جامعة المنصورة، 35516، مصر

الملخص

ركزت هذه الدراسة على تأثير المكملات الغذائية لنوعين من الطحالب البحرية الدقيقة على كتاكيت التسمين وهي سبيرولينا (SP) وكلوريلا (CV)، كلا علي حدا. هدفت هذه الدراسة إلى تحديد تأثيرهما على الأداء الإنتاجي، صفات الذبيحة، مقاييس الدم، القياسات المناعية وجودة اللحوم. تم تقسيم عدد 210 من كتاكيت التسمين عمر يوم من سلالة الروص 308 إلى سبع معاملات، تضمنت كل منها ثلاث مكررات. كانت معاملات النظام الغذائي (0.0، 2.0، 4.0، 6.0 جم / كجم مجموعة SP المجففة و 2.0، 4.0، 6.0 جم / كجم مجموعة CV المجففة) لمدة 42 يومًا. أظهرت النتائج تحسنا معنويا في معدل التحويل الغذائي و معدل الزيادة في وزن الجسم في المجموعات التي تم أضافة الطحالب اليها مقارنة مع مجموعة الكنترول. وأظهرت النتائج زيادة كبيرة في كرات الدم الحمراء والهيموجلوبين في معظم المجموعات المضافة اليها الطحالب حيث وجد أن أفضل النتائج ظهرت في كلا من المعاملة التي اضيف لها 4.0جم/كجم SP وكذلك المعاملة الت أضيف لها 6 جم/كجم CV. كما أظهرت النتائج زيادة الوزن النسبي bursa بشكل معنوي في جميع مجموعات الـCV وكانت أفضل نتيجة عند مستوى المجموعة 2.0 جم/كجم من الـCV. لم يكن للأنظمة الغذائية التي تحتوي على أنواع ومستويات مختلفة من الطحالب أي تأثير معنوي على ALT،البروتين الكلي، الألبومين، الكوليسترول، HDL، IgA، وSOD بين جميع المجموعات. ومع ذلك، انخفضت مستويات AST والدهون الثلاثية في سيرم الدم لمكملات دجاج التسمين عند مستوى 6 جرام/كجم من CV مقارنة مع المجموعات الأخرى. وبناء على ذلك، نستنتج أن المكملات الغذائية من SP أو CV لها آثار إيجابية على الأداء الإنتاجي، ومؤشرات الدم، وجودة اللحوم في دجاج التسمين. بشكل عام، يمكن الحصول على الفوائد القصوى عند مستويات تبلغ 4.0 جم/كجم من SP أو CV.