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Use of supplemental dietary coenzyme Q10 to improve testicular function and fertilization capacity in aged broiler breeder roosters

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ABSTRACT

In numerous studies it has been suggested that targeting mitochondria with specific compounds could efficiently inhibit various conditions associated with oxidative stress. The treatment of aged roosters with compounds such as coenzyme Q10 (CoQ10), may improve their reproductive performance by providing protection from oxidative stress. Therefore, this study was performed to assess the effect of supplemental dietary CoQ10 on the testicular function and fertility of aged broiler breeder roosters. A total of 36 roosters)47 weeks of age) were randomly divided into dietary treatments containing either 0, 300 or 600 mg CoQ10/kg diet. Three birds were allocated to each of four replicate groups in each dietary treatment. Between 47 and 54 weeks of age, ejaculates were obtained weekly from the three roosters in each replicate group. Samples in a replicate were pooled and analyzed as a single sample. Between 51 and 54 weeks of age, seminal plasma total antioxidant capacity (TAC), alanine amino transferase (ALAT) and aspartate amino transferase (ASAT) levels were assessed. Fertility, hatchability, and sperm penetration (SP) rates were likewise evaluated. Seminal volume, sperm concentration, sperm plasma membrane functionality, sperm plasma membrane integrity, seminiferous tubule diameter and seminiferous epithelium thickness exhibited quadratic increases in response to increasing levels of dietary CoQ10. Respectively, the 429.19, 433.33, 464.50, 613.50, 392.78 and 447.99 mg/kg dietary concentrations of CoQ10 provided the best results for each of the aforementioned variables. Also, other seminal traits, as well as testosterone concentration, fertility, and SP rates, displayed linear increases in response to the increasing levels of CoQ10. Dietary supplementation of CoQ10 linearly decreased seminal plasma ALAT and ASAT and linearly increased seminal plasma TAC. In conclusion, CoQ10 supplementation in the diet (a minimum of 300 mg CoQ10/kg diet) has the potential to improve the reproductive performance of aged broiler breeder roosters.

1. Introduction

Oxidative stress is commonly defined as an imbalance between the generation of reactive oxygen species (ROS) and their removal by the antioxidant system [1,2]. Because plasma membranes of avian spermatozoa have high amounts of polyunsaturated fatty acids, they are more sensitive to lipid peroxidation during oxidative stress [3]. The effects of lipid peroxidation include an irreversible loss of sperm plasma membrane integrity and motility, thereby decreasing rooster sperm fertilizing ability [4]. To counteract the adverse effects of ROS, the antioxidant system of rooster semen normally contain glutathione peroxidase, glutathione, catalase, superoxide dismutase and other natural antioxidants such as vitamin E and vitamin C [5]. However, the antioxidant activity in rooster semen decreases with age [1]. Furthermore, a highly significant positive correlation has been observed between seminal total antioxidant capacity (TAC) and rooster fertility [1]. Therefore, reduced TAC could impair sperm function due to a deficient antioxidant system or by increased ROS production.

Decades of genetic selection of broiler breeder lines (*Gallus gallus domesticus*) has significantly improved their growth.

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However, this has been associated with increases in their metabolic requirements and mitochondrial activity [6,7]. With increasing metabolic demands and heavy solicitation of energy from mitochondria, substantial amounts of free radicals are subsequently generated. For instance, it has been shown that broilers fed energy dense diets become more susceptible to oxidative stress, whereas restrictive feeding decreases their mitochondrial activity and the incidences of oxidative damage [8,9]. In studies concerning the sperm quality of aging fowl, it has been proven that the loss of the sperm quality associated with a loss of energy production by and a decrease in TAC [1,10]. In numerous studies it has been suggested that targeting mitochondria with specific nutrients from natural sources such as vitamins (vitamin C and E) [11], minerals (selenium) [12], and antioxidants (lycopene) [13], could efficiently control various conditions associated with mitochondrial dysfunction and oxidative stress. Therefore, the treatment of aged roosters with compounds that might protect their reproductive and non-reproductive tissues from oxidative stress is needed. One such novel compound is coenzyme Q10 (CoQ10), which is an antioxidant and is involved in mitochondrial oxidative phosphorylation [14]. In studies conducted on broiler chickens, it has been reported that CoQ10 supplementation in the diet increase their body weight gain and reduce the incidences of ROS and ascites mortality [15,16].

Coenzyme Q10 plays a vital role in energy metabolism and is concentrated in mitochondria that are located in the midpiece of spermatozoa [17]. Moreover, CoQ10 acts as an antioxidant, and is involved in the regeneration of endogenous antioxidants such as superoxide dismutase, which in turn inhibits lipid peroxidation [18]. *In vivo* studies on humans and rats have confirmed that including CoQ10 in diets improves TAC and semen quality [19,20]. It has been showed that dietary levels of CoQ10 are significantly correlated with sperm density and motility. Infertile men consequently have significantly lower seminal CoQ10 concentrations compared to fertile men [21].

In studies conducted on birds, it has been shown that TAC decreases during ageing, and that the addition of antioxidants, such as α-Tocopherol to diets improves TAC levels [22]. Coenzyme Q10 is a potent antioxidant that is even stronger than α-Tocopherol and can also regenerate α-Tocopherol from α-Tocopherol radicals [23,24]. Decreased TAC levels and increased ROS levels during aging by the inclusion of CoQ10 in diets of roosters may improve their TAC status and enhance their reproductive performance. Levels of CoQ10 have been shown to decline with age [25]. The efficacy of CoQ10 for the enhancement of reproductive performance in aged roosters has not been investigated. Therefore, the present study was conducted to investigate the effect of dietary CoQ10 on the sperm quality, seminal plasma TAC, testicular histology, and fertility rate of aged broiler breeder roosters.

2. Materials and methods

Approval for the current study was given by the Animal Welfare Committee of the Department of Animal Science, University of Tehran.

2.1. Chemicals

All chemicals, except for CoQ10 (Hangzhou Dingyan Chem Co., Ltd, Hangzhou, China), were purchased from Merck Co. (Darmstadt, Germany) and Sigma-Aldrich Co. (St. Louis, MO).

2.2. Birds and semen collection

Thirty-six mature Arbor Acres Plus broiler breeder roosters (weighing 5187.17 ± 92.99 g) were selected at 44 weeks of age from a commercial flock. The roosters were randomly assigned to 12 floor pens (1.25 m × 1 m) equipped with a pan feeder and an automatic bell drinker. Air temperature was set at 22 ± 2 °C and lighting cycle was 14 h light: 10 h dark photoperiod. Before starting the trial, roosters were fed a breeder diet without CoQ10 supplementation (Table 1). During the first two weeks (45 and 46 weeks of age) of the experiment, roosters were adapted to the semen collection procedure by the method of Burrows and Quinn [26]. After the adaptation period, three roosters at 47 weeks of age were randomly allocated in a completely randomized design, to each of four replicate pens in each of three treatment groups. Treatments included 0 (control), 300 and 600 supplemental levels of dietary CoQ10 (mg/kg of diet). According to previous studies, CoQ10 is absorbed better in the presence of oil [27], Therefore, the CoQ10 was dissolved in corn oil (1% of diet) before its addition to the diet. During the experiment, the amount of feed was controlled (146 g/day/bird) in accordance with the recommendations of the breeder company (Arbor Acres, Winston Salem, NC), and water was supplied for ad libitum consumption.

2.3. Sperm quality analyses

During the 47–54 weeks of age period semen ejaculate samples (n = 96) were obtained weekly from the three roosters in each replicate group, and pooled samples from each replicate group analyzed weekly [28]. Modified Beltsville poultry semen extender (mBPSE; pH and osmotic pressure settings were 7.2 and 310 mOsm/kg and, respectively) was used for all semen samples [3].

Ejaculate volumes were measured using a graduated microtube, and the evaluation of sperm concentration was conducted by adding a droplet of diluted semen (1:200 in distilled water) to a Neubauer chamber, and the number of spermatozoa was subsequently determined microscopically (400× magnification) [28]. Sperm motility was evaluated by placing diluted semen (1:20 in mBPSE) on a pre-warmed microscope slide (37 °C) covered with a coverslip. Using a light microscope (D-22976; Kruss, Hamburg, Germany) at 5 microscopic fields (400× magnification), the percentage of motile sperm was expressed as the percentage of spermatozoa exhibiting moderate to rapid progressive movement [29].

Sperm plasma membrane integrity and sperm morphology were evaluated by an eosin-nigrosin staining method [30]. Ten microliters of stain was placed on a pre-warmed microscope slide

Table 1
Ingredients and chemical composition of the diets fed to broiler breeder roosters (On a fed basis).

Ingredient	Amount (%)
Corn	65.42
Soybean meal, 42.6% CP	6.50
Wheat bran	23.80
Corn oil	1.00
Dicalcium phosphate	1.30
Mineral oyster shall	0.94
Common salt	0.32
$NaHCO_3$	0.10
Mineral premixes[1]	0.25
Vitamin premix[2]	0.25
DL-Met, 99%	0.12
Total	100
Calculated nutrient content	
AME (kcal/kg)	2700
CP (%)	11.54
Calcium (%)	0.73
Available phosphorus (%)	0.34
Sodium (%)	0.17
Met (%)	0.31

(37 °C). The stain was thoroughly mixed for 30 s with 10 μL of diluted semen (1:20 in mBPSE), and the mixture was then spread over the slide using a clean slide. After drying, the plasma membrane integrity was examined using oil immersion light microscopy (D-22976; Kruss, Hamburg, Germany) at 1000× magnification. Sperm with heads that were unstained were counted as having intact plasma membrane and were classified as live sperm, whereas and sperm with fully or partially stained heads were counted as having unintegrated plasma membranes, and were classified as dead sperm. Spermatozoa with separated, abaxial, or twisted heads, or with bent, coiled, or dual tails, or that possessed protoplasmic droplets were classified abnormal.

Sperm plasma membrane functionality was assessed by a hypo-osmotic swelling test [29]. A hypo-osmotic solution (100 mOsm/kg) was made by adding sodium citrate (1% w/v) to distilled water. Twenty-five microliters of diluted semen were mixed with 500 μL of the hypo-osmotic solution, and the mixture was then incubated at 37 °C for 30 min. The percentage of spermatozoa showing coiled mid-pieces and tail segments were determined after applying the eosin-nigrosine stain on a microscope slide. Spermatozoa (n = 200) were counted using oil immersion light microscopy assessed (D-22976; Kruss, Hamburg, Germany) at a 1000× magnification [31].

2.4. Activity of alanine amino transferase (ALAT), aspartate amino transferase (ASAT) and TAC in seminal plasma

The ALAT, ASAT and TAC activity of seminal plasma was determined between 51 and 54 weeks of age. To obtain the seminal plasma of the roosters, a portion of their ejaculate was centrifuged (1500 × g; 18 °C) for 15 min. The plasma was then collected and stored at −20 °C until analysis. All of the seminal plasma samples were analyzed at the same time to avoid inter-assay variations. The enzymatic activities of ALAT and ASAT were determined by a colorimetric enzymatic method using a commercial kit (Parsazmun, Tehran, Iran), with an intra-assay CV of 1.83 and 3.31%, respectively. Seminal plasma TAC was determined by a colorimetric enzymatic method using a commercial kit (Navandsalamat, Urmia, Iran), with an intra-assay CV of 3.87%.

2.5. Circulating testosterone concentrations

At 47, 50 and 54 weeks of age, two roosters from each treatment replicate every (eight roosters/treatment) were randomly selected and were bled from the brachial vein (2 mL). The blood samples were collected in heparinized tubes and centrifuged for 15 min (1800 × g; 18 °C). Plasma was subsequently collected and held at −20 °C until analysis. Plasma testosterone concentrations were measured using a commercially available ELISA kit (Monobind), with an intra-assay CV of 5.86%.

2.6. Testicular histology

At the end of the experiment, eight roosters per treatment were weighed and humanely killed by decapitation. The testes were then carefully removed, weighed, and the testis index was measured (testis weight (g)/body weight (kg)) [32]. After testis fixation in 10% buffered formalin (pH = 7), the samples were processed using a tissue processor system. After embedding in paraffin, the samples were cut (5 μm) and stained using a hematoxylin-eosin technique [33]. Then seminiferous tubule diameter and seminiferous epithelium thickness of 20 randomly selected cross-sections of the seminiferous tubules of each rooster were calculated [33].

2.7. Evaluation of fertility, hatchability rates and sperm penetration (SP) assay

The egg fertility, hatchability and sperm penetration rates of three roosters in each of the four replicate pens in each of the three treatments groups was determined. During the 51–54 weeks of age period, the semen samples of the roosters were gently pooled and diluted in the modified Beltsville extender. Each hen (51 weeks of age) was weekly inseminated (4 weeks) with diluted (approximately 1:4 in mBPSE) semen (0.2 mL diluted semen/hen; 200×10^6 spermatozoa/hen). Eggs were collected beginning on the second day after the first AI, for a period of 4 weeks. The eggs were incubated in a commercial incubator (Petersime, Zulte, Belgium) at a dry-bulb and wet-bulb temperature of 37.7 °C and 29 °C, respectively. Fertility rate (fertilized eggs divided by total eggs set × 100), hatchability of set eggs (chick number divided by total number of eggs set × 100) and hatchability of fertile eggs (hatched eggs divided by fertilized eggs × 100) were determined at the completion of each incubation period [34].

The number of SP holes in the inner perivitelline layer (IPL) is commonly used as an approximate indicator of the number of spermatozoa remaining in the sperm storage tubules (SSTs) [35]. The SP was measured weekly on day 3 following each insemination using the method described by Bramwell et al. (1995) and Sharideh et al. (2016a) [6,35]. Briefly, 12 eggs/treatment/week were opened and the yolk was separated from the albumen. The yolks were then immersed in a 1% NaCl solution for at least 5 min, and the germinal disc was removed. The germinal disc was transferred to a slide, and several drops of neutral phosphate-buffered formalin (10%) were placed on the IPL, which was then stained with Schiff's reagent and air-dried. The number of SP sites in one visual field (15.89 mm^2) was measured by light microscopy (D-22976; Kruss, Hamburg, Germany; 40× magnification).

2.8. Statistical analysis

All data were tested for normal distribution and normality of variances by Shapiro–Wilk and Levene's tests, respectively. When appropriate, percentage data (sperm motility, plasma membrane functionality, plasma membrane integrity and abnormal morphology) were subjected to arc-sine transformation. The ALAT, ASAT, TAC and SP data were analyzed using the GLM procedure of SAS 9.1 (SAS Institute, version 9.1, 2002, Cary, NC, USA) and data repeated in time were analyzed using repeated measures analysis of the Mixed procedure of SAS 9.1. Results were adjusted for multiple comparisons using the Tukey option and represented as means ± SEM. Fertility and hatchability rate data were analyzed by the GENMOD procedure via the chi-square test of SAS 9.1. Orthogonal contrasts were used to compare effects of the control treatment with the average of the 300 and 600 mg CoQ10/kg treatments, and polynomial contrasts were used to test for linear and quadratic effects of increasing dietary supplementation of CoQ10 on the dependent variable measured. When quadratic responses ($Y = a \pm bX \pm cX^2$; the response of the dependent variable (Y) to the graded levels of CoQ10 (X)) were observed, the optimal CoQ10 level was calculated by taking the first derivative of the quadratic equation.

3. Results

3.1. Sperm quality analyses

The effect of adding CoQ10 to the diet on the seminal properties examined is shown in Table 2. The diets containing 300 and 600 mg CoQ10/kg had a significantly higher ($P < 0.05$) ejaculate volume,

Table 2
The effects of the diet supplemented with different levels of coenzyme Q10 (CoQ10) on ejaculate volume and seminal characteristics in broiler breeder roosters (12 birds per treatment).

Treatment[a]	Traits[b]						
	Ejaculate volume (mL)	Sperm concentration (10^9/ml)	Sperm motility (%)	Plasma membrane functionality (%)	Plasma membrane integrity (%)	Abnormal morphology (%)	Testosterone ($ngmL^{-1}$)
Q-0	0.41	3.62	78.15	70.14	82.82	5.20	3.27
Q-300	0.55	3.95	83.53	78.06	88.06	5.23	4.40
Q-600	0.52	3.88	85.84	79.52	90.30	4.14	4.50
SEM	0.02	0.10	0.66	1.21	0.77	0.21	0.33
P-value							
Time	<.05	<.0001	<.0001	<.0001	<.0001	<.05	0.54
CoQ10*Time	0.93	0.75	<.05	0.26	0.08	0.90	0.62
Linear[c]	<.001	<.05	<.0001	<.0001	<.0001	<.05	<0.05
Quadratic[c]	<.05	<.05	0.06	<0.01	0.04	0.09	0.21
Control vs. CoQ10[c]	<0.01	0.05	<0.01	<0.01	<0.01	<0.05	<0.05

SEM: Standard error of the mean.
[a] The birds fed a standard basal diet supplemented with increasing levels of CoQ10 including 0 (Q-0), 300 (Q-300), and 600 (Q-600) mg CoQ10/kg diet for 8 successive weeks (47–54 weeks of age).
[b] Traits were evaluated after adaption period (47–54 weeks of age).
[c] The *P* values include the linear and quadratic responses (to describe the response in the dependent variable to dietary CoQ10 levels), and orthogonal contrast between CoQ10 levels (the roosters fed 300 300 and 600 mg CoQ10/kg diet) with control group (the roosters fed basal diet without CoQ10).

sperm concentration, sperm motility, and sperm plasma membrane functionality and integrity when compared to the control group. Whereas, roosters in the control group had a significantly higher ($P < 0.05$) percentage of morphologically abnormal spermatozoa compared to those fed CoQ10 at either level. Ejaculate volume ($Y = 0.4251 + 0.000588X - 0.000000685X^2$), sperm concentration ($Y = 3.5600 + 0.001872X - 0.00000216X^2$), sperm plasma membrane functionality ($Y = 70.1491 + 0.03716X - 0.00004X^2$) and sperm plasma membrane integrity ($Y = 82.8459 + 0.02454X - 0.00002X^2$) exhibited quadratic ($P < 0.05$) increases in response to the increasing levels of CoQ10. The best ejaculate volume, sperm concentration, sperm plasma membrane functionality and sperm plasma membrane integrity results were estimated at 429.19, 433.33, 464.50 and 613.5 mg CoQ10/kg diet, respectively. Dietary supplementation of CoQ10 linearly ($P < 0.05$) decreased the percentage of morphologically abnormal spermatozoa. Sperm motility exhibited a linear increase ($P < 0.001$) in response to increasing levels of CoQ10. With the exception of sperm motility ($P < 0.05$; Fig. 1), there were no significant interactive effects of CoQ10 and time for any of the other sperm quality features examined.

3.2. Seminal plasma of ALAT, ASAT and TAC

The effects of adding CoQ10 to the diets of the roosters on seminal plasma ALAT, ASAT, and TAC are shown in Table 3. The roosters fed 300 and 600 mg of supplemental CoQ10/kg had significantly lower ($P < 0.05$) seminal plasma ALAT and ASAT when compared to the control group. The roosters fed either level of CoQ10 had significantly higher ($P < 0.05$) seminal plasma TAC compared to the control group.

3.2.1. Circulating testosterone concentrations

The roosters fed 300 and 600 mg CoQ10/kg had significantly higher ($P < 0.05$) plasma testosterone concentrations compared to the control group (Table 2). The plasma testosterone concentration of roosters increased ($P < 0.05$) linearly in response to increased levels of CoQ10. However, there was no ($P > 0.05$) interactive effect between CoQ10 and time on testosterone concentration.

3.3. Testis index and histological parameters

The effect of dietary CoQ10 on testis index and morphology are shown in Table 4. Testis index was not affected ($P > 0.05$) by treatment, but there was a quadratic effect ($P < 0.05$) in response to CoQ10 level on seminiferous tubule diameter ($Y = 255.078 + 0.1590X - 0.0002024X^2$) and seminiferous epithelium thickness ($Y = 74.6007 + 0.07495X - 0.00008365X^2$) with the best results estimated at 392.78 and 447.99 mg CoQ10/kg diet, respectively.

3.4. Evaluation of fertility, hatchability and sperm penetration rates

Hatchability of fertile eggs in all treatment groups was not affected ($P > 0.05$; Table 5) by treatment, but there was a linear effect ($P < 0.05$; Table 5) in response to the level of CoQ10 for fertility, hatchability of total eggs, and SP rate. Roosters fed 300 and 600 mg CoQ10/kg had a significantly higher ($P < 0.05$) fertility, hatchability of total eggs, and SP rate in comparison to the control group.

4. Discussion

In this study, the addition of CoQ10 (300 and 600 mg CoQ10/kg diet) to diets of Arbor Acres Plus rooster diet improved their semen ejaculate volume, sperm concentration, and sperm motility by approximately 30%, 8% and 9%, respectively, in comparison to unsupplemented control group. Various dietary antioxidant materials, such as a turmeric by-product [36], dried ginger rhizomes [28], and vitamin C and E [11], have been shown to favor sperm quality in domestic fowl. *In vivo* studies performed on infertile men showed that dietary CoQ10 supplementation (150–400 mg CoQ10/man daily) increased the CoQ10 levels in their seminal plasma and sperm cells. This in turn, improved the sperm concentration and motility in humans [21,37,38]. Moreover, *in vitro* studies performed in bovine [39], human [40] and rooster [41] subjects showed that the supplementation of semen extender with CoQ10 improved their sperm quality. Cellular energy required for sperm generation and sperm motility is produced through oxidative phosphorylation in the mitochondria [42]. The levels of ATP produced by mitochondria have been demonstrated to decline with age [43]. This drop in mitochondrial energy generation leads to cellular dysfunction, physiological inabilities, and reproductive performance failure, which are usually correlated with aging [14]. The results of the current study suggest that as an antioxidant, CoQ10

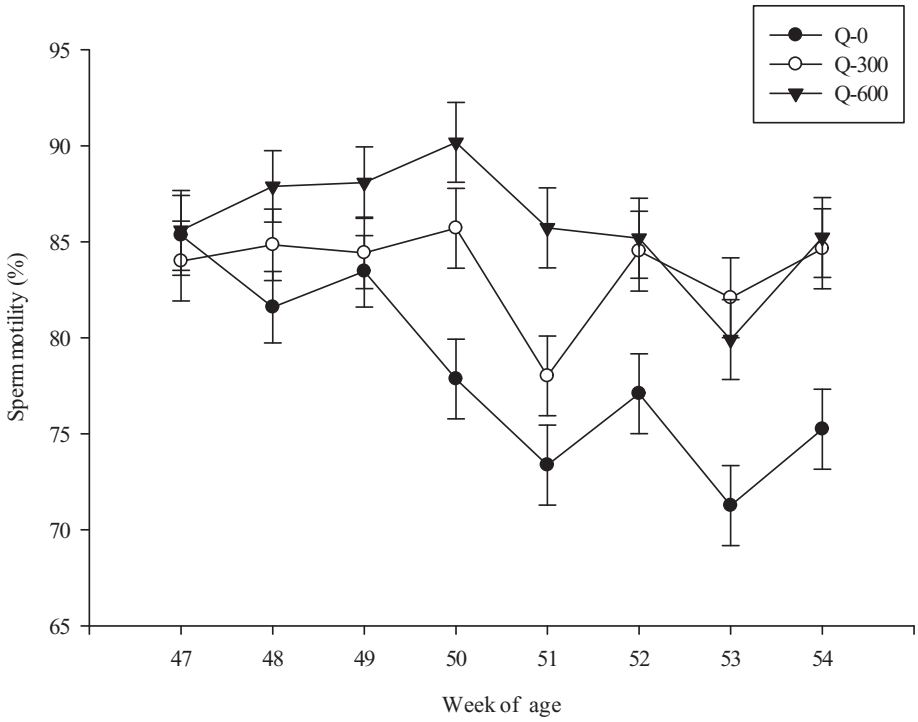

Fig. 1. Weekly sperm motility in broiler breeder roosters (12 roosters/treatment) fed the diet supplemented with different values of CoQ10 (Q-0, 0 mg CoQ10/kg diet; Q-300, 300 mg CoQ10/kg diet; Q-600, 600 mg CoQ10/kg diet).

prevented spermatozoa damage and improved mitochondrial performance in the roosters.

Furthermore, normal sperm morphology, sperm plasma membrane integrity, sperm plasma membrane functionality, and seminal plasma TAC in the roosters fed CoQ10 were higher than that of the control group. The excessive production of ROS associated with conditions such as aging and infection disease status, is related to increased percentages of morphologically abnormal spermatozoa, and decreased sperm plasma membrane integrity, sperm plasma membrane functionality, and sperm motility [42,44]. Kelso et al. (1996) showed that the changes in the lipid composition of sperm in aging fowl were correlated with a reduction in the activities antioxidant enzymes [1]. Balercia et al. (2004) showed that CoQ10 supplementation (400 mg CoQ10/man) in infertile men increased sperm membrane phosphatidylcholine concentration and enhanced sperm quality [37]. Similar to the current, a study performed on infertile men showed that supplemental dietary CoQ10 (200 mg CoQ10/man) improved antioxidant enzyme activity and decreased oxidative stress in seminal plasma [20]. These results suggest that the CoQ10 treatment (300 and 600 mg CoQ10/kg diet) protected the spermatozoa of the roosters from oxidative stress by providing antioxidant protection in their semen plasma.

Dead spermatozoa lose plasma membrane stability and release transaminase enzymes (ASAT and ALAT) in semen, thereby increasing their overall activity in semen [45]. Therefore, the activity of ASAT and ALAT in seminal plasma is a useful indicator of semen quality. In this study, CoQ10 treatment improved sperm membrane integrity and functionality, which in turn, decreased the activity of ALAT and ASAT in the seminal plasma.

In the present study, supplemental dietary of CoQ10 (300 and 600 mg CoQ10/kg diet) increased seminiferous tubule diameter, seminiferous epithelium thickness, and blood testosterone concentrations by approximately 10%, 19% and 36%, respectively. Testosterone production by the testes impacts testicular development and the behavior of roosters [36]. It also acts directly on the Sertoli and peritubular cells in the seminiferous tubules to stimulate spermatogenesis [46,47]. Oxidative stress is one of the most influential agents in the alteration of spermatogenesis in animals [2], and antioxidant materials, such as L-carnitine, exhibit positive results on sperm quality and testicular histology in aged roosters [48]. Diabetic rats administered CoQ10 (10 mg/kg body weight) experienced increased concentrations of pituitary gonadotropin levels, as well as improved testicular morphology and sperm quality [49]. It also has been shown that the pre-treatment of rats with L-carnitine and CoQ10 (10 mg/kg body weight) improved their testis weights and increased their blood testosterone concentrations when fed cholesterol rich diets [19]. Testosterone levels are

Table 3
The effects of the diet supplemented with different levels of coenzyme Q10 (CoQ10) on the seminal plasma of alanine amino transferase (ALAT), aspartate amino transferase (ASAT) and total antioxidant capacity (TAC) of broiler breeder roosters (12 birds per treatment).

Treatment[a]	Traits[b]		
	ALAT (U/l)	ASAT (U/l)	TAC (mmol/L Fe (II)
Q-0	6.44	59.76	4.29
Q-300	5.33	20.44	4.64
Q-600	3.12	15.41	6.17
SEM	0.76	9.36	0.52
P-value[c]			
Linear	<.05	<.01	<.01
Quadratic	0.57	0.16	0.36
Control vs. CoQ10	<0.05	<0.05	<0.05

SEM: Standard error of the mean.
[a] The birds fed a standard basal diet supplemented with increasing levels of CoQ10 including 0 (Q-0), 300 (Q-300), and 600 (Q-600) mg CoQ10/kg diet for 8 successive weeks (47–54 weeks of age).
[b] Traits were evaluated during the 50–54 weeks of age period.
[c] *P* values include the linear and quadratic responses (to describe the response in the dependent variable to dietary CoQ10 levels), and orthogonal contrast between CoQ10 levels (the roosters fed 300 300 and 600 mg CoQ10/kg diet) with control group (the roosters fed basal diet without CoQ10).

Table 4
The effects of the diet supplemented with different levels of coenzyme Q10 (CoQ10) on testis index and testicular histology in broiler breeder roosters (8 birds per treatment).

Treatment[a]	Traits[b]		
	Testis index	Seminiferous tubule diameter (μm)	Seminiferous epithelium thickness (μm)
Q-0	7.23	255.07	74.60
Q-300	7.25	284.57	89.55
Q-600	7.75	277.62	89.46
SEM	0.63	6.64	2.56
P-value[c]			
Linear	0.56	<.05	<.001
Quadratic	0.76	<.05	<.05
Control vs. CoQ10	0.80	<.05	<0.01

SEM: Standard error of the mean.
[a] The birds fed a standard basal diet supplemented with increasing levels of CoQ10 including 0 (Q-0), 300 (Q-300), and 600 (Q-600) mg CoQ10/kg diet for 8 successive weeks (47–54 weeks of age).
[b] Traits were evaluated at 54 weeks of age.
[c] *P* values include the linear and quadratic responses (to describe the response in the dependent variable to dietary CoQ10 levels), and orthogonal contrast between CoQ10 levels (the roosters fed 300 and 600 mg CoQ10/kg diet) with control group (the roosters fed basal diet without CoQ10).

Table 5
The effect of semen quality of broiler breeder roosters (12 roosters/treatment) fed the diet supplemented with different levels of coenzyme Q10 (CoQ10) on fertility (number of fertile eggs/total number of eggs set; 460 hatching eggs per treatment), hatchability on total eggs set (chick number/total number of eggs set), hatchability on fertile eggs set (hatched eggs/fertilized eggs) and sperm penetration (SP; 48 eggs/treatment) rates.

Treatment[a]	Traits[b]			
	Fertility (%)	Hatchability of eggs set (%)	Hatchability of fertile eggs set (%)	SP (Log_{10}+1)
Q-0	60.08	54.38	90.68	2.07
Q-300	65.33	60.29	92.39	2.28
Q-600	71.19	65.29	90.95	2.31
SEM	–	–	–	0.06
P-value[c]				
Linear	<.001	<.001	0.92	<.01
Quadratic	0.73	0.90	0.53	0.25
Control vs. CoQ10	<0.01	<0.01	0.81	<0.05

SEM: Standard error of the mean.
[a] The roosters fed a standard basal diet supplemented with increasing levels of CoQ10 including 0 (Q-0), 300 (Q-300), and 600 (Q-600) mg CoQ10/kg diet for 8 successive weeks (47–54 weeks of age).
[b] Traits were evaluated at 51–54 weeks of age.
[c] *P* values include the linear and quadratic responses (to describe the response in the dependent variable to dietary CoQ10 levels), and orthogonal contrast between CoQ10 levels (the roosters fed 300 300 and 600 mg CoQ10/kg diet) with control group (the roosters fed basal diet without CoQ10).

correlated with circulating antioxidants levels that protect testosterone producing testicle cells from oxidative stress [38]. The results of the present study indicate the administration of CoQ10 to aged roosters leads to an improvement in TAC status and allows for the return of the activity of testicular cells to normal levels, which in turn, increase circulating testosterone concentrations and subsequently improve sperm quality and quantity.

Successful fertilization is associated with the optimal concentration, motility, and plasma membrane integrity and functionality of spermatozoa [35,50,51]. It has been shown that the number of SP in IPL is positively correlated with fertility and sperm storage in the SST [6,52]. In the current study, all hens in each treatment were subjected to AI using the same sperm concentrations. Therefore, after the AI of the hens, the different SP between treatment groups were only associated with sperm quality variables such as sperm motility, plasma membrane integrity, and plasma membrane functionality. Coenzyme Q10 treatment improved sperm motility and plasma membrane integrity, and sperm plasma membrane functionality likely increased the population of useful spermatozoa in the SSTs. This would indicate that the elevated SP and fertility rates (by approximately 11% and 14%, respectively) observed following CoQ10 treatment (300 and 600 mg/kg diet) were the result of significant improvements in sperm quality.

In conclusion, in according with the quadratic effects of CoQ10 level on seminal volume, sperm concentration, sperm plasma membrane functionality, sperm plasma membrane integrity, seminiferous tubule diameter and seminiferous epithelium thickness it was demonstrated that the best results for these response variables were achieved at 429.19, 433.33, 464.50, 613.5, 392.78 and 447.99 dietary levels of CoQ10 (mg CoQ10/kg diet), respectively. Supplemental dietary CoQ10 to the diet (at last 300 mg CoQ10/kg diet) has, therefore, the potential to alleviate oxidative stress conditions and to improve the sperm quality, seminal plasma TAC, testicular histology, and fertility rate in aged broiler breeder roosters. Because mitochondrial function, and ATP levels and gene expression in the mitochondrial respiratory chain were not examined, further studies are necessary to determine the effects of CoQ10 on these indicators in broiler breeder roosters.

[1] Provides (per kg of diet): copper (CuSO4·5H2O), 10 mg; iodine (KI), 2 mg; iron (FeSO4·7H2O), 50 mg; manganese (MnSO4·H2O), 120 mg; selenium (Na2SeO3), 0.3 mg, Zn (ZnO), 110 mg.

[2] Provides (per kg of diet):vitamin A (retinyl acetate), 12,000 IU; cholecalciferol, 3,500 IU; vitamin E (DL-α-tocopheryl acetate), 100 IU; vitamin K, 5.0 mg; thiamin, 3.0 mg; riboflavin, 12 mg; D-pantothenic acid, 13 mg; niacin, 50 mg; pyridoxine, 6 mg; biotin, 0.66 mg; folic acid, 2 mg; vitamin B12, 0.03 mg.

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