






Arthrospira (Spirulina) Platensis Can Be Considered as a Probiotic Alternative to Reduce Heat Stress in Laying Japanese Quails

■ Author(s)

Hajati H¹  <https://orcid.org/0000-0002-9080-7742>
Zaghari M¹  <https://orcid.org/0000-0003-4440-3584>
Oliveira HC²  <https://orcid.org/0000-0001-7317-2004>

¹ Department of Animal Science, College of Agriculture and Natural Resource, University of Tehran 31587-11167, Karaj, Iran.

² Department of Animal Science, Universidade Federal de Viçosa, Viçosa, MG, 36570000, Brazil.

■ Mail Address

Corresponding author e-mail address
Hosna Hajati
Department of Animal Science, College of Agriculture and Natural Resource, University of Tehran 31587-11167, Karaj, Iran.
Phone: +989111566684
Email: H.hajati@ut.ac.ir

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Egg production; Heat stress; HSP70 gene; Japanese quails; *Spirulina platensis*.



ABSTRACT

This study was conducted to investigate the effects of *Arthrospira platensis* (*Spirulina platensis*, *SP*) on feed intake, feed conversion ratio, egg weight, hen day egg production, intestinal microflora, heat stress biomarkers, and HSP70 gene expression in laying Japanese quails (*Coturnix coturnix japonica*) suffering heat stress condition. A total of 250 female quails were allocated to 5 treatments, 5 replicates and 10 birds in each replicate in a completely randomized design. Experimental treatments included: 1) basal diet, 2) basal diet+ 0.03 % probiotic, 3) basal diet+ 0.1 % *SP*, 4) basal diet+ 0.3 % *SP*, 5) basal diet+ 0.5 % *SP*. During the last 6 days of the experiment, the quails were exposed to 8h of 34±1°C. The birds had free access to feed and water during the experiment. The results showed that using probiotic and different levels of *Spirulina* had no significant effect on laying performance of Japanese quails ($p>0.05$). Probiotic supplement increased *Lactobacil* bacteria population in laying quails' ileum under heat stress ($p<0.05$). Different levels of *SP* decreased *Escherichia coli* population in laying quails' ileum suffering heat stress ($p<0.05$). *SP* at the level of 0.5% caused the lowest blood Malondialdehyde level, heterophil, and H/L ratio ($p<0.05$). However, HSP70 gene expression in the heart or the liver of laying quails was not different ($p>0.05$). In conclusion, the results of the present study revealed that *SP* at the level of 0.5 % has the potential to be considered as a probiotic alternative in the diet of laying quails suffering heat stress condition.

INTRODUCTION

Heat stress threatens poultry productivity and economic benefits especially on summer days in many countries of the world (Attia *et al.*, 2011; Hajati *et al.*, 2015). Thermo-neutral temperature for adult quails is between 23-26°C (Sousa *et al.*, 2013), thus higher environmental temperatures may lead to heat stress in the birds. In fact, stress alter normal behavior, biochemical and physiological processes in birds that disrupts the body homeostasis (Sahin *et al.*, 2009). Oxidative stress induced by heat stress has detrimental effect on egg production by activation of the hypothalamic-pituitary-adrenal (HPA) axis, change the bird's neuroendocrine profile, and reduction of feed intake (Lara & Rostagno, 2013). Heat stress may lead to intestinal flora disorders in laying birds (Li *et al.*, 2015). It was documented that the expression of heat stress-related genes, like HSP70, increases in heat stress condition (Zhang *et al.*, 2014). In regard to some negative side effects of synthetic antioxidants in the body, poultry nutritionist are searching for organic compounds that can improve the health and welfare of the birds under heat stress condition. In addition, considering the effect of egg quality on human health, organic feeding of laying birds has high concern to ensure the quality of the eggs and support the health state of



consumers. It was reported that using additives such as probiotics may improve performance, and intestinal microbial ecology of birds suffering heat stress (Sugiharto *et al.*, 2017). Also, it was found that algae contain bioactive compounds, or phytochemicals that may help to consumer's health (Hafting *et al.*, 2012). *S. platensis* is a microalgae-rich in protein, vitamins, minerals, phytopigments (Farag, 2016), gamma linoleic acid, phycocyanins, phenolic acids, beta-carotene and chlorophyll (Mariey *et al.*, 2012). These substances have antioxidant (Fazilati *et al.*, 2016), antimicrobial, anti-cancer, and anti-inflammatory activity (Kulshreshtha *et al.*, 2008). Mariey *et al.* (2012) reported dietary inclusion of *S. platensis* at the levels of 0.1-0.2% improved egg production in laying hens. Recently, Park *et al.* (2018) documented that dietary *S. platensis* improved cecal *Lactobacillus* population and antioxidant enzyme activity in broiler chickens. However, there is no information about the potential effects of SP in heat-challenged laying quails. Therefore, the aim of the present study was evaluating the effects of *Arthrospira platensis* on feed intake, feed conversion ratio, egg weight, hen day egg production, intestinal microflora, heat stress biomarkers, and HSP70 gene expression in laying Japanese quails (*Coturnix coturnix japonica*) suffering heat stress condition.

MATERIALS AND METHODS

Birds, housing, and rearing conditions

A total of 250 Japanese laying quails (*Coturnix coturnix japonica*, 98 days old; 282±2 g) with 88.34% egg production was used in a completely randomized design with 5 treatments, 5 replicates (10 quails in each replicate). The experiment lasted 6 weeks. The quails were placed in wire cages (10 birds/cage) under lighting program of 16h/d light cycle with an average light intensity of 40 lux/m². During the normal condition (98-134 days of age), the rearing house temperature and relative humidity were kept in 22°C and 50–60%, respectively. In order to induce heat stress in the birds, during the last 6 days of the experiment (135-140 days of age), the quails were exposed to 1 h of 22 to 34±1°C (9:00 to 10:00 AM), 8h of 34±1°C (10:00 to 18:00), and 1 h (18:00 to 19:00) of 34±1 to 22°C. Also, relative humidity was set from 60% to 70%. The procedures of this study were approved by the Animal Research Ethical Committee of animal department, University of Tehran.

Diet formulation and additives

Regarding AOAC's (2007) method, before starting to formulate the experimental diets, feed ingredients

were analyzed for crude protein (CP), ether extract (EE), starch and total sugar. Then, metabolisable energy (ME_n) of the main ingredients was calculated based on analyzed values of the feedstuffs (NRC, 1994). The ME_n of corn was calculated by the following formula: ME_n = 36.21 × crude protein + 85.4 × ether extract + 37.26 × nitrogen free extract. The ME_n of soybean meal was calculated by the following formula: ME_n = 37.5 × crude protein + 46.39 × ether extract + 14.9 × nitrogen free extract. The probiotic used in this experiment containing *Bacillus subtilis* (21336) 4×10⁹CFU/g of the supplement (Biorun company, Iran). The ash, CP, EE, calcium, phosphorus, and total phenol content of *S. platensis* was 12.51± 0.6, 64.86 ±0.31, 4.73±0.11, 1.02±0.08, 1.41±0.09, 10.19 ± 0.04 mg GAE / g *S. platensis*, respectively. Experimental treatments included: 1) basal diet, 2) basal diet+ 0.03 % probiotic, 3) basal diet+ 0.1 % *S. platensis*, 4) basal diet+ 0.3 % *S. platensis*, 5) basal diet+ 0.5 % *S. platensis*. Basal diet (Table 1) were formulated using WUFFDA software according to nutritional requirements of laying Japanese quails described in NRC (1994).

Table 1 – The ingredients and nutrient composition of basal diet.

Ingredients (g kg ⁻¹)	98-140 days*
Yellow Corn	530.0
Soybean meal (44%)	356.3
Vegetable oil	35.0
Oyster shell	58.0
Mono calcium phosphate	11.0
Common salt (NaCl)	3.5
DL-Methionine	1.2
Vitamin and mineral premix ¹	5.0
Calculated contents	
ME (MJ kg ⁻¹)	12.12
Crude protein (g kg ⁻¹)	199.7
Calcium (g kg ⁻¹)	25.0
Available phosphorus (g kg ⁻¹)	3.60
Sodium (g kg ⁻¹)	1.5
Methionine (g kg ⁻¹)	4.4
Lysine (g kg ⁻¹)	11.0
Methionine + Cystine (g kg ⁻¹)	7.7
Threonine (g kg ⁻¹)	7.7
Analyses contents (g kg ⁻¹)	
Dry matter (DM)	896.3
Crude protein	198.9
Crud fat	51.4
Crude fiber	33.4

¹vitamin and mineral premix supplied the followings per kilogram of diet: A: 10000 IU; vitamin D : 3000 IU; vitamin E: 20 IU; vitamin K : 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: 0.2 mg and I: 0.3 mg. *Control group was fed the basal diet. The other groups fed the same basal diet supplemented with probiotic (0.3 g/kg), or SP powder at the levels of 1, 3, or 5 g SP/ kg diet.



During the experiment, a batch of basal diet was made weekly, and then divided into five equal portions, the definite dosage of probiotic and *S. platensis* added on top of each diet and mixed to make the five dietary treatments.

Measurements

Feed intake, feed conversion ratio, Egg weight and hen day egg production

Average feed intake per bird was measured weekly by subtracting the left-over feed from the quantity originally supplied to the quails in each pen. Feed conversion ratio (FCR) was calculated by dividing the daily feed intake (FI) to egg mass (average egg number × average egg weight) in each pen and adjusted for mortality. Every day, the eggs from each replicate were weighed, and then the average egg weight was calculated weekly. Hen day egg production was calculated by the following formula: egg production = number of egg production on each day/number of hens alive on that day × 100 (North & Bell, 1990).

Ileal microflora population

At 134(before heat stress) and 140(under heat stress) days of age, considering the average weight of the birds in each pen, two quails from each pen were selected and slaughtered by cervical dislocation. The ileum content (from Meckel's diverticulum to ileocecal junction) were quickly collected into sterile plastic containers under CO₂ and frozen at -80°C to determine *E. coli* and *Lactobacillus* counts according to Hu *et al.* (2012). Two ml sterilized saline were used to dilute 0.2 g of ileal content, then 10-fold serial dilutions (10⁻⁴, 10⁻⁵ and 10⁻⁶) were prepared. A 100 µl of the dilutions was transferred onto sterile plates. *Lactobacillus* count were assessed on DeMan, Rogosa and Sharpe (MRS) agar at 37°C after 48 h, and *Escherichia coli* O157:H7(*E. coli*) colonies were measured on MacConkey agar at 37°C after 24 h. The bacterial count expressed as 1 g colony forming units (CFU) per gram of ileal content.

Malondialdehyde concentration, Glutathione Peroxidase activity, heterophil, lymphocyte, H/L ratio, HSP70 gene expression

At 134 (before heat stress) and 140 (under heat stress) days of age, two quails considering the weight near to the average weight of each pen were selected to collect 2 ml of blood sample from the jugular vein using a syringe 20-gauge needle. Then, blood samples were centrifuged at 3,000 × g for 10 min, and sera were collected. Malondialdehyde (MDA) content

was measured using thiobarbituric acid (TBA) and spectrophotometer (n= 50, Mihara and Uchiyama 1978). Absorbance of samples was recorded at 532 nm wavelength using a spectrophotometer.

Also, blood hemolysate of two quails from each pen was prepared on the 134th and 140th days of age. A commercially Glutathione Peroxidase (GPx) kit (Randox, Crumlin, UK) was used to determine GPx activity according to the methodology of Paglia and Valentine (1967). The absorbance of the samples was recorded at 340 nm wavelength using a spectrophotometer (n=50).

To measure the percentages of heterophil, lymphocyte, and H/L ratio, blood smears of two birds from each cage were air-dried on the 134th and 140th days of age. Then, stained with Wright-Giemsa stain (Saikin Kagaku Institute Co. Ltd., Sendai, Japan), and counted to a total of 100 cells per slide, using a Zeiss (Jena, Germany) compound light microscope at a magnification of 100× with oil immersion (n=50).

For evaluating HSP70 gene expression, at the end of the experiment (140 d) heart and liver samples of birds were washed with normal saline, put into the liquid nitrogen tank and transferred to a -80°C freezer. Total RNA was extracted from homogenized heart and liver (100 mg, n=25) by TRIZOL reagent kit (Invitrogen Inc., CA, USA) according to the manufacturer's instruction. Assessing the quality and quantity of the extracted RNA by ND-1000 UV-Vis spectrophotometer, RNA integrity was assessed by gel-electrophoresis, melting curve and amplification plot of HSP70 gene was done (Figure 1a, b, c, d). Synthesis of the primers was done by SinaClon company. The primers for HSP70 (NM_001323199.1) and Beta-actin (AF199488) were as follows, respectively:

Forward 5' CCGCATCACTCCATCCTACG3',
Reverse 5' CCGCTTGGCATCAAACACAG 3',
Forward 5' CTGGCACCTAGCACAAATGAA 3',
Reverse 5' CTGCTTGCTGATCCACATCT 3',

PCR program include 40 cycles of 95°C for 15 s, 62°C for 30s, 72°C for 30s. Standard melt curve was implemented. All reactions were in triplicate to find the average Ct value. The β-actin gene has been used as reference gene. For data analysis and relative expression calculation the ΔΔCT methodology and 2^{-ΔΔCT} (Livak, 2001; Hajati *et al.*, 2015) assessed to find the fold change of treatments against control samples. Briefly, we calculated ΔCt by subtracting the Ct amount of HSP70 gene from Ct of β-actin for each sample, then ΔΔCt was calculated by subtracting ΔCt of each group from ΔCt of control group.

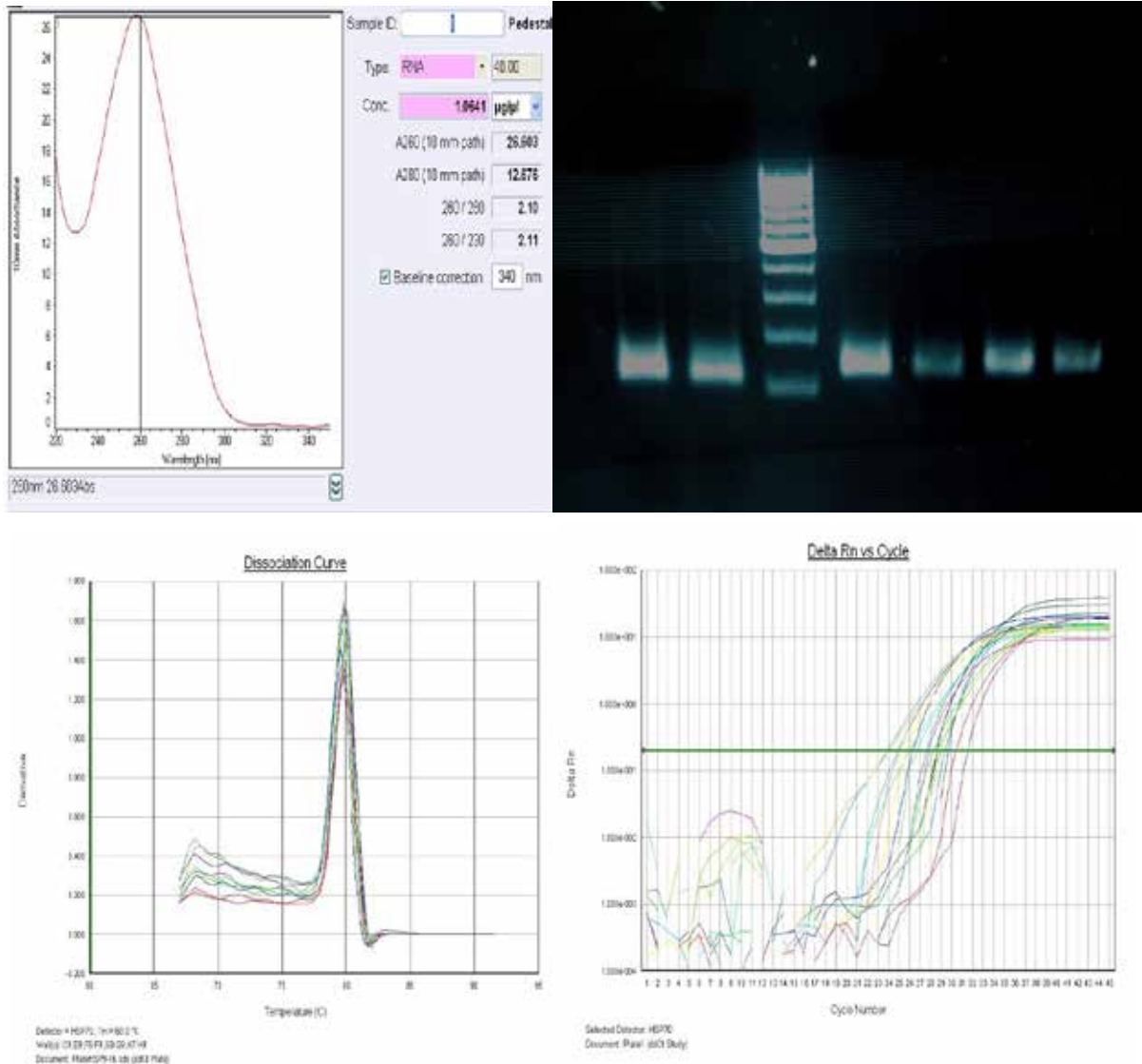


Figure 1-a – assessing the quality and quantity of the extracted RNA by ND-1000 UV-Vis spectrophotometer; b: PCR products of Beta-actin with 123 bp (1, 2, 3 columns) and HSP70 with 115 bp (4, 5, 6 columns); c: melting curve of HSP70 gene products; d: amplification plot of HSP70 gene.

STATISTICAL ANALYSIS

All the data were analyzed by SAS 9.1.3, ANOVA procedure (SAS 2006). Mean comparison was done by Duncan test, and the results were considered statistically significant when $p < .05$. Statistical model of this experiment was as follow: $X_{ij} = \mu + T_i + e_{ij}$, where, μ was overall mean, T_i was the effect of treatment, e_{ij} was experimental error.

RESULTS AND DISCUSSION

Feed intake, feed conversion ratio, egg weight, and hen day egg production

Table 2 shows that the supplementation of different levels of dietary *SP* and probiotic had no

significant effect on feed intake (g/bird/day), FCR, egg weight (g) and hen day egg production (%) of laying Japanese quails ($p > 0.05$). In agreement with these results, Rezaei-pour *et al.* (2015) found that the supplementation of multi strains probiotic (Primalac) had no significant effect on feed intake and FCR of Japanese quails. Also, Hajiaghapour & Rezaei-pour (2018) reported that probiotic supplementation did not alter feed intake, egg weight and egg mass in quail breeders. Dogan *et al.* (2016) reported that there was no significant difference on feed intake, FCR, egg production, and egg weight in quails fed with different levels of *SP*. Abouelezz (2017) used *SP* in the feed (1 %) and drinking water (0.25 %) of the laying Japanese quails. The researcher reported there was no significant difference on egg production of the birds.


Table 2 – Effects of probiotic and *Arthrospiraplatensis* (*Spirulina platensis*) on feed intake (g/bird/day), feed conversion ratio (g/g), egg weight (g), and hen day egg production (%) of laying Japanese quails from 98-140 d of age.

p value	SEM ²	SP ¹ (%)			probiotic	Control	
		0.5	0.3	0.1			
0.516	1.714	33.89	33.81	33.68	33.05	33.21	Feed intake (g/bird/day)
0.068	0.073	3.20	3.15	3.14	3.09	3.11	Feed conversion ratio (g/g)
0.607	0.398	12.44	12.48	12.39	12.51	12.32	Egg weight (g)
0.988	2.938	85.05	85.84	86.57	85.41	86.49	Hen day egg production (%)

¹*SpirulinaPlatensis*, ²Standard error of mean.

However, Mariey *et al.* (2012) reported that using dietary *S. platensis* (0, 0.10, 0.15 or 0.20%) improved egg production, egg weight and daily egg mass in local layer hens in Egypt. This is not in agreement with our findings. The differences may be due to the different types of birds and climate conditions.

Ileal microflora population

As shown in Table 3, before heat stress condition (134 d), supplementation of different levels *SP* or

probiotic decreased *E. coli* count in ileum content of the laying quails ($p < 0.05$). Adding 0.5% *SP* caused the lowest ileal *E. coli* count of the quails. However, there was no difference in *Lactobacillus* count of the birds on d 134 ($p > 0.05$). Also, different levels of *SP* or probiotic decreased *E. Coli* count in ileum content of the laying quails suffering heat stress condition on 140 d ($p < 0.05$). Probiotic supplement increased *Lactobacillus* count in ileal content of the birds under heat stress on 140 ($p < 0.05$).

Table 3 – Effects of probiotic and *Arthrospiraplatensis* (*Spirulina platensis*) on ileal *Lactobacillus* and *Escherichia coli* in laying Japanese quails before (134 d) and under heat stress (140 d) condition.

p value	SEM ¹	SP (%)			probiotic	Control	
		0.5	0.3	0.1			
134 d (before heat stress)							
0.0001	0.263	0.2 ^c	0.6 ^c	0.8 ^c	2.0 ^b	3.0 ^a	<i>Escherichia coli</i> (log 10 CFU g ⁻¹)
0.539	0.487	1.68	1.9	1.42	2.42	1.96	<i>Lactobacillus</i> (log 10 CFU g ⁻¹)
140 d (under heat stress)							
0.0001	0.349	2.6 ^c	4.4 ^b	5.6 ^b	3.6 ^{bc}	7.6 ^a	<i>Escherichia coli</i> (log 10 CFU g ⁻¹)
0.028	0.288	2.36 ^b	2.8 ^b	2.74 ^b	4.8 ^a	3.0 ^b	<i>Lactobacillus</i> (log 10 CFU g ⁻¹)

Means within the same row with uncommon superscript differ significantly ($p < 0.05$). ¹Standard error of mean.

Researchers found that feeding diets containing *Bacillus subtilis* decreased cecal *E. coli* count in Japanese quails (Manafi *et al.*, 2016). Qureshi *et al.* (1996) reported that *SP* had antimicrobial activity that improved chicken defence system. It was documented that the extract of *SP* was able to inhibit the growth of *Klebsiellapneumoniae*, *Shigellashigae*, *E. coli*, *S. aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi* (Mala *et al.*, 2009). The antibacterial activity of the algae extract could be due to the presence of different chemicals such as 1-Octadecene, 1-Heptadecane (Mishra & Sree 2007), flavonoids, triterpenoids, phenolic compounds, fatty acids (Demule *et al.*, 1996, Lampe *et al.*, 1998), acrylic acid (Pradhan *et al.*, 2014) free hydroxyl group (Yu *et al.*, 2009). In agreement with our result, Shanmugapriya *et al.* (2015) found that using dietary *SP* decreased *E. coli* count in the ileal and caecal digesta of broiler chickens, however, dietary *SP* increased Lactic acid bacteria count in the intestine. It is interesting to note

that gastrointestinal disorders which caused diarrhea may increase in birds faced with stressors, and performance of these birds may decrease significantly (Manafi, 2015). It was reported that antibiotic alternatives such as prebiotic and probiotic are more effective under heat stress condition (Sohail *et al.*, 2012).

MDA concentration, GPx activity, heterophil, lymphocyte, H/L ratio, HSP70 gene expression

The results showed that adding different levels of dietary *SP* and probiotic had no significant effect on blood MDA concentration, GPx activity, heterophil (%), Lymphocyte (%), and H/L ratio in laying Japanese quails before heat stress condition (134 d, $p > 0.05$). On d 140, different levels of dietary *SP* and probiotic decreased MDA concentration ($p < 0.05$), percentage of heterophil ($p < 0.05$), and H/L ratio ($p < 0.05$) in quails suffering from heat stress condition (Table 4). Supplementation of *S. platensis* increased the



Table 4 – Effects of probiotic and *Arthrospiraplantensis* (*Spirulina platensis*) on blood malondialdehyde (MDA) concentration (µmol/l), glutathione peroxidase activity (U/L), heterophil (%), Lymphocyte (%), and H/L ratio in laying Japanese quails before (134 d) and under heat stress (140 d) condition.

p value	SEM ²	SP ¹ (%)			probiotic	Control	
		0.5	0.3	0.1			
134 d (before heat stress)							
0.278	0.361	2.00	2.08	2.15	2.12	2.23	MDA (µmol/l)
0.723	34.21	404.2	413.3	424.1	419.6	442.3	GPx (U/L)
0.188	1.583	28.1	28.7	29.3	29.5	31.3	Heterophil (%)
0.474	1.917	62.3	62.4	61.7	61.2	59.4	Lymphocyte (%)
0.119	0.071	0.45	0.45	0.47	0.48	0.52	H/L ratio
140 d (under heat stress)							
0.038	0.058	2.07 ^c	2.54 ^{bc}	2.73 ^{bc}	3.52 ^b	6.09 ^a	MDA (µmol/l)
0.804	42.11	502.5	511.3	521.6	523.2	532.7	GPx (U/L)
0.031	1.681	25.80 ^c	27.08 ^{bc}	27.60 ^{bc}	29.44 ^b	32.32 ^a	Heterophil (%)
0.034	1.603	63.40 ^a	62.20 ^{ab}	61.48 ^{ab}	60.20 ^{bc}	58.20 ^c	Lymphocyte (%)
0.0001	0.012	0.40 ^c	0.43 ^{bc}	0.44 ^{bc}	0.48 ^b	0.55 ^a	H/L ratio

Means within the same row with uncommon superscript differ significantly ($p < 0.05$). ¹*Spirulina Platensis*, ²Standard error of mean.

percentage of lymphocyte in laying quails on d 140 ($p < 0.05$). Adding *S. platensis* and probiotic did not have significant effect on HSP70 gene expression in heart (Figure 2) or liver (Figure 3) of laying quails under heat stress condition ($p < 0.05$). However, HSP70 gene expression decreased numerically in the heart and liver of the birds and the lowest amount HSP70 gene expression was related to the groups fed with dietary *S. platensis* at the levels of 0.3 or 0.5 %.

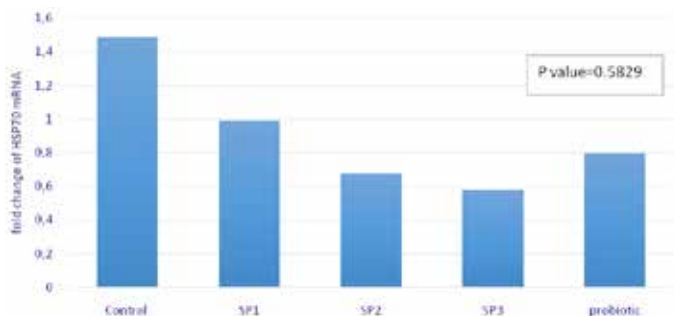


Figure 2 – Relative mean fold change of HSP70 mRNA in heart of laying Japanese quails under heat stress condition. SP1: SP powder at the level 0.1 %, SP2: SP powder at the level of 0.3 %, SP3: SP powder at the level of 0.5 %. (n=25)

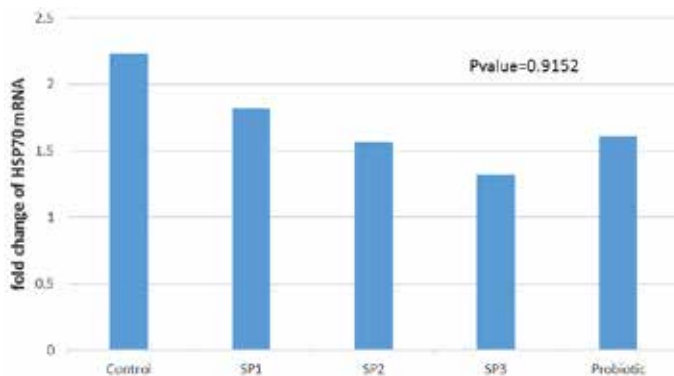


Figure 3 – Relative mean fold change of HSP70 mRNA in liver of laying Japanese quails under heat stress condition. SP1: SP powder at the level 0.1 %, SP2: SP powder at the level of 0.3 %, SP3: SP powder at the level of 0.5 %. (n=25)

One of the final products of polyunsaturated fatty acids peroxidation in the cells is MDA, which consider a marker of oxidative stress. Mahmoud *et al.* (2004) found a strong relationship between lipid oxidation and HSP70 synthesis in stressed cells. Georgieva *et al.* (2006) documented that increased levels of MDA and decreased activities of serum antioxidant enzymes such as GPx show imbalance in body oxidants/antioxidants system, and the birds suffer oxidative stress. Antioxidant nutrients and enzyme defenses are fundamental protectors under stressful condition (Ojha *et al.*, 2010). In present study, *SP* supplementation at the level of 0.5 % yielded the lowest MDA, heterophil %, and H/L ratio (as an acceptable index of stress) in laying quails. In agreement with this result, Mirzaie *et al.* (2018) reported that using dietary *SP* under high ambient temperature reduced H/L ratio in broiler chickens under heat stress. The antioxidant effect of *SP* in the quail’s body may be related to the presence of wide range of antioxidant compounds in *SP*. They include β -carotene, astaxanthin, phycocyanin, phycoerythrin, and sulfated polysaccharides such as fucoidans and heterofucans (Chu, 2011; Klein & Buchholz, 2013). It is interesting to notice that phycocyanin is almost 16 times more effective than trolox (vitamin E analog) and 20 times more efficient than vitamin C as an antioxidant (Romay *et al.*, 2000). Researchers reported that using dietary astaxanthin decreased the amount of thio-barbituric acid-reactive substances (TBARS) in egg yolks (Yang *et al.*, 2006). Also, salicylic, trans-cinnamic, synaptic, chlorogenic, quinic, and caffeic acids are phenolic substances of *SP* that have antioxidant properties (Miranda *et al.*, 1998). It is well documented that heat stress in quails may



increase lipid peroxidation and HSP expression (Sahin *et al.*, 2009). Among heat shock proteins, HSP70 shows the highest up-regulation under stressful situations. This may be due to the higher amount or activity of the heat shock transcription factor (Craig & Gross, 1991).

In a previous study, it was found that polyphenol content of organic additives lowered HSP70 gene expression in broiler chickens suffering from heat stress condition (Hajati *et al.*, 2015). The observed HSP70 gene expression numerically reduction might be related to the presence of β -carotene, vitamin C, vitamin E, selenium, manganese, or phenolic compound (10.19 ± 0.04 mg GAE / g) in *S. platensis* with radical scavenging, hydrogen or electron donating, metal chelating activity (Balasundrum *et al.*, 2006), cell signaling pathways and gene expression effects (Rodrigo *et al.*, 2011).

CONCLUSIONS

Dietary *S. platensis* supplementation did not have significant effect on egg production or HSP70 gene expression in the heart or liver of Japanese quails. However, adding *S. platensis* at the level of 0.5% caused the lowest intestinal *E. coli* population, blood MDA level, heterophil, H/L ratio, and HSP70 in quails' ileum under heat stress ($p < 0.05$). Thus, it seems that *S. Platensis* had the potential to decrease heat stress in laying quails and may be considered as a probiotic alternative in heat stress condition, however, further research is needed to clear its effects in detail.

DISCLOSURE STATEMENT

The authors declare that there is no potential conflict of interest.

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