

## Effect of Different Levels of *Spirulina platensis* on Growth Performance, Intestinal Morphology, Gut Microflora, Carcass Characteristics and Some Blood Parameters in Broiler Chickens

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### Abstract

The effect of different levels of *Spirulina platensis* on growth performance, intestinal morphology, gut microflora and some blood parameters in broiler chickens was investigated in this study. A total of 300 Ross 308 broiler chicks with initial weight of  $36.28 \pm 0.38$  g were used in a completely randomized design with 5 treatments, 4 replicates and 15 broiler chicks in each replicate. Experimental diets included control diet (with no additive), 3 levels of algae (1, 1.5, 2 g/kg diet), and the birds were fed by one level of prebiotic (1g/kg diet) as a positive control from 1 to 42 day of age. Results showed that Feeding broilers with 2g/kg algae feed caused the highest weight gain compared to other experimental groups ( $P<0.05$ ). The highest feed intake related to treatments include 1.5 and 2 g/kg algae diet and the lowest related to treatment include 2g algae/ kg diet ( $P<0.05$ ). The concentration of white blood cells, IgY, IgM, Ca and Phosphate in blood serum of broilers fed with 2 g/kg alga diet was higher than other groups, however, the concentration of malondialdehyde (MDA) was lower ( $P<0.05$ ). Supplementation of diet

with alga increased the number of lactobacillus in gut ( $P<0.05$ ). Also, villus height and the ratio of villus height/crypt depth in duodenum, jejunum and ileum of broilers fed with 2g/kg algae was higher than other groups ( $P<0.05$ ). In conclusion, *S. platensis* improved growth performance, villus height, white blood cell (WBC) count and decreased MDA in serum of broiler chickens, so it can be considered as a useful additive in broiler chickens diet.

**Keywords:** Broiler chicken, Gut microflora, Intestinal morphology, *Spirulina platensis*

### Introduction

Using of antibiotic growth promoters as feed additives has been banned by the European Union in 2006 due to cross-resistance against pathogens and residues in tissues, so scientists searched for alternatives to antibiotics (Hajati et al., 2011). In this view, medicinal plants and essential oils extracted from plants are becoming more important due to their antimicrobial characteristics and the stimulating effect on animal digestive systems (Ciftci et al., 2005). Beneficial ef-

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fects of herbal additives in farm animal may be due to the positive effects on feed intake and digestive secretions, immune stimulation, antibacterial, coccidiostatical, antihelminthical, antiviral or anti-inflammatory activity (Fotea et al., 2010).

*Spirulina* (Blue green algae) is a microscopic single cell algae which grows in fresh water and has a simple structure but a complex composition. It is a concentrated source of food containing various nutrients, antioxidants, and probiotics properties. Moreover, it is an important source of the blue photosynthetic pigmented protein C-PC, which has strong antioxidant and anti-inflammatory properties. Interestingly, *Spirulina* is known for its wide ranging biological activities, like prevention of anemia because of high iron and vitamin contents (Huang et al., 2005), inhibition of herpes simplex infection (Ferrira-Hermosillo et al., 2011). It was demonstrated that the ethanolic extract of *S. platensis* include alkaloids, flavonoids, glycosides, tannins and phenolic compounds, steroids and saponins (Anbarasan et al., 2011). Kaoud (2012) conducted a trial to investigate the effects of dietary supplementations of prebiotic (Lactose and Myco) and *S. platensis* on broiler performance, carcass yield, and organs weights. The researcher reported that the body weight, average daily weight gain, feed conversion ratio (FCR), and carcass yield of birds were significantly increased by the dietary inclusion of the prebiotic and *S. platensis* ( $P < 0.05$ ) as compared to the control group. Bonos et al. (2016) investigated the effects of dietary *Spirulina* on

*growth performance*, meat oxidative stability and fatty acid profile of broiler chickens. They found that body weight gain (at 21<sup>th</sup> day and 42<sup>th</sup> day), feed conversion ratio, mortality, breast and thigh meat lipid oxidation did not differ among the groups. Also, the fatty acid profile of the thigh meat was enriched in polyunsaturated fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid after *Spirulina* supplementation. Lokapirnasari et al. (2016) examined the effect of *Spirulina* as feed additive to myocardial necrosis and leukocytes which were infected by Avian Influenza H5N1 virus. They reported that there was no significant difference ( $P > 0.05$ ) in myocardial necrosis and significant difference ( $P < 0.05$ ) in leukocyte in the treatment of *Spirulina*. The researchers conclude that *Spirulina* can be used as feed additive to increase immunity of broiler chicken (Lokapirnasari et al., 2016).

Considering the beneficial effects of dietary *S. platensis* in broilers, this study was conducted to evaluate the effect of different levels of *S. platensis* on *growth performance*, intestinal morphology, gut microflora and some blood parameters in broiler chickens.

## Materials and Methods

### *Diets and management*

This experiment was carried out using a total of 300 Ross 308 (mixed-sex) broiler chicks. One day old chicks (initial weight,  $36.28 \pm 0.38$  g) were obtained from a local hatchery and divided into 20 groups of 15 birds each. On arrival, chicks were weighed and randomly housed in wood shavings cov-

ered floor pens (each 1×1.2 m). There were 5 experimental diets including 0, 1, 1.5, 2 g/kg algae diet, and 1 g prebiotic/kg diet as a positive control. The feeding program consisted of a starter (1 to 10 day old), grower (11 to 22 day old), and finisher diet (23 to 42 day old). The basal diet was in mash form and

prepared with the same batch of ingredients for starter, grower, and finisher periods and was formulated to meet the nutrient requirements according to Ross 308 guideline. All birds had free access to feed and water during the whole rearing period. The ingredients and chemical composition of the basal diets are

**Table 1.** Ingredients and nutrient composition of basal experimental diets.

Ingredient (g/kg)	1-10 d	11-28 d	29-42 d
Corn, ground	522.4	562.8	665
Soybean meal	410.1	361.5	259.9
Soybean oil	23.5	37.7	35.7
Dicalcium phosphate	18.2	15.8	17.2
Oyster shell	12.3	11.2	11.6
Common salt	3.6	2.8	2.9
Minerals mix <sup>1</sup>	2.5	2.5	2.5
Vitamins mix <sup>2</sup>	2.5	2.5	2.5
DL-Methionine	2.8	1.8	1.7
L-Lysine hydrochloride	2.1	1.5	1.4
NaHCO <sub>3</sub>	0.0	1.0	1.0
<u>Calculated composition</u>			
ME (kcal/kg)	2880	3000	3100
CP (%)	22.5	21	18.89
Ca (%)	0.96	0.88	0.78
AP (%)	0.48	0.44	0.38
Lysine (%)	1.39	1.14	0.97
Methionine (%)	0.66	0.55	0.48
Methionine+Cystine	1.05	0.90	0.78

<sup>1</sup>Mineral mix supplied the following per kg of diet: Cu, 20 mg; Fe, 100 mg; Mn, 100 mg; Se, 0.4; Zn, 169.4 mg.

<sup>2</sup>Vitamins mix supplied the following per kg of diet: vitamin A, 18,000 IU; vitamin D<sub>3</sub>, 4,000 IU; vitamin E, 36mg; vitamin K<sub>3</sub>, 4 mg; vitamin B<sub>12</sub>, 0.03 mg; thiamine, 1.8 mg; riboflavin, 13.2 mg; pyridoxine, 6 mg; niacin, 60 mg; calcium pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.2 mg; choline chloride, 500 mg.

shown in Table 1. Feed was prepared weekly and stored in airtight containers. Temperature was initially set at 34°C on day 1 and decreased linearly by 0.5°C per day. During the study, the birds received a lighting regime of 23 hours light and 1 hour dark (23L: 1D) from 1 to 42 day.

#### *Spirulina platensis* analysis

*S. platensis* algae samples were cultivated on July of 2017 in ACECR, Sari. Briefly, *S. platensis* was grown in modified Zarrouk's medium. Algae were incubated in a pond (12 m<sup>2</sup>) with paddle-wheels at mean temperature and irradiance of 29°C, 4 klux, respectively. Harvesting was performed after 12-14 days. The composition of *S. platensis* was measured by AOAC (Association of Official Analytical Chemists) procedures (AOAC, 1990).

#### *Growth performance*

The experimental period lasted 42 day. On day 1, 28, and 42, birds were pen weighed and feed consumption was recorded. Feed conversion ratio was calculated for each period.

#### *Carcass characteristics*

At 42 day old, two birds per pen close to the mean weight for the pen were selected and killed by cervical dis-location, to determine the carcass traits. The edible carcass (without viscera or feet), breast, drumstick + thigh, liver, empty gizzard and abdominal fat were weighed and expressed as percentages of live body weight.

#### *Gut morphology*

Intestinal tissues were obtained immediately after slaughter at day 42. Segments were removed from the duodenum, jejunum and

ileum as follows. Intestine from the gizzard to pancreatic and bile ducts was referred to as the duodenum, the jejunum was defined as the portion of intestine extending from the bile duct entrance to Meckel's diverticulum and the ileum was defined as the region from Meckel's diverticulum to a point 40 mm proximal to the ileo-cecal junction. The relative length of duodenum, jejunum and ileum to 100 g live body weight was calculated. Jejunum samples (3 cm) were taken at the midpoint of each section and immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine and embedded in paraffin wax. Transverse sections were cut (3 µm), stained with hematoxyline-eosin and analyzed under a light microscope (Model U-TV0.5 XC-2, Olympus corporation, BX51, Japan) to determine morphometric indices using image-analysis software (DP2-BSW). Measurements for the villi height were taken from the tip of the villus to the villus-crypt junction.

#### *Ileal microflora*

The ileums were excised and contents were collected by gently pressing the fingers to move the content into tubes at 42 day of age. Digesta of birds within a replicate were pooled and put on ice until they were transported to the laboratory for enumeration of microbial populations. One gram of ileal content was homogenized in 9 ml sterile water. Each sample was serially diluted. Using these diluted sub-samples, *Lactobacillus* was enumerated on De Man-Rogosa-Sharpe (MRS) agar after incubation at 37°C in an anaerobic

chamber for 48 hours (Guban et al., 2006) and coli-forms and *E. coli* was counted on CHROM agar ECC (EF322-Paris France) after incubation at 37°C in an aerobic chamber for 48 hours (Sallam, 2007).

#### *Hematological Parameters*

At day 42, two birds from each replicate were selected and their blood samples were collected using sterile syringes (2 ml) to draw blood from the wing vein. Samples were collected in EDTA-containing tubes. Blood smears were prepared on slides and painted by Giemsa method. The white blood cell counts were determined by an improved Neubauer hemocytometer method (Jain, 1993). To prevent coagulation, blood samples were mixed with EDTA and centrifuged at 3000g for 10 minutes. Plasma glucose concentration was measured using commercial laboratory kits (zistshimi and parsazmoon) with god-pap method at 546 nm wavelengths. triglyceride, cholesterol, LDL-cholesterol and HDL-cholesterol were measured using commercial laboratory kits (Friedewald et al., 1972).

#### *Statistical analysis*

Data were analyzed by GLM procedures (SAS, 2001). Means were compared using Duncan's new multiple range test. The level of significance was reported at  $P < 0.05$ .

## **Results**

### *Spirulina platensis analysis*

The chemical composition (crude fat, crude protein, total carbohydrate, fiber, calcium, total phosphorus and ash) of the algae is shown in Table 2.

#### *Growth performance*

The effects of dietary supplementation of *S. platensis* on feed intake, body weight gain, feed conversion ratio, mortality and European production efficiency factor (EPEF) are shown in Table 3. In starter period, feed intake and body weight gain of broiler chicks fed with diet supplemented with 2g/kg *Spirulina* diet or prebiotic was higher than control group ( $P < 0.05$ ), however, feed conversion ratio of the chicks consumed 2g/kg *Spirulina* diet or prebiotic was lower than control group ( $P < 0.05$ ). In grower period, the body weight gain of broiler chicken fed with 1.5 or 2 g/kg *Spirulina* diet was higher than other groups ( $P < 0.05$ ). In finisher period and also whole period of rearing, broiler chicken fed with 2g/kg *Spirulina* or prebiotic had higher feed intake and body weight gain, however, their feed conversion ratio was lower than control group ( $P < 0.05$ ). Broiler chickens fed with 1.5 or 2g/kg *Spirulina* diet or prebiotic had lower mortality and higher European production efficiency factor in whole period of rearing.

#### *Carcass characteristics*

The effects of dietary supplementation of *S. platensis* on carcass characteristics are shown in Table 4. Supplement in diets with different

**Table 2.** Composition of the *Spirulina platensis* analyzed by AOAC methods.

Crude Protein (%)	Crude Fat (%)	Calcium (%)	Phosphorus (%)	Ash (%)
74	1.73	1.02	1.41	12.51

**Table 3.** Effects of *Spirulina platensis* on growth performance of broilers at different periods.

Performance	Control	<i>Spirulina platensis</i> (g/kg diet)					SEM	Pr>F
		1	1.5	2	Prebiotic			
<b><u>1 to 10 day old</u></b>								
FI (g)	218.35 <sup>b</sup>	224.11 <sup>ab</sup>	240.21 <sup>a</sup>	242.87 <sup>a</sup>	220.57 <sup>ab</sup>	7.75	0.03	
BWG (g)	200.03 <sup>d</sup>	203.20 <sup>c</sup>	209.63 <sup>b</sup>	212.38 <sup>a</sup>	202.91 <sup>c</sup>	0.987	0.041	
FCR (g/g)	1.31 <sup>a</sup>	1.22 <sup>ab</sup>	1.17 <sup>b</sup>	1.13 <sup>c</sup>	1.29 <sup>a</sup>	0.01	0.0003	
<b><u>11 to 28 day old</u></b>								
FI (g)	1237.28 <sup>b</sup>	1240.06 <sup>b</sup>	1278.75 <sup>ab</sup>	1317.83 <sup>a</sup>	1234.38 <sup>b</sup>	12.46	0.02	
BWG (g)	1151.5 <sup>d</sup>	1171.5 <sup>c</sup>	1238.25 <sup>b</sup>	1281.75 <sup>a</sup>	1167.75 <sup>cd</sup>	11.64	<0.0001	
FCR (g/g)	1.53 <sup>b</sup>	1.44 <sup>ab</sup>	1.40 <sup>bc</sup>	1.45 <sup>c</sup>	1.58 <sup>a</sup>	0.013	0.014	
<b><u>29 to 42 day old</u></b>								
FI (g)	3790.78 <sup>b</sup>	3844.17 <sup>b</sup>	3918.96 <sup>a</sup>	3960.7 <sup>a</sup>	3834.95 <sup>b</sup>	17.00	0.001	
BWG (g)	1375.90 <sup>c</sup>	1303.40 <sup>c</sup>	1441.05 <sup>b</sup>	1488.71 <sup>a</sup>	1375.76 <sup>c</sup>	10.69	<0.05	
FCR (g/g)	2.09 <sup>a</sup>	2.06 <sup>a</sup>	1.99 <sup>b</sup>	1.92 <sup>c</sup>	2.08 <sup>a</sup>	0.017	<0.05	
<b><u>1 to 42 day old</u></b>								
FI (g)	3790.78 <sup>b</sup>	3844.17 <sup>b</sup>	3918.96 <sup>a</sup>	3960.7 <sup>a</sup>	3834.95 <sup>b</sup>	17.00	0.001	
BWG (g)	2237.19 <sup>c</sup>	2266.25 <sup>c</sup>	2375.19 <sup>b</sup>	2468.06 <sup>a</sup>	2243.03 <sup>c</sup>	21.4	0.0011	
FCR (g/g)	1.83 <sup>a</sup>	1.83 <sup>a</sup>	1.78 <sup>b</sup>	1.73 <sup>c</sup>	1.84 <sup>a</sup>	0.01	<0.0001	
Mortality (%)	4.00 <sup>a</sup>	3.25 <sup>b</sup>	3.25 <sup>b</sup>	2.25 <sup>c</sup>	3.75 <sup>ab</sup>	0.4	0.05	
EPEF <sup>*</sup>	279.4 <sup>c</sup>	285.3 <sup>ab</sup>	307.4 <sup>b</sup>	332.0 <sup>a</sup>	279.3 <sup>c</sup>	4.11	0.002	

Means within the same row with uncommon superscript differ significantly ( $P < 0.05$ ).

\* European production efficiency factor

levels of *Spirulina* or prebiotic caused higher carcass yield at day 42 ( $P < 0.05$ ), however, breast (%), drumstick + Thigh (%) and abdominal fat pad (%) did not show significant differences among the groups ( $P > 0.05$ ).

#### *Gut Gut morphology*

The effects of dietary supplementation of *S. platensis* on gut morphology of broiler chicken are shown in Table 5. The villus height of duodenum in broiler chicken fed with 2g/kg *Spirulina* diet or prebiotic was higher than control group ( $P < 0.05$ ). The crypt depth and villus: crypt of duodenum in broilers fed with 1.5 or 2g/kg *Spirulina* diet or prebiotic was

lower and higher than control group, respectively ( $P < 0.05$ ). The villus height of duodenum in broiler chicken fed with 1.5, 2g *Spirulina*/kg diet or prebiotic was higher than control group ( $P < 0.05$ ). The crypt depth of broilers consumed different levels of *Spirulina* or prebiotic was lower than control group ( $P < 0.05$ ). The villus: crypt of duodenum in broilers fed with 1.5 or 2g/kg *Spirulina* diet or prebiotic was higher than control group, respectively ( $P < 0.05$ ). The villus height of ileum in broiler chicken fed with different levels of *Spirulina* was higher than control group ( $P < 0.05$ ).

#### *Ileal microflora*

As shown in Table 6, dietary supplementation of different levels of *S. platensis* or prebiotic decreased coliforms count of ileum content

( $P < 0.05$ ), however, the count of ileal *Lactobacillus subtilis* increased in broilers fed with different levels of *S. platensis* or prebiotic

**Table 4.** Effects of *Spirulina platensis* on carcass characteristics of broilers.

	Control	<i>Spirulina platensis</i> (g/kg diet)				SEM	Pr>F
		1	1.5	2	prebiotic		
<u>42 day</u>							
Carcass (%)	62.02 <sup>c</sup>	65.93 <sup>a</sup>	65.81 <sup>a</sup>	65.88 <sup>a</sup>	64.11 <sup>b</sup>	0.34	0.04
Breast (%)	24.97	24.91	24.9	24.91	24.94	0.016	0.67
Drumstick + Thigh (%)	15.84	15.71	15.78	15.78	15.80	0.023	0.63
Abdominal fat (%)	1.32	1.30	1.29	1.28	1.28	0.006	0.24

Means within the same row with uncommon superscript differ significantly ( $P < 0.05$ ).

**Table 5.** Effects of *Spirulina platensis* on gut characteristics of broilers at 42 day.

	Control	<i>Spirulina platensis</i> (g/kg diet)				SEM	Pr>F
		1	1.5	2	prebiotic		
<u>42 day old</u>							
<u>Duodenum</u>							
<u>Morphology (µm)</u>							
Villus height	1857.61 <sup>b</sup>	1867.5 <sup>b</sup>	1995.75 <sup>a</sup>	2007.5 <sup>a</sup>	1861.5 <sup>b</sup>	17.93	0.0006
Crypt depth	199.37 <sup>a</sup>	185.52 <sup>bc</sup>	178.5 <sup>c</sup>	178.25 <sup>c</sup>	191.6 <sup>ab</sup>	2.23	0.0025
Villus: crypt	9.34 <sup>c</sup>	10.07 <sup>b</sup>	11.186 <sup>a</sup>	11.286 <sup>a</sup>	9.73 <sup>bc</sup>	0.18	<0.0001
<u>Jejunum Morphology</u>							
<u>(µm)</u>							
Villus height	1748.35 <sup>c</sup>	1833.25 <sup>b</sup>	1838.5 <sup>b</sup>	1927.5 <sup>a</sup>	1767.03 <sup>c</sup>	15.54	0.0001
Crypt depth	194.45 <sup>a</sup>	181.5 <sup>c</sup>	173.00 <sup>d</sup>	170.75 <sup>d</sup>	187.25 <sup>b</sup>	1.95	<0.0001
Villus: crypt	9.09 <sup>d</sup>	10.10 <sup>c</sup>	10.63 <sup>b</sup>	11.294 <sup>a</sup>	9.34 <sup>d</sup>	0.17	<0.0001
<u>Ileum Morphology</u>							
<u>(µm)</u>							
Villus height	851.93 <sup>c</sup>	1087.97 <sup>a</sup>	1081.05 <sup>ab</sup>	931.49 <sup>bc</sup>	1043.41 <sup>ab</sup>	2.078	0.0086
Crypt depth	121.67	147.94	157.26	134.60	142.04	0.875	0.0769
Villus: crypt	7.124	7.362	6.894	7.087	7.432	0.199	0.9117

Means within the same row with uncommon superscript differ significantly ( $P < 0.05$ ).

( $P < 0.05$ ).

#### Hematological parameters

The effects of dietary supplementation of *S. platensis* on hematological parameters of broiler chicken are shown in Table 7. Adding different levels of *Spirulina* or prebiotic to broilers diet increased hematocrit and phosphorus levels in blood ( $P < 0.05$ ), but it decreased the levels of MDA, cholesterol and triglyceride in blood of broilers ( $P < 0.05$ ). The concentration of calcium, white blood cell number and IgY

titer increased in broilers fed with 1.5 and 2g/kg *Spirulina* diet or prebiotic ( $P < 0.05$ ). Broilers consumed 2g/kg *Spirulina* diet or prebiotic had higher levels of blood total protein and IgM titer ( $P < 0.05$ ).

## Discussion

### Growth performance

Today there is increasing interest for using natural feed additives such as non-digestible ingredients which are known as prebiotic or

**Table 6.** Effects of *Spirulina platensis* on ileal microbial population (log CFU/g of digesta) of broilers at 42 day

	<i>Spirulina platensis</i> (g/kg diet)					SEM	Pr>F
	Control	1	1.5	2	prebiotic		
Coliforms	7.94 <sup>a</sup>	7.21 <sup>b</sup>	7.11 <sup>b</sup>	6.05 <sup>c</sup>	7.27 <sup>b</sup>	0.12	<0.0001
<i>Lactobacillus subtilis</i>	5.5 <sup>c</sup>	6.67 <sup>b</sup>	6.58 <sup>b</sup>	7.58 <sup>a</sup>	6.87 <sup>b</sup>	0.13	<0.0001

Means within the same row with uncommon superscript differ significantly ( $P < 0.05$ ).

**Table 7.** Effects of *Spirulina platensis* on hematological parameters in broiler chickens.

	<i>Spirulina platensis</i> (g/kg diet)					SEM	Pr>F
	Control	1	1.5	2	prebiotic		
<b>Hematology, 42 day old</b>							
WBC ( $10^3$ /ml)	153.11 <sup>d</sup>	154.22 <sup>c</sup>	156.18 <sup>b</sup>	159.18 <sup>a</sup>	153.1 <sup>d</sup>	0.52	<0.0001
RBC ( $10^6$ /ml)	2.17	2.30	2.35	2.34	2.32	0.02	0.23
Hematocrit (%)	29.2 <sup>c</sup>	30.37 <sup>c</sup>	33.41 <sup>b</sup>	35.42 <sup>a</sup>	30.22 <sup>d</sup>	0.53	<0.0001
MDA ( $\mu$ m/L)	1.72 <sup>a</sup>	0.915 <sup>c</sup>	0.895 <sup>c</sup>	0.892 <sup>c</sup>	1.14 <sup>b</sup>	0.07	<0.0001
Total protein	36.69 <sup>c</sup>	37.75 <sup>bc</sup>	41.56 <sup>a</sup>	39.47 <sup>b</sup>	37.5 <sup>bc</sup>	0.48	0.0011
Cholesterol	184 <sup>a</sup>	127.25 <sup>b</sup>	119.5 <sup>b</sup>	120.37 <sup>b</sup>	126.5 <sup>b</sup>	5.81	<0.0001
TG	46.65 <sup>a</sup>	38.00 <sup>b</sup>	26.78 <sup>c</sup>	26.04 <sup>c</sup>	37.75 <sup>b</sup>	1.8	<0.0001
Glucose	166.72	168.78	165.29	166.00	167.02	0.78	0.74
Calcium	2.64 <sup>c</sup>	2.95 <sup>b</sup>	3.05 <sup>a</sup>	3.107 <sup>a</sup>	2.60 <sup>c</sup>	0.05	<0.0001
Phosphorus	2.6 <sup>d</sup>	2.82 <sup>b</sup>	3.11 <sup>a</sup>	3.02 <sup>a</sup>	2.71 <sup>c</sup>	0.04	<0.0001
IgY	0.195 <sup>c</sup>	0.230 <sup>b</sup>	0.245 <sup>ab</sup>	0.225 <sup>a</sup>	0.207 <sup>c</sup>	0.006	0.0001
IgM	0.545 <sup>b</sup>	0.552 <sup>b</sup>	0.585 <sup>a</sup>	0.6 <sup>a</sup>	0.517 <sup>c</sup>	0.007	<0.0001

Means within the same row with uncommon superscript differ significantly ( $P < 0.05$ ).

phytobiotics as growth promoters. Indeed, using antibiotics as growth promoters had been banned in 1999 by the European Union (European Commission) because of their detrimental effects such as microbial resistance to antibiotics, residues in chicken meat which might be harmful to human health and expansion of pathogenic microorganisms (Kaoud, 2015). In the present study, supplemental algae improved feed intake, body weight gain and feed conversion ratio of broilers. This is in contrast with the findings of Gognet et al. (2001) and Toyomizu et al. (2001). They did not find any significant effect of adding dietary *Spirulina* in the performance of broilers. However, Shanmugapriya et al. (2015) reported improvement of body weight gain and feed conversion ratio in broilers consumed *Spirulina* algae. Also, Mariey et al. (2014) reported that a low dietary level of *Spirulina* biomass (0.02 or 0.03%) improved performance of broiler chickens.

#### *Carcass characteristics*

Present experiment showed that *Spirulina* supplementation improved the carcass yield of broiler chickens. Raach-Moujahed et al. (2011) reported that *Spirulina* improved the carcass yield of Arbor Acres broiler at a rate of 2.5% of incorporation. Bellof and Alarcon (2013) reported that in organic farming, adding dietary *Spirulina* improved carcass performance parameters of broilers significantly.

#### *Gut morphology*

The critical role of villi height in the absorption of intestinal nutrients has been reported by Mekbungwan et al. (2002). Furbeyre et al. (2017) reported that *Spirulina* increased villus height of jejunum in weaned piglet.

Also, Shanmugapriya et al. (2015) reported increased body weight gain and villus length in addition to fatty acid modification in broiler meat (Shanmugapriya et al., 2015). Present study revealed that dietary supplementation of *Spirulina* increased villus height in all segments of small intestine (duodenum, jejunum, ileum), which can increase nutrient uptake and cause higher digestibility of nutrients. So higher body weight gain in broilers consumed *Spirulina* may due to its positive effect on gut morphology.

#### *Ileal microflora*

Present study showed that *Spirulina* algae had positive effect on ileal micro flora. Previous researchers found that feeding dietary *Spirulina* may increase the Lactobacillus population and enhance the absorbance of dietary vitamins (Mariey et al., 2014).

*Spirulina* is one of the most important micro algae showing antimicrobial activity against many pathogenic bacteria and fungi (Kumar et al., 2013) and it contains active ingredients such as tocopherols, C-phycoerythrin, and extracellular polysaccharide (Pradhan et al., 2012; Ciftci et al., 2005) which have antimicrobial activities against *Escherichia coli*, *Pseudomonas* sp., *Enterobacter* sp., *Salmonella typhi*, *Klebsiella pneumoniae* and *Proteus vulgaris* (Pradhan et al., 2012). *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Aeromonas liquefaciens* (Shanmugapriya et al., 2015).

#### *Hematological Parameters*

This study showed that supplementing *Spirulina* in broiler diet increased RBC and hematocrit of birds. Zhang et al. (2001) found that

the polysaccharides of *Spirulina* increased the level of red blood cells, white blood cells, and hemoglobin in the blood, and also increased nucleated cells in bone marrow of dogs. They reported that Polysaccharide extract of *S. platensis* has chemo-protective and radio-protective capability, and may be a potential adjunct to cancer therapy (Zhang et al., 2001). It is reported that using phycocyanin after oxalate treatment significantly increased catalase and glucose-6-phosphate dehydrogenase activity ( $p < 0.001$ ) in RBC lysate suggesting phycocyanin as a free radical quencher (Farooq et al., 2006). So, the reduction in MDA concentration in this trial may due to free radical quenching effect of *Spirulina*. Huang et al. (2005) reported that *Spirulina* polysaccharides decreased blood glucose and could protect the vascular of alloxan induced diabetic rats. This is in contrast with the findings of the present study as we don't see any different in blood glucose concentration. A reduction in serum cholesterol and increasing in IgG observed in the current study is in agreement with Zeweil et al. (2016). The antioxidant materials such as phycocyanin and phenolic compounds, and poly-unsaturated fatty acids in the microalgae *Spirulina* may be the cause of the properties of *Spirulina* on the decrease of serum lipids levels (Colla et al., 2008). Nagaoka et al. (2005) reported that *S. platensis* concentrates or phycocyanin, a pigment extracted of *Spirulina*, caused hypocholesterolemic activity in rats. In conclusion, *Spirulina* improved growth performance, intestinal villus height, ileum Lactobacillus count, carcass yield and humoral immunity of broiler chickens. We rec-

ommend the addition of 2g *Spirulina*/Kg diet to improve performance and EPEF of broiler chickens. It seems that further study is needed to clarify the exact effect of *Spirulina* on physiological mechanisms in broilers body.

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