



## Evaluation of the Effects of *Spirulina platensis* Extract on Productive Performance, Egg Quality and Serum Composition in Laying Japanese Quails Under Dexamethasone-induced Stress

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### Abstract

In a 3×2 factorial arrangement, the effects of dietary *Spirulina platensis* extract (0, 0.4 and 0.6 g/kg of diet) and dexamethasone (0 and 1 g/kg of diet) and their interactions were investigated on production performance, egg quality and hematological parameters in Japanese quails. A total of 150 laying Japanese quails, aged 8 wk, were divided into six treatments, five replicates with five birds each. Dietary dexamethasone supplementation decreased egg production, feed intake, egg weight and egg mass ( $P < 0.05$ ). Moreover, the birds fed with dietary dexamethasone demonstrated a greater feed conversion ratio (FCR) compared to quails-fed diets without dexamethasone ( $P < 0.05$ ). Dexamethasone treatment significantly also reduced shell thickness and yolk color in layer quail hens ( $P < 0.05$ ). Supplemental *Spirulina platensis* extract significantly increased egg production, feed intake and egg mass ( $P < 0.05$ ). Adding *Spirulina platensis* extract to the diet of laying Japanese quails improved the FCR compared to quails-fed diets without *Spirulina platensis* extract ( $P < 0.05$ ). *Spirulina platensis* (0.4 g/kg of diet) added diets reduced serum triglyceride of the quails ( $P < 0.05$ ). Eggshell thickness in quails fed on diets supplemented with *Spirulina platensis* extract was significantly greater compared to quails fed diets without *Spirulina platensis* extract ( $P < 0.05$ ). The yolk color index in birds maintained on the diets supplemented with *Spirulina platensis* extract (0.4 and 0.6 g/kg of diet) was significantly greater than the birds fed diets without *Spirulina platensis* extract ( $P < 0.05$ ). Based on the findings of the present research, the addition of dexamethasone (1 g/kg) to the diet of laying quails induced stress and also dietary supplementation of *Spirulina platensis* extract at 0.4 and 0.6 g/kg of diet improved egg production, egg mass, FCR, eggshell thickness and yolk color index.

### Introduction

Today, attention to Japanese quail has increased due to various reasons such as low maintenance cost, short generation interval, resistance to diseases and high egg production. On the other hand, the demand for meat and quail eggs is increasing due to their acceptable taste and traditional medical recommendations (Rafieian-Naeini *et al.*, 2021). Today, birds are exposed to all kinds of stress, including oxidative stress, due to dense space and rapid growth (Oke *et al.*, 2024). The use of dexamethasone has been accepted as

an effective method to induce stress (Vicuna *et al.*, 2015). Oxidative stress by dexamethasone can affect physiological and biochemical processes and have an adverse effect on intestinal function and metabolism (Miao *et al.*, 2021). Oxidative stress increases susceptibility to disease and impairs immune function and as a result, may reduce growth and production indices (Berenjian *et al.*, 2021). One of the topics of interest in poultry research in recent years is the methods of preventing and minimizing the harmful effects of stress on the performance and health of

birds. Several compounds were used to reduce the negative effects of stress on the health and performance of birds, including probiotics, prebiotics, medicinal plants and microalgae (Oliveira *et al.*, 2023). In this regard, the use of additives such as microalgae due to their antioxidant and antimicrobial properties in poultry nutrition is an effective solution (Kumar *et al.*, 2022). Microalgae are single-celled, photosynthetic organisms that grow in saltwater or alkaline waters and contain several bioactive compounds, such as long-chain fatty acids, essential amino acids, and carotenoids (Abdelfatah *et al.*, 2024). Many recent reports identified high antioxidant activity in algae as well as its beneficial effects in improving the immune system, excreting heavy metals from the body, protecting kidneys and liver, reducing allergies, growth of cancer tumors and function of viruses in animals (Gupta *et al.*, 2011; Nassar *et al.*, 2023). *Spirulina platensis* was introduced as microalgae with a considerable nutritional, probiotic and antioxidant properties exerting positive effects on intestinal morphology and immune system in Japanese quails mainly attributable to phycocyanin pigment and polysaccharides (Abdel-Hack *et al.*, 2024; Alghamdi *et al.*, 2024). Phycocyanin has anti-inflammatory, hepatoprotective, neuroprotective, immune system regulating and anticancer activities (Omar *et al.*, 2022).

Considering the importance of stress control, the current study evaluated the impact of dietary *Spirulina platensis* extract supplementation on production performance, biochemical parameters and quality of eggs of Japanese quails under dexamethasone-induced stress.

## Materials and Methods

### Birds, design, and treatments

A total of one hundred fifty, 8-week-old laying Japanese quails were randomly allocated into 30 battery cages (45 × 50 × 40 cm) with five laying birds per cage. The experimental factors comprised dietary *Spirulina platensis* extract (0, 0.4 and 0.6 g/kg of diet) and dexamethasone (DEX) challenge (0 and 1 g/kg of diet). Dietary DEX (Iran Hormone Pharmaceutical Co, Iran) was used to induce stress in the birds. The experiment lasted for 12 weeks, from 8 to 20 weeks of age. Before starting the experiment, the quails were acclimatized to the experimental environment for 2 weeks. During the research period, all quails were kept under the same management conditions so that all birds had free access to feed and water and were exposed to a 16L:8D lighting regimen. The amount of humidity and temperature of the room during the breeding period was determined by a digital thermometer and hygrometer, respectively. The room temperature and relative humidity were maintained at 26 ± 2 °C and 60 ± 5%, respectively. The basal diet was formulated according to NRC (1994). The

composition and the calculated nutrient content of the basal diet are summarized in Table 1.

### Microalgae extract

Microalgae extract was produced according to the method proposed by Grossmann *et al.* (2018) and the phenolic content of the extract was determined by Folin–Ciocalteu method using gallic acid as a standard (McDonald *et al.*, 2001). The amount of total flavonoid was measured by the aluminum chloride colorimetric method (Quettier-Deleu, 2000). The antioxidant potential of the extract was determined using the scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH<sup>•</sup>) radical (Herald *et al.* 2012), first 10 mL of acetate buffer was mixed with 1 mL of tripyridyltriazine (TPTZ) dissolved in hydrochloric acid, and then 1 mL of ferric chloride solution was added to the above solution. A sample of 50 µL of the extract was added to the solution to start the reaction (Benzie and Strain, 1996). One mL of the extract was mixed with 2 mL ethanolic DPPH. The absorbance was measured at 517 nm after 30 min of dark incubation. Total phenolic, flavonoids, antioxidant activity and DPPH assay of *Spirulina platensis* and *Chromochloris zofingiensis* extract are shown in Table 2.

**Table 1.** Ingredients and nutrient composition of the basal diet

Ingredients	%
Corn	55.42
Soybean meal	35.07
Fish meal	3.00
Sunflower oil	0.16
Wheat bran	1.10
Dicalcium phosphate	1.44
Calcium carbonate	2.86
Mineral premix <sup>1</sup>	0.25
Vitamin premix <sup>2</sup>	0.25
Salt	0.30
DL-methionine	0.15
<i>Chemical composition</i>	
Metabolizable energy (Kcal/kg)	3000
Crude protein (%)	22.23
Crude fiber (%)	3.25
Methionine (%)	0.65
Methionine + Cystine (%)	0.87
Lysine (%)	1.22
Calcium (%)	2.50
Available phosphorus (%)	0.57
Sodium (%)	0.16

<sup>1</sup>One kg mineral premix contains: Mn, 50,000 mg; Fe, 25,000 mg; Zn, 50,000 mg; Cu, 5,000 mg; I, 500 mg; Se, 100 mg.

<sup>2</sup>One kg vitamin premix contains: vitamin A, 3,500,000 IU; vitamin D3, 1,000,000 IU; vitamin E, 9000 IU; vitamin K3, 1000 mg; vitamin B1, 900 mg; vitamin B2, 3,300 mg; vitamin B3, 5,000 mg; vitamin B5, 15,000 mg; vitamin B6, 150 mg; vitamin B9, 500 mg; vitamin B12, 7.5 mg; Biotin, 500 mg; Choline chloride, 250,000 mg.

**Table 2.** Total phenolic, flavonoids, antioxidant activity and DPPH assay of *Spirulina platensis* extract

Items	<i>Spirulina platensis</i> extract
Total phenols (mg/g)	20.15
Total flavonoid (mg/g)	5.56
FRAP (g of FeSO <sub>4</sub> /100 g)	78.65
DPPH scavenging activity, %	46.34

FRAP, ferric reducing ability of plasma.

DPPH, 1,1-diphenyl- 2-picrylhydrazyl.

**Laying performance**

The eggs produced in all the cages were collected and weighed daily, and the data was used to calculate mean egg weight and production percentage. Egg production was calculated by dividing the number of eggs produced in each cage by the number of quails. To determine egg mass, egg weight was multiplied by egg production. The amount of feed consumed was divided by the egg mass to determine the feed conversion ratio (FCR).

**Egg quality parameters**

On day 84 of the experiment, three eggs were randomly taken from each experimental group to measure egg length and width using calipers and data were used to calculate the egg shape index (width-to-length ratio). In order to calculate the relative albumen weight, the weight of the albumen was divided by the weight of the egg and the resulting number was multiplied by 100. The same method was applied to determine the relative weight of yolk and shell too. The yolk color score was evaluated by comparing it with the Roche color fan. Yolk index and albumen index were measured using the method described by Funk (1948):

$$\text{Yolk index} = \text{Yolk height (mm)} / \text{Yolk diameter (mm)} \times 100$$

$$\text{Albumen index} = (\text{Albumen height} / (\text{albumen length} + \text{albumen width})/2) \times 100$$

The eggshell was washed with water, dried at room temperature and weighed at a precision of 0.01 g. The shell thickness was recorded as the average of the four measurements at diagonal points using a micrometer (PK-0505SUE, Japan). Haugh unit (HU) was determined using the following equation (Card and Nesheim, 1972).

$HU = 100 \log (H + 7.57 - 1.7 \times W^{0.37})$ , where, W = egg weight (g) and H = height of the albumen (mm). Cholesterol and triglyceride content in the egg yolk was measured according to the method described by Hwang et al. (2003).

**Serum biochemical parameters**

Blood samples (2 mL) were randomly collected at slaughtering time from 10 quails per treatment. After collection, the blood was left for 2 h. The blood was then centrifuged at 3,000 rpm for 10 min at 4°C, and

the serum was extracted, transferred to test tubes without ethylenediaminetetraacetic acid (EDTA) anticoagulant, and stored at -20 °C for further biochemical analysis. Biochemical parameters including cholesterol, triglyceride, high-density lipoprotein (HDL), uric acid, phosphorus, calcium, albumin and total protein were measured using laboratory kits (Pars Azmoon, Tehran, Iran) by an autoanalyzer (Alcyon 300, USA).

**Statistical analysis**

The current research was conducted in a completely randomized design with a factorial arrangement of 3×2 with six treatments and five replicates. The factors consisted of dietary *Spirulina platensis* extract at three levels and dexamethasone at two levels. Statistical analysis was performed using the General Linear Models (GLM) of SAS (SAS Institute, 2003), and Tukey-Kramer test (at  $P < 0.05$ ) was used to compare mean differences between treatments.

**Results**

Dietary inclusion of *Spirulina platensis* extract (0.4 and 0.6 g/kg) in the diet increased egg production, egg mass and feed intake compared to quails-fed diets without *Spirulina platensis* (Table 3,  $P < 0.05$ ). Feed conversion ratio (FCR) improved in the quails fed on the diet supplemented with *Spirulina platensis* extract (0.4 and 0.6 g/kg) ( $P < 0.05$ ). The addition of dexamethasone to the diet of laying quails decreased egg production, egg mass and feed intake ( $P < 0.05$ ). Birds fed on the diet containing dexamethasone showed greater FCR compared to birds fed diets without dexamethasone ( $P < 0.05$ ). The interaction effects of *Spirulina platensis* extract and dexamethasone on egg production, egg mass, feed intake and FCR were not significant.

The addition of *Spirulina platensis* extract (0.4 and 0.6 g/kg) to the diet caused a significant increase in egg weight, where the lowest egg weight was obtained in the quails-fed diets without *Spirulina platensis* (Table 4,  $P < 0.05$ ). *Spirulina platensis* extract (0.4 g/kg) improved albumen relative weight ( $P < 0.05$ ). The yolk index of the eggs from quails-fed diets supplemented with 0.6 g/kg of *Spirulina platensis* extract was significantly lower than the control quails ( $P < 0.05$ ). The addition of *Spirulina platensis* extract (0.4 and 0.6 g/kg) to the diet of laying quails caused a significant increase in the yolk color index ( $P < 0.05$ ). Shell thickness in the eggs of quails fed with a diet containing 0.4 and 0.6 g/kg of *Spirulina platensis* extract was significantly higher compared to quails-fed diets without *Spirulina platensis* ( $P < 0.05$ ). Egg weight, yolk color and shell thickness were significantly decreased in the eggs from quails fed with the diet containing dexamethasone ( $P < 0.05$ ). Shape index and yolk relative weight of the eggs from quails consumed

feeds with dexamethasone were significantly higher than the quails fed diets without dexamethasone ( $P < 0.05$ ). The interaction effects of *Spirulina platensis* extract with dexamethasone on yolk relative weight, yolk index and yolk color were significant ( $P < 0.05$ ). The highest yolk relative weight was observed in the treatment containing dexamethasone and without *Spirulina platensis* extract. Yolk index in quail eggs fed with a diet containing 0.6 g/kg of *Spirulina platensis* extract and without dexamethasone was significantly lower than the control group. The egg yolk color yolk of quails fed diets supplemented with 0.6 g/kg of *Spirulina platensis* extract and without dexamethasone was significantly higher than those from the control quails. Dietary inclusion of *Spirulina platensis* extract and dexamethasone had no effects on egg yolk cholesterol and triglyceride contents (Table 5).

Blood triglyceride levels decreased in the birds given the *Spirulina platensis* extract (0.4 g/kg) supplemented diet ( $P < 0.05$ , Table 6). The addition of dexamethasone (1 g/kg of diet) to the diet decreased serum albumin ( $P < 0.05$ ). The interaction effect of *Spirulina platensis* with dexamethasone on serum albumin and calcium was significant ( $P < 0.05$ ). The highest amount of albumin was obtained in the serum of quails fed with a diet containing *Spirulina platensis* extract (0.4 g/kg) and without dexamethasone, and the lowest amount of albumin was observed in the serum of birds receiving 0.4 g/kg of *Spirulina platensis* extract and dexamethasone (1 g/kg of diet). The amount of calcium in the serum of quails receiving a diet supplemented with 0 g/kg of *Spirulina* and 1 g/kg of dexamethasone was higher than the group that used a diet containing 1 g/kg of dexamethasone and 0.6 g/kg of *Spirulina*.

**Table 3.** Effects of *Spirulina platensis* extract on productive performance of laying quails under stress induced by dexamethasone

Items	EP (%)	EM (g/bird per d)	ADFI (g/bird)	FCR (g feed/g egg)
SPE (g/kg)				
0	60.77 <sup>b</sup>	6.53 <sup>b</sup>	27.18 <sup>b</sup>	4.11 <sup>a</sup>
0.4	74.53 <sup>a</sup>	8.46 <sup>a</sup>	28.83 <sup>a</sup>	3.50 <sup>b</sup>
0.6	76.63 <sup>a</sup>	8.70 <sup>a</sup>	29.66 <sup>a</sup>	3.45 <sup>b</sup>
SEM	0.811	0.090	0.232	0.047
P-value	<.0001	<.0001	<.0001	<.0001
DEX (g/kg)				
0	77.08 <sup>a</sup>	8.88 <sup>a</sup>	30.18 <sup>a</sup>	3.43 <sup>b</sup>
1	64.21 <sup>b</sup>	6.91 <sup>b</sup>	26.94 <sup>b</sup>	3.95 <sup>a</sup>
SEM	0.662	0.073	0.189	0.039
P-value	<.0001	<.0001	<.0001	<.0001
SPE × DEX				
0 × 0	67.85	7.48	28.84	3.88
0 × 1	53.69	5.58	25.52	4.35
0.4 × 0	79.64	9.39	30.10	3.24
0.4 × 1	69.43	7.53	27.57	3.77
0.6 × 0	83.74	9.77	31.59	3.16
0.6 × 1	69.52	7.62	27.73	3.73
SEM	1.146	0.128	0.329	0.067
P-value	0.162	0.495	0.156	0.750

EP, egg production; EM, egg mass; ADFI, average daily feed intake; FCR, feed conversion ratio; SPE, *Spirulina platensis* extract (0, 0.4 and 0.6 g/kg of diet); SEM, standard error of the mean.; DEX, dexamethasone (0 and 1 g/kg of diet).

<sup>a,c</sup> Different superscripts within a column indicate a significant difference ( $P < 0.05$ ).

**Table 4.** Effects of *Spirulina platensis* extract on egg quality of laying quails under stress induced by dexamethasone

Items	EW(g)	SI(%)	RAW	RYW	RSW	YI(%)	AI(%)	YC	HU	SW(g)	ST(mm)
SPE (g/kg)											
0	10.79 <sup>b</sup>	77.30	57.17 <sup>b</sup>	32.44	8.70	42.91 <sup>a</sup>	9.66	7.35 <sup>b</sup>	89.08	0.91	0.19 <sup>b</sup>
0.4	11.34 <sup>a</sup>	77.52	60.34 <sup>a</sup>	32.08	8.15	41.00 <sup>b</sup>	10.01	7.85 <sup>a</sup>	89.20	0.91	0.20 <sup>a</sup>
0.6	11.33 <sup>a</sup>	77.01	58.73 <sup>ab</sup>	32.58	8.36	40.36 <sup>b</sup>	10.04	8.00 <sup>a</sup>	88.61	0.95	0.20 <sup>a</sup>
SEM	0.070	0.813	0.817	0.401	0.191	0.473	0.206	0.083	0.735	0.029	0.004
P-value	<.0001	0.907	0.043	0.673	0.155	0.003	0.368	<.0001	0.840	0.556	0.010
DEX (g/kg)											
0	11.54 <sup>a</sup>	76.08 <sup>b</sup>	59.45	31.68 <sup>b</sup>	8.24	41.85	9.95	7.92 <sup>a</sup>	89.21	0.95	0.20 <sup>a</sup>
1	10.76 <sup>b</sup>	78.47 <sup>a</sup>	58.05	33.05 <sup>a</sup>	8.57	41.00	9.85	7.54 <sup>b</sup>	88.72	0.90	0.19 <sup>b</sup>
SEM	0.057	0.663	0.667	0.328	0.156	0.386	0.168	0.068	0.600	0.024	0.003
P-value	<.0001	0.020	0.155	0.008	0.148	0.136	0.692	0.0009	0.567	0.146	0.010
SPE × DEX											
0 × 0	11.19	75.69	58.60	30.80 <sup>b</sup>	8.27	44.82 <sup>a</sup>	9.67	7.32 <sup>c</sup>	90.82	0.89	0.19
0 × 1	10.39	78.92	55.74	34.08 <sup>a</sup>	9.12	41.01 <sup>b</sup>	9.65	7.37 <sup>c</sup>	87.34	0.92	0.18
0.4 × 0	11.82	76.45	60.08	32.24 <sup>ab</sup>	8.13	41.01 <sup>b</sup>	10.21	8.17 <sup>ab</sup>	88.37	0.99	0.21

**Table 4.** Effects of *Spirulina platensis* extract on egg quality of laying quails under stress induced by dexamethasone

Items	EW(g)	SI(%)	RAW	RYW	RSW	YI(%)	AI(%)	YC	HU	SW(g)	ST(mm)
0.4 × 1	10.86	78.59	60.60	31.93 <sup>ab</sup>	8.17	40.99 <sup>b</sup>	9.82	7.52 <sup>c</sup>	90.03	0.83	0.19
0.6 × 0	11.63	76.11	59.65	32.01 <sup>ab</sup>	8.30	39.72 <sup>b</sup>	9.98	8.27 <sup>a</sup>	88.44	0.96	0.21
0.6 × 1	11.04	77.91	57.80	33.15 <sup>ab</sup>	8.42	41.00 <sup>b</sup>	10.10	7.72 <sup>bc</sup>	88.78	0.94	0.20
SEM	0.099	1.149	1.156	0.568	0.270	0.669	0.291	0.118	1.040	0.041	0.005
P-value	0.209	0.808	0.347	0.018	0.284	0.003	0.673	0.017	0.060	0.103	0.913

EW, egg weight; SI, shape index; RAW, relative albumen weight; RYW, relative yolk weight; RSW, relative shell weight; YI, yolk index; AI, albumen index; YC, yolk color; HU, haugh unit; SW, shell weight; ST, shell thickness; SPE, *Spirulina platensis* extract (0, 0.4 and 0.6 g/kg of diet); SEM, standard error of the mean.; DEX, dexamethasone (0 and 1 g/kg of diet).

<sup>a,c</sup>Different superscripts within a column indicate a significant difference ( $P < 0.05$ ).

**Table 5.** Effects of *Spirulina platensis* extract on egg yolk cholesterol and triglyceride contents of quails under stress induced by dexamethasone

Items	Cholesterol (mg/g)	Triglyceride (mg/g)
SPE (g/kg)		
0	43.2	424.63
0.4	53.00	388.88
0.6	43.25	443.88
SEM	5.558	44.731
P-value	0.378	0.683
DEX (g/kg)		
0	48.66	456.50
1	44.33	381.75
SEM	4.538	36.523
P-value	0.508	0.165
SPE × DEX		
0 × 0	38.75	429.75
0 × 1	47.75	419.50
0.4 × 0	54.50	424.75
0.4 × 1	51.50	353.00
0.6 × 0	52.75	515.00
0.6 × 1	33.75	372.75
SEM	7.860	33.2597
P-value	0.229	0.589

SPE, *Spirulina platensis* extract (0, 0.4 and 0.6 g/kg of diet); SEM, standard error of the mean.; DEX, dexamethasone (0 and 1 g/kg of diet).

**Table 6.** Effects of *Spirulina platensis* extract on blood constituents of laying quails under stress induced by dexamethasone

Items	TG (mg/dL)	TC (mg/dL)	HDL (mg/dL)	TP (g/dL)	ALB (g/dL)	UA (mg/dL)	Ca (mg/dL)	P (mg/dL)
SPE (g/kg)								
0	2886.88 <sup>a</sup>	271.56	41.34	4.37	2.13	5.19	32.37	7.73
0.4	1705.91 <sup>b</sup>	210.38	45.58	4.12	2.36	5.59	33.25	5.78
0.6	2841.03 <sup>a</sup>	236.06	46.11	4.31	2.23	5.11	28.37	6.71
SEM	205.22	20.487	4.479	0.235	0.122	0.425	2.501	0.550
P-value	0.017	0.118	0.715	0.736	0.443	0.698	0.361	0.442
DEX (g/kg)								
0	2498.62	230.21	43.86	4.29	2.40 <sup>a</sup>	5.39	32.50	7.10
1	2457.27	248.46	44.82	4.25	2.08 <sup>b</sup>	5.21	30.16	6.38
SEM	201.05	16.727	3.657	0.192	0.099	0.347	2.045	0.861
P-value	0.904	0.444	0.855	0.891	0.033	0.721	0.430	0.559
SPE × DEX								
0 × 0	2950.95	250.38	44.52	4.22	2.07 <sup>ab</sup>	5.43	28.25 <sup>a</sup>	8.12
0 × 1	2822.82	292.75	46.64	4.52	2.20 <sup>ab</sup>	4.96	36.50 <sup>a</sup>	7.35
0.4 × 0	2025.08	206.88	42.38	4.25	2.82 <sup>a</sup>	5.72	35.50 <sup>a</sup>	5.97
0.4 × 1	1386.74	213.88	40.29	4.40	1.90 <sup>b</sup>	5.47	31.00 <sup>a</sup>	5.60
0.6 × 0	2519.82	233.37	44.70	4.00	2.32 <sup>ab</sup>	5.02	33.75 <sup>a</sup>	7.22
0.6 × 1	3162.25	238.75	47.53	4.23	2.15 <sup>ab</sup>	5.20	23.00 <sup>b</sup>	6.20
SEM	217.51	28.97	6.334	0.332	0.172	0.601	3.542	1.492
P-value	0.326	0.772	0.916	0.679	0.020	0.862	0.043	0.976

SPE, *Spirulina platensis* extract (0, 0.4 and 0.6 g/kg of diet); SEM, standard error of the mean.; DEX, dexamethasone (0 and 1 g/kg of diet); TG, triglycerides; TC, total cholesterol; HDL, high density lipoprotein; TP, total protein, ALB, albumin; UA, uric acid; Ca, calcium; P, phosphorus.

<sup>a,c</sup>Different superscripts within a column indicate a significant difference ( $P < 0.05$ ).

## Discussion

Dexamethasone is a synthetic glucocorticoid with immunosuppressive and anti-inflammatory effects that is used to induce stress in animals (Eid *et al.*, 2008; Duff *et al.*, 2019). Results of the current experiment show that the birds receiving long term dietary dex. Suffering considerable physiological stress indicated by decreased egg production, egg mass and feed intake. Furthermore, birds receiving the dex -dex-containing diet demonstrated greater FCR along with a significant decrease in egg weight. These results agree Miao *et al.* (2021) findings which attributed the dex-related adverse effects on birds' performance to impaired intestinal function and metabolism disruptions. It was also reported that, during physiological stress, increased production of free radicals cause destruction of lipids in cell walls, a phenomenon termed as oxidative stress (Moustafa *et al.*, 2021). In biological systems, the production of free radicals, especially reactive oxygen species, is an unavoidable issue, and with the activity of antioxidant defense mechanisms, their harmful effects are neutralized to some extent (Pasri *et al.*, 2024). Nevertheless, if the production of free radicals increases or the activity of antioxidant systems decreases, the damage caused by them increases and can lead to a decrease in performance and health indicators. It has been reported that the growth rate in broiler chickens under stress caused by dexamethasone decreased due to the breakdown of the lining of the intestinal wall and the decrease in the absorption rate of nutrients (Vicuna *et al.*, 2015). Fouad *et al.* (2016) revealed that stress-causing factors have a negative effect on the reproductive performance of laying hens by reducing feed consumption, ovary weight and oviduct length which may result in reduced egg production and egg weight. The use of dexamethasone caused a significant decrease in body weight, feed intake and increased feed conversion ratio (Ademou *et al.*, 2018). Stress-causing factors activate the hypothalamus-pituitary-adrenal axis and, as a result, can cause the release of corticotropin-releasing factor, which is a strong anorexic peptide in birds (Berenjian *et al.*, 2021). It was also demonstrated that dietary dexamethasone significantly reduced feed intake and daily weight gain (Osho and Adeola, 2020). Daily injections of dexamethasone in laying hens caused a significant decrease in egg production and egg weight and a significant increase in the amount of triglycerides, glucose and heterophil to lymphocyte ratio (Eid *et al.*, 2008).

The results of the present research showed that dietary *Spirulina platensis* extract improved production indicators such as feed conversion ratio and egg production. Microalgae are natural feeds with favorable nutritional value and therefore have been proposed as a promising compound in animal

feeding (Abdel-Moneim *et al.*, 2022). Microalgae is the source of antioxidant and antibiotic properties and for these reasons, microalgae can replace antibiotics as safe and natural feeds (Elbaz *et al.*, 2022). The effective role of microalgae on the activity of digestive enzymes, liver function and intestinal microbial population was stated among the reasons for the improvement of growth performance in birds fed with *Spirulina platensis* extract (Alghamdi *et al.*, 2024; Abdelfatah *et al.*, 2024). It was shown that adding Spirulina to the diet of Japanese quails increased body weight gain (Abdel-Hack *et al.*, 2024). It has also been reported that algae derivatives had a positive effect on the reproductive performance of laying quails (Rahmatnejad *et al.*, 2024), the essential amino acid content or other beneficial compounds of microalgae that are beneficial to health may have a positive effect on production. On the other hand, microalgae affect the egg production process by changing the intestinal microbial population and reducing the production of toxic metabolites in the digestive system (Mens *et al.*, 2022). Spirulina increases the population of beneficial aerobic bacteria such as lactobacilli in the digestive tract by providing food for aerobic beneficial bacteria and, therefore, plays an effective role in improving the digestibility of nutrient sources and better use of feed components and improves the performance of poultry (Salvia *et al.*, 2014; Kang *et al.*, 2013; Park *et al.*, 2018). The improvement of growth and production indicators in poultry using microalgae can be, to some degree, attributable to the presence of bioactive peptides in these compounds. Bioactive peptides have many beneficial effects, such as antioxidant, antihypertensive, immune system regulation, anti-clotting, antimicrobial and cholesterol-lowering properties (Fan *et al.*, 2014).

In the present study, supplementing the diet of laying quails with *Spirulina platensis* extract improved the yolk color index and had positive effects on eggshell thickness. A wide range of physiological, genetic, nutritional and management factors influence egg quality. Eggs with bold yolks are more acceptable in the market, in other words, yolk color is an effective factor in the marketability of laying poultry products (Kotrbaček *et al.*, 2013). Different pigment sources can be used to create a suitable color index in the yolk. Of course, artificial pigment sources are used in most industrial poultry farms. If natural sources can be replaced with synthetic sources to create the right color in the yolk, it can create more confidence in the consumer (Del campo *et al.*, 2007). Feeding laying hens with a diet containing microalgae increased the color of the egg yolk (Park *et al.*, 2015). Feeding Japanese quail with Spirulina powder improved eggshell weight, probably due to increased intestinal calcium absorption (Oliveira *et al.*, 2024). Selim *et al.* (2018) stated that

dietary supplementation with microalgae powder had a positive impact on shell thickness quality. The effect of microalgae on shell thickness may be due to the mineral content of these compounds (Park *et al.*, 2015).

The amount of cholesterol in food sources is one of the important factors in consumer health, especially with regard to cardiovascular diseases. Researchers have tried to reduce the amount of cholesterol in egg yolk (Spence *et al.*, 2010). Previous studies showed that adding *Spirulina* to the diet of chickens reduced yolk cholesterol (Hajati and Zaghari, 2019). The results of the present research showed that dietary *Spirulina platensis* extract reduced serum triglyceride levels in quails. In a recent study, supplementing the diet of the heat-stressed Japanese quail with *Spirulina platensis* reduced the negative effects of stress on serum triglyceride levels (Nassar *et al.*, 2023). In this connection, it has been stated that the addition of *Spirulina* to the quail diet decreased serum triglycerides and cholesterol. This effect has been attributed to a pigment called phycocyanin (Abdel-Hack *et al.*, 2024). Mariey *et al.* (2012) observed that the inclusion of *Spirulina* microalgae in broiler diets increased the concentration of total protein, albumin,

glucose and globulin and decreased the triglyceride and cholesterol concentrations. The increased plasma albumin, globulin and total protein may be related to the high amount of protein in microalgae (An *et al.*, 2010). The richness of microalgae in terms of linoleic acid can lead to the reduction of serum lipids due to the role of this fatty acid in cholesterol and triglyceride catabolism (Deng and Chao, 2010).

## Conclusion

In general, the addition of dexamethasone to the diet of laying Japanese quails decreased egg production, feed intake and egg weight and increased the feed conversion ratio, so it can be said that the use of dexamethasone in feeding laying quails is effective in inducing stress, as well as supplementing the diet of quails with *Spirulina platensis* extract improves egg production and feed conversion ratio, and it is also effective in increasing the yolk color index and egg shell thickness.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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