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Supplementation of *Spirulina platensis* in feed and water for the performance of broiler

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ABSTRACT

Spirulina is multicellular, blue green algae, which contains protein (50-70%), carbohydrate (12-13%), fat (6%) fat and substantial amount of minerals, vitamins, carotenoids and some fatty acids. Considering nutrient content and prospective health benefits, an experiment was conducted with 144-day old broiler (Cobb-500). Chicks were divided into 6 groups having 24 birds in each (8 per cage). Experimental groups were (1)-0.25% vitamin in feed, (2)-0.25% vitamin in feed + 0.5g/L Spirulina in water from laboratory, (3)-0.25% vitamin in feed + 0.5g/L commercial Spirulina in water, (4)-0.0% vitamin + 0.25% Spirulina in feed, (5)-0.25% vitamin + 0.25% Spirulina in feed, (6)-0.0% vitamin + 0.0% Spirulina in feed. The diets were iso-caloric (3170Kcal/kg) and iso-nitrogenous (22.7% CP). After 35 days feeding trial body weight was 1263, 1294, 1308, 1338, 1372 and 1188g/bird in group 1, 2, 3, 4, 5 and 6 respectively where numerically higher weight was observed in group 5 and lower in group 6, but overall significant variation observed among the groups ($p<0.05$). Feed intake was 2475, 2302, 2320, 2311, 2342 and 2378 g/bird in group 1, 2, 3, 4, 5 and 6 respectively ($p<0.5$). Feed conversion ratio (Kg feed intake/kg live weight gain) was higher in group 6 (2.00) and lower in group 5 (1.70), but was overall significant variation observed among the groups ($p<0.05$). It may be concluded that Spirulina would be supplemented in feed and water separately or at a time for better performance and health status of broiler.

Introduction

Feed additives are using in the diet of poultry for improving the quality of feed, the quality of food from animal origin; improve the performance and health status of animal. Spirulina (*Spirulina platensis*) could be a potential feed additive because it contains higher amount of nutrients like 50.0-70.0% protein, 12.0-13.0% carbohydrate,6.0% lipid, 7.0% minerals and a lot of vitamins (Shuvo, 2001). It also contains a considerable amount of phosphorous, magnesium, zincs, pepsin and 6.0-11.0% polysaccharide, 44.6-54.1%palmitic acid, 8.0-31.7% gama-linolenic acid, 10.8-30.7% linoleic acid and 1.0-15.5% oleic acid (Shuvo, 2001). It is rich in B vitamins, minerals, trace elements, chlorophylls and enzymes (Kelly *et al*., 1995). The cell wall of Spirulina consists of polysaccharide which has a digestibility of 86.0% and easily absorbed in human body (Clement *et al*., 1967; Bourges *et al*., 1971).

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Spirulina increases the redness as well as yellowness of broiler meat when added in diet (Habib *et al*., 2008). Spirulina was added to forage for poultry and livestock feeding, their growth rate was improved significantly (Toyomizu *et al.,* 2001). Ross & Dominy. (1990) demonstrated that the addition of Spirulina up to 12.0% in broiler diet can replace protein source especially soybean meal without hampering growth and feed efficiency. Some study demonstrated that alga may be useful as a protein source is controversial (Soler *et al.,* 2000; Lacaz-Ruiz, 2003; Becker, 2007). Author already works in broiler with the Spirulina and found positive results (Islam *et al.,* 2021a; Islam *et al.,* 2021b; Khan *et al.,* 2021).

It has high growth potential and abundantly growing throughout the world. It assimilates CO_2 and emits O_2 during photosynthesis. So, algae would have potential use in poultry feed to increase production and improvement of product quality after growing in inorganic media (Kosaric media) contained different macronutrient like $NaHCO_3$, K_2HPO_4, $NaNO_3$, K_2SO_4, NaCl, $MgSO_4.7H_2O$, $CaCl_2$, and $FeSO_4.2H_2O$ as well as A_5 micronutrient solution which contains H_3BO_4, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, MoO_3, $CoCl_2.6H_2O$.

During Spirulina culture inorganic mineral of media converted into the organic mineral as well as Spirulina content would be higher amount of vitamins. So, algae have potentiality for growth, health benefits, environmental impact as well produces organic mineral as form of algae. Considering above benefits the research has conducted to know the effect of cultured Spirulina as replacer of dietary vitamin mineral premix either in drinking water or feed and compare to the imported commercial Spirulina for the performance of broiler.

Materials and Methods

The experimental work was conducted in the poultry rearing unit of Sahjalal Animal Nutrition Field Laboratory, under the Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh.

Culture of micro algae Spirulina platensis

Microalgae, Spirulina platensis was imported from China as well as cultivated in the laboratory of the Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. The pure Spirulina stock was imported from Malaysia.

Experimental design and layout

Intended experiment was carried out as per completed randomized design (CRD). One hundred and forty-four Cobb-500 commercial straight run chicks were divided into six dietary groups having three replications in each (eight birds in each replication). The six dietary treatment groups are as follows:

Group 1- (0.25% vitamin in feed); Group 2-(0.25% vitamin in feed + 0.5g/L Spirulina in water from laboratory cultivated); Group 3-(0.25% vitamin in feed + 0.5g/L commercial Spirulina in water); Group 4- (0.0% vitamin + 0.25% Spirulina in feed); Group 5- (0.25% vitamin + 0.25% Spirulina in feed); Group 6-(0.0% vitamin + 0.0% Spirulina in feed)

Formulation of experimental diets

The dietary ingredients were collected from the local market. After weighing, major ingredients like maize, protein concentrate, rice polish, soybean meal were well mixed. Soybean oil was mixed with this mixture step by step. The ingredients and nutritional composition of different dietary groups are showing in table 1 and 2, respectively.

General management of birds

A number of 144 Cobb-500 commercial straight run day old broiler chicks were reared in their experiment having average weight of 54.30 g/bird, divided into six groups, one control group and five treatment groups with 24 chicks per group.

Light, temperature and ventilation

A 100-watt electric bulb was assigned in each cage to control the temperature and light. Electric light was provided in the trial house for 24 hours and the brooding temperature was maintained near about 34^0C for first week and decreased gradually at the rate of 3^0C in each week. The broilers were vaccinated against following diseases shown in

Table 1: Formulation of different diet (%) as per experimental groups

Ingredients	Groups					
	1	2	3	4	5	6
Maize	54.50	54.50	54.50	54.50	54.50	54.70
Protein Conc.	12.50	12..50	12.50	12.50	12.50	12.50
Rice polish	3.00	3.00	3.00	3.00	3.00	3.10
Soya meal	23.00	23.00	23.00	23.00	22.70	22.95
DCP	1.50	1.50	1.50	1.50	1.50	1.50
Soybean oil	4.50	4.50	4.50	4.50	4.55	4.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50
DL Methionine	0.15	0.15	0.15	0.15	0.15	0.15
Choline Chloride	0.05	0.05	0.05	0.05	0.05	0.05
Mineral Mixture	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin	0.25	0.25	0.25	0.00	0.25	0.00
Spirulina	0.00	0.00	0.00	0.25	0.25	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

N.B: Group 1- (0.25% vitamin in feed), Group 2-(0.25% vitamin in feed + 0.5g/L Spirulina in water from laboratory), Group 3-(0.25% vitamin in feed + 0.5g/L commercial Spirulina in water), Group 4- (0.0% vitamin + 0.25% Spirulina in feed), Group 5- (0.25% vitamin + 0.25% Spirulina in feed), Group 6-(0.0% vitamin + 0.0% Spirulina in feed) % Spirulina in feed.

Composition of vitamin (Renata Limited, Bangladesh): Vitamin A, 4,800,000 I.U/kg; Vitamin D3, 1,000,000 I.U/kg; Vitamin-E 8,000 mg/kg, Vitamin-K3 1600 mg/kg, Vitamin-B1 600 mg/kg, Vitamin-B2 2000 mg/kg, Vitmin-B3 1600 mg/kg, Vitamin-B6 1600 mg/kg, Vitamin B12 4 mg/kg, Vitamin-PP 12,000 mg/kg, Biotin 20 mg/kg.

Table 3. Birds from 3 replicate cages from each treatment were separately vaccinated. The vaccination schedule is given bellow:

Live weight and body weight gain

Broilers were weighted in a group at the beginning of the trial and then every week at the age of day 0, 7, 14, 21, 28 and 35. Weighing was done using electric balance before supplying feed at morning of each week. The average body weight gain of broilers in each replication was calculated by deducting initial body weight from final body weight.

Feed consumption and feed conversion efficiency

Feed offered were recorded when supplied in cages and refusal in every week. Feed intake, feed conversion ratio and feed conversion efficiency were calculated

Table 2: Chemical composition of different diets as per groups

Parameter	Groups					
	1	2	3	4	5	6
CP (%)	22.72	22.72	22.72	22.72	22.6	22.72
CF (%)	4.55	4.55	4.55	4.55	4.53	4.55
ME(Kcal/kg)	3164	3164	3164	3164	3162	3172
Ca (%)	1.10	1.10	1.100	1.10	1.10	1.10
P (%)	0.53	0.53	0.53	0.53	0.54	0.53

N.B: 1-(0.25% vitamin in feed), 2-(0.25% vitamin in feed+0.5g/L Spirulina in water from laboratory), 3-(0.25% vitamin in feed+0.5g/L commercial Spirulina in water), 4-(0.0% vitamin + 0.25% Spirulina in feed), 5-(0.25% vitamin+0.25% Spirulina in feed), 6-(0.0% vitamin + 0.0% Spirulina in feed).

Blood analysis

Blood from healthy chicken was collected using 10ml falcon tube directly from the heart after slaughtering at day 35 of age from randomly selected birds of each replication. After collection of blood samples each tube was placed in a rack at room temperature. To avoid blood clot, EDTA was used and tubes were put in the centrifuge machine.

Table 3: Vaccination schedule during growth trial

Age in days	Diseases	Name of the vaccine	Route and Doses
4	Newcastle Disease (ND)	Avinew R	One drop in each eye
11	Infectious Bursal Disease (IBD)	Gumboro	One drop in each eye
20	Newcastle Disease (ND) Booster	Avinew R	One drop in each eye

Blood was centrifuged at 1500 rpm for 20 minutes to obtain plasma and transferred into sterile 1.5ml apendrop tube. The plasma was preserved in deep freezer and the tubes were marked properly with permanent marker for easy identification during blood analysis for cholesterol.

Cholesterol

For quantitative determination of cholesterol in blood plasma was analyzed by following procedure with biochemistry analyzer:

a. Test reagent - Cholesterol LS (Liquid. CHOD-POD);
b. Assay conditions
c. Wave length: 505 nm. (500-550)
d. Cuvette: 1 cm light path
e. Temperature: 37ºC (15-25ºC)
f. Instrument was adjusted to 0 with blank of reagent.
g. Pipette into cuvette:

Mixed and incubated for 5 minutes at 37ºC or 10 minutes at room temperature (15-25ºC). Absorbance (A) of the sample was recorded and calibrated against the blank till the color was stable at least 60 minutes.

Calculation:

$$\text{Cholesterol (mg/dl)} = \frac{(A)Sample}{(A)Standard} \times 200(Calibratorconc.)$$

Conversion factor: mg/dl × 0.0258 = mmol/L

Chemical analysis

Samples feed ingredients (in duplicate) ware analyzed to determine dry matter (DM), crude protein (CP) and total ash (Ash) following the method described by AOAC (1990).

Statistical analysis

All data were analyzed by using statistical SPSS program for one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (Duncan, 1955) done to know the differences among the means at significance level of 5.0% (Steel and Torrie, 1980).

Results

Growth of birds

Live weight of birds at different age was different among the groups (p>0.05). During completion of feeding trial, group 5 attained 1372g weight which is higher than negative control group (6).

Consumption of feed in different groups found different and varied significantly (p<0.05), where higher feed intake was in control (2475g) and lowest in group 2 (2302g) after completion of feeding trial. Feed conversion ratio found lower in group 5 (1.70) and 4(1.73) comparison to control (p<0.05). But, group 1, 2, 3 and 6 found statistically similar (p>0.05). The plasma of birds from different dietary groups found insignificant difference (p<0.05) among them regarding blood cholesterol level (p>0.05). No bird belongs to any group died during 35 days of feeding trail.

Table 4: Growth performance (per bird) and cholesterol level in blood of birds

Parameters	**Groups**					
	1	**2**	**3**	**4**	**5**	**6**
Initial weight (g/b)	55±0.4	54±0.2	54±0.2	55±0.4	54±0.4	54±0.4
Live weight (g/b)	1263^{ab}±45	1294^{ab}±44	1308^{ab}±47	1338^{a}±56	1372^{a}±53	1188^{b}±62
Weight gain (g/b)	1208^{ab}±45	1241^{ab}±44	1253^{ab}±48	1284^{a}±56	1322^{a}±53	1134^{b}±62
Feed intake (g/b)	2475^{a}±38	2302^{b}±38	2320^{b}±58	2311^{b}±45	2342^{b}±38	2378^{ab}±25
FCR(FI/LWG)	1.96^{ab}±.05	1.78^{abc}±.02	1.77^{abc}±.01	1.73^{bc}±.02	1.70^{c}±.01	2.00^{a}±.03
Cholesterol (mg/DL)	40^{a}±04	43^{a}±04	43^{a}±04	44^{a}±04	42^{a}±03	37^{a}±02

abcMeans with dissimilar superscripts are significantly different (p<0.05)

Table 5: Production cost per kg live weight gain of broiler

Parameters	Groups					
	1	2	3	4	5	6
Feed cost/kg	42	42	42	42	42	42
Feed+Chick (BDT/kg)	82.5	75.0	74.5	72.5	71.5	83.0

Economic analysis of using Spirulina

Production cost was calculated mainly based on cost of feed. The cost per kilogram feed was lowest in group 5 and 4 than group 1. Production cost (BDT) per kg live weight of broiler was lowest in group 5 (71.5) followed by group 4 (72.5), 3 (74.5), 2 (75.0), 1(82.5) and 6(83.0).

From the above result, it is clear that the diet without vitamin might compensate their performance due to dietary addition of Spirulina as an efficient alternative organic source of vitamin and mineral in broiler.

Discussion

The result obtained in the present study showed that addition of dietary Spirulina would compensate the growth of broiler when vitamin was excluded from the diet. Compensation of growth due to lacking vitamin mineral premix would be due to Spirulina in broiler diets could improve palatability, reduce toxicity and improved digestibility, antioxidant activity, hypercholesterolemia, anticancer, immune-stimulant, antiviral properties (Rodriguez-Hernández *et al.,* 2001; Derner *et al*., 2006; Colla *et al.,* 2007). Other research has shown that vitamin is normally not required when Spirulina has been included in the feed (Venkataraman *et al*., 1994). Negative control group had no vitamin or Spirulina in the diet. As a result; the dietary Spirulina group shows a significant difference ($p<0.05$) from negative control group in the live weight of broilers. Dietary Spirulina levels at 50-100 g/kg of feed ration would maintain typical growth, but exceeding 200 g/kg level will cause declining rate of growth (Toyomizu *et al*., 2001). But in this experiment the level of Spirulina is lower than the research to show any effects on intake of feed. Saxena *et al*., 1983 found that body weights of chicks fed Spirulina levels of 11.1 and 16.6% of diet were not different from the control group, receiving groundnut cake.

Chickens receiving dietary Spirulina have been found to be of better health than their supplemented counterparts (Venkataraman *et al*., 1994). At the inclusion rate of 12.0%, broilers showed slower growth in comparison with those fed 0, 1.5, 3.0, or 6.0% algae. Toyomizu *et al.* (2001) fed broilers with maximum 8.0% *Spirulina* sp. and found no significant differences in their performances at 16 days of age. The reason is for increasing the functionality of macrophage and mononuclear phagocyte system that indicate the enhanced disease resistance with increasing dietary Spirulina levels in broiler chickens (Qureshi and Ali, 1996; Al-Batshan *et al.,* 2001). In the present experiment, feed intake of broilers in different groups were decreased ($p<0.05$). The findings from Ross and Dominy (1990) found that supplemented diets in broiler birds with up to 20% blue-green algae, and found the 3-week-old broilers experienced depressed growth when algae inclusion levels were higher than 10%.

Kharde *et al.* (2012) and Shanmugapriya *et al.* (2015) reported that feed conversion ratio improved by the dietary addition of the Spirulina as compared to that of the control diet. Another study from Baojiang (1994) reported that the Spirulina has beneficial effect on intestinal flora of broiler that helps feed utilization process as well as conversion of animal feed to animal protein.

Another research from Torres *et al.* (1998) and Fong *et al.* (2000) found that triglycerides and cholesterol concentrations were reduced significantly in rats or mice when Spirulina were included in their diets. Tsuchihashi *et al.* (1987) and Mariey *et al.* (2012) also reported that the significant reduction in plasma cholesterol in case of broiler chickens fed dietary Spirulina could be attributed to reducing the absorption and/or synthesis of cholesterol in the gastro-intestinal tract by Spirulina supplementation that increase Lactobacillus population.

Production cost was considering only the cost of feed because other costs were similar for all the groups. The cost per kilogram feed was lowest in groups 5 and 4 than group 1. The study from Qureshi *et al.* (1996) found that chicken health was improved with low levels of Spirulina (10 g/kg in the ration). It is revealed from the above discussion that, the diet lacking of vitamin premix that might be compensated by Spirulina as an efficient alternative to organic sources of vitamin in the broiler diet. Moreover, Spirulina also increased the feed intake of broiler without any toxicity.

Conclusion

It may be concluded that vitamin premix might be replaced by *Spirulina* for enhancing the production performance of commercial broiler. Its administration in drinking water from locally cultured or imported from Myanmar would have similar effect on the production performance of commercial broiler. Further investigation is needed for the verification of the economic and feasibility to recommend in the diets of different kinds of poultry species.

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