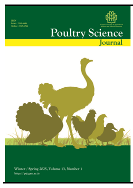

Poultry Science Journal
ISSN: 2345-6604 (Print), 2345-6566 (Online)
http://psj.gau.ac.ir

The Effect of *Spirulina platensis* and Vitamin C on Some Reproductive Parameters in Japanese Quails Under Induced Stress

Tahmine Hayatolgheibi, Seyyed Mojtaba Mousavi & Bahman Parizadian Kavan

Department of Animal Science, Faculty of Agriculture, Lorestan University, Khorramabad, Iran

Poultry Science Journal 2025, 13(1): 93-105

Keywords
Quail
Stress
Microalgae
Hatchability
Histomorphometry

Corresponding author
Seyyed Mojtaba Mousavi
Mousavi.sym@lu.ac.ir

Article history
Received: May 13, 2024
Revised: September 27, 2024
Accepted: October 10, 2024

Abstract

The objective was to determine the effects of dietary supplementation of *Spirulina platensis* (*Sp*) and Vitamin C (Vit C), as a potential strategy to alleviate the negative effects of stress on the bird's fertility. A total of 240 six-week-old quails were randomly assigned in a completely randomized design with a 3×2×2 factorial arrangement. The experimental treatments included different amounts of *Spirulina platensis* extract (zero, 0.4, and 0.6 g/kg), Vit C (zero and 300 mg/kg), and dexamethasone (DEX) (zero and 1 mg/kg). At the end of the experiment, an analysis was conducted on the biometric features of the oviduct and ovary, as well as the histomorphometry of the oviduct and uterus. Additionally, the concentrations of estrogen and progesterone hormones were measured, along with an assessment of fertility and hatchability rates. The quails that received a diet containing 0.4 g/kg of *Sp* exhibited a higher ovary height (8.42 mm; $P < 0.05$). Moreover, the administration of 1 mg/kg DEX and 0.4 g/kg *Sp* resulted in the highest weight and diameter of the magnum, along with the greatest isthmus diameter ($P < 0.05$). The consumption of Vit C had a positive effect on the uterine diameter and primary folds length in stressed quails. Also, The incorporation of *Sp* and Vit C led to an improvement in the primary fold diameter of the isthmus ($P < 0.05$). However, the treatments did not have any impact on the levels of estrogen and progesterone hormones, as well as the fertility and hatchability rates. In conclusion, the findings of this research indicate that the addition of *Spirulina* extract and Vit C to the diet had positive impacts on the morphology of the uterus and oviduct in quails experiencing physiological stress. However, these supplements did not show a notable influence on the reproductive capabilities of the quails.

Introduction

Birds, like other animals, frequently encounter a variety of stressors encompassing both social and environmental factors. The impact of stress on birds' reproductive capabilities and egg hatching is a significant concern within breeding populations (King'Ori, 2011). This is because certain modern breeding methods employed in aviculture can induce stress (Wang *et al.*, 2017).

Environmental stressors often result in the generation of reactive oxygen species (ROS) within the organism, causing oxidative stress and consequently diminishing the reproductive capacity of poultry (Agarwal *et al.*, 2005; Liu and Meng, 2019). When poultry experience stress, they exhibit specific physiological responses. One such response is the activation of the sympathetic adrenomedullary pathway (SAM), which leads to the release of catecholamines from the adrenal cortex (Lee *et al.*, 2022). Another response involves the activation of the hypothalamus-pituitary-adrenal (HPA) axis, which stimulates the release of glucocorticoids (such as cortisol and corticosterone) from the adrenal cortex. These glucocorticoids then initiate metabolic processes that contribute to the maintenance of the bird's physiological equilibrium or homeostasis (Lee *et al.*, 2022). Prolonged adrenocortical responses can lead to numerous negative impacts on both poultry productivity and animal welfare (Satterlee *et al.*, 2007).

Please cite this article as Tahmine Hayatolgheibi, Seyyed Mojtaba Mousavi & Bahman Parizadian Kavan. 2025. The Effect of *Spirulina platensis* and Vitamin C on Some Reproductive Parameters in Japanese Quails Under Induced Stress. Poult. Sci. J. 13(1) 93-105.

DOI: 10.22069/psj.2024.22533.2106

Research findings demonstrated that exogenous glucocorticoids led to developmental depression in cultured Japanese quail embryos at an early developmental phase (Kaltner *et al.,* 1993). Dexamethasone (DEX) is a man-made glucocorticoid that is employed to trigger oxidative stress and explore stress reactions in birds (Gao *et al.*, 2010; Njagi *et al.*, 2012; Berenjian *et al.*, 2021; Osho and Adeola, 2020; Bai *et al.*, 2023). In this research, DEX was utilized to mimic the actions of glucocorticoids and induce physiological stress (Osho and Adeola, 2020).

To support the success of poultry breeders, it is imperative to identify strategies that reduce avian stress and enhance the production of high-quality day-old chicks in poultry hatcheries (Schmidt *et al.*, 2009; Behboodi *et al.*, 2021). The use of antioxidant compounds, such as herbal extracts, vitamins, and minerals has demonstrated the ability to mitigate oxidative stress and enhance bird's reproduction performance (Agarwal *et al.*, 2005; Ruder *et al.*, 2008; Abd El-Hack *et al.*, 2020; Khalil-Khalili *et al.*, 2021).

Spirulina platensis, is known for its antioxidant activity due to the presence of phenolic compounds and natural pigments such as β-carotene, β-cryptoxanthin, chlorophyll, equinone, myxo xanthophyll, phycoerythrin, phycocyanin, xanthophylls, and xanthine (Hajati and Zaghari, 2019). Additionally, the addition of *Spirulina platensis* (*Sp*) to the diet of broiler chickens has been found to alleviate the negative effects caused by high ambient temperature at a biochemical level (Mirzaie *et al.*, 2018). Aljumaily and Taha (2019) conducted a study demonstrating that the incorporation of *Spirulina* extract into Japanese quail embryos decreases oxidative stress and enhances the functionality of the immune system during the incubation stage (Aljumaily and Taha, 2019). The ability of *Sp* to combat oxidative stress in heat-stressed quails and its positive impact can be attributed to the presence of polyphenolic and flavonoid compounds, which exhibit antioxidant activity and are abundantly present in *Sp* (Nassar *et al.*, 2023).

Vitamin C (Vit C) is recognized as a vital antioxidant, and although birds can produce it within their bodies, this synthesis may be insufficient in times of stress. As a result, it is advisable to include Vit C supplementation in the diet of broilers during stressful conditions (Khan *et al.*, 2012; Rafiee *et al.*, 2016). This study focused on contrasting *Spirulina* with Vit C, a renowned antioxidant, and investigating how their supplementation influences quail fertility. Therefore, the objective of the present study was to determine the effects of dietary supplementation of *Spirulina* extract and Vit C on the reproductive parameters of Japanese quails under induced stress by DEX.

Materials and methods

Birds and management

In this study, a total of 240 six wk-old Japanese quail (*Coturnix japonica*) were randomly allocated in a fully randomized design with a factorial arrangement of 3×2×2, comprising 12 treatments, four replicates, and five birds per group (four females and one male each) for an 8 wk experimental period. The birds were housed in 48 wired-floor cages, each measuring 35 cm in length, 35 cm in diameter, and 30 cm in height. The ambient temperature was maintained at 22 ± 1℃. The birds were exposed to a 16-h light/10-h dark with a relative humidity of 55±5% until the end of the experiment (14-week-old). Throughout the experimental period, the birds had *ad libitum* access to feed and fresh water. The diets were formulated according to the requirements specified by the National Research Council (NRC, 1994; Table 1). The experimental groups were subjected to various levels of the factors, including *Spirulina* extract at three levels (zero, 0.4, and 0.6 g/kg of the diet), Vit C (Royan darou Co., Tehran, Iran) at two levels (zero and 300 mg/kg of the diet), and DEX (Iran Hormone Co., Iran) at two levels (zero and 1 g/kg of the diet). Stress was induced in the birds through dietary supplementation of DEX (1 mg/kg) (Osho and Adeola, 2020). DEX tablets were dissolved in oil and incorporated into a basic diet. The basic diet was first prepared by mixing all ingredients except Vit C, DEX, and *Sp* extract. This mixture was then divided into 12 parts and the amounts of Vit C, DEX, and *Sp* extract corresponding to each treatment were added to each portion, followed by further mixing. All treatments lasted for 8 successive weeks.

Preparation of *Spirulina* extract

To *Spirulina* extract, 10 g of ground algae powder and 100 mL of 80% methanol solvent were added to an Erlenmeyer flask, which was then placed on a shaker for 72 hours at room temperature. Afterward, the mixture was filtered through filter paper. To concentrate the extract and eliminate the methanol, the methanol-containing solution was transferred to a rotary evaporator under vacuum conditions (Modaresi, 2012). Table 2 displays the total phenolic content, flavonoids, antioxidant activity, and DPPH (2,2-diphenyl-1-picrylhydrazyl) assay results of the *Spirulina* extract.

able 1. Ingredients and chemical composition of basal diet

Ingredient	Amount (%)
Corn	55.42
Soybean meal (44% CP)	35.07
Fish meal (60% CP)	3.00
Corn oil	0.16
Wheat bran	1.10
Common salt	0.30
Dicalcium phosphate	1.44
DL-Met, 99%	0.15
Mineral premix[1]	0.25
Vitamin premix[2]	0.25
CaCO3	2.86
Total	100
Calculated nutrient content	
ME (kcal/kg)	2997
CP (%)	22.23
Crude fiber (%)	3.25
Crude fat (%)	5.77
Ash (%)	3.97
Methionine (%)	0.65
Met + Cys (%)	0.87
Lysine (%)	1.22
Calcium (%)	2.50
Available phosphorus (%)	0.57
Sodium (%)	0.16

[1] Provides per kilogram of diet: Copper ($CuSO_4.5H_2O$), 4 mg; iodine (KI), 2 mg; iron ($FeSO_4.7H_2O$), 50 mg; manganese ($MnSO_4.H_2O$), 45 mg; Zn (ZnO), 70 mg; and cobalt, 0.25 mg.
[2] Provides per kilogram of diet: vitamin A (retinyl acetate), 16,000 IU; vitamin D3 (cholecalciferol), 4,000 IU; vitamin E (DL-a -tocopheryl acetate), 200 IU; vitamin K3 (menadione), 8.0 mg; vitamin B1 (thiamin), 3.0 mg; vitamin B2 (riboflavin), 10 mg; vitamin B3 (niacin), 60 mg; vitamin B5 (Dpantothenic acid), 13 mg; vitamin B6 (pyridoxine), 4 mg; vitamin B9 (folic acid), 3 mg; vitamin H2 (biotin), 0.25 mg; vitamin B12 (cobalamin), 0.03 mg.

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

Items	*Spirulina* extract
Total phenols (mg/g)	20.15
Total flavonoid (mg/g)	5.56
FRAP (g of $FeSO_4$/100 g)	78.65
DPPH˙scavenging activity (%)	46.34

FRAP, ferric reducing ability of plasma. DPPH, 1,1-diphenyl- 2-picrylhydrazyl.

Biometric parameters of the liver, oviduct, and ovary

At the end of the experiment, two Japanese quails from each replicate (8 / treatment) were humanely slaughtered. The liver, oviduct, and ovaries were removed and weighed with a digital scale, while their dimensions were measured using a digital caliper (Rafieian-Naeini *et al.*, 2021).

Histomorphometry of magnum, isthmus, and uterus

In the final week of the experiment, histological analysis was conducted on the magnum, isthmus, and uterus of two randomly selected birds in each replication (six birds per treatment). To prepare the tissue samples for analysis, two-centimetre sections of the oviduct, including the magnum, isthmus, and uterus, were collected. The tissue specimens were subsequently immersed in 10% neutral buffered formalin for fixation before being prepared for paraffin embedding. Following embedding, the tissues were sliced into 7 µm thick sections using a microtome (DIA PATH®, SDSGS9001, Italy). These sections were subsequently stained with hematoxylin and eosin (H&E) to facilitate histological observations. To evaluate the height and thickness of the magnum and isthmus, a light microscope at X40 magnification was used. The scale was adjusted, and measurements were made using ISCapture software (Rafieian-Naeini *et al.*, 2021).

Table 3. Morphometric characteristics of the liver, ovary, and uterus of quails received *Spirulina platensis* and Vitamin C under induced stress.

Parameter	Live weight (g)	Liver weight (g)	Ovary weight(g)	Ovary length (mm)	Ovary diameter(mm)	Ovary height (mm)	Uterus weight (g)	Uterus length (mm)	Uterus diameter (mm)	Uterus height (mm)
Spirulina (g/kg)										
0	191.94 [b]	7.01	0.98	19.18	11.68	7.70 [ab]	2.04	37.46	16.07	6.10
0.4	255.55 [a]	7.01	1.13	19.63	13.10	8.42 [a]	1.94	33.20	16.53	6.39
0.6	244.99 [a]	6.98	0.88	18.50	12.03	7.38 [b]	1.90	33.43	14.64	0.34
SEM	8.47	0.31	0.12	0.85	0.66	0.314	0.14	2.07	0.82	
Vit C (mg/kg)										
0	220.48 [b]	7.04	0.93	18.77	12.01	7.81	2.00	35.40	15.55	6.39
300	241.10 [a]	6.97	1.07	19.44	12.53	7.85	1.93	33.99	15.94	0.28
SEM	6.91	0.25	0.10	0.70	0.54	0.25	0.12	1.69	0.67	
DEX (mg/kg)										
0	242.31 [a]	7.15	1.03	19.16	12.28	8.04	1.88	34.53	23.00	6.68
1	219.28 [b]	6.85	0.96	19.06	12.26	7.62	2.04	34.86	16.26	0.28
SEM	6.91	0.25	0.10	0.70	0.54	0.25	0.12	1.69	0.67	
P-value										
Spirulina	0.001	0.9954	0.3602	0.6492	0.3071	0.0676	0.77	0.27	0.25	0.32
Vit C	0.042	0.8479	0.3304	0.5070	0.4987	0.9202	0.68	0.56	0.68	0.76
DEX	0.024	0.4067	0.6366	0.9170	0.9846	0.2502	0.33	0.89	0.28	0.25
*Spirulina**Vit C	0.044	0.7142	0.9163	0.8812	0.7594	0.7446	0.70	0.82	0.06	0.98
*Spirulina**DEX	0.959	0.7177	0.0741	0.0153	0.2975	0.7298	0.10	0.71	0.21	0.44
Vit C*DEX	0.190	0.60	0.27	0.42	0.35	0.40	0.16	0.97	0.09	0.97
*Spirulina**Vit C*DEX	0.022	0.2210	0.2525	0.5799	0.6774	0.0781	0.40	0.79	0.37	0.39

Means in the same column with different superscripts differ significantly ($P < 0.05$). *SEM* = Standard error of means.

The assessment of sex hormones

Following the completion of the experiment, two birds were randomly selected from each repetition. Blood samples were collected from slaughtered quails to assess the concentration of estrogen and progesterone hormones in female quails. Subsequently, the blood samples were subjected to centrifugation at 3,000 × g for 15 min. The plasma samples were then stored at a temperature of -20 °C to preserve their integrity for subsequent hormone evaluation using commercially the ELISA kits (Monobind Inc. USA) (Sharokhyan Rezaee *et al.*, 2022).

Fertility and hatchability

In the final three weeks of the experiment, the eggs were collected weekly (maintained in standard conditions of 12 ± 1 °C and relative humidity of 55%) and subsequently transferred to the incubator (Cocks® machine, Iran). After 14 days of incubation in the setter baskets at a temperature of 37.7°C and 65% humidity, the eggs were moved to the hatcher basket and kept at a temperature of 37.2°C and 70% humidity for three days. On the 17th day of incubation, after the hatching process, the number of eggs containing dead embryos was counted and the percentage of fertility was calculated (Rafieian-Naeini *et al.*, 2021).

Fertility (%) = [(eggs containing embryos + the number of hatched chicks)] / (the total number of eggs) × 100

Hatchability of fertile eggs (%) = (the number of hatched chicks) / (the number of fertile eggs) × 100

Hatchability of set eggs (%) = (the number of hatched chicks) / (the total number of eggs) × 100

Statistical analysis

To ensure the normal distribution of the data, Kolmogorov-Smirnov and Shapiro-Wilk tests were conducted. Continuous data were analyzed using the GLM method, while fertility, hatchability, and embryonic mortality data were analyzed using the GENMOD method. The analysis was performed using SAS 9.4 and means were compared by Duncan's multiple range test. $P < 0.05$ was considered as statistically significant.

Results

Biometric parameters of the liver, oviduct, and ovary

The findings indicate that the ingestion of 0.4 g/kg *Spirulina* resulted in an enhancement of ovary height (8.42 mm). Furthermore, ovary height was influenced by the interaction of Vit C and DEX. Also, the combination of 0.4 g/kg *Spirulina* and 1 mg/kg DEX led to the greatest ovary length (21.55 mm; $P < 0.05$). The ingestion of Vit C resulted in a beneficial impact on the uterus diameter in quails under physiological stress (Table 3).

However, the main factors (*Sp*, Vit C, and DEX) and their interaction did not have any significant effect on the parameters of weight, length, diameter, and height of the uterus ($P > 0.05$; Table 3). Table 4 reveals that the weight and diameter of the magnum, as well as the diameter of the isthmus, were influenced by the interaction between *Spirulina* and DEX.

Table 4. Morphometric characteristics of magnum and isthmus in female quails received *Spirulina platensis* and Vitamin C under induced stress.

Parameter	Magnum weight(g)	Magnum length (mm)	Magnum diameter (mm)	Magnum height (mm)	Isthmus weight (g)	Isthmus length (mm)	Isthmus diameter (mm)	Isthmus height (mm)
***Spirulina* (g/kg)**								
0	2.76	135.33	6.76	3.77	0.85	55.19	5.01	3.14
0.4	2.72	137.85	6.69	3.83	0.83	53.78	4.78	2.97
0.6	2.98	152.99	6.60	4.00	0.71	56.45	5.01	3.40
SEM	0.22	6.72	0.35	0.17	0.16	2.27	0.23	0.19
Vit C (mg/kg)								
0	2.87	141.82	6.52	3.87	0.823	54.95	4.93	3.19
300	2.77	142.29	6.84	3.86	0.77	55.32	4.88	3.15
SEM	0.18	5.48	0.29	0.14	0.13	1.85	0.19	0.16
DEX (mg/kg)								
0	2.72	140.26	6.41	3.94	0.63	54.48	4.78	3.09
1	2.92	143.85	6.95	3.79	0.96	55.7	5.03	3.24
SEM	0.18	5.48	0.29	0.14	0.13	1.85	0.19	0.16
P-value								
Spirulina	0.67	0.14	0.95	0.63	0.79	0.71	0.79	0.31
Vit C	0.67	0.95	0.44	0.98	0.81	0.88	0.84	0.87
DEX	0.45	0.64	0.19	0.46	0.09	0.62	0.38	0.51
*Spirulina**Vit C	0.64	0.14	0.71	0.97	0.30	0.11	0.63	0.90
*Spirulina** DEX	0.006	0.141	0.017	0.17	0.204	0.46	0.041	0.55
Vit C*DEX	0.29	0.65	0.42	0.42	0.58	0.85	0.21	0.75
*Spirulina**Vit C*DEX	0.32	0.71	0.82	58	0.32	0.60	0.53	0.66

SEM = Standard error of means.

The administration of 0.4 g/kg *Spirulina* and 1 mg/kg DEX resulted in the highest weight (3.37 g) and diameter of the magnum (7.76 mm), along with the greatest isthmus diameter (5.30 mm; $P < 0.05$; Table 5).

Table 5. The interaction between *Spirulina* and DEX on the weight and diameter of the magnum, as well as isthmus diameter and ovary length in quails received *Spirulina platensis* and Vitamin C under induced stress.

***Spirulina*×DEX**	**Magnum weight (g)**	**Magnum diameter (mm)**	**Isthmus diameter (mm)**	**Ovary length (mm)**
Sp0.0×DEX0	2.67 ^{ab}	6.53 ^{ab}	4.79 ^{ab}	19.47 ^{ab}
Sp0.0×DEX1	2.84 ^{ab}	6.93 ^{ab}	5.22 ^{ab}	18.90 ^{ab}
Sp0.4×DEX0	2.08 ^{b}	5.62 ^{b}	4.27 ^{b}	17.72 ^{b}
Sp0.4×DEX1	3.37 ^{a}	7.76 ^{a}	5.30 ^{a}	21.55 ^{a}
Sp0.6×DEX0	3.42 ^{a}	7.03 ^{ab}	5.29 ^{a}	20.29 ^{a}
Sp0.6×DEX1	2.54 ^{ab}	6.16 ^{b}	4.56 ^{ab}	16.72 ^{b}
SEM	0.31	0.50	0.33	1.21

Sp = *Spirulina* and DEX = dexamethasone, and the numbers written in front of them show the consumption amount of each of them. Means in the same column with different superscripts differ significantly ($P < 0.05$). *SEM* = Standard error of means.

Histomorphometry of magnum, isthmus, and uterus

The study also investigated the effects of DEX and the combined effect of *Spirulina* extract, Vit C, and DEX on the average histological parameters of the isthmus in female quails. The findings revealed that only the diameter of the primary folds in the isthmus was influenced by DEX and the interaction of *Spirulina*, Vit C, and DEX ($P < 0.05$; Table 6). The incorporation of *Spirulina* and Vit C led to an improvement in the primary fold diameter of the isthmus (Figure 1). Furthermore, the combined effect of 0.4 g/kg *Spirulina* and 1 mg/kg DEX had a positive impact on the magnum weight ($P < 0.05$; Table 7).

Table 6. Comparison of histological parameters of the isthmus in female quails received *Spirulina platensis* and Vitamin C under induced stress.

Parameter	**Isthmus weight (g)**	**Primary folds length (µm)**	**Primary folds diameter (µm)**	**Secondary folds length (µm)**	**Secondary folds diameter (µm)**	**Serosa layer diameter (µm)**
Spirulina (g/kg)						
0	0.85	1092.61	266.48	745.80	271.35	47.51
0.4	0.83	1092.8	267.53	745.81	270.16	47.71
0.6	0.71	1092.13	267.53	745.92	270.17	47.71
SEM	0.16	1.96	0.35	2.03	0.52	0.14
Vit C (mg/kg)						
0	0.82	1092.48	266.78	745.97	271.02	47.57
300	0.77	1092.06	267.58	745.71	27.10	47.71
SEM	0.13	1.60	0.28	1.65	0.43	0.12
DEX (mg/kg)						
0	0.63	1092.28	267.7642	746.696	270.1763	47.7133
1	0.96	1092.26	266.5996	744.995	270.9542	47.5788
SEM	0.13	1.60	0.4362	1.6594	0.4326	0.12054
P-value						
Spirulina	0.7996	0.9784	0.0658	0.9989	0.2022	0.5406
Vit C	0.8196	0.8536	0.0577	0.9135	0.1440	0.4350
DEX	0.973	0.9947	0.069	0.4733	0.2117	0.4350
Spirulina×Vit C	0.3069	0.9642	0.0413	0.9734	0.1198	0.5146
Spirulina×DEX	0.2045	0.9961	0.0007	0.9734	0.1916	0.4350
Vit C×DEX	0.5827	0.8467	0.0007	0.5666	0.0601	0.2612
Spirulina×Vit C×DEX	0.2096	0.9845	0.0001	0.7821	0.1916	0.2513

SEM = Standard error of means.

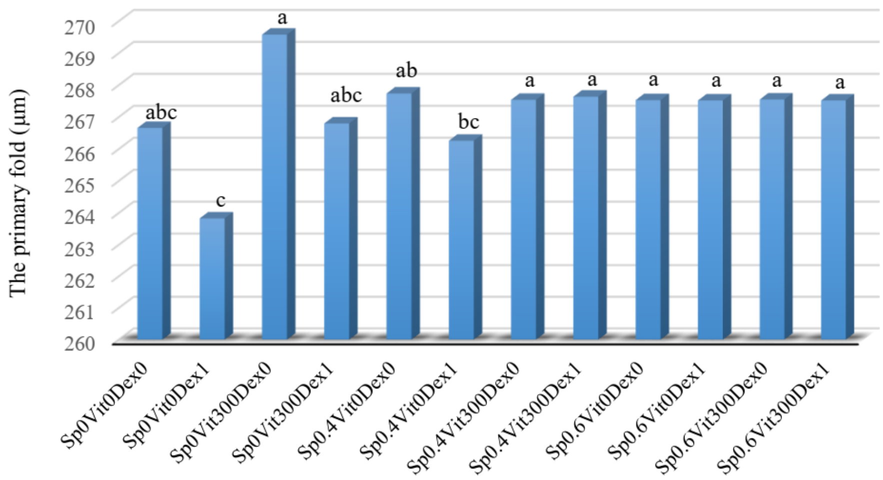

Figure 1. The interaction of *Spirulina*, DEX and Vit C on the diameter of the primary folds in the isthmus of female quails received *Spirulina platensis* and Vitamin C under induced stress. Sp = *Spirulina*, Vit C = Vitamin C, DEX = dexamethasone, and the numbers written in front of them show the consumption amount of each of them. Means in the same column with different superscripts differ significantly ($P < 0.05$).

Table 7. Comparison of histological parameters of magnum in female quails received *Spirulina platensis* and Vitamin C under induced stress.

Parameter	Magnum weight (g)	Primary folds length (µm)	Primary folds diameter (µm)	Secondary folds length (µm)	Secondary folds diameter (µm)	Serosa layer diameter (µm)
Spirulina (g/kg)						
0	2.76	976.98	521.41	449.26	431.66	25.76
0.4	2.72	976.03	52.58	449.9	430.95	25.76
0.6	2.98	976.02	52.55	449.09	430.92	25.77
SEM	0.22	8.68	5.10	1.83	1.73	0.02
Vit C (mg/kg)						
0	2.87	976.49	521.07	449.21	431.29	25.76
300	2.77	976.19	52.62	449.08	431.05	25.76
SEM	0.18	7.09	4.16	1.49	1.41	0.02
DEX (mg/kg)						
0	2.92	976.13	52.75	449.34	431.29	25.76
1	2.72	976.56	52.94	448.96	431.06	25.77
SEM	0.18	7.09	4.16	1.49	1.41	0.02
P-value						
Spirulina	0.6796	0.9960	0.9909	0.9969	0.9434	0.9854
Vit C	0.6771	0.9960	0.9386	0.9527	0.9052	0.9372
DEX	0.4510	0.9658	0.9735	0.8600	0.9094	0.8335
*Spirulina**Vit C	0.6455	0.9658	0.9956	0.9964	0.9900	0.9979
*Spirulina**DEX	0.0060	0.9993	0.9993	0.9731	0.9875	0.9704
Vit C*DEX	0.2907	0.9464	0.9581	0.9104	0.9285	0.8955
Spirulina×Vit C×DEX	0.3116	0.9997	0.9998	0.9983	0.9988	0.9982

SEM = Standard error of means.

Figure 2 illustrates the light micrograph of the magnum, isthmus, uterus, and testis tissues. The primary fold, secondary fold, serous layer, and seminiferous tube are depicted in the sections.

The comparison of the mean histological characteristics of the uterine revealed that solely the primary fold length and the secondary fold length were affected by the administration of Vit C ($P < 0.05$). Consequently, it can be inferred that Vit C exhibited a beneficial impact on the primary and the secondary fold length.

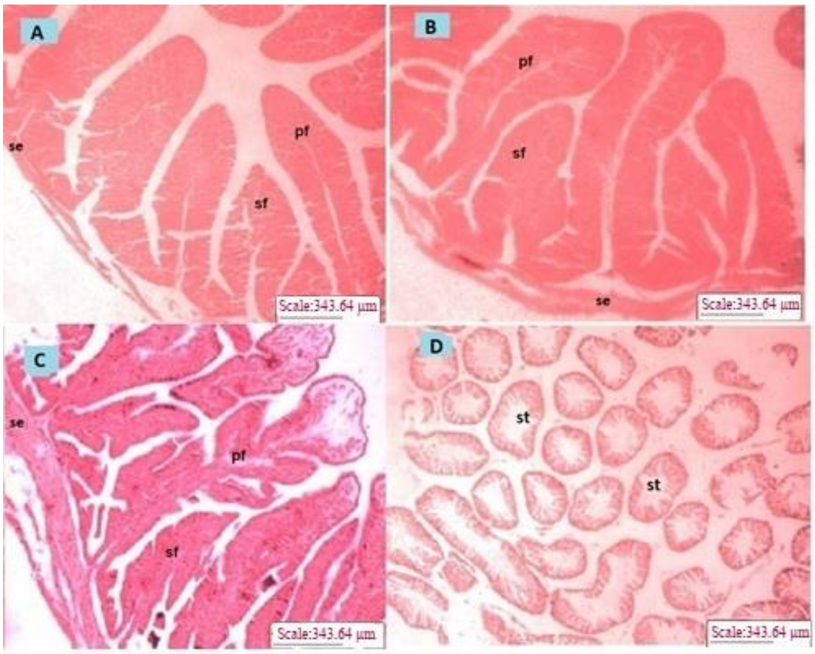

Figure 2. Light micrograph of magnum (A), isthmus (B), uterus (C), and testis (D) tissues in female quails received *Spirulina platensis* and Vitamin C under induced stress. pf; primary fold, sf; secondary fold, se; serous layer and st; seminiferous tube.

The interactions between *Spirulina* and Vit C, along with Vit C and DEX, were found to have notable impacts on the morphology of the uterus (Table 8). Vit C intake positively influenced ($P < 0.05$) the length of uterine primary folds in quails under stress (Table 8).

Table 8. Mean histological parameters of the uterus in female quails received *Spirulina platensis* and Vitamin C under induced stress.

Parameter	Uterus weight (g)	Primary folds length (µm)	Primary folds diameter (µm)	Secondary folds length (µm)	Secondary folds diameter (µm)	Serosa layer diameter (µm)
Spirulina (g/kg)						
0	2.04	1607.20	293.19	943.26	242.28	64.37
0.4	1.94	1569.50	252.59	818.10	234.24	52.89
0.6	1.90	1394.90	268.53	832.84	239.35	61.47
SEM	0.14	90.63	14.91	65.62	17.40	4.31
Vit C (mg/kg)						
0	2.00	1368.0 [b]	277.21	772.18 [b]	239.75	59.64
300	1.93	1679.6 [a]	266.03	961.67 [a]	237.84	59.44
SEM	0.12	74.00	12.17	53.63	14.25	3.52
DEX (mg/kg)						
0	1.88	1488.3	262.05	848.90	215.44 [b]	62.80
1	2.04	1563.3	280.56	888.44	261.05 [a]	56.41
SEM	0.12	74.00	12.17	53.63	14.25	3.52
P-value						
Spirulina	0.25	0.21	0.15	0.28	0.84	0.15
Vit C	0.68	0.04	0.54	0.02	0.79	0.97
DEX	0.28	0.43	0.29	0.67	0.03	0.21
Spirulina×Vit C	0.06	0.21	0.20	0.32	0.01	0.03
Spirulina×DEX	0.21	0.12	0.91	0.10	0.71	0.30
Vit C×DEX	0.09	0.04	0.21	0.10	0.07	0.90
Spirulina×VitC×DEX	0.86	0.24	0.48	0.31	0.15	0.34

Means in the same column with different superscripts differ significantly ($P < 0.05$). *SEM* = Standard error of means.

Fertility, hatchability, and sex hormones

Table 9 indicates that the weight of newly hatched chicks remained unaffected by Vit C, DEX, and their interaction ($P > 0.05$). However, the use of DEX negatively impacted the fertility and total yield of chickens ($P < 0.05$). The results of our study indicate that the administration of *Spirulina*, Vit C, DEX, and their combination did not have a significant impact on the sex hormone levels ($P > 0.05$; Table 9).

Table 9. Comparison of fertility parameters and sex hormone levels of quails received *Spirulina platensis* and Vitamin C under induced stress.

Parameter	Chickens weight (g)	Fertility (%)	Hatchability of fertile eggs (%)	Hatchability of set eggs(%)	Estradiol (pg/ml)	Progesterone (ng/ml)
Spirulina (g/kg)						
0	7.6954	0.3081	0.3306	0.2375	322.92	2.34
0.4	8.366	0.2363	0.3281	0.2100	356.88	2.34
0.6	7.8676	0.2594	0.1356	0.1194	342.32	2.33
SEM	0.1951	0.088	0.081	0.073	27.26	0.01
Vit C (mg/kg)						
0	7.7931	0.334	0.329	0.224	326.46	2.34
300	8.129	0.2017	0.200	0.153	354.96	2.34
SEM	0.1535	0.072	0.066	0.059	22.26	0.01
DEX (mg/kg)						
0	7.8835	0.421 [a]	0.358	0.282 [a]	345.18	2.34
1	7.8237	0.114 [b]	171	0.095 [b]	336.24	2.34
SEM	0.1432	0.072	0.066	0.059	22.26	0.01
P-value						
Spirulina	0.460	0.843	0.170	0.497	0.67	0.83
Vit C	0.904	0.204	0.179	0.405	0.37	0.65
DEX	0.308	0.004	0.055	0.032	0.77	0.65
Spirulina×Vit C	0.338	0.858	0.796	0.594	0.80	0.83
Spirulina×DEX	0.153	0.848	0.501	0.909	0.63	0.83
Vit C×DEX	0.229	0.163	0.244	0.29	0.10	0.65
Spirulina×VitC*DEX	0.740	0.361	0.586	0.600	0.26	0.83

Means in the same column with different superscripts differ significantly ($P < 0.05$). *SEM* = Standard error of means.

Discussion

Biometric parameters of the liver, oviduct, and ovary

Liver damage and abnormal lipid metabolism caused by heat stress can negatively impact steroid metabolism and vitellogenesis, leading to a decrease in the weight and number of eggs (Wang *et al.*, 2017). To mitigate reduced fertility during stress, protecting the liver during oxidative stress can be a potential solution (Magnoli *et al.*, 2012). Interestingly, the present study found that liver weight was not affected by any of the main factors of the experiment and their interaction. Chronic administration of corticosterone did not result in any adverse effects on the quail liver (Magnoli *et al.*, 2012). *Spirulina platensis* has mitigated oxidative stress and apoptosis in the liver of animals that were fed diets contaminated with aflatoxin (García-Martínez *et al.*, 2007). In a study conducted by Abd Elzaher *et al.* (2023), the *Spirulina*-fed quails demonstrated lower levels of liver enzyme (ALT) and kidney function markers (Creatinine and urea) compared to the control group (Abd Elzaher *et al.*, 2023). In the current study, the combination of 0.4 *Spirulina* demonstrated the most favorable impact on ovary length under stressful conditions. However, the primary factors of the experiment did not exert any influence on the weight, length, and diameter of the ovary. It is plausible that the levels of glucocorticoids utilized in this study were insufficient to induce alterations in ovarian quantity.

Histomorphometry of magnum, isthmus, and uterus

In this study, the addition of *Spirulina* and Vit C resulted in an enhancement of the primary folds diameter of the isthmus. The positive effects of vitamin C on the isthmus can be linked to an enhanced antioxidant response during stressful situations (Eladl *et al.*, 2022). Consistent with our results, the application of CoQ10, an antioxidant, resulted in a notable enhancement in the thickness and height of isthmus and magnum folds in comparison to the control group (Rafieian-Naeini *et al.*, 2021). *Spirulina platensis* is rich in beneficial fats like oleic acid and maintains an optimal balance between omega-3 and omega-6 fatty acids (Mariey *et al.*, 2012). In a study by Berenjian *et al.* (2021), it was revealed that incorporating omega-3 fatty acids into the diet had a positive influence on gut morphology in laying hens experiencing physiological stress. Nevertheless, the researchers found that these fatty acids were unable to counteract the detrimental effects of physiological stress on the hens' overall performance (Berenjian *et al.*, 2021).

Notably, it has been proposed that ACTH may possess additional activities beyond its adrenal functions (Tetel *et al.*, 2022). Consequently, ACTH might directly impact the oviduct or ovarian tissues, thereby modifying egg quality (Oluwagbenga *et al.*, 2023). However, this possibility was not explored in our investigation due to the inhibitory feedback of glucocorticoids on the secretion of ACTH from the diencephalon and pituitary. Moreover, the blue-green algae *Sp*, owing to its elevated levels of bioactive antioxidant compounds, has the potential to mitigate the detrimental effects of oxidative stress induced by high ambient temperatures (Moustafa *et al.*, 2021).

Fertility, hatchability, and sex hormones

In times of stress, the inhibitory effect of glucocorticoids on the hypothalamus and anterior pituitary can result in a reduction in the release of gonadotropin-releasing hormone (GnRH) in the hypothalamus. This disturbance can subsequently impact the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland (Zhang *et al.*, 2022). Furthermore, exposure to acute and chronic heat stress results in disrupted endocrine gland functions, reduced energy bioavailability in cells, and a negative effect on reproductive performance (Farghly *et al.*, 2018). Nevertheless, the findings from our study suggested that the administered treatments did not exert a substantial influence on the levels of sex hormones.

As mentioned, our study results demonstrated that the application of DEX had a detrimental effect on the fertility and hatchability of the set eggs. Nonetheless, the interaction between *Spirulina*, Vit C, and DEX did not produce a substantial effect on the fertility and hatchability results. The findings from this study aligned with those reported by Ahmadi Nia *et al.* (2021), which indicated that adding 0.6% *Spirulina* to the diets of laying hens did not affect the growth of ovarian follicles (Ahmadi Nia *et al.*, 2021). Similar to our results, the administration of CoQ10, as an antioxidant, did not yield a noteworthy impact on the hatchability and fertility of quails challenged with cadmium (Rafieian-Naeini *et al.*, 2021). Unlike our research findings, adding *Spirulina* to the feed or water of Japanese quails (Abouelezz, 2017; Habibi *et al.*, 2024) and laying hens (Mariey *et al.*, 2012; Samia *et al.*, 2018; Abd El Wahab *et al.*, 2019; Ismail *et al.*, 2023) improved fertility and hatchability rates. Furthermore, administering *Spirulina* extract to Japanese quail embryos during incubation was shown to lower oxidative stress and boost the immune system, which helps in directing energy towards hatching and supporting essential organs (Aljumaily and Taha, 2019). The ingestion of *Spirulina platensis* by rabbits has led to a rise in both the pregnancy and towining rates. Furthermore, it has resulted in an elevation of the plasma levels of E_2 and P_4 hoemones on the 15th day following mating (El-Ratel, 2017). This phenomenon could potentially be ascribed to the existence of all essential fatty acids (Mendes *et al.*, 2003), essential amino acids (Farag *et al.*, 2016), vitamins (Hoseini *et al.*, 2013), essential unsaturated fatty acids (Peiretti and Meineri, 2008; Singh *et al.*, 2023) and pigments (Bermejo *et al.*, 2008) in *Spirulina platensis*.

The differences noted in the results could be due to various factors, such as the amount of *Spirulina* given, the particular strain used, the methods of cultivation, the types of animals examined, and the variations in experimental settings. However, further investigations are essential to deepen our understanding of how *Spirulina* can alleviate the detrimental effects of stress on the reproductive processes of poultry.

Conclusion

In conclusion, the findings of this study demonstrated that the dietary inclusion of DEX, *Spirulina* extract, and Vit C in stressed quails did not have a significant effect on fertility and hatchability rates. However, it did exhibit certain beneficial effects on some of the biometric and histology parameters of reproductive organs.

Ethical Approval

All experimental procedures were conducted under the guidelines and regulations set by the Animal Ethics Committee of Lorestan University (LU. ECAR.2022.57), Iran.

Acknowledgments

We would like to thank the vice-chancellor for research at Lorestan University, for providing the facilities and the financial support to carry out this work.

References

Abd El-Hack ME, Abdelnour SA, Taha AE, Khafaga AF, Arif M, Ayasan T, Swelum AA, Abukhalil MH, Alkahtani S & Aleya L. 2020. Herbs as thermoregulatory agents in poultry: An overview. Science of the Total Environment, 703: 134399. DOI: 10.1016/j.scitotenv.2019.134399

Abd El Wahab E, Abaza I & Saad A. 2019. Effect of dietary supplementation of Spirulina Platensis and organic selenium on some biological traits of local laying hen under heat stress conditions. Egyptian Poultry Science Journal, 39: 207-217. DOI: 10.21608/EPSJ.2019.29817

Abd Elzaher HA, Ibrahim ZA, Ahmed SA, Salah AS, Osman A, Swelum AA, Suliman GM, Tellez-Isaias G, Alagawany M & Abd El-Hack ME. 2023. Growth, carcass criteria and blood biochemical parameters of growing quails fed

Arthrospira platensis as a feed additive. Poultry Science, 103205. DOI: 10.1016/j.psj.2023.103205

Abouelezz F. 2017. Evaluation of spirulina algae (Spirulina platensis) as a feed supplement for Japanese quail: nutiritional effects on growth performance, egg production, egg quality, blood metabolites, sperm-egg penetration and fertility. Egyptian Poultry Science Journal, 37: 707-719. DOI: 10.21608/epsj.2017.7535

Agarwal A, Gupta S & Sharma RK. 2005. Role of oxidative stress in female reproduction. Reproductive biology and endocrinology, 3: 1-21. DOI: 10.1186/1477-7827-3-28

Ahmadi Nia E, Mehri M & Shirmohammad F. 2021. Effects of dietary Spirulina platensis algae powder on performance, egg quality, ovarian follicles and immune system in Lohmann LSL laying hens. Iranian Journal of Animal Science, 51: 349-359. DOI: 10.22059/IJAS.2021.314088.653810

Aljumaily T & Taha A. 2019. Effects of Spirulina platensis algae extract early feeding on Japanese quail embryos. Advances in Animal and Veterinary Sciences, 7: 30-37. DOI: 10.17582/journal.aavs/2019/7.1.30.37

Bai K, Hao E, Huang C-x, Yue Q-x, Wang D-H, Shi L, Chen Y-f, Chen H & Huang Rl. 2023. Melatonin alleviates ovarian function damage and oxidative stress induced by dexamethasone in the laying hens through FOXO1 signaling pathway. Poultry Science, 102: 102745. DOI: 10.1016/j.psj.2023.102745

Behboodi HR, Sedaghat A, Baradaran A, & Nazarpak HH. 2021. The effects of the mixture of betaine, vitamin C, St John's wort (Hypericum perforatum L.), lavender, and Melissa officinalis on performance and some physiological parameters in broiler chickens exposed to heat stress. Poultry Science, 100: 101344. DOI: 10.1016/j.psj.2021.101344

Berenjian A, Sharifi SD, Mohammadi-Sangcheshmeh A & Bakhtiarizadeh MR. 2021. Omega-3 fatty acids reduce the negative effects of dexamethasone-induced physiological stress in laying hens by acting through the nutrient digestibility and gut morphometry. Poultry Science, 100: 100889. DOI: 10.1016/j.psj.2020.12.002

Bermejo P, Piñero E & Villar ÁM. 2008. Iron-chelating ability and antioxidant properties of phycocyanin isolated from a protean extract of Spirulinaplatensis. Food chemistry, 110: 436-445. DOI: 10.1016/j.foodchem.2008.02.021

El-Ratel I. 2017. Reproductive performance, oxidative status and blood metabolites of doe rabbits administrated with spirulina alga. Egypt Poultry Science, 37: 1153-1172. DOI: 10.21608/epsj.2017.5388

Eladl AH, Farag VM, El-Shafei RA, Aziza AE, Awadin WF & Arafat N. 2022. Immunological, biochemical and pathological effects of vitamin C and Arabic gum co-administration on H9N2 avian influenza virus vaccinated and challenged laying Japanese quails. BMC Veterinary Research, 18: 408. 8. DOI: 10.1186/s12917-022-03495-y

Farag MR, Alagawany M, El-Hack M & Kuldeep D. 2016. Nutritional and healthical aspects of Spirulina (Arthrospira) for poultry, animals and human. International Journal of Pharmacology, 12: 36-51. DOI: 10.3923/ijp.2016.36.51

Farghly M, Alagawany M & Abd El-Hack M. 2018. Feeding time can alleviate negative effects of heat stress on performance, meat quality and health status of turkey. British Poultry Science, 59: 205-210. DOI: 10.1080/00071668.2017.1413233

Gao J, Lin H, Wang X, Song Z & Jiao H. 2010. Vitamin E supplementation alleviates the oxidative stress induced by dexamethasone treatment and improves meat quality in broiler chickens. Poultry Science, 89: 318-327. DOI: 10.3382/ps.2009-00216

García-Martínez D, Rupérez FJ, Ugarte P & Barbas C. 2007. Tocopherol fate in plasma and liver of streptozotocin-treated rats that orally received antioxidants and Spirulina extracts. International Journal for Vitamin and Nutrition Research, 77: 263-271. DOI: 10.1024/0300-9831.77.4.263

Habibi H, Rahmatnejad E, Tohidifar SS, Afshar A, Kameli A, Jafari M & Mohammadi M. 2024. Improving performance, reproduction, and immunity in laying Japanese quail with algal derivatives. Poultry Science, 103: 103295. DOI: 10.1016/j.psj.2023.103295

Hajati H & Zaghari M. 2019. Effects of Spirulina platensis on growth performance, carcass characteristics, egg traits and immunity response of Japanese quails. Iranian Journal of Applied Animal Science, 9: 347-357.

Hoseini SM, Khosravi-Darani K & Mozafari MR. 2013. Nutritional and medical applications of spirulina microalgae. Mini Reviews in Medicinal Chemistry, 13: 1231-1237. DOI: 10.2174/1389557511313080009

Ismail F, Sherif K, Rizk Y, Hassan M, Mekawy A & Mahrose K. 2023. Dietary supplementation of spirulina and canthaxanthin boosts laying performance, lipid profile in blood and egg yolk, hatchability, and semen quality of chickens. Journal of Animal Physiology and Animal Nutrition, 107: 650-658. DOI: 10.1111/jpn.13729

Kaltner H, Schrott M, Schmahl W & Wittmann J. 1993. Developmental retardation of the Japanese quail embryo under the influence of dexamethasone. Research Communications in Chemical Pathology and Pharmacology, 79: 259-273.

Khalil-Khalili AA, Zhandi M, Zaghari M, Mehrabani-Yeganeh H, Yousefi AR & Tavakoli-Alamooti M. 2021. The effect of dietary organic selenium on reproductive performance of broiler breeder roosters under dexamethasone-induced stress. Theriogenology, 161: 16-25. DOI: 10.1016/j.theriogenology.2020.11.016

Khan R, Naz S, Nikousefat Z, Selvaggi M, Laudadio V & Tufarelli V. 2012. Effect of ascorbic acid in heat-stressed poultry. World's Poultry Science Journal, 68: 477-490. DOI: 10.1017/S004393391200058X

King'Ori A. 2011. Review of the factors that influence egg fertility and hatchability in poultry. International Journal of Poultry Science, 10: 483-492.

Lee C, Kim JH & Kil DY. 2022. Comparison of stress biomarkers in laying hens raised under a long-term multiple stress condition. Poultry Science, 101: 101868. DOI: 10.1016/j.psj.2022.101868

Liu Hl & Meng Fx. 2019. Effect of oxidative stress on antral follicular atresia in animals and its mechanism. Journal of Nanjing Agricultural University, 42: 6-13.

Magnoli A, Monge M, Nazar F, Magnoli C, Cavaglieri L, Bagnis G, Dalcero A & Marin R. 2012. Combined effects of aflatoxin B1 and corticosterone treatment on selected performance indices and liver histopathology in Japanese quail. Poultry Science, 91: 354-361. DOI: 10.3382/ps.2011-01763

Mariey Y, Samak H & Ibrahem M. 2012. Effect of using Spirulina platensis algae as a feed additive for poultry diets: 1-productive and reproductive performances of local laying hens. Egyptian Poultry Science Journal, 32: 201-215.

Mendes RL, Nobre BP, Cardoso MT, Pereira AP & Palavra AF. 2003. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorganica Chimica Acta, 356: 328-334. DOI: 10.1016/S0020-1693(03)00363-3

Mirzaie S, Zirak-Khattab F, Hosseini SA & Donyaei-Darian H. 2018. Effects of dietary Spirulina on antioxidant status, lipid profile, immune response and performance characteristics of broiler chickens reared under high ambient temperature. Asian-Australasian Journal of Animal Sciences, 31: 556. DOI: 10.5713/ajas.17.0483

Modaresi M. 2012. A comparative analysis of the effects of garlic, elderberry and black seed extract on the immune system in mice. Journal of Animal and Veterinary Advances, 11: 458-461.

Moustafa ES, Alsanie WF, Gaber A, Kamel NN, Alaqil AA & Abbas AO. 2021. Blue-green algae (Spirulina platensis) alleviates the negative impact of heat stress on broiler production performance and redox status. Animals, 11: 1243. DOI: 10.3390/ani11051243

Nassar FS, Alaqil AA, El-Sayed DA, Kamel NN & Abbas AO. 2023. Effects of dietary intervention using spirulina at graded levels on productive performance and physiological status of quail birds reared under elevated temperatures. Agriculture, 13: 789. DOI: 10.3390/agriculture13040789

Njagi LW, Nyaga PN, Bebora LC, Mbuthia PG & Minga UM. 2012. Effect of immunosuppression on Newcastle disease virus persistence in ducks with different immune status. International Scholarly Research Notices. DOI: 10.5402/2012/253809

Oluwagbenga E, Tetel V, Tonissen S, Karcher D & Fraley G. 2023. Chronic treatment with glucocorticoids does not affect egg quality but increases cortisol deposition into egg albumen and elicits changes to the heterophil to lymphocyte ratio in a sex-dependent manner. Frontiers in Physiology, 14: 434. DOI: 10.3389/fphys.2023.1132728

Osho S & Adeola O. 2020. Chitosan oligosaccharide supplementation alleviates stress stimulated by in-feed dexamethasone in broiler chickens. Poultry Science, 99: 2061-2067. DOI: 10.1016/j.psj.2019.11.047

Peiretti P & Meineri G. 2008. Effects of diets with increasing levels of Spirulina platensis on the performance and apparent digestibility in growing rabbits. Livestock Science, 118: 173-177. DOI: 10.1016/j.livsci.2008.04.017

Rafiee F, Mazhari M, Ghoreishi M & Esmaeilipour O. 2016. Effect of lemon verbena powder and vitamin C on performance and immunity of heat-stressed broilers. Journal of Animal Physiology and Animal Nutrition, 100: 807-812. DOI: 10.1111/jpn.12457

Rafieian-Naeini HR, Zhandi M, Sadeghi M, Yousefi AR & Benson AP. 2021. Effects of coenzyme Q10 on reproductive performance of laying Japanese quail (Coturnix japonica) under cadmium challenge. Poultry Science, 100: 101418. DOI: 10.1016/j.psj.2021.101418

Ruder EH, Hartman TJ, Blumberg J & Goldman MB. 2008. Oxidative stress and antioxidants: exposure and impact on female fertility. Human reproduction update, 14: 345-357. DOI: 10.1093/humupd/dmn011

Samia MM, Rizk A & El-Sayed OA. 2018. Effect of supplementing diet with Spirulina Platensis algae or turmeric on productive and reproductive performance of Golden Montazah layers. Egyptian Poultry Science Journal, 38.

Satterlee D, Cole C & Castille S. 2007. Maternal corticosterone further reduces the reproductive function of male offspring hatched from eggs laid by quail hens selected for exaggerated adrenocortical stress responsiveness. Poultry Science, 86: 572-581. DOI: 10.1093/ps/86.3.572

Schmidt J, Satterlee D & Treese S. 2009. Maternal corticosterone reduces egg fertility and hatchability and increases the numbers of early dead embryos in eggs laid by quail hens selected for exaggerated adrenocortical stress responsiveness. Poultry Science, 88: 1352-1357. DOI: 10.3382/ps. 2008-00513

Sharokhyan Rezaee M, Farzinpour A, Farshad A & Hatfaludi T. 2022. The regulative effect of Urtica dioica on sex hormones imbalance: elevated follicle-stimulating hormone/luteinizing hormone ratio≥ 4.5 is associated with low performance in aged breeder quails. Italian Journal of Animal Science, 21: 142-152. DOI: 10.1080/1828051X.2021.2007801

Singh M, Mollier RT, Patton RN, Pongener N, Bordoloi LJ, Kumar R, Katiyar R, Khan MH, Rajkhowa D & Mishra VK. 2023. Linseed oil in boar's diet improved in vivo fertility and antioxidant status. Reproduction in Domestic Animals, 58: 27-38. DOI: 10.1111/rda.14249

Tetel V, Tonissen S & Fraley G. 2022. Sex differences in serum glucocorticoid levels and heterophil: lymphocyte ratios in adult pekin ducks (Anas platyrhynchos domesticus). General and comparative endocrinology, 317: 113975. DOI: 10.1016/j.ygcen.2021.113975

Wang X, Liu L, Zhao J, Jiao H & Lin H. 2017. Stress impairs the reproduction of laying hens: an involvement of energy. World's Poultry Science Journal, 73: 845-856. DOI: 10.1017/S0043933917000794

Zhang J, Wang C, Li X, Zhang Y & Xing F. 2022. Expression and functional analysis of GnRH at the onset of puberty in sheep. Archives Animal Breeding, 65: 249-257. DOI: 10.5194/aab-65-249.