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## Original Article

# The effect of supplementing rosemary essential oil and selenium yeast in the diet on sperm parameters and antioxidant activity of semen in older broiler breeder roosters

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## Abstract

**Background:** Aging is associated with various physiological changes that lead to oxidative stress and the production of reactive oxygen species (ROS), negatively affecting sperm structure and function. **Aims:** This study aimed to investigate the impact of incorporating rosemary essential oil and selenium yeast into the diet on sperm quality parameters, antioxidant enzyme activity, and lipid peroxidation rates of semen in aged broiler breeder roosters. **Methods:** Thirty-six 57-week-old Ross 308 roosters were randomly assigned in a 3×2 factorial arrangement, in six treatments with six replications. The treatments included three levels of rosemary essential oil (0, 100, and 200 mg/kg) and two levels of selenium yeast (0 and 0.3 mg/kg) for 7 weeks. Sperm parameters, antioxidant enzyme activities, and lipid peroxidation levels in seminal plasma were evaluated. **Results:** The results showed significant effects of rosemary essential oil and selenium yeast on sperm parameters. 100 mg rosemary essential oil plus 0.3 mg selenium yeast/kg of diet showed the most significant improvement in sperm quality ( $P<0.05$ ). However, there was no significant difference in sperm morphological abnormalities among the treatments. Additionally, seminal plasma antioxidant enzyme activities, including glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) significantly increased and malondialdehyde (MDA) decreased, particularly in the treatment containing 100 mg rosemary essential oil plus 0.3 mg selenium yeast/kg diet ( $P<0.05$ ). **Conclusion:** Overall, 100 mg/kg rosemary essential oil combined with 0.3 mg/kg selenium yeast in the diet is recommended for aged broiler breeder roosters to enhance their reproductive function.

**Key words:** Antioxidants, Rooster, Rosemary, Selenium yeast, Sperm

## Introduction

The aging process in broiler breeder roosters presents a significant economic challenge in poultry production. The physiological changes associated with aging directly affect reproductive performance, drawing considerable attention from researchers (Rengaraj and Hong, 2015; Khang *et al.*, 2022). Age-related changes are linked to oxidative stress and the production of reactive oxygen species (ROS), which adversely affect sperm structure and function (Raei *et al.*, 2021), as well as reducing egg hatchability, leading to financial losses in the poultry industry (Behnamifar *et al.*, 2021). Several key reproductive traits in older roosters, including semen volume, sperm concentration and motility, testosterone levels, and overall antioxidant capacity, decline with age,

while lipid peroxidation of semen increases (Min *et al.*, 2018; Barbarestani *et al.*, 2024).

While ROS production plays a role in various sperm biological functions, excessive ROS can lead to negative effects, including reduced sperm motility, membrane damage, and acrosomal injury. Compounds with antioxidant activity are commonly used to mitigate the impact of oxidative stress (Dutta *et al.*, 2019).

*Rosmarinus officinalis* a perennial plant from the Lamiaceae family, contains bioactive compounds, such as flavonoids (including genkwanin, cirsimaritin, and homoplantagin), phenolic diterpenes (including carnosic acid, carnosol, and rosmarinic acid), and triterpenes including ursolic acid (Borrás-Linares *et al.*, 2014). The antioxidant potential of rosemary extract and oil is often attributed to phenolic diterpenes that

neutralize hydroxyl radicals, lipid peroxy radicals, and singlet oxygen (Gallego *et al.*, 2013). Additionally, the main bioactive compounds in rosemary essential oil, such as 1, 8-cineole, camphor, rosmarinic acid, and carnosic acid, reduce oxidative stress and enhance antioxidant status (Gülçin *et al.*, 2016). In one study, adding rosemary essential oil to rooster semen extenders increased sperm motility (Touazi *et al.*, 2018). Furthermore, including camphor, one of rosemary's essential oil compounds, in the diet of aging broilers improved semen characteristics and antioxidant enzyme activity (Raei *et al.*, 2021). The addition of rosemary essential oil to the diet of mice (El-Demerdash *et al.*, 2021) and rabbits (El-Gogary *et al.*, 2018) has also been shown to reduce blood lipid peroxidation and enhance antioxidant status.

Selenium, an essential trace element, plays a vital role in various biological functions, including growth, immunity, antioxidant status, and reproduction, and is a key component of selenoproteins (Ozturk Kurt and Ozdemir, 2022; Wróblewski *et al.*, 2024). It is a crucial component of several antioxidant enzymes, including glutathione peroxidase, thioredoxin reductase, and iodothyronine deiodinase. As part of these enzymes, selenium protects cells from the harmful effects of free radicals (Li *et al.*, 2023). Selenium plays a key role in spermatogenesis and sperm plasma membrane integrity by directly enhancing antioxidant status (Wang *et al.*, 2021a). Incorporating organic selenium into the diet has been shown to improve reproductive performance and antioxidant capacity in poultry (Wang *et al.*, 2021b). One organic form of selenium, selenium yeast, is less toxic and has superior digestibility and bioavailability as compared with sodium selenite (Arnaut *et al.*, 2021). Studies have demonstrated that selenium yeast supplementation reduces ROS production and, consequently, oxidative cellular damage in poultry. In previous studies, dietary organic selenium improved semen quality, and fertility, and reduced lipid peroxidation in roosters (Chauchu-Noo *et al.*, 2021; Sabzian-Melei *et al.*, 2022). Additionally, supplementation of organic selenium in the diet of Thai native roosters decreased malondialdehyde (MDA) concentration and increased sperm concentration (Authaida *et al.*, 2023). In a study by Kamrani *et al.* (2021) selenium supplementation improved fresh and frozen-thawed sperm parameters in roosters treated with dexamethasone. Moreover, selenium-enriched probiotics have been shown to increase testosterone levels and reduce oxidative stress in male rats with fertility problems (Khattab *et al.*, 2023).

While numerous studies have investigated the impact of antioxidants on sperm quality and fertility in broiler breeder flocks, no research has yet combined rosemary essential oil and selenium yeast in the diet of broiler breeder roosters. Given the positive effects of antioxidants on poultry fertility, this study was designed to investigate the impact of different levels of rosemary essential oil and selenium yeast in a factorial arrangement on sperm parameters, antioxidant enzyme

activities, and lipid peroxidation rates in aged broiler breeder roosters.

## Materials and Methods

### Location and housing of the roosters

This study was conducted at the Animal Husbandry Station of the University of Agricultural Sciences and Natural Resources of Khuzestan. The Ross 308 roosters (57-week-old) with a body weight of  $5100 \pm 150$  g were selected and housed in pens with bedding covered with straw. The roosters were kept under controlled environmental conditions with a standard temperature of 21–23°C and a lighting program consists 14 h of light and 10 h of darkness. They were fed a diet formulated according to the guidelines for the Ross 308 breed (Table 1).

**Table 1:** Ingredients and the chemical composition of basal diet

Ingredient	%
Corn	65.43
Soybean meal	8
Wheat bran	22.88
Soybean oil	0.5
Methionine	0.09
Lysine	0.03
Dicalcium phosphate	1.25
Calcium carbonate	0.95
Common salt	0.37
Vitamin premix <sup>a</sup>	0.25
Trace mineral premix <sup>b</sup>	0.25
<b>Chemical composition (%)</b>	
Metabolism energy (kcal/kg)	2850
Crude protein	12.2
Lysine	0.44
Methionine + cysteine	0.47
Threonine	0.36
Arginine	0.65
Calcium	0.76
Available phosphorus	0.33
Sodium	0.15

<sup>a</sup>Supplied per kilogram of diet: vitamin A: 15000 IU, vitamin E: 100 IU, vitamin K3: 4 mg, vitamin B12: 25 mg, vitamin D: 3000 IU, riboflavin: 7.5 mg, niacin: 50 mg, pantothenic acid: 18 mg, pyridoxine: 5.5 mg, biotin: 50 mg, and folic acid, 1.5 mg, and <sup>b</sup> Supplied per kilogram of diet: Fe: 90 mg, Mn: 120 mg, Zn: 110 mg, I: 2 mg, and Se: 0.3 mg

### Preparation of materials

Rosemary essential oil was purchased from Barij Essences Company in Kashan, and selenium yeast was obtained from Kavoshgar Sepehr Javan Company in Dezful, Iran. The composition of the rosemary essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS), revealing the components as shown in Table 2.

### Experimental design

Thirty-six Ross 308 roosters were assigned within a completely randomized design with a 3×2 factorial arrangement, comprising three levels of rosemary

essential oil (0, 100, 200 mg/kg) and two levels of selenium yeast (0, 0.3 mg/kg) in the diet for 7 weeks. Three replicates were considered for the study.

**Table 2:** Chemical composition of rosemary essential oil identified by GC-MS

Compound name*	Relative percentage
1, 8-cineole	34.78
Camphor	18.86
$\alpha$ -pinene	13.16
$\beta$ -pinene	12.66
Camphene	11.61
Limonene	3.17
p-cymene	8.58
Borneol	19.36
$\alpha$ -terpineol	20.04
$\beta$ -myrcene	13.33
Bornyl acetate	1.46
$\alpha$ -campholenic	17.46

\*The major constituents of rosemary essential oil are primarily monoterpenes and oxygenated monoterpenes, which contribute to its characteristic aroma

### Semen collection and evaluation of sperm parameters

Semen was collected from roosters by abdominal massage over a seven-week period, starting after two weeks of dietary treatment, and the quantitative and qualitative parameters of semen were evaluated. Semen volume was measured by aspirating the ejaculate into a needleless insulin syringe. For sperm concentration, a semen sample was diluted with distilled water at a 1:200 ratio and counted using a hemocytometer under a light microscope at  $\times 400$  magnification (Shahverdi *et al.*, 2015).

To assess total and progressive motility, the semen samples were diluted with a 0.9% sodium chloride solution at a 1:20 ratio. A drop of the diluted sample was placed on a microscope slide, covered with a coverslip, and observed at  $\times 400$  magnification in at least 10 microscopic fields to determine spermatozoa motility parameters (Akhlaghi *et al.*, 2014).

The integrity of the sperm plasma membrane was evaluated using the hypo-osmotic swelling test (HOST). To do so, 10  $\mu$ L of semen was incubated with 100  $\mu$ L of a hypo-osmotic solution (57.6 mM fructose and 19.2 mM sodium citrate) at 38°C for 20 min. The final osmolarity of the hypo-osmotic solution used in the HOST was approximately 150 mOsm/L. Then, one drop of sample was placed on a slide and smeared. After drying, at least 200 cells were examined using a phase-contrast microscope at  $\times 400$  magnification. The sperms with swollen and coiled tails were considered intact plasma membranes (Mehdipour *et al.*, 2020).

### Evaluation of antioxidant enzymes and lipid peroxidation

An aliquot of ejaculate was centrifuged at 800  $\times$ g for 15 min, and seminal plasma was separated and stored at -20°C until analysis. Total antioxidant capacity (TAC) was measured using the Randox kit (Crumlin, UK). In

this assay, 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) was incubated with hydrogen peroxide and peroxidase, producing the green-blue ABTS+ radical cation, which was measured at 600 nm (Miller *et al.*, 1993).

MDA concentration in semen was determined using the TBA (thiobarbituric acid) method, a commonly used assay for lipid peroxidation (Salih *et al.*, 2021). Briefly, 1 ml of semen was mixed with 1 ml of 20% trichloroacetic acid (TCA) and centrifuged at 800  $\times$ g for 10 min to precipitate proteins. The supernatant was heated with 1 ml of TBA (0.67% w/v) at 100°C for 10 min. After cooling, the MDA concentration was measured using a UV-visible spectrophotometer at 532 nm and reported as nMol/ml.

The activity of superoxide dismutase (SOD) was determined using the method of Sun *et al.*, (1988), which involves the inhibition of nitroblue tetrazolium (NBT) reduction by xanthine-xanthine oxidase, which serves as a superoxide generator. The SOD activity, reported as mg/protein, was expressed as the enzyme concentration that caused a 50% inhibition of the NBT reduction rate. (Paoletti *et al.*, 1986).

Glutathione peroxidase (GPx) activity was measured using the Randox kit (Crumlin, UK), as described by Paglia and Valentine (1967). The sample was mixed with t-butyl hydroperoxide in a 4:1 ratio and a solution containing water, buffer (0.025 M Na<sub>2</sub>EDTA and 0.25 M KH<sub>2</sub>PO<sub>4</sub>), 10 mg glutathione (GSH), NADPH, GSH reductase, and K-buffer. The GPx activity was measured by recording the rate of absorbance change over 60 s at 10 s intervals as described by the manufacturer.

Catalase (CAT) activity was measured using the Kiazist kit (Crumlin, UK). In this assay, catalase exhibits peroxidase activity in the presence of methanol, and the produced formaldehyde reacts with Purpald to form a purple color. The absorbance of the purple color was measured at 540 nm.

### Statistical analysis

The data were analyzed using the SAS software (version 9.4). The normality of the data was tested using the Shapiro-Wilk method. The data were analyzed in a 3 $\times$ 2 factorial arrangement for the dietary treatments using PROC GLM model. Duncan's multiple range test was used to compare mean values at a significance level of (P<0.05).

### Results

The effect of rosemary essential oil and selenium yeast on the quality parameters of sperm in aged broiler breeder roosters is presented in Table 3. The main effects of rosemary essential oil, selenium yeast, and the interaction effects between rosemary essential oil and selenium yeast on the quantitative and qualitative sperm parameters were significant (P<0.05). All experimental treatments with rosemary essential oil and selenium yeast resulted in increased semen volume, and sperm concentration, as well as improved total and progressive

**Table 3:** The dietary effects of rosemary essential oil and selenium yeast on sperm quality parameters in aged broiler breeder roosters

Treatments	Sperm parameters						
	Volume (ml)	Concentration ( $\times 10^9$ )	Total motility (%)	Progressive motility (%)	PMI <sup>1</sup> (%)	Abnormal (%)	
<b>Main effects</b>							
<b>Rosemary (mg/kg)</b>							
0	0.42 <sup>b</sup>	3.61 <sup>b</sup>	77.51 <sup>b</sup>	42.23 <sup>b</sup>	75.32 <sup>b</sup>	12.94	
100	0.47 <sup>a</sup>	3.72 <sup>a</sup>	82.85 <sup>a</sup>	45.64 <sup>a</sup>	79.83 <sup>a</sup>	12.85	
200	0.46 <sup>a</sup>	3.69 <sup>ab</sup>	80.32 <sup>a</sup>	44.73 <sup>a</sup>	78.92 <sup>a</sup>	12.86	
SEM	0.01	0.03	0.95	0.67	0.90	0.07	
<b>Selenium yeast</b>							
0	0.43 <sup>b</sup>	3.61 <sup>b</sup>	77.96 <sup>b</sup>	42.82 <sup>b</sup>	73.52 <sup>b</sup>	12.94	
0.3	0.47 <sup>a</sup>	3.73 <sup>a</sup>	82.50 <sup>a</sup>	45.58 <sup>a</sup>	77.63 <sup>a</sup>	12.83	
SEM	0.008	0.02	0.77	0.54	0.73	0.05	
<b>Rosemary</b>	<b>Selenium</b>						
0	0	0.37 <sup>c</sup>	3.45 <sup>b</sup>	71.39 <sup>d</sup>	38.08 <sup>c</sup>	69.92 <sup>d</sup>	13.14
0	0.3	0.48 <sup>ab</sup>	3.77 <sup>a</sup>	83.64 <sup>ab</sup>	46.39 <sup>a</sup>	80.71 <sup>ab</sup>	12.75
100	0	0.43 <sup>b</sup>	3.68 <sup>a</sup>	80.46 <sup>bc</sup>	33.32 <sup>ab</sup>	77.67 <sup>bc</sup>	12.87
100	0.3	0.50 <sup>a</sup>	3.76 <sup>a</sup>	85.25 <sup>a</sup>	46.96 <sup>a</sup>	82.00 <sup>a</sup>	12.84
200	0	0.48 <sup>ab</sup>	3.71 <sup>a</sup>	82.03 <sup>ab</sup>	46.07 <sup>ab</sup>	81.03 <sup>ab</sup>	12.82
200	0.3	0.45 <sup>b</sup>	3.67 <sup>a</sup>	78.60 <sup>c</sup>	43.39 <sup>b</sup>	76.82 <sup>c</sup>	12.91
SEM		0.014	0.04	1.34	0.94	1.28	0.09
<b>P-values</b>							
Rosemary		0.009	0.04	0.001	<0.002	0.002	0.62
Selenium		0.0003	0.002	0.0002	0.001	0.001	0.17
Selenium $\times$ Rosemary		0.0003	0.0008	<.0001	<.0001	<.0001	0.06

<sup>1</sup>Plasma membrane integrity, <sup>a, b, c</sup> Within each column, values with different superscript(s) differ significantly ( $P < 0.05$ ), and SEM: Standard error of the means

**Table 4:** The dietary effects of rosemary essential oil and selenium yeast on seminal plasma antioxidant enzyme activity and lipid peroxidation in aged broiler breeder roosters

Treatments	Items*					
	GPx (U/mg)	SOD (U/mg)	CAT (U/mg)	TAC (mmol/mg)	MDA (nmol/ml)	
<b>Main effects</b>						
<b>Rosemary (mg/kg)</b>						
0	11.11 <sup>b</sup>	15.28 <sup>ab</sup>	13.55 <sup>b</sup>	2.40 <sup>b</sup>	2.63 <sup>a</sup>	
100	11.89 <sup>a</sup>	16.47 <sup>a</sup>	14.30 <sup>a</sup>	2.93 <sup>a</sup>	2.19 <sup>b</sup>	
200	11.21 <sup>ab</sup>	16.14 <sup>ab</sup>	13.82 <sup>ab</sup>	2.67 <sup>ab</sup>	2.26 <sup>b</sup>	
SEM	0.18	0.30	0.16	0.10	0.07	
<b>Selenium yeast</b>						
0	10.95 <sup>b</sup>	14.94 <sup>b</sup>	13.55 <sup>b</sup>	2.39 <sup>b</sup>	2.57 <sup>a</sup>	
0.3	11.86 <sup>a</sup>	16.99 <sup>a</sup>	14.23 <sup>a</sup>	2.95 <sup>a</sup>	2.15 <sup>b</sup>	
SEM	0.15	0.25	0.13	0.08	0.05	
<b>Rosemary</b>	<b>Selenium</b>					
0	0	10.12 <sup>d</sup>	13.59 <sup>d</sup>	12.77 <sup>c</sup>	1.81 <sup>c</sup>	3.22 <sup>a</sup>
0	0.3	12.11 <sup>ab</sup>	16.98 <sup>ab</sup>	14.33 <sup>ab</sup>	3.00 <sup>ab</sup>	3.03 <sup>c</sup>
100	0	11.36 <sup>bc</sup>	14.93 <sup>cd</sup>	13.79 <sup>b</sup>	2.69 <sup>b</sup>	2.34 <sup>bc</sup>
100	0.3	12.41 <sup>a</sup>	18.02 <sup>a</sup>	14.82 <sup>a</sup>	3.18 <sup>a</sup>	2.04 <sup>bc</sup>
200	0	11.37 <sup>bc</sup>	16.31 <sup>bc</sup>	14.10 <sup>ab</sup>	2.68 <sup>b</sup>	2.14 <sup>bc</sup>
200	0.3	11.06 <sup>c</sup>	15.97 <sup>bc</sup>	13.53 <sup>b</sup>	2.67 <sup>b</sup>	2.38 <sup>b</sup>
SEM		0.27	0.43	0.23	0.14	0.1
<b>P-values</b>						
Rosemary		0.03	0.04	0.02	0.01	0.002
Selenium		0.001	<.0001	0.005	0.0007	0.0004
Selenium $\times$ Rosemary		0.004	0.001	0.002	0.006	<.0001

<sup>a, b, c, d</sup> Within each column, values with different superscript(s) differ significantly ( $P < 0.05$ ). SEM: Standard error of the means. \* GPx: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase, TAC: Total antioxidant capacity, and MDA: Malondialdehyde

motility, and plasma membrane integrity of spermatozoa compared with the control group. 100 mg rosemary essential oil plus 0.3 mg selenium yeast/kg diet had the

highest impact on improving these parameters. However, sperm morphological abnormalities were not affected by the experimental groups. The findings on the impact of

rosemary essential oil and selenium yeast on antioxidant enzyme activities and lipid peroxidation levels in the plasma of semen from aged broiler breeder roosters are shown in Table 4. The main and interaction effects of rosemary essential oil and selenium yeast on GPx, SOD, and CAT enzyme activities, as well as TAC and MDA concentration in semen plasma, indicated a significant increase in antioxidant parameters and a decrease in lipid peroxidation ( $P < 0.05$ ). 100 mg rosemary essential oil plus 0.3 mg selenium yeast per kg exhibited the highest antioxidant activity ( $P < 0.05$ ).

## Discussion

Oxidative stress caused by ROS accumulates with age and leads to an increase in sperm morphological abnormalities and a decrease in its motility, membrane integrity, and function (Bonanno *et al.*, 2016). According to the present findings, antioxidants initially restore oxidative balance by reducing oxidative damage in semen, subsequently preserving sperm membrane integrity and reversing the semen volume decline associated with aging. Therefore, the improvement in sperm quality in roosters under experimental treatments could be related to the antioxidant effects of selenium yeast and rosemary essential oil. In line with our results, adding rosemary essential oil to the diet of laying hens improved semen quality and reproductive efficiency and positively affected the antioxidant status under summer conditions (Habib *et al.*, 2017). In the study by Khnissi *et al.* (2023), adding rosemary to the diet of rams increased sperm concentration and motility. Rosemary essential oil is a rich source of natural antioxidants, such as phenolic compounds, which can neutralize the free radicals produced (Kaurinovic and Vastag, 2019). The rosmarinic acid and carnosic acid compounds in this plant contain two aromatic rings with two alcohol groups, capable of donating hydrogen ions to oxidizing agents and reducing lipid peroxidation caused by reactive oxygen species (Brewer, 2011). In the study by Touazi *et al.* (2018), rosemary extract under *in vitro* conditions improved sperm motility parameters of roosters at low doses but had an adverse effect at higher doses. In mice, rosemary extract preserved the sperm membrane health and increased its motility. Antioxidant compounds seem to increase sperm motility by reducing the free radicals, preventing lipid peroxidation of the membrane lipids, and maintaining the integrity of the sperm plasma membrane (Tousson *et al.*, 2018). In Barki rams, dietary supplementation with rosemary essential oil improved testicular parameters, accessory reproductive glands, and semen quality (Ali *et al.*, 2024).

Rosemary oil contains compounds that neutralize free radicals and can enhance intracellular antioxidant systems, including catalase, glutathione, and glutathione peroxidase (Christopoulou *et al.*, 2021). In agreement with the present study, Wang *et al.* (2017a) reported that rosemary extract increases the activities of SOD and CAT and reduces MDA levels. Rosemary protects the

liver from oxidative stress by preventing lipid peroxidation, activating antioxidant status, and stabilizing cellular membrane permeability. Our findings align with those of Bakirel *et al.* (2008) who observed that 200 mg/kg rosemary extract significantly reduced serum MDA levels in rabbits. Essential oils can influence lipid metabolism in animal tissues by increasing the activity of antioxidant enzymes, preventing the production of reactive oxygen species, and reducing the peroxidation of polyunsaturated fatty acids (Nehme *et al.*, 2021). In another study, adding rosemary essential oil at a dose of 0.5 g/kg to the diet of rabbits increased the total antioxidant capacity and reduced MDA levels in blood plasma (El-Gogary *et al.*, 2018). Lower MDA levels indicate delayed or inhibited lipid oxidation, suggesting improved oxidative status in birds treated with rosemary. The polyphenolic compounds in rosemary can enhance antioxidant enzymes and reduce free radicals in the body (Alavi *et al.*, 2021).

Adding rosemary essential oil to the Japanese quail diet showed an increase in spermatogenesis, and a reduction in lipid peroxidation in testicular tissue. Several active molecules, such as 1, 8-cineole, camphor, beta-thujone, chrysanthenone, beta-cubinen, and camphene, known for their antioxidant activity, have been identified in the phytochemical analysis of rosemary essential oil (Türk *et al.*, 2016). In a study by Deniz *et al.* (2022), the effects of 200 and 400 mg/kg of rosemary essential oil in laying quail diet on the oxidative stability of egg yolk were examined. They found that rosemary supplementation at both levels significantly reduced MDA levels in the egg yolk. The significant reduction in MDA value under the influence of rosemary can be explained by the fact that the phenolic hydroxyl groups in the plant delay the formation of hydroxyl peroxides by donating hydrogen to peroxide radicals at the initial stage of lipid oxidation (Barakat and Ghazal, 2016).

In the present study, the addition of selenium yeast to the diet of aging broiler roosters improved both quantitative and qualitative sperm parameters as well as its antioxidant activity. Selenium is an essential trace element that regulates an important component of the antioxidant defense mechanism by controlling the body's glutathione pool and the selenium-containing antioxidant enzyme. It is widely accepted that selenium is a powerful antioxidant, protecting cells and tissues from oxidative stress by preventing the formation of excess ROS (Huang *et al.*, 2022).

Selenium is an essential trace element that regulates an important component of the antioxidant defense mechanism by controlling the body's glutathione pool and the selenium-containing antioxidant enzyme (Huang *et al.*, 2022). It is widely accepted that selenium is a powerful antioxidant, protecting cells and tissues from oxidative stress by preventing the formation of excess ROS. Selenium yeast is an important mineral composed of various enzymes with mechanisms that defend against oxidative stress (Samo *et al.*, 2018). Selenium contains the enzyme GPx, a key factor in enhancing resistance to

lipid peroxidation and oxidative stress (Ingold and Conrad, 2018). Animals fed a selenium-deficient diet significantly reduced GPx activity and damaged testes germ cells (Kaur and Bansal, 2004). Some researchers have shown that selenium supplementation in animal diets increases SOD activity (Horky *et al.*, 2013). Similarly, selenium intake increases antioxidant capacity in roosters by elevating levels of SOD and GPx (Long *et al.*, 2022). Using selenium yeast at levels of 0.3 and 0.45 mg/kg diet, improved rooster semen quality traits, including sperm motility and membrane integrity, ejaculate volume, as well as increase seminiferous tubule diameter and the thickness of the seminiferous epithelium, which aligns with the results of our study (Sabzian-Melei *et al.*, 2022). Glutathione peroxidase plays a role in sperm maturation and has high activity in the sperm tail structure, confirming the beneficial role of selenium in male fertility (Zubair *et al.*, 2023). These mechanisms may explain the improved semen quality in roosters receiving selenium supplementation.

The increase in sperm motility and the percentage of live sperm in roosters fed with selenium is likely related to the increased activity of the GPx enzyme, which provides additional protection against the spontaneous lipid peroxidation of the sperm plasma membrane, thereby enhancing sperm motility and plasma membrane integrity (Carrillo-Nieto *et al.*, 2018). Darwish *et al.* (2024), demonstrated that using selenium-enriched probiotics increased sperm count, motility, and fertility gene expression in rats. In another study, 0.3 mg/kg of selenium yeast was added to the diet of goats, antioxidant status was improved with increased GPx and SOD activity in serum and testicular tissue (Bano *et al.*, 2019).

In the study by Hosny *et al.* (2020), adding 0.3 mg/kg selenium-enriched yeast to the diet of heat-stressed rabbits resulted in increased sperm concentration and plasma membrane integrity, as well as enhanced total antioxidant capacity of plasma and GPx activity. Additionally, the MDA level in plasma was significantly reduced, which is consistent with the findings of the present study in roosters. This effect can be attributed to the antioxidant activity of selenium, which mitigates the negative effects of free radicals on lipid peroxidation of the sperm plasma membrane (Wang *et al.*, 2017b). Sperm contains selenium, and its deficiency, depending on severity, leads to reduced motility, increased morphological abnormalities, and consequently infertility (Pieczyńska and Grajeta, 2015).

The enzyme GPx, which contains selenium in its chemical structure, prevents the formation of free radicals, thus preventing tissue damage from oxidation (Surai *et al.*, 2018). Selenium yeast may serve as a potential dietary supplement to reduce liver and kidney damage caused by copper in broiler chickens, possibly activating the antioxidant molecules and reducing inflammatory stress. Furthermore, selenium yeast increased cellular antioxidant biomarkers, including SOD, CAT, GPx, and GSH, while decreasing MDA content in liver and kidney tissues (Habotta *et al.*, 2022).

GPx can protect growing sperm from DNA damage caused by oxidative stress. It is also a structural component of the sperm mitochondrial sheath in the midpiece, making it essential for sperm stability and motility (Qazi *et al.*, 2019). Our study demonstrated this role of selenium, where roosters receiving selenium had higher GPx activity than those fed a control diet.

It has been reported that increased ROS and DNA damage due to oxidation are expected under conditions of low selenoprotein expression, leading to disturbances in cellular homeostasis and disruption of the cell cycle. Under selenium supplementation, the expression of specific selenoproteins is elevated, and these may play a crucial role in effectively combating harmful ROS levels in oxidative stress-related challenges (Touat-Hamici *et al.*, 2014). During the early stages of spermatogenesis, it is believed that GPx protects developing germ cells from DNA damage caused by oxidative stress. Later, by binding to proteins in the midpiece of sperm and becoming a structural component of the mitochondrial sheath, it provides integrity for the sperm piece, which is essential for sperm survival and motility (Safarinejad and Safarinejad, 2009; Wang *et al.*, 2021).

Given the antioxidant role of selenium yeast and rosemary essential oil, the increase in antioxidant enzymes and reduction of lipid peroxidation in this experiment shows a synergistic effect of these two substances. Due to antioxidant compounds and support of the intracellular antioxidant system, rosemary plays a significant role in preventing membrane lipid peroxidation and maintaining sperm quality. Rosemary essential oil traps cellular free radicals and prevents the accumulation of nitric oxide. Selenium-enriched yeast can serve as a precursor for synthesizing selenoproteins, antioxidant enzymes that prevent cellular damage from free radical activity. Selenium yeast increases antioxidant enzyme activity, thereby preventing the accumulation of free radicals. As a result, these reasons suggest that rosemary essential oil and selenium-enriched yeast improved the sperm quality of roosters in our study.

In conclusion, the present study revealed that feeding older broiler breeder roosters with rosemary essential oil at 100 and 200 mg/kg and selenium yeast at 0.3 mg/kg of diet, and interaction effect of 100 mg/kg rosemary essential oil plus 0.3 mg/kg selenium yeast had the most significant impact on sperm motility parameters, plasma membrane integrity, antioxidant enzyme activity, and lipid peroxidation. Therefore, adding 100 mg of rosemary essential oil in combination with 0.3 mg of selenium yeast per kg of diet is generally recommended to improve the quantitative and qualitative sperm parameters and the antioxidant activity of semen in older broiler breeder roosters to enhance their productivity.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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