



## Dietary coenzyme Q<sub>10</sub> may improve the growth performance and antioxidant status in quails exposed to cold stress

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**ABSTRACT.** In this study, the effects of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) on growth performance, antioxidant status and organ weights in cold-stressed Japanese quails (*Coturnix coturnix japonica*) were investigated. During the experiment, a 2 × 3 factorial design was employed with two environmental temperatures (ET) and three levels of CoQ<sub>10</sub> (0, 20 and 40 mg/kg). A total of 180 one-day-old male quails were randomly allocated into 6 groups with 6 replicates with 5 birds in each replicate. The birds were fed in two separate rooms at either 22 ± 2 °C for 24 h/day (thermoneutral, TN) or 12 ± 2 °C for 8 h/day (cold stress, CS; between 09:00–17:00) followed by 22 ± 2 °C for 16 h/day. CoQ<sub>10</sub> addition into diet increased final body weight, body weight gain and cumulative feed intake only in CS regardless of the used dose. It was stated that CoQ<sub>10</sub> supplementation did not exert influence on serum and liver superoxide dismutase (SOD) activity and liver total antioxidant status (TAS) in TN conditions, but increased these parameters in CS; however in liver the higher CoQ<sub>10</sub> dose was required to obtain the statistically positive effect. When quails were exposed to CS a higher dose of CoQ<sub>10</sub> caused a more pronounced decreased in serum malondialdehyde (MDA) level than the lower one; however the effect of CoQ<sub>10</sub> on liver MDA level was shown regardless of the used dose. The obtained results show that CoQ<sub>10</sub> supplementation reverses the negative effects of CS on growth performance, antioxidant status and organ weights in quails. The caused may effects partly associated with direct antioxidant properties of CoQ<sub>10</sub> as well as the synergistic efficacy of CoQ<sub>10</sub> with SOD activity.

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### Introduction

Animals can be exposed to climatic, nutritional, environmental, social, physical or physiological stressors in the breeding systems (Siegel, 1995). The cold stress (CS) has been shown to increase negative effects on animal health and behavioural characteristics (Dhanalakshmi et al., 2007). Adult poultry exposed to low temperature tries to adjust body temperature by increasing the amount of con-

sumed energy and regulating metabolism. However, young poultry is vulnerable to low temperatures and cannot survive in a cold environment without adequate shelter and warming (Mozo et al., 2005).

Climatic stressors such as CS and heat stress cause damage to proteins, lipids, DNA and carbohydrates in the cell due to the induction of oxidative stress in broilers (Sahin and Kucuk, 2003; Şahin and Gümüşlü, 2004). Additionally, oxidative stress caused by low and/or high temperatures can directly

affect body temperature and disrupt both the physical and mental activity of poultry (Dhanalakshmi et al., 2007). Indeed, it was shown that animal performance and behavioural traits were reduced by the activation of the hypothalamic–pituitary–adrenal (HPA) axis during exposure to stressors such as cold temperature. The CS is also known to reduce immunity and reproduction as well as to increase mortality in poultry (Mujahid and Furuse, 2009). At ambient temperatures below 18 °C, the birds may have problems maintaining a proper body temperature, which may cause serious illnesses, permanent tissue damage and even death (Dhanalakshmi et al., 2007). Low temperatures also disturb the physiological responses associated with oxygen consumption and stimulate changes in energy production and the formation of reactive oxygen species (ROS) (Şahin and Gümüşlü, 2004). Various studies indicate that exposure to CS can cause a significant deterioration in the antioxidant defence system (Mujahid and Furuse, 2009). CS preferentially reduces the total antioxidant status (TAS) and increases the levels of malondialdehyde (MDA) in serum and some internal organs such as the liver, brain and heart (Mujahid and Furuse, 2009). It can also change the activity of superoxide dismutase (SOD), which is an important antioxidant enzyme that plays a key role in disposing oxygen-derived reactants under oxidative stress conditions. Sialic acid (N-acetylneuraminic acid), commonly found in living organisms, is a derivative of neuraminic acid, an acidic sugar with a nine-carbon backbone. In animals, sialic acid is found mainly in the form of glycoproteins building cell membranes (Schauer, 1982). Another important compound that participates in defence mechanisms in oxidative stress is ceruloplasmin, which participates in the oxidation of Fe<sup>2+</sup> to Fe<sup>3+</sup>. These certain plasma proteins synthesis and release can be elevated during the acute phase reaction (Schauer, 1982).

CoQ<sub>10</sub>, which has in its structure 50 carbon atoms and 10 isoprene units, is located in the hydrophobic part of the double phospholipid layer in the mitochondrial membrane. It exhibits antioxidant properties and can be synthesized endogenously in the body (Ernster and Forsmark-Andrée, 1993). However, CoQ<sub>10</sub> also plays a pro-oxidative role in generating the main superoxide anion/hydrogen peroxide second messenger system (Linnane et al., 2007). In poultry, it can be seen that substances such as lycopene, selenium and  $\alpha$ -tocopherol, which possess strong antioxidant properties, are widely used in order to prevent or reduce the negative effects of oxidative stress (Rozbicka-Wieczorek et al., 2012; Konieczka et al., 2015). Indeed, due to its strong

antioxidant efficacy, CoQ<sub>10</sub> can effectively inhibit the oxidation of lipids, proteins and DNA joining electron and proton transport in oxidative phosphorylation (Ernster and Forsmark-Andrée, 1993; de Barcelos and Haas, 2019). Studies have shown that CoQ<sub>10</sub> is the first antioxidant which acts under oxidative stress conditions in the organism (Overvad et al., 1999). Moreover, high levels of CoQ<sub>10</sub> have been found in organs, such as the heart and brain, and muscle, which all required high energy supply. It was also stated that CoQ<sub>10</sub> plays an important role in the energy production from carbohydrates and lipids in cells and in lowering the serum total cholesterol level by inhibiting the synthesis of this steroid in the liver (Modi et al., 2006).

The results available in the literature provide very limited information on the relationship between CS disruptive effects and the radical-scavenging activity of CoQ<sub>10</sub> regarding growth efficiency and antioxidant status in poultry. Therefore, in the present study the effects of dietary CoQ<sub>10</sub> supplementation on growth performance, antioxidant status and organ weights in cold-stressed Japanese quails were examined. Attempts were also made to show possible relationships between CoQ<sub>10</sub> and other factors involved in the body antioxidant defence.

## Material and methods

### Animals, treatments and management

All procedures used in the present study were approved by the Institutional Animal Care and Use Committee of Veterinary Faculty of Dicle University (Diyarbakir, Turkey). One hundred and eighty, one-day-old male Japanese quails (*Coturnix coturnix japonica*) were purchased from the poultry unit of the Veterinary Faculty of Dicle University (Diyarbakir, Turkey) and randomly allocated to six groups. Each of the experimental group was replicated in 6 cages of 5 birds each. The birds were housed in our animal facility under a 24-hour light cycle and in the temperature of 34 ± 2 °C in the first days of life. The room temperature gradually decreased to 22 ± 2 °C by the end of the 15<sup>th</sup> day. Then, throughout the whole 28-day long experiment, the birds were fed in temperature-controlled two separate rooms at two environmental temperatures: 22 ± 2 °C for 24h/day (thermoneutral (TN) conditions) and 12 ± 2 °C for 8 h/day (between 09:00–17:00) followed by 22 ± 2 °C for 16 h/day (cold stress conditions, CS). Feed and fresh water were offered *ad libitum* throughout the whole experiment.

### Diet, sample and data collection

All birds were fed the same basal diet (grower from day 15 to day 28 and finisher from day 29 to day 42), but with different levels of CoQ<sub>10</sub> supplementation (0, 20 or 40 mg/kg of diet). CoQ<sub>10</sub> was supplied by Sigma-Aldrich (Product No. C9538; Sigma Aldrich, St. Louis, MO, USA). Nutrient and chemical compositions of the used diets are shown in Table 1.

**Table 1.** Ingredient and nutrient composition of the basal diet<sup>1</sup>

Indices	Grower (15–28 day)	Finisher (29–42 day)
Ingredients, %		
maize	54.9	59.2
soybean meal (44%)	35.1	31.4
soy oil	5.8	5.3
limestone	1.6	1.58
dicalcium phosphate	1.5	1.4
sodium chloride	0.4	0.42
DL-methionine	0.2	0.2
vitamin-mineral premix <sup>2</sup>	0.5	0.5
Determined analyses, dry matter basis, %		
crude protein	22.05	19.78
crude fat	6.03	6.22
crude fibre	4.71	3.88
calcium	1.13	0.93
phosphorus	0.78	0.72
Calculated compositions <sup>3</sup>		
metabolizable energy, kcal/kg	2925	3020
lysine, %	1.17	1.10
methionine + cysteine, %	0.88	0.83

<sup>1</sup>CoQ<sub>10</sub> at doses of 20 or 40 mg/kg (Cat. No. C9538; Sigma-Aldrich, St. Louis, MA (USA)) was added to basal diet at the expense of maize;

<sup>2</sup>Vitamin premix provides the following per kg, in mg: all-trans-retinyl acetate 1.8, cholecalciferol 0.025, all- $\alpha$ -tocopherol acetate 1.25, menadione 1.1, riboflavin 4.4, thiamine 1.1, pyridoxine 2.2, niacin 35, Ca-pantothenate 10, vitamin B<sub>12</sub> 0.02, folic acid 0.55, d-biotin 0.1; Mineral premix provides the following per kg, in mg: Mn 40, Fe 12.5, Zn 25, Cu 3.5, I 0.3, Se 0.15, choline 175; <sup>3</sup>calculated value according to tabular values listed for the feed ingredients

Cumulative feed intakes (CFI) and body weights were recorded weekly, and body weight gains (BWG) and feed conversion ratios (FCR) were calculated on a weekly basis. At the end of the study, after the 8–10-h fasting period, 12 quails from each group (2 quails from each cage) were slaughtered to determine the carcass, liver, heart and testis weights, and to measure the serum and liver MDA level, SOD activity, TAS and ceruloplasmin and sialic acid contents, and serum aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity and total cholesterol level. The carcasses were obtained after the feather, feet and visceral organs were separated. The carcasses were kept at 4 °C for 18 h, and then cold carcass weights (CCW) were measured and cold carcass yields (CCY) were calculated.

The blood samples were collected into the biochemical tubes and centrifuged at 4 °C at 5000 g for 10 min to obtain serum samples. Liver samples were immediately weighed, washed with 0.9% NaCl solution and homogenized in 1.15% KCl solution (1:10 w/v) in an ice bath using a handheld homogeniser (2000 g for 1 min) (SHM 1; Stuart, Staffordshire, UK). The obtained homogenates were centrifuged at 5000 g for 60 min at 4 °C and the supernatants were collected. Protein analysis in the obtained tissue homogenates was performed according to the Lowry method (1951) with the use of UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan). Serum samples and liver homogenates were stored at –80 °C until further analysis.

### Laboratory analyses

The analysis of SOD activity in serum and tissue homogenates was performed according to the Sun et al. (1988). Serum and tissue MDA levels were determined by using the single heating method of Yoshioka et al. (1979) based on thiobarbituric acid (TBA) reactivity. Briefly, 0.5 ml of tissue homogenate or serum was mixed with 2.5 ml of trichloroacetic acid solution (TCAA; 20%) and 1 ml of thiobarbituric acid (0.67%), and then placed in a boiling water bath for 30 min at 95 °C. After cooling in tap water, the reaction mixture was vortexed and 4 ml of n-butanol was added to it and all vials were then centrifuged for 10 min at 3000 g. Then, the organic layer was collected and its absorbance at 535 nm against n-butanol was measured. Finally, MDA was calculated by the absorbance coefficient of the MDA-TBA complex (absorbance coefficient  $\epsilon = 1.56 \times 10^5$  M/cm) and was described as  $\mu\text{mol/mg}$  protein for tissue homogenate or  $\mu\text{mol/l}$  for serum.

Serum and liver TAS were determined using an automated measurement method with a commercially available kit developed by Rel (TAS Assay kit, Rel Assay Diagnostics, Gaziantep, Turkey). The results were expressed as millimoles of Trolox equivalent per mg of protein in tissue homogenates or millimoles of Trolox equivalent per liter of serum. Serum and tissue total sialic acid levels were measured spectroscopically using the thiobarbituric acid method according to Warren (1959). Serum and liver ceruloplasmin levels were determined by measurement of p-phenylenediamine oxidase activity according to Sunderman and Nomoto (1970). Serum ALT (Cat. No. A2221; Archem, Istanbul, Turkey) and AST (Cat. No. A2212; Archem, Istanbul, Turkey) activities and total cholesterol (Cat No. A2091;

Archem, Istanbul, Turkey) level were measured with commercially available kits on a biochemistry autoanalyzer (D280; Sinnowa, Nanjing, China).

Chemical analyses of the basal diet for crude protein (#988.05), ether extract (#932.06), crude fibre (#962.09), crude ash (#936.07), Ca (#968.08) and P (#965.17) were conducted in triplicate using methods described by the AOAC (1990). Energy and amino acid (methionine and lysine) ingredients were computed from tabular values arranged for the feedstuffs.

### Statistical analysis

The study was based on the 2 × 3 factorial design. Two-way analysis of variance (ANOVA) was applied to assess the effects of two main factors: environmental temperature (ET; TN conditions and CS) and CoQ<sub>10</sub> addition (0, 20 and 40 mg/kg), and the significance of the interaction between main factors (ET × CoQ<sub>10</sub> interaction) with use of SAS Software ver. 9 (2002) (Statistical Analysis System Institute, Cary, NC, USA). If ANOVA indicated significant interaction effect, means were compared using Duncan's multiple range test. Data are presented as means with standard error of the mean (SEM), and the value of  $P < 0.05$  was considered statistically significant. SEM was estimated by dividing the standard deviation by the square root of replication number.

## Results

### Growth performance

The exposure to CS adversely affected the performance parameters of quails in comparison to birds housed at TN conditions, which was reflected by decreased final body weight (FBW), BWG, CCW and CCY and increased CFI and FCR (Table 2).

Dietary CoQ<sub>10</sub> supplementation significantly increased FBW, BWG, CFI, CCW and CCY and decreased FCR; however the significant ET × CoQ<sub>10</sub> interaction for FBW, BWG and CFI showed that CoQ<sub>10</sub> addition significantly increased examined parameters only in CS regardless of the used dose.

### Serum biochemical parameters

The effect of ET was stated for serum TAS, ceruloplasmin content, AST and ALT activities and cholesterol content. The ceruloplasmin level, AST and ALT activities and cholesterol content were increased, whereas TAS value was decreased when birds were exposed to CS. Inversely the CoQ<sub>10</sub> addition increased TAS and decreased cholesterol level in quails' serum. A tendency for CoQ<sub>10</sub> addition to increase SOD activity and decrease ALT activity ( $P = 0.067$  and  $P = 0.072$ , respectively) was also observed. For serum SOD activity the statistically significant ET × CoQ<sub>10</sub> interaction was stated, showing that CoQ<sub>10</sub> addition into the diet did not exert influence on SOD activity in TN conditions, but increased it in CS regardless of the used dose (Table 3).

MDA serum level was significantly increased in CS in comparison to TN conditions ( $P = 0.001$ ). The effect of CoQ<sub>10</sub> was also significant with decreased values of MDA in CoQ<sub>10</sub> supplemented groups ( $P = 0.001$ ). The ET × CoQ<sub>10</sub> interaction ( $P = 0.0001$ ) showed that when quails were exposed to CS a higher dose of CoQ<sub>10</sub> (40 mg/kg) caused a more pronounced decrease in MDA level than the lower one (20 mg/kg) (Figure 1A).

### Liver biochemical parameters

The CS caused a significant decrease in TAS with simultaneous increase in sialic acid content (Table 4). The CoQ<sub>10</sub> addition increased SOD activity, TAS and ceruloplasmin content in liver.

**Table 2.** Effect of dietary coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) supplementation (0, 20 and 40 mg/kg) on performance in cold-stressed quails

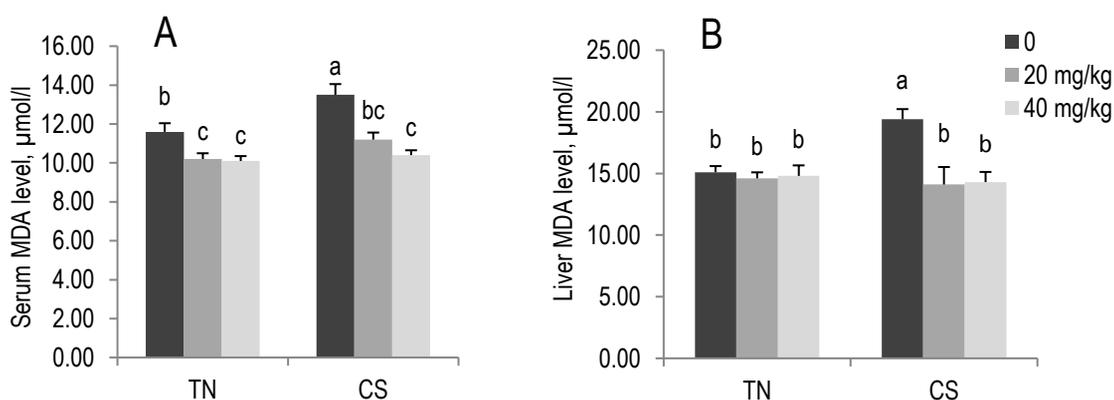
Indices	ET						SEM	Statistical significance, <i>P</i> -value		
	TN			CS				ET	CoQ <sub>10</sub>	ET × CoQ <sub>10</sub>
	0	20	40	0	20	40				
FBW, g	190 <sup>ab</sup>	188 <sup>bc</sup>	192 <sup>a</sup>	176 <sup>d</sup>	184 <sup>c</sup>	187 <sup>bc</sup>	1.016	0.0001	0.0001	0.0001
BWG, g	161 <sup>ab</sup>	159 <sup>abc</sup>	164 <sup>a</sup>	147 <sup>d</sup>	156 <sup>c</sup>	158 <sup>bc</sup>	1.248	0.0001	0.0001	0.0001
CFI, g	648 <sup>c</sup>	650 <sup>c</sup>	652 <sup>c</sup>	684 <sup>b</sup>	710 <sup>a</sup>	714 <sup>a</sup>	1.098	0.0001	0.0001	0.0001
FCR	4.02	4.04	3.94	4.64	4.56	4.47	0.036	0.0001	0.002	0.274
CCW, g	112	121	126	102	111	118	1.819	0.0001	0.0001	0.707
CCY, %	59	65	65	58	60	63	1.084	0.011	0.0001	0.276

ET – environmental temperature; TN – thermoneutral; CS – cold stress; 0, 20, 40 – levels of CoQ<sub>10</sub> supplementation (mg/kg diet); SEM – standard error mean; FBW – final body weight; BWG – body weight gain; CFI – cumulative feed intake; FCR – feed conversion ratio; CCW – cold carcass weight; CCY – cold carcass yield; the data are presented as means ± standard error mean; <sup>a-d</sup> – means in the same row with different superscript are significantly different

**Table 3.** Effect of dietary coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) supplementation (0, 20 and 40 mg/kg) on serum antioxidant parameters and cholesterol levels in cold-stressed quails

Indices	ET						SEM	Statistical significance, <i>P</i> -value		
	TN			CS				ET	CoQ <sub>10</sub>	ET × CoQ <sub>10</sub>
	0	20	40	0	20	40				
SOD, % inhibition/l	28.0 <sup>ab</sup>	27.2 <sup>ab</sup>	27.4 <sup>ab</sup>	15.2 <sup>b</sup>	28.8 <sup>a</sup>	30.6 <sup>a</sup>	2.930	0.430	0.067	0.015
TAS, mmol Trolox equiv./l	3.3	4.3	4.4	2.5	3.8	4.0	0.208	0.007	0.0001	0.726
Ceruloplasmin, g/dl	11.3	11.7	9.9	14.9	13.1	14.5	1.176	0.005	0.761	0.477
Sialic acid, µg/ml	167	164	165	184	172	137	11.817	0.944	0.156	0.178
AST, U/l	262	255	257	344	305	302	22.143	0.003	0.493	0.668
ALT, U/l	24	20	20	34	27	26	2.508	0.001	0.072	0.673
Cholesterol, mg/dl	83	78	78	108	95	85	4.229	0.0001	0.012	0.120

ET – environmental temperature; TN – thermoneutral; CS – cold stress; 0, 20, 40 – levels of CoQ<sub>10</sub> supplementation (mg/kg diet); SEM – standard error mean; SOD – superoxide dismutase; TAS – total antioxidant status; AST – aspartate aminotransferase; ALT – alanine aminotransferase; the data are presented as means ± standard error mean; <sup>a,b</sup> – means in the same row with different superscript are significantly different

**Figure 1.** Effect of different doses of coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) diet supplementation on serum (A) and liver (B) malondialdehyde (MDA) levels in two environmental temperature (thermoneutral (TN; 22 ± 2 °C for 24 h/day) and cold stress (CS; 12 ± 2 °C for 8 h/day followed by 22 ± 2 °C for 16 h/day)). The data are presented as means ± standard error of mean; a,b,c – bars with different superscript are significantly different**Table 4.** Effect of dietary coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) supplementation (0, 20 and 40 mg/kg) on liver antioxidant parameters in cold-stressed quails

Indices	ET						SEM	Statistical significance, <i>P</i> -value		
	TN			CS				ET	CoQ <sub>10</sub>	ET × CoQ <sub>10</sub>
	0	20	40	0	20	40				
SOD, % inhibition/mg protein	24.4 <sup>ab</sup>	24.1 <sup>ab</sup>	25.2 <sup>ab</sup>	20.1 <sup>b</sup>	26.2 <sup>ab</sup>	29.5 <sup>a</sup>	1.521	0.632	0.015	0.036
TAS, mmol Trolox equiv./mg protein	1.35 <sup>ab</sup>	1.40 <sup>a</sup>	1.40 <sup>a</sup>	1.17 <sup>b</sup>	1.32 <sup>ab</sup>	1.44 <sup>a</sup>	0.096	0.044	0.001	0.003
Ceruloplasmin, g/mg protein	21.3	22.4	25.9	26.0	23.1	28.1	1.488	0.069	0.033	0.468
Sialic acid, µg/mg protein	102.4	92.0	96.5	141.5	126.0	116.7	9.728	0.003	0.376	0.704

ET – environmental temperature; TN – thermoneutral; CS – cold stress; 0, 20, 40 – levels of CoQ<sub>10</sub> supplementation (mg/kg diet); SEM – standard error mean; SOD – superoxide dismutase; TAS – total antioxidant status; the data are presented as means ± standard error mean; <sup>a,b</sup> – means in the same row with different superscript are significantly different

However the interaction between main factors showed that both SOD activity and TAS were positively influenced by CoQ<sub>10</sub> only in CS and this effect was only significant for the higher dose of CoQ<sub>10</sub> (40 mg/kg) in comparison to non-supplemented group.

The analysis of main factors showed that liver MDA content was increased in CS ( $P = 0.038$ ) and decreased when CoQ<sub>10</sub> supplementation was introduced ( $P = 0.004$ ); however the ET × CoQ<sub>10</sub>

interaction indicated that effect of CoQ<sub>10</sub> was shown only in CS ( $P = 0.012$ ) regardless of the used dose (Figure 1B).

### Organ weights

The testis weight was adversely affected in quails exposed to CS, but no such effect was stated for heart weight (Table 5). In contrary, there was observed a tendency ( $P = 0.88$ ) to higher liver weight in CS. The CoQ<sub>10</sub> did not influence the examined

**Table 5.** Effect of dietary coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) supplementation (0, 20 and 40 mg/kg) on organ weights in cold-stressed quails

Indices	ET						SEM	Statistical significance, <i>P</i> -value		
	TN			CS				ET	CoQ <sub>10</sub>	ET × CoQ <sub>10</sub>
	0	20	40	0	20	40				
Liver, g	3.55 <sup>b</sup>	4.16 <sup>ab</sup>	4.13 <sup>ab</sup>	4.63 <sup>a</sup>	3.84 <sup>b</sup>	4.16 <sup>ab</sup>	0.183	0.088	0.746	0.001
Heart, g	1.93	1.96	1.94	1.88	1.85	1.92	0.068	0.308	0.917	0.818
Testis, g	1.81	1.78	1.85	1.23	1.13	1.18	0.205	0.001	0.945	0.980

ET – environmental temperature; TN – thermoneutral; CS – cold stress; 0, 20, 40 – levels of CoQ<sub>10</sub> supplementation (mg/kg diet); SEM – standard error mean; the data are presented as means ± standard error mean; <sup>a,b</sup> – means in the same row with different superscript are significantly different

organs weight. However the ET × CoQ<sub>10</sub> interaction showed that the liver weight of quails exposed to CS was significantly decreased by dietary CoQ<sub>10</sub> supplementation when group with the 20 mg/kg addition was compared to non-supplemented one.

## Discussion

In the present study, the effects of CoQ<sub>10</sub> dietary supplementation on growth performance, organ weights and some oxidative stress parameters in cold-stressed quails were investigated. Low ambient temperature (<18 °C) is widely known as the environmental stress factor that affects the growth performance, health and well-being of poultry (Şahin and Gümüşlü, 2004). The growth performance parameters such as FBW, BWG, CCW and CCY were lower, while CFI, FCR and liver weight were increased in quails exposed to CS compared to birds kept under TN conditions. However, the quails' diet supplementation with CoQ<sub>10</sub> at a dose of 20 and 40 mg/kg diet eliminated the negative effects of CS, which was reflected in the increase in FBW and BWG, and in the decrease in liver weight. Some researchers have already shown the positive effects of the CoQ<sub>10</sub> addition into the diet on growth performance parameters (Fathi, 2015; Nemati et al., 2017; Kalantar et al., 2019), but others reported that CoQ<sub>10</sub> supplementation did not affect the FI in broilers exposed to CS (Nemati et al., 2017). It is well known that low ambient temperature effectively increases the energy requirement necessary for thermoregulation, which results in increased feed consumption in animals (Hangalapura, 2006). That was confirmed by Collin et al. (2003) who indicated that CoQ<sub>10</sub> supplementation increased FI by increasing the thermogenic effect of digestion, absorption and utilization of nutrients in quails under cold environmental conditions. The results of the present study clearly show that the growth performance of quails fed diet with additional CoQ<sub>10</sub>

is better than in birds fed the non-supplemented diet under stressful conditions. It is thought that these positive effects of CoQ<sub>10</sub> may be caused by an improvement in mitochondrial function and a reduction in ascites due to the direct antioxidant capacity of this compound (Geng and Guo, 2005).

Cold stress interferes with the body antioxidant system and the course of enzymatic and non-enzymatic reactions causing oxidative damage in various tissues (Şahin and Gümüşlü, 2004; Hangalapura, 2006). An imbalance between the oxidant and antioxidant systems affects the functioning of tissues, causing oxidative damage. Lipid peroxidation by free oxygen radicals is an important cause of cell membrane damage (Halliwell and Gutteridge, 2015). Antioxidants play a vital role in protecting the cells from the damaging effects of oxidative stress. In order to assess the oxidative and antioxidative status of the organism, the level of MDA, the activity of SOD, TAS, and the contents of ceruloplasmin and sialic acid are measured (Matés and Sánchez-Jiménez, 1999). MDA is the last product of polyunsaturated fatty acids peroxidation and is an important indicator of the lipid oxidative damage. Serum and tissue MDA levels are measured as an indicator of free radicals in the oxidative involvements of metabolism. CoQ<sub>10</sub> is able to efficiently inhibit fat, protein and DNA oxidation by participating in electron and proton transport *via* oxidative phosphorylation (Ernster and Forsmark-Andrée, 1993), it also generates the major superoxide anion/hydrogen peroxide second messenger system (Linnane et al., 2007), due to its strong antioxidant properties conditioned by the specific structure of the chain. In a constructive study, it was shown that heart and brain MDA levels of chickens exposed to low temperatures were 1.2 to 2.1 times higher than in the control group (Mujahid and Furuse, 2009). It has been also reported that the antioxidant agents effectively reduce serum and/or tissue MDA levels in poultry exposed to both heat

stress and cold stress (Tuzcu et al., 2008). In the present study, the serum and liver MDA levels were significantly decreased by CoQ<sub>10</sub> supplementation which was in agreement with Fathi (2015) and Geng and Guo (2005), who used dietary CoQ<sub>10</sub> at the level of 40 mg/kg of broiler's diet. This is presumably due to the reduction in free radicals production caused by CoQ<sub>10</sub>, a strong antioxidant (Bentinger et al., 2010).

Exposure to low temperature provokes increases in oxygen consumption and requires an increase in energy production. One of the main ROS is superoxide radical, called endogenous free radical, which is mainly produced in mitochondria (Halliwell and Gutteridge, 2015). It is known that the accumulation of ROS causes damage to cells, especially DNA, proteins and lipids, under conditions of oxidative and their elimination from the body requires endogenous and/or exogenous ROS scavengers. It has been also stated that changes in the activity of antioxidant enzymes could be used to assess TAS and the degree of oxidative stress in poultry (Öztürk-Ürek et al., 2001). Panda et al. (2007) found that dietary vitamin C supplementation effectively enhances TAS levels in stress-exposed White Leghorn layers. On the other hand, biological macromolecules are protected by antioxidant enzymes such as SOD that catalyses the dismutation of superoxide into oxygen and hydrogen peroxide. The physiological importance of SOD has been confirmed by severe pathological consequences in genetically modified mice lacking this enzyme. It was reported that CS effectively reduced the levels of SOD in the quail intestine (Fu et al., 2013). In the present study, SOD activity and TAS in serum and liver were significantly reduced in birds exposed to CS; however CoQ<sub>10</sub> supplementation reversed the negative effect of CS by increasing SOD activity in serum, and SOD activity and TAS in the liver, but in liver only the higher dose of CoQ<sub>10</sub> (40 mg/kg) reached significant effect. It is suggested that CoQ<sub>10</sub> supplementation during CS, as well as vitamins E, C and L-carnitine during heat stress, enhance both SOD activity and TAS levels to minimize oxidative stress in quails by inhibiting the production and increasing elimination of ROS (Öztürk-Ürek et al., 2001). Furthermore, it was reported that the activity of endogenous antioxidants (such as SOD) in the liver and spleen was also significantly increased by the curcumin application to iron overload rats. This increment of SOD activity upon curcumin treatment could be attributed to the relationship between copper content in the body and serum ceruloplasmin content.

## Conclusions

In conclusion, the present study showed that the cold environmental temperature negatively affected growth performance, antioxidant status and organs weight in quails, whereas dietary coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) supplementation significantly reversed cold stress negative effects on body weight, feed intake and antioxidant status of serum and liver. It can be also suggested that the observed CoQ<sub>10</sub> dietary supplementation effects can be partly associated with direct antioxidant properties of CoQ<sub>10</sub> as well as the synergistic efficacy of CoQ<sub>10</sub> with SOD activity. However, further studies are needed to investigate the effects of CoQ<sub>10</sub> supplementation on antioxidant system in quails exposed to cold stress.

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