Effects of coenzyme Q10 on reproductive performance of laying Japanese quail (*Coturnix japonica*) under cadmium challenge

Hamid Reza Rafieian-Naeini,[*] Mahdi Zhandi,[*,1] Mostafa Sadeghi,[*] Ali Reza Yousefi,[†] and Andrew Parks Benson[‡]

[*]*Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Alborz, Karaj, Iran;* [†]*Department of Pathology and Experimental Animals, Razi Vaccine and Serum Research Institute, Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran; and* [‡]*Department of Poultry Science, University of Georgia, Athens, GA 30602-2772, USA*

ABSTRACT Japanese quail is an increasingly important bird of economic importance for commercial egg and meat production, particularly in developing countries. There is a need for research aimed at improving efficiency of these birds during stressful challenges, such as oxidative stress. Coenzyme Q10 (**CoQ10**), a highly functional antioxidant, protects cells against oxidative stress. This study was conducted to determine the effects of CoQ10 on reproductive performance of Japanese quail under cadmium (**Cd**) challenge. A total of 216 six-wk-old Japanese quail were randomly allocated into 3 groups for an 8 wk experimental trial. The treatments include a negative control (**NC**): feeding basal diet; a positive control (**PC**): feeding basal diet and cadmium administration (1 mg/100 g BW, at 10 and 11 wk of age), and (CdQ10): feeding CoQ10 supplemented (900 mg/kg diet) basal diet and Cd administration. At 11 and 13 wk of age, egg production, body weight, mortality, oviduct, and ovarian biometry, were recorded. Histology and histopathology of isthmus and magnum, fertility, hatchability, hatchling quality, and HSP70 mRNA transcript abundance in the utero-vaginal junction (**UVJ**) were evaluated. Positive control and CdQ10 group had no significant effect on live body weight, stroma weight, follicle size, hatchability, and fertility; however, Cd administration increased ($P < 0.01$) mortality rate in the PC group compared to the NC and CdQ10 groups. CdQ10 quail produced more eggs and had a higher hatchling quality compared to the PC group ($P < 0.01$). The thickness and height of isthmus and magnum folds in the CdQ10 group was increased compared to the PC group ($P < 0.01$) and overall oviduct weight was increased with CoQ10 supplementation ($P < 0.01$). Compared to PC, the CdQ10 group had a reduction in infiltration of inflammatory cells. Relative abundance of HSP70 mRNA in UVJ was influenced by interactive effect of treatment × time ($P < 0.05$). In conclusion, dietary supplementation of CoQ10 showed beneficial effects on some reproduction characteristics of female Japanese quail under Cd-induced oxidative stress.

Key words: antioxidant, avian, histology, *HSP70*, oxidative stress

2021 Poultry Science 100:101418
https://doi.org/10.1016/j.psj.2021.101418

INTRODUCTION

Japanese quail belong to the order *Galliformes* and the *Phasianidae* family, genus *Coturnix* and species *japonica*. From an economical perspective, this bird has the advantage of low maintenance cost (due to its relatively small body size), short generation interval (3−4 generation per year) (Baer et al., 2015), resistance to diseases and high egg production. Due to their unique flavor, costumer demand for quail meat and eggs is increasing (Vali, 2008) and the number of quail in industrial farming has progressively increased over the last few decades (Lukanov, 2019).Quail have been estimated to produce around 10% of all table eggs (Lukanov, 2019), there are several articles related to the effect of heat stress on egg production and the egg quality indicators of quail (Sahin et al., 2008; Mehaisen et al., 2019). But yet there is a paucity of reports concerning ameliorating the impact of stress on reproductive efficiency for this economically important bird.

Environmental pollution has increased over the past decades, leading to potential risks to all biological systems, including poultry sector (Alagawany et al., 2018).

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Received June 17, 2021.
Accepted July 26, 2021.

[1]Corresponding author: mzhandi@ut.ac.ir

Cadmium (**Cd**), a powerful inducer of oxidative stress, has been shown to cause ventral body wall defects in chick embryos treated at Hamburger−Hamilton stages 16−17 (Cuypers et al., 2010; Thompson et al., 2010). With continuous exposure to levels of Cd, it is being deposited in different tissues day after day, causing toxic effects on various tissues of poultry (Khafaga et al., 2019; Zhu et al., 2021). Some agricultural practices, as phosphatic fertilizers, sewage sludge disposal, and town-refuse composts applications, may increase Cd accumulation in soil and lead to heavy metal transfer to crops (The bulk of the avian ration) and to the food chain. Polluted drinking water and ambient air of industrial cities contribute relatively little to the daily intake of Cd in both animals and human (Smith, 2009).

Cadmium induces deleterious changes in gonadal tissue, endocrine disruption, and follicular atresia in different animal models (Zhang et al., 2008). In quail, the exposure to Cd has considerable adverse effects on reproductive and productive performance. It has been found that injection of the 10 mg Cd/kg BW in quail induced 41.7% mortality within 24 h (Rahman et al., 2007); however, the body weight loss in the quail exposed to Cd was decreased only at the end of a 28-d trial, indicating a cumulative effect of the metal may be associated with action of the metallothioneins (Sant'Ana et al., 2005). The decreased hatchling weight found in Cd-injected quail is thought to be the result of Cd toxicity in the developing embryo (Rahman et al., 2007), which could be mitigated by supplementation of antioxidative components.

During past decade, several studies with different levels of success have been conducted to find the effects of dietary antioxidative components on improving avian reproductive performance against oxidative stress (Kazemizadeh et al., 2019; Zhandi et al., 2020a,b; Khalil-Khalili et al., 2021). Co-enzyme Q10 (**CoQ10**), also known as a ubiquinone, is a vitamin-like nutrient and lipid soluble compound primarily located in the mitochondria where it plays a significant role in the production of adenosine triphosphate (Crane, 2000). CoQ10 also has the unique ability to act as a potent fat-soluble antioxidant free radical scavenger, thus limiting damage associated with oxidative stress (Sohet and Delzenne, 2012). In addition to neutralizing free radicals directly, CoQ10 is capable of restoring antioxidants like ascorbate (vitamin C) and α-tocopherol (vitamin E) (Navas et al., 2007). Less than 5% of orally ingested CoQ10 is thought to reach the circulation and high plasma concentrations of CoQ10 are needed to promote uptake by peripheral tissues (Bhagavan and Chopra, 2007), however there are no reports of significant adverse effects of CoQ10 supplementation (Hathcock and Shao, 2006).

Dietary supplementation of CoQ10 linearly improved fertility, hatching ability and sperm penetration (**SP**) percentage in aged broiler breeder hens (Sharideh et al., 2020). In addition, CoQ10 supplementation significantly altered the structure of the magnum and isthmus, by increasing fold height and surface epithelia (Sharideh et al., 2020). Supplementing 900 mg CoQ10 /Kg of diet improved production and reproductive traits of broiler breeder hens (Sharideh et al., 2020). Increases in antioxidant activity as a result of CoQ10 supplementation may reduce damage by reactive oxygen species (**ROS**) and may lead to improvements in the reproductive performance of hens (Sharideh et al., 2020).

Based on the literature, dietary supplementation of CoQ10 may improve reproductive performance of quail under Cd challenge, mainly through its antioxidative properties. Although previous studies have shown CoQ10 supplementation improved the reproductive performance of aged hens (Sharideh et al., 2020), the protective effect elicited by dietary CoQ10 supplementation following a Cd challenge has not been assessed using the avian model. Developmental studies in experimental toxicology are important to evaluate the effects of exposure to environmental pollutants on production and reproductive efficiency (Hutchinson et al., 2000). The current study, therefore, was conducted to investigate the effect of dietary supplementation of CoQ10 on quail reproductive performance following Cd administration.

MATERIALS AND METHODS

The current study was approved by the Animal Care Committee and the ethics of the institutional committee of Animal Science, University of Tehran, Karaj, Iran.

Birds and Management

This study was carried out using 216 six wk-old Japanese quail (*Coturnix japonica*) with an average body weight of 210 ± 20 g. Following a 2-wk acclimation to the experimental conditions, the birds were randomly allocated into 3 replicates per group (18 females and 6 males each). The quails were housed in 80 × 80 cm cages with an ambient temperature of 25 ± 2°C and ventilation rate of 540 m^3/h. The quails were on a 16L:8D lighting schedule at an intensity of 10 lux and were fed a basal diet (30 g, in morning and noon meal) formulated to meet their nutritional requirements (National Research Council, 1994; Table 1) along with free access to freshwater. Treatment included a negative control (**NC**): feeding basal diet; a positive control (**PC**): feeding basal diet and cadmium administration (1 mg per 100 g BW (Sharideh et al., 2020)), and (**CdQ10**): feeding a CoQ10 supplemented (900 mg per kg diet) basal diet and Cd administration. The Cd (1 mg per 100 g BW) was subcutaneously injected at 10 and 11 wk of age (**woa**) (Rahman et al., 2007). As a placebo, saline was injected into the NC quail. An outline of the experimental design indicating treatments and times of acute stress induction is shown in Figure 1.

Egg Production, Body Weight, and Mortality

Body weight (**BW**) of each replicate was measured weekly. The number of produced eggs, egg weight, and

Table 1. Ingredients and chemical composition of the diets fed to Japanese quail breeder (As fed basis).

Ingredient	Amount (%)
Corn	54.25
Soybean meal, 44% CP	34.80
Dicalcium phosphate	1.45
CaCO3	5.25
Common salt	0.20
NaHCO3	0.17
Vegetable oil	3.23
DL-Met, 99%	0.15
Mineral premix[1]	0.25
Vitamin premix[2]	0.25
Total	100
Calculated nutrient content	
AME (kcal/kg)	2900
CP (%)	20
Calcium (%)	2.5
Available phosphorus (%)	0.35
Sodium (%)	0.15
Lysine (%)	1.59
Methionine (%)	0.45
Met + cys (%)	0.77
Threonine (%)	0.77

[1]Provides (per kg of diet): Choline (C5 H14 N O), 300 mg; iron (FeSO4.7H2O),50 mg; manganese (MnSO4.H2O), 120 mg; Zn (ZnO), 110 mg; copper(CuSO4.5H2O), 10 mg; selenium (Na2SeO3), 0 mg; iodine (KI), 2 mg.

[2]Provides (per kg of diet): vitamin A (retinyl acetate), 11,000 IU; vitamin D3 (cholecalciferol), 3500 IU; vitamin E (DL-α -tocopheryl acetate), 150 IU; vitamin K3(menadione), 5.0 mg; vitamin B1 (thiamin), 3.0 mg; vitamin B2 (riboflavin), 12 mg; vitamin B3 (niacin), 55 mg; vitamin B5 (Dpantothenic acid), 15 mg; vitamin B6(pyridoxine), 4 mg; vitamin B9 (folic acid), 2 mg; vitamin H2 (biotin), 0.25 mg; vitamin B12 (cobalamin), 0.03 mg.

dead birds (if any) were recorded daily. Egg mass was also calculated weekly as hen week production (%) multiplied by egg weight (g) divided by 100.

Necropsy

Necropsy was carried out on 6 randomly selected birds per group (2 birds per replicate) at 11 and 13 woa according to the method described by Genchev and Mihaylov (2008).

Biometric Parameters of Oviduct and Ovary

Following slaughter, the oviduct and stroma portion of the ovary (ovary without yellow follicles) were weighed with a digital balance (0.01 g). In addition, three of the largest ovarian follicles were removed from the ovaries, weighed, and their diameters measured across the stigma with a digital caliper (0.01 mm).

Histology and Histopathology of Isthmus and Magnum

About 2 cm segments of the oviduct, including both the magnum and isthmus, were taken for histological analysis. The collected tissue samples were fixed in 10% neutral buffered formalin, and following tissue processing, embedded in paraffin wax. The paraffin-embedded tissues were cut into 7-μm-thick sections using a microtome (Rotary microtome, Didsabz company, model DS4055, Urmia, Iran) and then stained with hematoxylin and eosin (**H&E**). For histological observations, height and thickness of magnum and isthmus were assessed by optical microscopy at X40 magnification, but X100 magnification was used for magnification for tunica muscularis and serosa thicken measurements. Setting scale and measurements were conducted using image J 1.52a software.

Fertility, Hatchability, and Hatchling quality

During the last 3 wk of the experiment (11−13 woa), the eggs were collected weekly and transferred into an incubator (DQ100SH; Belderchin Damavand, Tehran, Iran). After 14 d at 37.7°C and 65% humidity, the eggs were transferred to the hatchery basket and placed at 37.2°C and 70% humidity for 3 days. On the 17th day of incubation, after hatching, the number of eggs containing no/dead embryo were counted and the percent of fertility was calculated. Hatchability (chick number divided by total number of eggs incubated × 100) and fertility (total of hatched and fertilized eggs divided by

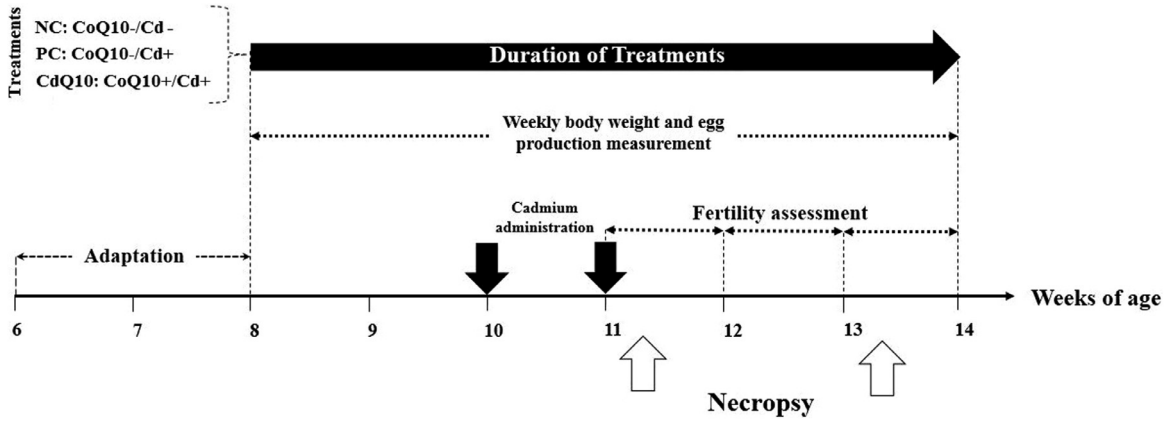

Figure 1. An outline of the experimental design indicating initiation of dietary co-enzyme Q10 (CoQ10; 900 mg per kg diet) supplementation and times of acute stress induction (subcutaneous injection of 1 mg Cadmium (Cd)/100g BW). Note: Following 2 weeks of adaptation to experimental condition, the quails (n = 216) were divided randomly into 3 experimental groups (n = 72 quails per group, kept in 3 separate pens) and either not treated with Cd and CoQ10 (negative control; NC), treated with Cd but not CoQ10 supplementation (positive control; PC), or treated with Cd and CoQ10 (CdQ10) for 6 successive weeks (8−14 weeks of age).

total eggs incubated × 100) rates were calculated. In addition, the hatchlings were weighed and evaluated by the Tona chick quality table (Tona et al., 2003). After opening unhatched eggs, embryonic mortality was determined and catagorized as early death, late death, or pipnig (Seker et al., 2005).

RNA Extraction, cDNA Synthesis, and RT-PCR

Utero-vaginal junction (**UVJ**) samples were dissected immediately after slaughter and placed in cryovials and frozen in liquid nitrogen. Samples were stored at −80°C for RNA extraction. Total RNAs were extracted from UVJ using TRIzol reagent (Yektatajhiz, Tehran, Iran), according to the manufacturer's instructions. The concentration of RNA produced was evaluated by spectrophotometry (NanoDrop 1000). In order to treat and remove possible contamination from the sample, 6 μL of RNA was mixed with 1 μL of 10X buffer solution, 2.5 μL of DEPC-treated water and 0.5 μL of DNAs were mixed in a thermal cycler (SimpliAmp, Waltham, MA) at 37°C for 30 min. In the next step, 1 μL of EDTA was added to the above solution and the resulting solution was placed in a thermal cycler for 5 min at 65°C (Rasouli-Gharehsaghal et al., 2020).

RNA was reverse transcribed into cDNA using 0.5 to 8 μL of RNA (according to concentration), 10 μL mixed buffer (2X), 2 μL enzyme mix and reached 20 μL volumes with DEPC-treated water using a cDNA Synthesis kit (Parstous, Iran). The reaction was incubated for 1 h at 42°C in SimpliAmp Thermal Cycler. The cDNA mixture was kept at −20°C until it was used in a real-time PCR reaction.

The sense and antisense primers used for the amplification of *HSP70* were 5'-TTGCTTATGGTGCTGCTGTG-3' and 5'-GCGTTTGTTGGTGGGAATG-3', respectively. Gene expression data were normalized against the 40s ribosomal protein S17 (**RS17**) with sense primer: 5'-AACAAGCGGGTGTGTGAGG-3' and antisense primer: 5'-AGCATCTCCTTGGTGTCTGG-3' using the same cycling conditions for amplification of *HSP70*. The reaction was subjected to real-time PCR according to the instructions provided for the use of the Light Cycler Nano System with the FastStart Essential DNA Green Master (Roche Applied Science, Pleasanton, CA). Briefly, following a denaturing step at 95°C for 15min, PCR was advanced by using a thermal protocol consisting of 94°C for 20 s, 58°C for 30 s, and 72°C for 30 s in a 12μL PCR reaction containing 0.5 μL of cDNA, 1 μL of each primer, 6 μL of Q-PCR Master Mix SYBRGreen (SMOBIO, Hsinchu, Taiwan) and 3.5 μL DEPC-treated water. Relative quantification (Expression) of the mRNA was calculated for each sample using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001; Nasirikhah et al., 2019).

Statistical Analysis

Data were analyzed by SAS 9.4. Normal distribution of the data was checked by Kolmogorov-Smirnov and Shapiro-Wilk test. The continuous data was analyzed by GLM procedure; however, the binary distributed data, such as fertility and habitability were analyzed by GENMOD procedure using a logit odds ratio link function. Tukey's multiple comparison tests was used to determine significant difference between experimental groups. Data are expressed as mean and SEM and $P \leq 0.05$ and $0.05 \leq P \leq 0.10$ were considered as statistically significant difference and tendency, respectively.

RESULTS

Egg Production, Body Weight, and Mortality

The effects of dietary supplementation of CoQ10 on BW, egg production and mortality of female quail under Cd stress are presented in Table 2. Positive control and CdQ10 group had no significant effect on BW. However, the highest mortality rate and lowest egg production were noted in PC group, and CoQ10 supplementation ameliorated these adverse effects of Cd administration. The CdQ10 quail had a lower mortality rate and higher egg production compared to the PC group ($P < 0.05$, Table 2). The interactive effect of treatment and time on egg production and mortality rate are represented in Figures 2 and 3, respectively. There was no difference in egg production among the experimental groups prior to Cd challenge. Although egg production was decreased in both groups challenged with Cd (PC and CdQ10), the decrease in egg production was less severe in the CdQ10 group ($P < 0.05$) after the first Cd challenge. It is worth noting that this ameliorating effect of CoQ10 was not

Table 2. Effects of dietary supplementation of CoQ10 on BW, egg production, and mortality of Japanese quail under cadmium challenge.

	Experimental groups				*P* value		
Parameter	NC	PC	CdQ10	SEM	Treatment	Wk	Treatment × Wk
Body weight (g)	276.15	284.56	277.02	3.65	0.20	<0.01	0.39
Egg production (%)	78.28[a]	38.29[c]	48.72[b]	0.07	<0.01	<0.01	<0.01
Mortality (%)	0[c]	32[a]	18[b]	1.00	<0.01	0.21	>0.05

[a,b]Within rows, values with different superscripts are statistically different ($P < 0.05$).

Note: Details of experimental design and treatments have been represented in Figure 1.

NC: negative control (no CoQ10 supplementation and no cadmium administration); PC: positive control (subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; CdQ10, supplementation of CoQ10 and Cd administration.

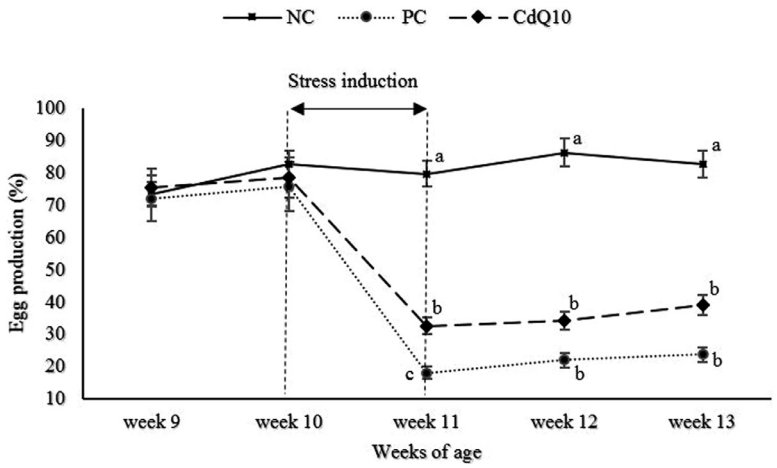

Figure 2. Interactive effect of treatment and time on egg production in laying quails under cadmium challenge. Note: Details of experimental design and treatments have been represented in Figure 1; negative control (NC): no CoQ10 supplementation and no cadmium administration; positive control (PC): subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; (CdQ10): supplementation of CoQ10 and Cd administration. Points with common superscripts (a,b) are not statistically different ($P > 0.05$).

seen following the second Cd challenge (Figure 2). The highest and lowest mortality rate following both the first and second Cd administrations were noted in the PC and NC groups, respectively ($P < 0.05$). As with egg production, there was a significant difference between CoQ10 and PC concerning mortality after the first Cd administration, however, this ameliorating effect of CoQ10 supplementation was not significantly different from PC following the second Cd administration (Figure 3).

Biometric Parameters of Oviduct and Ovary

Effects of dietary supplementation of CoQ10 on uterus, oviduct, ovary stroma, and ovarian follicle weight and diameter under Cd challenge are shown in Table 3. The highest and lowest oviduct weight was noted in CdQ10 and PC group, respectively ($P < 0.05$). However, stroma weight and other measured follicle parameters were not different among the treatments.

Histology and Histopathology of Isthmus and Magnum

Figures 4 and 5 show representative light micrographs of the quail reproductive tracts from the three different experimental groups. Cd administration induced a severe infiltration of inflammatory mononuclear cells in the lamina propria layer and subcellular epithelium. Degeneration and atrophy of the tubular glands, reduction in the height of the mucosal folds, and overlapping of the magnum and isthmus lumens were noted in PC quail (Figure 4). However, the severity of infiltration of mononuclear inflammatory cells in CdQ10 group varied from mild to moderate. Glandular degeneration and atrophy were less common in the CdQ10 quail than the cadmium-treated group. Histological parameters of isthmus and magnum sections are shown in Table 4. Cadmium administration decreased isthmus primary and secondary fold height and thickness as well as the thickness of

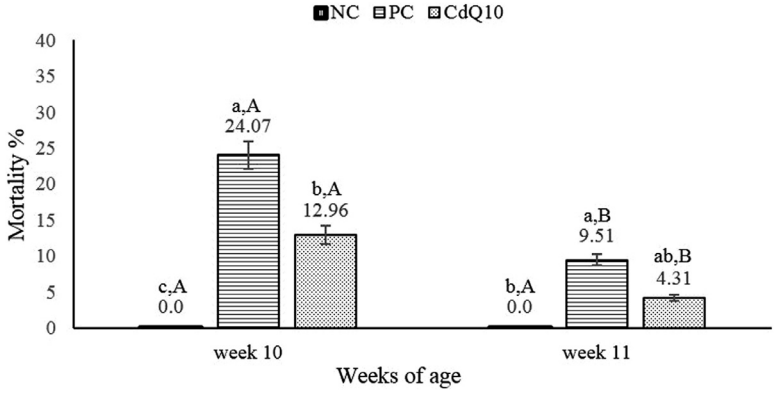

Figure 3. Effects of dietary supplementation of CoQ10 on mortality of Japanese quail under cadmium challenge. Note: Details of experimental design and treatments have been represented in Figure 1; negative control (NC): no CoQ10 supplementation and no cadmium administration; positive control (PC): subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; (CdQ10): supplementation of CoQ10 and Cd administration. Within each week, values with different superscripts (a,b) are significantly different ($P < 0.05$). Within each experimental group, values with different superscripts (A,B) are significantly different ($P < 0.05$).

Table 3. Effects of dietary CoQ10 supplementation on uterus, oviduct, ovary stroma, and ovarian follicles weight and diameter of Japanese quail under cadmium challenge.

	Experimental groups				*P* value		
Parameter	NC	PC	CdQ10	SEM	Treatment	Wk	Treatment × Wk
Oviduct weight (g)	6.46[ab]	5.94[b]	8.29[a]	0.54	<0.01	<0.01	0.24
Stroma weight (g)	1.15	1.08	1.28	0.08	0.24	0.23	0.34
F1 diameter (mm)	16.04	15.02	18.45	1.58	0.29	<0.01	0.85
F1 weight* (%)	0.18	0.13	0.16	0.016	0.11	<0.01	0.23
F2 diameter (mm)	14.77	12.98	13.74	1.18	0.61	<0.05	0.63
F2 weight* (%)	0.09	0.08	0.07	0.001	0.47	<0.05	0.38
F3 diameter (mm)	10.78	9.74	10.56	1.08	0.78	0.29	0.57
F3 weight* (%)	0.03	0.03	0.04	0.008	0.25	0.55	0.53

[a,b]Within each row, values with different superscripts are significantly different ($P < 0.05$).

Note: Details of experimental design and treatments are represented in Figure 1.

NC: negative control (no CoQ10 supplementation and no cadmium administration); PC: positive control, subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; CdQ10, supplementation of CoQ10 and Cd administration.

*Follicle weights reported as percentage of carcass weight and they were multiplied by 10.

tunica muscularis in the PC group compared to the NC group ($P < 0.05$). CoQ10 supplementation ameliorated these adverse effects as all the forementioned histological measurements were higher in the CdQ10 group compared to the PC group ($P < 0.05$). A similar trend was also noted in magnum, where the lowest primary and secondary fold heights and thicknesses were in the PC group ($P < 0.05$) and CoQ10 supplementation ameliorated these measured effects of Cd administration. Interestingly, CoQ10 supplementation significantly increased serosa thickness in both the magnum and isthmus, but tunica muscularis thickness was not influenced by the treatment ($P > 0.05$).

Fertility, Hatchability, and Hatchling Quality

The effect of CoQ10 on fertility, hatchability and hatchling quality of Japanese quail under cadmium challenge are shown in Table 5. Positive control and CdQ10 group had no significant effect on fertility or hatchability. Hatchling quality in PC was severely decreased by Cd administration, but CoQ10 supplementation mitigated the adverse effect of Cd as there was no difference between NC and CdQ10 groups ($P > 0.05$). The interactive effect of treatment and time on hatchling quality revealed that following Cd administration at wk 10 and 11 of the experiment the only significant difference was observed for the PC groups at wk 13 (Figure 6).

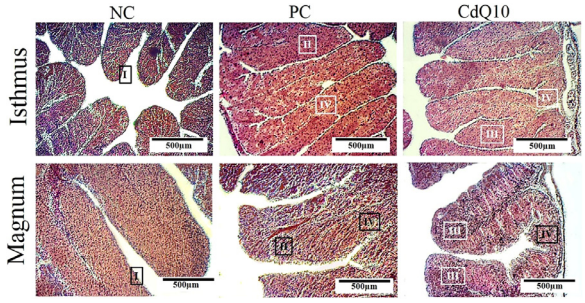

Figure 4. Histological lesions of the isthmus and magnum in quails under cadmium challenge. NC (feeding basal diet), PC (feeding basal diet and cadmium) and CdQ10 (feeding basal diet supplemented with 900 mg CoQ10 per kg diet and Cd administration). The tissue sections were stained with hematoxylin and eosin. Isthmus: (NC) intact Epithelium of lumen, (PC) high infiltration of mononuclear cells, atrophied tubular glands with degeneration, CdQ10) moderate infiltration of inflammatory cells, atrophied tubular glands. Magnum: (NC) intact epithelium of lumen, (PC) indicates a decrease in lumen diameter and degeneration of glandular cells and loss of epithelium. Infiltration of inflammatory cells is also highly visible, CdQ10) the penetration of inflammatory cells was reduced, the epithelium is more intact than PC. I: Intact epithelium, II: High infiltration of inflammatory cells, III: Moderate infiltration of inflammatory cells, IV: Atrophied tubular glands with degeneration. Quails were exposed to 1 mg cadmium (Cd)/100 g BW and/or 900 mg CoQ10/Kg feed for 8 wks.

HSP70 Gene Expression

The results of *HSP70* mRNA transcript abundance in UVJ of the Japanese quail are shown in Figure 7. Although abundance of *HSP70* mRNA transcript was lower in the PC group compared to the NC at wk 11 of the experiment ($P < 0.05$), there was no significant effect between the experimental groups at wk 13 of the experiment ($P > 0.05$). The results also showed an increase in the abundance of *HSP70* mRNA transcript in the PC group at wk 13 as compared to wk 11 of the experiment ($P < 0.05$).

DISCUSSION

Cd has deleterious effects on the reproductive system. Oxidative stress is believed to be crucial in the etiology of Cd-induced reproductive toxicity (Samuel et al., 2011). Cd administration not only increased mortality and decreased egg production in our quail study, but it also caused histological changes of the reproductive tract and impaired quail hatchling quality. The forementioned adverse impacts of Cd in our study were effectively ameliorated by CoQ10 supplementation.

Rahman et al. (2007) reported a decrease in quail BW following Cd injection at 3 mg/kg of BW but did not report BW results at the concentration equivalent to the current study (10 mg/kg BW), presumably due to the high rate of mortality (41%) in this group. Similar to Rahman et al. (2007), the current study had a high rate

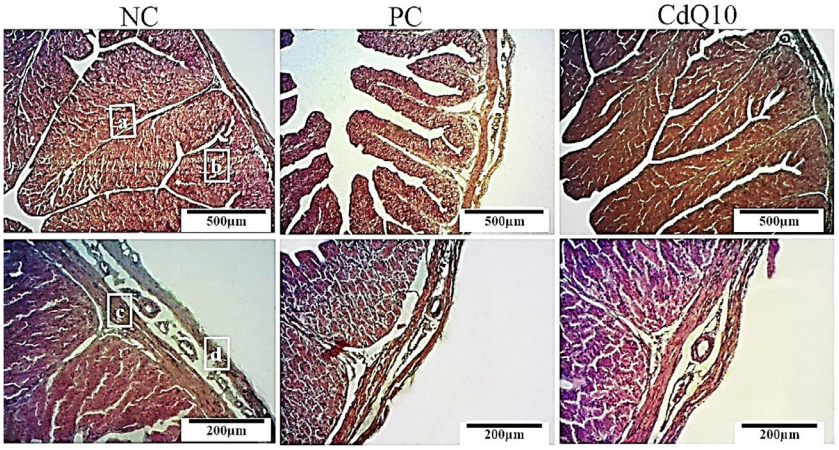

Figure 5. Light micrograph of the isthmus in NC (feeding basal diet), PC (feeding basal diet and cadmium) and CdQ10 (feeding basal diet supplemented with 900 mg CoQ10 per kg diet and Cd administration) quails. The magnum sections were stained with hematoxylin and eosin. Upper photoes show folds with X40 magnafacation (Scale bar = 500 μm) and lower photoes depict tunica muscularis and serosa with X100 magnification (Scale bar = 200μm). (A) Primary fold, (B) secondary fold, (C) tunica muscularis, and (D) serosa. Quails were exposed to 1 mg cadmium (Cd)/100 g BW and/or 900 mg CoQ10/Kg feed for 8 wk.

of mortality (24%) following the first Cd administration thus removing quail highly sensitive to Cd from the BW calculations. Nevertheless, the current results align with Sant'Ana et al. (2005) where there were no observed changes in BW following the first 2 wk of Cd treatment and BW was only impacted following 28 d of chronic Cd exposure. CoQ10 supplementation significantly reduced mortality as a result of Cd administration by serving as a potent antioxidant that diminished any oxidative stress induced by Cd (Choudhury et al., 1991). This protective antioxidant role also explains how CoQ10 supplementation reduces ascites in broilers (Geng et al., 2004).

Egg production was significantly decreased following Cd administration, with a more severe effect following the first administration at 10 woa. Butt et al. (2018) also reported that Cd impaired egg production as a result of alterations in the egg formation pathway; while Sharideh et al. (2020) reported CoQ10 supplementation improved egg production. Data concerning the adaptation of animals to stressors, as reviewed by Davies (2016), show that when animals are subjected to repeated oxidative stress, they often show an high response in the first few days after exposure, and then later, following subsequent exposure(s), a diminished response. This may explain why the initial Cd administration provoked a more deleterious effect in the quail. For example, heat-stressed broilers can adapt to chronically high environmental temperatures and associated oxidative stress (Pamok et al., 2009). The potent antioxidant capacity of CoQ10 protected against the Cd induced oxidative stress and this likely contributed to the improved egg production in the CoQ10 quail (Saha et al., 2019).

The laying success of poultry is closely related to the development of ovarian follicles and reproductive organs (Hanafy and Elnesr). Also the ovarian stroma, follicular diameter and follicular weight were not affected by Cd

Table 4. Effects of dietary CoQ10 supplementation on isthmus and magnum folds, tunica muscularis, and serosa thickness of Japanese quail under cadmium challenge.

	Experimental groups				*P* value		
Parameter	NC	PC	CdQ10	SEM	Treatment	Wk	Treatment × Wk
Isthmus							
Primary fold height (μm)	1324.33^{a}	906.28^{b}	1331.99^{a}	54.53	<0.01	0.20	0.24
Secondary fold height (μm)	878.15^{a}	603.13^{b}	842.61^{a}	50.66	<0.01	0.25	0.80
Primary fold thickness (μm)	425.48^{a}	248.72^{c}	314.76^{b}	14.44	<0.01	0.83	0.69
Secondary fold thickness (μm)	415.74^{a}	267.93^{b}	368.26^{a}	15.54	<0.01	0.33	0.63
Tunica muscularis thickness (μm)	96.88^{a}	64.88^{b}	104.61^{a}	5.37	<0.01	0.63	0.01
Serosa thickness (μm)	19.00^{b}	18.68^{b}	26.85^{a}	1.87	<0.01	0.91	0.54
Magnum							
Primary fold height (μm)	1732.51^{a}	1148.27^{c}	1460.31^{b}	51.84	<0.01	0.45	0.47
Secondary fold height (μm)	1229.57^{a}	567.61^{c}	995.49^{b}	30.23	<0.01	0.45	0.35
Primary fold thickness (μm)	789.94^{a}	493.97^{c}	635.88^{b}	24.59	<0.01	0.57	0.57
Secondary fold thickness (μm)	621.86^{a}	320.59^{b}	641.90^{a}	19.04	<0.01	0.73	0.019
Tunica muscularis thickness (μm)	57.16	66.96	69.09	3.93	0.11	<0.01	0.74
Serosa thickness (μm)	17.94^{b}	18.30^{b}	23.67^{a}	1.47	<0.05	0.94	0.52

^{a,b}Within each week, values with different superscripts are significantly different ($P < 0.05$).

Note: Details of experimental design and treatments are represented in Figure 1.

NC: negative control (no CoQ10 supplementation and no cadmium administration); PC: positive control, subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; CdQ10, supplementation of CoQ10 and Cd administration.

Table 5. Effects of CoQ10 on fertility, hatchability, hatchling quality, and embryonic death stages of Japanese quail under cadmium challenge.

	Experimental groups				*P* value		
Parameter	NC	PC	CdQ10	SEM	Treatment	Wk	Treatment × Wk
Fertility	93.3%(98/105)	88.6%(86/97)	86.7%(85/98)	0.36	0.159	0.48	0.27
Hatchability	69.5%(73/105)	59.7%(58/97)	66.3%(65/98)	0.21	0.25	0.68	0.79
Hatchling quality	98.23[a]	84.99[b]	95.03[a]	2.81	<0.01	0.30	<0.05
Early embryonic death	60%(15/25)	32.14%(9/28)	30%(6/20)	0.44	0.06	-	-
Middle embryonic death	40%(10/25)	60.71%(17/28)	65%(13/20)	0.43	0.17	-	-
Piping	0%(0/25)	7.14%(2/28)	5%(1/20)	0.79	0.60	-	-

[a,b]Within each row, values with different superscripts are significantly different ($P < 0.05$).

Note: Details of experimental design and treatments are represented in Figure 1.

NC: negative control (no CoQ10 supplementation and no cadmium administration); PC: positive control, subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; CdQ10, supplementation of CoQ10 and Cd administration.

administration or CoQ10 supplementation, but both reproductive tract weight and histology were significantly changed by treatment. The relative size of stroma is stable and rarely affected by the exogenous influencers (Nad et al., 2007). In addition, Massányi et al. (1997) reported that the lack of change in follicle weight of rabbits exposed to Cd is related to tissue edematization. Consistent with the present findings, a single injection of Cd (15 mg/Kg BW) significantly decreased the weight of the oviduct in Indian koel (Sarkar et al., 1976). In this study, histopathological alterations were found in the isthmus and magnum of Cd treated quail. CoQ10 supplementation ameliorated these Cd-induced histological alterations and reduced the severity of mononuclear inflammatory cell infiltration. Furthermore, the CoQ10 supplementation resulted in less glandular degeneration and atrophy. These findings show the adverse effect of Cd on egg production and hatchling quality is likely exerted through histopathological alterations of the reproductive tract.

Primary and secondary fold height and thickness, as well as tunica muscularis and serosa thickness of both the isthmus and magnum were significantly affected by Cd administration, but CoQ10 supplementation ameliorated these histological changes. Sex hormones, including estradiol and progesterone, have been reported to increase both the diameter of the oviduct and height of folds and villi (Perche et al., 1989; Saha et al., 2019), and Zhang et al. (2008) revealed that the release of ovarian progesterone and estradiol can be inhibited by Cd challenge in rat. Cd exposure induces oxidative stress, cell apoptosis, and follicular atresia in the ovary of laying hens (Zhu et al., 2021), all of which impair synthesis of sex hormones. Thus, the disruption of ovarian sex hormone release associated with Cd could be a contributing factor to the histological alterations and impairment in egg production. Supplementation of CoQ10 in an aged mouse model restored cumulus cell number, stimulated glucose uptake, and increased progesterone production (Ben-Meir et al., 2019). Sharideh et al. (2020) reported that dietary supplementation of CoQ10 increased egg production and enhanced fold height and thickness of the magnum and isthmus. Thus, the protective effect of CoQ10 mitigating Cd administration in this study may also be related to its antioxidant properties in the ovary.

Our findings indicated that there was no significant difference in hatchability and fertility associated with Cd administration. Pribilincova and Marettova (1996) also reported that fertilization rate and hatchability were not affected by Cd at an inclusion of either 3 ppm or 20 ppm to feed. However, Rahman et al. (2007) demonstrated that exposure to Cd suppressed quail reproduction for only 3 d postinjection, while Marettová et al. (2012) reported that fertility and embryo death in

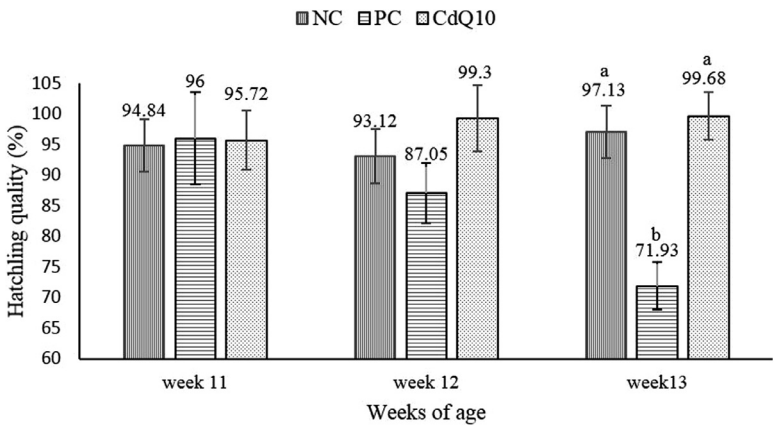

Figure 6. Effects of dietary supplementation of CoQ10 on hatchling quality of Japanese quail under cadmium challenge. Note: Details of experimental design and treatments have been represented in Figure 1; negative control (NC): no CoQ10 supplementation and no cadmium administration; positive control (PC): subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; (CdQ10): supplementation of CoQ10 and Cd administration. Within each week, values with different superscripts (a,b) are significantly different ($P < 0.05$).

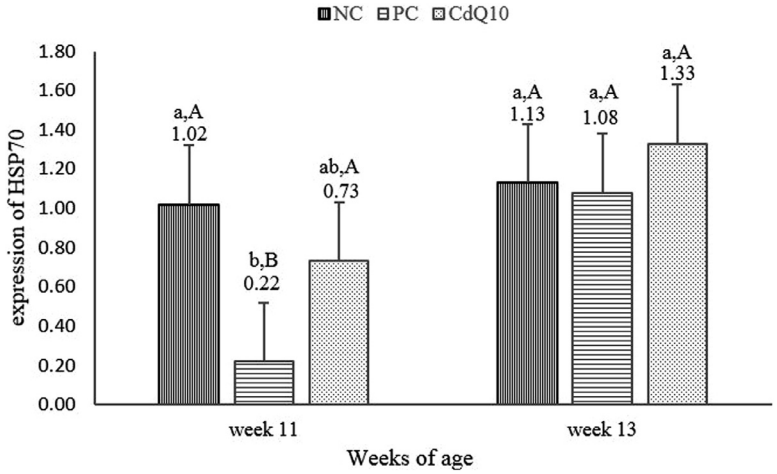

Figure 7. Interactive effects of treatment and time on relative abundance of *HSP70* mRNA transcript in utero-vaginal junction (UVJ) of Japanese quail under cadmium challenge dietary supplemented with co-enzyme Q10. Note: Details of experimental design and treatments have been represented in Figure 1; effects of treatment, time and the interaction of treatment by time was, 0.06, <0.01, and >0.05, respectively. Negative control (NC): no CoQ10 supplementation and no cadmium administration; positive control (PC): subcutaneous injection of 1 mg cadmium (Cd)/100 g BW; (CdQ10): supplementation of CoQ10 and Cd administration. Within each week, values with different superscripts (a,b) are significantly different ($P < 0.05$). Within each experimental group, values with different superscripts (A,B) are significantly different ($P < 0.05$).

the 20 mg kg^{-1} Cd group was not significantly different from the control group. It is worth noting that by advancing the experimental period, the quality of the hatched quails in the Cd-treated group decreased sharply. These findings may be due to Cd accumulation in the yolk which can disrupt embryonic chick development (Marettová et al. (2012).

Analysis of *HSP70* gene expression revealed that *HSP70* mRNA transcripts were expressed in the UVJ, where *HSP70* protein has previously been localized to the epithelial surface (Hiyama et al., 2014). Heat Shock Protein 70-binding sites have been detected on the entire surface of the sperm (Matsuzaki and Sasanami, 2017), implying that *HSP70* derived from surface epithelium of the UVJ may play an important role in sperm re-activation and facilitating sperm migration toward the infundibulum (Hiyama et al., 2014). Heat Shock Protein 70 can also serve as biomarkers for oxidative damage (El Golli-Bennour and Bacha, 2011); however, there is no direct and simple correlation between *HSP70* response and the level of oxidative stress (Wirth et al., 2002). The abundance of *Hsp70* mRNA treanscript was lower in the PC group, compared to the other treatments, for 3 d after second Cd injection. However, 2 wk subseqeunt after the second Cd administration, there was no significant difference between the experimental groups. Consistent with the current results, it has been shwon that Cd challange upregulated hepatic and renal expression of *HSP70* mRNA (Ali et al., 2003). However, Cd challenge reportedly does not stimulate this response in brain and nervous tissue (Liberge and Barthelemy, 2007). The effects of oxidative stress on the abundance of *HSP70* mRNA transcript in UVJ are not fully understood. Xu et al., 2018 demonstrated that CoQ10 upregulates the expression of *HSP70* through PKC/MAPK pathways in chicken myocardial cells and this may explain why the expression of *HSP70* was lower in the PC group, compared to the CoQ10 group, following the second Cd administration. The increased expression of *HSP70* mRNA transcript in PC quails following 2 wk after second Cd administration aligns with capacity of animals to adapt to a recurring oxidative stress (Pamok et al., 2009; Davies, 2016).

CONCLUSIONS

Dietary supplementation of CoQ10 had benefical effects on reducing mortality and increasing the length and thickness of magnum and isthmus folds in Cd-challenged quail. However, CoQ10 supplementation did not improved quail fertility or hatchability. Cadmium adminstration reduced ovarian expression of *HSP70* mRNA following the second Cd injection, but CoQ10 supplementation mitigated this reduction following Cd adminstration. Although Cd administration impaired egg production and quality of hatchlings, CoQ10 supplementation ameliorated these adverse effect likely due to its antioxidant properties and associated protective effect on the oviduct and of Cd-associated histopathological alterations.

ACKNOWLEDGMENTS

The authors also thank Mr. Qais Rahmati (RIP) and Ms. Saba Irani for their technical assistance. The authors gratefully acknowledge the funding provided by College of Agriculture and Natural Resources, University of Tehran [grant number 73130581.6.25].

DISCLOSURES

The authors declare no conflict of interest.

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