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Research Article
Effect of Dietary Supplement of Algae (*Spirulina platensis*) as an Alternative to Antibiotics on Growth Performance and Health Status of Broiler Chickens

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Abstract
Objective: The aim of the current study was to evaluate the effects of adding *Spirulina platensis* as a dried powder to the commercial broiler feed on the growth performance, microbial population in the excreta and biochemical and hematological parameters of broiler chicks in comparison to an antibiotic-based diet. **Materials and Methods:** A total of 150 one-day-old broiler chicks (Cobb 500) were used in a 28-day experiment. Broiler chicks were allocated to five treatments with three replications (10 chicks/replication) based on a completely randomized design. Diet was the same for all treatments but dried *Spirulina* powder (DSP) was provided in the feed as follows: control with no DSP (T_5), 0.5% DSP (T_1), 1% DSP (T_2), 1.5 % DSP (T_3) and antibiotic with no DSP (T_4). **Results:** The results showed that the birds fed 0.5 and 1.5% DSP diets achieved superior body weights, body weight gain and feed conversion ratios compared to those of the control and antibiotic-treated groups. The weight of the lymphoid organ and internal organs of different groups showed that there was no significant ($p>0.05$) difference among the groups. The consequences of the biochemical test were also not significant ($p>0.05$) among the treatments. The results of the hematological studies showed no significant ($p>0.05$) differences due to supplementation of DSP, except for the number of red blood cells (RBC), number of lymphocytes and mean corpuscular hemoglobin concentration (MCHC) which all were significantly affected ($p<0.05$) compared with the control and antibiotic-treated groups. However, addition of DSP to the broiler chicks' diets showed significant ($p<0.05$) differences in the bacterial colony count among the groups. **Conclusion:** The results of this study demonstrate that *Spirulina platensis* supplementation positively affected the growth performance and health status in broilers, indicating that *Spirulina platensis* can be safely used to replace antibiotics as a growth promoter.

Key words: Broiler chicks, growth performance, hematological parameters, microbial population, *Spirulina platensis*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The term "antibiotic growth promoter" is used to describe any medicine that destroys or inhibits bacteria and is administered at a low, subtherapeutic dose. The mechanism of action of antibiotics as growth promoters is related to the interactions with the intestinal microbial population[1,2]. The ban on using antibiotics as feed additives has accelerated research into the use of alternative feed additives in poultry production[3]. Phytogenic additives are plant-derived products used in animal feed to improve the performance of agricultural livestock[3,4]. This class of additives has recently gained increasing interest, especially for use in modern poultry production[5]. Phytogenic supplements can improve the health status of broilers and their production performance, among other things, by stimulating the microbiota of the gastrointestinal tract or by improving the digestibility of nutrients[6,7]. Generally, plant extracts have no problems of resistance[8] and broilers fed herbal feed additives are accepted well by the consumers[9]. *Spirulina platensis* is a cyanobacterium, which is generally regarded as a rich source of protein, essential amino and fatty acids, vitamins and minerals. *Spirulina* is also known to be rich in thiamin, riboflavin, pyridoxine, vitamin-B12, vitamin C, gamma linolenic acid, phycocyanins, tocopherols, chlorophyll, β-carotenes and carotenoids[10]. Over the past few years, many additional pharmacological properties have been found for *Spirulina*. Many studies suggest that *Spirulina* has therapeutic effects. It helps to reduce cholesterol and cancer by enhancing the immune system. It also helps increase intestinal lactobacilli, reduce nephrotoxicity by heavy metals and drugs and radiation protection[11]. Additionally, *Spirulina* is well known to have antioxidant properties, which are attributed to molecules, such as phycocyanin, beta-carotene and tocopherol. It has been found that *Spirulina* is capable of inhibiting carcinogenesis and organ-specific toxicity due to its antioxidant properties[12]. It has been shown that *Spirulina* enhances immune function and reproduction, increases growth and has been used throughout the world as a feed component in quality broiler[14] and layer diets to enhance yolk color and flesh[15]. The present study was designed to compare the efficacy of different concentrations of dried *S. platensis* powder in broiler feed on the growth performance, microbial population in the excreta and biochemical and hematological parameters in broiler chickens.

MATERIALS AND METHODS

Experimental design, broilers, housing and diets: One hundred and fifty Cobb 500 one-day-old broiler chicks (body weight 40.0±0.22 g). (mean±standard deviation) were used in the current study. Broiler chicks were allocated to five treatments with three replicates each with 10 chicks per replication based on a completely randomized design. Diet was the same for all treatments but dried *Spirulina* powder (DSP) was provided in the feed as follows: control with no DSP (T_5), 0.5% DSP (T_1), 1% DSP (T_2), 1.5% DSP (T_3) and antibiotic with no DSP (T_4)Throughout the study period, the chicks were raised in an open-sided broiler house with rice husk-littered floor pens. The proportion of *S. platensis* supplemented to basal diets was based on Shanmugapriya *et al.*[16]. Feeds (as mash form) and water were provided *ad libitum* throughout the study period. A coccidiostat was not included in the feeds. The chemical compositions of the *S. platensis* powder and basal diets [formulated to meet the Bangladesh National Standards for Broiler Feed (SNI, 2010)] provided to the broiler chicks are presented in Table 1 and 2, respectively.

Table 1: Chemical composition of *Spirulina platensis* (as dry basis)

Items	Composition (%)
Moisture	91.8
Crude ash	11.9
Crude fat	0.63
Crude protein	52.4
Crude fiber	34.2

Table 2: Ingredients and nutrient composition (as dry basis) of basal diet used in the study

Items	Composition (%, unless otherwise noted)
Maize	45.50
Soybean meal (CP 46%)	17.00
Wheat flour	10.00
Bread flour	5.00
Rice bran	4.45
Crude palm oil	3.50
Corn gluten meal (CP 62%)	3.60
Distiller dried grains (CP 27%)	3.00
Meat bone meal (CP 49%)	2.80
Chicken feather meal (CP 79%)	2.00
Bone meal (CP 22%)	1.50
Lysine	0.55
Methionine	0.37
L-threonine	0.08
Salt	0.15
Premix[1]	0.50
Analyzed composition	
Metabolizable energy (kcal kg^{-1})[2]	3.30
Dry matter	89.60
Crude protein	21.90
Crude fat	6.40
Crude fiber	5.62
Ash	6.39

[1]Mineral-vitamin premix per kg of diet; Ca: 2.250 g, P: 0.625 g, Fe: 3.570 mg, Cu: 0.640 mg, Mn: 5.285 mg, Zn: 0.003 mg, Co: 0.001 mg, Se: 0.013 mg, I: 0.016 mg, Vitamin A: 375 IU, Vitamin D: 150 IU and Vitamin E: 0.080 mg, [2]Metabolizable energy was calculated according to formula (Bolton, 1967): 40.81 CP: Crude protein

Antibiotic or *S. platensis* was added at the expense of the feeds. *S. platensis* was obtained from the Bangladesh Council of Scientific and Industrial Research (BCSIR). The microalgae were grown in fresh water. The experiment was conducted according to the standard procedures of rearing of farm animals as stated in law number 12, 2013, of the People 's Republic of Bangladesh.

The chicks were vaccinated with the commercial Newcastle disease virus (NDV) and infectious bronchitis (IB) vaccines through eye drops at 4 days and 21 days. The Gumboro vaccines were given through drinking water at days 9 and 17 of the experiment, respectively. Body weight and feed intake were determined weekly. The feed conversion ratio (FCR) was determined as the feed intake per weight gain. To determine the hematological profile, blood was obtained from the wing veins and collected in vacutainers containing ethylene diamine tetra acetic acid (EDTA) at day 25. The rest of the blood was collected in the vacutainers with no anticoagulant, let to clot at room temperature and centrifuged at 2000 rpm for 15 min to produce serum. The serum was frozen until serum biochemistry was performed[17]. At day 28, a total of 45 chicks were slaughtered, defeathered and eviscerated. The internal organs were immediately taken out and weighed. Digesta were collected from the cecum of the broilers for microbiological analyses.

Complete blood counts were determined using a hematology analyzer. Total cholesterol and sugar in serum were measured with the enzymatic colorimetric/color method. The counts of certain bacteria in the intestinal digesta of the broilers were determined according to Sugiharto *et al.*[18] with a few modifications. For enumeration of *E. coli* and salmonella bacteria, samples were cultured on EMB and SS agar. After aerobic incubation at 38°C for 24 h, *E. coli* and salmonella bacteria were counted as metallic sheen and colorless colonies with black centers, respectively.

Data analysis: The data was subjected to statistical analysis by applying a one-way ANOVA using the statistical package for social sciences (SPSS) version 16. Differences between means were tested using Duncan's multiple comparison test, LSD and the significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Production performances of broiler chickens

Final life weight: Data presented in Table 3 show that the effect of treatments on the final live weight (gram per broiler chicken) was not significant ($p>0.05$). The relative final live weights (g) of broiler chickens in the dietary groups T_1, T_2, T_3, T_4 and T_5 were 1538.89±18.69, 1522.22±55.68, 1604.22±62.88, 1461.11±36.12 and 1550.00±63.45, respectively. The largest ending weight was found in group T_3 (1604.22±62.88) and the lowest ending weight was observed in the T_4 (1461.11±36.12) group. However, although the final live weight of broilers fed the *Spirulina* diets increased, this result was not significant ($p>0.05$) compared to that of the control and antibiotic-treated groups.

These results are in agreement with those obtained by Ross *et al.*[19], who found that there was no adverse effect of dietary *Spirulina* on final body weight. In addition, these results are contradictory with those of previous researchers[20-21], who reported that dietary *Spirulina* significantly ($p<0.05$) improved the weight gain of chickens compared with control groups. However, Ross and Dominy[19] and Nikodémusz *et al.*[22] reported that birds fed dietary *Spirulina* had positive effects on productive performance. In this regard, Raju *et al.*[23] concluded that dietary inclusion of *Spirulina* at a level of 0.05% can partially offset the adverse effects of aflatoxin on the growth rate of broiler chickens.

Feed consumption (FC): Different treatment groups (Table 3) showed significant ($p<0.05$) differences in FC of broiler chickens. The control group consumed more feed (1928.43±37.35 g) and the 1% (T_2) dried *Spirulina* powder treated group consumed a lower amount of feed (1778.80±28.93 g). The antibiotic treated group T_4

Table 3: Production performance of broiler chickens treated with *Spirulina* and antibiotics

Treatments	T_1	T_2	T_3	T_4	T_5	Mean±SE	$LSD_{(0.05)}$
Final Live weight (g broiler^{-1})	1538.89±18.69	1522.22±55.68	1604.22±62.88	1461.11±36.12	1550.00±63.45	1535.29± 22.71	71.374^{NS}
FC (g)	$1819.63±20.74^{bc}$	$1778.80±28.93^{c}$	$1873.67±25.73^{ab}$	$1867.53±21.98^{abc}$	$1928.43±37.35^{a}$	1853.61±17.13	39.028*
FCR	$1.28±0.03^{b}$	$1.30±0.02^{b}$	$1.29±0.02^{b}$	$1.42±0.01^{a}$	$1.45±0.01^{a}$	1.34±0.023	0.026*
DP% (skinless)	68.66±1.66	71.54±3.58	71.67±2.01	69.19±1.90	70.50±3.15	69.91±1.09	3.64^{NS}
Survivability (%)	100.00±0.00	99.00±0.01	100.00±0.00	99.00±0.01	100.00±0.00	99.60±0.01	0.002^{NS}

Here, T_1: 0.5% DSP supplementation, T_2: 1% DSP supplementation, T_3: 1.5% DSP supplementation, T_4: Antibiotic and T_5: Control. Values are expressed as Mean±S.E. (n = 15) and are the results from the one-way ANOVA (SPSS, Duncan method), Means with different lowercase superscripts are significantly different ($p<0.05$), Means with the same lowercase superscripts are not significantly ($p>0.05$) different, SE: Standard error, LSD: Least significant difference, NS: Non significant, *means significant at 5% level of significance ($p<0.05$)

(1867.53±21.98 g) showed no significant (p>0.05) difference in total FC and weekly FC compared to all other treatment groups.

These results are in agreement with those of previous researchers[24,15], who recorded no significant (p>0.05) effects of dietary *Spirulina* supplementation on performance parameters. In contrast, other researchers[20,21] reported that dietary *Spirulina* supplementation significantly (p<0.05) improved feed consumption of broiler chickens.

Feed conversion ratio (FCR): The feed conversion ratio (FCR) was significantly (p<0.05) lower for birds fed the diet supplemented with 0.5% (1.26±0.03) dried *Spirulina* powder than that of the control birds (1.45±0.01). However, the FCR was significantly (p<0.05) higher in the T_4 group (1.42±0.01) (supplemented with antibiotics) compared to the T_2 (1.29±0.02) and T_3 (1.27±0.02) groups (Table 3).

These results are in agreement with previous studies conducted by Kharde *et al.*[20] and Shanmugapriya and SaravanaBabu[21] reported that dietary *Spirulina* significantly (p<0.05) improved the feed efficiency of broiler chickens compared with the control groups. These results contradict the findings of previous researchers[15,19,24,25], who found no significant (p>0.05) effects of dietary *Spirulina* supplementation on performance parameters. However, Ross and Dominy[19] and Nikodémusz *et al.*[22] reported that birds fed dietary *Spirulina* had beneficial effects on productive performance. Contradictory results are possibly due to the different *Spirulina* inclusion levels and quality in the present trials. In addition, secondary parameters such as feed composition, housing conditions and production systems may contribute to the variation in the results of the present study.

Survivability: The survivability rate shown in Table 3 was higher for the *Spirulina*-supplemented group (100±0.00) than that of the antibiotic-supplemented group but was not significantly (p>0.05) different from the control group (100±0.00).

These results are in agreement with those of Qureshi *et al.*[25], who found a lower nonspecific mortality rate in turkey poults fed *Spirulina* at the level of 1000-10000 mg kg^{-1} compared to poults fed a basal diet. These results are also supported by the other researchers[15,24] who recorded no significant (p>0.05) effects of dietary *Spirulina* supplementation on performance parameters.

Dressing percentage (DP): T_3 (1.5% *Spirulina*-supplemented group) had a greater (p>0.05) carcass percentage (71.67±2.01%) compared with the antibiotic-treated group (69.19±1.90%) as well as the 0.5% (T_1), 1% (T_2) and control (T_5) group, whose DPs were 68.66±1.66, 71.54±3.58 and 69.91±1.09, respectively (Table 3).

In the present study, the effects of *Spirulina* on broiler performance parameters, including average DP, was in agreement with previous studies[27,28]. Furthermore, Bell of and Alarcon[29] reported that under organic farming, dietary *Spirulina* supplementation significantly (p<0.05) improved carcass performance parameters of broilers. However, dried *S. platensis* supplement displayed a greater growth-promoting effect and increased the carcass yield percentage.

Sugar: The effects of dietary dried *Spirulina* powder supplementation on the concentration of sugar in broiler chickens are presented in Table 4. Feeding dietary *Spirulina* made no significant (p>0.05) differences in blood sugar levels among the treatments. Although, the highest amount (10.40±0.85 mmol L^{-1}) of plasma sugar was found in T_3 (1.5% *Spirulina*), this level was not significantly different from the levels from the antibiotic, control and other groups.

The increase in plasma glucose concentration of hens fed dietary *Spirulina* may be attributed to its excellent nutritional profile and high carotenoid content. In this regard, El-Khimsawy[30] reported that vitamin A plays an important role for the synthesis of glucose molecules in the body.

Total cholesterol: Total cholesterol concentration (mg dL^{-1}) in the serum of different groups ranged from 117.44±4.74 to 132.89±4.81. Statistical analysis revealed an insignificant (p>0.05) difference among the groups. The cholesterol levels of the different treatments were 119.00±1.07 (T_1), 117.44±4.74 (T_2), 132.00±12.81 (T_3), 130.00±11.64 T_4 and 132.89±4.81 T_5. Although, not significantly different, the cholesterol concentration found in broilers from the T_5 group (132.89±4.81) was comparable to that of broilers in the T_3 (132.00±12.81) and T_4 (130.00±11.64) groups (Table 4).

Table 4: Effect of *Spirulina* on the serum biochemical level of different broiler chickens under different treatments

Parameters	T_1	T_2	T_3	T_4	T_5	Mean±SE	$LSD_{(0.05)}$
Sugar (mmol L^{-1})	10.18±0.25	9.37±0.27	10.40±0.85	10.08±0.60	10.33±0.27	10.07±0.218	0.726^{NS}
Cholesterol (mg dL^{-1})	119.00±1.07	117.44±4.74	132.00±12.81	130.00±11.64	132.89±4.81	126.27±3.615	11.775^{NS}

Here, T_1: 0.5% DSP supplementation, T_2: 1% DSP supplementation, T_3: 1.5% DSP supplementation, T_4: Antibiotic and T_5: Control. Values are expressed as Mean±S.E. (n = 15) and are the results from the one-way ANOVA (SPSS, Duncan method, Means with different superscripts are significantly different (p<0.05). Means with the same superscripts are not significantly (p>0.05) different, SE: Standard error, NS: Non significant, LSD: Least significant difference

Results of the present study were similar to previous studies conducted by Kanagaraju and Omprakash[31] and Swee Weng *et al.*[32], who found that the addition of 1% *Spirulina* resulted in significantly lower serum cholesterol levels than found in the control group in quails. These results are contradictory with the findings of Abdel-Daim[10], Kannan *et al.*[33] and AbouGabal *et al.*[34]. Additionally, *Spirulina platensis* supplementation at a level of 1% significantly improved the blood parameters[21]. This contradictory result was found due to certain adverse environmental effects and heat stress during the summer season. Furthermore, Jamil *et al.*[35] concluded that ALT and AST decreased significantly ($p<0.05$) when animals were fed *Spirulina platensis* compared with the control group.

Relative giblet weight (liver, heart and gizzard): The largest results for relative giblet weight were observed in the T_1 group and the lowest in the T_4 group. However, there was no significant ($p>0.05$) difference in the relative weight of livers between the groups (Table 5).

The comparative weight of the liver (g) of broiler chicks in the dietary groups T_1, T_2, T_3, T_4 and T_5 were 7.39±0.72, 6.11±0.56, 7.44±0.27, 6.52±0.36, 6.72±0.05, respectively. The qualified weight of the heart of different groups showed that there was no significant ($p>0.05$) difference between the groups and the values ranged from 6.11±0.56 to 7.44±0.27 (Table 5).

The comparative weight of the gizzard of different groups did not show any significant ($p>0.05$) difference in groups T_1 (41.28±1.64), T_2 (41.00±3.12), T_3 (36.33±1.72) and T_5 (37.61±3.64) compared to the T_4 group (35.83±2.03) (Table 5).

The relative weights of the giblet organs, namely, the liver, heart and gizzard, revealed no increase in any group. The present results are akin to that of Hernandez *et al.*[9], who observed no difference in the mean weight of the proventriculus, gizzard, intestine, liver and pancreas in broilers fed two herbal extracts. In another study[36], a decoction of neem was evaluated as a total replacement for antibiotics and coccidiostat in a 6-week feeding trial in broilers.

Weight of intestine: The results of the different groups showed that there was no significant ($p>0.05$) difference among the groups in the weight of the intestine and the values ranged from 101.78±0.22 to 116.83±6.75 (Table 5).

The present results are akin to that of Hernandez *et al.*[9], who observed no difference in the mean weight of the proventriculus, gizzard, intestine, liver and pancreas in broilers fed two herbal extracts.

Immune organs: The effects of dried *Spirulina* powder supplementation on immune organs of Cobb 500 strain broiler chicks during the period from 0 to 28 days of age are summarized in Table 6. The comparative weight of the spleen (g) of broiler chicks in the dietary groups T_1, T_2, T_3, T_4 and T_5 were 1.94±0.27, 1.73±0.19, 1.78±0.36, 1.61±0.20, 1.44±0.14, respectively. The highest weight was 1.94±0.27 g in the T_1 group and the lowest weight was 1.44±0.14 g in the T_5 group. On the other hand, the relative weight of the spleen for different groups showed that there was no significant ($p>0.05$) difference among the groups and the values ranged from 1.44±0.14 to 1.94±0.27 g.

Table 5: Effect of dietary supplementation of *Spirulina* on liver, gizzard, intestine and heart weight of different treatments

Parameters	T_1	T_2	T_3	T_4	T_5	Mean±SE	$LSD_{(0.05)}$
Liver							
weight (g)	37.89±3.13	37.33±1.61	35.56±2.25	34.00±2.50	35.83±1.62	36.12±0.944	3.252^{NS}
Gizzard weight (g)	41.28±1.64	41.00±3.12	36.33±1.72	35.83±2.03	37.61±3.64	38.41±1.145	3.612^{NS}
Intestine weight (g)	101.78±0.22	103.67±7.98	116.83±6.75	108.11±2.14	101.78±7.21	107.04±2.599	8.148^{NS}
Heart weight (g)	7.39±0.72	6.11±0.56	7.44±0.27	6.52±0.36	6.72±0.05	6.84±0.221	0.650^{NS}

Here, T_1: 0.5% DSP supplementation, T_2: 1% DSP supplementation, T_3: 1.5% DSP supplementation, T_4: Antibiotic and T_5: Control. Values are expressed as Mean±S.E. (n = 15) and are the results from the one-way ANOVA (SPSS, Duncan method), Means with different superscripts are significantly different ($p<0.05$). Means with the same superscripts are not significantly ($p>0.05$) different, SE: Standard error, NS: Non significant, LSD: Least significant difference, *means significant at 5% level of significance ($p<0.05$)

Table 6: Effects of supplementation of dried *Spirulina* powder to broiler diets on certain immune organs

Parameters	T_1	T_2	T_3	T_4	T_5	Mean ±SE	$LSD_{(0.05)}$
Spleen wight (g)	1.94±0.27	1.73±0.19	1.78±0.36	1.61±0.20	1.44±0.14	1.70±0.104	0.351^{NS}
Bursa weight (g)	1.72±0.30	1.56±0.31	2.44±0.11	1.72±0.64	1.67±0.00	1.82±0.157	0.496^{NS}

Here, T_1: 0.5% DSP supplementation, T_2: 1% DSP supplementation, T_3: 1.5% DSP supplementation, T_4: Antibiotic and T_5: Control. Values are expressed as Mean±S.E. (n = 15) and are the results from the one-way ANOVA (SPSS, Duncan method), Means with different superscripts are significantly different ($p<0.05$), Means with the same superscripts are not significantly ($p>0.05$) different, SE: Standard error, LSD: Least significant difference, NS: Non significant, *means significant at 5% level of significance ($p<0.05$)

Table 7: Effect of supplementation of dried *Spirulina* powder (DSP) to broiler diets on blood parameters

Parameters	T_1	T_2	T_3	T_4	T_5	Mean±SE	$LSD_{(0.05)}$
Hemoglobin ($g\ dL^{-1}$)	8.76±0.79	8.53±0.43	8.45±0.33	7.22±0.37	8.03±0.25	8.20±0.231	0.673^{NS}
RBC (million/mm^3)	$3.23±0.35^a$	$3.07±0.17^{ab}$	$2.72±0.23^{ab}$	$2.48±0.01^b$	$2.51±0.04^b$	2.81±0.113	0.292*
WBC ($10^3/mm^3$)	6277.78±116	6155.56±908	5922.22±800.3	8833.33±1291.1	7366.67±1578.4	6911.11±494.635	1501.605^{NS}
Neutrophil (%)	62.78±2.35	62.89±0.80	62.00±2.03	68.78±2.39	67.00±1.89	64.69±1.038	2.802^{NS}
Lymphocyte (%)	$33.78±2.69^a$	$33.00±0.69^{ab}$	$33.89±2.04^a$	$26.67±2.51^b$	$28.56±1.45^{ab}$	31.18±1.105	2.854*
Monocyte (%)	1.78±0.11	1.67±0.19	1.67±0.19	1.78±0.11	1.56±0.22	1.69±0.069	0.243^{NS}
Eosinophil (%)	2.67±0.13	2.44±0.11	2.44±0.15	2.78±0.17	2.89±0.19	2.64±0.124	0.427^{NS}
PCV (%)	26.46±2.36	$26.67±1.22^a$	26.07±1.28	23.58±1.15	24.56±0.65	25.47±0.634	2.047^{NS}
MCV (Fl)	80.45±2.27	81.85±1.5	78.18±2.57	76.97±2.02	78.87±1.01	79.26±0.871	2.773^{NS}
MCH (pg)	30.12±0.12	29.84±0.13	29.53±0.30	29.82±0.28	30.16±0.32	29.89±0.112	0.352^{NS}
MCHC ($g\ dL^{-1}$)	$31.41±0.43^a$	$31.46±0.10^a$	$30.72±0.29^{ab}$	$30.24±0.19^b$	$30.22±0.17^b$	30.81±0.177	0.378*

Here, T_1: 0.5% DSP supplementation, T_2: 1% DSP supplementation, T_3: 1.5% DSP supplementation, T_4: Antibiotic and T_5: Control, Values are expressed as Mean±S.E (n = 15) and are the results from the one-way ANOVA (SPSS, Duncan method), Means with different lowercase superscripts are significantly different (p<0.05), Means with the same lowercase superscripts are not significantly (p>0.05) different, SE: Standard error, LSD: Least significant difference, *means significant at 5% level of significance (p<0.05)

Table 8: Bacterial colony count in *Spirulina* experiment in broiler chickens

Treatments	*E. coli* (EMB) $\times 10^4$ (CFU mL^{-1})	*Salmonella* (SS) $\times 10^4$ (CFU mL^{-1})
T_1	$13.07±2.78^{ab}$	$12.40±1.55^b$
T_2	$14.88±2.85^{ab}$	$12.25±2.37^b$
T_3	$11.98±0.71^b$	$15.70±0.20^b$
T_4	$10.57±0.46^b$	$13.65±0.90^b$
T_5	$20.25±3.19^a$	$22.50±2.18^a$
Mean±SE	14.15±1.25	15.30±1.19
$LSD_{(0.05)}$	3.278*	2.338*

Here, T_1: 0.5% DSP supplementation, T_2: 1% DSP supplementation, T_3: 1.5% DSP supplementation, T_4: Antibiotic and T_5: Control, Values are expressed as Mean±S.E. (n = 15) and are the results from the one-way ANOVA (SPSS, Duncan method), Means with different lowercase superscripts are significantly different (p<0.05), Means with the same lowercase superscripts are not significantly (p>0.05) different, SE: Standard error, LSD: Least significant difference, *Means significant at 5% level of significance (p<0.05)

The weight of the bursa was higher in the T_3 group (2.44±0.11 g) compared to the T_1 (1.72±0.30 g), T_2 (1.56±0.31 g), T_4 (1.72±0.64 g) and T_5 (1.67±0.00 g) groups, respectively. However, these values were not significantly different among the treatments (Table 6). It can be concluded that the addition of *Spirulina platensis* to broiler diets improved the weight of the bursa and spleen compared with the control. However, these values were not different among the groups. In accordance with the present results, Kaoud[37] reported that the relative and absolute weights of the thymus and bursa were induced for the groups fed diets containing *Spirulina* compared to the control group. These results may be considered a good indicator of the healthy status of chicks fed dietary *Spirulina*. In this respect, Bennett and Stephens[38] reported that the bursa functions were half of the immune system and its size reflects the overall health status of bird. These authors added that stressed or sick birds have small-sized bursa but healthy or productive birds have large-sized bursa. Bursa size is a biological indicator of how flocks are well-managed and preserved from disease. Additionally, the addition of less than 1% *Spirulina* in chicken diets significantly enhanced the defense systems for antigen processing, greater T-cell activity and increased microbial killing[39]. In addition, an increased content of Zn concentration in *Spirulina* plays a role in inducing the cellular immunity of birds[40].

Hematological parameters: Table 7 shows the effect of dietary levels of dried *Spirulina* powder (0.5, 1 and 1.5%) in feed and their impact on some blood parameters. Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of dried *Spirulina* powder, except for RBCs, lymphocytes and MCHC, which were significantly affected (p<0.05). Birds fed diets supplemented with dried S*pirulina* powder (at levels of 0.5, 1 and 1.5%) had higher values of RBCs, lymphocytes and MCHC but in the case of the antibiotic and control groups, this trends was lower than the *Spirulina*-treated groups.

These results are in line with the findings of Abdel-Daim *et al.*[10], Kannan *et al.*[33] and AbouGabal *et al.*[34]. The increment in the blood indices may be related to the rich mineral content (Fe, Cu and Zn) in *Spirulina*[41,42]. It is well known that iron plays an important role in hemoglobin and red blood cell biosynthesis to prevent anemia and is essential for metabolic enzyme biosynthesis, such as cytochromes, superoxide dismutase and glutathione reductase[43]. These results are in agreement with a previous study conducted by Badway[43]. However, 1% of *Spirulina platensis* supplementation significantly (p<0.05) improved the blood parameters[21]. However, Kamruzzaman[44] concluded that the mean hemato-biochemical values of Hb, ESR, PCV, heterophils, eosinophils, basophils, triglyceride, HDL, LDL, SGPT and SGOT differed significantly (p<0.01) in the different groups supplemented with probiotics in broiler rations.

Intestinal microflora: The microbial load (total count, *E. coli* and *Salmonella* for its beneficial effect) in broilers fed different levels of dried *Spirulina* powder is given in Table 8. *E. coli* count was significantly (P<0.05) lower in birds fed 0.5%, 1% and 1.5% dried *Spirulina* powder and antibiotics (13.07±2.78, 14.88±2.85, 11.98±0.71 and 10.57±0.46, respectively) than the control birds (20.25±3.19). *Salmonella* sp. count was significantly (p<0.05) lower in birds fed 0.5, 1 and 1.5% dried *Spirulina* powder and antibiotics (12.40±1.55, 12.25±2.37, 15.70±0.20 and 13.65±0.90) than the control birds (22.50±2.18).

These results are in accordance with the earlier findings of Wakwak *et al.* and Kulshreshtha *et al.* [46]. In addition, the results of the current study support the results of Baojiang[48], who found that *Spirulina* is useful for the beneficial intestinal flora.

CONCLUSION

It is concluded that a dietary inclusion level of 1.5% *S. platensis* powder was very effective. Thus, *S. platensis* could be used as an alternative for antibiotics in broiler rations. This study therefore recommends conducting field trials on commercial poultry farms to fixed the inclusion level of *S. platensis*.

SIGNIFICANCE STATEMENT

This study discovered the beneficial effects of *S. platensis* supplementation that can positively affect the growth performance and health status of broiler chickens. This study will help the producer raise antibiotic-free broiler chickens and will help the researcher uncover the critical areas of antibiotic-free broiler chicken production that many researchers have not yet been able to explore. Thus, a new theory from this study may be derived.

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