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Beneficial effects of dietary coenzyme Q10 on the productive and reproductive variables of broiler breeder hens

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ABSTRACT

The aim of this study was to evaluate the effects of supplementary CoQ10 in the diets of aged broiler breeder hens on productive and reproductive variables. A total of 128 hens)44 weeks of age) were randomly assigned to one of 16 groups (eight hens per group). The hen-groups (with equal mean egg production and egg weight) were randomly assigned to one of four diet-groups to provide four pen/groups per treatment. There was no CoQ10 supplementation or supplemental amounts of either 300, 600 or 900 mg CoQ10/kg added to the basal diet. Egg production, weight, and mass were determined weekly. To assess fertility, hatchability, and sperm penetration (SP) rate, the hens were artificially inseminated on a weekly basis (from 47–54 weeks of age). The hens were weighed and killed at the end of the experiment for evaluation of the ovarian morphology, oviduct histology, utero-vaginal junction (UVJ) total antioxidant capacity (TAC), and *Pdss2*, *GDF9*, and *BMP15* mRNA transcript abundances in the germinal disc regions. The results indicated that there was a linear response curve to increasing amounts of supplemental dietary CoQ10 on fertility, hatchability of eggs, SP rates, TAC of the UVJ, fold height and surface epithelia of the magnum and isthmus, and abundance of *GDF9*, *BMP15* and *Pdss2* mRNA transcripts in the germinal disc region. In conclusion, the findings of the present study indicate diet supplementation with CoQ10 had beneficial effects on the productive and reproductive variables of aged hens.

1. Introduction

Following a single natural mating or artificial insemination, sufficient numbers of viable and functional spermatozoa are stored in sperm storage tubules [SSTs; located at the utero-vaginal junction (UVJ)] for several days and the storage of the functional spermatozoa is necessary for fertilization after each ovulation (Breque et al., 2003). In the SSTs, spermatozoa are protected against lipid peroxidation by a highly efficient antioxidant system (Breque et al., 2006). Total antioxidant capacity (TAC) markedly decreases with age, resulting in the damage of spermatozoa stored in the SSTs, which decreases duration of hen fertility after artificial insemination (Breque et al., 2003; Gumulka and Kapkowska, 2005). Also, in studies with meat type birds there was a decrease in reproductive performance with aging that is associated with increased oxidative stress (Breque et al., 2006) and decreases in energy utilization due to mitochondrial dysfunction (Iaffaldano et al., 2018). The inclusion of coenzyme Q10 (CoQ10) in broiler diets improves antioxidant

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capacity and mitochondrial function, and reduces mortality due to ascites pathophysiology disorders (Geng and Guo, 2005; Gopi et al., 2015). Increases in antioxidant activity as a result of CoQ10 may result in a lesser damage by reactive oxygen species (ROS), and ultimately may lead to improvements in the reproductive performance of aged broiler breeder hens.

Coenzyme Q10, which is ubiquitous to almost all body tissues, is a highly functional antioxidant (Ben-Meir et al., 2015). Coenzyme Q10 is an important electron shuttling component of the mitochondrial electron transport system. In addition, when there are lesser amounts of CoQ10, there can be inhibition of cellular oxidation of lipids, proteins and DNA (Ozcan et al., 2016). Results of several studies indicate there are tissue-specific enzymes that are responsible for CoQ10 production in the mitochondria, such as decaprenyl-diphosphate synthase subunit 2 (*Pdss2)*, that decrease with age, which in turn results in a decrease in CoQ10 concentrations and a greater incidence of mitochondrial dysfunction (Miles et al., 2004; Ben-Meir et al., 2015). The mitochondria of cells with a longer half-life, therefore, tend to generate more ROS and less adenosine triphosphate (ATP) than cells with a shorter half-life (Ozcan et al., 2016). Lee et al. (2014) reported that a lesser ATP production in pig oocytes had a negative effect on the abundance of *growth differentiation factor 9* (*GDF9*) and *bone morphogenetic protein 15* (*BMP15*) mRNA transcripts. Coenzyme Q10 has the potential to restore mitochondrial performance in aged animals (Takahashi and Takahashi, 2013). With CoQ10 supplementation, there is preservation of ovarian reserves and improved oocyte mitochondrial functions in aged rats (Ozcan et al., 2016).

The germinal disc (GD) of the bird oocyte is structurally and functionally equivalent to the mammalian oocyte and contains important components, such as *BMP15* and *GDF9* that are integral for fertilization and early embryonic development (Elis et al., 2007; Han et al., 2015). Adenosine triphosphate is required for spindle formation and chromosomal alignment during oocyte maturation, as well as for the processes of fertilization and early embryo development (Bentov et al., 2010). Enriching the diets of the aged hens with mitochondrial nutrients, such as CoQ10, may result in an improvement in ATP production and oocyte quality, which can subsequently increase egg hatchability rates. The specific hypothesis for the present study was dietary supplementation with CoQ10 would improve reproductive performance in aged broiler breeder hens. The present study, therefore, was conducted to investigate the effect of dietary CoQ10 on egg production, fertility and hatchability, rate of sperm penetration (SP) in the perivitelline layer (IPL), and mRNA transcript abundance of some genes in the GD region, and the TAC of the UVJ in aged broiler breeder hens.

2. Materials and methods

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

2.1. Birds and treatment

A total of 128 broiler breeder hens (Arbor Acres Plus), at 44 weeks of age and with an average body weight of 3950 ± 293 g, were randomly selected from a commercial flock. The hens were randomly assigned to one of 16 pens (1.25 × 2.5 m; eight hens/pen). The pens (containing a pan feeder and an automatic bell drinker) were in a facility that was maintained at 22 ± 2 °C and that provided a 14L:10D photoperiodic regimen. The birds were adapted to new conditions by being fed a basal breeder hen diet without supplemental CoQ10 for 2 weeks (Table 1). Egg production and mean body weight (BW) in each pen were controlled during the acclimation period. The hen groups (with equal mean egg production and egg weight) were then randomly assigned to one of four diet groups to provide four pens/group/treatment. The diets contained no supplemental CoQ10 (Control) or supplemental amounts of either 300, 600 or 900 mg CoQ10 (Hangzhou Dingyan Chem Co., Ltd, Hangzhou, China) per kg of diet, for 8 consecutive weeks. During the experiment (44–54 weeks of age), the amount of the feed was controlled, in accordance with the recommendations of Arbor Acres Plus, that produced the genetic line used in the present study. Water was supplied *ad libitum* throughout the study.

2.2. Egg production

From 47–54 weeks of age, rate of lay, egg weight, and egg mass (laying rate × egg weight/100) were calculated weekly.

2.3. Evaluation of rates of fertility, hatchability, and SP

The roosters (Arbor Acres Plus; 44 weeks of age) were first adapted to the semen collection procedure used in this study Burrows and Quinn, 1937). Semen samples of 12 roosters were collected and pooled (sperm motility ≥ 80%) and diluted in modified Beltsville extender (Sharideh et al., 2019b). All treatment hens were inseminated weekly (from 47–54 weeks of age) with diluted semen (0.2 mL diluted semen/hen; 200 × 10^6 spermatozoa/hen). Eggs were collected at the beginning of the second day after the first AI, for a period of 7 weeks. The eggs were incubated weekly in a commercial incubator (Petersime, Zulte, Belgium) at a dry-bulb and wet-bulb temperature of 37.7 and 29 °C, respectively. Percentage fertility (fertilized eggs divided by total eggs incubated × 100), hatchability of eggs (chick number divided by total number of eggs incubated × 100) and hatchability of fertile eggs (hatched eggs divided by fertilized eggs × 100) were determined at the completion of each incubation period (by breaking the eggs to determine whether the ovum was unfertilized or fertilized; Sharideh et al., 2019a).

The SP rate was determined weekly (from 47–54 weeks of age), on the third day following each insemination (200 × 10^6 spermatozoa/hen), by using the method described by Bramwell et al. (1995) and Sharideh et al. (2016). Briefly, the eggs (12 eggs/treatment/week) were broken 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 GD was removed. The GD was transferred to a slide, several drops of formalin (10 %) were added on to the

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

Ingredient	Amount (%)
Corn	66.40
Soybean meal, 42.6% CP	19.5
Wheat bran	3.40
Corn oil	1.00
Dicalcium phosphate	1.30
Mineral oyster shall	7.32
Common salt	0.32
$NaHCO_3$	0.10
Mineral premixes[a]	0.25
Vitamin premix[b]	0.25
DL-Met, 99%	0.16
Total	100
Calculated nutrient content	
AME (kcal/kg)	2800
CP (%)	14
Calcium (%)	3.2
Available phosphorus (%)	0.33
Sodium (%)	0.18
Met (%)	0.39

[a] 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.

[b] 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.

IPL, and the IPL was stained with Schiff's reagent and air-dried. The number of SP sites in one visual field (15.89 mm^2) were determined using light microscopy (D-22976; Kruss, Hamburg, Germany; 40× magnification).

2.4. Tissue collection and weighing

At the end of the experiment, eight hens per treatment were weighed and humanely killed by decapitation. The ovarian morphology, oviduct histology, TAC of the UVJ, and the abundance of *Pdss2*, *BMP15*, and *GDF9* mRNA transcripts in the GD region (largest ovarian follicles) of the hens were evaluated. The weight and diameter of the ovarian follicles were determined (using a digital balance and digital calipers, respectively) as follows: large yellow follicle numbers and weights (> 11 mm), small yellow follicle numbers (5–10 mm), and large white follicle numbers (2–4 mm) (Romero et al., 2009). The largest ovarian follicle (F1) and the stroma (ovary without the large yellow follicles) were also weighed. Immediately after bird euthanasia, the GD regions and the overlying layer of granulosa cells, were collected from the preovulatory follicle (F1) as previously described (Elis et al., 2008). The GD region samples were collected, quickly frozen in liquid nitrogen, and then stored at −80 °C prior to the mRNA transcript abundance analysis. For UVJ TAC assay, the UVJ mucosal layer was carefully removed with a scalpel and placed in in 2 mL cryotubes. Samples were then quickly frozen in liquid nitrogen and stored at −80 °C until further analysis. Segments (~2 cm) were taken from the isthmus and magnum of the oviduct, for histo-morphometric evaluation. The segments were then gently washed with a 1% NaCl solution to remove the remaining contents, and the washed samples were then fixed in 10% formalin.

2.5. RNA extraction, cDNA synthesis and mRNA transcript abundance (real time quantitative PCR)

Total RNA was isolated from GD regions using a YTzol kit (Yekta Tajhiz Azma Co, Tehran, Iran), according to the manufacturer's protocol. Total RNA was utilized for reverse transcription (20 μL final volume) following the manufacturer's instruction (Cinaclon Co, Tehran, Iran) and cDNA was stored at −80 °C for subsequent conducting of the real time quantitative PCR procedures. The nucleotide sequences of all genes investigated in the GD region were obtained from the Gen Bank database (https://www.ncbi.nlm.nih.gov). Primers were designed using Primer3 Plus online software (https://www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi) and assessed using Oligo Analyzer (https://eu.idtdna.com/calc/analyzer), OligoCalc (http://biotools.nubic.northwestern.edu/OligoCalc.html) and Primer BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast). Primers for real time quantitative PCR (rtPCR) were synthesized by Cinaclon (Tehran, Iran) Co., Ltd. Primer sequences for *Pdss2* (with an intra-assay CV of 4.27 %), GDF9 (with an intra-assay CV of 4.13 %), BMP15 (with an intra-assay CV of 3.46 %), and *β-actin* (as a stable housing keeping gene; with an intra-assay CV of 4.08 %) are shown in Table 2. Using a Rotor-Gene Q System (QIAGEN Hilden, Germany) the PCR amplifications were conducted in

Table 2
Gene-specific primers for real-time quantitative reverse transcription PCR.

Gene name	Primer sequence	Product size (bp)	Annealing temperature (°C)	GenBank Accession
BMP15	F: CTCCTCTTCCTCAACGACAC R: GATCCAATGGTCCCAACCC	183	64	AY725199.1
GDF9	F: ACTCGGTGGATTTGCTCTTC R: CGCTGGGACATACTTGGCT	168	64	AY672110.1
Pdss2	F: AGCTCTCTTTCCAGCACAAC R: ATCTCCACTCAGAACAGCCA	191	64	NM_001318441.1
β-actin	F: AAGCCAACAGAGAGAAGA R: AGAGTCCATCACAATACCA	128	56	L08165.1

GDF9: growth differentiation factor 9; *BMP15:* bone morphogenetic protein 15; *Pdss2:* decaprenyl-diphosphate synthase subunit 2.

a final 15 μL volume reaction mixture containing 1 μL of cDNA, 7.5 μL of RealQ plus 2x master mix green [without Rox (Ampliqon, Denmark)], 0.6 μL (10 μmol/L) of each primer, and 5.3 μL of sterilized water. The cycling condition were as follows: For BMP15, GDF9 and *Pdss2*, 15 min at 95 °C, followed by 40 cycles of denaturation at 95 °C, 15 s; 60 °C, 30 s; and 72 °C, 30 s. For *β-actin*, 40 cycles of 95 °C, 15 s; 56 °C 30 s; and 72 °C, 30 s.. At the end of each PCR, a melting curve analysis was performed at a rate of 0.18 $^{\circ}Cs^{-1}$ for all genes to assess the specificity of the products. All of the real-time reactions were performed in triplicate and relative abundances of mRNA were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.6. Total antioxidant capacity (TAC) assay

For TAC assay of the UVJ, tissue samples on ice were homogenized in a cold 1.15 % KCl2 (1:10, w/v) solution. The remaining fraction of each homogenate was centrifuged (1,200 *g* for 10 min at 4 °C), and the supernatant was retained for detection of the TAC. The TAC was determined using a colorimetric enzymatic method utilizing a commercial kit (Navandsalamat, Urmia, Iran) with an intra-assay CV of 3.87 %.

2.7. Isthmus and magnum histology

After isthmus and magnum segments (~2 cm segments have been collected from the middle parts of isthmus and magnum) were fixed in formalin (10 %), these were dehydrated in an increasing alcohol gradient. The segments were then cleared in xylene and embedded in paraffin, and the samples were subsequently cut (5 μm) and stained by hematoxylin-eosin technique. The fold height and surface epithelium of the magnum and isthmus were determined using light microscopy (to record digitally, coupled to a Dino-Eye Eyepiece Camera; AnMo Electronics, New Taipei, Taiwan) at 40 × (for fold height) and 400 × (for surface epithelium) magnification. A cross sectional image was then analyzed using Image J software (National Institutes of Health; Bobadilla-Mendez et al., 2016).

2.8. Statistical analysis

All data were assessed for a normal distribution and normality of variances using the Shapiro–Wilk and Levene's tests, respectively. The data repeated in time (egg production, egg weight and egg mass) were analyzed using Proc Mixed of SAS 9.1 (SAS Institute, 2002, Cary, NC), and other data were analyzed using Proc GLM of SAS 9.1. The results were represented as LSmeans ± SEM. Fertility and hatchability rate data were analyzed using the GENMOD procedure utilizing the chi-square test of SAS 9.1. Orthogonal polynomial contrasts were used to determine the linear, quadratic, and cubic effects of increasing supplemental dietary CoQ10 concentrations on the dependent variables.

3. Results

The data for evaluating effects of dietary CoQ10 on egg production, weight and mass from 47–54 weeks of age are shown in Table 3. In comparison to the control group, treatment with CoQ10 resulted in a lesser egg weight. Specifically, supplementation with CoQ10 to the diet of the hens reduced egg weight in a quadratic response curve, but tended to increase egg production.

The data for evaluating effects of adding CoQ10 to the diet on fertility, hatchability, SP rate, and TAC of the UVJ are shown in Table 4. Fertility, hatchability of incubated eggs, and SP percentages were increased in a linear response curve as a result of CoQ10 supplementations. With inclusion of CoQ10 in the diet, there was a linear response curve for TAC of the UVJ. The supplementation with CoQ10, however, had no effect on the hatchability of fertile eggs.

The data for evaluating effects of including CoQ10 in the diet on the abundance of the *GDF9*, *BMP15* and *Pdss2* mRNA transcripts in the GD of F1 follicles are shown in Table 5. Abundances of the *BMP15* and *Pdss2* mRNA transcripts in the CoQ10 groups were greater compared to the control group. There was a linear response curve for abundance of the *GDF9, BMP15* and *Pdss2* mRNA transcripts as the amounts of supplemental CoQ10 increased.

Table 3
The effects of different amounts of coenzyme Q10 (CoQ10) on egg production, weight and mass in broiler breeder hens for 8 successive weeks.

CoQ10 amount	Egg production (%)	Egg weight (g)	Egg mass (g)
0	71.18	66.61	48.35
300	75.25	64.70	49.32
600	74.45	65.63	49.10
900	74.87	65.87	49.69
SEM	1.24	0.25	0.80
P-value[a]			
Con compared with CoQ10	0.060	0.009	0.26
Linear	0.180	0.4744	0.29
Quadratic	0.45	0.0049	0.80
Cubic	0.10	0.078	0.56

SEM: Standard error of the mean.
[a] *P* values include the linear, quadratic and cubic responses, and orthogonal contrast among CoQ10 amounts (300, 600 and 900 mg CoQ10/kg diet) with control group (Con; hens fed basal diet without CoQ10).

Table 4
The effects of different amounts of coenzyme Q10 (CoQ10) on fertility (number of fertile eggs/total number of eggs set; 1000 hatching eggs per treatment), hatchability of incubated eggs (chick number/ total number of eggs set), hatchability of fertile eggs (hatched eggs/fertilized eggs), number of sperm penetration (SP; 84 eggs/treatment) in the perivitelline layer, and total antioxidant capacity (TAC; at the end of the experiment) at the utero-vaginal junction in broiler breeder hens during an 8 successive week period.

CoQ10 amount	Fertility (%)	Hatchability of fertile eggs (%)	Hatchability of set eggs (%)	SP (log10 + 1)	TAC (mmol/L Fe (II)
0	62.93	91.89	58.21	2.18	3.05
300	66.91	92.72	61.99	2.22	3.61
600	67.82	93.88	63.37	2.27	3.72
900	70.39	93.48	66.19	2.38	3.77
SEM	–	–	–	0.04	0.17
P-value[a]					
Con compared with CoQ10	0.003	0.17	0.0013	0.03	0.004
Linear	0.0011	0.14	0.0002	0.0043	0.0089
Quadratic	0.7830	0.49	0.7908	0.5475	0.1728
Cubic	0.3888	0.60	0.5620	0.8148	0.6458

SEM: Standard error of the mean.
[a] *P* values include the linear, quadratic and cubic responses, and orthogonal contrast between CoQ10 amounts (300, 600 and 900 mg CoQ10/kg diet) with control group (Con; hens fed basal diet without CoQ10).

Table 5
Relative abundance of mRNA transcripts (at the end of the experiment) of growth differentiation factor 9 (*GDF9*), bone morphogenetic protein 15 (*BMP15*), and decaprenyl-diphosphate synthase subunit 2 (*Pdss2*) in the germinal disc regions of the hens fed a diet supplemented with different amounts of coenzyme Q10 (CoQ10) after 8 weeks of treatment.

CoQ10 amounts	*GDF9*	*BMP15*	*Pdss2*
0	0.89	1.05	0.82
300	0.92	1.35	0.96
600	1.02	1.34	0.90
900	1.17	1.69	1.14
SEM	0.07	0.14	0.08
P-value[a]			
Con compared with CoQ10	0.10	0.02	0.05
Linear	0.0089	0.0087	0.0106
Quadratic	0.3918	0.8805	0.5037
Cubic	0.9986	0.3486	0.1069

SEM: Standard error of the mean.
[a] *P* values include the linear, quadratic and cubic responses, and orthogonal contrast between CoQ10 amounts (300, 600 and 900 mg CoQ10/kg diet) with control group (Con; hens fed basal diet without CoQ10).

The data for evaluating effects of adding CoQ10 to the diet on ovarian and oviduct morphology are included in Tables 6 and 7. Dietary supplementation of CoQ10 did not affect ovarian morphology. In comparison to the control group, the supplementation of dietary CoQ10 resulted in linear increases in the fold height and surface epithelia of the magnum and isthmus. Specifically, adding CoQ10 to the diet resulted in a cubic increase in the fold height of the magnum.

Table 6
Number and weight of ovarian follicles, along with the weight of other parts of the genital tract, in broiler breeder hens fed a diet supplemented with different amounts of coenzyme Q10 (CoQ10) after 8 weeks of treatment.

CoQ10 amount	Number of ovarian follicles			Weight of ovarian follicles		Stroma (g)	Ovary (g)	Oviduct (g)
	LYF (> 11 mm)	SYF (5–10 mm)	LWF (2–4 mm)	LYF (g)	F1 (g)			
0	4.50	17.62	32.25	42.07	16.65	13.56	73.61	73.53
300	4.62	17.25	31.12	41.97	17.33	12.60	71.91	67.31
600	5.00	16.50	32.25	47.25	18.12	13.81	79.18	69.06
900	4.62	17.37	33.00	44.47	18.43	13.25	76.87	70.81
SEM	0.29	1.25	2.12	2.77	1.11	1.10	3.82	2.79
P-value[a]								
Con compared with CoQ10	0.47	0.69	0.95	0.62	0.31	0.79	0.5	0.17
Linear	0.5767	0.7919	0.7257	0.6909	0.2269	0.9542	0.3246	0.6116
Quadratic	0.4070	0.6236	0.6632	0.3846	0.8674	0.8582	0.9345	0.1651
Cubic	0.4577	0.7252	0.7849	0.2232	0.9089	0.4352	0.2591	0.5291

LYF: Large yellow follicles; SYF: small yellow follicles; LWF: large white follicles; F1: the largest yellow follicles.
SEM: Standard error of the mean.
[a] *P* values include the linear, quadratic and cubic responses, and orthogonal contrast between CoQ10 levels (300, 600 and 900 mg CoQ10/ kg diet) with control group (Con; hens fed basal diet without CoQ10).

Table 7
Fold height and surface epithelia of the magnum and isthmus in broiler breeder hens fed a diet supplemented with different amounts of coenzyme Q10 (CoQ10) after 8 weeks of treatment.

CoQ10 amount	Magnum		Isthmus	
	Fold height (mm)	Surface epithelium height (μm)	Fold height (mm)	Surface epithelium height (μm)
0	3.38	19.73	2.56	20.19
300	4.24	28.42	3.00	26.03
600	3.94	28.92	3.37	27.28
900	4.37	29.00	3.23	28.62
SEM	0.14	2.19	0.12	2.08
P-value[a]				
Con compared with CoQ10	0.0003	0.001	0.0009	0.007
Linear	0.0013	0.0126	0.0020	0.0091
Quadratic	0.1928	0.0749	0.0664	0.3098
Cubic	0.0196	0.4514	0.5462	0.6457

SEM: Standard error of the mean.
[a] *P* values include the linear, quadratic and cubic responses, and orthogonal contrast between CoQ10 levels (300, 600 and 900 mg CoQ10/ kg diet) with control group (Con; hens fed basal diet without CoQ10).

4. Discussion

The supplementation of CoQ10 to the diet resulted in greater fertility, hatchability, and SP rates and also TAC of the UVJ. Results of a previous study indicated that decreases in hen fertility with age coincide with lesser numbers of SP in the perivitelline layer (Gumulka and Kapkowska, 2005). The number of SP in the perivitelline layer is positively correlated with fertility and population of functional spermatozoa in the SST (Donoghue, 1996). The decreasing population of functional spermatozoa after 45 weeks of age has been correlated with multiple factors such as a decrease in testicular mass, sperm generation, antioxidant capacity, and testosterone concentrations of broiler breeder roosters (Sarabia Fragoso et al., 2013; Sharideh et al., 2019a). The decrease in the TAC of UVJ as hens age coincides with increases in the lipid peroxidation in the plasma membranes of sperm and decreases in the storage of sperm in the SST. Dietary supplementation of some antioxidants such as vitamin E, can improve the TAC of the SST and the number of SP in the IPL of aged hens (Brillard and Bakst, 1990; Breque et al., 2003). Coenzyme Q10 is involved in the regeneration of some antioxidants such as superoxide dismutase and vitamin E, which in turn, inhibits lipid peroxidation (Navas et al., 2007). Results of *in vitro* studies in roosters indicate supplemental CoQ10 in semen extenders improves sperm quality and inhibits lipid peroxidation of the plasma membranes of sperm (Masoudi et al., 2018; Sharideh et al., 2019b). In the present study, supplemental dietary CoQ10 has a positive effect on fertility in aged hens by improving the TAC in the SST, and by increasing the capacity for sperm preservation in the SST (determined by the number of SP in the IPL).

The addition of CoQ10 to the diets in the present study resulted in a reduction in egg weight and enhanced fold height and surface epithelium of the magnum and isthmus. Results of some studies indicate fertility and hatchability rates that decrease with age are related to egg quality factors such as egg weight (Iqbal et al., 2016). Egg quality is affected by the development of the epithelium of the oviduct (Fertuck and Newstead, 1970). Supplementation of the diets of aged hens with vitamins C and E (Franchini et al., 2002)

or green tea (Wang et al., 2018) improved the quality of eggs and increased fold height and surface epithelia of the magnum and isthmus portions of the oviducts. Reactive oxygen species are produced to a greater extent in aged as compared with younger hens, which results in damage to the cells of tissues (Breque et al., 2006). Coenzyme Q10, a potent antioxidant, can scavenge active ROS in many cells and thereby decrease the amount of oxidative damage of cells (Hosseinzadeh et al., 2017). Consequently, dietary supplementation with CoQ10 can enhance the TAC of aged broiler breeder hens, which in turn protects the magnum and isthmus cells from the ROS-induced damage.

There was a linear response curve for abundances of *GDF9*, *BMP15* and *Pdss2* mRNA transcripts in the GD of the present study as a result of increases in the amounts of dietary CoQ10. A *Pdss2* deficiency in the oocytes of animals is associated with a reduction in ovarian follicular reserves and ATP production, which in turn, results in premature failure of ovarian functions and female infertility (Ben-Meir et al., 2015). Adenosine triphosphate has an important function in oocyte maturation, fertilization, and subsequent embryo development. Lee et al. (2014) reported that a lesser ATP production in pig oocytes has a negative effect on the abundance of *GDF9* and *BMP15* mRNA transcript abundance. Coincident with decreased amounts of CoQ10 in aging tissues, there is a decrease in the amount of ATP produced by mitochondria (Conley et al., 2007). Results of some studies indicate CoQ10 supplementation enhances CoQ10 concentrations in some tissues, such as the testes and ovaries (Safarinejad, 2009; Ozcan et al., 2016). Furthermore, Kamisoyama et al. (2010) reported that CoQ10 supplementation in laying hen diets resulted in increases in the CoQ10 concentrations of the eggs the hens produced. In the present study, therefore, dietary supplementation of CoQ10 may increase the CoQ10 concentrations in tissues, which in turn, may restore mitochondrial functions and affect the abundance of *Pdss2*, *BMP15* and *GDF9* mRNA transcripts.

Maternal factors such as oocyte quality, fertility and hatchability are markedly affected by incubation condition and the capacity of the oviduct to store and maintain sperm after each ovulation (Zhang et al., 2018). In the present study, supplementation with dietary CoQ10 resulted in an improved fertility, hatchability of incubated eggs, and SP rates. In meat type birds, metabolic disturbances increase with age, and have a negative effect on reproductive performance (Iaffaldano et al., 2018). Supplemental CoQ10 had a counteractive effect on metabolic disturbances in aged hens. In an *in vitro* study with oocytes of cattle, inclusion of CoQ10 in the media improved oocyte developmental competence (Gendelman and Roth, 2012). Furthermore, in a report from a study performed with aged mice, it was suggested that the administration of CoQ10 may reverse a decrease in oocyte quality and quantity and improve the abundance of the *Pdss2* mRNA transcript (Ben-Meir et al., 2015). In the present study, the use of supplemental dietary CoQ10 probably improved ATP production by enhancing the gene expression for enzymes such as *Pdss2* that are responsible for CoQ10 production. This can subsequently improve abundance of *GDF9* and *BMP15* mRNA transcript abundances, which can lead to and improved oocyte quality and an increase in the hatchability of incubated eggs.

5. Conclusions

In conclusion, the findings of the present study indicate diet supplementation with CoQ10 had beneficial effects on the productive and reproductive performance of aged hens. These benefits were associated with the enhancement of TAC in the UVJ, and increases in folds of the magnum and isthmus, and the number of SP in the IPL. Other associated effects included increased fertility and hatchability, and the expression of genes, as indicated by mRNA transcript abundances that effect ovulation rate, normal follicular development, and fertility. Dietary supplementation with CoQ10, therefore, is a potential management factor for improving the reproductive performance of aged broiler breeder hens. Further studies are needed concerning the reproductive effects of CoQ10 in broiler breeder hens, to support these findings and clarify the mechanisms for CoQ10 actions.

Declaration of Competing Interest

The authors have no conflict of interest to disclose for this study.

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