Vol. 21, No. 3, Autumn, 2025, 42-53 *Original Article*
DOI: 10.22055/ivj.2025.535387.2818
Journal Homepage: http://www.ivj.ir/

Effect of Dietary Supplementation with Rosemary Essential Oil and Selenium on Fertility Gene Expression in Broiler Breeder Roosters

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Received: 18.07.2025 Accepted: 08.10.2025

Abstract

Advancing age in broiler roosters reduces reproductive potential. Natural antioxidants, such as rosemary oil and selenium, are expected to enhance reproductive function by reducing oxidative stress. This study aimed to investigate how these antioxidants affect the expression of fertility genes (StAR and PVRL3) in aging roosters. Forty-two Ross 308 broiler roosters, all over 50 weeks old, were used in a factorial experiment in a completely randomized design over 10 weeks. Treatments were: 1) Control diet, 2) Basic diet +100mg/kg rosemary essential oil, 3) +200mg/kg rosemary essential oil, 4) +0.3mg/kg selenium-enriched yeast, 5) +100mg/kg rosemary essential oil and 0.3mg/kg selenium-enriched yeast, and 6)+200mg/kg rosemary essential oil and 0.3mg/kg selenium-enriched yeast. At the end of the treatment, three samples of testicular tissue from each treatment were collected and stored at -80°C. The expression levels of the StAR and PVRL3 genes were measured using real-time quantitative PCR. The results indicated that rosemary oil did not significantly affect StAR gene expression. However, a dose of 200 mg/kg significantly reduced PVRL3 expression, whereas the 100 mg/kg dose did not show a significant effect. Selenium supplementation at a dosage of 0.3 mg/kg significantly increased the expression of the StAR and PVRL3 genes. Adding 100 mg/kg of rosemary along with 0.3 mg/kg of selenium significantly increased the expression of the PVRL-3 gene. However, when 200 mg of rosemary was added in the presence of selenium, a decrease in PVRL-3 gene expression was observed, and selenium did not prevent this decline. This suggests that using 200 mg of rosemary essential oil may be undesirable. Based on these results, adding 100 mg/kg of rosemary along with 0.3 mg of selenium-enriched yeast to the diet of older broiler breeder roosters is recommended.

Key words: Aged rooster, PVRL3, Rosemary, Selenium

Introduction

In broiler chickens, a rooster can successfully inseminate scores of hens; therefore, the reproductive performance of the roosters is one of the most significant

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production characteristics (Sun et al, 2019). Various factors such as body weight loss and oxidative stress, decrease in circulating testosterone, decrease in reproductive activity, low sperm rate, and impaired sperm quality are the causes of reproductive disorders in old flocks. Antioxidant function of rooster sperm reduces with age, and oxidative stress becomes evident when oxidant impact surpasses antioxidant defenses (Bansal and Bilaspuri, 2011). Oxidative stress intensifies the production of reactive oxygen species (ROS), causing lipid peroxidation (LPO), apoptosis, and DNA damage (Budai et al, 2014). ROS are highly reactive oxidants with free electrons and may participate in radical substitution reactions that form more radicals. Potent reactive radicals such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), peroxyl (ROO^-), and hydroxyl radicals (OH^-) are harmful to reproduction, but peroxynitrite anion ($ONOO^-$) and nitric oxide's nitrogen radicals (NO) possess physiological roles in fertilization and other animal reproduction determinants (Maneesh and Jayalekshmi, 2006). Sperm plasma membrane contains elevated levels of polyunsaturated fatty acids (PUFAs) (Khan, 2011), and the main rooster semen PUFA (22:4n-6, docosatetraenoic acid) is highly peroxidizable. Such sperm parameters, combined with age-related declines in seminal antioxidant function and activity of gonadal axis, render bird species susceptible to compromised fertility with aging (Surai et al, 2011).

Males' fertility peaks at around 37 weeks of age and declines with age above about 40 weeks of age (in reference to genetic strain). Low fertility leads to the reduction of egg production for hatch and whopping economic losses (Lagares et al, 2017). In this regard, it was found that supplements with higher levels of antioxidants will prove to be beneficial in an effort to improve the reproductive function in aged broiler breeder roosters. Over the recent decades, the issue has been of particular research interest into effective means of maintaining fertility within stress-charged roosters. One such popular technique is plant antioxidant compound, vitamins, and minerals supplementation that has been of critical significance to rooster breeding. It has been shown through experiments that antioxidant compound supplementation through diet or sperm extenders significantly reduces the adverse impact of oxidative stress while optimizing rooster fertility (Zhandi et al, 2020; Khalil-Khalili et al, 2021).

Medicinal plants have been widely used in the poultry industry due to their beneficial properties (such as antimicrobial, antioxidant, anti-inflammatory, and etc.) in the recent years (Shahraki Mojahed et al, 2024; Sabahi et al, 2020; Nazari et al, 2023; Rabieh et al, 2020; Mosavi et al, 2022; Nazari et al, 2024). Antioxidants are found in high concentrations within medicinal plants and can neutralize free radicals, converting them into harmless compounds (Parlet et al, 2005). Antioxidants within plants have also been researched highly for potential application in the treatment of sexual dysfunction and fertility enhancement within the recent years (Zarghi et al, 2015). Supplementing the diet of aged cocks with rosemary oil as an antioxidant appears to enhance fertility. The secondary metabolites of rosemary include flavonoids (genkwanin, isoscutellarein, and homoplantaginin), phenolic diterpenes (carnosic acid, carnosol, and rosmarinol), and triterpenes (ursolic acid) (Borras-Linares et al, 2014). Phenolic diterpenes are the major bioactive antioxidant compounds of rosemary extract (Rostami et al, 2017). They exhibit diverse biological activities such as antioxidant, anti-inflammatory, and anticancer activity (Borghei-Rad et al, 2017).

Selenium (Se) is an animal- and human-required trace element and serves a special

role in the preservation of spermatogenesis and male fertility and has structural and enzymatic functions and is exceptionally well known to carry out catalytic and antioxidant function (Qazi et al, 2019). Selenium is incorporated into enzymes like glutathione peroxidases (GPx) and Selenoproteins that are responsible for the antioxidant defense (Pappas et al, 2008). The principal role of glutathione peroxidases is the elimination and detoxication of hydrogen peroxide and lipid hydroperoxides to avoid oxidative damage to the sperm (Lemoine et al, 2011). Glutathione peroxidase occurs in rooster seminal plasma and semen (Bréque et al, 2003). Selenium plays a vital role in supporting healthy testicular function and structure, as well as ensuring sperm to move properly and work effectively. It has been postulated in some studies that selenium supplementation enhances the probability of fertility success and Sertoli cell count (Shi et al, 2010).

One such fertility-related gene is PVRL3 (PoliovirusReceptor-Related3). As a member of the family of cell adhesion proteins, the PVRL3 gene is of great relevance in mechanisms of spermatogenesis and fertility. It is particularly pertinent to sperm development and Sertoli cell-spermatid adhesion formation. Research has proven that the lack of expression or dysfunction of PVRL3 can lead to defects in sperm morphology, which ultimately leads to male infertility. PVRL3 not only contributes to sperm production but also sperm function and quality (Adeldust et al, 2021). Another key gene that impacts fertility is StAR (Steroidogenic Acute Regulatory Protein). This gene is responsible for spermatogenesis and fertility. StAR is responsible for cholesterol transport into the mitochondria, a critical step of steroid hormone biosynthesis such as testosterone and progesterone, which are crucial to spermatogenesis and fertility (Stocco et al, 2001).

According to the above-mentioned properties of rosemary oil and selenium, this study aimed to establish the effect of rosemary essential oil and selenium as antioxidants on the fertility-associated StAR and PVRL3 gene expression in aged broiler roosters. We hypothesized that rosemary oil and selenium would modulate StAR and PVRL3 expression and improve reproductive potential in aged roosters. The aim of this study is therefore to understand potential nutritional treatments for stimulating reproductive function by observing changes in gene expression.

Materials and methods

Farming Management

Forty-two Ross 308 broiler roosters, each over 50 weeks of age, were used in a 10-week factorial experiment (3 × 2) in a completely randomized design including rosemary oil at three levels (0, 100, 200 mg/kg) and selenium at two levels (0, 0.3 mg/kg).The experiment included 6 treatments with 7 birds per treatment group in were kept in single wood shavings-lined cages with one feeder and one drinker per cage. The condition in which they were reared was under strict control, where temperature was maintained at 21°C throughout, humidity at 60%and a light and dark regime of 14 hours light and 10 hours darkness. The feeding regimen was structured based on the Ross 308 management guide (2016) (Table 1). The complete design and description of the treatments are provided in Table 2.

Table 1: Composition and nutrients of experimental diets for roosters

Ingredient (g)	Amount kg/100 kg
Maize (8/5 % crude protein)	65.43
Wheat bran	22.88
Soybean meal (49% Crude Protein)	8.00
Calcium hydrophsphate	1.25
Calcium Carbonate	0.95
Nacl	0.37
Methionine	0.09
Lysine	0.03
Soybean oil	0.5
Vitamin[1]	0.25
Mineral[2]	0.25
Composition	
ME(Kcal/kg)	2850
CP (%)	12.2
Ca (%)	0.76
L-Lysine (%)	0.44
Methionine + Cystine (%)	0.47
Theronine(%)	0.36
Arginine	0.65
Sodium (%)	0.15
Availablephosphorus (%)	0.33

*1. Supplied per kilogram of diet: vitamin A, 15,000 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.
2. Supplied per kilogram of diet: Fe, 90 mg; Mn, 120 mg; Zn, 110 mg; I, 2mg and Se, 0.3 mg

Table 2: Structure of the dietary treatments in a 3 × 2 factorial arrangement

Treatment Group	Rosemary Essential Oil (mg/kg)	Selenium Enriched Yeast (mg/kg)
1 (Control)	0	0
2	100	0
3	200	0
4	0	0.3
5	100	0.3
6	200	0.3

Testicular Tissue Sampling

At the end of the experiment, three animals from each treatment group were sacrificed to collect testicular tissue samples in order to explore the StAR and PVRL3 genes. To achieve this, a portion of the testis was quickly excised using a sterile knife and placed in 1.5 ml RNase-free microtubes. The samples were sent to the labratory in liquid nitrogen and stored at –80°C.

RNAextractionandcDNAsynthesis

The RNA isolated from the tissues was purified with the Denazist kit according to the manufacturer's instructions. The quality of the isolated RNA was assessed by electrophoresis on a 2% agarose gel and Its purity and concentration by the ratios A260/A280 and A260/A230 was determined quantified by NanoDrop spectrophotometer (Thermo Scientific NanOdrOp. 2000C. USA). The purified RNA was kept at -80°C for future use. Finally, the reverse transcription reaction was carried out with Sinaclon's cDNA Synthesis Kit. And for each sample, about 8 high-quality microRNAs were used using random primers included in the kit. The cDNA products were stored at -20°C for subsequent analysis.

Real-Time PCR Using the SYBR Green Method

To analyze the expression of target genes, primer sequences for the StAR, β-actin (Amin Altawash et al, 2019) and PVRL3 (Adeldust et al, 2022) genes were considered according to the specifications presented in Table 3 and synthesized by Sinaclone Company (Iran).

Table 3: List of sequences and properties of primers used in this study

Gene name	Sequence	Piece length (bp)	Annealing temperature	Accession number
Star	F: 5'- TTCAGCGAGATGGAGATGTCC-3' R: 5'- GGAACACCTTACCCACGTCC-3'	160	60	NN_204686.2
PVRL3	F: 5'- CATGTGGACCAGGCTGGATG-3' R: 5'- GTCTTCTGATCACTCCTCTGACC-3'	150	60	XM_416630.5
β-actin	F: 5'- ACGTCGCACTGGATTTCGAG-3' R: 5'- AAAGATGGCTGGAAGAGGGC-3'	145	60	X00182

Before performing the first PCR reactions with the designed primers, primer specificity to the target genes was ensured by using a Thermalcycler Mini (Germany). Subsequently, real-time PCR was done in duplicate for genes β-actin, StAR and PVRL3, where each gene was done in separate plates using the StepOnePlus Real-Time PCR System (USA). Table 3 illustrates the step-by-step details of the real-time PCR process.

To confirm the target gene specific amplification, PCR reaction was carried out first using the designed primers in thermal cycler (Thermalcycler Mini, Germany). Real-time PCR was carried out afterwards in the samples in two technical replicates for StAR, PVRL3 and reference gene β-actin. The reactions were also carried out in single plates in the Step One Plus Real-Time PCR System (USA). The reaction in a volume of 25 µL contained 12.5 µL of Master Mix Green, 1 µL of each of the forward and reverse primers (Concentration 10 µmolar), 2 µL of template cDNA (50 ng), and 8.5 µL of double-distilled water. The samples were then cycled on a Bio-Rad thermocycler using the following thermal cycling profile for gene expression analysis: the initial step was 95°C for 10 minutes of denaturation followed by 40 cycles of 95°C for 30 seconds of denaturation, 60°C for 30 seconds of annealing, and 72°C for 45 seconds of extension. The final extension of 72°C for 5 minutes was performed. Melting curve analysis was performed to verify primer specificity.

To compare the data of Real-time PCR, an initial report was initially made utilizing the Step One ABI software and exported in Excel (v 2016). Second, the relative expression levels of the target gene were normalized to the reference gene β-actin. Relative gene expression was calculated using Pfaffl method (2004) by the formula $2^{-\Delta\Delta CT}$. After the Calculation of fold change values, all experimental values were obtained by using SAS software (v 9.4) with a factorial 2×3 design of the complete randomized design with the help of the GLM procedure. Treatment means were tested at a significance level of 5% by using the LSD test based on the given equation.

$y_{ijk}= \mu + C_i + T_j + (CT)_{ij} + e_{ijk}$

In this equation: y_{ijk} represents the value of each observation, µ is the overall mean, C_i denotes the effect of the different rosemary levels, T_j indicates the effect of the different selenium levels, $(CT)_{ij}$ corresponds to the interaction effect between rosemary and selenium and e_{ijk} is the experimental error.

Result

Results of RNA quantity and quality assessment

The RNA integrity and yield of purified RNA were accurately ascertained by gel electrophoresis and Nanodrop spectrophotometry. Pureness was revealed in Nanodrop absorbance readings with A260/A280 values ranging consistently between 1.8–2.2 denoting negligible protein contaminations. RNA integrity was also claimed by clear sharp 28S and 18S rRNA bands on agarose gel with not even a trace of degradation. These assays ensured the quality of the RNA samples and suitability for downstream investigations. Successful cDNA synthesis was subsequently guaranteed, with sufficient templates for subsequent gene expression investigation.

Evaluation of the replication efficiency of the studied genes

The PCR products were observable as sharp, well-defined bands of the defined size on the agarose gel following electrophoresis (Figure 1). As you can see, the StAR gene has a band of 160pb, the PVRL3 gene has a band of 150pb, and the β-actin gene has resulted in a band of 145 base pair on the agarose gel. The sharpness and intensity of the bands were evidence of the specificity and efficiency of the amplification, indicating high-quality PCR output in the absence of non-specific amplification.

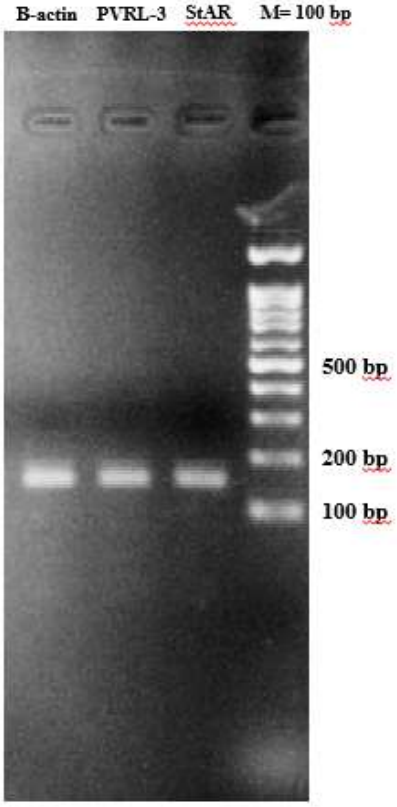

Figure 1. Agarose gel electrophoresis showing PCR products of β actin, PVRL3 and StAR genes with band lengths of 150pb, 145pb and 160pb respectively. The molecular marker (Ladder) is 100 bp.

Reviewing the results of the Real-time PCR reaction

Melting Curves of the StAR, PVRL3, and β-actin genes are shown in sections A, B, and C of Figure 2, respectively. Specificity of PCR amplification was also confirmed through the presence of single sharp peaks during melting curve analysis of Real-time PCR reactions. There are no additional peaks and no signs of nonspecific product or primer dimers. These results confirm that the primers actually designed had successfully achieved specific amplification, and the Real-time PCR assays were extremely reliable for additional gene expression analysis.

The effect of adding rosemary essential oil and selenium on the expression of StAR and PVRL-3 genes

Results on the effect of rosemary essential oil and selenium supplementation on StAR and PVRL-3 gene expression are presented in Table 4. Adding rosemary essential oil to the maternal rooster's diet had no significant effect on the expression of the StAR gene ($P>0.05$). However, the inclusion of 200 mg rosemary essential oil significantly reduced PVRL-3 gene expression ($P<0.05$), but 100 mg did not influence this gene ($P>0.05$). In addition, real-time PCR indicated that the inclusion of 0.3 mg selenium-enriched yeast significantly increased the expression of both genes ($P<0.05$). Overall, the impact of selenium and rosemary essential oil was leaded to a significant increase in the expression of both genes ($P<0.05$).

Co-treatment with 100 or 200 mg rosemary essential oil and selenium caused significant increase StAR gene expression ($P<0.05$). Co-treatment with 100 mg rosemary essential oil and 0.3 mg selenium-enriched yeast caused significant increase PVRL-3 expression ($P<0.05$), while 200 mg rosemary essential oil together with selenium caused decreased PVRL-3 expression, and selenium could not abrogate this decrease. It appears that the addition of rosemary essential oil at 200 mg/kg is not favorable, and even selenium, which was favorable, could not overcome this adverse effect.

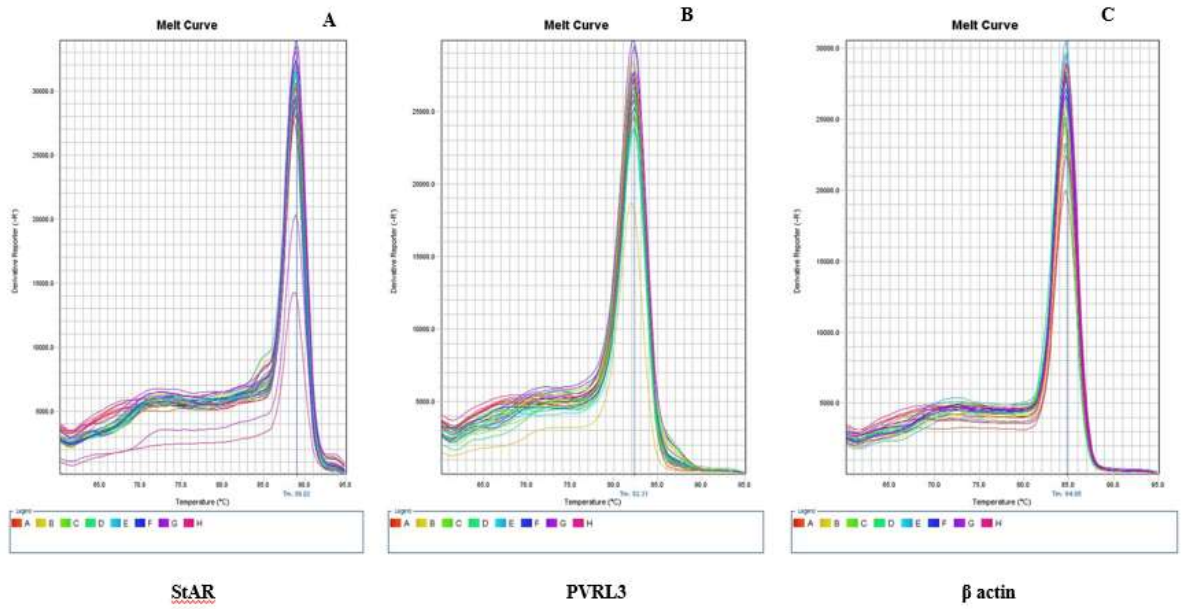

Figure 2. Melting curves of StAR (A), PVRL3 (B) and β actin (C) genes

Table 4: Effect of rosemary essential oil and selenium on the expression of StAR and PVRL-3 genes (Alpha = 0.05)

Treatments Group	Main effects		StAR	PVRL-3
	0		1.48	1.30[a]
Rosemary essential oil	100 200		1.30 1.29	1.22 [a] 0.76 [b]
SEM			0.07	0.18
Selenium	0		0.68 [b]	0.85 [b]
	0.3		2.03 [a]	1.34 [a]
SEM			0.14	0.06
Interactions				
Basal diet (control)	0	0	1 [b]	1 [b]
Basal diet + 100 mg/kg rosemary essential oil	0	100	0.49 [b]	0.75 [b]
Basal diet + 200 mg/kg rosemary essential oil	0	200	0.56 [b]	0.81 [b]
Basal diet + 0.3 mg/kg selenium	0.3	0	1.96 [a]	1.61 [a]
Basal diet + 100 mg/kg rosemary essential oil + 0.3 mg/kg selenium	0.3	100	2.11 [a]	1.70 [a]
Basal diet + 200 mg/kg rosemary essential oil + 0.3 mg/kg selenium	0.3	200	2.02 [a]	0.72 [b]

*Different letters in each column indicate significant differences($P<0.05$).

Discussion

Fertility is one of the key determinants of the economic profitability in poultry flocks and is determined by several factors such as breed, quality of nutrition, age of flock, and quality of semen (Miazi et al, 2012). During the collection of eggs, producers will observe a decline in fertility (Khalil-Khalili et al, 2021), which is primarily due to cocks aging and the consequent loss of reproductive ability. Current studies have shown that natural antioxidant compounds present in dietary supplements reverse the negative effect of aging on fertility in roosters and affect gene expression related to fertility.

Our research revealed that supplementation with selenium increased the expression of the StAR and PVRL-3 genes. Other studies have repeatedly demonstrated that organic selenium is of higher bioavailability compared to the inorganic substances (Hadrup and Ravn-Haren, 2021). Selenium yeast contains mostly organic selenium compounds such as seleno-methionine and analogs (Schrauzer, 2001). It is worth noting that the selenium yeast lowered the production of reactive oxygen species (ROS) and inhibited oxidative cellular injury among various animal species (Yang et al, 2022; Liu et al, 2020; Samo et al, 2020). Generally, selenium yeast significantly lessens oxidative damage to testis and maintains regular reproductive function in roosters under oxidative stress (Xiong et al, 2025). The results of adding selenium nanoparticles to the diet demonstrated improvements in sperm quality and reproductive performance in male rainbow trout breeders. In fact, dietary selenium enhanced sperm volume, motility time, and concentration in males. Furthermore, the antioxidant character of the selenium nanoparticles in diet was the determining factor for the enhanced fertilization and hatching rates (Jahaabad et al, 2020).

One important consideration in this case is the fact that selenium is a fundamental component of the glutathione peroxidase enzyme, which plays an important role in detoxifying lipid peroxides and protecting cells against oxidative damage from reactive oxygen species (ROS) (Zachara, 1992). It has been determined from the previous research that selenium-supplemented yeast is able to counteract testicular toxicity accumulated via oxidative stress (Cao et al, 2020). Results indicate that selenium yeast can reverse oxidative damage to testicles in roosters caused by oxidative stress and activate the Nrf2/HO-1 signaling pathway (Xiong et al, 2025).

Healthy production of spermatozoa and androgens by the testis is crucial to male reproductive function (McBride and Coward, 2016). In addition, the function of molecules involved in testosterone generation, such as StAR, has already been highlighted previously in the regulation of steroidogenesis (Heng et al, 2017). In a research study, main markers in terms of testosterone generation, such as StAR, were explored. The western blot examination revealed that testis from rooster exposed to oxidative agents had significantly lower StAR expression levels compared to controls. This was in concordance with previously mentioned mRNA expression patterns for StAR molecules. Importantly, pretreatment of cells with selenium yeast avoided such changes. These findings strongly suggest that selenium yeast restores impaired expression of molecules associated with testosterone production and supports normal reproductive performance in roosters. Therefore, maintenance of testosterone production is of utmost significance in the preservation of male reproductive health (Xiong et al, 2025).

The results of the present study indicated that the addition of rosemary essential oil did not have an impact on the StAR gene expression, but with a high concentration (200 mg), the PVRL-3 gene expression was significantly reduced. Researchers have

explored the antioxidant effect of rosemary in the preservation of testicular integrity and fertility and the potential role in the improvement of reproductive performance and in the reduction of oxidative stress. Rosemary has potent antioxidant activity responsible for lowering oxidative stress in testicular tissues. Rosemary supplementation has been shown in studies to boost total antioxidant capacity, leading to sperm quality and testosterone enhancement in animal models (Mansouri Torghabeh et al, 2022; Alahmadi and Alahmadi, 2024). In diabetic mice, rosemary extract also lowered malondialdehyde (MDA) content, a sign of oxidative stress, and increased glutathione and superoxide dismutase contents, signs of protection against oxidative damage (Alahmadi and Alahmadi, 2024). In addition, supplementation with rosemary essential oil has also been associated with semen quality improvement in semen parameters like an improvement in the concentration and motility of sperm and an increase in testosterone levels in rams (Ali et al, 2024).

Conversely, some research reveals that rosemary in high concentrations may be antifertility as, in male rats, spectacular morphological changes in testicular morphology were observed with increasing dosage (El-Din et al, 2012). This is to imply that in spite of the beneficial antioxidant effect of rosemary, its effect on fertility is dose- and context-dependent.

This study found no significant effect of rosemary on fertility genes. However, when an optimal dosage of rosemary essential oil was administered alongside selenium yeast, there was an enhanced expression of fertility genes. This suggests a synergistic action between the two compounds. In summary, selenium is known to support testicular fertility and health, while rosemary may produce adverse effects at higher dosages. Therefore, caution should be exercised when using both of these substances simultaneously. More research is necessary to confirm their potential synergy.

Acknowledgments

We acknowledge the Research deputy of Agricultural Sciences and the Natural Resources University of Khuzestan for financial support.

Conflict of Interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Funding

This research was financially supported by the Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran.

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Received: 18.07.2025
Accepted: 08.10.2025

DOI: 10.22055/ivj.2025.535387.2818

تأثیر افزودن اسانس رزماری و سلنیوم به جیره غذایی بر بیان ژن‌های باروری در خروس مادر گوشتی

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تاریخ دریافت: ۱۴۰۴/۴/۲۷ تاریخ پذیرش: ۱۴۰۴/۷/۱۶

چکیده

در مرغ‌های گوشتی، عملکرد تولیدمثلی خروس‌ها با افزایش سن کاهش می‌یابد. آنتی‌اکسیدان‌های طبیعی مانند روغن رزماری و سلنیوم می‌توانند با کاهش استرس اکسیداتیو، عملکرد تولیدمثلی را بهبود بخشند. مطالعه حاضر تأثیر این آنتی‌اکسیدان‌ها را بر بیان ژن‌های باروری (StAR و PVRL3) در خروس‌های مسن بررسی کرده است. ۴۲ خروس در ۶ گروه و ۷ تکرار به صورت آزمایش فاکتوریل در قالب طرح کاملاً تصادفی مورد آزمایش قرار گرفتند. تیمارها شامل: ۱) جیره شاهد، ۲) جیره حاوی ۱۰۰ میلی‌گرم/کیلوگرم اسانس رزماری، ۳) جیره حاوی ۲۰۰میلی‌گرم/کیلوگرم اسانس رزماری، ۴) جیره حاوی ۰/۳ میلی‌گرم بر کیلوگرم مخمر غنی‌شده با سلنیوم، ۵) جیره حاوی ۱۰۰میلی‌گرم/کیلوگرم اسانس رزماری +۰/۳میلی‌گرم بر کیلوگرم مخمر غنی‌شده با سلنیوم و ۶) جیره حاوی ۲۰۰میلی-گرم/کیلوگرم اسانس رزماری +۰/۳میلی‌گرم بر کیلوگرم مخمر غنی‌شده با سلنیوم بودند. در آخر، بافت بیضه از سه حیوان هر تیمار جداسازی و در دمای ۸۰- نگهداری شدند. بیان ژن StAR و PVRL3 با واکنش Real-timeqPCR انجام و نرمال‌سازی نسبت به ژن مرجع β-actin سنجیده شد. نتایج نشان داد، افزودن اسانس رزماری به جیره خروس تأثیر معنی‌داری بر بیان ژن StAR نداشت، اما دوز بالا (۲۰۰میلی‌گرم/کیلوگرم) باعث کاهش معنی‌دار بیان ژن PVRL-3 شد، در حالی که دوز ۱۰۰ میلی‌گرم تأثیر معنی‌داری نداشت. مکمل سلنیوم (۰/۳ میلی‌گرم) بیان هر دو ژن StAR و PVRL-3 را به طور معنی‌داری افزایش داد. ترکیب سلنیوم با ۱۰۰ میلی‌گرم رزماری نیز بیان این ژن‌ها را افزایش داد، اما ترکیب با ۲۰۰میلی‌گرم رزماری اثر منفی دوز بالا را جبران نکرد. سلنیوم با کاهش استرس اکسیداتیو، سلامت تولیدمثلی و بیان ژن‌های مرتبط با تستوسترون را بهبود می‌بخشد، در حالی که رزماری در دوزهای پایین مفید است اما دوزهای بالا ممکن است باروری را کاهش دهد؛ بنابراین استفاده همزمان آن‌ها باید با احتیاط افزوده شود.

کلمات کلیدی: خروس مسن، PVRL3، رزماری، سلنیوم

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