Coenzyme Q10 enhances testicular functions and sexual behavior through regulating steroidogenic-related gene expression and inflammatory pathways of Japanese quail (*Coturnix japonica*) aganist cadmium

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ABSTRACT A progressively essential bird of high economic significance for meat production and commercial egg is the Japanese quail, and so more experiments are necessary to improve the productivity of these birds under stressful encounters. The current work was performed to define the influences of Coenzyme Q10 (**CoQ10**) on growth, sexual behavior, and testicular characteristics (size of the gonad, traits of spermatic features, levels of plasma testosterone, steroidogenic-related gene expression) of Japanese quail (Coturnix coturnix japonica) aganist cadmium (**Cd**) administration. Chicks of quail (n = 250), 14 days old of age were distributed into 4 groups: basal ration (Group 1), basal ration and CoQ10 at 100 mg/kg ration (Group 2), basal ration and Cd at 50 mg/kg ration (Group 3), and CoQ10 + Cd (Group 4). Several parameters relating to productive performance, such as weight of the body, weight gain, feed intake, and the conversion ratio of feed, were evaluated. Constant visual scanning of the quails was performed to observe their sexual behaviors. Sperm characteristics, plasma testosterone levels, and testicular inflammatory markers of interleukin-6 (**IL-6**) and tumor necrosis factor-α (**TNF-α**) were also determined. Oxidative-antioxidant redox status in the testes was evaluated by analyzing MDA level, GPx, and SOD activities. Steroidogenic-related gene expression in the testes (CYP17A1, StAR, 3β-HSD, and Cyp19) was also examined. In addition, testicular apoptosis was assessed by monitoring the alterations in the local expression of stress-induced (**HSP70**), proteins of anti-apoptotic marker (**Bcl-2**), and pro-apoptotic markers (caspase-3 and Bax). Cd administration hurts body performance, sexual behavior, and testicular efficiency parameters.

Interestingly, CoQ10 supplementation improved reproductive performance. Moreover, it significantly increases the % of birds exhibiting sexual behavior and enhances testicular functions, which were damaged by Cd administration, by enhancing plasma testosterone level, antioxidative enzyme activity, and sperm quality traits while reducing the MDA and pro-inflammatory markers. Furthermore, downregulation of pro-apoptotic factor expression, though it increased the expression of the anti-apoptotic protein, was recorded. Correspondingly, CoQ10 revealed a marked upregulation in the expression of steroidogenic-related genes. Conclusion: It is established that the consumption of CoQ10 in the ration of Japanese quail, following a Cd adminstration, improves productive performance, sexual behavior, and several testicular function parameters as a potent antioxidant.

Key words: coenzyme Q10, oxidative stress, sexual behavior, steroidogenic-related genes, testicular functions

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INTRODUCTION

Japanese quail fit to Galliformes order and family Phasianidae, Coturnix genus, and japonica species. From an economical perception, this bird has the benefit of a low keeping fee (because of its relatively small size), short intermission of generations (3−4 ages each year), high immunity opposition to diseases, and high

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production of eggs (Rafieian-Naeini et al., 2022). The customer request for quail meat is growing because of their distinctive taste; also, over the preceding few years, the quantity of quail in the industrial farming system has gradually improved (Hafez). About 10% of all table eggs have been reported to be produced by quails, and numerous studies associated stress's impact on the manufacture of eggs and the features of egg characteristics of quails (Butt et al., 2018; Lukanov, 2019). Nonetheless, research is insufficient concerning reducing the harmful effects of stress on the efficiency of reproductive functions of this economically essential bird.

Contamination of the environment has amplified over the preceding decades, resulting in possible hazards to all forms of biological organisms, including poultry production (Khafaga et al., 2019; Rafieian-Naeini et al., 2022). Cadmium (**Cd**), a dominant trigger of oxidative stress, with Cd constant exposure, will accumulate in various tissues, causing harmful impacts on several tissues of avians (de Angelis et al., 2017; He et al., 2020). Certain agricultural performs, such as discarding sewage sludge, phosphatic fertilizers, and composts from town-refuse might raise Cd deposition in soil, which results in the transmission of heavy metals to crops (poultry diet main bulk) and subsequently to the food chain. Industrial cities pollute ambient air and drinking water contributing to Cd daily consumption in humans and animals (Khafaga et al., 2019). Cadmium encourages endocrine dysfunction and several harmful variations in gonadal tissue in different models of animals (Tahir et al., 2017; Chen et al., 2018). Cd exposure to quails has substantial adverse impacts on both productive and reproductive efficiency (Rafieian-Naeini et al., 2022); this heavy metal accumulative impact might be related to the action of metallothionein (Zhu et al., 2020). This could be alleviated by administrating antioxidative constituents.

Throughout past years, numerous studies with various success levels have been performed to discover the impacts of dietary antioxidants on enhancing the poultry reproductive efficiency against oxidative stress (Lukanov, 2019; Rafieian-Naeini et al., 2022). Coenzyme Q10 (**CoQ10**), likewise identified as ubiquinone, is a lipid-soluble substance and a vitamin-like nutrient mainly situated in mitochondria, where it has a significant part in the manufacture of adenosine triphosphate (Abdulidha et al., 2020). CoQ10 also has the exceptional capability to perform as a potent free radical scavenger fat-soluble antioxidant, therefore restraining the damage related to oxidative stress (Mollaei et al., 2022). As well as directly deactivating free radicals, CoQ10 can retain antioxidants like vitamin C (ascorbate) and vitamin E (α-tocopherol) (Bayril et al., 2020). Below 5% of administrated orally, CoQ10 is assumed to spread to the blood circulation, and higher levels of CoQ10 in the plasma are required to stimulate peripheral tissue absorption (Abdulidha et al., 2020). Nevertheless, there are no records of significant harmful impacts of CoQ10 administration (Abdeen et al., 2020).

CoQ10 ration administration linearly enhanced hatching capability, fertility, and penetration of the sperm percent in hens of higher age. In addition, supplementing CoQ10 in the diet improved broilers' reproductive and production qualities (Sharideh et al., 2020). Improving the antioxidative status due to dietary supplementation of CoQ10 may decrease injury by reactive oxygen species (**ROS**). It could lead to enhancements in the efficiency of reproduction (Mollaei et al., 2022). Poultry sexual behavior is connected to the male execution of several courtship displays such as tidbitting, waltzing, and flapping wings, followed by mating, semen ejaculation, and deposition in female cloaca (Mattsson and Brunström, 2017). Numerous hormones control semen production, sexual maturity, and male poultry sexual behavior. Inside the bird's testis, testosterone is the primary sex hormone; variations in excretion of this hormone can be associated with testes' functions and sexual behavior (Lovas et al., 2010). Cadmium which causes oxidative stress in birds leads to a change in their standard behavioral patterns. On the other hand, CoQ10 has antioxidant effects, which ameliorate cadmium's adverse effects, reflecting on birds' expression of standard behavioral patterns (Rafieian-Naeini et al., 2022).

Grounded on the above literature, CoQ10 dietary administration may recover reproductive efficiency of quail aganist Cd adminstration, primarily by its antioxidative effects. Though previous research has revealed that CoQ10 administration enhanced the reproductive functions of aged birds (Sharideh et al., 2020), the defensive impact prompted by CoQ10 diet administration on testicular functions after a Cd adminstration, experiment has not been evaluated under the poultry model. Moreover, little information is identified about the direct impact of cadmium on the sexual behavior of quails and if CoQ10 could alleviate these adverse effects, and if birds exhibit their normal testicular functions and sexual behavioral patterns. The existing experiment, consequently, was performed to study the impact of CoQ10 ration administration on quail testicular functions and sexual behavior through regulating steroidogenic-related gene expression, oxidative stress, apoptotic, and inflammatory pathways of *Coturnix japonica* (Japanese quail) aganist cadmium.

MATERIALS AND METHODS

Chemicals and Reagents

Co enzyme-Q 10 (CoQ10) was obtained from MEPACO, Cairo, Egypt. Cadmium was purchased from El-Gomhoria Company for Pharmaceutical Chemicals, Egypt. Testosterone level kit of ELISA were bought from DRG Diagnostics (Marburg, Germany). In contrast, interleukin-6 (**IL-6**) and tumor necrosis factor-α (**TNF-α**) kits were obtained from My BioSource (CA). Malondialdehyde (MDA), superoxide dismutase (**SOD**), and glutathione peroxidase (**GPx**) analysis kits were purchased from Biodiagnostic (Giza, Egypt).

Reagent of TRIzol (Invitrogen, Life Technologies, Carlsbad, CA). A kit for cDNA production was obtained from Fermentas (Waltham, MA). Except else prominent, all substances were bought from Sigma-Aldrich (St Louis, MO).

Ethical Approval

National Research Council (**NRC**) for usage and maintenance of animals and poultry for experimental procedures were followed. The local board permitted the practices of this study for Ethics for Care and Use of Laboratory Animals of Alexandria University, Egypt (Permit #2022/013/11).

Animals and Management

A whole of 250 chicks (14 days of age) *Coturnix coturnix japonica* (Japanese quail) were purchased from a commercial farmhouse. Quails were divided into 4 groups (Every group was represented with 4 replicates with 25 quails per replicate). Group 1 was administered with a basal ration following the NRC (1994) guidelines (Table 1). The quails of Group 2 were delivered with a basal ration dispensed with CoQ10 at a 100 mg/kg diet (Taha and Al-Tikriti, 2021). Group 3 was fed a basal ration administered with cadmium at a 50 mg/kg diet (Butt et al., 2018). Group 4 was fed a basal ration dispensed with CoQ10 and cadmium as groups 2 and 3, respectively. Chicks were given a balanced diet that was made following the NRC (1994) rules (Table 1). Birds were permitted free entrees to water. The experiment was carried out at the Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Egypt.

Table 1. Composition and nutrient contents of the basal diet.

Ingredients	Growing (2−6 wk)	Laying (7−9 wk)
Corn (7.8% CP)	52.3	53.7
Soybean meal (42.9% CP)	36	28.5
Gluten (59.2% CP)	7.7	5.12
Oil	1	1.1
Limestone	1.65	6.1
Mono-calcium-phosphate (MCP)	0.55	0.7
Wheat bran	0	4
Lysine	0.05	0.05
Methionine	0.06	0.08
Threonine	0.04	0
Choline	0.05	0
Mycotoxin adsorbent	0.05	0.05
Salt	0.25	0.3
Vitamin premix[1]	0.15	0.15
Mineral premix[2]	0.15	0.15
Total	100	100
Chemical analysis		
Moisture%	13.1	11.8
Crude protein%	24.1	20.2
Ether extract%	4.4	4.1
Ash%	6.3	14.4
Se mg/ kg	0.25	0.29
Ca*	0.8	2.5
P*	0.32	0.37
ME Kcal/kg*	2,972.5	2,816.72

*Calcium, phosphorus, and metabolizable energy were calculated according to NRC.

[1]Vitamin premix provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E, 20 mg; vitamin K3, 2.5 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; niacin, 30 mg; Ca-D-pantothenate, 8 mg; vitamin B6, 3.5 mg; vitamin B12, 0.015 mg; folic acid, 1 mg; D-biotin, 0.025 mg; vitamin C, 50 mg; choline chloride, 300 mg.

[2]Mineral premix provided per kilogram of diet: manganese, 60 mg; zinc, 25 mg for growing and 50 mg for laying; iron, 120 mg for growing and 60 mg for laying; copper, 5 mg; cobalt, 0.15 mg; iodine, 0.3 mg; selenium, 0.2 mg. Each 1 kg mineral premix contains: Mn sulfate (243.9 gm), Zn oxide (31.09 gm for growing and 62.189 gm for laying), Iron carbonate (248.96 gm for growing and 124.48 gm for laying), Copper oxide (6.259 gm), Cobalt oxide (0.21 gm), Pot iodide (0.39 gm), Sodium selenite (0.438 gm) and carrier (limestone) up to 1 kg.

Productive Performance

Each week, the weight of the body was determined till the age of 6 wk, gaining of body weight of quail (stated in grams) was evaluated as the change between 2 successive weights. Also, feed conversion ratio (**FCR**) and feed intake were determined.

Sexual Behavior

The activity of mating behavior observation was performed following the recommendations (Mattsson and Brunström, 2017) by constant visual scanning. Observations were undertaken for 2 successive weeks, from the sexually mature age (6th wk) till 8 wk. Quails were monitored for 4 h per day, 3 days per wk for 2 wk. Every day was allocated into 2 stages, morning (from 6 a.m. to 12 p.m.) and afternoon (from 12 p.m. to 6 p.m.). The monitoring was performed 2 h for every phase of the day, which means 2 h in the morning and 2 h in the afternoon with switching. On the first day, monitoring was performed from 6:8 a.m. and 12:2 p.m.; then, on the second day, 8:10 a.m. and 2:4 p.m. were ongoing for the remaining weeks. The hourly observation was divided into 5 min intermissions of scanning all quails and starting with a new 5-min monitor of all behavior until the end of the observation (El-Kazaz el al., 2020).

These behavior patterns were documented by one individual performing the monitoring from outdoor pens. To minimize interruption by the observer, 5 min acclimatization periods were practiced before the beginning of every observation (see Supplementary Table 1). Quails were retained in a flock of mixed sex until sexual maturity age. Then they were sexed at a ratio of 1:1. The experimental rooms had natural light to maintain the birds in reproductive condition. Food and water were always available.

Necropsy

Necropsy was performed on 10 randomly chosen male quails from each group at 8 wk of age; by the end of the study, they were gathered and weighed and then euthanized through cervical dislocation. Blood samples were gathered and placed instantly into heparinized tubes by heart puncture. Samples were centrifuged at 3,000 rpm for 10 min using a centrifuge (Centabun 23, Germany).

Plasma was stored/frozen for testosterone analysis. Left and right testes were assembled from each quail, tapped dry of fluid, then weighed by a digital balance to evaluate gonadosomatic index. The vas deferens took spermatozoa from each male quail for sperm quality trait assessment.

Each bird's testes were collected, cleaned, and washed with cold saline; the testes were weighed individually. In cold phosphate buffer saline (**PBS**), part of each testis was homogenized and centrifuged at 4°C for ten min at 1,435 × *g*. The supernatants were stored at −20°C for biochemical evaluations. The other part of the testes was frozen at −80°C for RT-PCR examination.

Gonadosomatic Index

Gonadosomatic index percent was determined as the relationship between testicular weights and the body's total weight. Gonadosomatic index corresponds to testes weight divided by entire body weight and multiplied by one hundred.

Sperm Traits

Samples of the spermatozoa were added onto glass slides (37°C). Live/dead sperms (viability) were examined by the stain of eosin−nigrosin. By using Giemsa stain, sperm morphology was evaluated. Motility, morphology, and viability parameters were verified as %. Then, subsequent data were estimated and documented at 100× or 400× amplification: individual and mass sperm motility. Count of sperms was examined by the counting chamber of hemacytometer (Rodríguez-Martínez, 2013).

Plasma Testosterone Analysis

An indirect enzyme immunoassay assay kit (Monobind, 100 North Point Drive, Lake Forest, CA) evaluated plasma testosterone concentration according to the methods defined by Tietz (1995).

Oxidative Stress Markers

Tissues of the testes were tested for oxidative stress estimation following homogenization in phosphate buffer saline. Supernatants were evaluated for MDA (Okaichi et al., 2005) and GPx from the rate of NADPH oxidation (Flohé and Gunzler, 1984) beside SOD by a commercial kit (Biodiagnostic, Giza, Egypt) (Nishikimi et al., 1972). Protein levels of testes were measured for the calibration of biochemical assessments by the Bradford method (BioRad Laboratories, Watford, UK; Bradford, 1976).

Testicular Pro-inflammatory Cytokines Biomarkers

An enzyme-linked immunoassay (**ELISA**) kit (Sigma-Aldrich) has been used for interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in tissues of the testes, following the manufacturers instruction.

Gene Expression Analysis

RNA of around 100 mg of testicular tissues was acquired with (Invitrogen, Life Technologies) TRIzol reagent and Nanodrop for quantification. For DNA production, samples of RNA of 1.8 or more A260/A280 were utilized by a kit for cDNA synthesis (Fermentas). SYBR Green Master Mix and the primers (**GAPDH**) of the household gene were shown in Table 2 additional to amplify cDNA. Recordsin on magnification were investigated by $2-\Delta\Delta T$ methods (Livak and Schmittgen, 2001).

Table 2. Primers for gene expression by RT-PCR.

Gene	Direction	Primer sequence	Accession number
CASP3	Sense	CTGGACTGCGGTATTGAGAC	NM_012922.2
	Antisense	CCGGGTGCGGTAGAGTAAGC	
Bax	Sense	GGCGAATTGGCGATGAACTG	NM_017059.2
	Antisense	ATGGTTCTGATCAGCTCGGG	
Bcl-2	Sense	GATTGTGGCCTTCTTTGAGT	NM_016993.1
	Antisense	ATAGTTCCACAAAGGCATCC	
HSP70	Sense	TCAGAGCTGCTATGTCGCTG	NM_153629.1
	Antisense	GCAGCGGTCGCTATACTCAT	
CYP17A1	Sense	ACTGAGGGTATCGTGGATGC	NM_012753.2
	Antisense	TCGAACTTCTCCCTGCACTT	
StAR	Sense	CTGCTAGACCAGCCCATGGAC	NM_031558.3
	Antisense	TGATTTCCTTGACATTTGGGTTCC	
3β-HSD	Sense	CCCATACAGCAAAAGGATGG	M38178
	Antisense	GCCGCAAGTATCATGACAGA	
Cyp19	Sense	GCTTCTCATCGCAGAGTATCCGG	M33986
	Antisense	CAAGGGTAAATTCATTGGGCTTGG	
GAPDH	Sense	TCAAGAAGGTGGTGAAGCAG	NM_017008.4
	Antisense	AGGTGGAAGAATGGGAGTTG	

Abbreviations: Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CASP3, caspase 3; CYP17A1, cytochrome P450 17A1; Cyp19, cytochrome P450 aromatase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HSP70, heat shock protein 70; StAR, steroidogenic acute regulatory protein; 3β-HSD, 3-beta-hydroxysteroid dehydrogenase/delta-5-delta-4 isomerase type I.

Table 3. Impact of CoQ10 (100 mg/kg diet) on body weight (g) of Japanese quail aganist cadmium (Cd) (50 mg/kg diet) adminstration.

	Treatment				
Productive performance	Control	CoQ10	Cd	CoQ10+Cd	*P* value
Initial body weight (2 wk)	46.18 ± 0.95	47.37 ± 0.57	45.16 ± 0.09	46.33 ± 1.57	NS
Body weight (3 wk)	108.35 ± 1.42[a]	112.78 ± 2.98[a]	91.96 ± 1.36[c]	100.78 ± 2.18[b]	<0.0007
Body weight (4 wk)	161.33 ± 1.12[b]	174.90 ± 1.94[a]	136.21 ± 1.64[d]	154.17 ± 3.88[c]	<0.0001
Body weight (5 wk)	212.23 ± 1.85[b]	233.59 ± 1.61[a]	206.30 ± 1.92[d]	201.89 ± 2.61[c]	<0.0001
Final body weight (6 wk)	262.13 ± 2.75[b]	271.52 ± 2.54[a]	215.75 ± 2.09[d]	252.52 ± 2.09[c]	<0.0001
Total gain (g.)	215.95 ± 2.85[b]	224.15 ± 1.52[a]	170.59 ± 2.18[d]	206.19 ± 1.99[c]	<0.0001
Total feed intake (g.)	769.10 ± 0.00[b]	784.99 ± 0.00[a]	729.59 ± 0.00[d]	760.99 ± 0.00[c]	<0.0001
Feed conversion ratio (FCR)	3.56 ± 0.02[c]	3.50 ± 0.04[d]	4.28 ± 0.05[a]	3.69 ± 0.03[b]	<0.0001

The data presented as mean ± standard error.
[a-d]Means bearing different superscript letters within the same row are significantly different ($P < 0.05$).

Statistical Analysis

Statistical analysis was achieved by SAS software (SAS, 1994). One-way ANOVA was utilized for examination of growth performance, data of behavioral patterns, testosterone hormone, sperm parameters, and testicular oxidative/antioxidant markers. The behavioral records were shown in the percentage of quails performing various behavioral patterns. Duncan's test was consumed for post-hoc examines if treatment impacts were substantial. The overall significance was set at $P < 0.05$. Values were presented as the mean ± standard error.

RESULTS

Effect of CoQ10 on Productive Efficiency

The impact of cadmium (Cd) and CoQ10 on the performance of growth parameters is presented in Table 3. Cd induced a notable decrease in weekly and total body weight, intake of food, and total gaining of significance ($P < 0.05$) of quails and a marked enhancement in feed conversion ratio compared with other groups, respectively. While CoQ10 showed the best growth performance parameters as there was a significant increase in weekly and total body weight, intake of food, and weight ($P < 0.05$) of quails and a marked reduction in FCR. Moreover, the addition of CoQ10 with Cd (Cd + CoQ10 group) alleviates the harmful impact of cadmium on the growth performance of quails as there was a substantial improvement ($P < 0.05$) in weight of the body, total weight gain, and intake of food and a marked reduction ($P < 0.05$) in FCR relative to birds fed a diet with Cd.

Effect of CoQ10 on Sexual Behavior

Patterns of sexual behavior differ markedly among the administered groups. As summarized in (Table 4) cadmium administration caused a marked ($P < 0.001$) reduction in the percent of birds exhibiting sexual behavioral patterns such as Waltzing, tidbitting, wing flapping, mounting, treading, rear approach, and complete copulation compared with control, CoQ10 and Cd + CoQ10 groups. On the contrary, CoQ10 significantly increased ($P < 0.001$) in birds showing sexual behavioral patterns than other birds in other groups. Moreover, CoQ10 significantly improved the percent of birds exhibiting those behavioral patterns in Cd + CoQ10 concerning the Cd group treated quail's group.

Effect of CoQ10 on Testosterone Hormone

Exposure of the birds to Cd led to a marked ($P < 0.001$) decline in plasma values of testosterone (Table 6) hormone compared with Cd + CoQ10 and CoQ10 groups. In contrast, CoQ10 administration in the Cd + CoQ10 and CoQ10 quails markedly ($P < 0.001$) improved plasma testosterone levels concerning the Cd-adminstred quail group, as shown in Figure 1.

Effect of CoQ10 on Gonadosomatic Index and Sperm Morphology

The cadmium administration caused a marked ($P < 0.05$) decline in the weight of testes relative to the control and CoQ10 birds, resulting in a small

Table 4. Impact of CoQ10 (100 mg/kg diet) on the percent of birds that exhibit sexual behavioral patterns of male Japanese quails aganist cadmium (Cd) (50 mg/kg diet) adminstration.

	Treatment				
Behavioral patterns	Control	CoQ10	Cd	CoQ10+Cd	*P* value
Waltzing	12.48 ± 0.26[b]	15.22 ± 1.94[a]	7.15 ± 0.96[d]	10.15 ± 1.96[c]	<0.0001
Wing flapping	82.23 ± 5.33[b]	89.73 ± 9.12[a]	59.52 ± 3.05[d]	67.58 ± 6.45[c]	<0.0001
Tidbitting	4.25 ± 0.74[b]	5.33 ± 0.78[a]	1.95 ± 0.86[d]	2.23 ± 0.57[c]	<0.005
Rear approach	63.42 ± 4.96[b]	75.84 ± 6.58[a]	48.42 ± 9.21[d]	58.43 ± 6.85[c]	<0.0001
Mounting	70.12 ± 3.34[b]	79.74 ± 8.12[a]	50.18 ± 5.47[d]	66.25 ± 6.21[c]	<0.0001
Treading and copulation	80.35 ± 7.44[b]	88.82 ± 6.24[a]	51.25 ± 3.85[d]	71.25 ± 4.03[c]	<0.0001

The data presented as mean ±standard error.
[a-d]Means bearing different superscript letters within the same row are significantly different ($P < 0.05$).

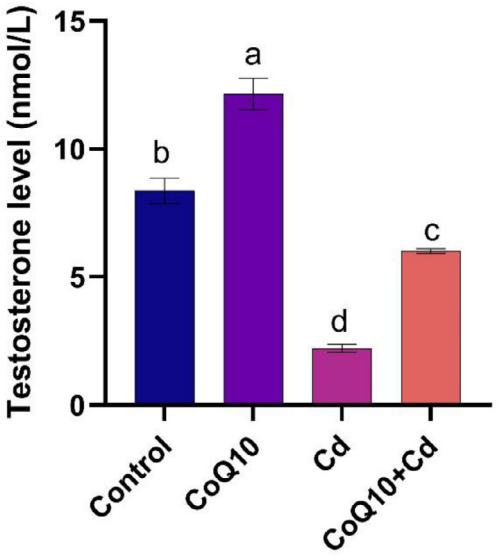

Figure 1. Impact of CoQ10 (100 mg/kg diet) on plasma testosterone level of Japanese quail aganist cadmium (Cd) (50 mg/kg diet) adminstration. The data presented as mean ± standard error. Means bearing different superscript letters within the same row are significantly different ($P < 0.001$).

gonadosomatic index. On the other hand, CoQ10 noticeably ($P < 0.05$) enhanced this parameter in Cd + CoQ10 relative to the Cd group. Comparable data were revealed from the count of spermatozoa and percent of sperm abnormalities, sperm motility, and sperm live/dead in Cd and Cd + CoQ10 groups, correspondingly (Table 5). The concentrations of the sperm cell, in addition to the percent of the mass and individual motility and percent of live spermatozoa, were noticeably ($P < 0.05$) decreased in Cd administered quails. However, the percent of sperms abnormality was substantially ($P < 0.05$) increased in Cd-supplemented quails compared to control birds. These data were improved in the co-administered group of Cd + CoQ10.

Impact of CoQ10 on Testicular Oxidant/ Antioxidant Parameters and Pro-inflammatory Cytokines

MDA (oxidative stress marker) values and pro-inflammatory markers (IL-6 and TNF-α) are noticeably ($P < 0.001$) augmented in the testicular tissue of Cd-treated quails. On the contrary, the amounts of antioxidant markers (GPx and SOD) markedly ($P < 0.001$) reduced relative to the CoQ10 and control birds. In contrast, CoQ10 administration in the Cd + CoQ10 group substantially ($P < 0.001$) ameliorated the oxidative and inflammatory injury in the tissue of the testes (Table 6).

Effect of CoQ10 on the Expression of Apoptotic and Function-Related Genes Fold Change

Caspase 3 (**CASP3**), B-cell lymphoma 2 (**Bcl2**), Bcl-2-associated X protein (**Bax**), cytochrome P450 17A1 (CYP17A1), heat shock protein 70 (**HSP70**), steroidogenic acute regulatory protein (**StAR**), cytochrome P450 aromatase (**Cyp19**) and 3-beta-hydroxysteroid dehydrogenase/delta-5-delta-4 isomerase type I (3β-HSD) mRNA expression in testicular tissue of control, CoQ10, Cd, and Cd + CoQ10 quails were shown in Figure 2. Cd substantially ($P < 0.05$) enhanced the gene fold changes of pro-apoptotic genes (Bax and CASP3) relative to control birds. Moreover, compared to Cd

Table 5. Impact of CoQ10 (100 mg/kg diet) on the testes index weight and sperm parameters of Japanese quail aganist cadmium (Cd) (50 mg/kg diet) adminstration.

Groups	Control	CoQ10	Cd	CoQ10+Cd	*P* value
Testes index weight	2.15 ± 0.18^{b}	2.23 ± 0.21^{a}	1.09 ± 0.07^{d}	1.85 ± 0.13^{c}	<0.0001
Mass motility (%)	78.55 ± 3.47^{b}	82.27 ± 4.04^{a}	52.36 ± 3.19^{d}	65.17 ± 2.61^{c}	<0.0001
Individual motility (%)	74.27 ± 4.01^{b}	79.64 ± 3.22^{a}	48.41 ± 2.98^{d}	63.27 ± 3.16^{c}	<0.0001
Dead sperms (%)	22.46 ± 2.12^{c}	19.46 ± 2.36^{d}	43.18 ± 3.22^{a}	33.56 ± 2.21^{b}	<0.0001
Abnormal sperms (%)	28.36 ± 1.97^{c}	23.43 ± 2.01^{d}	47.56 ± 4.81^{a}	38.11 ± 2.49^{b}	<0.0001
Sperms count ($x10^{9}$/mL)	1.85 ± 0.09^{b}	2.03 ± 0.11^{a}	0.76 ± 0.03^{d}	1.43 ± 0.08^{c}	<0.003

The data presented as mean ± standard error.
[a-d]Means bearing different superscript letters within the same row are significantly different ($P < 0.05$).

Table 6. Impact of CoQ10 (100 mg/kg diet) on the testicular oxidative/antioxidant markers and pro-inflammatory cytokines of Japanese quail aganist cadmium (Cd) (50 mg/kg diet) adminstration.

Groups	Control	CoQ10	Cd	CoQ10+Cd	*P* value
MDA (nmol/ mg protein)	32.15 ± 2.18^{c}	29.23 ± 2.21^{cd}	51.09 ± 4.07^{a}	40.15 ± 2.87^{b}	<0.0001
GPx (U/ mg protein)	44.12 ± 3.21^{ab}	47.14 ± 3.02^{a}	19.87 ± 1.25^{d}	32.74 ± 2.17^{c}	<0.0001
SOD (U/mg protein)	64.27 ± 3.55^{b}	69.64 ± 4.71^{a}	32.33 ± 3.14^{d}	53.14 ± 2.24^{c}	<0.0001
TNF-α (pg/ mg protein)	22.96 ± 1.72^{c}	20.46 ± 1.54^{c}	53.44 ± 3.55^{a}	35.56 ± 2.01^{b}	<0.0001
IL-6 (pg/ mg protein)	18.61 ± 0.95^{c}	17.43 ± 0.87^{c}	33.84 ± 2.24^{a}	26.47 ± 1.09^{b}	<0.0001

The data presented as mean ± standard error.
[a-d]Means bearing different superscript letters within the same row are significantly different ($P < 0.001$).

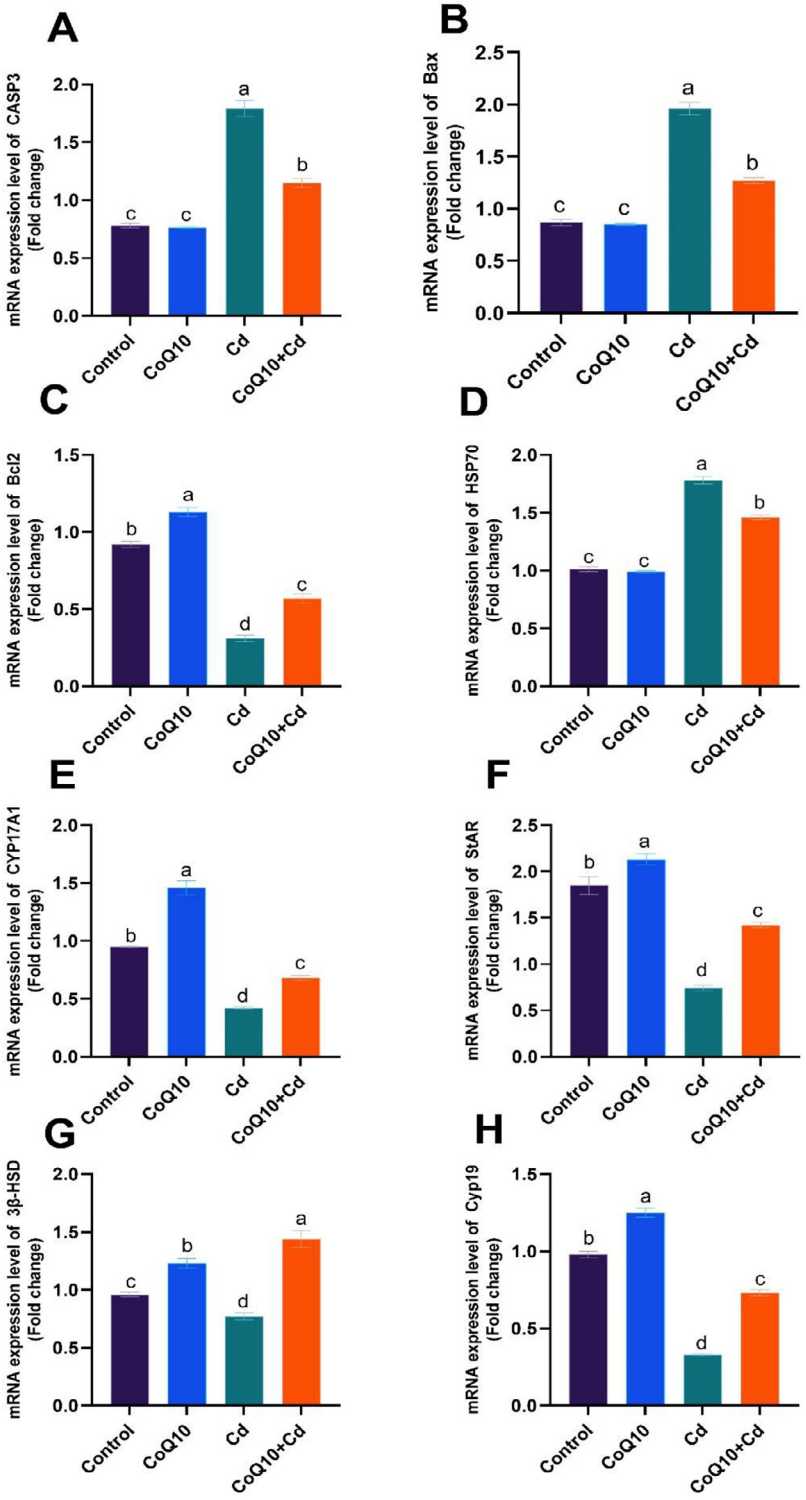

Figure 2. Impact of CoQ10 (100 mg/kg diet) on expression of fold changes of testicular caspase 3 (CASP3) (A), Bcl-2-associated X protein (Bax) (B), B-cell lymphoma 2 (Bcl2) (C), heat shock protein 70 (HSP70) (D), cytochrome P450 17A1 (CYP17A1) (E), steroidogenic acute regulatory protein (StAR) (F), 17βhydroxysteroid dehydrogenase (17β-HSD) (G), and cytochrome P450 aromatase (Cyp19) (H), of Japanese quail aganist cadmium (Cd) (50 mg/kg diet) adminstration. The data presented as mean ± standard error. Means bearing different superscript letters within the same row are significantly different ($P < 0.05$).

birds, CASP3 and Bax considerably ($P < 0.05$) were downregulated in Cd + CoQ10 treated quails. Nevertheless, fold changes of the anti-apoptotic gene, Bcl2, were markedly improved because of CoQ10 administration in CoQ10 and Cd + CoQ10 birds compared to Cd birds.

HSP70 mRNA expression in the testicular tissue enhanced ($P < 0.05$) in Cd-administered quails compared with the control, Cd + CoQ10, and CoQ10 birds. CoQ10 considerably improved the expression of cytochrome P450 17A1 (CYP17A1), StAR, 3β-HSD, and Cyp19 mRNA levels in testes relative to Cd- administered birds. In contrast with Cd, the Cd + CoQ10 markedly improved the expression fold changes of 3β-HSD, CYP17A1, StAR, and Cyp19 mRNA levels.

DISCUSSION

Environmental pollution with heavy metals has increased over time, resulting in enormous hazards to all biological organisms and poultry (Khafaga et al., 2019; Rafieian-Naeini et al., 2022). Cadmium (Cd) is considered a potent prompting of oxidative stress, so in our experiment, we consumed coenzyme Q10 as an antioxidative agent to contrary the harmful effects of cadmium.

The impact of CoQ10 on the bodyweight of quail with cadmium administration was demonstrated in (Table 3). The overall results indicated a considerable decrease in body weight, feed intake, and weight gain in quails with Cd exposure and a marked enhancement in feed conversion ratio. Comparable data were recorded by Rafieian-Naeini et al. (2022), who reported a significant reduction in body weight of Japanese quails following cadmium exposure. Moreover, Al-Waeli et al. (2013) revealed that supplementation of a hundred mg of cadmium per kg of feed produced marked negative impacts on growth, such as decreased body mass, reduced food intake, and enhanced FCR. Berzina et al. (2007) recorded growth retardation in birds given a Cd-enriched ration.

Moreover, the results agree with the reports that Cd decreased feed consumption (Lisunova et al., 2006) and lowered feed efficiency (Erdogan et al., 2005). Also, Rahman et al. (2007) revealed a reduction in quail body weight after cadmium injection. Sant'Ana et al. (2005) recorded no variations in body weight after the first 2 wk of Cadmium addition. Body weight was only affected after 28 days of chronic Cd administration. The harmful impact of Cd on growth performance might be because of the antagonistic relationships between this metal and some micronutrients, especially zinc and iron. Cadmium has an inhibitory effect on the activity of the zinc-containing enzyme, such as carboxypeptidase and mannosidase (Chen et al., 2018).

Moreover, in the upkeep of this statement, it has been recorded that chronic Cd administration leads to an exhaustion of muscular and liver glycogen because of its impact on the enzymes related to energy metabolism and glycogenesis (Tourry et al., 1985) and causes oxidative stress in kidney and liver (Yiin et al., 1999). The CoQ10 supplementation, as a consequence of Cd addition, serves as a powerful antioxidant that restores oxidative stress caused by Cadmium (Choudhury et al., 1991). CoQ10 supplementation significantly increases the weight of the body, intake of food, and gaining of weight also, it considerably decreases FCR. These data agreed with Gopi et al. (2014), who revealed that CoQ10 dietary exposure significantly improved body weight and weight gain with a higher production performance. This defensive antioxidant impact also elucidates how CoQ10 addition decreases broilers' ascites (Geng et al., 2004). It also explains that adding CoQ10 with cadmium alleviates its oxidative effect, which causes a significant enhancement in the growth performance of birds due to its antioxidant effect.

Exposure to cadmium induces dramatic changes in neuroendocrine/behavioral function. We recorded that cadmium induced a significant decrease in the manifestation of mating and sexual behavior in quails as there was a substantial decline in the percent of male birds exhibiting sexual behavior than other groups. Clark et al. (1994) stated that cadmium supplementation significantly decreases erectile function, with a reduction in the behavior of copulation and function of the testicular tissue (demonstrated by declines in the level of plasma testosterone). These results may be attributed to testicular damage and a significant decrease in serum testosterone concentration as an effect of cadmium exposure. The impacts of Cd on the behavior of copulation are prominent and might not be only caused by a decrease in blood testosterone concentrations (de Angelis et al., 2017; He et al., 2020). Various researches maintain this proposition, demonstrating that after castration of sexually practiced males in adulthood is followed by a gradual failure in the mating behavior (Clark, 1993; Davidson et al., 1978). In our study, Coenzyme Q10 supplementation significantly increases the percentage of birds exhibiting sexual behavior as it is one of the most commonly used antioxidants for male infertility treatment and improves erectile function (Tsao et al., 2021). Moreover, the addition of CoQ10 with cadmium exhibited a considerable improvement in the percent of birds showing sexual behavior than birds exposed to cadmium only as CoQ10 protects those birds from lipid peroxidation. These outcomes are possible because of cadmium's oxidative stress augmentation, including lower GPx and SOD activates (Cupertino et al., 2017; Saleemi et al., 2019).

Energy manufacture inside the mitochondria, which is required to sustain the sperm cell energy system and defend it against the harm of lipid peroxidation, involves coenzyme Q10 (CoQ10), as it is a part of the ubiquinone family which is known to have unique antioxidant characteristics. It is one of the furthermost extensively consumed antioxidants for male infertility treatment (Tsao et al., 2021). Cd is one of the heavy metals that animals are subjected to via polluted foods and soil; Cd is reported to have many harmful effects on testicular tissue (Zhu et al., 2020).

Cadmium provided a remarkable reduction in reproductive organ weight because of the reduction in blood testosterone level, motility of spermatocyte, sperm concentration, and percent of spermatozoa viability. It substantially enhanced the percent of spermatozoa abnormalities in Cd-given quails, which might induce its hazardous effect. Accordingly, the results indicate that Cd reduces the quality of the sperms, resulting in male quail's infertility. The mechanism of heavy metal harmfulness on the tissues of the testes can be associated with the triggering of oxidative damages. Our data approve with Chen et al. (2018) and Cupertino et al. (2017), who stated that Cd's long-term exposure reduces harmful impacts on sperm motility and sperm characters caused by Cd. Furthermore, overproduction of free radicals, which surpasses cellular ability, causes oxidative harm and decreases spermatozoa fertility and capability (Elmallah et al., 2017). The spermatocyte holds a high

quantity of polyunsaturated fatty acids. It is vulnerable to damage triggered by increased oxidative stress and causing plasma membrane peroxidation, leading to a reduced sperm cell count and a decline in motility (Tohamy et al., 2021). Cd-intoxicated rats revealed a notable surge in MDA, which reflects the significance of lipid degradation and lipid peroxidation generating radicals and decrease of the antioxidant enzymatic activities of GPx and SOD, which resonate the level of oxidative damage and antioxidant activity and consequent lipoperoxidation (Tahir et al., 2017; Saleemi et al., 2019). These data succeed the results of Rafieian-Naeini et al. (2022) that revealed declined levels of GSH in the liver of laying Japanese quails under the impact of Cd. The decline in the activity of GPx and SOD in testicular tissue of Cd-administered rats (5 and 10 mg/kg bwt) led to a reduction in antioxidant capacity in the testis (Elmallah et al., 2017). These results are possibly validated to cadmium's oxidative stress augmentation, declined GPx and SOD, and the activity of antioxidant enzymes and subsequent lipoperoxidation (Cupertino et al., 2017). Cd-given rats exhibited an over the manufacture of IL-6 and TNF-α pro-inflammatory markers. TNF-α is a primary immune and inflammatory response marker, and IL-6 is a cytokine pleiotropic secreted by monocytes and tissue macrophages (Butt et al., 2018), which indicates an inflammatory impact resulting from Cd administration.

Results of the current work revealed that Cd remarkably enhanced pro-apoptotic (Bax and CASP3) and reduced anti-apoptotic (Bcl2) mRNA fold changes expression in the tissues of the testes. These data showed an enhancement in apoptosis caused by heavy metal-induced oxidative stress, as evidenced by Rafieian-Naeini et al. (2022). In addition, testicular oxidative stress damage can similarly trigger sperm apoptosis, initiate sperm disorders and disturb spermatocyte production (Atta et al., 2020). Likewise, Chen et al. (2018) revealed a substantial upregulation of CASP3 in the testicular tissue in Cd-administrated rats, triggering oxidative deterioration. Oxidative stress is also connected with a noticeable enhancement in Hsp70 proteins (Tohamy et al., 2021). Hsp70 is a crucial protein that assists in protecting the cells from stress (Atta et al., 2020). In the current work, tissues of the testes demonstrated upregulation in HSP70 mRNA fold changes expression in Cd-administered quails. Correspondingly, Cupertino et al. (2017) recorded a substantial stimulation of HSP70 in testicular tissue when the animals were subjected to cadmium.

The data indicated that the Cd group revealed a marked downregulation in the expression of steroidogenic-related genes containing CYP17A1, STAR, 3β-HSD, and Cyp19 relative to other treated groups and the control quails. However, CoQ10 with Cd co-addition exhibited a noticeable stimulation and maintenance of steroidogenic gene expression. The steroidogenic enzymes are obligatory for testosterone manufacture; Cd reduced CYP17A1, STAR, 3β-HSD, and Cyp19. Consequently, the Cd group exhibited a remarkable decline in plasma testosterone concentration in the present experiment. The data propose that testicular cadmium-induced oxidative stress contributes to Leydig cell hyperplasia and shrinkage in testosterone biosynthesis (Zhu et al., 2020). Hormones of steroid nature are synthesized from cholesterol via numerous steroid cytochrome P450 hydroxylases-triggered reactions (Atta et al., 2020). Under the influence of steroidogenic acute regulating protein (StAR), cholesterol relocates from the exterior to the mitochondria's inward membrane, which includes a rate-limited steroidogenesis phase (Cupertino et al., 2017). Afterward, steroidogenesis initiates by converting P450 (P450scc/CYP11A1/Cyp11a1) cholesterol to pregnenolone, a crucial particle in producing the body's steroid hormones (Miller and Auchus, 2011). Downregulation of CYP17A1, STAR, 3β-HSD, and Cyp19 resulted in a decline in the biosynthesis of testosterone hormone under the influence of Cd administration, as demonstrated by Chen et al. (2018). Heavy metal toxicity declined the synthesis of steroids by Leydig cell's via CYP17A1 or StAR protein deprivation (He et al., 2020), likewise, chronic exposure to oxidative stress reduced the expression of StAR in rat testicular tissue (Cupertino et al., 2017).

CoQ10 and cadmium co-administration markedly enhanced the estimated parameters such as the reproductive organ weights, regulated testosterone concentrations, and improved sperm characteristics, which were accredited to the normalization of spermatogenesis, which led to the enhanced numbers of spermatocytes (Taha and Al-Tikriti, 2021). The favorable impact of CoQ10 on adult male quail fertility might be caused by its antioxidant properties (Tsao et al., 2021). CoQ10 treatment regulates the apoptotic outcome of Cd, revealed by our data, in which Cd substantially upregulates the expression of CASP3, Bax, and HSP70 genes with marked downregulation to Bcl-2. These results contradicted (Abdeen et al., 2020); they showed the antioxidant, anti-inflammatory, and anti-apoptotic impacts of CoQ10. CoQ10's potential role in hunting the ROS induced by Cd inside the testicular tissue was linked with the reduced oxidative stress marker (MDA) and pro-inflammatory markers (TNF-α and IL-6); the improved levels of anti-oxidative enzymatic activates of GPx and SOD. According to our data, Cd's molecular mechanism that encouraged fertility dysfunction was concise in steroidogenic-related gene expression downregulation, including STAR, CYP17A1, and 3β-HSD.

Numerous research works are focused on the mechanism dynamics of the defensive activities in which CoQ10-administered quails revealed a decline in serum TNF-α and IL-6 expression and hepatic Inos (Rafieian-Naeini et al., 2022). Bayril et al. (2020) reported that CoQ10 has anti-inflammatory impacts in birds subjected to cold stress by constraining nitric oxide, tumor necrosis factor-α (TNF-α), IL-1β, and mitogenic protein kinases; which may indicate that CoQ10 as a probably potent anti-inflammatory drug (Abdulidha et al., 2020). In addition, CoQ10 is considered to be a powerful antioxidative agent; predominantly, the reactive oxygen

species gathering, and subsequently testicular oxidative injury in Leydig cells by a particular chemical oxidant, might fail their response and performance to produce testosterone. CoQ10 is an antioxidant inside the cell that safeguards mitochondrial layer protein, layer LDL-C and phospholipids, and free radical-triggered oxidative damage (Taha and Tikriti, 2021). Moreover, CoQ10 is an antioxidant when supplied to mature males by falling MDA levels in seminal plasma and blood, testis histology clearances, and development make strides in sex hormone (Mollaei et al., 2022; Sarica et al., 2007).

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DISCLOSURES

There are no conflicts of interest to declare.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.102517.

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