Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2018, IX(19)

Influence of dietary *Spirulina Platensis* supplementation on growth, carcass characteristics, egg traits, and immunity response of *Japanese* quails

DOI: 10.7904/2068–4738–IX(19)–29

Mojtaba ZAGHARI, Hosna HAJATI*

Department of Animal Science, College of Agriculture and Natural Resource, University of Tehran, Karaj 31587–11167, IRAN
Corresponding author: H.hajati@ut.ac.ir

Abstract. Traditionally microorganism sources and their derivations help to improve the performance and productive system and immunity in birds. Thus, two trials had been conducted to evaluate the effects of *Spirulina Platensis* (SP) on growth, carcass characteristics, egg traits, immunity response of *Japanese* quails. In trial 1, a total number of 275 one–day–old *Japanese* quails were randomly assigned into 5 groups with 5 replicates (pen) with 11 birds/per replicate. Experimental diets contained 5 levels of SP (0, 2.5, 5, 10, 20 g / kg diet). In trial 2, a total number of 240 *Japanese* laying quails were randomly assigned into 4 groups with 6 replicates (pen) with 10 female 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). Quails were evaluated for antibody titre against sheep red blood cell (SRBC) on 35 days. The results showed that using 5 g SP/kg diet had higher body weight gain and European production efficiency factor during 1–35 d of age (P<0.05). Using different levels of SP decreased shell thickness, albumen height, haugh unit and yolk height in laying quails (P<0.05). But suing SP linearly increased (p<0.05) egg yolk color when compared with control groups. Dietary inclusion of SP at levels of 3 and 5 g/kg diet decreased plasma cholesterol concentration per/g yolk (P<0.05). Different levels of SP caused higher total antibody titer against SRBC (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, SP at levels of high levels can be advised for improving the growth performance and immune responses of *Japanese* quails.
Keyword: *Egg traits; Growth; Immunity; Quails; Spirulina Platensis*.

Introduction

Regards to increasing world population and demand for animal products, poultry breeders are paying attention to find alternative dietary protein which can be used in human food and animal feed [ALTMANN *et al*, 2018]. It is important to consider that this alternative should have all nutrients required for efficient growth the poultry [NEUMANN *et al*., 2017].

Studies have shown that *Spirulina Platensis* (SP) may be one option for using in poultry diet as an alternative dietary protein source [NEUMANN *et al*., 2017; ALTMANN *et al*, 2018]. SP is a blue–green microalgae or cyanobacteria from the *Cyanophyceae* family [DEGNECHEW and BUZAYEHU, 2018]. SP 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 [KHAN *et al*., 2005; MARIEY *et al*., 2012, PENTEA et al.; 2016]. American food and drug administration and European food safety authority consider SP as GRAS (generally recognized as safe) [GONG and BASSI, 2016].

Selim and collab. reported that dietary supplementation of SP improved laying performance and egg quality of layer hens [SELIM *et al*. 2018]. The substances such as zeaxanthin, xanthophylls and ß–carotene that present in SP may be accumulated in the egg yolk and improve the yolk color [TAKASHI, 2003, VARDANIAN *et al.;* 2018].

Phenolic compounds, sulphated polysaccharides and phycocyanin content of SP have immunomodulatory and anti–viral activities [CHEN *et al*., 2014; FINAMORE *et al*., 2017]. The microalgae can improve macrophage and mononuclear phagocyte system in chickens [Al–BATSHAN *et al*., 2001; CAUNII *et al.;* 2015; IANCULOV *et al.;* 2004]. SP can stimulate the production of antibodies and cytokines

which improve the immunity state [BLINKOVA *et al.*, 2001; SAMFIRA *et al.;* 2014; BUTNARIU; 2012; BUTU *et al.;* 2014a,].

It was hypothesized that SP may alleviate effects on growth performance, carcass characteristics, egg traits and immune response. Thus, this study was conducted to evaluate the effects of dietary inclusion of SP on growth performance, carcass characteristics, egg traits and immune response of *Japanese* Quails.

Material and methods

Experiment 1. All the used procedures were approved by standard ethical committee of Tehran University (Tehran–Iran: No. T79/35923) for care and treatment of animals. In trials 1, a total of 275 one–day–old *Japanese* quails (12.14±0.23 g) were used in a completely randomized design with 5 treatments, 5 replicates (11 quail chicks in each replicate). The birds were randomly allocated to 25 pens (40 × 50 cm^2) with wood shavings litter.

Table 1.

The ingredients and nutrient composition of diets in experiment 1 (1–35 d).

Nutrient composition	Basal diet	Inclusion of 2.5 g SP / kg	Inclusion of 5 g SP / kg	Inclusion of 10 g SP / kg	Inclusion of 20 g SP / kg
Ingredients (%)					
Corn	49.9	50.0	50.0	51.0	50.59
Soybean meal (44%)	41.91	42.0	42.0	40.1	39.40
Corn gluten meal	3.0	2.55	2.30	3.0	2.5
SP	0.0	0.25	0.5	1	2
Vegetable oil	2.0	2.0	2.0	1.71	1.90
Oyster shell	1.47	1.46	1.46	1.47	1.46
Mono calcium phosphate	0.67	0.69	0.69	0.68	0.69
Common salt	0.35	0.35	0.35	0.35	0.35
DL–Methionine	0.11	0.11	0.11	0. 1	0.11
Lysine	0.01	0.01	0.01	0.01	0.01
L–Threonine	0.08	0.08	0.08	0.08	0.08
Vitamin and mineral premix [1]	0.5	0.5	0.5	0.5	0.5
Sand	0	0	0	0	0.42
Calculated contents (%)					
ME (Kcal/kg DM)	2910	2910	2910	2910	2910
Crude protein	24.23	24.23	24.23	24.23	24.23
Calcium	0.8	0.8	0.8	0.8	0.8
Available phosphorus	0.3	0.3	0.3	0.3	03
Sodium	0.15	0.15	0.15	0.15	0.15
Methionine	0.5	0.5	0.5	0.5	0.52
L–Lysine HCL	1.30	1.30	1.31	1.30	1.31
Methionine + Cystine	0.89	0.89	0.90	0.89	0.91
Threonine	1.00	1.01	1.02	1.02	1.05

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; I, 0.2 mg.

Experimental diets including basal diet (with no additive) 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. Birds had free access to feed ad water during the experiment.

The ingredients and composition of the experimental diets are shown in Table 1.

The initial temperature of birds' house was set on 36°C and gradually decreased to 22 °C on d 35.

The birds had 24 h' light.

Experiment 2. In trial 2, a total of 240 *Japanese* laying quails (49 days old; 260±2 g) with 83.84 % egg production was used in a completely randomized design with 4 treatments, 6 replicates (10 quails in each replicate).

The birds were randomly allocated to 24 commercial cages. The relative humidity and average temperatures were maintained in 18–20 ºC and 50–60 %, respectively.

Experimental diets included basal diet (with no additive) and diets supplemented with 3 levels of SP (1, 3 or

Available on-line at http://www.bjbabe.ro
Banat's Journal of Biotechnology
2018, IX(19)

5 g / kg diet). The diets were formulated to meet the nutrient requirements of the quail as recommended by NRC [NRC 1994].

The ingredients and composition of the experimental diets are shown in Table 2.

Table 2.

The ingredients and nutrient composition of basal diet in experiment 2 (8– 20 wks of age).

Ingredients (%)	8–20 wks*	Calculated contents (%)	8–20 wks*
Corn	53.0	ME (Kcal/kg DM)	2900
Soybean meal (44%)	35.63	Crude protein	19.97
Vegetable oil	3.50	Calcium	2.50
Oyster shell	5.8	Available phosphorus	0.36
Mono calcium phosphate	1.1	Sodium	0.15
Common salt	0.35	Methionine	0.44
DL–Methionine	0.12	Lysine	1.10
Vitamin and mineral premix [1]	0.5	Methionine + Cystine	0.77
		Threonine	0.77

[1]vitamin and mineral premix supplied the followings per kilogram of diet: vitamin A (retinyl acetate), 150 µg; vitamin D_3 (cholecalciferol), 1250 µg ; vitamin E, 50 mg; vitamin K_3, 3 mg; thiamin, vitamin B_{12}, 2 mg; pantothenate, 50 mg; folic acid, 1 mg; riboflavin, 8 mg; biotin, 200 µg ; Cu, 10 mg; Fe, 80 mg; Zn, 80 mg; KI, 1 mg. *Control group was fed the basal diet. The other groups fed the same basal diet supplemented with SP powder at the levels of 1, 3, or 5 g SP/ kg diet.

The experiment lasted 12 weeks, and birds had 16 hours' light. SP algae were cultivated on July, 2017. Briefly, SP was grown in modified Zarrouk's medium.

Algae were incubated in a pond (12 m^2) with paddle–wheels at mean temperature and irradiance of 29 °C. Harvesting was performed after 12–14 days. After drying and grounding, ash, crude protein, crude fat, calcium and phosphorus content of SP was measured by AOAC procedures [AOAC, 1990]. Total phenol content of SP was determined based on previous researches [ASSIS *et al.*, 2014; BUTNARIU and SAMFIRA, 2012; IANCULOV *et al.;* 2005]. 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 [LI *et al.*, 2007].

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) [BUTNARIU *et al.;* 2012].

Growth performance. In trial 1, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of the quails were measured weekly. Body weight of hens was recorded at the beginning and at the end of the experiment. FCR calculated by dividing the daily feed intake (FI) to BWG in each pen and adjusted for mortality.

Feed intake was measured weekly by subtracting the left–over feed from the quantity originally supplied to the animals. Mortality was daily registered. Any bird that died, it was weighed and FCR were calculated by dividing FI to egg mass plus dead birds. At the end of the experiment, European production efficiency factor (EPEF) was calculated as *bello [(Body weight×% survival rate)/FCR×rearing period (d)]×100*.

Carcass characteristics. At the end of the trials 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 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*–colour fan. 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* [NORTH and BELL, 1990; DIMITRIU *et al.;* 2016; GEORGIEVA *et al.;* 2018; BUTNARIU and CAUNII, 2013]. The egg surface area, unit surface shell weight, shape index, haugh unit of the eggs were calculated with the formula described by Carter [Carter 1975]: $S=3.9782\ W^{0.75056}$, S=Egg surface area (cm^2), W=Egg weight (g), Unit surface shell weight (g/cm^2)=Egg weight (mg) / Egg surface area (cm^2), Shape index (%)=[Width (cm) / Height (cm)] × 100, Haugh unit was calculated with the formula described by Kul and Seker [KUL and SEKER 2004]: Haugh unit (HU)= $100 \log (H + 7.57–1.7\ W^{0.37})$, H=Albumen height (mm), W=Egg weight (g). At the end of the experiment, yolk cholesterol was measured by method described by Pasin and [PASIN *et al.* 1998].

Immune system variables. In trial 2, sheep red blood cells (SRBC) were used as T–dependent antigens to quantify the antibody responses. At 10–week, two laying quails from each replicate were intramuscularly (i.e.) treated with SRBC (5 % suspension in PBS, 0.2 mL / bird), followed by the second injection 7 d later.

Blood samples were collected 7 d after the first injection and 7 d after the second injection. The serum of samples was collected, heat inactivated at 56 °C for 30 min and then analyzed for total antibodies against SRBC and IgG (mercaptoethanol–resistant) as primary and secondary humoral responses described by Cheema and [CHEEMA *et al.* 2003; PUTNOKY *et al.;* 2013, BUTNARIU *et al.;* 2014; BUTNARIU and GIUCHICI, 2011].

Toe web swelling test. In trial 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 cutaneous basophil hypersensitivity (CBH) response to PHA–P was measured in 2 quails from each replicate at the end of the trial. Each bird received 100 µg of PHA–P in 0.1 mL of sterile phosphate–buffered saline (PBS, 0.15 M at pH=7.4), that was injected intradermally in inter digital skin between the second and third toes of the left foot. The right foot was injected with 0.1 mL of PBS as a sham control. The thickness of each injection site was measured using a micrometer before injection and at 12 or 24 h after injection. The CBH response to PHA–P was calculated using the following formula: *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)]* [AKHLAGHI *et al.*, 2013].

Spirulina algae analysis. Table 3 illustrated the chemical composition (dry matter, crude fat, crude protein, crude fiber, calcium, total phosphorus and ash), and total polyphenolic content of SP used in this study.

Table 3.

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

Statistical Analysis. The data of the two experiments were analyzed by ANOVA using GLM procedures [SAS Institute Inc., 2004]. Means were compared using Duncan's new multiple range test [DUNCAN, 1955]. All data were expressed as means ± standard deviation (±SD). All reports of statistically significance were regarded to probability of P< 0.05. The statistical model that used in this study was completely randomized design (CRD) as follow: $Yij = \mu + Ti + eij$.

Results and discussion

Growth performance. Results of the effects of SP on growth performance and European production efficiency factor of quails is shown in Table 4. Algae

inclusion at the level of 20 g/kg diet significantly increased FI and FCR of quails during the first, second, third weeks and whole period of rearing (P<0.05).

Table 4.

Effects of *Spirulina Platensis* on growth performance of quails (*Coturnix coturnix Japonica)* during 1– 35 d

	Control	*Spirulina Platensis* (g/kg diet)				SEM	P–value
		2.5	5	10	20		
1 to 7 d							
FI (g)	38.16^{b}	38.33^{b}	40.10^{ab}	41.26^{ab}	43.80^{a}	0.461	0.0314
BWG (g)	19.72	20.05	21.11	21.79	20.22	0.494	0.5030
FCR (g/g)	1.93^{b}	1.91^{b}	1.90^{b}	1.89^{b}	2.16^{a}	0.012	0.0180
8 to 14 d							
FI (g)	112.33^{b}	115.83^{b}	115.4^{b}	118.0^{b}	126.06^{a}	2.485	0.028
BWG (g)	46.94	49.11	49.58	42.97	45.31	0.479	0.5247
FCR (g/g)	2.39^{b}	2.35^{b}	2.32^{b}	2.76^{a}	2.78^{a}	0.013	0.0120
15 to 21 d							
FI (g)	147.03^{b}	149.93^{b}	157.43^{a}	159.26^{a}	161.6^{a}	3.580	0.0238
BWG (g)	53.83	55.33	60.46	60.40	58.13	0.491	0.2169
FCR (g/g)	2.26^{b}	2.25^{b}	2.19^{b}	2.22^{b}	2.34^{a}	0.018	0.0498
22 to 28 d							
FI (g)	165.27	170.02	170.5	171.24	175.94	5.582	0.2911
BWG (g)	45.73	45.83	48.14	46.28	47.91	0.476	0.9698
FCR (g/g)	3.62^{a}	3.70^{a}	3.54^{b}	3.70^{a}	3.67^{a}	0.017	0.0408
29 to 35 d							
FI (g)	180.00	181.33	184.66	185.33	187.06	7.591	0. 170
BWG (g)	45.22	46.43	48.73	46.20	47.69	0.470	0.8644
FCR (g/g)	3.98^{a}	3.90^{a}	3.78^{b}	4.01^{a}	3.92^{a}	0.027	0.0494
1 to 35 d							
FI (g)	642.81^{c}	655.46^{bc}	668.10^{b}	675.11^{ab}	694.48^{a}	8.24	0.0056
BWG (g)	211.45^{b}	216.76^{b}	228.03^{a}	217.65^{b}	219.26^{b}	2.33	0.0063
FCR (g/g)	3.039	3.024	2.930	3.102	3.168	0.051	0.140
EPEF*	198.73^{b}	204.84^{b}	222.20^{a}	200.68^{b}	201.47^{b}	4.11	0.0029

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

Using 5g SP per kg diet increased BWG of quails during whole period of rearing (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 5, using SP at levels of 2.5 or 5 g/ kg diet increased carcass yield of the birds compared with control group numerically.

Table 5.

Effects of *Spirulina Platensis* on carcass characteristics of quails (*Coturnix coturnix Japonica)* at 35 d.

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

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. [1]Carcass was calculated as the ratio of carcass weight (breast + drumstick + thigh + wings + neck + back without skin & viscera) to live body weight; [2]Relative weight of organ to carcass weight (without skin & viscera) as percentage; [3] Relative weight of organ to carcass weight (without skin) as percentage.

Also, consuming SP at levels of 2.5 or 5 g /kg diet increased relative weight of breast in quails (P<0.05). Feeding SP did not have any significant effect on the relative weights of drumstick + thigh, liver, and gizzard (P>0.05).

Egg traits. Regard to Table 6, 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).

Table 6.

Effects of *Spirulina Platensis* 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
SP1	87.64	12.29	33.69	26.40	6.57^{ab}	3.99^{b}	1.57^{ab}	0.306^{c}	78.40
SP3	87.86	12.32	34.18	26.48	6.48^{ab}	4.07^{b}	1.55^{ab}	0.317^{b}	77.54
SP5	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 cholestero (mg/g)
Control	26.02	0.470	91.94^{a}	5.04^{a}	25.56	10.10^{a}	5.53^{d}	7.74^{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

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

At the present study, all levels of SP decreased eggshell thickness, haugh unit, albumen height and yolk height (P<0.05). SP supplementation at the levels of 1 or 3g / 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). As indicated in the Table 6, SP at the levels of 3 or 5 g / kg diet decreased cholesterol level per g yolk (P<0.05).

Immune system variables. Adding different levels of SP to laying quails increased total antibody titer against SRBC as a primary response to SRBC injection (Table 7).

Table 7.

Effects of *Spirulina Platensis* on cutaneous basophil hypersensitivity (CBH) response, and antibody titer (log_2) against sheep red blood cell (SRBC) in laying *Japanese* quails (*Coturnix coturnix Japonica)*

Items	Control	*Spirulina Platensis*			SEM	P–value
		1 g/ kg	3 g / kg	5 g / kg		
SRBC injection, wk 10	Primary response (7 d after injection)					
Total anti–SRBC	1.2^{c}	2.4^{b}	2.8^{ab}	3.6^{a}	0.132	0.0001
IgG	1.00	1.8	1.8	2.0	0192	0.860
SRBC injection, wk 11	Secondary response (14 d after injection)					
Total anti–SRBC	3.0^{c}	4.8^{ab}	5.2^{a}	5.8^{a}	0.187	0.0002
IgG	1.8^{b}	3^{a}	3.6^{a}	3.6^{a}	0.107	0.0038
Hypersensitivity (mm), wk 12						
12 h after	0.28^{c}	0.71^{b}	0.77^{ab}	0.87^{a}	0.014	0.0312
24 h after	0.13^{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.

Also, quails fed diet supplemented with SP had higher total antibody titer against SRBC and IgG titer as the secondary response to SRBC injection (P<0.05).

Toe web swelling test. As shown in Table 7, 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 and [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 and collab. found that SP had 94.4 % dry matter, 61.74 % crude protein, 5.09 % crude fat, and 9 % ash [RADHAKRISHNAN *et al.* 2017]. Gutiérrez–Salmeán and collab. [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; BAGIU *et al.; 2012*; BUTNARIU and CORADINI, 2012].

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^{-1} GAE [MACHU *et al.*, 2015]. The reason of differences in SP content may be related to origin geography, cultivation ad harvesting situations, season, climate [COLLA *et al.*, 2007], condition of extraction and type of solvent [MACHU *et al.*, 2015, PETRACHE *et al.;* 2014, BUTNARIU *et al.;* 2014, BARBAT 2013, BUTU *et al.;* 2015].

Growth. Based on the results present study using SP in quails diet tend to increase feed consumption of the birds; as the level of 20 g/kg diet increased FI of quails meaningfully (Table 4). It seems that SP increased appetite of the quails and there was an upward trend in FI of the quails with increasing levels of SP.

Although there was an upward trend in BWG and EPEF of quails up to 5 g SP / kg diet, the trend was not continued at higher dosage of the algae. This can be interpreted that at higher dosage, quails cannot use nutrients as efficiently as lower levels of dietary SP. The FCR of quails was not affected by the experimental diets that can be explained by parallel increasing of FI and BWG in quails fed SP. It has been observed that using microalgae in broilers diet had no significant effects on BW [EVANS *et al.*, 2015].

In line with these results, Cheong and collab. [CHEONG *et al.*, 2015] reported that feeding SP to quails increased FI and BWG of the birds. Improvement of quails BWG up to 5g SP/ kg diet might be due to increased feed efficiency [KAOUD, 2015], increased villi height of intestine [SHANMUGAPRIYA *et al.*, 2015a], or higher nutrient digestibility [EVANS *et al.*, 2015].

However, the excessive intake of SP may be resulted in metabolic disturbances and affected the liver function cause to retarded growth rate [SHANMUGAPRIYA *et al.*, 2015b]. It has been reported that there was no significant different in FI and FCR of quails fed SP [DOGAN *et al.*, 2016].

However, in another study Kharde and collab. [KHARDE *et al.* 2012] found that SP supplementing BW and feed efficiency of broilers. In agreement with our result, Park and collab. [PARK *et al.* 2018] reported that SP improved 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, [BUTU *et al.;* 2014c. SAMFIRA *et al.;* 2015, BUTNARIU *et al.;* 2015b; BUTU *et al.;* 2014b].

Carcass characteristics. Using SP has not affected carcass yield of quails compared with control group significantly. 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 and collab. [SUGIHARTO *et al.* 2018] and Altmann and collab. reported that using SP had not any significant effect on broilers carcass characteristics [ALTMANN *et al.* 2018]. Also, Cheong and collab. found that SP had no effect on carcass yield and breast percentage [CHEONG *et al.* 2015].

However, Mariey and collab. found that SP at the levels of 0.2 or 0.3 g / kg increased dressing percentage [MARIEY *et al.* 2014]. Also, Saada and collab. reported that SP increased carcass output and liver weight of broiler chickens. On the other hand [SAADA *et al.* 2016], Razafindrajaona and collab. 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 [RAZAFINDRAJAONA *et al.* 2008]. 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 and collab. observed increased liver weight in broiler chickens fed with higher level of SP (4 or 8 kg / 100 kg) [TOYOMIZU *et al*. 2001]. The difference between results may be due to the different levels of SP used in the trials.

Egg traits. In the current study, 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 and collab. who reported that dietary inclusion of algae had no effect on egg production and egg weight of *Leghorn hens* [CARRILLO *et al*. 2008]. However, Mariey and collab. found that laying hens fed with *Spirulina* (1–2 g/kg) had higher egg production [MARIEY *et al*. 2012]. Variety in results may be due to the birds' species, different levels of functional substances, method of using in birds diet, *etc*. The egg shell thickness, albumen height, haugh unit and yolk height decreased in birds fed with different levels of algae. Adding 5 g SP per kg diet had adverse effect on albumen weight and egg shell weight. The reduction in haugh unit may be due the presence of caroteniods [SKŘIVAN *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 haugh unit [MARIEY *et al*., 2012; 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 description how SP influence the egg shell thickness [SELIM *et al*., 2018]. Poultry producers usually add colourants to hen diets to improve the attractiveness of the eggs as a marketing strategy [FRANCHINI and PADOA, 1996]. Also, pigment enrichment of egg yolk has the following advantages: preventing macular degeneration, anti–oxidant and anti–carcinogenic effects, and safeguard effect for retina [SINGH *et al*., 2012]. SP possess phytopigments such as phycobilins, phycocyanin, and allophycocyanin [BERMEJO *et al*. 2008].

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. It is interesting to notice that breed, strain, and housing conditions may influence a hen's ability to deposit pigments in the yolk [PONSANO *et al*., 2004].

In present study, different levels of SP increased egg yolk colour compared with control group. In agreement with this result, Zahroojian and collab. reported that dietary SP increased egg yolk color due to its high carotenoids content [ZAHROOJIAN *et al*. 2013].

Our results showed that SP decreased cholesterol level per g yolk. This reduction may be due to docosahexaenoic acid [PARK *et al*., 2015], plant sterols [LIRETTE *et al*., 1993], or fiber content of algae [LAHAYE and JEGOU, 1993] that have negative effect on cholesterol absorption from intestine. Also, Chen and collab. [CHEN *et al*. 2011] reported that DHA from a microalga source can inhibit 3–hydroxy–3–methylglutaryl–coenzyme A (HMG–CoA) reductase activity, which reduces cholesterol synthesis.

Immune system. In present study, adding different levels of SP to laying quails diet caused higher humoral immunity responses. 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]. In agreement with our result, Katayama and collab. reported higher IgG levels in broiler chickens fed SP [KATAYAMA *et al*. 2016]. 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, 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. Results of this paper are in agreement with Raju and collab. who reported that SP increased the humoral and cellular immune responses of broiler chickens [RAJU *et al.* 2004].

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 and spleen growth [HIRAHASHI *et al.*, 2002, GROZEA et al.; 2017].

Conclusions

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. Regards to the little information about the effective mechanisms of SP in quail's body, further investigations are needed.

Declaration of conflict of interests

The authors report that they have no other financial or personal relationships that could inappropriately influence or bias the content of the paper.

Abbreviations: BWG: body weight gain; **CBH:** cutaneous basophil hypersensitivity; **EPEF**: European production efficiency factor; **FCR**: feed conversion ratio; **FI**: feed intake**; SRBC**: sheep red blood cells; **PHA**: phytohemagglutinin; PBS: phosphate–buffered saline; **SP**: *Spirulina Platensis*

References

1. Akhlaghi, A.; Zamiri, M.J.; Jafari Ahangari, Y.; Atashi, H.; Ansari Pirsaraei, Z.; Deldar, H.; Eghbalian, A.N.; Akhlaghi, A.A.; Navidshad, B.; Yussefi Kelarikolaei, K. Hashemi, S.R. Oral exposure of broiler breeder hens to extra thyroxine modulates early adaptive immune responses in progeny chicks. *Poultry Science*, **2013**. 92(4): 1040–1049.
2. Al–Batshan, H.A.; Al–Mufarrej, S.I.; Al–Homaidan, A.A.; Qureshi, M.A. Enhancement of chicken macrophage phagocytic function and nitrite production by dietary Spirulina platensis. *Immunopharmacology and immunotoxicology*, **2001**. 23(2): 281–289.
3. Altmann, B.A.; Neumann, C.; Velten, S.; Liebert, F.; Mörlein, D. Meat Quality Derived from High Inclusion of a Micro–Alga or Insect Meal as an Alternative Protein Source in Poultry Diets: A Pilot Study. *Foods*, **2018**. *7*(3), 34.
4. AOAC, Official method of analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC, USA. **1990**.
5. Assis, L.M.D.; Machado, A.R.; Motta, A.D.S.D.; Costa, J.A.V.; Soares, L.A.D.S. Development and characterization of nanovesicles containing phenolic compounds of microalgae *Spirulina strain* LEB–18 and *Chlorella pyrenoidosa*. **2014**.
6. Bagiu, R.V.; Vlaicu, B.; Butnariu, M. Chemical Composition and in Vitro Antifungal Activity Screening of the *Allium ursinum* L. (Liliaceae), *International Journal of Molecular Sciences*, **2012**, 13(2): 1426–1436.
7. Barbat, C.; Rodino, S.; Petrache, P.; Butu, M.; Butnariu, M. Microencapsulation of the allelochemical compounds and study of their release from different, *Digest journal of nanomaterials and biostructures*, **2013**, 8(3), 945–953.
8. Belay, A. Production of high quality Spirulina at Earthrise farms. *Algal biotechnology in the Asia–Pacific region.* **1994**. 92–102.
9. Bensehaila, S.; Doumandji, A.; Boutekrabt, L.; Manafikhi, H.; Peluso, I.; Bensehaila, K.; Kouache, A. Bensehaila, A. The nutritional quality of *Spirulina platensis* of Tamenrasset, Algeria. *African Journal of Biotechnology*, **2015**. *14*(19), pp.1649–1654.
10. Bermejo, P.; Piñero, E.; Villar, Á.M. Iron–chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulinaplatensis. *Food Chemistry*, **2008**.*110*(2), 436–445.

11. Beutler, B. Innate immunity: an overview. *Molecular immunology*, **2004**. 40(12): 845–859.
12. Blinkova, L.P.; Gorobets, O.B.; Baturo, A.P. Biological activity of Spirulina Zh Mikrobiol Epidemiol Immunobiol. 2001; (2): 114–8. Review. In this review information of Spirulina platensis (SP), a blue–green alga (photosynthesizing cyanobacterium) having diverse biological activity is presented. Due to high content of highly. *Zh Mikrobiol Epidemiol Immunobiol*, **2001**. *2*, 114–8.
13. Bonos, E.; Kasapidou, E.; Kargopoulos, A.; Karampampas, A.; Christaki, E.; Florou–Paneri, P.; Nikolakakis, I. Spirulina as a functional ingredient in broiler chicken diets. *South African Journal of Animal Science*, **2016**. 46(1): 94–102.
14. Butnariu, M. An analysis of *Sorghum halepense's* behavior in presence of tropane alkaloids from *Datura stramonium* extracts, *Chemistry Central Journal*, **2012**, Volume: 6, Article Number: 75.
15. Butnariu, M.; Caunii, A. Design management of functional foods for quality of life improvement, *Annals of Agricultural and Environmental Medicine*. **2013**, 20(4): 736–741.
16. Butnariu, M.; Caunii, A.; Putnoky, S. Reverse phase chromatographic behaviour of major components in *Capsicum Annuum* extract. *Chemistry Central Journal*, **2012**, 6, Article Number:146.
17. Butnariu, M.; Coradini, C.Z. Evaluation of Biologically Active Compounds from *Calendula officinalis* Flowers using Spectrophotometry, *Chemistry Central Journal*, **2012**, 6, Article Number: 35.
18. Butnariu, M.; Negrea, P.; Lupa, L.; Ciopec, M.; Negrea, A.; Pentea, M.; Sarac, I.; Samfira, I. Remediation of Rare Earth Element Pollutants by Sorption Process Using Organic Natural Sorbents. *International journal of environmental research and public health*, **2015**, 12(9), 11278–11287b.
19. Butnariu, M.; Rodino, S.; Petrache, P.; Negoescu, C.; Butu, M. Determination and quantification of maize zeaxanthin stability, *Digest journal of nanomaterials and biostructures*, **2014**, 9(2), 745–755.
20. Butnariu, M.; Samfira, I. Free Radicals and Oxidative Stress, *Journal of Bioequivalence & Bioavailability*, **2012**, 4(3): 1.
21. Butnariu, M.V.; Giuchici, C.V. The use of some nanoemulsions based on aqueous propolis and lycopene extract in the skin's protective mechanisms against UVA radiation, *Journal of Nanobiotechnology*, **2011**, Volume: 9, Article Number: 3.
22. Butu, M.; Butnariu, M.; Rodino, S.; Butu, A. Study of zingiberene from *Lycopersicon esculentum* fruit by mass spectometry, *Digest journal of nanomaterials and biostructures*, **2014**, 9(3), 935–941a.
23. Butu, M.; Rodino, S.; Butu, A.; Butnariu, M. Screening of bioflavonoid and antioxidant activity of *Lens culinaris* medikus, *Digest journal of nanomaterials and biostructures*, **2014**, 9(2), 519–529b.
24. Butu, M.; Rodino, S.; Pentea, M.; Negrea, A.; Petrache, P.; Butnariu, M. IR spectroscopy of the flour from bones of European hare, *Digest journal of nanomaterials and biostructures*. **2014**, 9(4), 1317–1322c.
25. Carrillo, S.; López, E.; Casas, M.M.; Avila, E.; Castillo, R.M.; Carranco, M.E.; Calvo, C. Perez–Gil, F. Potential use of seaweeds in the laying hen ration to improve the quality of n–3 fatty acid enriched eggs. In *Nineteenth International Seaweed Symposium* (pp. 271–278). **2008**. Springer, Dordrecht.
26. Carter, T.C. The hen's egg: Estimation of shell superficial area and egg volume, using measurements of fresh egg weight and shell length and breadth alone or in combination. **1975**. 541–543.
27. Caunii, A.; Negrea, A.; Pentea, M.; Samfira, I.; Motoc, M.; Butnariu, M. Mobility of Heavy Metals from Soil in the Two Species of the Aromatic Plants, *Revista de Chimie*, **2015**, 66(3): 382–386.
28. Cheema, M.A.; Qureshi, M.A.; Havenstein, G.B. A comparison of the immune response of a 2001 commercial broiler with a 1957 random bred broiler strain when fed representative 1957 and 2001 broiler diets. *Poultry science*, **2003**. 82(10): 1519–1529.
29. Chen, H.W.; Yang, T.S.; Chen, M.J.; Chang, Y.C.; Eugene, I.; Wang, C.;

Ho, C.L.; Lai, Y.J.; Yu, C.C.; Chou, J.C. and Chao, L.K.P. Purification and immunomodulation activity of C–phycocyanin from *Spirulina platensis* cultured using power plant flue gas. *Process Biochemistry*, *2014*. *49*(8), pp.1337–1344.

30. Chen, J.; Jiang, Y.; Ma, K.Y.; Chen, F.; Chen, Z.Y. Microalga decreases plasma cholesterol by down–regulation of intestinal NPC1L1, hepatic LDL receptor, and HMG–CoA reductase. *Journal of agricultural and food chemistry*, **2011**. *59*(12), 6790–6797.
31. Cheong, D.S.W.; Kasim, A.; Sazili, A.Q.; Hishamuddin, O.M.A.R.; Teoh, J.Y. Effect of supplementing Spirulina on live performance, carcass composition and meat quality of Japanese quail. *Walailak Journal of Science and Technology (WJST)*, **2015**. *13*(2), 77–84.
32. Colla, L.M.; Reinehr, C.O.; Reichert, C.; Costa, J.A.V. Production of biomass and nutraceutical compounds by Spirulina platensis under different temperature and nitrogen regimes. *Bioresource technology*, *98*(7), **2007**. 1489–1493.
33. Corrier, D.E.; DeLoach, J.R. Evaluation of cell–mediated, cutaneous basophil hypersensitivity in young chickens by an interdigital skin test. *Poultry Science*, **1990**. 69(3): 403–408.
34. Degnechew, G.D.; Buzayehu, D.B. Applications of Arthrospira platensis as an Alternative Source of Food, Maintaining Nutritional Security and Awareness Creation; there by Reducing Problems of Malnutrition in the Society. *World News of Natural Sciences*, **2018**. *19*, 1–8.
35. Dimitriu, D.C.; Stoleru, V.; Corciova, A.; Vlase, L.; Stan, T.; Jitareanu, A.; Munteanu, N.; Rotaru, L.; Patras, A.; p–Coumaric acid content in sweet pepper under farming methods. *Environmental Engineering and Management Journal*, **2016**, 15(8), 1841–1848.
36. Dogan, S.C.; Baylan, M.; Erdogan, Z.; Akpinar, G.C.; Kucukgul, A.; Duzguner, V. Performance, egg quality and serum parameters of Japanese quails fed diet supplemented with Spirulina platensis. *Fresen Environ Bull*, **2016**. *25*(25), 5857–62.
37. Duncans, D. B. Multiple range and multiple F. test. *Biometrics*, **1955**. *11*(1).
38. Evans, A.M.; Smith, D.L.; Moritz, J.S. Effects of alga incorporation into broiler starter diet formulations on nutrient digestibility and 3 to 21 d bird performance. *Journal of Applied Poultry Research*, **2015**. 24(2): 206–214.
39. Finamore, A.; Palmery, M.; Bensehaila, S.; Peluso, I. Antioxidant, immunomodulating, and microbial–modulating activities of the sustainable and ecofriendly spirulina. (2017). *Oxidative medicine and cellular longevity*, *2017*.
40. Franchini, A.; Padoa, E. I pigmenti nell'alimentazione del pollo da carne. *Rivista di Avicoltura*, **1996**. *65*, 22–30.
41. Georgieva, NA; Kosev, VI; Genov, NG; Butnariu, M. Morphological and biological characteristics of white lupine cultivars (*Lupinus Albus* L.). *Romanian Agricultural Research*. **2018**, 35, 109–119.
42. Gong, M.; Bassi, A. Carotenoids from microalgae: a review of recent developments. *Biotechnology advances*, **2016**. *34*(8), 1396–1412.
43. Grozea, A.; Drasovean, A.; Lalescu, D.; Gal, D.; Cziszter, L.T.; Cristina, R.T. The Pike Perch (*Sander lucioperca*) Background Color First Choice in the Recirculating Aquaculture Systems. *Turkish journal of fisheries and aquatic sciences*, **2016**, 16(4), 891–897.
44. Gutiérrez–Salmeán, G.; Fabila–Castillo, L.; Chamorro–Cevallos, G. Aspectos nutricionales y toxicológicos de Spirulina (arthrospira). *Nutricion hospitalaria*, **2015**. *32*(1), 34–40.
45. Hirahashi, T.; Matsumoto, M.; Hazeki, K.; Saeki, Y.; Ui, M.; Seya, T. Activation of the human innate immune system by Spirulina: augmentation of interferon production and NK cytotoxicity by oral administration of hot water extract of *Spirulina platensis*. *International Immunopharmacology*, **2002**. 2(4): 423–434.
46. Ianculov, I.; Gergen, I.; Palicica, R.; Butnariu, M.; Dumbrava, D.; Gabor, L. The determination of total alkaloids from *Atropa belladona* and *Lupinus sp* using various spectrophotometrical and gravimetrical methods, *Revista de chimie*, **2004**, 55(11), 835–838.

47. Ianculov, I.; Palicica, R.; Butnariu, M.; Dumbrava.; D. Gergen, I. Obtinerea in stare cristalina a clorofilei din cetina de brad (*Abies alba*) si de pin (*Pinus sylvestris*). *Revista de Chimie*, **2005**, 56(4), 441–443.
48. Kaoud, H.A. Effect of Spirulina platensis as a dietary supplement on broiler performance in comparison with prebiotics. *Science Arena Publications* Specialty *Journal* of *Biological Sciences*, **2015**. 1(2): 1–6.
49. Katayama, Sh.; Kayahara, Y.; Watanabe, T. Enhancement of Immunological Responses by Dietary Arthrospira platensis and Possibility of Field Applications as Alternative to Antibiotics in Broiler Chicken. *American journal of animal and veterinary sciences*. **2016**. 11(1): 18–24.
50. Khan, Z.; Bhadouria, P.; Bisen, P.S. Nutritional and therapeutic potential of Spirulina. *Current pharmaceutical biotechnology*, **2005**. 6(5): 373–379.
51. Kharde, S.D.; Shirbhate, R.N.; Bahiram, K.B.; Nipane, S.F. Effect of Spirulina supplementation on growth performance of broilers. *Indian Journal of Veterinary Research*, **2012**. *21*(1), 66–69.
52. Kul, S.; Seker, I. Phenotypic correlations between some external and internal egg quality traits in the *Japanese quail* (*Coturnix coturnix japonica*). *International Journal of Poultry Science*, **2004**. 3(6): 400–405.
53. Lahaye, M.; Jegou, D. Chemical and physical–chemical characteristics of dietary fibers from Ulva lactuca (L.) Thuret and *Enteromorpha compressa* (L.) Grev. *Journal of Applied Phycology*, **1993**. 5(2): 195.
54. Li, H.B.; Cheng, K.W.; Wong, C.C.; Fan, K.W.; Chen, F.; Jiang, Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food chemistry*, **2007**. 102(3), 771–776.
55. Lindberg, J.E.; Lindberg, G.; Teräs, J.; Poulsen, G.; Solberg, S.Ø.; Tybirk, K.; Przedrzymirska, J.; Sapota, G.P.; Olsen, M.L.; Karlson, H. Jóhannsson, R. *Nordic Alternative Protein Potentials: Mapping of regional bioeconomy opportunities*. Nordic Council of Ministers. **2016**. Pp 131.
56. Lirette, A.; Crober, D.C.; Lawson, P.D.; Firth, N.L.; Robinson, A.R. Effect of oat bran, cotton seed hulls and guar gum on chicken egg and blood lipids during the early laying period. *Canadian Journal of Animal Science*, **1993**. 73(3): 673–677.
57. Luescher–Mattli, M. *Current Medical Chemistry–Anti–Inflammatory Agents*.; **2003**, 2: 219–225.
58. Machu, L.; Misurcova, L.; Vavra Ambrozova, J.; Orsavova, J.; Mlcek, J.; Sochor, J.; & Jurikova, T. Phenolic content and antioxidant capacity in algal food products. *Molecules*, **2015**. *20*(1), 1118–1133.
59. Mariey, Y.; Samak, H.; Abou–Khashba, H.; Sayed, M.; Abou–Zeid, A. Effect of using Spirulina platensis alga as a feed additive for poultry diets: 2 productive performance of broiler. Egypt. *Poultry Science*, **2014**. 34(1): 245–258.
60. Mariey, Y.A.; Samak, H.R.; Ibrahem, M.A. Effect of using Spirulina platensis alga as a feed additive for poultry diets: 1– productive and reproductive performances of local laying hens. *Poultry Science*, **2012**. 32(1): 201–215.
61. Neumann, C.; Velten, S.; Liebert, F. Improving the dietary protein quality by amino acid fortification with a high inclusion level of micro algae (*Spirulina platensis*) or insect meal (*Hermetia illucens*) in meat type chicken diets. *Open Journal of Animal Sciences*, **2017**. *8*(01), 12.
62. North, M.O.; Bell, D.D. Commercial chicken production manual. Van Nostrand Reinhold. 4th edition. **1990**.
63. Park, J.H.; Lee, S.I.; Kim, I.H. Effect of dietary *Spirulina* (Arthrospira) platensis on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. *Poultry science*. **2018**. 97:2451–2459.
64. Park, J.H.; Upadhaya, S.D.; Kim, I.H. Effect of dietary marine micro alga (*Schizochytrium*) powder on egg production, blood lipid profiles, egg quality and fatty acid composition of egg yolk in layers. *Asian–Australasian journal of animal sciences*, **2015**. 28(3): 391.
65. Pasin, G.; Smith, G.M.; O'mahony, M. Rapid determination of total cholesterol in egg yolk using commercial diagnostic cholesterol

reagent. *Food Chemistry*, **1998**. 61(1–2): 255–259.
66. Pentea, M.; Hulea, C.; Stancu, A.; Butnariu, M.; Cristina, R.T. Developing the Plastination Laboratory for the Technique S10. *Materiale plastice*. **2016**. 53(1), 150–152.
67. Petrache, P.; Rodino, S.; Butu, M.; Pribac, Pribac, G.; Pentea, M.; Butnariu, M. Polyacetylene and carotenes from Petroselinum sativum root, *Digest Journal of Nanomaterials and Biostructures*, **2014**, 9(4):1523–1527.
68. Ponsano, E.H.G.; Pinto, M.F.; Neto, M.G.; Lacava, P.M. *Rhodocyclus gelatinosus* biomass for egg yolk pigmentation. *Journal of applied poultry research*, **2004**. *13*(3), 421–425.
69. Putnoky, S.; Caunii, A.; Butnariu, M. Study on the stability and antioxidant effect of the *Allium ursinum* watery extract, *Chemistry Central Journal*, **2013**, Volume: 7, Article Number: 21.
70. Radhakrishnan, S.; Bhavan, P.S.; Seenivasan, C.; Muralisankar, T. Nutritional profile of Spirulina platensis, *Chlorella vulgaris* and *Azolla pinnata* to novel protein source for aquaculture feed formulation. *Austin Journal of Aquaculture and Marine Biology*, **2017**. *2*(1), 1–8.
71. Raju, M.V.L.N.; Rama Rao, S.V.; Radhika, K.; Chawak, M.M. Effects of Spirulina platensis or furazolidone on the performance and immune response of broiler chickens fed with aflatoxin contaminated diet. *Indian Journal of Animal Nutrition*, **2004**. *21*(1), 40–44.
72. Razafindrajaona, J.; Rakotozandriny, J.N.; Rakotozandrindrainy, R.; Tsivingaina, A.; Ramapiherika, K.D.; Randria, J. Influence de l'incorporation dans les provendes de la spiruline de Madagascar (Spirulina platensis var. Toliarensis) sur la croissance des poulets de chair. In *International Symposium on Spirulina–Toliara Sud–Ouest de Madagascar*. **2008**. Pp 195.
73. Saada, D.A.; Chabane, O.A.; Bekada, A.; Bouderoua, K.; Keddam, R.; Laala, B.; Mourot, J. Selselet–Attou, G.; Valorization ESsay of Arthrospira platensis in Alimentation of Broilers. *Global Veterinaria*. **2016**. 16 (1): 71–85.
74. Samfira, I.; Butnariu, M.; Rodino, S.; Butu, M. Structural investigation of mistletoe plants from various hosts exhibiting diverse lignin phenotypes, *Digest Journal of Nanomaterials and Biostructures*, **2014**, 8(4), p. 1679–1686.
75. Samfira, I.; Rodino, S.; Petrache, P.; Cristina, R.T.; Butu, M.; Butnariu, M. Characterization and identity confirmation of essential oils by mid infrared absorption spectrophotometry. *Digest journal of nanomaterials and biostructures*, **2015**, 10(2), 557–566.
76. SAS, ed. Statistical Analysis Systems user's guide (9.1 ed.). SAS Institute Inc.; Raleigh, North Carolina, USA. **2004**.
77. Selim, S.; Hussein, E.; Abou–Elkhair, R. Effect of Spirulina platensis as a feed additive on laying performance, egg quality and hepatoprotective activity of laying hens. *European Poultry Science*, **2018**. 16: 82.
78. Shanmugapriya, B.; Babu, S.S.; Hariharan, T.; Sivaneswaran, S.; Anusha, M.B. Dietary administration of Spirulina platensis as probiotics on growth performance and histopathology in broiler chicks. International Journal *of Recent Scientific Research*; **2015**a. 6(2): 2650–3.
79. Shanmugapriya, B.; Babu, S.S.; Hariharan, T.; Sivaneswaran, S.; Anusha, M.B.; Raja, P.U. Synergistic effect of Spirulina platensis on performance and gut microbial load of broiler chicks. *Indo–Asian Journal of Multidisciplinary Research*. **2015**b. 1, 149–155.
80. Simsek, N.; Karadeniz, A.; Karaca, T. Effects of the Spirulina platensis and Panax ginseng oral supplementation on peripheral. *Revue Méd. Vét*, **2007**. *158*(10), 483–488.
81. Singh, V.; Pathak, V.; Akhilesh, K.V. Modified or enriched eggs: A smart approach in egg industry: A. *American Journal of Food Technology*, **2012**. 7(5): 266–277.
82. Skřivan, M.; Englmaierová, M.; Skřivanová, E.; Bubancová, I. Increase in lutein and zeaxanthin content in the eggs of hens fed marigold flower extract. *Czech Journal of Animal Science*, **2015**. *60*(3), 89–96.
83. Sugiharto, S.; Yudiarti, T.; Isroli, I.; Widiastuti, E. Effect of feeding

duration of *Spirulina platensis* on growth performance, haematological parameters, intestinal microbial population and carcass traits of broiler chicks. *South African Journal of Animal Science*, **2018**. *48*(1), 98–107.

84. Takashi, S. Effect of administration of Spirulina on egg quality and egg components. *Animal Husbandry*, **2003**. *57*, 191–195.
85. Tornabene, T.G.; Bourne, T.F.; Raziuddin, S.; Ben–Amotz, A. Lipid and lipopolysaccharide constituents of cyanobacterium *Spirulina platensis* (Cyanophyceae, Nostocales). *Marine Ecology Progress Series*, **1985**.121–125.
86. Vardanian, A.; Kurzbaum, E.; Farber, Y.; Butnariu, M.; Armon, R. Facilitated enumeration of the silicate bacterium *Paenibacillus mucilaginosus* comb. nov. (formerly *Bacillus mucilaginosus*) via tetrazolium chloride incorporation into a double agar–based solid growth medium. *Folia microbiologica*, **2018**, 63(3), 401–404.
87. Zahroojian, N.; Moravej, H.; Shivazad, M. Effects of dietary marine alga (Spirulina platensis) on egg quality and production performance of laying hens. *Journal of Agricultural Science and Technology*, **2013**. 15: 1353–1360.

Received: May 28, 2018
Article in Press: September 26, 2018
Accepted: Last modified on: November 20, 2018