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REGULAR ARTICLE

Replacement of vitamin-mineral premix by Spirulina and its effect on the performance of broiler

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Abstract

The main purpose of this study was to explore the effects of Spirulina (*Spirulina platensis*) as a feed supplement by replacing Vitamin-Mineral Premix on the performance of broiler. The study explored that final body weight was 1039, 1070, 1044, 1065, 1117 and 893 g/bird in group 1, 2, 3, 4, 5 and 6 where significantly (p<0.5) higher weight observed in Spirulina group (5) but lower in negative control group (6). Feed intake observed more or less similar in all groups (1483±24g; p>0.5). Feed conversion ratio (kg FI/kg LWG) observed significantly different like 1.72, 1.65, 1.69, 1.62, 1.61 and 1.99 in group 1, 2, 3, 4, 5 and 6 respectively (p<0.5) where higher in negative group (6) and control group (1), but is lowest and similar in 75% Spirulina group (4) and highest (100%) Spirulina group (5). %Ash of different dietary groups were 46.35, 46.54, 48.93, 49.93, 55.07 and 46.92 of group 1, 2, 3, 4, 5 and 6 respectively. The %ash of bone was gradually increased among dietary groups and the best result was obtained in 100% Spirulina group 5 (55.07). It also revealed that diets containing no vitamin-mineral premix with 100% Spirulina improve the performance of broiler. The findings suggest that poultry farmer can use spirulina as a feed item for broiler for more yield.

Key words: Broiler; *Spirulina platensis*; replacement; feed

Introduction

The blue-green algae (*Spirulina platensis*) is one of the nutritional algae which has been used as a food source for human and animal. The protein content of spirulina is ranging from 55-65% with essential amino acids [1,2]. It provides good energy ranges from 2.5-3.29 kcal/gram with 41% phosphorous [1,3]. It also is being used as a feed component for broiler and layer diets since it is a good source of some vitamins like vitamin B12, thiamin, pyridoxine, vitamin C, riboflavin, and carotenoids which enhance yolk color and flesh [2]. About 30% of the total production of algae is being used as animal feed production [1]. Some studies have already been done by some researcher regarding suitability of algae as an animal and

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poultry feed [1,5-10]. Moreover, the broiler is a very popular poultry item in Bangladesh [11-13] and other countries which supplied a major portion of meat demand. The villagers of Bangladesh are rearing broiler by establishing the small firm of the home yard [14,15] and earn money which helps to alleviate poverty [15-18] and ensuring food security [18-20]. Many people of the remote village take broiler poultry farming as a profession [2]. Not only that broiler meat act as a main meat source in the urban area as well as rural areas [3]. But few types of research have been done on an issue of replacement of by Spirulina and its effect on the performance of broiler. The aim of this research was to explore the effects of Spirulina *(Spirulina platensis)* as a feed supplement by replacing Vitamin-Mineral Premix on the performance of broiler.

Materials and methods

The experiment was conducted in the Shahjalal Animal Nutrition Field Laboratory of the Department of Animal Nutrition, Bangladesh Agricultural University (BAU), Bangladesh.

Collection of Spirulina platensis, stock culture and Kosaric Medium (KM)

Microalgae, *Spirulina platensis* was collected from the imported stock which was maintained at Animal Nutrition Laboratory, Department of Animal Nutrition, Bangladesh Agricultural University, Mymensingh. The pure stock culture of *Spirulina platensis* was maintained in the laboratory in Kosaric medium (KM) [4]. Kosaric media (KM) was prepared for the culture of *S. platensis*. Different concentrations of nutrients of the kosaric medium are shown in Table 1. For the preparation of Kosaric Medium, the mentioned amount of ingredients from 1-8 was weight with the help of electric balance and taken in a 5.0 L glass jar [1]. The 5ml micronutrient solution was added with the help of pipette in the flask and distilled water was added to make the volume 5.0L, mixing autoclaving (sterilized at 121^{o}C for 15 minutes with moist heat) and cooling was carried out and kept it for culture.

Statement of research work and Source of chicks

The experiment was conducted in the poultry rearing unit of Shahjalal Animal Nutrition Field Laboratory, Bangladesh Agricultural University, Mymensingh for a period of 28 days by using 180 DOC (Day old chick) to find out the effect of Spirulina algae as a replacement of vitamin-mineral premix on the performance of broiler. Day-old straight run broiler chicks (COBB 500) were reared for the experiment. The chicks were produced and marketed by Kazi Farm Ltd.

Table 1. Composition of Kosaric Medium (Modified after Zarrouk [3]) for *Spirulina platensis*

Chemicals/compounds	Concentration of chemicals in stock solution g/L
$NaHCO_3$	9.0
K_2HPO_4	0.250
$NaNO_3$	1.250
K_2SO_4	0.50
NaCl	0.50
$MgSO_4$ $7H_2O$	0.10
$CaCl_2$	0.02
$FeSO_4$ $2H_2O$	0.005
A_5 micronutrient solution	0.5ml/L
a) Composition of A_5 micronutrient solution	5g
i) H_3BO_4	2.86
ii) $MnCl_2.4H_2O$	1.81
iii) $ZnSO_4$.$7H_2O$	0.22
iv) $CuSO_4.7H_2O$	0.08
v) MoO_3	0.01
vi)$CoCl_2.6H_2O$	0.01

Test substance

Spirulina (*Spirulina platensis)* is a multicellular, blue-green micro-alga is very small and microscopic and 300-500µm in length. These blue-green algae contain 50-70% protein, 10-12% carbohydrate (in dry condition), 6% fat, 7% minerals and a lot of vitamins [5]. A considerable amount of phosphorous, magnesium, zincs and pepsin found in Spirulina [6]. It also consists 6-11% polysaccharide, the predominant are palmitic (16:0, 44.6-54.1%) gamma linolenic or GLA (18:3, 8.0-31.7%), linoleic (18:2, 10.8-30.7%) and oleic acids (18:1,1-15.5%). It is rich in B vitamins, minerals, trace elements, chlorophylls and enzymes [7].

Experimental design and layout

The experiment was conducted in a completely randomized design (CRD). One hundred and eighty chicks (DOC) were randomly divided into six treatment groups having three replicates in each (ten birds in each replication). The six dietary treatment groups were:

1. Control [100% Vitamin-mineral premix (2.5g/kg) + 0% Spirulina (0g)]
2. 75% Vitamin-mineral premix (1.9g/kg) + 25% Spirulina (0.6g/kg)
3. 50% Vitamin-mineral premix (1.3g/kg) + 50% Spirulina (1.2g/kg)
4. 25% Vitamin-mineral premix (0.6g/kg) + 75% Spirulina (1.9g/kg)
5. 0% Vitamin-mineral premix (0g) + 100% Spirulina (2.5g/kg)
6. 0% Vitamin-mineral premix (0g) + 0% Spirulina (0g)

Formulation of experimental diets

The ingredients were ground individually using a grinding machine. After weighing, a part of the required amount of ground wheat, soybean meal, rice polish, oyster shell, meat and bone meal and fish meal were mixed thoroughly. Soybean oil was mixed with this mixture step by step. Then the salt, Di-calcium phosphate, Choline Chloride, Lysine, DL-Methionine were added except Vitamin-Mineral Premix and Spirulina [8]. Group 1 was controlled and no Spirulina was used. In group 2, 3, 4 and 5 Spirulina was added at replacing the amount of vitamin-mineral premix and mixed homogenously, then sun-dried. In group 6, there was neither vitamin-mineral premix nor Spirulina in the diet considered as the negative control diet [26]. It was done by mixing all those premixes with a small amount of mixed feed and then with the gradual increase in quantity by adding the remaining mixed feed [9]. The control diet consists of wheat, soybean meal, soybean oil, rice polish, fish meal, common salt, Choline chloride, Di–calcium phosphate, Vitamin-mineral premix, meat and bone meal, Lysine, and DL-methionine [10]. Spirulina was calculated on DM basis added minerals in the media. Calculation (in kg) is given below:

Where, Control diet vitamin-mineral = 2.5 g/kg; Media mineral = 12.12 g/kg;12.12 g mineral in 1 kg media;
0.6g mineral in = (0.6/12.12)=0.05 kg=50g (ml) media

1. Control - 2.5g vitamin-mineral premix/kg + 0 g Spirulina
2. 1.9g premix/kg + 0.6g Spirulina/kg (contains 50ml media)
3. 1.3g premix/kg + 1.2g Spirulina/kg (contains 100ml media)
4. 0.6g premix/kg + 1.9g Spirulina/kg (contains 150ml media)
5. 0g premix/kg + 2.5g Spirulina/kg (contains 200ml media)
6. Negative control = 0g premix + 0g Spirulina

The ingredients which are used for ration formulation in different dietary treatments are shown in Table 3.

General management of birds and Preparation of house

One hundred and eighty Cobb-500 commercial day-old broiler chicks were reared in this experiment having an average weight of 50.30 g/b, divided into six groups, one control group and five treatment groups with 30 chicks per group. The chicks were collected from Kazi Farms Ltd. Mymensingh, Bangladesh by grading, sexing and weighing individually to maintain homogeneity of experimental units. Eighteen cages were considered for this trial each having floor space of 0.91 m^2 (120 cm × 76 cm).

The management house and cages were done as described previously [5]. Wall and wire nets were thoroughly disinfected by spraying Vircons-S solution (Antec International Limited, England) in water and kept free to dry up properly.

Table 2. The layout of the experiment (Level of premix + Spirulina).

Replications	Diets					
	1 Control (100% premix + 0% Spirulina)	2 (75% premix + 25% Spirulina)	3 (50% premix + 50% Spirulina)	4 (25% premix + 75% Spirulina)	5 (0% premix + 100% Spirulina)	6 (0% premix + 0% Spirulina)
1	10	10	10	10	10	10
2	10	10	10	10	10	10
3	10	10	10	10	10	10
Birds/ treatment	30	30	30	30	30	30
Total birds						180

Table 3. Formulation of diet (kg/100kg) in different dietary groups.

Groups	1	2	3	4	5	6
Premix	100%	75%	50%	25%	0%	0%
Spirulina	0	25%	50%	75%	100%	0%
Wheat	55.00	55.00	55.00	55.00	55.00	55.00
Rice polish	3.70	3.70	3.70	3.70	3.70	3.70
Meat & bone meal	8.00	8.00	8.00	8.00	8.00	8.00
Soybean meal	23.00	23.00	23.00	23.00	23.00	23.00
Soybean oil	7.00	7.00	7.00	7.00	7.00	7.00
Oyster shell	1.00	1.00	1.00	1.00	1.00	1.00
Lysine	0.40	0.40	0.40	0.40	0.40	0.40
DCP	1.00	1.00	1.00	1.00	1.00	1.00
Metheonine	0.40	0.40	0.40	0.40	0.40	0.40
[a]Vit-min premix	0.25	0.19	0.13	0.06	0.00	0.00
[b]Spirulina	0.00	0.06	0.12	0.19	0.25	0.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25

Note: 1-Control (100%premix + 0%Spirulina); 2-75% premix + 25 % Spirulina; 3-50% premix + 50% Spirulina; 4-25%premix + 75% Spirulina; 5-0% premix + 100% Spirulina; 6-0% premix + 0% Spirulina

[a]Vitamin mineral premix: Vitamin A, 4,800,000 I.U/kg; Vitamin D3, 1,000,000 I.U/kg; Vitamin-E 8,000 mg/kg, Vitamin-K_3 1600 mg/kg, Vitamin-B_1 600 mg/kg, Vitamin-B_2 2000 mg/kg, Vitmin-B_3 1600 mg/kg, Vitamin-B_6 1600 mg/kg, Vitamin B_{12} 4 mg/kg, Vitamin-PP 12,000 mg/kg, Biotin 20 mg/kg, Iron 9600 mg/kg, Copper 2400 mg/kg, Manganese 19,200 mg/kg, Cobalt 120 mg/kg, Zinc 16,000 mg/kg, Iodine 240 mg/kg, Selenium 80 mg/kg, Antioxidant 4000 mg/kg, Lysine 1.2%, Methionine 2%.

Source: NOVAVIT-L (NOVA Nutrition, Belgium)

[b]Spirulina: 0.06kg(50ml); 0.12kg(100ml); 0.19kg(150ml); 0.25kg(200ml).

Table 4. Nutrient composition (g/100 kg air dry sample) of different treatment groups

Groups	1	2	3	4	5	6
Premix	100%	75%	50%	25%	0%	0%
Spirulina	0	25%	50%	75%	100%	0%
DM	89.12	89.12	89.12	89.12	89.12	89.12
CP	23.71	23.71	23.71	23.71	23.71	23.71
Ca	1.05	1.05	1.05	1.05	1.05	1.05
P	0.79	0.79	0.79	0.79	0.79	0.79
*Lysine	1.53	1.53	1.53	1.53	1.53	1.53
*Metheonine	0.74	0.74	0.74	0.74	0.74	0.74
CF	4.32	4.32	4.32	4.32	4.32	4.32
ME (kcal/kg)	2950	2950	2950	2950	2950	2950

*Calculated value

N.B: 1-Control (100%premix + 0%Spirulina); 2-75% premix + 25 % Spirulina; 3-50% premix + 50% Spirulina; 4-25% premix + 75% Spirulina; 5-0%premix + 100% Spirulina; 6-0% premix + 0% Spirulina

Table 5. Vaccination schedule followed for the experimental broilers

Age of birds (Days)	Disease	Name of vaccine	Route of administration and dose
7	Newcastle Disease (ND)	Avinew [R]	One drop in each eye
14	Infectious Bursal Disease (IBD)	Gumboro	One drop in each eye

Source: District Veterinary Hospital, Aqua, Mymensingh, Bangladesh.

Chick's management

Freshly dried rice husk was spread on the floor under the cages at a depth of 4 cm. After 3 weeks of age, the upper layer of the litter with droppings was removed and replaced by new litter material. The management was done by following standard methodology [7,12].

Vaccination and Clinical observation

The broilers were vaccinated against Newcastle Disease and Infectious Bursal Disease (Gumboro). Birds from three replicate cages from each treatment were separately vaccinated against ND at the 7th day of age, Gumboro disease vaccine at 14th day of age and no vaccine at all respectively. The vaccine which followed during the experimental period is given Table 5.

Record keeping and collection of data

Broilers were weighted in a group at the beginning of the trial and then every week at the age of day 7, 14, 21 and 28. Weighing was done using electric balance before supplying feed at the morning of each week by following standard method [7] and as described by Dadgar et al. [1].

Bone ash% content

Bone ash% was determined from the shank bone of the birds. At day 28, slaughtering the birds and cut the shank of them from randomly selected birds of each replication. After collection of shank bones; those were boiled for 1 hour and then skinning them [13], washed properly and air dried; then weighed the crucibles and shank bones and dried in the oven at 105°C for 24 hours. After 24 hours of drying, the oven dry weight of bones was taken; then kept the bones in the muffle furnace at 600°C for 5 hours and then calculated the DM% and %ash by the following the formula:

W_1= Oven dry wt. - Crucible wt.

$$DM\% = \frac{W1}{\text{Sample wt}} \times 100$$

$$\%Ash = \frac{\text{Ash wt} - \text{Crucible wt}}{\text{Sample wt.}} \times 100$$

Blood analysis

Blood analysis was done by following standard methods as explained previously [7].

Economic analysis

The cost of broiler production for each treatment was calculated based on the cost of feed ingredients. Cost for management and other costs are not considered as those are same for all groups.

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 [7].

Statistical analysis

All data were analyzed by using statistical SPSS program for one way analysis of variance (ANOVA) and Duncans Multiple Range Test.

Results and discussion

Performance of Broilers

The results of growth performance (body weight, feed consumption and feed conversion ratio), mortality, % ash of bone and cholesterol & triglyceride status in blood of broilers are presented in this chapter.

Live weight

The live weight of broilers of different dietary groups at different ages is shown in Table 8. During day 8-14, 15-21 and 22-28 of ages, the live weights of birds are not significantly different ($p>0.05$) in 1, 2, 3, 4 & 5 dietary treatment groups but have significantly difference ($p<0.05$) with group 6. At the end of the trial, group 5 attained 1117g live weight, which is higher than negative control (6), and almost similar to group 2 (1070g), group 4 (1065g), group 3 (1044g) and group 1 (1039g). In this experiment, the negative control group was used for observing the effect of no vitamin-mineral premix in broiler diet. Neither vitamin-mineral premix nor Spirulina in the diet, birds of the negative control group (6) was lame and unable to move. Their feed intake was irregular; so, their live weight and live weight gain were low at the end of the trial. The result showed that, dietary Spirulina can compensate the performance of broiler by eliminating vitamin-mineral premix level in the diet. The probable reason may be the Spirulina in broiler diets could improve better qualities which give better performance and recover the deficiency of vitamin-mineral premix [29,10]. Other studies that replaced groundnut cake [14] or fishmeal [15] with Spirulina in chicken diets found no variation in growth. On the other hand, the negative control group (6) had no vitamin-mineral premix or Spirulina in the diet. As a result, the dietary Spirulina group shows a higher significant difference ($p<0.05$) from the negative control group in the live weight of broilers [16]. The other groups of birds 1, 2, 3 and 4 had no significant differences (p>0.05) among them. But significantly different (p<0.05) from group 5 to group 6.

Table 8. Weight (g/bird) of birds at different age.

Groups	1	2	3	4	5	6	Level of Sign.
Premix	100%	75%	50%	25%	0%	0%	
Spirulina	0	25%	50%	75%	100%	0%	
Initial wt (Day 7)	*155^{a} ±0.5	154^{a} ±1.7	155^{a} ±0.6	155^{a} ±0.2	155^{a} ±0.3	155^{a} ±1.0	NS
Relative to 1	100	100	100	100	100	100	
Day 14	344^{ab} ± 10	345^{ab} ±11	343^{ab} ±17	352^{ab} ±26	365^{b} ±15	328^{a} ±11	5%
Relative to 1	100	100	100	102	106	95	
Day 21	678^{b} ±03	700^{b} ±20	687^{b} ±08	689^{b} ±41	719^{b} ±62	603^{a} ±38	5%
Relative to 1	100	100	101	102	105	88	
Day 28	1039^{b} ±34	1070^{b} ±59	1044^{b} ±50	1065^{b} ±36	1117^{b} ±79	893^{a} ±18	5%
Relative to 1	100	103	101	103	111	86	

N.B: 1-Control (100% Premix + 0% Spirulina); 2-75% Premix + 25 % Spirulina; 3-50% Premix + 50% Spirulina; 4-25% Premix + 75% Spirulina; 5-0% Premix + 100% Spirulina; 6-0% Premix + 0% Spirulina
* Mean ± SD; ^{abc}Means with dissimilar superscripts are significantly different (p<0.05); **NS**-Not Significant

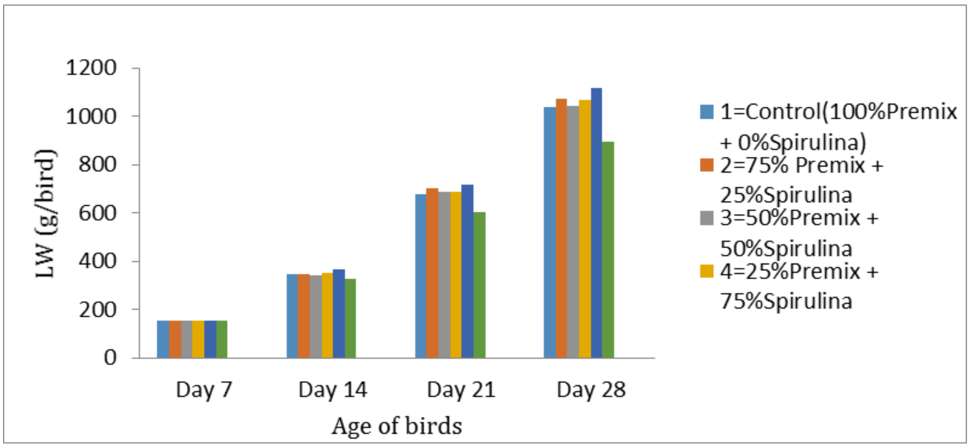

Fig. 1. Live weight of birds at different age

Live weight gain

Live weight gain of the birds receiving different groups is given in Table 9. Weekly weight gain of birds was not significantly differed (p>0.05) among the groups 1, 2, 3, 4 at day 8-14, 15-21 and 22-28 of age, where the highest weight gain was observed in group 5 during day 8-14 (210g) and day 22-28 (397g). On day 15-21, there was no significant difference (p>0.05) among groups 1, 2, 3, 4 and 5; but had a significant difference (p<0.05) with group 6 [7]. During trial highest weight gain (397g) was observed in day 22-28 of age in 100% Spirulina group. Relatively higher weight gain observed in group 5 (397g) than control (361g) and negative control (290g) during 28 days trial which were significantly different (p<0.05) [17].

Feed intake

The weekly feed intake of broilers receiving different diets is presented in Table 10. Feed intake of the bird under different groups during day 8-14, 15-21 and 22-28 of age differed significantly (p<0.05) from each other but feed intake of group 5 (447g) was highest at day 15-21 of age. At the end of the trial group, 2 (779g) resulted in significantly higher feed intake than other groups. Cumulative feed intake was highest in group 5 (1537g) and lowest in group 4 (1464g) at the day 8-28 of age.

Table 9. Live weight gain of birds at different age.

Groups Premix *Spirulina*	1 100% 0	2 75% 25%	3 50% 50%	4 25% 75%	5 0% 100%	6 0% 0%	Level of Sign.
Day 8-14	*189[ab] ± 10	190[ab] ±11	189[ab] ±16	196[ab] ±26	210[b] ±16	173[a] ±12	5%
Relative to 1	100	100	100	104	111	91	
Day 15-21	334[b] ±14	355[b] ±27	344[b] ±11	340[b] ±13	355[b] ±46	275[a] ±47	5%
Relative to 1	100	104	101	100	104	81	
Day 22-28	361[ab] ±31	370[ab] ±50	373[ab] ±22	377[ab] ±26	397[b] ±54	290[a] ±42	5%
Relative to 1	100	117	118	119	125	80	
Cumulative weight gain							
Day 8-21	523[b] ±04	546[b] ±19	531[b] ±07	533[b] ±41	564[b] ±62	448[a] ±37	5%
Relative to 1	100	103	100	101	106	85	
Day 8-28	884[b] ±35	915[b] ±57	889[b] ±48	910[b] ±35	962[b] ±79	738[a] ±18	5%
Relative to 1	100	108	105	107	114	84	

N.B: 1-Control (100% Premix + 0% Spirulina); 2-75% Premix + 25 % Spirulina; 3-50% Premix + 50% Spirulina; 4-25% Premix + 75% Spirulina; 5-0% Premix + 100% Spirulina ; 6-0% Premix + 0% Spirulina
*Mean ± SD; [abc] Means with dissimilar superscripts are significantly different (p<0.05); NS-Not Significant

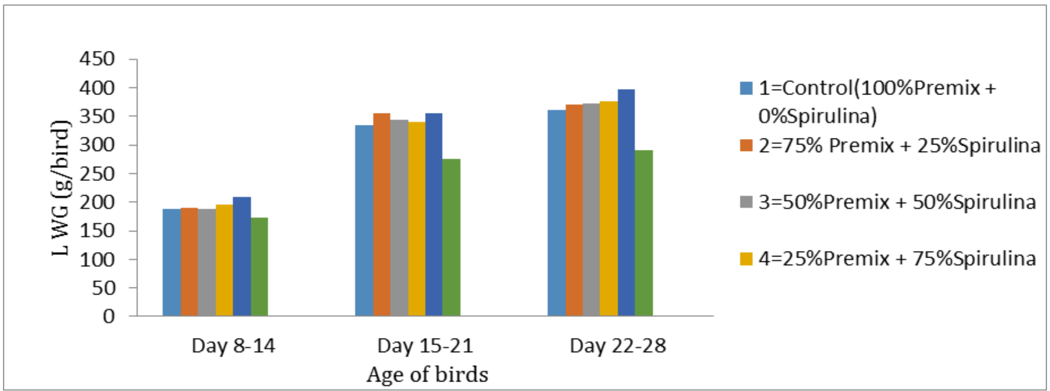

Figure 2. Live weight gain (LWG) of birds at different age.

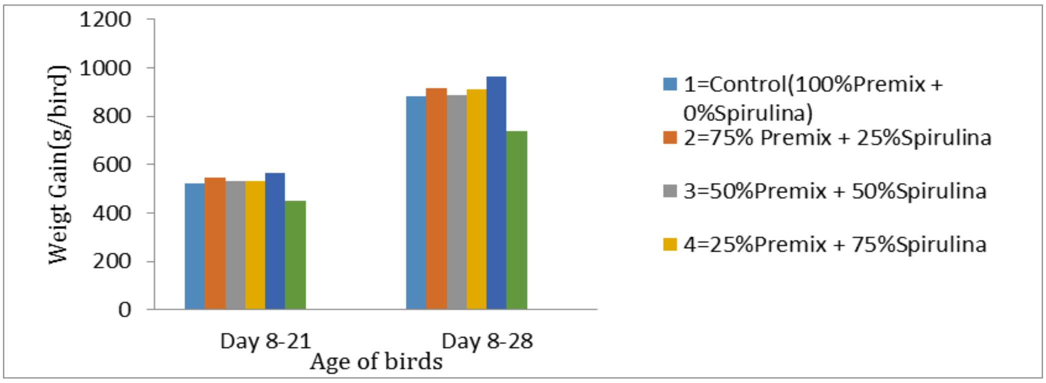

Figure 3. Cumulative weight gains of birds at different age.

Table 10. Feed intake of birds at different age.

Groups Premix *Spirulina*	1 100% 0	2 75% 25%	3 50% 50%	4 25% 75%	5 0% 100%	6 0% 0%	Level of Sign.
Day 8-14	*340^{c} ±00	305^{b} ±05	307^{b} ±06	315^{b} ±26	317^{b} ±01	256^{a} ±14	5%
Relative to 1	100	90	90	93	93	75	
Day 15-21	420^{a} ±00	421^{a} ±21	437^{a} ±34	427^{a} ±25	447^{a} ±44	453^{a} ±08	NS
Relative to 1	100	100	104	102	106	108	
Day 22-28	754^{ab} ±06	779^{b} ±29	733^{ab} ±18	722^{a} ±09	773^{b} ±44	762^{ab} ±18	5%
Relative to 1	100	103	97	96	103	101	
Cumulative feed intake							
Day 8-21	*760^{a} ± 00	726^{a} ±16	744^{a} ±32	742^{a} ±37	764^{a} ±43	709^{a} ±13	NS
Relative to 1	100	96	98	98	100	93	
Day 8-28	1514^{bc} ±06	1505^{abc} ±36	1478^{ab} ±39	1464^{a} ±29	1537^{c} ±38	1471^{ab} ±21	5%
Relative to 1	100	97	96	97	100	97	

N.B: 1-Control (100% Premix + 0% Spirulina); 2-75% Premix + 25 % Spirulina; 3-50% Premix + 50% Spirulina; 4-25% Premix + 75% Spirulina; 5-0% Premix + 100% Spirulina ; 6-0% Premix + 0% Spirulina
*Mean ± SD; ^{abc} Means with dissimilar superscripts are significantly different ($p<0.05$); NS-Not Significant
In the present study, feed intake of broilers in different groups was significantly increased. This is might be due to in case of low energy because feed intake was more to recover the energy requirements.

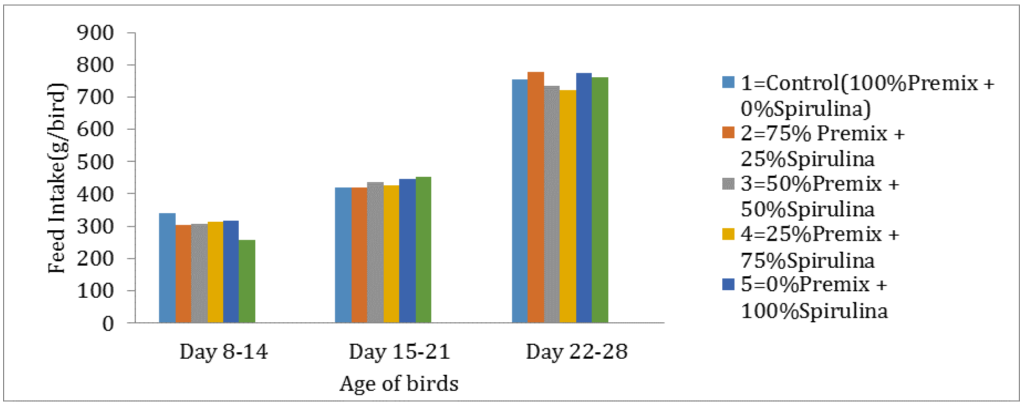

Figure 4. Feed intake of birds at different age.

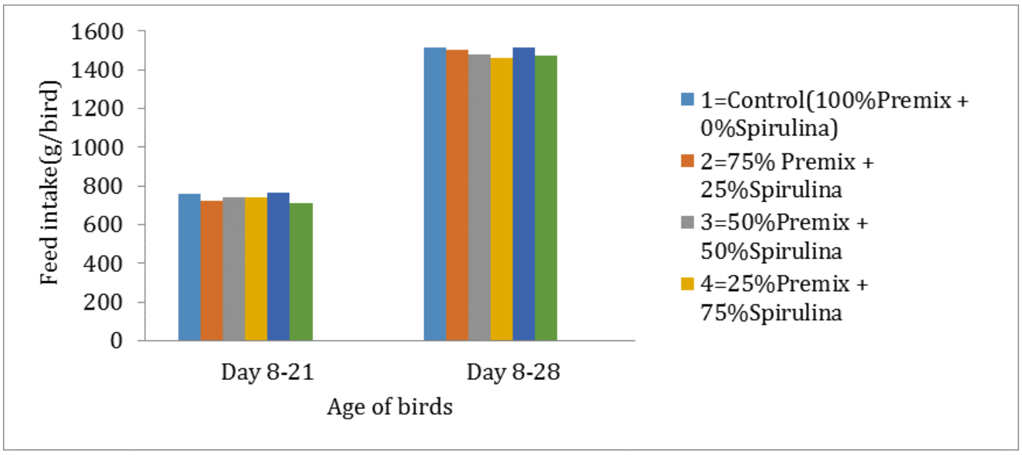

Figure 5. Cumulative feed intake of birds at different age.

Feed Conversion Ratio (FCR)

The feed conversion ratio (FCR) of broilers receiving different dietary treatments is shown in Table 11. The feed conversion ratio (FCR) of broilers were not significant (p>0.05) at day 8-14. At day 15-21, FCR of group 1, 2, 3, 4 and 5 were not significantly different (p>0.05) among them but had a significant difference (p<0.05) with group 6 [18]. Again, there was no significant difference (p>0.05) of FCR in any group at day 22-28.

The highest feed conversion ratio was observed in all groups (p<0.05) at day 22-28. At the end of trial numerically highest FCR was attained in group 6 (1.99). Cumulative FCR was significantly different at day 8-21 and 8-28. Best feed conversion ratio was observed in group 6 (1.99) compared to control (1.72), group 2 (1.65), group 3 (1.69), group 4 (1.61) and group 5 (1.62). As mentioned above, the feed conversion ratio is related to feed intake and live weight gain in all groups of birds [16]. In group 1, 2, 3, 4 and 5, birds received the proper nutrients available in their diet. So, the feed intake of these groups is good and the FCR was not significantly different (p>0.05). But in group 6, birds were deficient in their needed vitamins and minerals, so their feed intake was more to recover the nutrient requirements [8]. So that, their FCR was higher than another group though there was no significant difference among them (p>0.05).

Table 11. FCR of birds at different age.

Groups Premix *Spirulina*	1 100% 0	2 75% 25%	3 50% 50%	4 25% 75%	5 0% 100%	6 0% 0%	Level of Sign.
Day 8-14	*1.32^{a} ± 0.07	1.38^{a} ±0.19	1.46^{a} ±0.19	1.38^{a} ±0.06	1.39^{a} ±0.14	1.48^{a} ±0.08	NS
Relative to 1	100	99	104	99	99	82	
Day 15-21	1.50^{ab} ±0.05	1.46^{ab} ±0.07	1.50^{ab} ±0.17	1.48^{ab} ±0.27	1.53^{ab} ±0.08	1.68^{b} ±0.28	5%
Relative to 1	100	102	105	99	102	136	
Day 22-28	2.10^{a} ± 0.19	2.14^{a} ±0.35	2.09^{a} ±0.59	1.92^{a} ±0.11	1.96^{a} ±0.41	2.40^{a} ±0.10	NS
Relative to 1	100	88	86	79	80	114	
Cumulative FCR							
Day 8-21	*1.45^{ab} ± 0.01	1.47^{ab} ±0.07	1.54^{ab} ±0.12	1.43^{a} ±0.02	1.45^{ab} ±0.05	1.59^{b} ±0.12	5%
Relative to 1	100	102	107	100	101	111	
Day 8-28	1.72^{a} ± 0.07	1.65^{a} ±0.14	1.69^{a} ±0.28	1.61^{a} ±0.03	1.62^{a} ±0.41	1.99^{b} ±0.07	5%
Relative to 1	100	92	94	90	91	116	

N.B: 1-Control (100% Premix + 0% Spirulina); 2-75% Premix + 25 % Spirulina; 3-50% Premix + 50% Spirulina; 4-25% Premix + 75% Spirulina; 5-0% Premix + 100% Spirulina; 6-0% Premix + 0% Spirulina
*Mean ± SD; ^{abc} Means with dissimilar superscripts are significantly different (p<0.05) ; NS-Not Significant

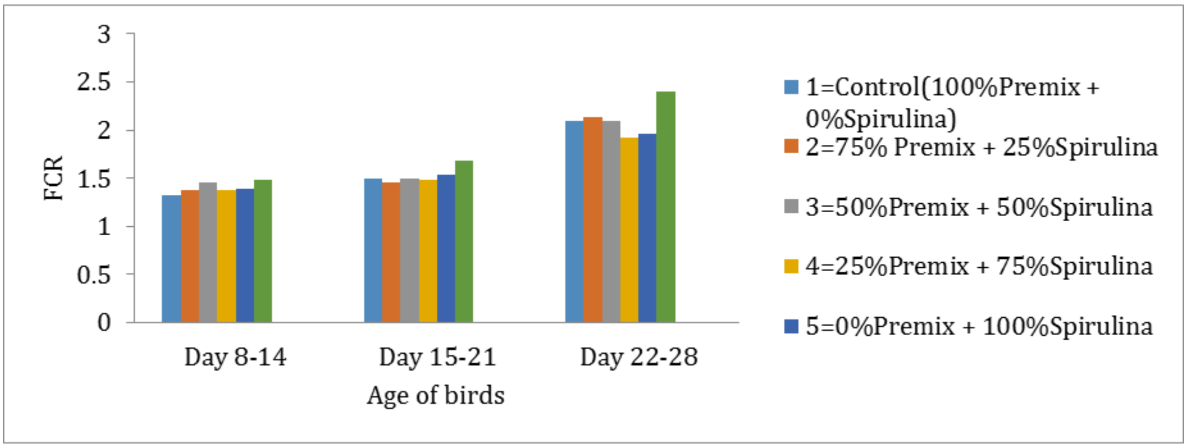

Figure 6. FCR of birds at different age.

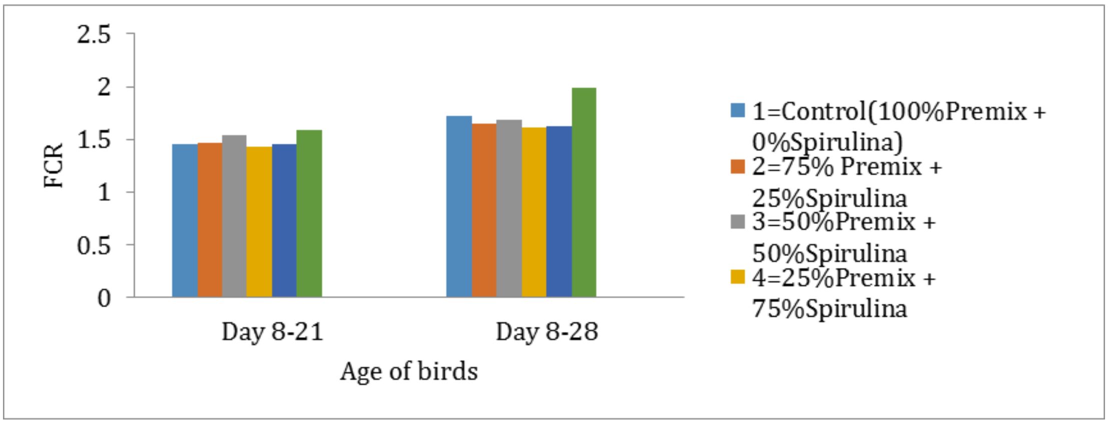

Figure 7. Cumulative FCR of birds at different age.

Bone Ash (%) content

After the experiment, the shank bone from every group of the bird was taken for determining %ash of the bones. In this experiment, the %ash of bone is gradually increased in group 1 (46.35), 2 (46.54), 3 (48.93), 4 (49.93) and 5 (55.07). But in group 6, there is an average percentage of ash (46.92) in the bone because of deficiency of vitamin and mineral in their diet. Ash% is higher in group 5 (55.07) and lower in group 1 (46.35). So, it can be said that with increasing the level of Spirulina in broiler diet, ash% was increased and the best result was obtained when supply the 100% Spirulina [4].

Blood analysis

The plasma of birds from different dietary groups showed that they had significant differences ($p<0.05$) among them. Blood cholesterol was higher (42) in group 5 and triglycerides level was higher (128) in group 1 in this experiment. Other groups of birds from group 2, 3, 4 were not shown any significant differences ($p>0.05$) among them. But group 1, 5 and 6 had significant differences among them [5]. Cholesterol level was gradually increased from group 1(36) to group 5 (42) and lower in group 6 (26). In case of triglycerides, the highest level (128) in group 1 and lowest level (49) in group 6. There were no significant differences ($p>0.05$) between group 2 and 3. There were great significant differences ($p<0.05$) among the group 1, 4, 5 and 6 [7].

From Table 12, it can be shown that both the level of cholesterol and triglycerides of blood were lower in group 6; 26 and 49 respectively. So, it could be assumed that Spirulina can provide an adequate amount of cholesterol and triglycerides in the blood of the birds in the Spirulina treated groups [19].

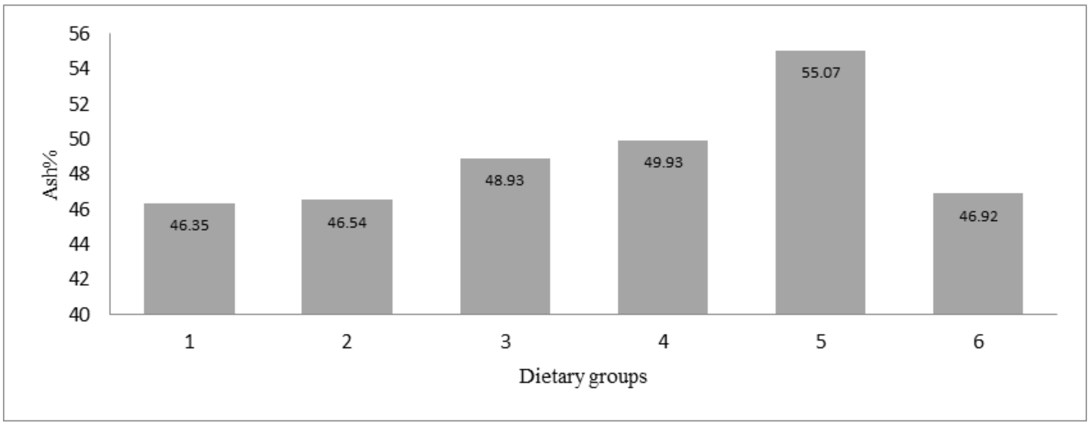

Figure 8. Ash% content of bone.

Table 12. Cholesterol and triglycerides in blood of birds of different groups

Groups	1	2	3	4	5	6	Level of Sign.
Cholesterol	*36[b] ±03	38[ab] ±04	37[ab] ±01	39[ab] ±01	42[a] ±01	26[c] ±03	5%
Relative to 1	100	110	105	111	118	73	
Triglycerides	128[a] ±26	104[bc] ±12	92[bc] ±10	83[c] ±09	112[b] ±12	49[d] ±09	5%
Relative to 1	100	81	71	83	87	38	

N.B: 1-Control (100% premix + 0% Spirulina); **2**-75% premix + 25 % Spirulina; **3**-50% premix + 50% Spirulina; **4**-25% premix + 75% Spirulina; **5**-0%premix + 100% Spirulina ; **6**-0% premix + 0% Spirulina
*Mean ± SD; [abc] Means with dissimilar superscripts are significantly different ($p<0.05$)**; NS**-Not Significant.

Table 13. Production cost per kg live weight gain of broiler.

Group	1	2	3	4	5	6
FCR	1.79	1.65	1.69	1.61	1.62	2.06
Feed cost/kg	41.84	41.78	41.72	41.65	41.6	41.6
Production/kg	100	99.94	99.88	99.81	99.75	99.75

N.B: 1-Control (100% premix + 0% Spirulina); 2-75% premix + 25 % Spirulina; 3-50% premix + 50% Spirulina; 4-25% premix + 75% Spirulina; 5-0%premix + 100% Spirulina; 6-0% premix + 0% Spirulina

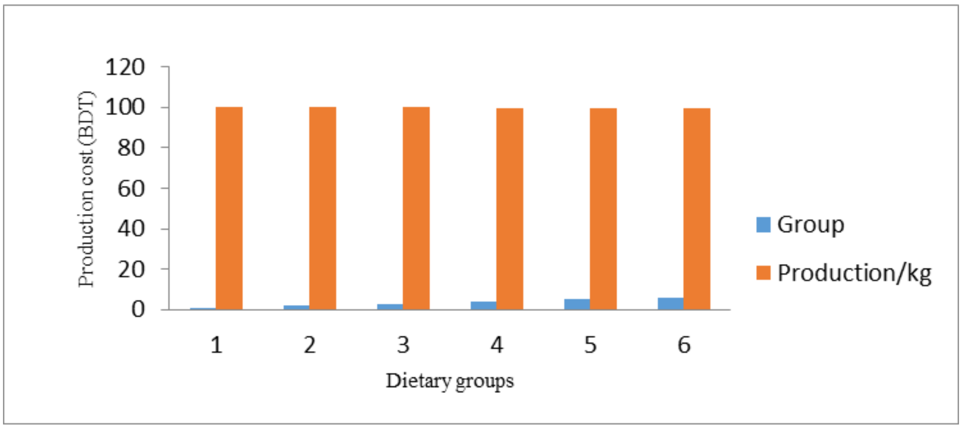

Figure 9. Production cost per kg live weight gain.

Mortality and Economic analysis of using Spirulina

No bird belongs to any group died during 28 days of feeding trial. Production cost was calculated by considering the cost of feed [19]. The cost per kilogram feed was lowest in group 5 and 6 than control. Production cost per kilogram live weight of broiler was lowest (P<0.05) in group 5 (100% Spirulina) followed by group 4 (99.81), 3 (99.88), 2 (99.94) and control (100). Chicken health can improve with low dietary Spirulina levels of 10 g/kg in the ration, indicating greater production cost efficiency [20].

From the above discussion, it is clear that the diet which contains no vitamin-mineral premix might be compensated by the addition of 100% Spirulina as an efficient alternative organic source of vitamin-mineral for the supplementation in broiler diet to produce natural organic products [21,23,24,25,]. Moreover, Spirulina enhances to increase feed intake without toxicity [26,27,28,29,30]. Further research is needed for the confirmation about of economic and feasibility to recommend in diets of different poultry species [31-33].

Conclusion

It may be concluded that diets containing no vitamin-mineral premix with 100% Spirulina improve the performance of broiler. Further research is needed for the confirmation of economic and feasibility to recommend in diets of poultry.

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