Journal of Bioscience and Environment Research 1 (2): 4-11

Content available at Genesis Publishing Consortium Limited

Journal of Bioscience and Environment Research

Journal homepage: www.genesispcl.com/journals/jber

Original Research

Dried *Chlorella* powder supplementation: Impact on broiler chicken growth, health, and intestinal microflora

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Article info

Article history
Received: 14 June 2024
Revised: 14 July 2024
Accepted: 02 September 2024
Published: 13 September 2024

Keywords
Dried *Chlorella* powder
Nutrient absorption
Broiler performance
Microbial population
Growth promoters

Abstract

The use of dried Chlorella as an immune and growth stimulant to enhance nonspecific host defense mechanisms or as an antimicrobial to inhibit bacterial growth has been reported. This study aimed to assess the effects of dried Chlorella powder (DCP) supplementation on the growth, health, and intestinal microflora of commercial broiler chicks, comparing a diet containing DCP with an antibiotic-based diet. A total of 120 pieces day-old Cobb 500 broiler chicks were reared at Sher-e-Bangla Agricultural University Poultry Farm, Dhaka, and randomly divided into four experimental groups of three replicates each, with 10 chicks per replication. One group was fed a control diet, while the remaining three groups were fed diets with 0.5% and 1.0% DCP, and antibiotics, respectively. Results indicated significant (P<0.05) improvements in body weight and dressing percentage with DCP inclusion compared to control-fed broilers. A linear increase in body weight was observed with higher DCP levels, with birds on the 1% DCP diet achieving superior body weights (1665.13±8.82) compared to the control and antibiotic groups. Feed Conversion Ratio (FCR) and feed consumption were also significantly (P<0.05) improved, with the best FCR at 1% DCP (1.37±0.01) and the highest FCR in the control group (1.45±0.00). The highest feed consumption was noted in the control group. No significant (P>0.05) differences were observed in the relative weight of spleen and bursa among the groups. DCP had no significant (P>0.05) effects on liver, gizzard, intestine, and heart weights. Hematological studies revealed no significant (P>0.05) differences, except for Hemoglobin and Red Blood Cells (RBC), which were significantly (P<0.05) increased by DCP compared to control and antibiotic groups. DCP supplementation significantly (P<0.05) reduced *E. coli* and *Salmonella* sp. counts while increasing *Lactobacillus* sp. counts. Additionally, treatments with DCP significantly (P<0.05) boosted Newcastle disease (ND) titre levels compared to the control group. The study showed that DCP can be effectively replaced antibiotics in broiler diets, enhancing growth, health, and immune response, thereby promoting sustainable and safer poultry production practices.

1. Introduction

In the poultry industry, antibiotic growth promoters (AGPs) have been used for decades to enhance gut health and control sub-clinical diseases (Abreu *et al.*, 2023; Miyakawa *et al.*, 2024). However, the use of synthetic growth enhancers and supplements presents several challenges. These additives are expensive, often unavailable, and can have adverse effects on both birds and humans (Abd El-Hack *et al.*, 2022). Sub-therapeutic levels of antibiotics given to poultry as growth enhancers can result in the development of antibiotic-resistant bacteria, posing significant hazards to animal and human health (Kasimanickam *et al.*, 2021).

Concerns about AGPs impact on human health have led to global restrictions (Salim *et al.*, 2018). AGPs work by interacting with the intestinal microbial population, improving nutrient absorption, reducing toxin production, and decreasing subclinical infections (Broom, 2017; Krajmalnik-Brown *et al.*, 2012).

The use of antibiotics as feed additives has come under severe criticism due to the risk of promoting antibiotic-resistant bacteria, which threatens human health (Ben *et al.*, 2019; Ma *et al.*, 2021; Manyi-Loh *et al.*, 2018). Concerns have been raised that the use of antibiotics for therapeutic and growth promotion purposes could lead to increased resistance in bacteria of both human and animal origin, particularly gram-negative bacteria such as *Salmonella* spp. and *Escherichia coli* (Butaye *et al.*, 2003; Rahman *et al.*, 2022). Consequently, the poultry industry is moving towards reducing the use of synthetic antibiotics (Mehdi *et al.*, 2018; Selaledi *et al.*, 2020).

In response to these concerns, the European Union (EU) has banned the use of antibiotic growth promoters as additives in animal nutrition (Castanon, 2007). This has led to the exploration of alternative feed additives, referred to as Natural Growth Promoters (NGPs) or non-antibiotic growth promoters. These include acidifiers, probiotics, prebiotics, phytobiotics, feed enzymes, immune stimulants, and antioxidants (Ayalew *et al.*, 2022; Kikusato, 2021). Plant materials, rich in bioactive compounds, have been used for medical treatment since prehistoric times and are now gaining

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doi: https://doi.org/10.69517/jber.2024.01.02.0002

attention for their potential to positively affect poultry health and productivity (Ivanova *et al.*, 2024; Jamil *et al.*, 2022).

Plants contain important bioactive components such as alkaloids, flavonoids, glycosides, saponins, phenols, terpenoids, essential oils, and polypeptides. These compounds can positively affect poultry health and productivity by providing a natural defense against bacterial attacks (Awuchi, 2019; Ivanova *et al.*, 2024; Riaz *et al.*, 2023). Herbs, seeds, spices, and plant extracts have been shown to stimulate appetite, improve digestion, and promote the growth of beneficial gut bacteria while reducing pathogenic bacteria (Dahl *et al.*, 2023; Frankič *et al.*, 2009). Supplementing poultry diets with plant materials rich in these active substances can enhance immune responses and serve as an effective alternative to antibiotic growth promoters (Ayalew *et al.*, 2022; Seidavi *et al.*, 2021).

Plant extracts, known as phytogenic feed additives, are generally free from antibiotic-resistant bacteria and are well-accepted by consumers when used in broiler diets. These plant-derived products are safer, less toxic, and residue-free compared to synthetic antibiotics, making them ideal feed additives (Ayalew *et al.*, 2022; Ivanova *et al.*, 2024; Upadhaya and Kim, 2017). Phytogenics enhance animal growth and health by improving digestibility, nutrient absorption, and eliminating gut pathogens. Notably, *Chlorella vulgaris*, a nutrient-rich microalga, offers essential amino acids, vitamins, and minerals (Karásková *et al.*, 2015). It has demonstrated benefits like growth promotion, antioxidant activity, and immunomodulation, making it a promising alternative to antibiotic growth promoters in poultry diets (Abdel-Wareth *et al.*, 2024).

The use of antibiotic growth promoters in poultry feed has raised significant health concerns due to the development of antibiotic-resistant bacteria, necessitating the search for effective alternatives. This study hypothesized that DCP supplementation in broiler diets can improve growth performance, hematological properties, and organ characteristics while effectively controlling *E. coli* and *Salmonella* populations, thus serving as a viable alternative to antibiotics. The research aims to evaluate the effects of DCP on broiler growth, health, and intestinal microflora, and to determine the optimal inclusion level of DCP in broiler diets for maximum benefits. The finding could lead to the adoption of dried *Chlorella* powder as a natural and effective alternative to antibiotic growth promoters in poultry feed, enhancing broiler health and productivity while mitigating antibiotic resistance concerns.

2. Materials and Methods

2.1 Ethical approval

No ethical approval is required for this study.

2.2 Study area and periods

The research work was conducted at Sher-e-Bangla Agricultural University poultry farm, Dhaka, Bangladesh for a period of 28 days from 06th July to 5th August, 2018 (Figure 1).

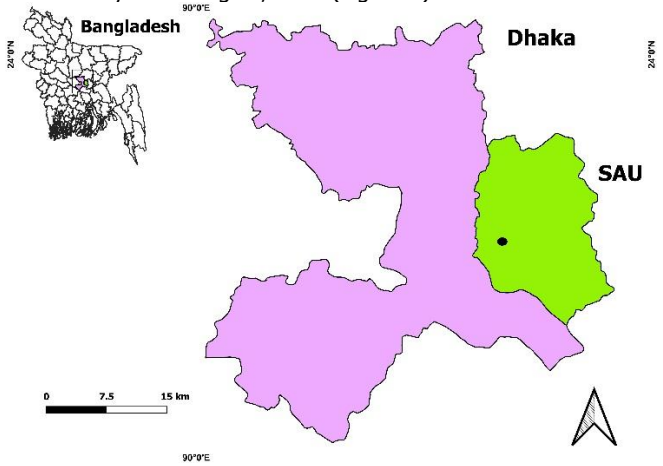

Figure 1. Map of the study area, from where the samples were collected.

2.3 Collection of experimental broilers and management

A total of 120 pieces day-old Cobb 500 broiler chicks from Kazi hatchery, Gazipur, Dhaka, were brooded for one week on a basal diet at the university poultry farm. After brooding, 60 chicks were assigned to two dietary treatments of dried *Chlorella* powder (DCP), and the remaining 60 were assigned to an antibiotic treatment and a control group (Table 1). The study consisted of four treatments, T_1, the control group, received a basal diet; T_2 received the basal diet supplemented with the antibiotic Doxivet; T_3 was fed a basal diet with 0.5% dried *Chlorella* powder (0.5 kg DCP per 100 kg feed); and T_4 received a basal diet with 1% dried *Chlorella* powder (1 kg DCP per 100 kg feed). Each treatment was replicated three times with 10 birds per replication, totaling 120 broiler chicks.

Table 1. Layout of the experimental design.

Treatment	Replications			Total
	R_1	R_2	R_3	
T_1	10	10	10	30
T_2	10	10	10	30
T_3	10	10	10	30
T_4	10	10	10	30
Total	40	40	40	120

2.4 Preparation of experimental house

The experimental room was properly cleaned and washed by using tap water. Ceiling walls and floor were thoroughly cleaned and disinfected by spraying diluted iodophor disinfectant solution (3 ml/liter water). After proper drying, the house was divided into 12 pens of equal size using wood materials and wire net. The height of wire net was 36 cm. A group of 10 birds were randomly allocated to each pen (replication) of the 4 (four) treatments. The stocking density was $1m^2$/10 birds.

2.5 Experimental diets

Commercial Kazi broiler starter and grower feeds were purchased, containing 19-21% protein as per the feed company's manual. Feeds were supplied four times daily, with ad libitum drinking water twice daily, following the Cobb 500 Manual. Dried *Chlorella* powder (DCP), imported from the USA, was used in the commercial basal diets (Table 2).

Table 2. Nutritional composition of *Chlorella vulgaris* (dry matter basis).

Nutrient component	Amount
Dry matter	94.80
Metabolizable energy	3 kcal/kg
Crude protein	60.60
Fat	12.80 g
Crude fat	13
Ash	4.50
Lysine	4.88
Methionine	1.20
Ca	0.01
P	1.06
K	1.12
Mg	0.36
Cu	1.40 mg/kg
Fe	224.00 mg/kg
Zn	33.70 mg/kg
Vitamin A	589 IU/kg
Vitamin E	207.48 IU/kg
Thiamine (B_1)	12.90 mg/kg
Riboflavin (B_2)	45.50 mg/kg
Vitamin C	740 mg/kg

Source: Data were collected from the manufacturer (Daesang Corporation, Icheon, Korea).

2.6 Management procedures

Body weight and feed intake were recorded weekly, and survivability was tracked for each replication up to 28 days. The experiment ran from July 6 to August 5, 2018, with an average temperature of 31.5 °C and 80% relative humidity. Chicks were brooded together for one week, then distributed randomly into pens with 10 chicks per $1m^2$ pen. Due to the hot climate, brooding temperature was adjusted as needed, using an electric bulb for

stimulation during the day and providing extra heat only at night when necessary. Daily room temperature and humidity were recorded every six hours. Rice husk was used as litter, stirred daily to prevent gas accumulation and parasite infestation, with fresh litter added as needed. Birds were given feed and water ad libitum, with feeders cleaned weekly and drinkers washed daily. Lighting was provided 24 hours for the first two weeks, then reduced to 22 hours with 2 hours of darkness. Biosecurity measures included vaccination, sanitation, and supplementation with vitamins and electrolytes (Table 3). The south-facing, open-sided broiler shed allowed for easy cross-ventilation, and ventilation was adjusted using polythene screens. Strict sanitation measures, including the use of disinfectant (Virkon), were maintained throughout the experiment.

Table 3. Vaccination schedule for the experimental chicken.

Age of birds	Name of disease	Name of vaccine	Route of administration
3 days	IB + ND	MA-5 + Clone-30	One drop in each eye
9 days	Gumboro	G-228E (inactivated)	Drinking Water
17 days	Gumboro	G-228E (inactivated) booster dose	Drinking Water
21 days	IB + ND	MA-5 + Clone-30	Drinking Water

IB= infectious bronchitis; ND=Newcastle disease.

2.7 Study parameters

Weekly live weight, weekly feed consumption and death of chicks to calculate mortality percent. FCR was calculated from the final live weight and total feed consumption per bird in each replication. After slaughter gizzard, liver, spleen, intestine, heart and bursa were measured from each broiler chicken. The dressing yield was calculated for each replication to find out the dressing percentage. The blood sample was analyzed from each replication to measure, Complete blood count (CBC), sugar and cholesterol levels. Feces sample was collected to measure microbial load in the gut.

2.8 Data collection and calculation

The initial and weekly live weights were recorded for each replication to obtain the final live weight per bird. Dressing yield was calculated by subtracting the weight of blood, feathers, head, shank, digestive system, liver, and heart from the live weight. Daily feed consumption was tracked to get weekly and total feed consumption per bird. Mortality was recorded daily up to 28 days of age. For dressing procedures, three birds from each replicate were randomly selected and sacrificed at 28 days. The birds were fasted for 12 hours with water provided ad-libitum, weighed before slaughter, and bled out. Carcasses were washed, eviscerated, and dissected, with the dressing yield calculated by removing specific parts from the live weight. Blood samples were collected and analyzed for glucose, cholesterol, and complete blood count. The average body weight gain was determined by subtracting the initial weight from the final weight,

Body weight gain=Final weight−Initial weight.

Feed intake was calculated as total feed consumption divided by the number of birds in each replication, and the feed conversion ratio (FCR) was calculated as total feed consumption divided by weight gain,

Feed conversion ratio=$\frac{Total\ feed\ consumption}{Weight\ gain}$

2.9 Statistical analysis

The data was subjected to statistical analysis by applying one way ANOVA using statistical package for social sciences (SPSS) version 16. Differences between means were tested using Duncan"s multiple comparison test and significance was set at $P<0.05$.

3. Results and Discussion

3.1 Production performances of broiler chicken

3.1.1 Final live weight

The relative final live weight (g) of broiler chickens in the dietary groups T_1, T_2, T_3 and T_4 were 1610.47±4.67, 1627.47±5.67, 1631.80±5.77 and 1665.13±8.82 respectively. The significantly ($P<0.05$) highest result was found in T_4 (1665.13±8.82) and lowest result was in T_1 (1610.47±4.67) group (Table 4). However, final live weight of broiler fed with *Chlorella* diets increased significantly ($P<0.05$) compared to that of the control and antibiotic treated groups.

These results are in agreement with those obtained by Kang *et al.* (2013) who found that several *Chlorella*-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks enhanced body weight. In addition, these results are in contradictory with those of previous researchers Peiretti and Meineri (2008), and Takekoshi *et al.* (2005) reported that dietary *Chlorella* did not significantly ($P>0.05$) improve weight gain of chickens compared with the control groups. However, Choi *et al.* (2017) and Abou-Zeid *et al.* (2015) reported that birds fed dietary *Chlorella* had beneficial effects on productive performance.

Table 4. Production performance of broiler chicken treated with DCP and antibiotic.

Treatment	T_1	T_2	T_3	T_4	Mean± SE
Final live weight (g/bird)	1610.47±4.67^b	1627.47±5.67^b	1631.80±5.77^b	1665.13±8.82^a	1633.72±6.57*
Feed consumption (g)	2338.33±3.17^a	2281.13±10.07^c	2312.47±1.28^b	2287.30±8.90^c	2304.81±7.44^b
Feed conversion ratio (FCR)	1.45±.00^a	1.40±0.01^b	1.42±.01^b	1.37±0.01^c	1.41±0.01*
DP% (Skinless)	67.51±0.29^b	69.03±1.42^{ab}	71.09±0.45^a	71.39±0.54^a	69.76±0.59*

Mean with different superscripts are significantly different ($P<0.05$); SE= standard error.

3.1.2 Feed consumption (FC)

Chlorella treated T_4 (2287.30±8.90) and antibiotic treated T_2 (2281.13±10.07) group consumed significantly ($P<0.05$) lower amount of feed, and T_1 control group consumed significantly ($P<0.05$) higher amount of feed (2338.33±3.17). Antibiotic treated group T_2 consumed numerically lower amount of feed compared to T_4 group (Table 4).

These results are in contrast with the findings of previous researchers who found that DCP had no effect on feed intake between experimental groups compared with that of control group (Abou-Zeid *et al.*, 2015; Kang *et al.*, 2013). Finding of this experiment of FC are in agreement with those of previous researchers who recorded that dietary micro algae spirulina significantly ($P<0.05$) improved Feed consumption (FC) of broiler chickens in different inclusion levels (Hassan *et al.*, 2022; Mirzaie *et al.*, 2018).

3.1.3 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) in different groups were significantly ($P<0.05$) different and the highest FCR was in T_1 (1.45±0.00) group and lowest FCR was in T_4 (1.37±0.01) group (Table 4). These results are in agreement with those of previous researchers Khalilnia *et al.* (2023) and El-Shall *et al.* (2023) who reported that dietary *Chlorella* significantly ($P<0.05$) improved feed efficiency of broiler chickens compared with the control groups.

The improvement of feed conversion ratio in *Chlorella* and prebiotic treated broilers could be related to better equilibrium in the intestinal flora (Bedford, 2000). These results are in contradictory with those of previous researchers Kang *et al.* (2013) and Oh *et al.* (2015) who showed that there were no significant effects on feed efficiency between the Chlorella treated and control groups.

3.1.4 Dressing percentage

The T_4 (71.39±0.54) and T_3 (71.09±0.45) DCP supplemented group had greater ($P<0.05$) dressing percentage compared with the control (67.51±0.29) group (Table 4). These findings are in accordance with the findings of El-Deek *et al.* (2011) who showed that thermal or enzymatic treatments, using different levels of algae in broiler finisher diets had significant effect on dressing percentages (ranged between 73.1 to 73.8%) at 39 days of age. These results are

contradictory with the Abdelnour *et al.* (2019) who recorded non-significant (P>0.05) effects of dietary *Chlorella* supplementation on dressing percentages as compared to control group.

3.1.5 Weekly body weight gain

The mean body weight gains (g) of broiler chicks at the end of 4th week in different groups were 652.50±13.61, 726.83±3.33, 659.03±1.35 and 673.50±.00 respectively (Table 5). At the end of 1st week the body weight gain in different groups were non-significant (P>0.05). T_2 group had the higher body weight gain than other groups. At the end of 4th week the body weight gain in different groups were significantly different (P<0.05). T_2 group had the higher body weight gain (726.83±3.33) than other group. According to Choi *et al.* (2017) broilers fed the PC_2 treatment group (1.0% EFL with *Chlorella*) (P< 0.05) exhibited higher BWG than in the primary NC treatment group.

Table 5. Effects of feeding different level of DCP and antibiotic on body weight gain (BWG) (g/bird) of broiler chickens at different weeks.

Parameters	1st week b. w. g	2nd week b. w. g	3rd week b. w. g	4th week b. w. g
T1	196.23±2.03^{NS}	337.57±0.33^{b}	466.83±11.84^{b}	652.50±13.61^{b}
T2	198.23±1.45^{NS}	342.23±1.86^{a}	497.83±1.33^{a}	726.83±3.33^{a}
T3	195.90±4.00^{NS}	335.57±0.88^{b}	419.97±1.27^{c}	659.03±1.35^{b}
T4	195.57±1.20^{NS}	335.23±1.20^{b}	427.50±4.58^{c}	673.50±0.00^{b}
Mean±SE	196.48±1.08^{NS}	337.65±0.99*	453.03±9.85*	677.97±9.31*

Values are Mean±SE (n=12); analyzed from one way ANOVA (SPSS, Duncan method). Mean with different superscripts are significantly different (P<0.05).

3.1.6 Weekly feed consumption (FC)

The mean FC (g) of broiler chicks at the end of 4th week in different groups were 1004.80±5.57, 1000.90±15.09, 991.23±5.12 and 1008.00±4.368 correspondingly (Figure 2). The overall mean FC of different groups showed that there were no significant (P>0.05) difference between different treatment groups. These findings are in accordance with Takekoshi *et al.* (2005) who indicated that dietary supplementation of *Chlorella* (*Chlorella pyrenoidosa*) did not affect the feed intake of mice. An *et al.* (2016) also showed Chicks fed diets with 0.15 or 0.5 % DCP had no effect on feed intake between experimental groups compared with that of control group.

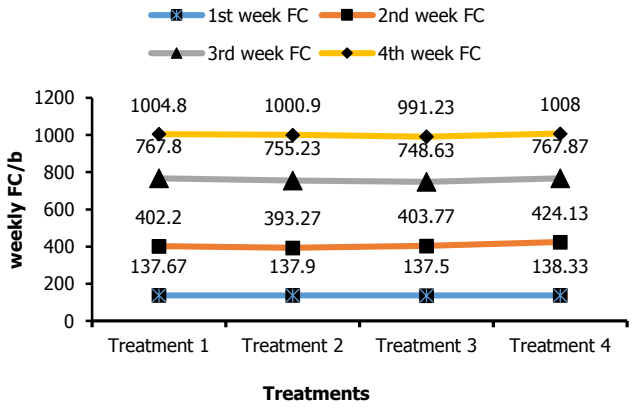

Figure 2. The effect of supplementation of DCP to broiler diets on feed consumption (g/bird) of broiler chickens at different weeks.

3.1.7 Weekly feed conversion ratio (FCR)

The mean body FCR of broiler chicks at the end of 4th week in different groups were 1.55±0.03, 1.36±0.01, 1.52±0.01 and 1.49±0.02 respectively. The overall mean FCR of different groups showed that there was a significant (P<0.05) difference in groups. T_2 showed the lowest FCR compared to control and other treatment groups (Table 6). These findings are in line with the findings of Abou-Zeid *et al.* (2015) dietary treatments improved feed conversion ratio compared to the birds fed control diet during starter, finisher and the whole experimental periods. In contrast these findings are opposite to the result of Oh *et al.* (2015) who reported that there were no significant effect on feed efficiency between the *Chlorella* treated and control groups. Kang *et al.* (2013) also reported that several *Chlorella*-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks did not affect feed conversion ratio.

Table 6. The effects of feeding DCP and antibiotics on FCR of broiler chickens at different weeks.

Treatment	1st w. FCR	2nd w. FCR	3rd w. FCR	4th w. FCR
T_1	0.70±.01^{NS}	1.26±0.02^{NS}	1.65±0.04^{b}	1.55±0.03^{a}
T_2	0.69±.01^{NS}	1.18±0.02^{NS}	1.50±0.01^{c}	1.36±0.01^{b}
T_3	0.70±.01^{NS}	1.20±0.04^{NS}	1.83±0.02^{a}	1.52±0.01^{a}
T_4	0.70±.00^{NS}	1.17±0.05^{NS}	1.77±0.01^{a}	1.49±0.02^{a}
Mean± SE	0.70±.00^{NS}	1.20±0.02^{NS}	1.69±0.04*	1.48±0.02*

Values are mean ± S.E (n=12); Mean with different superscripts are significantly different (P<0.05).

3.2 Blood glucose and cholesterol

Although the highest amount (11.53±0.54 m mol/L) of plasma glucose was found in T_2 but this was not statistically different (P>0.05) with control and other groups (Figure 3). The results of the present study are compatible with those observed by Kotrbáček *et al.* (2015) and An *et al.* (2016) observed incorporation of dietary *Chlorella* in broilers diet had no significant (P>0.05) effect on serum glucose level of broiler chicken. The increase in plasma glucose concentration of hens fed dietary *Chlorella* may be attributed to its excellent nutritional profile and high carotenoid content. Total cholesterol concentration (mg/dl) in the serum of T_1, T_2, T_3 and T_4 groups were 215.33±33.01, 214.67±10.17, 187.33±10.41 and 189.33±14.11 respectively. Statistical analysis revealed no significant (P>0.05) difference among the group (Figure 3). However the cholesterol level was lower in T_3 fed group (187.33±10.414) numerically but not statistically. Similar results had also been observed by Kotrbáček *et al.* (2015), who reported that dietary DCP did not affect the concentration of plasma triacylglycerol and cholesterol in laying hens. Study of Panaite *et al.* (2023) had shown contradictory result that *Chlorella* reduces cholesterol and increases the omega-3 content of eggs.

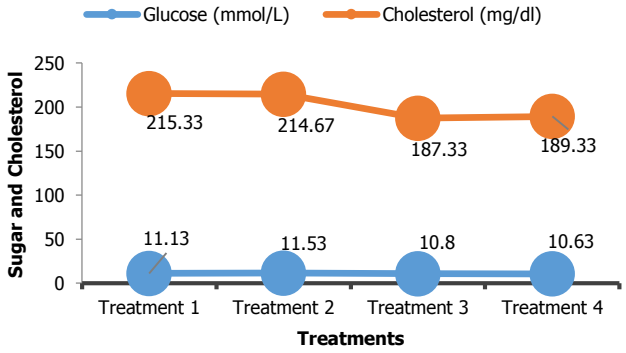

Figure 3. Effect of DCP on serum biochemical level of different broiler chicken under different treatments.

3.3 Relative giblet and intestine weight

The relative weight of liver (g) of broiler chicks in the dietary groups T_1, T_2, T_3 and T_4 were 37.33±0.16, 38.87±0.32, 40.13±0.91 and 38.50±1.53 respectively. The highest results were in T_3 and lowest was in T_1 group. However, there were no significant (P>0.05) difference in the relative weight of liver between the groups (Table 7). This results are in line with the findings of An *et al.* (2016) who reported that dietary *Chlorella* did not affect relative organ weights including liver, spleen, bursa of Fabricius and abdominal fat. Research of El-Deek *et al.* (2011) also accomplished that using different levels of algae in broiler finisher diets had insignificant (P>0.05) effect on gizzard weights. The comparative weight of heart (g) of broiler chicks in the dietary group T_1, T_2, T_3 and T_4 were 9.33±0.60, 9.50±0.50, 10.50±0.00 and 9.17±0.73 correspondingly. The qualified weight of hearts of different groups showed that there were no significant (P>0.05) differences between the groups (Table 10). Abdelnour *et al.* (2019) also reported that supplementing the rabbit diets with CLV did

not induce significant differences (P>0.05) in giblets, heart, kidney, lung, and liver as compared to the control animals. It means that fenugreek infusion having antimicrobial and antibiotics like properties have no influence on either increasing or decreasing the relative weights of giblet.

The results of different groups showed that there were no significant (P>0.05) differences between the groups and the values were ranged from 84.67±0.88 to 92.67±4.37 (Table 7). In the study of An *et al.* (2016), they showed *Chlorella* had no impact on visceral organs (liver, heart, gizzard, and intestines) of broiler chicks.

Table 7. Effect of dietary supplementation of DCP on liver, gizzard, intestine and heart weight of different treatments.

Parameters	T_1	T_2	T_3	T_4	Mean±SE
Liver weight (g)	37.33±0.17^{NS}	38.87±0.32^{NS}	40.13±0.91^{NS}	38.50±1.53^{NS}	38.71±0.49^{NS}
Gizzard weight (g)	36.67±0.33^{NS}	38.33±0.88^{NS}	38.33±0.44^{NS}	38.50±0.58^{NS}	37.96±0.34^{NS}
Intestine weight (g)	84.67±0.88^{NS}	90.00±3.79^{NS}	91.00±4.00^{NS}	92.67±4.37^{NS}	89.58±1.76^{NS}
Heart (g)	9.33±0.60^{NS}	9.50±0.50^{NS}	10.50±0.00^{NS}	9.17±0.73^{NS}	9.62±0.28^{NS}

Values are Mean±SE (n=12); mean with different superscripts are significantly different (P<0.05); NS=not significant.

3.4 Immune organs (spleen and bursa)

The comparative weight of spleen (g) of broiler chicks in the dietary group T_1, T_2, T_3 and T_4 were 1.67±0.44, 2.00±0.50, 2.27±0.27 and 2.50±0.00 respectively. The highest value was T_4 (2.50±0.00) and lowest value was T_1 (1.67±0.44). On the other hand, the relative weight of spleen of different groups showed that there were no significant (P>0.05) difference. The weight of bursa was higher in T_3 group (2.17±0.333) compared to the other group whose values were T_1 (1.67±0.17), T_2 (1.67±0.17) and T_4 (1.83±0.17) correspondingly. But these values are not significantly differing among the treatments (Figure 4).

An *et al.* (2016) reported that dietary *Chlorella* did not affect relative organ weights including spleen, bursa of Fabricius and abdominal fat. Schiavone *et al.* (2007) also found that using of 5g algae/kg feed insignificantly affected on the slaughter characteristics of the Muscovy ducks.

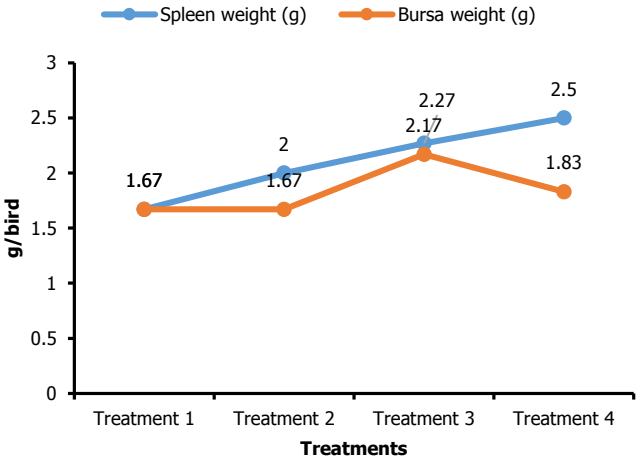

Figure 4. The effect of supplementation different level of DCP to broiler diets on some immune organs.

3.5 Hematological parameters

Concerning the treatment effect on blood constituents, the results indicated no significant differences due to supplementation of DCP, except Hemoglobin and RBC which were significantly affected (P<0.05). Birds fed diets supplemented with *Chlorella* (at levels of 0.5% and 1%) diet had higher values of Hemoglobin and RBC but in case of control group these trends were lower than *Chlorella* treated groups (Table 8).

Table 8. Effect of supplementation of DCP to broiler diets on blood parameters.

Parameters	T1	T2	T3	T4	Mean±SE
Hemoglobin(g/dl)	8.98±0.12^{b}	9.14±0.21^{ab}	9.78^{a}±0.25	9.39^{ab}±0.25	9.32±0.11*
RBC	3.47±0.28^{b}	4.39±0.20^{a}	4.20±0.13^{a}	4.49±0.28^{a}	4.14±0.13*
WBC	7.44±0.34^{NS}	7.67±0.17^{NS}	7.56±0.29^{NS}	8.00±0.33^{NS}	7.67±0.14^{NS}
Neutrophil	71.89±1.65^{NS}	69.78±1.29^{NS}	70.89±1.42^{NS}	71.33±1.27^{NS}	70.97±0.69^{NS}
Lymphocyte	62.33±3.86^{NS}	66.11±4.72^{NS}	73.22±3.76^{NS}	70.22±4.36^{NS}	67.97±2.12^{NS}
Monocyte	1.52±0.08^{NS}	1.55±0.10^{NS}	1.58±0.11^{NS}	1.44±0.13^{NS}	1.52±0.05^{NS}
Eosinophil	1.50±0.06^{NS}	1.59±0.06^{NS}	1.56±0.05^{NS}	1.55±0.06^{NS}	1.55±0.03^{NS}
PCV	28.66±0.94^{NS}	30.01±0.94^{NS}	30.14±0.96^{NS}	30.06±0.96^{NS}	29.72±0.47^{NS}
MCV	78.46±2.78^{NS}	81.81±1.50^{NS}	81.77±0.97^{NS}	81.52±1.33^{NS}	80.89±0.88^{NS}
MCH	30.43±0.37^{NS}	31.15±0.48^{NS}	30.13±0.50^{NS}	30.76±0.60^{NS}	30.62±0.25^{NS}
MCHC	31.65±0.44^{NS}	31.14±0.45^{NS}	31.22±0.31^{NS}	31.27±0.37^{NS}	31.32±0.19^{NS}

Values are Mean ± S.E (n=12); mean with different superscripts are significantly different (P<0.05); SE= standard error; NS= not significant; RBC=red blood cells; WBC= white blood cells; PCV=packed cell volume; MCV=mean corpuscular volume; MCH= mean corpuscular hemoglobin; MCHC= mean corpuscular hemoglobin concentration.

These results align with the earlier findings of An *et al.* (2010), who reported increased levels of total protein, albumin, glucose, and interferon-γ in the blood serum of mice fed with a hot water extract of *Chlorella*.

Phillips *et al.* (2023) reported that 0.5% biomass of fresh water *Chlorella* significantly enhanced the phagocytic activity of leucocytes and lymphatic tissue development of broiler chickens. These results are in accordance with the earlier findings of Kabir *et al.* (2004) and Kulshreshtha *et al.* (2008). In contrast, Kang *et al.* (2013) reported that Supplemental AGP and *Chlorella* had no effect on blood leucocytes of broiler chickens.

3.6 Intestinal microflora

E. coli count was significantly (P<0.05) decreased in birds fed 0.5%, 1% *Chlorella* and antibiotic (11.00±0.30, 11.23±0.44 and 11.68±0.34 respectively) than the control birds (15.58±0.87). *Salmonella* sp. count was also significantly (P<0.05) decreased in birds fed 0.5%, 1% DCP and antibiotic (5.70±1.55, 4.66±1.67 and 9.03±1.33 respectively) than the control birds (14.46±1.25). *Lactobacillus* count was significantly (P<0.05) increased in birds fed 0.5% and 1% *Chlorella*. The highest number of lactobacillus was counted in T_4 group (19.76±0.38) and the lowest in T_1 group (11.70±0.33) (Table 9).

Table 9. Bacterial colony count in DCP experiment in broiler chicken.

Parameters	*E. coli* × 10^6 (CFU/ml)	*Salmonella* × 10^6 (CFU/ml)	*Lactobacillus* × 10^6 (CFU/ml)
T_1	15.58±0.88^{a}	14.46±1.25^{a}	11.70±0.33^{d}
T_2	11.68±0.34^{b}	9.03±1.33^{b}	14.98±0.77^{c}
T_3	11.00±0.30^{b}	5.70±1.55^{b}	18.07±0.49^{b}
T_4	11.23±0.44^{b}	4.66±1.67^{b}	19.76±0.38^{a}
Mean±SE	12.37±0.41*	8.46±0.95*	16.12±0.58*

Values are Mean±SE (n=12); mean with different superscripts are significantly different (P<0.05); SE= standard error

These results of the experiment are in accordance with the earlier findings of Janczyk *et al.* (2009) who reported that feeding *Chlorella* vulgaris significantly increased the lactobacilli diversity in crop and ceca of laying hens with a stronger effect on the cecal bacterial population. Nigussie *et al.* (2021) reported that methanol extracts of *C. vulgaris* lowered *E. coli* and Salmonella. However, the population of cecal coliform bacteria in ducks fed diet with 2,000 mg/kg fermented *C. vulgaris* tended to be lower compared with their control-diet fed counterparts (linear effect at P=0.064), indicating that *C. vulgaris* may have a positive effect on improving cecal microflora (Oh *et al.*, 2015).

3.7 Antiviral activity

Concerning the treatment effect on HI titre the results indicated significant (P<0.05) differences due to supplementation of DCP. Remarkably better titres of ND were achieved in blood at day 15 (5.56±0.24) and day 29 (6.89±0.26) in the T_4 treatments compared to control group (Table 10). It is reported that either DCP or CGF improved immune functions in rodents and chickens (An *et al.*, 2016; Kang *et al.*, 2013). Kang *et al.* (2013) reported that dietary supplementation of *Chlorella* significantly (P< 0.05) increased the

plasma IgA, IgM and IgG concentration of chicks compared with AGP and control. In contrast, these results are contradictory with the earlier findings of An *et al.* (2016) who found that the antibody titers against NDV and IBV in chicks were not affected by DCP and CGF. Immurella, a polysaccharide compound in the *Chlorella* cells, is also an important factor to enhance the immune response of broilers fed *Chlorella*-supplemental diets (Pugh *et al.*, 2001).

Table 10. Effect of DCP on pre-vaccination ND HI titre in broiler chicken.

Parameters	Day 15 (log^2)	Day 20 (log^2)	Day 29 (log^2)
T_1	3.78±0.22^b	3.11±0.26^b	5.22±0.22^c
T_2	4.56±0.38^b	3.44±0.18^{ab}	5.89±0.20^b
T_3	5.89±0.26^a	4.00±0.24^a	6.67± 0.24^a
T_4	5.56±0.24^a	3.78 ±0.22^{ab}	6.89± 0.22^a
Mean±SE	4.94±0.20*	3.58 ±0.12*	6.17± 0.16*

Values are presented as mean±SE (n=12); Mean with different superscripts are significantly different (P<0.05); SE= standard error

4. Conclusions

The current study demonstrated that broilers fed with dried *Chlorella* powder, particularly at one percent inclusion, showed significant improvements in body weight, feed conversion ratio, hemoglobin, and red blood cell counts compared to control and antibiotic groups. Additionally, *Chlorella* supplementation resulted in lower cholesterol levels, higher *Lactobacillus* counts, and enhanced immune response, indicating its potential as a viable alternative to antibiotics in broiler diets. These findings suggest that *Chlorella* can effectively enhance growth performance and health in broilers

Acknowledgments

The authors would like to thank Department of Poultry Science, Sher-e-Bangla Agricultural University for logistic and laboratory facilities provided during the investigation. The first author sincerely acknowledges the financial grant support from Ministry of Science and Technology, People's Republic of Bangladesh as National Science and Technology (NST) fellowship.

Data availability

The data generated from this study might be shared with a valid request from the corresponding author.

Informed consent statement

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

Conceptualization: NAM, MAHB and KBMSI; **Data collection:** NAM and SA; **Data analysis:** NAM, PB and MZR; **Figure preparation:** NAM PB and MZR. All authors critically reviewed the manuscript and agreed to submit final version of the manuscript. All authors critically reviewed the manuscript and agreed to submit final version of the manuscript.

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