

Effects of dietary supplementation of different sources and levels of selenium on the semen quality and reproductive performance in aged broiler breeder roosters

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ABSTRACT Fertility has a great impact on economic outcome in poultry sector. However, several physiological stressors such as aging adversely affected fertilization capacity and hatching quantity and quality. This study investigated the effect of dietary supplementation of different sources and levels of inorganic and organic selenium on the semen quality and reproductive performance of aged broiler breeder roosters. A total of thirty-six 50-wk-old Ross 308 roosters were randomly allocated to 6 groups and fed with different levels of organic and inorganic selenium. Treatments were included in the basal diet (control: **CG**), dietary supplementation of 0.15 (**SeY0.15**), 0.30 (**SeY0.30**), and 0.45 (**SeY0.45**) mg/kg selenium-enriched yeast (**SeY**), dietary supplementation of 0.30 mg/kg commercial organic selenium (Selemax), and dietary supplementation of 0.30 mg/kg sodium selenite (**SS**) as an inorganic source during 12 consecutive weeks. Ejaculated volume, semen quality attributes of the collected semen samples were evaluated every week.

To assess fertility, hatchability and the hatched chick quality, the semen samples collected during last 2 wk of the trial were used to artificial insemination of hens. In order to measure seminiferous tube diameter and seminiferous epithelium thickness, testicular histology was also performed at the end of the experiment. Sperm motility, plasma membrane functionality and integrity, and ejaculation volume were higher in the SeY0.45 group compared to the other groups ($P < 0.05$). Fertility and hatchability rate as well as seminiferous epithelium thickness and seminiferous tube diameter were improved in the SeY0.45 compared with CG, SeY0.15 and SS groups ($P < 0.05$). Also hatchling quality from roosters with SeY0.45 was higher than CG and SS groups ($P < 0.05$). No significant differences were noted in embryonic mortality between groups ($P > 0.05$).

In conclusion, dietary supplementation of 0.45 mg SeY improved sperm quality and reproductive performance of aged broiler breeder roosters.

Key words: aging, fertility, rooster, selenium-enriched yeast, sperm

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INTRODUCTION

Fertility is one of the main factors affecting the economic outcome in poultry flocks and is influenced by several variables, such as breed, nutrition quality, flock age, and quality of inseminated semen (Miazi et al., 2012). Following the picking of eggs, the breeder chickens commonly experience a reduction in fertility (Khalil-Khalili et al., 2021). A combination of factors such as increasing body weight and oxidative stress, decreased

circulating testosterone, reduced reproductive behavior, low sperm production, and poor sperm quality is involved in the reproduction disorders of the aged flocks. The antioxidant activity in rooster semen decreases with age (Kelso et al., 1996), and dietary supplementation of a higher level of antioxidant may be suitable for enhancing the reproductive performance of aged broiler breeder roosters. During last decades, this issue has drawn the attention of many researchers to find out an appropriate strategy for maintain fertility of stressed roosters. As a most common approach, supplementing antioxidative components that are classified in herbal, vitamins and minerals were associated with advantages on reproduction of roosters. Supplementing antioxidative components, both in the diet and in semen extenders, has shown to have a great impact on mitigating the adverse

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effects of oxidative stress and improving fertility rate in roosters (Sharideh et al., 2020; Zhandi et al., 2020; Khalil-Khalili et al., 2021).

Selenium (Se) is an essential element in poultry nutrition and plays critical roles in the productive and reproductive performance of both male and female birds (Surai and Fisinin, 2014). From a nutritional point of view, Se is supplied in diet in organic (selenocysteine, selenocystine, and selenomethionine) and inorganic (selenites, selenides) forms. It has been assumed that the organic forms of Se have higher tissue retention rates and bioavailability than inorganic forms (Han et al., 2009; Sochor et al., 2012). Selenium is involved in the regulation of GSH-Px (Surai and Fisinin, 2014), which is highly expressed in the chicken seminal plasma and spermatozoa (Surai et al., 1998a,b). Since avian semen contain a high proportion of polyunsaturated fatty acids (PUFAs), which in turn are susceptible to lipid peroxidation, dietary Se is thought to have beneficial effect on improving quality of the produced sperm (Surai et al., 1998b). In this regard, there is evidence suggesting that addition of Se in the diet (0.3 mg Se/kg diet) of male chicken significantly increased Se-GSH-Px activity in the liver, testes, spermatozoa, and seminal plasma (Surai et al., 1998c). Furthermore, the organic Se supplementation (0.3 mg Se/kg diet) in the cockerel diets has been reported to enhance seminal plasma GSH-Px activity and semen quality attributes, mainly due to lowering lipid peroxidation (Ebeid, 2012).

Yeast can utilize soluble sugars and organic acids to produce biomass with high protein and its production is easy to manage (Esmaeili et al., 2012). By converting mineral selenium with low bioavailability and high toxicity to an organic form, yeast has a potential to produce safer and more biologically active Se source. Selenium-enriched yeast (SeY) is produced by fermenting *Saccharomyces Cerevisiae* bacteria in a selenium-rich media. Yeasts contain high amount of protein and compared to plant sources they can incorporate Se by replacement of Se in proteins. Improved methods of analysis revealed that almost (>90%) all of the Se in yeast is represented in selenomethionine (SeM) form (Schrauzer, 2006). It is worth noting that among different sources of Se, the SeM has the highest biological potency in the body. Selenium in yeast consists of up to 98% organic Se and due to its simple production, it is considered as a good source of SeM. Considering the fact that SeM is the natural form of Se in plant proteins and animal tissue, DL-SeMet was approved to be used as a feed additive in all animal species on August 4, 2014 by the European commission (Li et al., 2018).

According to the literature, supplementing Se to the diet may help improve the fertility of flocks through its antioxidative properties. However, there is no conclusive information on the effect of long-term utilization of different levels and sources of Se on semen quality and fertility of aged broiler breeder roosters. Therefore, the aim of the present study was to investigate the effects of dietary supplementation of different levels of SeY on sperm quality and reproductive performance of aged broiler

breeder roosters, and comparison the effects of SeY with other organic and inorganic Se sources.

MATERIALS AND METHODS

This study was approved by the Animal Care Committee and Animal Research Ethics Board from the Department of Animal Science, University of Tehran, Iran.

Chemicals

Chemicals used in this study were from Sigma Chemical Co. (St. Louis, MO) and Merck Co. (Darmstadt, Germany).

SeY Production

Production process of SeY has been previously reported (Rayman, 2004). In brief, the strain PTCC 5209 of *Saccharomyces Cerevisiae* were cultured in a medium containing different ingredients including KH_2PO_4 (5 g/L), Na_2HPO_4 (3.5 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 g/L), yeast extract (6 g/L), NH_4NO_3 (3 g/L), and glucose (10 g/L), and adjusted to pH = 5.8 (Yin et al., 2010). The culture process was performed in a shaker (150 strokes/min at 28°C for 48 h) at an inclusion rate of 35 g/L. After 10 h, a solution of sodium hydrogen selenite (NaHSeO_3 , 30 mg /L) as the inorganic source of Se was added to the medium. At the end, the suspension was centrifuged ($3,000 \times g$ for 15 min at 25°C) and the supernatant discarded and the solid phase washed 3 times with deionized water to remove residues of the medium and surface-bound Se. Then, the cells were dried and total Se content was determined using an optical emission spectrometer with induced coupled plasma, ICP-OES Perkin Elmer, Optima 7300 DV.

Birds Management and Experimental Design

A total of 36 Ross 308 broiler breeder roosters (50-wk-old) were kept in the individual floor pens (100 cm × 60 cm) and reared on a 14L:10D lighting schedule at an intensity of 40 lux. The roosters were habituated with experimental condition, adapted to abdominal massage and also semen collection procedure (Burrows and Quinn, 1937) for 2 wk. The birds had free access to water and received an average of 140 g standard basal diet (Aviagen, Alabama, USA; Table 1) per day. Then, the roosters were randomly divided into 6 experimental groups for 12 consecutive weeks. The treatments consisted of basal diet (control: CG), supplemented with 0.15 (SeY0.15), 0.30 (SeY0.30), and 0.45 (SeY0.45) mg/kg diet selenium-enriched yeast, supplementation of 0.30 mg/kg diet commercial organic selenium (Selexmax), and 0.30 mg/kg diet sodium selenite as an inorganic source (SS).

Table 1. Ingredient and chemical composition of basal diet fed to Ross 308 broiler breeder roosters.

Feed ingredient	Amount (g/kg)
Corn	695
Soybean meal, 42.6% CP	90
Wheat bran	195
Dicalcium phosphate	1.8
Calcium carbonate (CaCO ₃)	8.5
Sodium chloride	3.5
DL-Met, 99%	1.2
Vitamin premix ¹	2.5
Se-free Mineral premixes ²	2.5
Calculated nutrient content	
AME (kcal/kg)	2,754
CP (%)	12
Available phosphorus (%)	0.35
Sodium (%)	0.15
Selenium (%)	0.00001
Calcium (%)	0.7
Digestible Lys ³ (%)	0.46
Digestible Met ³ (%)	0.39
Digestible Met + Cys ³ (%)	0.49
Digestible Thr ³ (%)	0.37

¹Provides (per kg of diet): retinyl acetate (vitamin A), 11,000 IU; cholecalciferol (vitamin D₃), 3,500 IU; DL- α -tocopheryl acetate (vitamin E), 150 IU; menadione (vitamin K₃), 5.0 mg; thiamin (vitamin B₁), 3.0 mg; riboflavin (vitamin B₂), 12 mg; D-pantothenic acid (vitamin B₅), 13 mg; niacin (vitamin B₃), 50 mg; pyridoxine (vitamin B₆), 6 mg; biotin (vitamin B₇), 0.66 mg; folic acid (vitamin B_c), 2 mg; cobalamin (vitamin B₁₂), 0.03 mg.

²Provides (per kg of diet): copper (CuSO₄•5H₂O), 10 mg; iodine (KI), 2 mg; iron (FeSO₄•7H₂O), 50 mg; manganese (MnSO₄•H₂O), 120 mg; Zn (ZnO), 110 mg.

³Values are standardized ileal digestible [AMINODAT 4.0 (Evonik Industries, 2010)].

Assessment of Sperm Variables

Semen samples were collected from the roosters by abdominal massage technique (Burrows and Quinn, 1937) every weeks, during the experiment. Semen was sampled individually into 1.5 mL plastic micro-tube and ejaculate volume estimated with a micropipette. Eosin-nigrosin staining method was used to assess plasma membrane integrity and evaluation sperm morphology. Briefly, 10 mL of stain was mixed with 10 mL of diluted semen (1:20 in Lake extender), 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 (LX-400, Labomed, Los Angeles, CA) at 1,000 × magnification (Lukaszewicz et al., 2008). The sperm with unstained heads were considered as alive sperm with intact plasma membranes whereas those with fully or partially stained heads were considered as dead spermatozoa with unintegrated plasma membranes. Also, sperms with detached heads, abaxial heads, malformed heads, bent tails, coiled tails, double tails, and protoplasmic droplets were considered as abnormal. The stained smear was prepared in duplicate and 200 sperm per slide were evaluated (Lukaszewicz et al., 2008).

To assess sperm motility, a 10 μ L diluted sample (1:20 in 2.9% sodium citrate) was placed on a warm slide (37°C) and then covered with a coverlid. In five microscopic

fields, the percentage of motile sperm was evaluated using light microscope (Zeiss, Jena, Germany) at 400 × magnification, and the average of them was reported as total motility. Total sperm motility was expressed as the percentage of spermatozoa exhibiting moderate or progressive forward direction movement to the total counted sperms (Santiago-Moreno et al., 2009).

Using the hypo-osmotic swelling test (HOST), sperm plasma membrane functionality was assessed (Santiago-Moreno et al., 2009; Sharideh et al., 2020). Briefly, a 0.5 mL micro-tube containing 10 μ L raw semen and sodium citrate solution (200 μ L, 100 mOsm/kg) was placed in an incubator at 37°C for 30 min. The percentage of spermatozoa possessing swollen bubble around the curled flagellum was calculated by counting 200 sperm per slide with light microscope (Zeiss, Jena, Germany) at 1,000 × magnification (Zhandi et al., 2020).

Artificial Insemination, Fertility and Hatchability Evaluation

One-hundred and fifty, 54-wk-old Ross 308 broiler breeder hen were divided into 6 groups (25 birds per group) and each group were randomly assigned to one of the experimental groups. Semen samples collected during the last 2 wk of the experiment was used for artificial insemination (AI). The semen samples from each treatment pooled and diluted by Lake extender (containing 1.92 g sodium L-glutamate monohydrate, 0.5 g potassium acetate, 0.08 g magnesium acetate tetrahydrate, 0.8 g glucose, 0.3 g polyvinylpyrrolidone [Mr 10000], and 100 mL of water [343mOsm/kg, pH 7.08]) (Behnamifar et al., 2021) to a concentration of 400 × 10⁶ spermatozoa/mL (Ansari et al., 2018). Then, hens were inseminated twice a week with diluted semen (0.25 mL diluted semen/hen; 100 × 10⁶ spermatozoa/hen) of their corresponding experimental groups (Sharideh et al., 2020). All produced eggs were collected from the second day following insemination for 12 successive days. The eggs were then incubated at 37.5°C and 55% RH. At the end of the incubation period, by breaking unhatched eggs, fertility (percentage of fertilized per incubated eggs × 100), and hatchability (percentage of hatched eggs per total eggs set × 100) rates were calculated for each experimental group. All hatchlings were taken out of the incubator, counted, and then classified macroscopically as A-grade (of good quality) or culls (Jabbar and Ditta, 2017). Also, the embryonic mortality rate of fertilized but unhatched eggs was evaluated after breaking (percentage of dead embryos per total fertilized eggs set × 100).

Sperm Penetration Assay

Within 7 d of laying, 10 eggs per groups that were stored in optimum ambient condition used in the SP-holes assay. The SP assay was conducted as described by Bramwell et al. (1995). In brief, approximately 1 cm²

of the ovum perivitelline layer, directly upper the germinal disc was removed, washed, straightened on a microscope slide, fixed with 20% formalin, and stained with the Schiff's fuchsin-sulfite reagent. A light microscope was used to count the digestion holes made by sperms in the perivitelline layer (magnification: $\times 10$; [Figure 1A](#)). The number of SP sites in one visual field (15.89 mm^2) was measured.

Testis Histology Study

At 12th wk of the experiment, five roosters per group were randomly selected and euthanized to accomplish necropsy evaluation. In brief, left testis was cutted in half and fixed in 10% neutral buffered formalin, dehydrated in ethyl alcohol, cleared in xylene, and finally embedded in paraffin. The paraffin-embedded tissues were cut into $7\text{-}\mu\text{m}$ -thick sections using a microtome (Rotary microtome, Didsabz company, model DS4055, Urmia, Iran) and stained with hematoxylin and eosin (H&E). For histological observation, slides were investigated by optical microscopy at a final magnification of $\times 10$. Setting scale and measurements were performed using ImageJ 1.52a software. The diameter of the seminiferous tube and the thickness of the seminiferous epithelium were measured on at least 20 randomly sections of seminiferous tubules and mean of the values were reported ([Figure 1B](#)).

Statistical Analysis

The normality of the data was tested by the UNIVARIATE procedure and Shapiro–Wilk test of SAS 9.4, and all percentage data were normalized through $\text{ArcSin}\sqrt{x}$ transformation when appropriate. The MIXED procedure of SAS 9.4 was used to analyze repeated measurement data. Fertility, hatchability, embryonic mortality, and chick quality data were analyzed as binomial data ([Walsh and Brake, 1997](#)), where each individual egg or chick was taken as a binomial event, using GENMOD procedure of SAS 9.4. Mean of

experimental group was compared at the level of significance adjusted to $P < 0.05$ and the results were presented as the Mean \pm standard error of the mean.

RESULTS

Sperm Quality Parameters

The effect of treatments on sperm ejaculation volume, plasma membrane integrity, sperm total, and progressive motility, plasma membrane functionality, sperm abnormality, and sperm penetration (SP) are shown in [Table 2](#). There was no significant effect of treatment or interaction effect of treatment and time on ejaculation volume and other parameters; meanwhile, the highest ejaculated volume was observed in SeY0.45 group and the lowest ejaculated volume was for CG and SeY0.15 ($P < 0.05$).

Dietary supplementation of 0.45 mg SeY/ kg diet was associated with the highest percentage of viable sperms; however, implementation of other organic and inorganic Se did not improved plasma membrane integrity compared to the control group ($P > 0.05$). Although the highest total motility was for SeY0.45 groups ($P < 0.05$), sperm progressive motility was significantly higher in the SeY0.45 and Selemax groups compared to the control, SeY0.15, and SS groups. The highest and lowest sperm plasma membrane functionality was for SeY0.45 and CG groups, respectively ($P < 0.05$). Furthermore, the lowest percentage of sperm abnormality was noted in SeY0.45 group, while CG and SS groups had the highest percentage of abnormal sperms ($P < 0.05$). The number of SP holes was higher in SeY0.45 groups compared to other corresponding groups ($P < 0.05$; [Figure 1A](#)).

Fertility, Hatchability and Hatchelling Quality

The effects of different sources and levels of Se on fertility, hatchability, embryonic mortality rate, and hatching quality are shown in [Table 3](#). Fertility rate was higher in the SeY0.45 group compared to the CG and SS

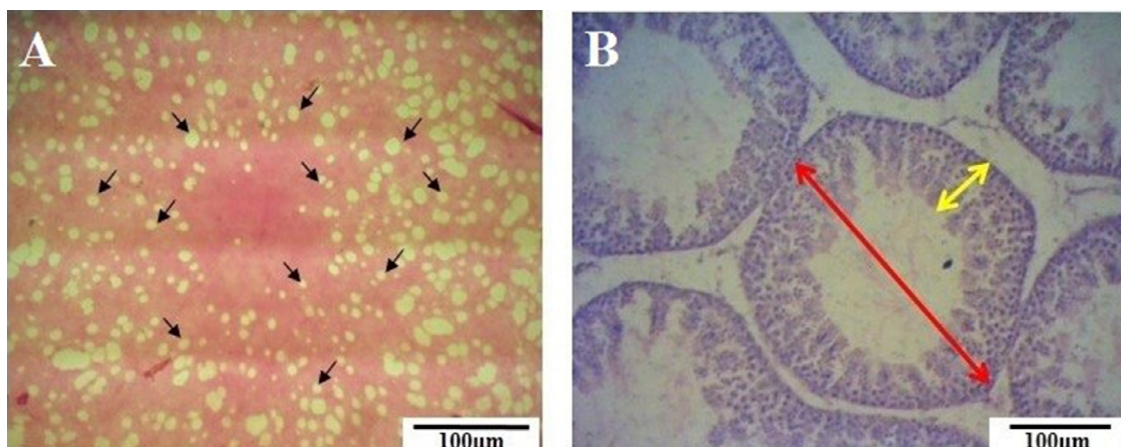


Figure 1. Light micrograph of the perivitelline layer (A) and seminiferous tube, (B) black arrows illustrate the digestion holes made by sperms in the perivitelline layer; red arrow shows seminiferous tube diameter and yellow arrow depict seminiferous epithelium. $\times 100$ magnification (Scale bar = $100 \mu\text{m}$).

Table 2. Effects of dietary supplementation of different selenium sources and levels on the sperm quality attributes of aged broiler breeder roosters.

Experimental group	Ejaculation volume (mL)	Plasma membrane integrity (%)	Total motility (%)	Progressive motility (%)	Plasma membrane functionality (%)	Abnormality (%)	Sperm penetration (SP)
CG	0.29 ^b	84.09 ^b	83.75 ^b	60.2 ^d	65.4 ^d	11.36 ^a	66.01 ^b
SeY0.15	0.28 ^b	83.27 ^b	83.33 ^b	64.79 ^{bc}	65.85 ^{cd}	8.47 ^b	68.25 ^b
SeY0.30	0.34 ^{ab}	85.22 ^b	86.66 ^{ab}	68.12 ^{ab}	69.34 ^b	7.02 ^c	66.10 ^b
SeY0.45	0.41 ^a	91.14 ^a	88.95 ^a	69.79 ^a	72.12 ^a	5.31 ^d	74.24 ^a
Selemax	0.31 ^{ab}	85.16 ^b	84.79 ^b	69.54 ^a	67.86 ^{bc}	6.87 ^c	69.83 ^b
SS	0.30 ^{ab}	83.88 ^b	83.12 ^b	62.29 ^c	67.23 ^{cd}	10.58 ^a	66.49 ^b
SEM	0.04	1.37	1.55	1.59	0.90	0.46	3.21
<i>P</i> -value							
Treatment	0.33	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01
Week	0.58	0.57	0.32	0.15	<0.01	0.92	0.57
Treatment × Week	0.95	0.38	0.40	0.06	0.57	0.70	0.38

*Treatments were included of feeding basal diet (control: CG), supplementation of 0.15 (SeY0.15), 0.30 (SeY0.15), and 0.45 (SeY0.15) mg/kg diet selenium-enriched yeast (SeY), supplementation of 0.30 mg/kg diet commercial organic selenium (Selemax), and supplementation of 0.30 mg/kg diet sodium selenite as an inorganic source (SS) during 12 consecutive weeks.

^{a-d}values within a column with different superscripts differ at $P < 0.05$.

groups ($P < 0.05$); also, hatchability in SeY0.45 was higher compared with the GC, SeY0.15 and SS groups ($P < 0.05$) but there were not any significant difference between SeY0.45, SeY0.30 and Selemax on hatchability. However, treatments had no significant effect on embryonic mortality rates ($P > 0.05$). The highest and lowest chick quality was for SeY0.45 and CG, respectively ($P < 0.05$).

Testis Histology

The effect of treatments on diameter of the seminiferous tube and the thickness of the seminiferous epithelium are given in Table 4 and Figure 1B. Both of the parameters were increased in SeY0.30, SeY0.45, and Selemax compared with the other groups ($P < 0.05$).

DISCUSSION

Selenium is an important component of enzymes and selenoprotein that plays antioxidative, anti-inflammatory, and antiviral role in biological systems (Pappas et al., 2008; Sochor et al., 2012). It is believed that Se supplementation may help to improve semen quality and fertility of aged broiler breeder roosters. In the present study, the effect of different levels SeY on semen quality and reproductive performance of aged

broiler breeder roosters was compared with the other organic and inorganic Se sources.

Our results showed that Se supplementation has considerable positive effect on semen quality attributes; however, dietary supplementation of 0.45 mg SeY/kg diet was associated with the highest improvement in ejaculation volume, sperm plasma membrane integrity, motility, plasma membrane functionality, and abnormality as compared to inorganic form of Se. These findings were in agreement with many studies proving that organic Se exerts its effect more effectively than the inorganic form (Edens, 2002; Surai, 2002; Lukaszewicz et al., 2008; Ebeid, 2009). Ebeid (2009) showed that organic selenium supplementation increased semen quality parameters including sperm concentration, motility, plasma membrane integrity, and antioxidant status of rooster semen. Generally, the better effect of organic Se than the inorganic form is thought to be associated with more absorption and tissue retention in pig and broiler breeder hen (Mahan and Parrett, 1996; Urso et al., 2015). For instance, following dietary supplementation of different Se sources, Surai (2000) showed that yolk and albumen Se concentrations were higher in eggs from hens fed with SeY than SS. In addition, the present results were supported with several previous findings showing 0.45 of organic Se supplementation improved reproduction performance of both hen and roosters (Emamverdi et al., 2019; Khalil-Khalili et al., 2021).

Table 3. The effects of different sources and levels of Se on sperm penetration, fertility, hatchability, embryonic mortality, and hatchling quality of aged broiler breeder roosters.

Experimental group	Fertility %	Hatchability %	Embryonic mortality %	Hatchling quality %
CG	54.44 (49/90) ^d	45.55 (41/90) ^{bc}	12.24 (6/49)	65.75 (27/41) ^d
SeY0.15	58.88 (53/90) ^{bc}	42.22 (38/90) ^c	7.54 (4/53)	89.41 (34/38) ^{abc}
SeY0.30	74.44 (67/90) ^{ab}	53.33 (48/90) ^{abc}	4.47 (3/67)	93.75 (45/48) ^{ab}
SeY0.45	77.77 (70/90) ^a	60.00 (54/90) ^a	5.71 (4/70)	94.44 (51/54) ^a
Selemax	70.00 (63/90) ^{abc}	58.88 (53/90) ^{ab}	6.34 (4/63)	81.13 (43/53) ^{bc}
SS	61.11 (55/90) ^{bcd}	44.44 (40/90) ^{bc}	9.09 (5/55)	77.50 (31/40) ^{cd}

Abbreviations: CG, basal diet used as control; SeY0.15, SeY0.30, SeY0.45, basal diet supplemented with 0.15, 0.30, and 0.45 mg/kg, respectively, of diet selenium-enriched yeast, supplementation of 0.30 mg/kg diet commercial organic selenium (Selemax), and supplementation of 0.30 mg/kg diet sodium selenite as an inorganic source (SS) for 12 wk.

^{a-d}Values within a column with different superscripts differ at $P < 0.05$.

Table 4. The effects of dietary supplementation with sodium selenite (SS), Selemax, and different levels of produced selenium-enriched yeast (SeY) on seminiferous tube diameter, seminiferous epithelium thickness, and sperm penetration rate of broiler breeder roosters.

Experimental group	Seminiferous tube diameter (μm)	Seminiferous epithelium thickness (μm)
CG	289 ^b	74.17 ^b
SeY0.15	296 ^b	76.50 ^b
SeY0.30	381 ^a	98.10 ^a
SeY0.45	387 ^a	100.18 ^a
Selemax	371 ^a	99.60 ^a
SS	300 ^b	80.40 ^b
SEM	6.41	8.13

Abbreviations: CG, basal diet used as control; SeY0.15, SeY0.30, SeY0.45, basal diet supplemented with 0.15, 0.30, and 0.45 mg/kg, respectively, of diet selenium-enriched yeast, supplementation of 0.30 mg/kg diet commercial organic selenium (Selemax), and supplementation of 0.30 mg/kg diet sodium selenite as an inorganic source (SS) for 12 wk.

^{a-d}Values within a column with different superscripts differ at $P < 0.05$.

Avian sperm plasma membrane contains large amounts of PUFAs that make it susceptible to peroxidative damages, and thereby, decreases their sperm quality and fertility. In addition, the source of antioxidant, avian spermatozoa have lower cytoplasm concentration than mammalian sperms. These evidences indicate the necessity of antioxidant supplementation, especially when the avian species are on stressful conditions. Glutathione peroxidase (**GSH-Px**) is an important selenoprotein that is expressed in seminal plasma and spermatozoa (Surai et al., 1998b; Surai and Fisinin, 2014). It has been reported that the addition of Se, especially organic form to the rooster's diet significantly increases the activity of GSH-Px in the testes, seminal plasma and spermatozoa, which in turn, could improve roosters sperm quality (Ebeid, 2009).

The GSH-Px is involved in sperm maturation and has a very high activity in the sperm tail structure, which confirms a beneficial role for Se in male fertility (Khalid et al., 2016). These mechanisms may explain the better semen quality in Se-supplemented roosters.

Selenium in the form of regenerated glutathione peroxidase (**Se-GSH-Px**) and thyridoxine converts lipid hydroperoxide (**LOOH**) produced by the reaction of vitamin E with released peroxy radicals released in the lipid detoxification process to non-toxic compounds. In fact LOOH are not stable and in the presence of transitional metal ions, they can produce new free radicals. Therefore, LOOH can be removed from the cell with a similar method such as hydrogen peroxide (H_2O_2), which cannot be catalase, and the only Se can react with these compounds and convert LOOH into low-risk compounds (Papazyan et al., 2006).

By improving antioxidative capacity, probably Se decreased oxidative reaction that usually occurs by free oxidative radicals (Khalil-Khalili et al., 2021). Neutralizing the free oxidative radicals and scavenging them are 2 of main mechanisms that are thought to be employed by the body antioxidative system against oxidative attack to plasma membrane and lipid peroxidation

(Ozougwu, 2016). In the present study, the higher plasma membrane functionality and integrity also indicate Se had influential impact on plasma membrane protection, specifically when SeY is supplemented. On the other hand, the higher total and progressive motility observed in this study may be related to role of Se in sperm structure and maturation and also to their protected plasma membrane. The higher plasma membrane functionality observed in SeY-fed roosters suggests a more intact middle piece and flagellum portion in this groups that are directly involved in spermatozoa motility. In addition, the motility of spermatozoa is directly correlated with the percentage of viable sperms that was higher in SeY0.45 groups. Given the role of Se and GSH-Px on spermatogenesis and developmental competence of rat sperm, the lower percentage of abnormal sperms in SeY0.45 is reasonable (Rezvanfar et al., 2013). The presence glutamic-oxaloacetic transaminase (**GOT**) enzyme system in bovine seminal plasma and in ejaculated sperm may provide further useful information on the question of how Se may affect sperm viability (Flipse, 1960). Łanocha-Arendarczyk et al. (2018) indicated that elevated concentrations of Se in mouse model increased plasma GOT levels. Glutamic-oxaloacetic transaminase and glutamic-pyruvate (**GPT**) are intracellular enzymes that in semen were associated with a higher bull sperm concentration at pre- and post-thaw stage (Chaudhary et al., 2017). As the plasma membrane of spermatozoa is permeable to GOT (Flipse, 1960) supplementing Se may increase GOT in seminal plasma and improve the sperm concentration due to the role of GOT in amino acid metabolism.

In the aged roosters, testis undergo several changes including decrease in the thickness of germinal epithelium, decrease in the diameter of the seminiferous tubules and thickening of the interstitial space due to the influx of collagen fibers and fibroblasts (Sarabia Fragoso et al., 2013). It is known that dietary supplementation of Se, especially organic form, had a beneficial effect on seminiferous tubule development and recovery of seminiferous tubules injury in male mice (Kushki et al., 2015). In this line, results of this study showed that histological parameters of testis including seminiferous tubule diameter and seminiferous epithelium thickness were improved in SeY0.45 group and other organic Se sources. There is a relationship between increased dietary Se supplementation and enhanced seminiferous tubule diameter and seminiferous epithelium thickness in chicken (Khalid et al., 2016). Supplementation of vitamin E and Se has shown to increase the diameter, thickness, and height of the germinal epithelium of the seminiferous tubules in rat and chicken (Mehranjani et al., 2009; Khalid et al., 2016).

It is reported that the reversal methionine oxidation in proteins can change their structure and functions. In order to adjust the redox signaling, Se assimilation and metabolism consists of the alternation of all dominant types of Se to H_2Se (Kaya et al., 2015). Organic selenium replaces sulfur in the amino acid structure of the phosphorus group. H_2Se synthesizes SeCys and

incorporates it in 26 synthesized selenoproteins which are integral parts of the antioxidant system of the body (Papazyan et al., 2006; Surai et al., 2017).

As the increase of seminiferous tubes is a simple way to monitor changes in human spermatogenesis (Xu et al., 2013), the higher ejaculate volume observed in a study on goat is probably related to higher spermatogenesis as result of increase in Sertoli and Leydig cells (Leal et al., 2018) which in turn, enhances diameter of seminiferous tubule and their epithelium thickness.

The higher fertility and hatchability rate observed in this study is explained by the improved sperm quality attributes in Se-supplemented quails. Dietary implementation of Se not only was associated with higher sperm quality, but also improved their SP potential. Fertility could be expected as the potential function of sperm to penetrate perivitelline membrane in bull sperm (Brahmkshtri et al., 1999). In this line, Surai and Taylor-Pickard (2008) showed that organic supplements of selenium (Sel-Plex) in Hubbard Ultra-Yield Layers diet improved fertility and hatchability. However, dietary supplementing 0.4 mg/kg of mineral selenium or zinc-selenomethionine to Cobb broiler breeder hen and rooster from 22 to 53 wk of age did not improved fertility rate (Urso et al., 2015). Genetic, dosage and type of the supplemented Se may affect fertility outcome in poultry (Khan et al., 2017).

The lipid portion of poultry embryonic tissues contains a high percentage of unsaturated fatty acids (Speake et al., 1998) and antioxidants components may have a beneficial role in decreasing embryonic death and increasing hatchelling quality (Surai, 2002). For instance canthaxanthin (CX) could elevate hatchelling quality and reduced embryonic death in chicks (Surai, 2012). The better hatchelling quality observed in SeY0.45 is probably related the higher embryonic competence donated from sperm of SeY.

However, our results showed that the feeding different Se sources were not associated with a lower of embryonic death. Probably, the quality of the fertilizing eggs has more impact on lowering mortality rate. In this regard, there are evidences showing that transferring some antioxidant such as canthaxanthin and Coenzyme Q10 from avian diet improved egg and hatchelling quality (Zhang et al., 2011; Rafeian-Naeini et al., 2021). It is worth noting that the mortality rate recorded in this study was in normal to low range, and the 3 to 6 records for mortal embryos is not adequate frequency to find significant difference in binary distributed data.

CONCLUSIONS

In this study the potential of dietary SeY supplementation, as an alternative Se source with high bioavailability potential, on testicular histology, semen quality and reproductive performance in aged broiler breeder roosters were investigated. Results showed that organic Se sources, especially SeY, enhanced semen attributes, increase diameter of the seminiferous tube and the

thickness of the seminiferous epithelium. These improvements were associated with a higher fertility and hatchability rates and better hatchling quality in the birds fed by 0.45 mg SeY /kg diet.

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DISCLOSURES

The authors declare no conflict of interest.

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