Original Article

Black Seed and L-carnitine Mitigate Heat Stress in Japanese Quails Egg Quality Organ Morphology

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How to Cite This Article Habibi, A., Ghazanfari, Sh., Karimi Torshizi, M. A., & Mohammadi-Sangcheshmeh, A. (2026). Black Seed and L-carnitine Mitigate Heat Stress in Japanese Quails Egg Quality Organ Morphology. *Iranian Journal of Veterinary Medicine, 20*(3), 445-458. http://dx.doi.org/10.32598/ijvm.20.3.1005700

http://dx.doi.org/10.32598/ijvm.20.3.1005700

ABSTRACT

Background: Heat stress negatively impacts egg production and health in quails, prompting research on dietary supplements, like black seed and L-carnitine for their potential to alleviate these effects and improve performance.

Objectives: This study evaluated the effects of black seed, L-carnitine, and their combination on egg production and intestinal and magnum morphology in laying Japanese quails under normal and heat stress conditions, with an additional assessment of vitamin E supplementation.

Methods: Five-hundred laying Japanese quails were divided into two temperature groups (normal and heat stress) and five dietary treatments (control, black seed, L-carnitine, black seed + L-carnitine, and vitamin E) in a 2×5 factorial design. The experiment consisted of acclimation (two weeks), heat exposure (five weeks), and recovery (three weeks). Egg production and intestinal and magnum morphology were measured throughout the entire period.

Results: During heat stress, control and black seed diets resulted in higher Haugh unit values, while the black seed + L-carnitine diet increased yolk percentage. Vitamin E improved eggshell strength under normal conditions, and after recovery, both vitamin E and black seed + L-carnitine diets produced stronger eggshells (P<0.05). Black seed + L-carnitine reduced the the ratio of villous length to crypt depthcompared to the controls. Vitamin E under heat stress and black seed + L-carnitine under normal conditions increased magnum fold height, while the control diet under heat stress had the lowest fold thickness. Vitamin E enhanced epithelial height under normal temperatures and reduced magnum gland diameter (P<0.05).

Conclusion: Black seed and vitamin E improved egg quality (Haugh unit, shell strength), while black seed + L-carnitine had mixed effects on morphology depending on temperature. Dietary strategies can optimize the performance of laying Japanese quails under different conditions.

Keywords: Black seed, Egg quality, Heat stress, L-carnitine, Laying Japanese quail

Article info:
Received: 30 Jul 2025
Accepted: 27 Oct 2025
Publish: 01 May 2026

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Introduction

The Japanese quail (*Coturnix japonica*) belongs to the family Phasianidae and is renowned for its egg-laying abilities, producing up to 300 eggs annually. Its high productivity, small size, and low feed requirements make it cost-effective for commercial egg production. Quail eggs are nutritionally superior to chicken eggs (Davodzadeh et al., 2017). However, heat stress adversely affects quail well-being, meat quality, production rate, and egg quality, particularly impacting their reproductive performance due to their susceptibility to high temperatures. Effective strategies, including antioxidant additives in their diet, are needed to enhance productivity and mitigate oxidative stress under such conditions (Lukanov, 2018; Habibi et al., 2024).

To combat heat stress, various nutritional interventions have been employed, including supplementation with vitamins (e.g. Vitamins C and E), minerals (e.g. selenium), electrolytes, and herbal extracts, all of which aim to improve antioxidant capacity, immune function, and thermoregulation (Sahin et al., 2009). Among these, antioxidant compounds play a crucial role in neutralizing reactive oxygen species induced by heat stress.

To enhance the reproductive and production characteristics of quails, dietary supplements, like L-carnitine and black seed have been suggested. L-carnitine, an amino acid-like compound, plays a crucial role in cellular energy metabolism and mitochondrial CoA regulation, improving yolk fat metabolism and follicular growth. It potentially increases egg weight through enhanced albumen deposition and eggshell calcification (Peebles et al., 2007). Under heat stress, L-carnitine improves growth, laying performance, and immune function due to its antioxidant properties (Rehman et al., 2017). Similarly, vitamin E, another antioxidant, enhances reproductive performance and protects cells from damage (Raya et al., 2007). *Nigella sativa* (black seed) is known for its health benefits in humans, but limited research exists on its effects on egg yield and reproductive activity in Japanese quails. Rich in dry matter (50.91-48.94%), fat (34.49-41.6%), and protein (16-26.7%), essential minerals, and vitamins, black seed contains bioactive compounds, like thymoquinone and oleic acid, which have strong antioxidant, anti-inflammatory, and antimicrobial properties (Isik et al., 2019). Studies suggest that incorporating 2% black seed or 0.5% black seed oil into quail diets enhances growth performance and egg production and reduces intestinal bacterial pathogens (Seidavi et al., 2020). Additionally, quails consuming 1 g/kg of black seed extract show higher egg yield and weight, improved feed efficiency, and better egg quality (Denli et al., 2004). Compared to other nutritional strategies, the advantage of black seed supplementation lies in its multi-functional bioactive components, which not only improve antioxidant capacity but also enhance immune response and gut health simultaneously. This multifaceted action may provide more comprehensive protection against heat stress-related damages, thereby improving reproductive performance more effectively than single-component additives.

The purpose of this study was to determine the effects of natural compounds, such as black seed and L-carnitine on egg quality and the morphological properties of the intestine and magnum of laying Japanese quails under both normal and heat stress conditions. Investigating these factors can provide valuable insights into developing effective strategies to manage and optimize egg quality in laying Japanese quails, thereby ensuring a sustainable and profitable poultry industry.

Materials and Methods

Composition of nutrients and essential oil in black seed

The nutrient composition of black seed was determined based on Association of Official Analytical Chemists (AOAC) procedures (AOAC, 2007). Black seed's nutrient profile included 96% dry matter, 5.34% ash, 8.17% crude fiber, 33.13% fat, 23.54% crude protein, 26.24% neutral detergent fiber (NDF), 17.26% acid detergent fiber (ADF), and 3.60 Kcal/kg dry matter of metabolizable energy. The essential oil content was estimated using gas chromatography (GC) coupled with mass spectrometry (MS) analysis, as shown in Table 1. Essential oils from black seed were extracted using the hydrodistillation method, which is a widely accepted procedure for isolating volatile compounds from plant material. The seeds were ground and subjected to hydrodistillation with a Clevenger-type apparatus for approximately 3 hours. The yield of essential oil extracted was approximately 0.5–1.0% (v/w) based on the dry weight of black seed. The extracted essential oils were analyzed using a gas chromatograph (Agilent 7890B GC system) coupled with a mass spectrometer (Agilent 5977A MSD). The GC was equipped with an HP-5MS capillary column (30 m × 0.25 mm internal diameter [i.d.], 0.25 µm film thickness). The oven temperature was programmed to increase from 50 °C to 250 °C at a rate of 5 °C/min. Helium was used as the carrier gas at a flow rate of 1.0 mL/min.

The mass spectrometer operated in electron ionization mode at 70 eV, scanning the mass range of m/z 40–450. This technique allowed for the identification and quantification of the main bioactive constituents of black seed essential oil with high precision.

Bird husbandry and experimental groups

A total of 500 laying Japanese quails (*C. japonica*) were procured from a local hatchery (Amol, Iran). All experimental protocols were reviewed and approved by the Animal Welfare Committee of the Department of Animal Science, University of Tehran, in accordance with institutional guidelines for the care and use of laboratory animals. The birds were randomly allocated to 10 treatment groups, each comprising 5 replicates with 10 quails per replicate. The study employed a completely randomized design with a 2×5 factorial arrangement, including two ambient temperature levels (normal and heat stress) and five experimental treatments: 1) control diet; 2) 1.5% black seed + control diet; 3) 250 ppm L-carnitine+control diet; 4) 1.5% black seed + 250 ppm L-carnitine+control diet; and 5) 200 ppm vitamin E + control diet. These treatments were administered under both normal and heat stress conditions.

The rearing period lasted for 10 weeks. At the beginning of the experiment, when the birds were 58 days old, they were weighed and placed in experimental cages based on their weight (267 g) and egg production (64%). During the first stage, lasting two weeks (from 58 to 72 days old), the laying Japanese quails were acclimated to the experimental diets. In the second stage, spanning five weeks (from 73 to 108 days old), the quails were exposed to both normal temperature and heat stress conditions. Heat stress involved maintaining an ambient temperature of 36 °C for 6 hours daily, from 10 am to 4 pm. In the third stage, the quails underwent a three-week recovery period under normal temperature conditions (from 109 to 130 days old). Throughout the 10-week period, the quails were individually housed in battery wire cages (50×30×50 cm^3) with individual feeders and nipple drinkers, ensuring ad libitum access to feed and water. The lighting regimen followed a continuous 16-hour light cycle. Utilizing corn and soybean meal as the primary ingredients, all diets were isocaloric and isonitrogenous, meeting or exceeding NRC (1994) nutritional requirements for laying Japanese quails (Table 2). Consistent care and control measures were implemented throughout the experiment.

Egg quality traits

To evaluate egg quality traits, two eggs were randomly selected from each replication (10 eggs per treatment) and transported to the laboratory. Eggshell percentage was determined by cracking the eggs, separating the shells, and incubating them at 65 °C for 72 hours. After cooling, shell weight was measured and expressed as a percentage of the total egg weight. Eggshells were divided into three sections (wide, narrow, and middle), and thickness was measured using a precise caliper (Mitutoyo, Tokyo, Japan) with 0.001 mm accuracy. The average of these measurements was recorded as the eggshell thickness. Eggshell strength was assessed using an eggshell strength tester (Okayama, Japan).

Albumen height was measured with a three-legged caliper, and Haugh units were calculated using the Equation 1:

1. $HU=100\ \log\ (albumen\ height-1.7\times(egg\ weight^{0.37})+7.57)$

Yolk and albumen percentages were determined by weighing them separately and expressing their weights as percentages of the total egg weight. Yolk color was evaluated using the Roche color scale. The yolk index was calculated by dividing the yolk diameter by the yolk height and multiplying by 100 (Torki et al., 2014). These traits were measured during both the heat stress and recovery periods.

Intestinal and magnum morphological assay and ovary weight

Following the completion of the heat stress and recovery periods, five birds were randomly selected from each treatment group. These birds were weighed and euthanized. After opening the abdominal cavity, the ovaries were collected and individually weighed. The weight of the ovaries was expressed as a percentage relative to the body weight (%). For histomorphometry experiments, five tissue samples were randomly selected from each treatment and transferred to the laboratory. The ventral cavity, including the oviduct and intestines, was carefully dissected. Three-centimeter sections from the small intestine (jejunum) were obtained at the end of both the heat stress and recovery periods.

Tissue sections from the magnum (part of the oviduct) were collected at the end of the recovery period. These tissue samples were first washed with a phosphate buffer solution and then fixed in a 10% formalin solution. The fixed tissue samples were then transported to a laboratory for histological analysis. Hematoxylin-eosin (H&E) staining was used to stain the tissue sections. Paraffin embedding was performed to prepare the tissue slides, and a microtome was used to obtain thin sections from the paraffin blocks. Various morphological characteristics were measured using a light microscope (Olympus CX31) connected to a computer. These measurements included villus height and width, crypt depth in the intestinal tissue, height and thickness of folds, epithelium height, depth and diameter of glands, as well as the thickness of the internal and outer muscle layers in the magnum tissue (Uni et al., 2001).

Statistical analysis

The data collected from the experiment were analyzed using a 2×5 factorial design within a completely randomized design (CRD). The main factors were ambient temperature (normal and heat stress) and the experimental treatments (control, black seed, L-carnitine, black seed + L-carnitine, and vitamin E). There were a total of 10 treatments, each replicated 5 times. The statistical analysis was performed using a general linear model (GLM) in SAS software (SAS, 2003), according to the following model:

$$Yij = \mu + Ai + Bj + (A + B)ij + eij$$

Where, Yij is the dependent variable; μ is the population mean, Ai is the effect of ambient temperature, Bj is the effect of experimental treatments, (A × B)ij is the two-way interaction of treatment, and eij is the random error. The significance of differences between the means was assessed using Tukey's test, with a significance level set at 5%.

Results

Egg quality traits

This study found a significant interaction between ambient temperature and experimental treatments that influenced egg quality traits in laying Japanese quails during heat stress (Table 3), but not during the recovery period (Table 4).

At the end of the heat stress period, the albumin percentage was higher in birds on the control diet under heat stress and those on a vitamin E diet under normal conditions compared to birds receiving a black seed + L-carnitine diet under heat stress ($P<0.05$). Additionally, albumin height and Haugh unit values were significantly higher in birds on the control diet and those consuming black seed under heat stress compared to those receiving L-carnitine ($P<0.05$). The yolk percentage was higher in birds receiving black seed + L-carnitine during heat stress than in birds on black seed alone under heat stress and those on vitamin E under normal conditions ($P<0.05$). Shell strength was greatest in birds on a vitamin E diet under normal conditions and lowest in those on the control diet under heat stress ($P<0.05$). No significant effects of temperature or treatments were observed on yolk color, shell percentage, or eggshell thickness. The yolk index increased under heat stress but was unaffected by experimental treatments ($P<0.05$) (Table 3).

During the recovery period, there were no significant effects of ambient temperature or experimental treatments on albumen height, albumen percentage, Haugh unit, yolk color, yolk index, yolk percentage, or eggshell percentage and thickness. However, eggshell strength decreased under heat stress ($P<0.05$). Birds receiving vitamin E and those on a black seed + L-carnitine diet demonstrated higher eggshell strength compared to the control group ($P<0.05$). A trend was observed for a lower eggshell percentage in birds raised under heat stress ($P=0.06$) (Table 4).

Intestinal morphological assay and ovary weight

The interaction between ambient temperature and experimental treatments did not significantly affect the intestinal morphology of laying Japanese quails during heat stress and recovery periods. However, exposure to high ambient temperatures resulted in a reduction in villus width ($P<0.05$). Other morphological characteristics, including villus length, crypt depth, and the ratio of villous length to crypt depth, remained unaffected by ambient temperature. During heat stress, quails receiving a black seed diet tended to have increased villus length ($P=0.06$), and those provided with black seed and L-carnitine exhibited greater villus width compared to the control diet and the vitamin E group ($P<0.05$). In the recovery period, no significant impact on villus length and width was observed from any treatments. Birds that received black seed and the black seed + L-carnitine treatment had greater crypt depth than the control group during both periods ($P<0.05$). The the ratio of villous length to crypt depth was higher in birds on the control diet, black seed, and L-carnitine during the heat stress period. In contrast, higher ratios were observed in the control and vitamin E groups during recovery compared to the black seed + L-carnitine group ($P<0.05$) (Table 5).

Table 1. GC-MS analysis of black seed essential oil

Serial No.	Chemical Name[a]	Inhibition Coefficient	%
1	3-methyl nonan	930	0.4
2	3,1 and 5- terimethyl banzen	960	0.3
3	Decan	1000	0.5
4	1-methyl 3-propyl banzen	1050	0.4
5	1-ethyl 2 and 3- dmethyl banzen	1085	0.2
6	Tetradecan	1403	0.2
7	Hexanone decan	1605	0.3
8	α-thujol	925	2.1
9	α –pinene	938	1.3
10	Sabinen	974	1.5
11	B-pinen	977	1.4
12	Mirsen	994	0.5
13	α –phellanderen	1002	0.7
14	Para-simen	1024	13.8
15	Limonene	1027	4.0
16	Gama-terpinen	1049	0.4
17	Phenshon	1093	1.3
18	Dihydrokaron	1202	0.4
19	Karon	1240	4.2
20	Thymoquinone	1239	0.8
21	Karakrol	1030	1.4
22	(+)-4-carene	1243	0.5
23	2,4-dimethylanisole	1220	0.4
24	Phenol, 2-methoxy-4-(1-propenyl)-	1225	0.3
25	Phenol, 2-methoxy-4-(1-propenyl)-, (E)-	1180	0.5
26	2-benzothiazolamine, N-methyl-	1214	0.2
27	Thymol	1246	1.2
28	Terpinen-4-ol	1219	0.6

[a]Identification by GC–M: National Institute of Standards and Technology (Gaithersburg, MD).

Table 2. Diet composition for laying Japanese quail (as-fed basis)

Item		Control	Black Seed
Ingredients (%)	Corn	60.8	59.55
	Gluten	5.13	4.52
	Soybean meal (44% CP)[a]	22.32	22.85
	Soybean oil	2	1.96
	Limestone	7.15	7.14
	Dicalcium phosphate	1.54	1.53
	Black seed	0	1.5
	Vitamin premix[c]	0.25	0.25
	Mineral premix[c]	0.25	0.25
	Common salt	0.2	0.2
	DL- methionine	0.26	0.24
	L- lysine HCL	0.09	0
Chemical composition (%)	Metabolizable energy (Kcal/kg)	2950	2950
	CP	18	18
	Ca	3.1	3.1
	Available P	0.449	0.449
	Na	0.129	0.129
	Cl	0.129	0.129
	Lys	0.85	0.85
	Met	0.583	0.583
	Met + Cys	0.82	0.82
	Met + Cys	0.82	0.82

[a]CP: Crude protein, [b]Mineral premix supplied per kilogram of diet: Mn (60 mg); Fe (80 mg), Zn (50 mg), Cu (10 mg), Co (2 mg), I (1 mg), Se (0.250 mg), and vehicle quantity sufficient to 500 mg. [C]Vitamin premix supplied per kilogram of diet: vitamin A (15,000,000 IU), vitamin D3 (1500,000 IU), vitamin E (15,000 IU), vitamin B1 (2.0 mg), vitamin B2 (4.0 mg), vitamin B6 (3.0 mg), vitamin B12 (0.015 mg), nicotinic acid (25 mg), pantothenic acid (10 mg), vitamin K3 (3.0 mg), and folic acid (1.0 mg).

Furthermore, the interaction between ambient temperature and experimental treatments significantly affected the relative ovary weight of the quails during both heat stress and recovery periods. During heat stress, quails on the control diet displayed higher relative ovary weights compared to all other treatments, except for the black seed + L-carnitine group ($P<0.05$). This trend continued into the recovery period, where the control diet group showed the highest relative ovary weight at high temperatures ($P<0.05$). The absence of dietary supplements in the control group may have led to a physiological response aimed at maintaining reproductive function during heat stress (Table 5).

Magnum morphological assay

The effects of ambient temperature and experimental treatments on the microscopic structure of magnum tissue in laying Japanese quails are illustrated in Figure 1 and detailed in Table 6. The interaction between temperature and treatments significantly influenced the histological prop-

Table 3. Effects of ambient temperature and experimental treatments on laying Japanese quail egg quality (73–107 days old, 5 weeks)

	Item	Albumen (%)	Albumen Height	Haugh Unit	Yolk Index	Yolk Color	Yolk (%)	Shell Strength (kg/cm^2)	Shell (%)	Shell Thickness ($\times 10^{-2}$ mm)
AT	HS	51.4	5.04	92.03	50.47^{a}	10.08	34.49	1.12^{b}	14.09	0.216
	NT	50.44	4.95	91.38	47.02^{b}	10.6	34.58	1.25^{a}	14.65	0.21
	SEM	0.903	0.11	0.561	1.17	0.226	0.91	0.016	0.255	0.004
Experiment treatments	Control	52.38^{ab}	5.22	92.89	50.17	10.9	34^{ab}	0.962^{c}	13.6	0.207
	Black seed	53.26^{a}	5.18	92.61	51.06	10	31.94^{b}	1.2^{b}	14.79	0.212
	L-carnitine	48.6^{ab}	4.75	90.41	48.04	9.9	37.03^{ab}	1.18^{b}	14.36	0.21
	Black seed+ L-carnitine	47.28^{b}	4.67	89.99	47.81	10.1	38.28^{a}	1.18^{b}	14.42	0.215
	Vitamin E	53.07^{a}	5.17	92.64	46.64	10.8	31.42^{b}	1.39^{a}	14.7	0.223
	SEM	1.42	0.174	0.888	1.86	0.357	1.43	0.026	0.404	0.006
AT × experiment treatments	HS×control	56.2^{a}	5.67^{a}	95.41^{a}	54.07	10.6	30.94^{abc}	0.93^{e}	12.84	0.212
	HS×black seed	54.84^{ab}	5.59^{a}	94.78^{ab}	53	9.6	30.2^{bc}	1.17^{bc}	14.94	0.216
	HS×L-carnitine	49.7^{ab}	4.36^{b}	88.37^{c}	46.54	9.8	36.27^{abc}	1.15^{bcd}	14.02	0.206
	HS×black seed+ L-carnitine	45.65^{b}	4.77^{ab}	90.61^{abc}	48.52	9.6	40.3^{a}	1.09^{cde}	14.04	0.218
	HS×vitamin E	50.62^{ab}	4.83^{ab}	90.99^{abc}	50.22	10.81	34.73^{abc}	1.26^{bc}	14.63	0.23
	NT×control	48.57^{ab}	4.78^{ab}	90.38^{abc}	46.27	11.2	37.06^{abc}	0.99^{de}	14.36	0.202
	NT×black seed	51.68^{ab}	4.76^{ab}	90.43^{abc}	49.12	10.4	33.67^{abc}	1.23^{bc}	14.64	0.208
	NT×L-carnitine	47.5^{ab}	5.14^{ab}	92.44^{abc}	49.53	10	37.79^{ab}	1.21^{bc}	14.69	0.214
	NT × black seed+ L-carnitine	48.92^{ab}	4.56^{ab}	89.38^{bc}	47.11	10.6	36.26^{abc}	1.28^{b}	14.81	0.212
	NT×vitamin E	55.51^{a}	5.5^{ab}	94.3^{abc}	43.05	10.8	28.11^{c}	1.52^{a}	14.76	0.216
	SEM	2.02	0.246	1.25	2.63	0.506	2.03	0.036	0.571	0.009
P	AT	0.452	0.548	0.419	0.044	0.113	0.945	<0.0001	0.129	0.329
	Experiment treatments	0.01	0.072	0.061	0.445	0.158	0.004	<0.0001	0.271	0.527
	AT×experiment treatments	0.024	0.001	0.001	0.247	0.848	0.018	0.019	0.574	0.822

Abbreviations: AT: Ambient temperature; NT: Normal temperature; HS: Heat stress; SEM: Pooled standard error of the mean.
Note: a-c Means in each column with different superscripts are different ($P<0.05$). Data represent the means of 5 cages (n=5).

erties of the oviduct magnum. Quails supplemented with vitamin E at high temperatures and those receiving black seed or black seed + L-carnitine at normal temperatures exhibited higher magnum fold heights compared to the groups that received black seed, L-carnitine, or black seed + L-carnitine at high temperatures ($P<0.05$). Quails on the control diet at high temperatures displayed the thinnest folds, except when compared to those receiving the black seed treatment at high temperatures ($P<0.05$). At normal temperatures, vitamin E supplementation resulted in increased epithelial height, outperforming other treatments except for black seed + L-carnitine under both temperature conditions ($P<0.05$). L-carnitine supplementation at high temperatures produced the thickest internal muscular layer ($P<0.05$). The group receiving black seed + L-carnitine at high temperatures also showed increased outer muscular layer thickness

Table 4. Effects of ambient temperature and experimental treatments on laying Japanese quail egg quality during recovery period (108–128 days old, 3 weeks)

	Item	Albumen (%)	Albumen Height	Haugh Unit	Yolk Index	Yolk Color	Yolk (%)	Shell Strength (kg/cm^2)	Shell (%)	Shell Thickness ($\times 10^{-2}$ mm)
AT	HS	47.83	4.7	89.87	50.65	8.08	37.78	1.18^{b}	14.38	0.198
	NT	49.48	4.62	89.53	50.74	8.08	35.48	1.27^{a}	15.03	0.197
	SEM	1.21	0.14	0.798	0.865	0.145	1.28	0.022	0.244	0.005
Experiment treatments	Control	48.75	4.33	88.15	51.36	8.2	36.64	0.975^{c}	14.59	0.205
	Black seed	49.58	4.97	91.3	49.18	8.3	35.89	1.25^{b}	14.52	0.194
	L-carnitine	47.45	4.65	89.59	50.75	8	38.2	1.23^{b}	14.34	0.211
	Black seed+ L-carnitine	51.61	4.7	90.03	53	7.6	33.99	1.29^{ab}	14.39	0.19
	Vitamin E	45.89	4.64	89.45	49.17	8.3	38.42	1.4^{a}	15.68	0.19
	SEM	1.91	0.221	1.26	1.36	0.23	2.03	0.035	0.386	0.007
AT × experiment treatments	HS×control	45.82	4.19	87.73	50.22	8.2	39.24	0.978	14.93	0.208
	HS×black seed	49.32	4.96	90.99	49.32	8.6	36.56	1.23	14.1	0.198
	HS×L-carnitine	48.67	4.96	91.08	49.92	8.2	37.38	1.21	13.94	0.212
	HS×black seed+ L-carnitine	47.16	4.6	89.39	53.67	7.2	38.48	1.2	14.34	0.184
	HS×vitamin E	48.19	4.78	90.17	50.1	8.2	37.21	1.31	14.58	0.19
	NT×control	51.69	4.46	88.58	52.51	8.2	34.05	0.972	14.24	0.202
	NT×black seed	49.84	4.98	91.61	49.04	8	35.21	1.27	14.94	0.19
	NT×L-carnitine	46.23	4.35	88.1	51.58	7.8	39.01	1.25	14.74	0.21
	NT×black seed+ L-carnitine	56.05	4.8	90.66	52.33	8	29.49	1.38	14.44	0.196
	NT×vitamin E	43.6	4.49	88.72	48.23	8.4	39.62	1.49	16.77	0.19
	SEM	2.71	0.313	1.78	1.93	0.325	2.88	0.05	0.546	0.011
P	AT	0.341	0.681	0.766	0.939	1.00	0.215	0.008	0.068	0.911
	Experiment treatments	0.298	0.387	0.530	0.255	0.182	0.537	<0.0001	0.106	0.246
	AT×experiment treatments	0.090	0.607	0.726	0.775	0.246	0.260	0.188	0.131	0.914

Abbreviations: AT: Ambient temperature; NT: Normal temperature; HS: Heat stress; SEM: Pooled standard error of the mean.
Note: a-c Means in each column with different superscripts are different ($P<0.05$). Data represent the means of 5 cages (n=5).

compared to the control, black seed, L-carnitine at high temperatures, and black seed + L-carnitine at normal temperatures ($P<0.05$). Moreover, vitamin E at high temperatures, along with control, black seed, L-carnitine, and black seed + L-carnitine at normal temperatures, enhanced magnum gland depth compared to L-carnitine at high temperatures ($P<0.05$). However, vitamin E supplementation reduced the magnum gland diameter relative to L-carnitine and black seed + L-carnitine ($P<0.05$).

Table 5. Effects of ambient temperature and experimental treatments on laying Japanese quail intestinal morphology and ovary weight (%) under heat stress and recovery periods

	Item	Heat Stress					Recovery				
		Villus Length (µm)	Villus Width (µm)	Crypt Depth (µm)	Vh / Cr ratio	Ovary (%)	Villus Length (µm)	Villus Width (µm)	Crypt Depth (µm)	Vh/Cr ratio	Ovary (%)
AT	HS	441.98	90.64^{b}	72.46	6.11	1.19^{a}	492.8	89.88^{b}	73.37	6.73	1.09
	NT	445.64	99.1^{a}	71.41	6.25	1.01^{b}	493.96	97.18^{a}	73.58	6.75	1.08
	SEM	8.99	1.66	0.71	0.14	0.02	5.85	1.38	1.02	0.11	0.022
Experiment treatments	Control	446.9	90.05^{b}	68.9^{c}	6.5^{a}	1.14^{ab}	489.19	91.72	68.8^{b}	7.12^{a}	1.22^{a}
	Black seed	479.08	99.29^{a}	73.89^{ab}	6.5^{a}	1.09^{abc}	503.54	95.45	77.6^{a}	6.51^{ab}	1.06^{b}
	L-carnitine	443.4	99.44^{a}	69.69^{bc}	6.37^{a}	1.03^{bc}	483.72	94.81	71.67^{ab}	6.78^{ab}	1.03^{b}
	Black seed+ L-carnitine	417.4	95.05^{ab}	76.94^{a}	5.44^{b}	1.22^{a}	485.06	95.46	77.62^{a}	6.26^{b}	1.12^{ab}
	Vitamin E	432.29	90.53^{b}	70.28^{bc}	6.16^{ab}	1^{c}	505.46	90.25	71.74^{ab}	7.06^{a}	0.999^{b}
	SEM	14.23	2.62	1.11	0.22	0.032	9.24	2.18	1.61	0.18	0.035
AT × experiment treatments	HS×control	444.3	86.67	69.94	6.37	1.39^{a}	489.63	88.44	69.72	7.03	1.35^{a}
	HS×black seed	467.69	93.24	75.47	6.22	1.17^{bc}	499.8	91.29	77.36	6.47	1.04^{b}
	HS×L-carnitine	445.8	94.08	69.7	6.4	1.06^{bcd}	482.64	91.67	72.1	6.73	1.01^{b}
	HS×black seed+ L-carnitine	419.52	91.75	75.74	5.56	1.24^{ab}	500.07	91.74	75.64	6.62	1.08^{b}
	HS×vitamin E	432.61	87.52	71.50	6.06	1.06^{bcd}	491.9	86.3	72.07	6.85	0.976^{b}
	NT×control	449.50	93.44	67.87	6.63	0.886^{d}	488.74	95	67.87	7.21	1.1^{b}
	NT×black seed	490.47	105.36	72.34	6.79	1.01^{cd}	507.27	99.61	77.84	6.56	1.08^{b}
	NT×L-carnitine	441	104.82	69.7	6.33	1.01^{cd}	484.79	97.95	71.24	6.83	1.05^{b}
	NT×black seed+ L-carnitine	415.29	98.36	78.14	5.32	1.2^{abc}	470.04	99.17	79.6	5.9	1.16^{ab}
	NT×vitamin E	431.97	93.55	69.06	6.25	0.94^{d}	519	94.2	71.4	7.27	1.02^{b}
	SEM	20.12	3.7	1.57	0.306	0.045	13.06	3.08	2.28	0.25	0.05
P	AT	0.776	0.001	0.304	0.475	<0.0001	0.889	0.001	0.882	0.919	0.851
	Experiment treatments	0.069	0.042	0.0002	0.011	0.0002	0.318	0.335	0.002	0.010	0.0005
	AT×experiment treatments	0.955	0.886	0.414	0.726	<0.0001	0.326	0.996	0.740	0.231	0.017

Abbreviations: AT: Ambient temperature; NT: Normal temperature; HS: Heat stress; Vh to Cr ratio: Villus height to crypt depth ratio; SEM: Pooled standard error of the mean.

Note: a-c Means in each column with different superscripts are different ($P<0.05$). Data represent the means of 5 cages (n=5).

Table 6. Effects of ambient temperature and experimental treatments on histological characterization of the oviduct magnum during the whole period (10 weeks) in laying Japanese quails

	Item	Fold Height (µm)	Fold Thickness (µm)	Epithelium Height (µm)	Internal Muscle Layer Thickness (µm)	Outer Muscle Layer Thickness (µm)	Gland Depth (µm)	Gland Diameter (µm)
AT	HS	3372.01^{b}	968.78^{b}	14.27^{b}	27.05^{a}	27.14	422.33^{b}	7.53
	NT	3625.51^{a}	1131.03^{a}	16.63^{a}	22.11^{b}	27.74	528.96^{a}	8.04
	SEM	29.22	13.14	0.38	0.6	0.51	11.14	0.24
Experiment treatments	Control	3470.42	987.09^{b}	12.92^{c}	19.4^{c}	27.23^{ab}	423.31^{b}	7.37^{ab}
	Black seed	3493.49	1032.21^{ab}	14.92^{bc}	23.36^{bc}	25.43^{b}	466.98^{b}	7.59^{ab}
	L-carnitine	3391.36	1042.03^{ab}	15.5^{b}	29.69^{a}	29.38^{a}	441.55^{b}	8.39^{a}
	Black seed+ L-carnitine	3572.39	1102.61^{a}	18.61^{a}	24.76^{b}	25.86^{b}	475.31^{b}	8.87^{a}
	Vitamin E	3566.14	1085.57^{a}	15.3^{bc}	25.7^{b}	29.3^{a}	571.07^{a}	6.7^{b}
	SEM	46.21	20.79	0.6	0.95	0.81	17.61	0.38
AT × experiment treatments	HS×control	3453.4^{bcd}	727.11^{e}	13.25^{cd}	20.41^{bcd}	21.5^{c}	363.53^{cd}	7.07
	HS×black seed	3305.02^{cd}	863.7^{ed}	13.3^{cd}	23.56^{bcd}	21.91^{c}	405.12^{bcd}	7.86
	HS×L-carnitine	3153.92^{d}	999.22^{cd}	17.13^{bc}	40.68^{a}	28.37^{b}	358.35^{d}	8.53
	HS×black seed+ L-carnitine	3309.29^{cd}	1075.04^{bc}	19.15^{ab}	24.5^{bcd}	34.27^{a}	392.95^{cd}	7.67
	HS×vitamin E	3638.45^{ab}	1178.84^{ab}	8.53^{e}	26.1^{b}	29.65^{ab}	591.7^{a}	6.51
	NT×control	3487.45^{bc}	1247.08^{a}	12.6^{ed}	18.4^{d}	32.97^{ab}	483.1^{abc}	7.66
	NT×black seed	3681.95^{ab}	1200.72^{ab}	16.54^{bcd}	23.15^{bcd}	28.95^{ab}	528.83^{ab}	7.31
	NT×L-carnitine	3628.81^{acb}	1084.85^{bc}	13.86^{cd}	18.69^{cd}	30.38^{ab}	524.75^{ab}	8.25
	NT × black seed+ L-carnitine	3835.5^{a}	1130.19^{abc}	18.06^{ab}	25.02^{bcd}	17.45^{c}	557.68^{a}	10.07
	NT×vitamin E	3493.83^{bc}	992.31^{cd}	22.05^{a}	25.3^{bc}	28.95^{ab}	550.44^{a}	6.88
	SEM	65.35	29.4	0.838	1.33	1.14	24.9	0.54
P	AT	<0.0001	<0.0001	0.0003	<0.0001	0.4161	<0.0001	0.1533
	Experiment treatments	0.0661	0.0068	<0.0001	<0.0001	0.0045	<0.0001	0.0054
	AT×experiment treatments	0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0026	0.0943

Abbreviations: AT: Ambient temperature; NT: Normal temperature; HS: Heat stress; SEM: Pooled standard error of the mean.

Note: a-c Means in each column with different superscripts are different (P<0.05). Data represent the means of 5 cages (n=5).

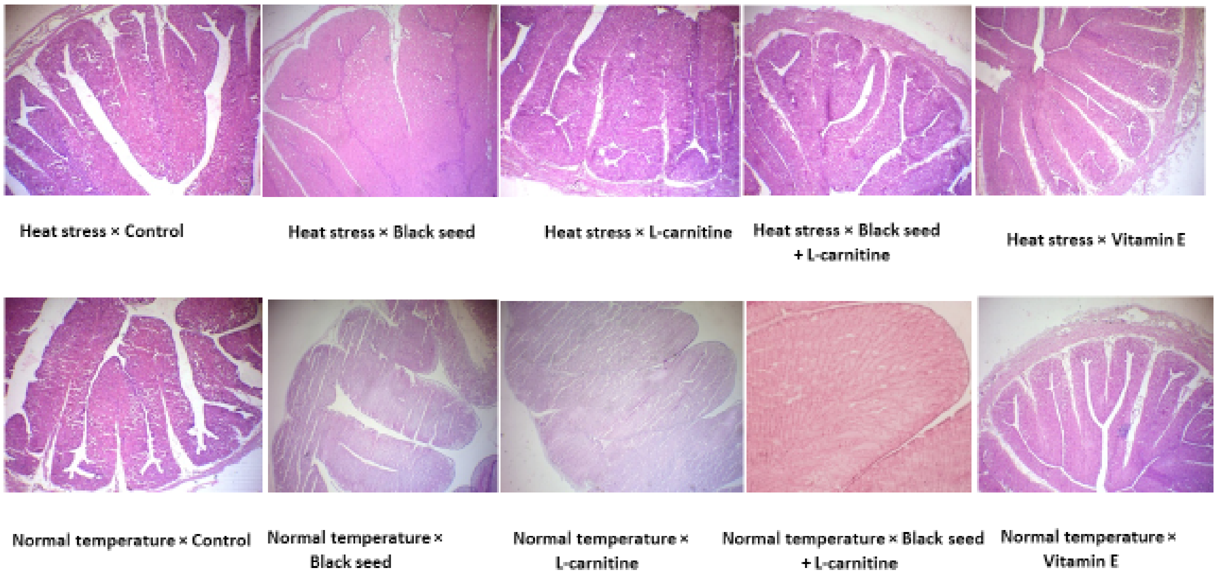

Figure 1. Effects of ambient temperature and experimental treatments on optical microscopic image of the magnum tissue during the whole period (10 weeks) in laying Japanese quails (Magnification ×40)

Discussion

Egg quality traits

This study examined how ambient temperature and dietary treatments affected egg quality in laying Japanese quails during heat stress. The results showed significant improvements in albumin and Haugh unit values for control and vitamin E diets compared to the black seed + L-carnitine diet. Eggshell strength peaked in the vitamin E group. The findings of this study align with previous research by Mehaisen et al., (2019) which indicated that heat stress reduces eggshell strength in laying Japanese quails by impacting electrolyte regulation and altering blood acid-base balance, resulting in blood alkalosis and dehydration. Such imbalances adversely affect eggshell quality, enzyme activity, and protein synthesis. In this study, birds supplemented with dietary additives exhibited enhanced eggshell strength compared to the control group. Specifically, vitamin E was found to significantly improve eggshell strength and weight due to its antioxidant properties and improved bioavailability of calcium supplements (Nemati et al., 2020). Moreover, black seed extract at a concentration of 1 g/kg was shown to increase albumin height and support eggshell gland integrity, particularly under heat stress (Denli et al., 2004). L-carnitine was found to aid energy metabolism, enhancing fatty acid oxidation and calcium metabolism—both critical for eggshell formation (Łukaszewicz et al., 2007; Nemati et al., 2020). Thus, implementing tailored dietary strategies that include vitamin E, black seed, and L-carnitine may improve eggshell strength, egg quality, and production efficiency in environments experiencing high temperatures.

Intestinal morphological assay and ovary weight

The results demonstrated that additive supplements significantly influence the intestinal structure, epithelial integrity, and villus morphology in laying Japanese quails. Heat-stressed quails often experience decreased intestinal epithelial integrity (Yu et al., 2010), and specific antioxidants can help mitigate the detrimental effects of heat stress on growth and overall physiology (Sahin et al., 2008). The findings are consistent with previous research by Aziza et al., (2019) which showed that the inclusion of black seed oil in Japanese quail diets resulted in longer villi compared to the positive control group, enhancing intestinal morphology and feed utilization efficiency. Moreover, L-carnitine supplementation has been associated with increased length, width, surface area, and depth of villous crypts, attributed to improvements in growth performance and feed digestion (Abu-Alya et al., 2021). Interestingly, the combination of L-carnitine and black seed led to the lowest ratio of villous length to crypt depth, suggesting possible disturbances in intestinal morphology. This outcome could be due to the energy-diverting effects of L-carnitine, which enhances lipid metabolism and energy production but may also detract from maintaining intestinal structure (Rouhanipour et al., 2021). The combination of these supplements may further complicate nutrient absorption dynamics and gut microbiota composition, potentially triggering stress responses and hormonal changes that adversely affect intestinal health (Habibian et al., 2015).

Regarding relative ovary weight, the study highlights the physiological adaptations of quails under heat stress conditions. The increased relative ovarian weight in the control group may represent a compensatory response to heat stress, as heat stress negatively impacts reproductive systems, resulting in reduced egg production, egg quality, and ovarian development. Supplementation with black seed, vitamin E, and L-carnitine may have enabled birds to manage heat stress more effectively, reducing the necessity to allocate resources for ovarian maintenance, leading to lower relative ovarian weights compared to the control group (Attia et al., 2010). The presence of essential fatty acids, antioxidants, and bioactive compounds in black seed (Abd El-Hack et al., 2018), together with L-carnitine's role in supporting energy metabolism and antioxidant protection (Rehman et al., 2017), suggests that these supplements can enhance reproductive performance and general health during stress conditions.

Magnum morphological assay

Heat stress is known to adversely impact the reproductive function of quails by disrupting hormone secretion rates and affecting gonadal sensitivity to hormones. High environmental temperatures negatively influence the production of various hormones, including estrogen, parathyroid hormone, thyroid hormone, gonadotropin, and calcitonin (Ayo et al., 2011). In this study, L-carnitine supplementation—particularly at a dosage of 100 mg/kg—was found to increase the thickness of the magnum's internal muscle layer in quails, corroborating findings from Rouhanipour et al. and Agarwal and Said, who noted L-carnitine's positive effects on magnum morphology. L-carnitine enhances fatty acid oxidation, facilitating the synthesis of estrogen and progesterone by regenerating the reducing equivalents necessary for cholesterol side-chain cleavage—a critical process for ovarian growth and follicular maturation (Agarwal & Said, 2004). Additionally, L-carnitine possesses antioxidant properties (Xu et al., 2003), and improved magnum morphology resulting from L-carnitine supplementation may lead to increased egg protein synthesis throughout the oviduct, thereby enhancing albumen quality. L-carnitine may also expedite yolk lipid storage, promoting follicular development and increasing metabolic activity in both the shell gland and magnum. Consequently, this process facilitates the accumulation of albumen and calcium in the eggshell, contributing to increased eggshell thickness and egg weight (Kazemi-Fard et al., 2015). While research specifically targeting the effects of black seed on reproductive health is limited, some studies have indicated its antioxidant and anti-inflammatory benefits. For instance, Saleh et al. (2019) evaluated the impact of cumin seed oil on reproductive morphology in laying hens and found no adverse effects. Further investigation, including clinical trials, is necessary to better understand the beneficial effects and modes of action of black seed on reproductive health.

Conclusion

This study evaluated the effects of black seed and L-carnitine on egg quality, as well as intestinal and magnum morphology of laying Japanese quails under both normal and heat stress conditions. The findings indicated that dietary supplementation with black seed and vitamin E significantly enhances egg quality, particularly in terms of Haugh unit values and eggshell strength, especially during heat stress. Additionally, these supplements improve intestinal health and ovarian weight through enhanced metabolic efficiency and antioxidant protection. While the combination of black seed and L-carnitine shows potential in improving certain morphological aspects, its effects vary with temperature conditions. Overall, implementing these dietary strategies can optimize quail performance and welfare under different environmental stressors, emphasizing the critical role of tailored nutrition in poultry management. The study highlights the need for further research to explore additional nutritional interventions and management practices to further optimize performance in laying quail production systems.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Animal Welfare Committee of the Department of Animal Science, University of Tehran, Tehran, Iran (Code: IR.AUSMT.REC.1400.16).

Funding

This study was extracted from the PhD dissertation of Atefe Habibi, approved by the Department of Animal Science, Faculty of Agricultural Technology (Aburaihan), University of Tehran, Pakdasht, Iran. This research was financially supported by the University of Tehran, Faculty of Agricultural Technology (Aburaihan), Pakdasht, Iran.

Authors' contributions

Conceptualization and statistical analysis: Shokoufe Ghazanfari; Methodology, data curation, and formal analysis: Atefe Habibi, Shokoufe Ghazanfari, and Abdullah Mohammadi-Sang Cheshmeh; Investigation: Atefe Habibi and Abdullah Mohammadi-Sang Cheshmeh; Sample collection, analysis, and writing the original draft: Atefe Habibi; Supervision, review and editing: Shokoufe Ghazanfari, Abdullah Mohammadi-Sang Cheshmeh, and Mohammad Amir Karimi Torshizi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to thank the Faculty of Agricultural Technology (Aburaihan), University of Tehran, for providing the research facilities and administrative support.

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