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Effects of dietary supplement of dried *Chlorella* powder as an alternative to antibiotic on growth performance and health status of broiler chicken

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Abstract

The objectives of the present study was to investigated the effect of different levels of Dried *Chlorella* Powder (DCP) on growth performance, microbial, biochemical and haematological parameters of broiler chicken from one day old to 4 weeks of age. A total of 240 days old Cobb 500 broiler chicken were randomly assigned to four treatment groups (T_1, T_2, T_3 and T_4), 60 birds per treatment group and were respectively fed with diets containing 0.25% and 0.50% of DCP. At 4 weeks of age, 36 birds per group were slaughtered to collect blood, gizzard, spleen, bursa, and liver. Results showed no significant difference ($p>0.05$) in feed intake, body weight, feed conversion ratio (FCR) and relative internal organ weight. In addition, total cholesterol, sugar level, and CBC count has no significant differences ($p>0.05$) in broiler chickens fed DCP as compared to control. However, significant difference ($p<0.05$) infaecal microbial load was found between groups. In conclusion, the results of this study demonstrate that DCP supplementation positively affected the growth performance and health status in broilers, indicating that dried *chlorella* powdercan be safely used to replace antibiotic as a growth promoter, thereby reducing the risk of antibiotic resistance issues.

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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 interactions with intestinal microbial population (Dibner and Richards, 2005). The ban on using antibiotics as feed additives has accelerated research into the use of alternative feed additives in poultry production (Kiczorowska *et al.,* 2016). Phytogenic additives are plant-derived products used in animal feed to improve the performance of agricultural livestock (Windisch *et al.*, 2008; Hashemi and Davoodi 2010). This class of additives has recently gained increasing interest, especially for use in modern poultry production (Al-Yasiry *et al.,* 2017). Sub-therapeutic levels of antibiotics given to poultry as growth enhancer may result to the development of antibiotic-resistant bacteria, which are hazardous to animal and human health (Sarica *et al.,* 2005).

Meanwhile, the use of organic supplements such as herbs and*Chlorella* are generally believed to be safer, healthier, and less subject to hazards. In the developed countries peoples use Chlorella in diet of human, livestock, poultry as herbal medicinal product. Thus,*Chlorella* and its products could be incorporated in poultry feed or water instead of antibiotic in order to stimulate or promote effective use of feed nutrients which result in more rapid gain, higher production and better feed efficiency. Moreover, *Chlorella* contains active substances that can improve digestion and metabolism and possess bacterial and immune-stimulant activities (Ghazalah and Ali, 2008). Therefore, alternatives to AGP need to be proposed to poultry producers in order to maintain animal health, productivity and carcass quality. Yoshizawa (1993) reported that algae extract activated the macrophages and increased the proinflammatory cytokine production of laboratory animals. However, huge numbers of algae species are available that produce novel compounds. It has been reported that supplementation of *Chlorella* in human and animal diets performed numerous biochemical and physiological functions, such as growth promotion, antioxidant functions and immunomodulation. In addition, antimicrobial properties of *Chlorella* are considered to be an effective alternative to AGP in the diets to maintain optimum health and productivity of the animal. Another microalga studied to a lesser extent is *Schizochytrium*. Supplementing layer diets with a commercial *Schizochytrium*-containing product was shown to increase the DHA (docosahexaenoic acid) content of the eggs (Abril and Barclay, 1998). DHA is an essential omega-3 fatty acid that is important, among others, in the formation of the brain and nervous system of infants. Similarly, supplementation of layer diets with *Porphyridium* (a red microalga) has been shown to reduce cholesterol and increase the omega-3 content of eggs (Ginzberg *et al.*, 2000).

Research has been conducted to the possibility of using *Chlorella* in poultry diets. Moreover, very limited information is available about the use of *Chlorella* as an alternative to antibiotic feed supplement to promote growth, immune characteristics, and intestinal microbial population of broiler chicken. Therefore, the present study was conducted to determine the effect of dietary supplementation of *Chlorella* on the growth performance, immune characteristics, and intestinal microflora population of broiler chickens compared with an AGP.

Materials and methods

Experimental design

The experiment was conducted at Sher-e-Bangla Agricultural University Poultry Farm, Dhaka Bangladesh. During 4 weeks, 1 day-old chicks Cobb 500 broiler chicken were fed with two types of diets containing Dried *Chlorella* Powder (DCP). *Chlorella* were collected from commercial source.Two hundred and forty day-old Cobb broiler chicks were were randomly assigned into four groups (T_1, T_2, T_3 and T_4) with 3 replications of 60 chicks in each treatment group. They were fed basal diet supplemented with Dried *Chlorella* Powder (DCP) at the levels of 0.25 (T_1) and 0.50% (T_2), antibiotic (T_3) and only basal diet (T_4) respectively. Each diet was formulated to fit crude protein (CP) and metabolisable energy (ME) (Table 1).

Table 1. Gross composition of experimental diets (%).

	Treatment			
Feed Ingredients	T_1	T_2	T_3	T_4
Maize	55	55.8	55.64	55.48
Wheat bran	12	11.4	11.66	11.5
Fish meal	8	7.6	7.8	7.82
Soya seed	20	19.7	19.9	20.2
Concentrate	3.75	4	4	4
Oyster shell	1	1	1	1
Dried *Chlorella* Powder	.25	.50	0	0
Total	100	100	100	100
Calculated analysis				
ME (kcal kgG[1])	2962.50	2956.00	2942.81	2955.12
CP (%)	20.11	19.88	19.58	19.81
Calcium (%)	0.97	0.95	0.93	094
Available phosphorus (%)	0.05	0.05	0.05	0.05
Sodium (%)	0.35	0.34	0.34	0.34
Lysine total (%)	1.09	1.06	1.01	1.04
Methionine total (%)	0.48	0.47	0.46	.48
CF (%)	4.99	4.97	4.89	4.88

CP: Crude protein, ME: Metabolizable energy, CF: Crude fibre

Data collection

Production parameters

The amount of feed offered and refused per pen was recorded daily. The amount of feed consumed was determined as the difference between feed offered and remaining feed. Birds were weighed each week individually and pen average was calculated. Body weight change was calculated as the difference between the final and initial body weight. Average daily body gain was calculated as body weight change divided by the number of experimental days. These data were used to determine feed conversion ratio by dividing feed intake by body weight gain. Internal organs weight: At 4 weeks of age, 3 randomly selected birds from each replication were weighed and slaughtered. After slaughtering, the birds were de-feathered and eviscerated. The gizzard, heart, pancreas and liver were weighed. The organ weights were used to determine their proportion of body weight.

Haematological and Bio-chemical analysis

To determine haematological 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 minutes to produce serum. The serum was frozen until serum biochemistry (Sugiharto *et al.*, 2017a). Complete blood counts were determined using a hematology analyser (Hycount 5 Plus Vet - 5-Part differential analyzer for veterinary applications.) Total cholesterol and sugar in serum were measured with the enzymatic colorimetric/colour method.

Feacal microbial sample analysis

At day 28, a total of 36 chicks were slaughtered, de-feathered and eviscerated. The internal organs were immediately taken out and weighed. Digesta were collected from the caecum of broilers to determine microbiological analyses.The counts of certain bacteria in the intestinal digesta of broilers were determined according to Sugiharto *et al.,* (2017b) with few modification. 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 center, respectively.

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, LSD and significance was set at $P<0.05$.

Results

Production performance

Feed consumption (FC), live weight (LW), Feed conversion ratio (FCR): The average highest feed was consumed by control group and lowest in *Chlorella* treatment group, but no significant (P>0.05) difference was found among *Chlorella* treated groups with control and antibiotic treated group (Table 2). No significant (P>0.05) difference was found in final live weight among chlorella treated groups with control and antibiotic treated group, but the highest LW was found in chlorella treated group and lowest in control group. Which LW would be better can be found out by FCR. No significant (P>0.05) difference was found in final live weight among chlorella treated groups with control and antibiotic treated group, but the highest LW was found in chlorella treated group and lowest in control group.

Table 2. Production parameter of different treatment.

Treatment	FC (g)/Bird	Live weight (g)/Bird	FCR	DP%	Survivability
T_1(0.25% DSP)	2084.57± 4.53	1632.33± 6.21	1.28± 0.12	65.68± 1.32	100.00± 0.00
T_2 (0.50% DSP	2124.10± 6.22	1623.33± 8.45	1.31± 0.32	64.03± 2.21	100.00± 0.00
T_3 (Antibiotic)	2091.23± 7.34	1571.67± 7.32	1.33± 0.23	60.44± 1.16	100.00± 0.00
T_4 (Control)	2104.57± 5.43	1560.00± 9.53	1.35± 0.21	64.82± 3.22	98.00± 0.02
Mean±SE	2101.18±10.52	1596.83±21.28	1.32±0.015	63.74±1.37	99.58±0.42
LSD (0.05)	31.44^{NS}	63.18^{NS}	0.04^{NS}	4.08^{NS}	1.18^{NS}

The values are mean ± SE of 60 chicks in each treatment group. Means with different superscripts (a, b, c, and d) within a column are significantly different from each other at $P < 0.05$ as determined by Duncan's multiple range test.

Dressing percent (DP) and Survivability percent (SP): The highest DP was found in chlorella treated group and the lowest in antibiotic treated grou. But, DP of all the treatment groups were not affected by chlorella and antibiotic comparing with control group. The highest survivability was found in antibiotic group which showed no significant (P>0.05) difference with chlorella treated and control group (Table 2).

Organ relative weights

The treatment groups were not affected significantly (P>0.05) either by *chlorella* or antibiotic in comparison with control group. Although, the highest weight was found in *chlorella* treatment group, but statistical difference was not significant (P>0.05).

Using different levels of algae in broiler finisher diets had no significant effect on gizzard and spleen percentages. The average gizzard weight of different treatment groups shown in Table 3, were not affected significantly (P>0.05). The height gizzard weight was found in antibiotic group (T_3- 40.0g) and lowest in chlorella treated group. Using different levels of algae in broiler finisher diets had insignificant effect on gizzard and spleen percentages. The mean bursa weight of different treatment groups were presented in Table 3, but no significant (P>0.05) difference was found among different treatment groups.

The chlorella treatment group showed height weight, whereas antibiotic group showed the lowest weight.

Weight of some important internal organs

Table 3. Weight of internal organs under different treatment.

Treatment	Liver weight (g)	Spleen Weight(g)	Gizzard Weight(g)	Bursa Weight(g)
T_1(0.25% DSP)	38± 0.13	1.83± 0.19	32.83± 1.13	2.50± 0.15
T_2 (0.50% DSP	37± 0.21	2.17± 0.14	34.83± 1.03	2.17± 0.13
T_3 (Antibiotic)	36.67± 0.11	1.8± 0.18	40.00± 1.10	1.67± 0.16
T_4 (Control)	40.67± 0.18	2.00± 0.10	33± 1.19	2.17± 0.19
Mean±SE	38.08±0.96	1.96±0.16	35.17±1.41	2.12±0.20
LSD (0.05)	2.79^{NS}	0.50^{NS}	3.66^{NS}	0.58NS

The values are mean ± SE of 60 chicks in each treatment group. Means with different superscripts (a, b, c, and d) within a column are significantly different from each other at P<0.05 as determined by Duncan's multiple range test.

Blood serum bio-chemical parameters

Effects of dietary Dried *Chlorella* Powder (DCP) supplementation on concentration of sugar of broiler chickens are presented in Table 4. Feeding dietary *Chlorella had* no significant (P>0.05) difference among the treatment. Although the highest amount (10.40±0.85) of plasma sugar are found in T_3 (1.5% *Chlorella*) but this was not statistically difference with control and other groups. Total cholesterol concentration (mg/dl) in the serum of different groups ranged from 117.44±4.74 to 132.89±4.81.

Statistical analysis revealed a nonsignificant (P>0.05) deference among the group. (Table 4).

Table 4. Effect of Dried *Chlorella* Powder (DCP) on Serum biochemical level of different broiler chicken under different treatment.

Parameters	T_1 (0.25% DSP)	T_2 (0.50% DSP)	T_3 (Antibiotic)	T_4 (Control)	Mean ±SE	LSD (0.05)
Sugar mmol/L	10.18±0.25	9.37±0.27	10.40±0.85	10.08±0.60	10.07±0.218	0.726^{NS}
Cholesterol mg/dl	119.00±1.07	117.44±4.74	132.00±12.81	130.00±11.64	126.27±3.615	11.775^{NS}

The values are mean ± SE of 60 chicks in each treatment group. Means with different superscripts (a, b, c, and d) within a column are significantly different from each other at P <0.05 as determined by Duncan's multiple range test.

Table 5. Bacterial colony count in *Spirulina* experiment in broiler chicken.

Treatment	*E. coli* (EMB) ×10⁴ (CFU/ml)	*Salmonella* (SS) ×10⁴(CFU/ml)
T_1(0.25% DSP)	13.08^{ab}±2.79	12.42^{b}±1.55
T_2 (0.50% DSP	14.88^{ab}±2.45	12.35^{b}±2.38
T_3 (Antibiotic)	11.98^{b}±0.72	15.71^{b}±0.21
T_4 (Control)	20.25^{a}±3.19	22.54^{a}±2.18
Mean ±SE	14.15±1.24	15.33±1.20
LSD (0.05)	3.28^{*}	2.33^{*}

The values are mean ± SE of 60 chicks in each treatment group. Means with different superscripts (a, b, c, and d) within a column are significantly different from each other at P <0.05 as determined by Duncan's multiple range test.

Table 6. The hematological report of blood sample treated by chlorella and antibiotic.

Treatments	Hb (Gm/dl)	RBC (mill/cum)	WBC (mill/cum	Neutro-phils(%)	Lymphocytes (%)	Mono-cytes (%)	Eosi-Nophils (%)	HCT/PCV (%)	MCV (Fl)	MCH(Pg)	MCHC (g/dl)
T_1(0.25% DSP)	13.17± 0.31	4.57^{a}	11800.00 3063.49	65.00^{a}	29.33^{a}	2.67^{a}	2.67^{a}	39.96^{a}	88.58^{a}	30.17^{a}	32.68^{a}
T_2 (0.50% DSP	12.21± 0.36	4.59^{a}	9233.333 066.49	65.67^{a}	35.33^{a}	1.67^{a}	3.67^{a}	37.20^{a}	87.90^{a}	30.85^{a}	32.80^{a}
T_3 (Antibiotic)	13.13± 0.33	4.57^{a}	9666.623 064.59	68.33^{a}	27.67^{a}	2.67^{a}	3.67^{a}	39.71^{a}	88.22^{a}	30.20^{a}	32.68^{a}
T_4 (Control)	12.67± 0.36	4.47^{a}	10166.673 074.49	66.33^{a}	30.00^{a}	2.33^{a}	2.67^{a}	37.71^{a}	88.21^{a}	30.17^{a}	32.77^{a}
Mean±SE	12.79± 0.35	4.55±0.08	9666.67± 3064.49	66.33±2.94	30.58±2.74	2.33±0.19	3.17±1.62	38.64±1.05	88.23±0.51	30.33±0.16	32.75±0.15
LSD (0.05)	1.08^{NS}	0.25^{NS}	3531.76^{NS}	9.66^{NS}	8.64^{NS}	0.471^{NS}	1.49^{NS}	3.24^{NS}	1.69^{NS}	0.46^{NS}	0.48^{NS}

The values are mean ± SE of 60 chicks in each treatment group. Means with different superscripts (a, b, c, and d) within a column are significantly different from each other at P<0.05 as determined by Duncan's multiple range test.

Hematological parameters

The hemoglobin, RBC, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, HCT/PCV, MCV, MCH and MCHC of different blood sample were not affected significantly (P>0.05) treated by *Chlorella* and antibiotic in comparison with control group. But, the highest hemoglobin (T1-13.17gm/dl), RBC (T2-4.59mill/cum), WBC (T1-11800 mill/cum), lymphocytes (T2-35.33%), Monocytes (T1-2.67%) PCV (39.96%), MCV (T1-88.58), MCH (T2-30.85Pg) and MCHC (T2-32.80) were found in the Chlorella treated groups, which is an indication of good health (Table 6).

Feacal microflora

The microbial load in broilers fed different levels of dried *Chlorella* powder is given in Table 5, *E. coli* count was significantly (P<0.05) decreased in birds fed 0.25%, and 0.50% dried *Chlorella* powder and antibiotic than the control birds. *Salmonella sp.* count was significantly (P<0.05) decreased in birds fed 0.25% and 0.50% dried *Chlorella* powder and antibiotic) than the control birds.

Discussion

In this study, feed intake was not comparable among the three groups, serum and production parameters were also non- significantly affected.In contrast to our finding, dietary supplementation of 0.50 % biomass of chlorella did not affect final body weight in broiler chicks in study of Kotrbáček *et al.,* (1994). On the other hand, Kang *et al.,* (2013) reported that several *Chlorella*-based supplements including DCP, liquid media or CGF added into the diets of broiler chicks enhanced body weight, but did not affect feed intake and feed conversion ratio. The above results are supported by Peiretti and Meineri (2008). They reported that final body weight (BW), weight gain, and feed efficiency were not affected by dietary supplementation of microalgae at different levels in the rabbit diet. In addition, Kotrbácek *et al.* (1994) concluded that combination of *Chlorella* with other biological feed additives did not affect the live weight of broilers. Kang *et al.,* (2013) observed a opposite result, they found that use of 1% chlorella significantly increase the final live weight of the broiler chicken.Thus, the improvement of chicken growth may be attributed to those essential nutrients contained in dried *Chlorella* powder.

In case of DP % and survivability % these results agree in part with those reported by *Venkatararaman et al.,* (1994)found that broiler dressing percentage and the weights of different organs were not affected by the addition of *Spirulina* algae dried powder to broiler diet. *El Deek et al., (1987)* and *El Deek and Brikaa (2009) also* found that using different levels of seaweed had insignificant effect on ducks carcass quality. But dissimilar results were found by *El Deek et al.,* (2011).Regardless of 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 in agreement in part with those reported by *El Deek and Brikaa* (2009)who found that the levels (0, 4, 8, 12%) of seaweed did not affect the performance of the ducks.*Schaivone et al.,* (2007)found that using of 5g algae / kg feed insignificantly affected on the slaughter characteristics, chemical structure, color and stability of oxidation properties and sensory of the Muscovy ducks.These results are corresponding with El Deek *et al.,* (2011) experiment. They accomplished that using different levels of algae in broiler finisher diets had insignificant effect on gizzard and spleen percentages (ranged between 2.12 to 2.35% and 0.12 to 0.15%, respectively), regardless of thermal or enzymatic treatments.These findings are partially supported by Venkatararaman *et al.,* (1994).They found that broiler dressing percentage and the weights of different organs were not affected by the addition of *Spirulina*algae dried powder to broiler diet. Schaivone *et al.,* (2007) found that using of 5g algae / kg feed insignificantly affected on the slaughter characteristics, chemical structure, color and stability of oxidation properties and sensory of the Muscovy ducks. The increase in plasma glucose concentration of hens fed dietary *Chlorella* may be attributed to its excellent nutritional profile and high carotenoid content. In this regard, El-Khimsawy (1985) reported that vitamin A plays an important role for synthesis glucose molecule in the body.

The present study give similar findings with the results of Kanagaraju and Omprakash (2016) and SweeWeng *et al.,* (2016), found that the addition of 1% *Spirulina* had significantly lower serum cholesterol level than that of the control group in quails.These results are contradictory with the findings of Kannan *et al.,* (2005), Abdel-Daim *et al.,* (2013) and AbouGabal *et al.,* (2015). Also, *Spirulina platensis* supplementation at level of 1% significantly improved the blood parameters (Shanmugapriya and SaravanaBabu, 2014). This contradictory result was found due to some adverse environmental effect and heat stress during the summer season. Furthermore, Jamil *et al.,* (2015) concluded that, ALT and AST decreased significantly (P<0.05) when fed with *Spirulina platensis* compared with the control group.In the current study, the total blood leucocytes of broiler chickens were not affected by dietary *Chlorella* supplementation compared with the control. In contrast to our results, An *et al.,* (2010) reported that total protein, albumin, glucose, and interferon-γ were increased in blood serum of mice fed hot water extract of *Chlorella*. They suggested that this extract may be useful in improving the immune function of animals. Similarly, Kotrbácek *et al.,* (1994) reported that 0.5% biomass of fresh water *Chlorella* significantly enhanced the phagocytic activity of leucocytes and lymphatic tissue development of broiler chickens. Therefore, our blood leucocytes results were not consistent with the report of An *et al.,* (2010) and Kotrbácek *et al.,* (1994). However, the numbers of WBC and lymphocytes were higher in broilers fed with dried *Chlorella* powder (DCP) in the present study, maybe due to the processing technique and the nutritional value of *Chlorella* forms (Robinson *et al.* and Komaki *et al.*,). These results are in accordance with the earlier findings of Wakwak *et al.,* (2003), Kabir *et al.,* (2004) and Kulshreshtha *et al.,* (2008). In addition, the current results confirmed those of Baojiang (1994) who found that *Spirulina* is useful for the beneficial intestinal flora.

However, due to the potential health benefits of dried *Chlorella* powder it helps to improve the growth and productivity of birds by increasing digestibility and prevent the growth of harmful microbial population.

Conclusion

It can be concluded that Dried *Chlorella* powder (DCP) supplementation up to 0.5% in chick's diet has no effect on growth performance,relative internal organ weight, biochemical analysis and haematological analysis data. The DCP supplementation also had a positive effect on total cholesterol and sugar levels. Therefore, DCP can be used by farmers to enhance bird's performance. Further studies are required to observe the dose dependent effect of DCP on global gene expression profiling in chicken liver.

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