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The Effects of Dietary Omega-3 and / or Coenzyme Q10 on Semen Quality and Reproductive Function of Aged Broiler Breeder Roosters

Research Article

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ABSTRACT

The aim of this study was to investigate the effects of dietary supplementation of WHAT with omega-3, coenzyme Q10 (CoQ10), and the combination of both on semen quality, reproductive performance, and plasma variables in broiler breeder roosters. A total of 48 broiler breeder roosters (Hubbard F15, 49 weeks of age) were randomly assigned to one of four experimental diets: a control (Con; basal diet consisting of corn-soybean base), a Salomega (S; basal diet supplemented with 30 g of Salomega per kg of feed), a CoQ10 (Q; basal diet supplemented with 400 mg of CoQ10 per kg of feed), and a combination diet (CSQ; basal diet supplemented with 30 g of Salomega and 400 mg of CoQ10 per kg of feed). Salomega (Agritech Co. Ireland) contains 50% fat and about 10% total omega-3 fatty acids. Each treatment was replicated four times with three roosters in each. The results showed that dietary supplementation of CoQ10 increased sperm concentration, sperm motility, sperm plasma membrane integrity, seminal plasma total antioxidant capacity (TAC), and blood testosterone ($P<0.05$) in comparison to the Con groups, and the synergistic effects were observed in the basal diet supplemented with 30 g of Salomega and 400 mg of CoQ10 per kg of feed (CSQ groups) compared to Con groups ($P<0.05$). In addition, the CSQ supplementation reduced plasma levels of glucose and alanine aminotransferase (ALT) activity ($P<0.05$) in comparison to the Con groups. Roosters fed the CSQ supplementation had significantly higher fertility, hatchability of total eggs, and sperm penetration rates in comparison to the Con group ($P<0.05$). It can be concluded that the dietary combination of omega-3 and CoQ10 had a synergistic effect to improve the reproductive performance of aged broiler breeder roosters in comparison to separately dietary supplementation with omega-3 or CoQ10.

KEY WORDS aged rooster, antioxidant, fertility, hatchability, sperm penetration.

INTRODUCTION

One of the main concerns in broiler breeder management is the mass production of fertilized eggs that can turn into chicks. Although reproductive performance and fertility in fowls are affected by both roosters and hens. The rooster is more efficient than hens because each rooster covers several hens (1:10 for natural mating) in a commercial flock (Akhlaghi *et al.* 2014a). Sarabia Fragoso *et al.* (2013) showed that with aging roosters, notably from 45 weeks of age, testis index, the number of sertoli cells, sperm production and testosterone concentrations decreased and subsequently declined in mating and fertility (Sarabia Fragoso *et al.* 2013). Recently studies on meat type fowls (broiler breeder rooster and turkey) showed that mitochondrial energy production, seminal antioxidant status, sperm polyunsaturated fatty acids (PUFAs), and unsaturated fatty acids (UFAs) decreased with aging (Iaffaldano *et al.* 2018; Kelso

et al. 1996). Studies demonstrated that targeting mitochondria with coenzyme Q10 (CoQ10) can efficiently control various conditions associated with mitochondrial dysfunction, metabolic disturbances, and oxidative stress in aging hens and roosters (Sharideh *et al.* 2020a; Sharideh *et al.* 2020b; Sharideh *et al.* 2020c). Serval researches showed that including essential fatty acids such as omega-3 in the fowl diet improved sperm PUFAs (Safari Asl *et al.* 2018; Zanussi *et al.* 2019). However, other studies reported that omega-3 supplementation has harmful or inefficient effects on boar reproductive performance (Paulenz *et al.* 1995; Castellano *et al.* 2010). So, the specific hypothesis for the present study was dietary supplementation of a potent antioxidant such as CoQ10 along with dietary omega-3 may neutralize the harmful effects of omega-3 or has a booster effect on reproductive performance.

Polyunsaturated fatty acids have 16 to 22 carbon atoms and more than one double bond (Feng *et al.* 2015). Omega-3 PUFAs eicosapentaenoic acid (EPAs) and docosahexaenoic acid (DHAs) had principal roles such as prostaglandins and some hormones' precursors, and regulatory effects in reproductive endocrinology (Wang *et al.* 2003; Feng *et al.* 2015). Rooster sperm membranes contain large amounts of PUFAs the spermatozoa membrane keeps the stableness and membrane flexibility during the fertilization process (Amini *et al.* 2015; Sharideh *et al.* 2019). However, sperm PUFAs are more sensitive to lipid oxidation during oxidative stress, resulting in damage to sperm function (Akhlaghi *et al.* 2014b). To confront the effects of reactive oxygen species (ROS), the rooster semen antioxidant system typically contains glutathione, catalase, superoxide dismutase, and other natural antioxidants such as vitamins E and C (Kelso *et al.* 1996; Moghbeli *et al.* 2016). However, the antioxidant activity in rooster semen decreases with age (Kelso *et al.* 1996; Sharideh *et al.* 2020a).

Coenzyme Q10, a lipid-soluble vitamin-like potent antioxidant, is a coenzyme that is abundant in the mitochondria (Kataoka *et al.* 2021). It acts as an electron-shuttling compound in the mitochondrial electron transport chain and oxidation-reduction process in all cell membranes (Navas *et al.* 2007). Coenzyme Q10 acts as a stronger antioxidant than vitamin E and also is involved in the regeneration of some endogenous antioxidants such as superoxide dismutase and vitamin E, which in turn inhibits lipid peroxidation of PUFAs (Navas *et al.* 2007). The potency of a dietary combination of CoQ10 and omega-3 to improve reproductive performance in aged roosters has not been investigated. So, the aim of this study was to investigate the effects of dietary omega-3, CoQ10, and the combination of both on semen quality, reproductive performance, and plasma metabolites in aged broiler breeder roosters.

MATERIALS AND METHODS

Approval for the current study was given by the Animal Welfare Committee of the Animal Science department, Gorgan University Agricultural Sciences and Natural Resources. All chemicals were purchased from Merck Co. (Darmstadt, Germany) and Sigma-Aldrich Co. (St. Louis, MO).

Birds and treatments

A total of 48 Hubbard F15 broiler breeder roosters (weighing 4576±90 g, 49 weeks of age) were selected from a commercial flock (Aq-Qala, Golestan province) and transferred to 16 separate floor pens (1.25 m×1 m). The floor pens were covered with straw 10-15 cm thick. Three roosters were placed in each pen equipped with a pan feeder and an automatic bell drinker. The light program was 14 h light:10 h dark photoperiod. The feed intake was adjusted based on the recommendations of the Hubbard F15 strain broiler breeding catalog, and water was supplied *ad libitum*. The roosters were randomly assigned to one of four experimental diets: a control (Con; basal diet consisting of corn-soybean base), a Salomega (S; basal diet supplemented with 30 g of omega-3 per kg of feed), a CoQ10 (Q; basal diet supplemented with 400 mg of CoQ10 per kg of feed), and a combination diet (CSQ; basal diet supplemented with 30 g of omega-3 and 400 mg of CoQ10 per kg of feed). Salomega contains 3% crude protein, 14.5% crude fiber, 50% fat, and 10% total omega-3 fatty acids. Each treatment was replicated four times with three roosters in each. After feeding a diet without CoQ10 and omega-3 for two weeks (49-51 weeks of age, adaptation period), the experimental treatments were applied at 51 weeks of age. During the adaptation period, the roosters were adapted to the semen collection procedure by dorso-abdominal massage (Burrows and Quinn, 1937). The roosters were fed a usual breeder diet with 2700 kcal of MEn/kg and 11.5% crude protein and 0.7% calcium. Coenzyme Q10 and Salomega (used as an omega-3 fatty acids source) were purchased from Webber naturals (Canada) and Agritech (Ireland), respectively.

Sperm quality analyses

At 54 and 58 weeks of age (after three weeksof dietary treatment), semen samples were collected from the three roosters in each replicate group, and semen samples from each replicate were pooled to perform further analyses (Sharideh *et al.* 2020a). Beltsville poultry semen extender (pH and osmotic pressure settings were 7.5 and 333 mOsm/kg, respectively) was used for diluted semen samples and hens' artificial insemination (Sexton, 1977).

Ejaculate volumes and weight of semen were recorded by a graduated microtube and digital balance, respectively (Akhlaghi *et al.* 2014a). To sperm concentration evaluation, the semen samples were diluted with distilled water (1:200). Then 10 µL of diluted semen were transferred to a Neubauer chamber, and the number of the spermatozoa was subsequently determined microscopically (400×magnification) (Sharideh *et al.* 2020a).

Sperm motility was evaluated by placing diluted semen (1:20 in Beltsville poultry semen extender) on a pre-warmed microscope slide (37 ˚C) covered with a coverslip, using a light microscope at 5 microscopic fields (400×magnification). The motile sperm was expressed as the percentage of spermatozoa displaying moderate to a fast progressive movement (Santiago-Moreno *et al.* 2009).

To assay sperm plasma membrane integrity, and eosin-negrosin staining method was used (Łukaszewicz *et al.* 2008). Ten µL of the stain was placed on a microscope slide and then mixed well with 10 µL of diluted semen (1:20) for 30 seconds and the mixture was then spread over the slide using a clean slide. It was then placed in an incubator at 37 ˚C. After drying, by counting 200 spermatozoa, sperm plasma membrane integrity was evaluated by oil immersion light microscopy (1000×magnification). In this staining method, due to membrane dead sperm defects, the sperm heads were fully or partially stained, while live sperms with intact plasma membrane were unstained.

Sperm plasma membrane function was assessed using a hypo-osmotic swelling test (HOST) (Santiago-Moreno *et al.* 2009). Ten µL of semen sample was mixed with 500 µL of hypo-osmotic solution (100 mOsm/kg; by adding 1 g sodium citrate/100 mL of distilled water), and the mixture was incubated at 37 ˚C for 30 minutes. Then a drop of the sample was stained (by eosin-negrosin staining) and spread over the slide using a clean slide (Sharideh *et al.* 2019). After staining, sperm plasma membrane function was evaluated by oil immersion light microscopy (1000×magnification; 200 sperm/slide). Based on this experiment, sperm with twisted tails were considered as healthy sperm with suitable membrane function, and sperm that did not react to the hypo-osmotic solution was considered as with dysfunctional membrane.

The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes, and total antioxidant capacity in seminal plasma

Seminal plasma TAC, and activity of AST and ALT were assessed at 54 and 58 weeks of age. To do this, part of the pooled semen (three roosters/replication) was centrifuged at 1500 g for 15 minutes and then immediately, the upper part of the semen was removed and kept at -20 ˚C until examination. Seminal plasma TAC and enzymatic activities were evaluated by a colorimetric enzymatic method using a commercial kit, Navandsalamat (Urmia, Iran) and Pars Azmoun (Tehran, Iran), respectively.

Plasma levels of testosterone, glucose, lipids, and enzymes activity

At 54 and 58 weeks of age, two roosters were selected from each of the 4 replicate pens (8 birds/treatment) and blood samples (2.5 mL) were collected (from the right brachial vein) into heparinized vacuum tubes by venipuncture to assay plasma levels of testosterone**,** glucose, lipids (cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides) and enzymes activity (alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST)). Immediately after blood collection, blood samples were centrifuged at 1500 ×g for 15 minutes, and then plasma samples were collected and stored at -20 ˚C for later analysis. Plasma enzymatic activity of ALP, ALT and AST as well as plasma concentrations of glucose, cholesterol, triglycerides were measured by a colorimetric enzymatic method using a commercial kit (Pars Azmoun, Tehran, Iran). Circulating testosterone concentrations were assessed using a commercially available ELISA kit (Monobind, USA).

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

Sperm fertility potential (fertility, hatchability and SP rate) of three roosters in each of the four replicate pens in each of the four dietary treatments groups were determined. For this purpose, 80 Hubbard F15 broiler breeder hens (20 hens/treatment) were used. The hens (54 weeks of age) were weekly inseminated with diluted semen (by Beltsville poultry semen extender; 200 × 10^6 spermatozoa/hen) (Sharideh *et al.* 2020c). The eggs were collected from the second day of the first artificial insemination until the end of 58 weeks of age (4 weeks). The eggs were sent to a commercial incubator every week for incubation. To calculate fertility, hatchability of fertile eggs, and hatchability of set eggs after 21 days of the incubation period, the hatched chicks from each treatment were counted and the eggs that did not hatch were broken and then their condition was recorded (Zhandi *et al.* 2016).

To assay SP in the inner perivitelline layer, 10 eggs/treatment were collected on the third day after each artificial insemination (from 54-58 weeks of age). The SP was weekly evaluated by using the method described (Schiff stain) by Bramwell *et al.* (1995) and Sharideh *et al.* (2016). The number of SP holes in one visual field (15.89 mm^2) was determined using light microscopy (40×magnification).

Statistical analysis

The data (repeated in time) of sperm quality, plasma levels of testosterone, glucose, lipids and enzymes activity and also SP were statistically analyzed using Proc Mixed of SAS 9.1 (SAS, 2003). The results were described as least squares means ± SEM, where they were compared by the Tukey's test at $P \leq 0.05$. The data of fertility and hatchability rate was analyzed using the GENMOD procedure utilizing the chi-square test of SAS 9.1.

RESULTS AND DISCUSSION

Effects of CoQ10 and omega-3 supplementation on the seminal properties examined are shown in Table 1. Dietary supplementation of CoQ10 increased sperm concentration, sperm motility, sperm plasma membrane integrity acompared to Con and S groups (P<0.05). The additional effects on sperm concentration, sperm motility, sperm plasma membrane integrity were observed in the CSQ supplementation compared to Con and S groups (P<0.05). The lowest plasma testosterone concentrations were observed in Con group compered to Q, S and CQS groups (P<0.05).

The effects of adding CoQ10 and omega-3 to the diets of the roosters on seminal plasma ALT, AST, and TAC are shown in Table 2. The basal diet supplemented with 30 g of Salomega and 400 mg of CoQ10 per kg of feed (CSQ group) increased seminal plasma TAC compared to Con, S, and Q groups (P<0.05). However, no obvious differences in activities of seminal plasma ALT and AST were observed (P>0.05). The effect of dietary CoQ10 and omaga-3 on plasma levels of glucose, triglyceride, cholesterol, HDL-c and LDL-c, and plasma enzymes activity (ALT, AST, and ALP) are shown in Table 3. Supplementation with either CoQ10 or CSQ decreased plasma levels of glucose (P<0.05), and also CSQ supplementation decreased plasma enzymes activity of ALT compared to Con, S, and Q groups (P<0.05). The highest plasma levels of cholesterol were observed in the CSQ supplementation compared to other group treatments and also the highest plasma levels of HDL-c were observed in the CSQ and Q groups in comparison to the Con and S groups (P<0.05).

Data associated with fertility, hatchability and sperm penetration rates are shown in Table 4. The highest SP rate were recorded in the CSQ supplementation (P<0.05). Dietary omega-3 and/or coenzyme Q10 increased fertility and hatchability of total eggs (P<0.05). However, no obvious differences in hatchability of fertile eggs were observed (P>0.05). In this study, dietary supplementation of CoQ10 increased sperm concentration, sperm motility, sperm plasma membrane integrity, and seminal plasma TAC, and the additional (synergistic) effects on sperm quality and seminal plasma TAC were observed in the CSQ supplementation compared to Con groups.

In vivo studies performed on bulls (Gholami *et al.* 2010), rams (Alizadeh *et al.* 2014), boars (Estienne, 2008), and breeder roosters (Feng *et al.* 2015) showed that dietary supplementation of omega-3 had efficacy effects to improve sperm quality. However, other studies suggested that omega-3 may have harmful or ineffective effects on boar sperm quality (Castellano *et al.* 2010; Paulenz *et al.* 1995), and the state that the harmful effects can attribute to several factors such as age, breed, and components of the diet. In this study, omega-3 supplementation had ineffective effects on sperm quality and seminal plasma TAC, and CoQ10 supplementation had moderate effects on the parameters. The CSQ supplementation had additional effects on sperm quality and seminal plasma TAC. A recent study performed on aging roosters showed that adding CoQ10 to the rooster diet increased sperm production, motility, membrane integrity, and seminal plasma TAC (Sharideh *et al.* 2020a). Therefore, supplemental dietary CoQ10 to the aging rooster has the potential to alleviate oxidative stress conditions, which in turn, contributes to improving the sperm quality. Kelso *et al.* (1996) and Iaffaldano *et al.* (2018) demonstrated that a significant decrease of sperm PUFA and UFA, in aging fowl were correlated with a reduction in the activities of antioxidant enzymes and the enzymatic activities involved in the biosynthesis of the PUFA from linoleic acid (Kelso *et al.* 1996; Iaffaldano *et al.* 2018). In aging roosters, the dietary supplementation of omega-3/omega-6 essential precursor linolenic acid has been efficient in increasing the PUFA sperm content, but the supplementation without antioxidant such as vitamin E had adverse effect on sperm quality (Safari Asl *et al.* 2018). It seems that omega-3 supplementation of aging rooster diet has a potential to improve PUFA sperm content, but unprotected the PUFA from oxidative stress conditions may cause the loss of sperm function.

The results of the current study suggest that, omega-3 supplementation may improve PUFA sperm content and dietary supplementation CoQ10 improved TAC and protect the PUFA content in sperm from oxidation. Therefore, dietary combination of omega-3 and CoQ10 had a synergistic effects on sperm quality.

In the current study, S, Q, and CSQ supplementation increased blood testosterone compared to Con group. Polyunsaturated fatty acids affecting prostaglandin synthesis (as precursors of prostaglandin), steroidogenesis (through direct effects on steroid acute regulator), and cell membrane properties (Wang *et al.* 2003; Feng *et al.* 2015), have a great effect on reproductive performance. Feng *et al.* (2015) showed that dietary supplementation PUFAs in young broiler breeder roosters had no significant effect on the testis index, although blood testosterone was increased (Feng *et al.* 2015).

Table 1 The effects of the diet supplemented with coenzyme Q10 (CoQ10; 0 and 400 mg/kg diet) and omga-3 (0 and 30 g/kg diet) on ejaculate volume (and weight), seminal characteristics and circulating testosterone concentrations in aged broiler breeder roosters (12 birds per treatment)

Variable	Treatments				SEM	P-value
	Con	S	Q	CSQ		
Ejaculate volume (mL)	0.185	0.233	0.257	0.220	0.034	0.4511
Ejaculate weight (mL)	0.165	0.215	0.258	0.215	0.032	0.205
Sperm concentration (10^9/mL)	1.93^{bc}	1.69^{c}	2.23^{ab}	2.47^{a}	0.14	0.0131
Sperm motility (%)	72.96^{bc}	69.96^{c}	78.25^{ab}	80.49^{a}	2.34	0.0323
Plasma membrane functionality (%)	67.68	71.76	71.02	72.69	1.50	0.1756
Plasma membrane integrity (%)	82.43^{b}	82.97^{b}	86.49^{a}	88.88^{a}	1.12	0.0035
Testosterone ($ngmL^{-1}$)	1.23^{b}	1.93^{a}	1.61^{a}	2.39^{a}	0.17	0.0056

CON: control group; S: basal diet supplemented with 30 g of Salomega per kg of feed); Q: basal diet supplemented with 400 mg of CoQ10 per kg of feed and CSQ: basal diet supplemented with 30 g of omega-3 and 400 mg of CoQ10 per kg of feed.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 2 The effects of the diet supplemented with coenzyme Q10 (CoQ10; 0 and 400 mg/kg diet) and omega-3 (0 and 30 g/kg diet) on the seminal plasma of total antioxidant capacity (TAC), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) of broiler breeder roosters (12 birds per treatment)

Variable	Treatments				SEM	P-value
	Con	S	Q	CSQ		
TAC (mmol/L Fe (II))	450.37^{b}	431.12^{bc}	463.50^{b}	469.75^{a}	8.04	0.038
AST (U/I)	134.41	132.44	99.19	110.53	18.00	0.5004
ALT (U/I)	9.00	6.47	11.24	11.31	1.74	0.18

CON: control group; S: basal diet supplemented with 30 g of Salomega per kg of feed); Q: basal diet supplemented with 400 mg of CoQ10 per kg of feed and CSQ: basal diet supplemented with 30 g of omega-3 and 400 mg of CoQ10 per kg of feed.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effects of the diet supplemented with coenzyme Q10 (CoQ10; 0 and 400 mg/kg diet) and omega-3 (0 and 30 g/kg diet) on the plasma levels of, glucose, lipids (cholesterol, HDL-c, and LDL-c), and enzymes activity (ALT, ALP, and AST) of broiler breeder roosters (12 birds per treatment)

Variable	Treatments				SEM	P-value
	Con	S	Q	CSQ		
Glucose (mg/dL)	223.64^{a}	210.21^{ab}	174.08^{c}	185.02^{cb}	11.61	0.039
Triglyceride (mg/dL)	31.61	32.15	32.49	3.02	0.37	0.10
Cholesterol (mg/dL)	104.84^{c}	103.43^{c}	108.80^{b}	115.85^{a}	0.99	< 0.0001
HDL-c (mg/dL)	26.45^{b}	26.80^{b}	27.96^{a}	28.32^{a}	0.23	0.0009
LDL-c (mg/dL)	60.79	62.82	61.93	63.36	0.87	0.2627
ALT (U/I)	6.29^{a}	7.25^{a}	6.16^{a}	4.29^{c}	0.61	0.041
ALP (U/I)	526.06	546.94	514.43	539.31	49.09	0.96
AST (U/I)	126.53	172.60	134.57	188.96	17.52	0.068

CON: control group; S: basal diet supplemented with 30 g of Salomega per kg of feed); Q: basal diet supplemented with 400 mg of CoQ10 per kg of feed and CSQ: basal diet supplemented with 30 g of omega-3 and 400 mg of CoQ10 per kg of feed.
HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; ALT: alanine aminotransferase; ALP: alkaline phosphatase and AST: aspartate aminotransferase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 The effect of semen quality of broiler breeder roosters (12 roosters/treatment) fed the diet supplemented with coenzyme Q10 (CoQ10; 0 and 400 mg/kg diet) and omega-3 (0 and 30 g/kg diet) on fertility (number of fertile eggs/total number of eggs set), 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) rates

Variable	Treatments				SEM	P-value
	Con	S	Q	CSQ		
SP	39.72^{c}	48.98^{bc}	51.53^{bc}	58.10^{ab}	4.37	0.0265
SP (log10+1)	2.53^{c}	2.63^{bc}	2.65^{bc}	2.69^{ab}	0.03	0.0073
Fertility (%)	74.08^{b}	90.14^{a}	88.72^{a}	91.81^{a}	-	< 0.0001
Hackability of set egg (%)	63.13^{b}	80.29^{a}	78.54^{a}	84.69^{a}	-	< 0.0001
Hackability of fertile egg (%)	85.22	89.06	88.52	92.24	-	0.1204

CON: control group; S: basal diet supplemented with 30 g of Salomega per kg of feed); Q: basal diet supplemented with 400 mg of CoQ10 per kg of feed and CSQ: basal diet supplemented with 30 g of omega-3 and 400 mg of CoQ10 per kg of feed.
HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; ALT: alanine aminotransferase; ALP: alkaline phosphatase and AST: aspartate aminotransferase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

A study was performed on aged broiler breeder roosters by Sharideh *et al.* (2020a) showed that supplemental dietary CoQ10 improved seminal plasma TAC, sperm quality, and blood testosterone concentrations (Sharideh *et al.* 2020a). CoQ10 and omega-3 raise testosterone levels by regulating the LH secretion and increasing the levels of enzymes involved in testosterone synthesis, respectively (Kataoka *et al.* 2021). The results of the current study indicate the CSQ supplementation to aged broiler breeder roosters' diets leads to an improvement in TAC and availability of essential fatty acids for steroidogenesis and allows for the return of the activity of testicular cells to normal levels, which in turn, enhance circulating testosterone concentrations and improve sperm quality. In the present study, supplementation with either CoQ10 or CSQ decreased plasma levels of glucose, and also CSQ supplementation decreased plasma enzyme activity of ALT. In addition, CSQ supplementation increased HDL-c and cholesterol. Amin *et al.* (2014) suggested that increased liver enzyme activity such as aminotransferases have a relationship with hepatic gluconeogenesis and / or inflammation in insulin resistance patients (Amin *et al.* 2014). A study performed on broiler breeder hens showed that CoQ10 supplementation increased adiponectin and proliferator-activated receptor-α genes' expression and suppressed gluconeogenesis pathway and subsequently reduced plasma levels of glucose (Sharideh *et al.*2020b). Similar to the current study, studies in rats and humans showed that supplemental dietary CoQ10 improved insulin sensitivity, serum lipid profile, and decreased serum levels of ALT (Amin *et al.* 2014; Gholami *et al.* 2018). Moreover, Ehr *et al.* (2017) showed that adding omega-3 to laying hen diets increased alpha-linolenic deposition into yolk (Ehr *et al.* 2017). Previous studies on meat type fowls (turkey and broiler breeder) suggested that decreased ROS production and improved antioxidant status can improve mitochondrial function and metabolic disturbances (Iaffaldano *et al.* 2018; Sharideh *et al.* 2020a; Sharideh *et al.* 2020b). Therefore, it seems that CSQ supplementation can improve antioxidant status and availability of essential fatty acids, which in turn, enhances metabolic disturbances. In the present experiment, the highest fertility, hatchability of total eggs, and SP holes in the inner perivitelline layer were recorded in the CSQ supplemented group. It has proved that the number of SP holes in the inner perivitelline layer is positively correlated with the fertility rate and population of useful spermatozoa in the sperm storage tubules (Bramwell *et al.* 1995; Donoghue, 1996; Sharideh *et al.* 2020b). Although successful fertilization is associated with sperm quality (Bramwell *et al.* 1995), the hatchability of fertile eggs is markedly affected by incubation conditions and maternal factors such as oocyte quality (Zhang *et al.* 2018). A positive correlation has been reported between the omega-3 content of sperm, seminal plasm TAC and fertility rate (Abayasekara and Wathes, 1999; Zanussi *et al.* 2019). In the present study, dietary supplementation of CSQ improved sperm quality likely increased the population of useful spermatozoa in the sperm storage tubules and consequently improved fertility and hatchability rates.

CONCLUSION

It can be concluded that the dietary combination of omega-3 and coenzyme Q10 had the greatest effects to improve the reproductive performance of aged broiler breeder roosters in comparison to separately dietary supplementation with omega-3 or CoQ10.

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