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Effects of *Spirulina platensis* on Growth Performance, Carcass Characteristics, Egg Traits and Immunity Response of Japanese Quails

Research Article

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ABSTRACT

In present study, two trials had been conducted to evaluate the effects of *Spirulina platensis* (SP) on growth performance, carcass characteristics, egg quantity and quality traits, and immunity response of Japanese quails. In trial 1, a total number of 180 one-day-old Japanese quails were randomly assigned into 6 groups, 3 replicates with 10 quail chicks per replicate. Experimental diets including basal diet (with no additive), diet contained probiotic, and diets contained 4 levels of *SP* (2.5, 5, 10, 20 g/kg diet). In trial 2, a total number of 250 Japanese laying quails were randomly assigned into 5 groups, 5 replicates with 10 laying quails per replicate. Experimental diets included of basal diet (no additive) and diets supplemented with three levels of SP (1, 3 or 5 g/kg diet). In experiment 1, using 5 g SP/kg diet caused higher body weight gain and European production efficiency factor during 1-35 d of age ($P<0.05$). Using SP at the levels of 2.5 or 5 g/kg diet increased breast percentage ($P<0.05$). In experiment 2, using different levels of SP decreased shell thickness, albumen height, haugh unit and yolk height in laying quails ($P<0.05$). However, Feeding different levels of SP increased ($P< 0.05$) egg yolk color compared to control group linearly. Dietary supplementation of SP at levels of 3 and 5 g/kg diet decreased cholesterol level per g yolk ($P<0.05$). Different levels of SP caused higher levels of total antibody against sheep red blood cells (SRBC) and IgG titers ($P<0.05$). Laying quails fed with 3 or 5 g SP/kg showed higher cutaneous basophil hypersensitivity after 12 or 24 h of phytohemagglutinin injection ($P<0.05$). In conclusion, we recommend using SP at the levels of 5 and 3 g/kg diet during growth and laying period of Japanese quails, respectively.

KEY WORDS egg quality, immunity, quail, *Spirulina platensis*.

INTRODUCTION

Nowadays, the incidence of diseases such as cancers, obesity, arteriosclerosis, infectious diseases, etc. are relatively high and poultry nutritionists pay much attention to use organic functional feed stuffs in poultry diet to improve poultry meat and egg consumers' health state. *Spirulina platensis* (SP) is a blue-green microalgae or cyanobacteria from the Cyanophyceae family (Degnechew and Buzayehu, 2018). It was reported that SP can be considered as probiotic due to its antimicrobial materials, positive effect on intestinal morphology and immune system (Shanmugapriya *et al*. 2015). *Spirulina platensis* possess high amount of protein (Lindberg *et al*. 2016), vitamins, minerals, carotene and xanthophylls phyropigments (Gutiérrez-Salmeán *et al*. 2015), gamma linoleic acid, phycocyanins, phenolic acids and chlorophyll (Mariey *et al*. 2012). American food and drug administration and European food safety authority consider SP as generally recognized as safe (GRAS) (Gong and Bassi, 2016). Studies have shown that SP may be one option for using in poultry diet as an alternative dietary protein source (Neumann *et al*. 2017; Altmann *et al*. 2018).

Interestingly, spirulina is known for its wide range of biological activities, like prevention of anemia because of high iron and vitamin contents (Hemalatha *et al*. 2012), inhibition of herpes simplex infection (Ferrira Hermosillo *et al*. 2011). Kanagaraju and Omprakash (2016) reported that the available energy content of spirulina is about 2.50-3.29 kcal/g and the availability of phosphorous in spirulina is about 41%. Kanagaraju and Omprakash (2016) used different levels of spirulina (0, 1, 2, 3%) in meat quails diet and found that using dietary spirulina in Japanese quails significantly improved body weight gain (BWG) and feed conversion ratio (FCR). Bonos *et al*. (2016) reported that spirulina could be a promising functional ingredient in broiler chicken nutrition. On the other hand, using natural colorants in laying hens diets is preferable compare to synthetic colorant that are more expensive and may have detrimental effect on consumers' health (Downham and Collins, 2000). The substances such as zeaxanthin, xanthophylls and ß-carotene that present in SP can accumulate in egg yolk and improve the yolk color (Takashi, 2003). Selim *et al*. (2018) reported that dietary supplementation of SP improved laying performance and egg quality of layer hens.

Nikodemusz *et al*. (2010) reported that farmed pheasants fed with diet contain spirulina had the best productive and reproductive performance compared with the control group. Zahroojian *et al*. (2013) found that using dietary spirulina improved egg yolk color of Hy-Line (w-36) hens. Also, improving immunity state of the birds in intensive production systems is critical, especially in regions with high incidence of infectious diseases. It was reported that phenolic compounds, sulphated polysaccharides and phycocyanin content of *SP* have immunomodulatory and anti-viral activities (Finamore *et al*. 2017). The microalgae can improve macrophage and mononuclear phagocyte system in chickens (Al-Batshan *et al*. 2001). Regard to the above back ground, the aim of the present study was to study the effect of *SP* on growth performance, carcass characteristics, egg quantity and quality traits, and immunity response of Japanese quails.

MATERIALS AND METHODS

Birds, diets, and management

Two experiments were done to evaluate the effects of *SP* in Japanese quails (*Coturnix coturnix Japonica*). In experiment 1, a total of 180 one-day-old Japanese quails (12.14±0.23 g) were used in a completely randomized design with 6 treatments, 3 replicates (10 chicks in each replicate). The birds were randomly allocated to 18 pens (40×50 cm^2) with wood shavings litter. All procedures for the use and care of animals were conducted after approval by the animal department of University of Tehran.

The main ingredients of the diets included corn, soybean meal and corn feed meal (Table 1). The diets were all made to be iso-caloric and iso-nitrogenous. Experimental diets including control diet (with no additive), positive control diet contained probiotic (0.2 g *Bacillus subtilis* spore/kg diet) and diets contained 4 levels of SP (2.5, 5, 10 or 20 g/kg diet) were fed to birds from 1 to 35 d of age. The initial temperature of birds' house was set on 36 ˚C and gradually decreased to 22 ˚C on d 35. The birds had 24 hours' light.

In experiment 2, a total of 250 Japanese laying quail (49 days old) with 83.84 percent egg production and 260.73 g body weight were used in a completely randomized design with 5 treatments, 5 replicates (10 quails in each replicate).

The birds were randomly allocated to 25 commercial cages. The relative humidity and average temperatures were maintained in 18-20 ˚C and 50-60%, respectively. Experimental diets including control diet (with no additive), positive control diet contained probiotic (0.3 g *Bacillus licheniformis*/kg diet) and diets contained 3 levels of SP (1, 3 or 5 g/kg diet) were fed to birds. The diets were formulated to meet the nutrient requirements of the quail as recommended by NRC (1994) (Table 1). The experiment lasted 12 weeks. The birds had 16 hours of light:8 hours of dark.

Spirulina platensis

Spirulina platensis algae samples were cultivated on July, 2017. Briefly, SP was grown in modified Zarrouk's medium (Figure 1). Algae were incubated in a pond (12 m^2) with paddle-wheels at mean temperature and irradiance of 29 ˚C, 4Klux, respectively. Harvesting was performed after 12-14 days. Ash, crude protein, crude fat, calcium, and phosphorus content of SP were measured by AOAC procedures (AOAC, 1990). Total phenol content of *SP* was determined based on previous researches (Assis *et al*. 2014). Three grams of SP was mixed with 75 mL of methanol and shook at 35 ˚C for 120 min at 230 rpm. Then centrifuged at 3200 g for 15 min, supernatant was evaporated by rotary and the residue was dissolved in 50 mL of distilled water. For separating non-phenolic compounds, $Ba(OH)_2$ and $ZnSO_4$ were used. The extract was filtered and phenolic content of SP measured by Folin-Ciocalteu reagent and spectrophotometry at 765 nm. Gallic acid (0-500 mg/L) was used for standard calibration curve and phenolic content was determined as mg of gallic acid equivalent per gram dry weight of SP (n=4).

Growth performance

In experiment 1, feed intake (FI) was measured weekly by subtracting the left-over feed from the quantity originally supplied to the animals. Body weight of quails was recorded at the end of each week.

Table 1 Ingredients and nutrient composition of basal diets

Ingredients (%)	Experiment 1 Starting and growing (1-35 d)	Experiment 2 Layer (8-20 weeks)
Corn	47.00	57.46
Soybean meal	43.5	0.00
Soy peptide	0.00	34.87
Corn feed meal	3.00	0.00
Vegetable oil	2.7	0.00
Oyster shell	1.2	5.60
Dicalcium phosphate	1.5	0.00
Mono calcium phosphate	0.00	1.10
Common salt	0.30	0.35
L-threonine	0.05	0.00
DL-methionine	0.25	0.12
Vitamin and mineral premix[1]	0.50	0.5
Calculated contents (%)		
Metabolizable energy (ME) (kcal/kg)	2925	2900
Crude protein	24	20
Calcium	0.95	2.52
Available phosphorus	0.44	0.36
Sodium	0.19	0.15
Lysine	1.31	1.15
Methionine + cystine	0.68	0.8

[1] Mineral premix supplied the followings per kilogram of diet: vitamin A: 10000 IU; vitamin D_3: 3000 IU; vitamin E: 20 IU; vitamin K_3: 2 mg; Thiamin: 2 mg; Pyridoxine hydrochloride: 4 mg; Cobalamin: 0.06 mg; Calcium-D-pantothenate: 20 mg; Nicotinic acid: 50 mg; Folic acid: 1 mg; Riboflavin: 8 mg; Biotin: 0.2 mg; Cu: 10 mg; Fe: 60 mg; Zn: 60 mg; Mn: 80 mg; Se: 0.3 mg and I: 0.2 mg.

Figure 1 *Spirulina platensis* rearing condition

Mortality was daily registered. FCR calculated by dividing the FI to body weight gain (BWG) in each pen and adjusted for mortality. At the end of the experiment, European production efficiency factor (EPEF) was calculated as bellow: [(body weight×% survival rate) / FCR × rearing period (d)] × 100.

Carcass characteristics

At the end of the trial 1, four quails from each pen with body weight close to the mean of each pen were selected for carcass analyses. Then, the birds were slaughtered by cervical dislocation and carcass yield was calculated as the ratio of carcass weight (without viscera) to live body weight.

Relative weights of breast, drumstick + thigh, liver and gizzard were calculated as [organ weight (g) / live body weight (g)] × 100.

Egg quantity and quality traits

In trial 2, hen day egg production, average egg weight, egg length, egg width, albumen weight, yolk weight, egg shell weight, shell thickness, albumen height, yolk diameter and yolk height were measured weekly. Briefly, during each week 14 eggs from each replicate were weighed individually and broken in a plate to evaluate its quality. Yolk color was determined by comparison with the DSM Ovo-color fan (Figure 2). Hen day egg production was calculated by the following formula:

Egg production (%)= [number of egg production on each day / number of hens alive on each day] × 100

The egg surface area (S), unit surface shell weight (USSW), shape index (SI), haugh unit (HU) of the eggs were calculated with the formula described by Carter (1975):

$S\ (cm) = 3.9782\ W^{0.75056}$
W= egg weight (g)
$USSW\ (g/cm^2) = W\ (mg) / S\ (cm^2)$
SI (%)= [width (cm)/height (cm)] × 100

Haugh unit was calculated with the formula described by Kul and Seker (2004):

$HU = 100 \log (\text{albumen height (mm)} + 7.57 - 1.7\ W^{0.37})$

During the last week of the experiment two, yolk cholesterol of 14 eggs from each replicate was measured by method described by Pasin *et al*. (1998).

Humoral and cellular immunity responses

Antibody response to SRBC

In experiment 2, sheep red blood cells (SRBC) were used as T-dependent antigens to quantify the antibody response. At week 10, two laying quails from each replicate were injected with SRBC (5% suspension in phosphate-buffered saline (PBS), 0.2 mL/bird) intramuscularly, followed by the second injection 7 d later. Blood samples were collected 7 d after the first and second injections. The serum of samples of the two laying quails in each pen was collected, heat inactivated at 56 ˚C for 30 min and then analyzed for total antibody, IgG (mercaptoethanol-resistant), IgM (mercaptoethanol-sensitive) anti-SRBC antibodies as described by Cheema *et al*. (2003).

Toe web swelling test

In experiment 2, the cutaneous basophilic hypersensitivity response to phytohemagglutinin P (PHA-P; Sigma Chemical Co., St. Louis, MO), as an indicator of a T-cell-induced delayed type hypersensitivity reaction, was assessed as described previously (Corrier and DeLoach, 1990). The CBH response to PHA-P was measured in 2 quails from each replicate at the end of 6 and 12 weeks of trial. Each bird received 100 µg of PHA-P in 0.1 mL of sterile PBS (0.15 M at pH=7.4), that was injected intra-dermally in inter digital skin between the second and third toes of the left foot. The right foot was injected with 0.1 mL of phosphate-buffered saline (PBS) as a sham control. The thickness of each injection site was measured using a pressure-sensitive micrometer before injection and at 12 or 24 h after injection. The cutaneous basophil hypersensitivity (CBH) response to PHA-P was calculated using the following formula (Hajati *et al*. 2018): Swelling index= [(thickness of left toe web after PHA-P injection-initial thickness of left toe web) - (thickness of right toe web after PBS injection-initial thickness of right toe web)].

Statistical analysis

Data of the two experiments were analyzed separately by analysis of variance using general linear method (GLM) procedures (SAS, 2001). Means were compared using Duncan's new multiple range test (Duncan, 1955). The level of significance was reported at $P < 0.05$. Statistical model of this experiment was as follow:

$Yij = \mu + Ti + eij$

Where:
Yij: response variable.
µ: overall mean.
Ti: fixed effect of treatment.
eij: effect of residual factors.

RESULTS AND DISCUSSION

Composition of the SP

Chemical analysis of SP is shown in Table 2. In present study, SP had 96.3% dry mater (DM), 64.86% crude protein (CP), 4.73% crude fat (CF), 1.02% calcium (Ca), 1.41% phosphorus (P), 12.51 % ash, and 10.19 mg (GAE)/g DM.

Growth performance traits

Results of the effects of SP on growth performance and European production efficiency factor of quails is shown in Table 3. Algae inclusion at the level of 20 g/kg diet significantly increased FI of quails during the first, second, third weeks and whole period of rearing (P<0.05). Also, feeding 20 g SP increased FCR during the first and second weeks of rearing (P<0.05). Using 5 g SP per kg diet increased BWG of quails during whole period of rearing (P<0.05). Feeding using 5 g *SP* per kg diet decreased FCR during third, fourth and fifth weeks of the trial (P<0.05). Quails fed with *SP* at the level of 5 g/kg diet had higher European production efficiency factor during the whole period of rearing (P<0.05).

Carcass characteristics

As shown in Table 4, using SP at the levels of 2.5 or 5 g/kg diet increased carcass yield of the birds compared with control group numerically. Consuming SP at the levels of 2.5 or 5 g/kg diet increased relative weight of breast in quails (P<0.05).

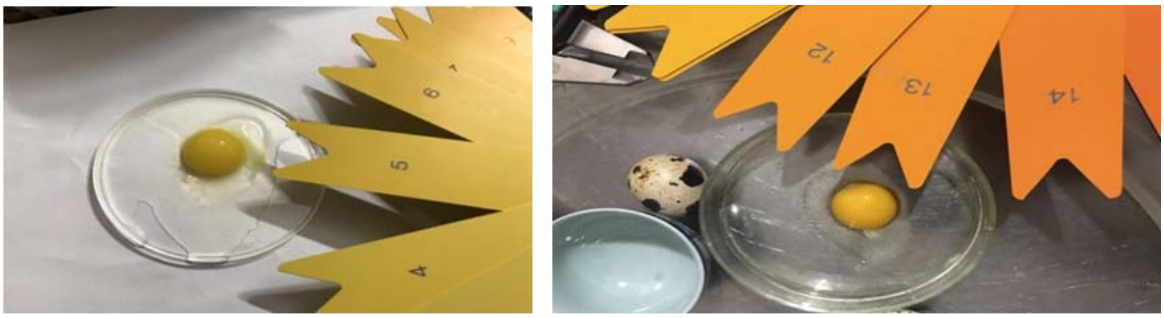

Figure 2 Evaluating yolk color with DSM yolk color fan

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

Dry matter (%)	Crude protein (%)	Crude fat (%)	Calcium (%)	Phosphorus (%)	Ash (%)	mg GAE/g SP
96.3±0.12	64.86±0.31	4.73±0.11	1.02±0.08	1.41±0.09	12.51±0.6	10.19±0.04

GAE: gallic acid equivalent.

Table 3 Effects of *Spirulina platensis* on growth performance of quails (*Coturnix coturnix Japonica)* during 1-35 d

Growth performance	Control	Probiotic	*Spirulina platensis* (g/kg diet)				SEM	P-value
			2.5	5	10	20		
1 to 7 d								
Feed intake (g)	38.16^{b}	38/50^{b}	38.33^{b}	40.10^{ab}	41.26^{ab}	43.80^{a}	0.461	0.0314
Body weight gain (g)	19.72	19.61	20.05	21.11	21.79	20.22	0.494	0.5030
Feed conversion ratio (g/g)	1.93^{b}	1.96^{b}	1.91^{b}	1.90^{b}	1.89^{b}	2.16^{a}	0.012	0.0180
8 to 14 d								
Feed intake (g)	112.33^{b}	118.4	115.83^{b}	115.4^{b}	118.0^{b}	126.06^{a}	2.485	0.028
Body weight gain (g)	46.94	48.88	49.11	49.58	42.97	45.31	0.479	0.5247
Feed conversion ratio (g/g)	2.39^{b}	2.42	2.35^{b}	2.32^{b}	2.76^{a}	2.78^{a}	0.013	0.0120
15 to 21 d								
Feed intake (g)	147.03^{b}	152^{bc}	149.93^{b}	157.43^{a}	159.26^{a}	161.6^{a}	3.580	0.0238
Body weight gain (g)	53.83	55.00	55.33	60.46	60.40	58.13	0.491	0.2169
Feed conversion ratio (g/g)	2.73^{a}	2.76^{a}	2.70^{ab}	2.60^{b}	2.63^{b}	2.77^{a}	0.018	0.0498
22 to 28 d								
Feed intake (g)	165.27	164.13	170.02	170.5	171.24	175.94	5.582	0.2911
Body weight gain (g)	45.73	47.74	45.83	48.14	46.28	47.91	0.476	0.9698
Feed conversion ratio (g/g)	3.62^{a}	3.43^{b}	3.70^{a}	3.54^{b}	3.70^{a}	3.67^{a}	0.017	0.0408
29 to 35 d								
Feed intake (g)	180.00	170.33^{b}	181.33	184.66	185.33	187.06	7.591	0. 170
Body weight gain (g)	45.22	42.48	46.43	48.73	46.20	47.69	0.470	0.8644
Feed conversion ratio (g/g)	3.98^{a}	4.00^{a}	3.90^{a}	3.78^{b}	4.01^{a}	3.92^{a}	0.027	0.0494
1 to 35 d								
Feed intake (g)	642.81^{c}	643.37^{c}	655.46^{bc}	668.10^{b}	675.11^{ab}	694.48^{a}	8.24	0.0056
Body weight gain (g)	211.45^{b}	213.72^{b}	216.76^{b}	228.03^{a}	217.65^{b}	219.26^{b}	2.33	0.0063
Feed conversion ratio (g/g)	3.039^{ab}	3.012^{ab}	3.024^{ab}	2.930^{b}	3.102^{a}	3.168^{a}	0.051	0.041
EPEF[2]	198.73^{b}	204.84^{b}	204.84^{b}	222.20^{a}	200.68^{b}	201.47^{b}	4.11	0.0029

EPEF: European production efficiency factor was calculated as:
EPEF= [(body weight×% survival rate) / (feed conversion ratio×rearing period (d))] × 100.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Feeding SP did not have any significant effect on the relative weights of drumstick + thigh, liver, and gizzard of quails on d 35 (P>0.05).

Egg quantity and quality traits

Regard to Table 5, SP did not have any significant effect on egg production, average egg weight, egg length, egg width, shape index, egg surface area, unit surface shell weight, and yolk diameter (P>0.05). At the present study, all levels of SP decreased eggshell thickness, HU, albumen height and yolk height (P<0.05). SP supplementation at the levels of 1 or 3 g/kg diet decreased egg yolk weight compared with control group (P<0.05).

Using 5 g SP per kg diet decreased albumen weight and egg shell weight (P<0.05). Different levels of SP increased egg yolk color compared with control group (P<0.05).

Table 4 Effects of *Spirulina platensis* on carcass characteristics of quails (*Coturnix coturnix Japonica)* at 35 d

Carcass traits	Control	Probiotic	*Spirulina platensis* (g/kg diet)				SEM	P-value
			2.5	5	10	20		
Carcass yield[1] (%)	67.89^{ab}	66.16^{abc}	69.06^{a}	68.33^{ab}	65.00^{b}	63.42^{bc}	1.107	0.0263
Breast[2] (%)	25.50^{b}	26.02^{b}	29.23^{a}	29.37^{a}	26.07^{b}	25.01^{b}	0.647	0.0010
Drumstick + thigh[2] (%)	19.30	18.22	17.33	17.62	18.08	17.60	0.530	0.1942
Liver[3] (%)	2.34	2.18	2.25	2.22	2.24	2.29	0.189	0.641
Gizzard[3] (%)	1.64	1.70	1.71	1.71	1.73	1.63	0.114	0.994

[1] Carcass was calculated as the ratio of carcass weight (breast+drumstick+thigh+wings+neck+back without skin and viscera) to live body weight.
[2] Relative weight of organ to carcass weight (without skin and viscera) as percentage.
[3] Relative weight of organ to carcass weight (without skin) as percentage.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of *Spirulina platensis* (SP) on egg quantity and quality traits of laying quails (*Coturnix coturnix Japonica)* during 8-20 wks of age

Treatment	Egg production (%)	Average egg weight (g)	Egg length (mm)	Egg width (mm)	Albumen weight (g)	Yolk weight (g)	Egg shell weight (g)	Shell thickness (mm)	Shape index
Control	87.41	12.21	34.08	26.50	7.16^{a}	4.45^{a}	1.61^{a}	0.366^{a}	77.84
Probiotic	87.79	12.16	33.78	26.52	6.88^{b}	3.95^{b}	1.67^{ab}	0.33^{b}	78.56
SP1[1]	87.64	12.29	33.69	26.40	6.57^{ab}	3.99^{b}	1.57^{ab}	0.306^{c}	78.40
SP3[1]	87.86	12.32	34.18	26.48	6.48^{ab}	4.07^{b}	1.55^{ab}	0.317^{b}	77.54
SP5[1]	86.83	12.28	34.03	26.38	6.18^{b}	4.51^{a}	1.51^{b}	0.303^{c}	77.55
SEM	0.766	0.033	0.260	0.281	0.147	0.136	0.0189	0.0115	0.119
P-value	0.9961	0.7896	0.3660	0.3373	0.0419	0.0466	0.048	0.0468	0.0543

Treatment	Egg surface area	Unit surface shell weight	Haugh unit	Albumen height (mm)	Yolk diameter (mm)	Yolk height (mm)	Yolk color	Yolk cholesterol (mg/g)
Control	26.02	0.470	91.94^{a}	5.04^{a}	25.56	10.10^{a}	5.53^{d}	7.74^{a}
Probiotic	26.11	0.471	92.12^{a}	4.92^{ab}	25.54	10.22^{a}	6.58^{d}	7.88^{a}
SP1	26.15	0.471	90.57^{b}	4.80^{b}	25.57	9.89^{b}	7.80^{c}	7.09^{ab}
SP3	26.20	0.472	90.73^{b}	4.83^{b}	25.79	9.92^{b}	9.45^{b}	6.56^{b}
SP5	26.13	0.471	90.09^{c}	4.71^{c}	25.48	9.71^{c}	10.69^{a}	6.70^{b}
SEM	0.151	0.017	0.159	0.030	0.52	0.048	0.140	0.167
P-value	0.2920	0.163	0.0004	0.0017	0.4097	0.0004	0.0001	0.0217

[1] SP1: 1 g SP/kg diet; SP3: 3 g SP/kg diet and SP5: 5 g SP/kg diet.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

As indicated in the Table 5, SP at the levels of 3 or 5 g/kg diet decreased cholesterol level per g yolk (P<0.05).

Antibody response to SRBC

As shown in Table 6, adding different levels of SP to laying quails increased total antibody titer against SRBC, IgG and IgM as a primary response to SRBC injection (P<0.05). Also, quails fed diet supplemented with SP had higher total antibody titer against SRBC and IgG titer as secondary response to SRBC injection (P<0.05).

Toe web swelling test

Regards to Table 6, quails fed diet supplemented with different levels of *SP* had higher cutaneous basophil hypersensitivity after 12 or 24 hours of PHA-P injection compared to control group (P<0.05).

Spirulina algae analysis

Bensehaila *et al*. (2015) stated that SP had 94.58% dry matter, 60.32% crude protein, 7.28% crude fat, 0.22 mg Ca/g SP, and 6.88% ash. Radhakrishnan *et al*. (2017) found that SP had 94.4% dry matter, 61.74% crude protein, 5.09% crude fat, and 9% ash. Gutiérrez-Salmeán *et al*. (2015) reported that SP had 63% crude protein and 4.3% crude fat. It seems that SP is an ingredient with high protein and low fat content, so lipid peroxidation occurs at relatively low rate in SP (Bensehaila *et al*. 2015). According to previous findings the amount of total phenolic content of *SP* after extraction by different solvents was in the range of 17-43.2 mg/g GAE (Machu *et al*. 2015). The reason of differences in SP content may be related to origin geography, cultivation ad harvesting situations, season, climate, condition of extraction and type of solvent (Machu *et al*. 2015).

Growth performance

In experiment 1, spirulina inclusion at the level of 20 g/kg diet increased feed intake of quails. This is in agreement with the findings of Cheong *et al*. (2015), who reported that feeding SP to quails during 15 to 35 days of age increased feed intake of the birds. In this study, we found that SP had positive effect on appetite of the quails and there was an upward trend in feed intake of the birds. Cheong *et al*. (2015) reported that dietary spirulina increased weekly weight gain of the quails, however, using this algae exceeding 4% had adverse effect on growth performance of the birds. In our study, using of 5 g SP per kg diet increased body weight gain of quails during 1-35 d of age (P<0.05). Jamil *et al*. (2015) used 2, 4, or 8 g spirulina/kg diet in broiler chickens. They found that the algae improved body weight and feed conversion ratio of broilers. Also, Shanmugapriya *et al*. (2015) reported that 1% inclusion of spirulina in broilers diet improved their growth performance. The mentioned researchers stated that spirulina increased intestine villi height, so it increased absorptive surface of broilers gut. Evans *et al*. (2015) reported that dietary algae inclusion up to 16% can decrease digesta passage rate and increase nutrient digestibility. Also, quails fed with SP at the level of 2.5 g/kg diet had higher European production efficiency factor (P<0.05). This is a critical economic index that poultry producers should notice it before starting their investments. In agreement with our result, Park *et al*. (2018) reported that SP improved European production efficiency factor (EPEF) in broilers. This can be explained by high nutrient composition and physiological function of SP that cause positive effect of SP in body metabolism related to growth performance (Park *et al*. 2018).

Carcass characteristics

At present study, using SP had not any significant effect on carcass yield of quails compared with control group. However, using *SP* at the levels of 2.5 or 5 g/kg diet increased the relative weight of breast. Also, feeding SP did not have any significant effect on relative weights of drumstick + thigh, liver and gizzard of the quails. In line with our result, Sugiharto *et al*. (2018) and Altmann *et al*. (2018) reported that using SP had not any significant effect on broilers carcass characteristics. Also, Cheong *et al*. (2015) found that SP had no effect on carcass yield and breast percentage. However, Mariey *et al*. (2014) found that SP at the levels of 0.2 or 0.3 g/kg increased dressing percentage. On the other hand, Razafindrajaona *et al*. (2008) stated that bad conditions of SP media may lead to accumulation of certain heavy metals such as lead, cadmium and mercury which can affect the carcass parameters. This may be the one reason for decreasing trend in carcass yield at higher levels of SP that was seen in this study. However, the increased relative weight of breast up to 5 g SP/kg diet may due to high nutrient content, feed efficiency and nutrient conversion to lean meat (Cheong *et al*. 2015). Our results revealed that SP had no effect on the relative weights of liver and gizzard, however, Toyomizu *et al*. (2001) observed increased liver weight in broiler chickens fed with higher level of SP (4 or 8%). The difference between results may be due to the different levels of SP used in the trials.

Egg quantity and quality traits

In experiment 2, SP did not have any significant effect on egg production, average egg weight, egg length, egg width, egg surface area, unit surface shell weight and yolk diameter. This is in agreement with Carrillo *et al*. (2008), who reported that dietary inclusion of algae had no effect on egg production and egg weight of Leghorn hens. However, Mariey *et al*. (2012) found that laying hens fed with spirulina (1-2 g/kg) had higher egg production.

Variety in results may be due to the birds' species, different levels of functional substances, method of using in birds' diet, etc. Adding 5 g SP per kg diet had adverse effect on albumen weight and egg shell weight. The egg shell thickness, albumen height, HU and yolk height decreased in birds fed with different levels of algae. The reduction in HU may be due the presence of caroteniods (Skrivan *et al*. 2015). In contrast to our result, some researchers reported that dietary inclusion of SP did not have any significant effect on the percentages of egg shell, albumen index, yolk index, egg shape index or the HU (Zahroojian *et al*. 2011; Mariey *et al*. 2012; Canogullari Dogan *et al*. 2016; Selim *et al*. 2018).

On the other hand, some researchers reported the positive effect of microalgae on egg shell thickness in layer hens (Park *et al*. 2015; Selim *et al*. 2018). However, there is no scientific study for how *SP* influence the egg shell thickness (Selim *et al*. 2018).

Poultry producers usually add colorants to laying hen diets to improve the attractiveness of the eggs as a marketing goal. Also, pigment enrichment of egg yolk has the following advantages: preventing macular degeneration, antioxidant and anti-carcinogenic effects, and safeguard effect for retina (Singh *et al*. 2012). *Spirulina platensis* possess phytopigments such as phycobilins, phycocyanin, and allophycocyanin (Bermejo *et al*. 2008). Shimkus *et al*. (2009) stated that SP have high amount of phycocyanin, as 27% of its composition may refer to phycocyanin. Researchers stated that after feeding layers with diets containing carotenoids, it can efficiently deposit in the egg yolk (Kotrbacek *et al*. 2013). In laying hens, the muscle and skin xanthophylls stores are transferred to the ovaries with the onset of sexual maturity, and some parts of them are excreted in the egg yolk.

Table 6 Effects of *Spirulina platensis* on cutaneous basophil hypersensitivity (CBH) response, and antibody titer (log2) against sheep red blood cell (SRBC) in laying Japanese quails (*Coturnix coturnix Japonica*)

Items	Control	Probiotic	*Spirulina platensis*			SEM	P-value
			1 g/kg	3 g/kg	5 g/kg		
SRBC injection, wk 10				Primary response			
Total anti-SRBC	1.2^{c}	2.0^{bc}	2.4^{b}	2.8^{ab}	3.6^{a}	0.132	0.0001
IgG	1.00^{b}	1.4^{ab}	1.8^{a}	1.8^{a}	2.0^{a}	0.192	0.046
IgM	0.2^{b}	0.6^{b}	0.6^{b}	1.0^{ab}	1.6^{a}	0.233	0.048
SRBC injection, wk 11				**Secondary response**			
Total anti-SRBC	3.0^{c}	4.0^{bc}	4.8^{ab}	5.2^{a}	5.8^{a}	0.787	0.0002
IgG	1.8^{b}	2.8^{a}	3^{a}	3.6^{a}	3.6^{a}	0.707	0.0038
IgM	1.2	1.2	1.8	1.6	2.2	1.009	0.492
Hypersensitivity (mm), wk 12							
12 h after	0.28^{c}	0.33^{bc}	0.71^{abc}	0.77^{ab}	0.87^{a}	0.018	0.0312
24 h after	0.13^{d}	0.27^{c}	0.49^{b}	0.54^{b}	0.70^{a}	0.032	0.048

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In present study, different levels of *SP* increased egg yolk color compared with control group. In agreement with this result, Zahroojian *et al*. (2013) reported that dietary *SP* increased egg yolk color due to its high carotenoids content. They also recommended that using SP at the level of 2.0-2.5% cause nice yolk color similar to a synthetic pigment. Our results showed that SP decreased cholesterol level per g yolk. This can help to prevent diseases such as obesity and arteriosclerosis in consumers. In line with our result, Canogullari Dogan *et al.* (2016) reported that the mean egg yolk cholesterol levels dropped by 19.65 and 18.93% in the 1.0 or 2.0% SP supplemented groups compared with control group in quails. This reduction may be due to docosahexaenoic acid (Park *et al*. 2015), phytosterols or fiber content of algae that have negative effect on cholesterol absorption from intestine. Chen *et al*. (2011) reported that DHA from a microalgae source can inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, which reduces cholesterol synthesis.

Antibody response to SRBC

In present study, adding different levels of SP to laying quails diet caused higher humoral immunity responses. In agreement with our result, Katayama *et al*. (2016) reported higher IgG levels in broiler chickens fed SP. They stated that SP might affect a particular cytokine production that increased IgG level. In addition, SP can regulate the production of cytokines by peripheral blood mononuclear cells (Beutler, 2004). It was reported that some nutrients in SP such as lipopolysaccharide (Tornabene *et al*. 1985), vitamins, minerals, essential fatty acids (Belay *et al*. 1994) may be activated the macrophages, thus improve the humoral immunity state.

Toe web swelling test

Results of the present study showed that adding SP to laying quails diet improved cellular immunity of the quails.

This might due to increasing the T-cell proliferation and phagocytic functions of macrophages (Al-Batshan *et al*. 2001), or increased the activity of bone marrow stem cells (Simsek *et al*. 2007). Results of this paper are in agreement with Raju *et al*. (2004), who reported that SP increased the humoral and cellular immune responses in broiler chickens. Previous researches revealed that sulphated polysaccharides isolated from water extract of spirulina, named as calcium-spirulan (CaSp) showed immune-modulatory and anti-viral activities (Luescher-Mattli, 2003). It was reported that polysaccharides and phycocyanin content of spirulina increased immunity in mice by enhancing bone marrow reproduction, thymus growth, and spleen (Hirahashi *et al*. 2002; Zhang *et al*. 2001).

CONCLUSION

In conclusion, using 5 g/kg SP improved European production efficiency factor of quails during 1-35 d of age. Using SP in laying quails improved yolk color, humoral and cell immunity of quails. The microalgae also decreased egg yolk cholesterol. So, we recommend using SP at the levels of 5 and 3 g/kg diet during growth and laying period of Japanese quails, respectively. Regards to the little information about the effective mechanisms of SP in quail's body, further investigations are needed.

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