Journal homepage
www.ajas.uoanbar.edu.iq
Anbar Journal of Agricultural Sciences
(University of Anbar – College of Agriculture)

EFFECT OF SELENIUM SUPPLEMENTATION AND EXPRESSION OF SOME GENES ON TESTIS TISSUES IN ROOSTERS

A. Kh. Ahmed [1,2] N. Kh. Mehdi [3] Sh. Melak [4] Z. Peng [2]*

[1] Department of Animal Production, College of Agriculture, Tikrit University, Iraq.
[2] Department of Animal Genetics, Breeding, and Reproduction, College of Animal Science and Technology, Northeast Agricultural University, Harbin, 150030, China.
[3] Department of Biology, Education College for Women, University of Anbar, Ramadi, Iraq.
[4] Animal Production Research Institute, Agriculture Research Center, Dokki, 12619. Giza, Egypt.

***Correspondence to:** Zheng Peng, Department of Animal Genetics, Breeding, and Reproduction, College of Animal Science and Technology, Northeast Agricultural University, Harbin, 150030, China.
Email: Zhengpeng@neau.edu.cn

Article info

Received: 2024-12-18
Accepted: 2025-02-02
Published: 2025-06-30

DOI-Crossref:
10.32649/ajas.2025.154898.1464

Cite as:
Ahmed, A. Kh., Mehdi, N. Kh., Melak, Sh., and Peng, Z. (2025). Effect of selenium supplementation and expression of some genes on testis tissues in roosters. Anbar Journal of Agricultural Sciences, 23(1): 455-469.

©Authors, 2025, College of Agriculture, University of Anbar. This is an open-access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

Abstract

Selenium is a vital trace element that plays a key role in physiological functions, antioxidant enzyme activity, and reproductive health in birds. This study evaluated the effects of different levels of selenium in the diet and drinking water on the expression of genes related to inflammation, antioxidants, apoptosis, and testicular metabolism in poultry. The results showed that selenium positively influenced immune responses in chickens. Higher selenium levels in water or in combination with feed (T3 and T4) significantly reduced IL-1β gene expression. Conversely, IL-1RN expression increased in the feed-only treatment (T2) but decreased when selenium was provided through water or both sources. Notably, SelPF15 gene expression rose significantly across T2, T3, and T4 compared to the control (T1). Additionally, SelPP expression increased in T2 and T3 treatments but decreased in T4. Analysis of programmed cell death revealed a decrease in Fas gene expression with

selenium treatments, while FaslG and Caspase-3 expressions increased significantly. Superoxide dismutase (SOD 3) expression was higher in T3 and T4 but lower in T2. Significant effects on NOX5 and AGT were observed with feed and water combinations, but not when both were supplemented together. Lastly, POR expression declined with selenium treatments, while AKT expression increased across all treatments, suggesting that enhanced selenium availability boosts these metabolic pathways. Overall, the findings indicate that increased selenium significantly improves immune response, apoptosis, antioxidant activity, and testicular structure in poultry.

Keywords: Selenium, Testis, Expression genes, Chickens, Ultrastructure.

تأثير مكملات السيلينيوم في التعبير الجيني لبعض الجينات على نسيج الخصية في ديكة الدجاج

احمد خالد احمد [1،2] نغم خضير مهدي [3] شريف ملك [4] زانك بينك [2]*

[1] قسم الإنتاج الحيواني، كلية الزراعة، جامعة تكريت، العراق.

[2] قسم الوراثة الحيوانية، التربية والتكاثر، كلية علوم وتكنولوجيا الحيوان، جامعة شمال شرق الزراعة، هاربين، 150030، الصين.

[3] قسم علوم الحياة، كلية التربية للبنات، جامعة الأنبار، الرمادي، العراق.

[4] معهد بحوث الإنتاج الحيواني، مركز البحوث الزراعية، الدقي، 12619، الجيزة، مصر.

***المراسلة الى:** زانك بينك، كلية الزراعة، قسم الوراثة الحيوانية، التربية والتكاثر، كلية علوم وتكنولوجيا الحيوان، جامعة شمال شرق الزراعة، هاربين، 150030، الصين.

البريد الالكتروني: Zhengpeng@neau.edu.cn

الخلاصة

السيلينيوم هو عنصر حيوي يلعب دورًا رئيسيًا في الوظائف الفسيولوجية ونشاط إنزيم مضادات الأكسدة والصحة الإنجابية في الطيور. هدفت هذه الدراسة إلى تقييم كيف يؤثر تغيير مكملات السيلينيوم في النظام الغذائي ومياه الشرب على التعبير الجيني المتعلق بالالتهاب ومضادات الأكسدة وموت الخلايا وايض خلايا الخصية في الدواجن. أشارت النتائج إلى أن مكملات السيلينيوم أثرت بشكل إيجابي على الاستجابات المناعية في الدجاج. أدت مستويات السيلينيوم الأعلى في الماء أو مع العلف (T3 وT4) إلى تقليل التعبير الجيني IL−1β بشكل كبير. وعلى العكس، زاد التعبير عن IL−1RN في معاملة التغذية فقط (T2) ولكنه انخفض مع زيادة السيلينيوم

في الماء أو كلا المصدرين. ومن الجدير بالذكر أن التعبير الجيني لـ SelPF15 ارتفع بشكل ملحوظ للمعاملات T2 وT3 وT4 مقارنة بالمجموعة الضابطة (T1). بالإضافة إلى ذلك، زاد التعبير عن لـ SelPP في علاجات T2 وT3، ولكنه انخفض في T4. كشف تحليل موت الخلايا المبرمج عن انخفاض التعبير الجيني لـ Fas مع علاجات السيلينيوم، في حين زاد التعبير لـ FasIG وCaspase-3 بشكل ملحوظ. كان التعبير عن إنزيم (SOD 3) أعلى في T3 وT4 ولكنه أقل في T2. وقد لوحظت تأثيرات كبيرة على لـ NOX5 وAGT مع معاملات T3, T2، ولكن ليس مع T4 مقارنة مع T1. وأخيرًا، انخفض التعبير عن لـ POR مع اضافة السيلينيوم، في حين زاد التعبير عن لـ AKT في جميع معاملات الاضافة، مما يشير إلى أن زيادة توفر السيلينيوم تعزز هذه المسارات الأيضية. بشكل عام، تشير النتائج إلى أن زيادة السيلينيوم تعمل على تحسين الاستجابة المناعية، وتنظيم مسار موت الخلايا المبرمج، ونشاط مضادات الأكسدة، وبنية الخصية في الدواجن بشكل كبير.

كلمات مفتاحية: السيلينيوم، الخصية، جينات التعبير، الدجاج، البنية الدقيقة.

Introduction

Selenium (Se) is a chemical element and an essential nutrient (24). It is well established that it is an important trace element (25), and is closely related to cytokines during inflammation (23). In addition to its role in the immune system and apoptosis, selenium is administered to protect animals against metal toxicity and damage to the male reproductive system (10). Due to its multiple protective roles and important functions in regulating cellular homeostasis, metabolism, and antioxidant defense, Selenium supplementation in animal sciences has become increasingly important (20). A previous study found that feeding chickens with selenium increased glutathione peroxidase (GSH-Px) activity in their testes and seminal plasma, demonstrating that dietary selenium has beneficial effects on the antioxidant system in sperm (21). Selenium is an essential nutrient required in the diet, and its deficiency can lead to reproductive disorders (18) and abnormal mitochondria in sperm (2). Accordingly, a recent study indicated that selenium deficiency can cause reproductive decline in poultry due to increased oxidative stress (19). Recent studies have also shown the effectiveness of adding antioxidants in improving the physiological and immune properties of poultry tissues (14 and 15).

The testis is the most important reproductive gland of male animals, and testicular growth is crucial for male fertility. It comprises two main tissue types: parenchymal and interstitial (17). The parenchyma consists of seminiferous tubules where adjacent Sertoli cells form tight junctions, creating the blood-testis barrier (BTB) (9). The BTB, known as the Sertoli cell epithelial barrier, is one of the tightest blood-tissue barriers in the mammalian body (13). It has a crucial role in maintaining spermatogenesis by protecting spermatogenic tubules from harmful substances in the blood, preventing sperm antigens from entering the bloodstream and triggering autoimmune reactions, and providing nutrients and a stable environment necessary for germ cell proliferation, division, and maturation (3). The testicular tissue is distributed between the seminiferous tubules and consists mainly of Leydig cells, which are responsible for the secretion of male hormones (5).

This study aimed to evaluate the effects of increasing selenium supplementation in the diet and drinking water on the gene expression of some inflammatory response genes, antioxidants, apoptosis, and metabolism of testicular tissue in poultry.

Materials and Methods

Animal and Tissue Sampling: Healthy male Ross 308 chickens were obtained from the experimental chickens. Two hundred male chickens were randomly divided into four groups, with five replications per treatment (10 chickens per replicate), and housed in cages until they reached six weeks of age. The first control group (T1) was fed a standard diet, and the second experimental group (T2) was fed the experimental diet (a basal diet supplemented with 0.3 mg/kg of inorganic selenium) and untreated water. The third experimental group (T3) received a standard feed and treated water (250 ppm selenium solution), and the fourth experimental group (T4) received 250 ppm selenium solution in the water and was fed the experimental feed (basal diet + 0.3 mg/kg inorganic selenium).

After six weeks of feeding, ten roosters were randomly selected from each group, euthanized, and their testes promptly removed and weighed. The testes were cut into two parts, one prepared for histological observation, and the other frozen in liquid nitrogen at −80 °C for real-time polymerase chain reaction (RT-PCR) analysis. All experimental methods and sample collection for this study were carried out in compliance with the guidelines provided by the Institutional Animal Care and Use Committee at Northeast Agricultural University (NEAU).

Preparation of testicular tissue samples using TEM: Testicular tissue samples were prepared for transmission electron microscopy (TEM) by cutting the testes into 1–2 mm^3 blocks and post-fixing them in 2.5% glutaraldehyde in a 100 mM phosphate buffer at pH 7.0 for 2–24 hours. The sections for histological evaluations were prepared according to Watson (22). Tissue sections from 5 male chickens were used in each group, and at least 60 tubules (n = 20) were used to evaluate the circular cross-sections. The total cell numbers in the circular tubule cross sections were used as the denominator for calculating cell proportions. Sections were observed and photographed under a transmission electron microscope (Hitachi, Tokyo, Japan).

Extracting RNA from testis tissues: RNA from each testis tissue (−80 °C) was isolated using the TRIzol reagent method according to the manufacturer's instructions (Invitrogen Corporation, Carlsbad, CA, USA). RT-PCR and PCR were used to prepare cDNA from this procedure, and the following PCR reactions performed with 0.24 µmol/l of each sense and antisense primers, 0.06 mmol/l of rTaq polymerase, 0.8 mmol/l deoxynucleotide mixes, and 10× PCR buffer. The reaction was performed by predenaturation at 94 °C for 5 min, denaturation at 94 °C for 30s, and annealing at 61.5 °C for 30s. This was followed by 30 cycles of extension at 72 °C for 40s and a final extension at 72 °C for 10 minutes, with the reaction held at 4 °C after completion. The results were analyzed on a 1.5% agarose gel via electrophoresis.

Quantitative real-time PCR analysis: The analysis was conducted using quantitative real-time PCR on an ABI PRISM 7500 detection system (Applied Biosystems, USA). The reactions were performed in a 20-ml reaction mixture comprising 10ml PCR

Master Mix (SYBR Green I 2×) (TaKaRa, China), 2ml diluted cDNA, 0.4ml each of the primers (10 mM), 0.4ml ROX Reference Dye II (50x), and 6.8ml pure PCR water.

Thermal reaction conditions: Thermal reaction conditions involved initial termination at 95 °C for 30s and amplification for 40 cycles including 5 °C for 5s (termination) and 60 °C for 20s (extension). The primers were specifically designed for the experiment by the first author. β-actin was used as an internal reference gene. The oligonucleotide sequences of the forward and reverse primers of the target genes are shown in Table 1. The relative estimate was expressed as the ratio of the target to the control genes using the delta-delta Ct (ΔΔCt) method of Livak and Schmittgen (11).

Table 1: List of gene-specific primers.

Name of Target Gene	Nucleotide Sequence of Primers (5′→3′)	ID: NCBI	Fragment Size (bp)
FASLG	AGGAAGCAAGGAAGGCAGCA GGAAGAGCACATTGGAGTA	NM_001031559.1	171
Fas	TCTCGGTGTGAACATTGCGAGTGTCT GAAGTTGAAGTAC	NM_001199487	81
AGT	CCAAAAGCAAAGGCCAAAGC CGAACATCCACTGCAACCA	XM_419584.6	79
Caspase-3	CCACGCTCAGGGGAAGATGTAT CGGTATCTCGGTGGAAGTTCTTA	NM_204725	178
Gpx-3	AGGGCCAGGAGAGGGA CCGTAGTCGTAGATGGTGC	NM_001163232.2	64
POR	GGGCTGGGGAACAAGACTTA CCTCCAGTCTCTTGTCCACA	NM_001195796.1	67
SOD3	ATCCAAGCAGCGCGTTACT CCCATCAGTCTCATTATCAGCC	XM_015285700.2	98
NOX5	CCCTTTGCCTCCATCCTGC CCGGTTGATCCAGATGAAGT	NM_001305472.1	129
IL-1β	GGCACTGGGCATCAAGGGCT AGGGAGGTGCAGATGAA	NM_204524.1	210
IL-1RN	GCCTCCGCGCCGTTCACCT GGAGGTGCAGAGGAA	HE608245	93
SelPF (15)	AGCTTGCAGGGAACTTGGC CCACATACTTCTAGGACAGCTC	NM_001012926.3	168
SelPP1	CGGTGGTCGCTCTCCTC CCCTCATTCTCTAACTTCACTC	NM_001031609.2	91
AKT	ACAGAACTCACGGCATCCA CCCGGTCTTCAGAAAATACACGC	NM_205055.1	143
β-actin	CCGAGAGAGAAATTGTGCGTGAC TCGGGGCACCTGAACCTCTC	L08165	166

Statistical analysis: The experimental data are presented as mean ± SD. The statistical analysis was performed using CoStat software (CoHort Software, Monterey, CA, USA). Differences between the two groups were detected by one-way analysis of variance (ANOVA) and the t-test values of $P \leq 0.05$ or $P \leq 0.01$.

Results and Discussion

Figure 1 illustrates the effect of increasing selenium intake on the immune response of roosters. Figure 1A shows that increasing selenium through water or through both

water and feed (T3 + T4) resulted in a significant decrease in interleukin IL-1β ($P \leq 0.05$) gene expression. In contrast IL-1RN gene expression increased markedly ($P \leq 0.05$) in the T2 feed treatment while it decreased significantly for T3 and T4 compared to the control treatment.

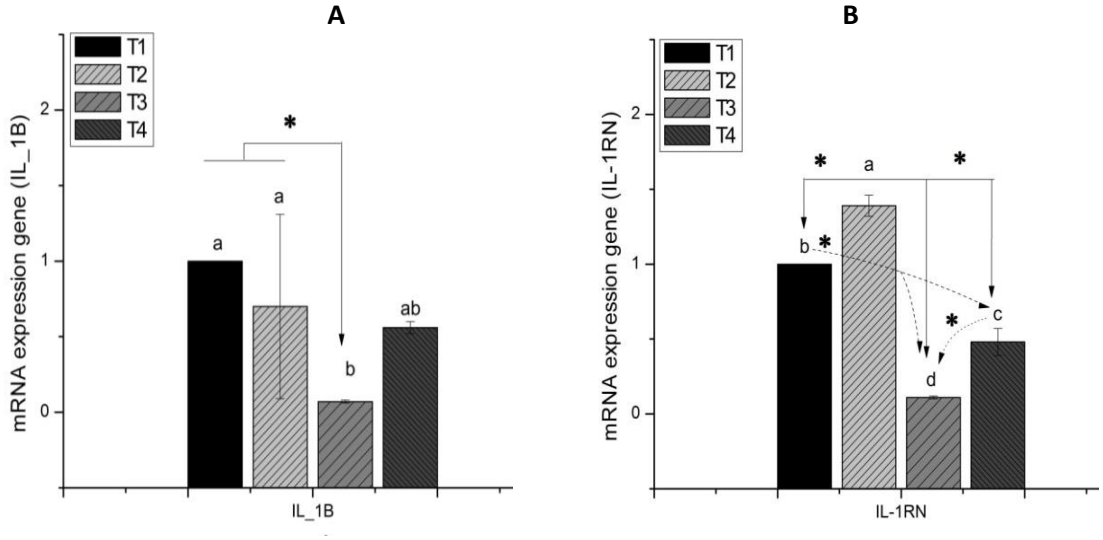

Fig. 1: Effect of dietary selenium supplementation on the mRNA levels of immune response genes in the testicular tissues of poultry under different treatments.

(A) Interleukin IL-1β, and (B) IL-1RN gene expressions. Values are means ± standard error (SD). Different lowercase letters indicate significant differences between treatments. *$P \leq 0.05$.

The results in Figure 2 indicate an increase in SelPF15 gene expression following a significant ($P \leq 0.05$) addition of selenium to the feed, water, or to both water and feed (T2, T3, +T4) compared to the control T1. The results also show a significant increase ($P \leq 0.05$) in SelPP gene expression in treatments T2 and T3, while the higher selenium in the water and feed (T4) led to a significant decrease compared to the control treatment. This indicates that higher selenium amounts negatively affect SelPP gene expression (Fig. 2B).

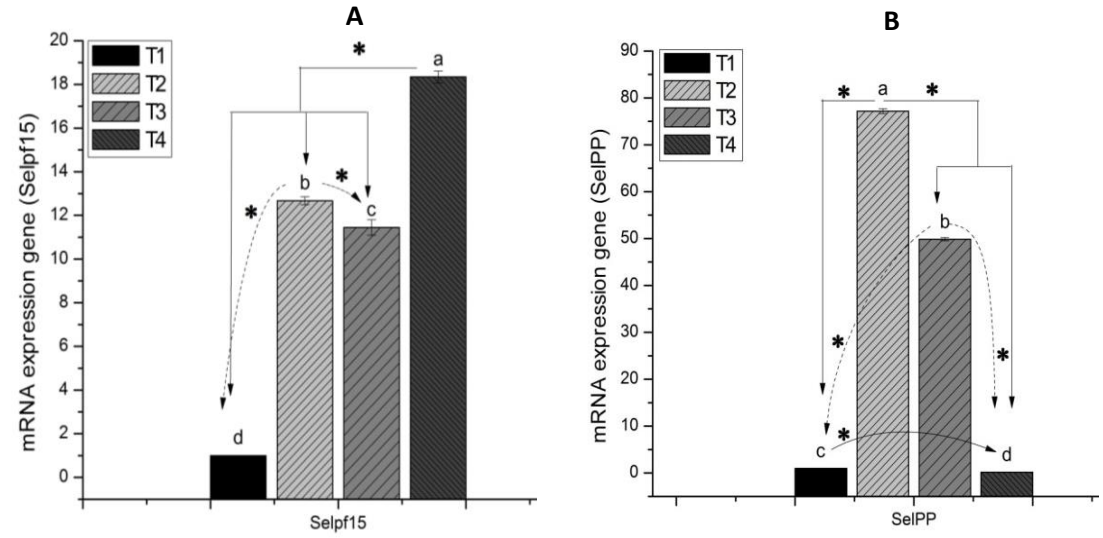

Fig. 2: Effect of dietary selenium supplementation on the mRNA levels of selenoprotein genes in the testicular tissues of poultry under different treatments.

(A) SelPF15, and (B) SelPP gene expressions. Values are means ± standard error (SD). Different lowercase letters indicate significant differences between treatments. *P ≤ 0.05.

Figure 3 shows the effect of increasing selenium dosage on programmed cell death, with Fas gene expression decreasing significantly (P ≤ 0.05) compared to the control (Fig. 3A). However, increasing selenium in the feed, drinking water, or in both resulted in a significant increase in FaslG gene expression over the control treatment (Fig. 3B). Additionally, as seen in Fig. 3C, there was a significant increase in caspase-3 gene expression from the selenium addition compared to the control treatment.

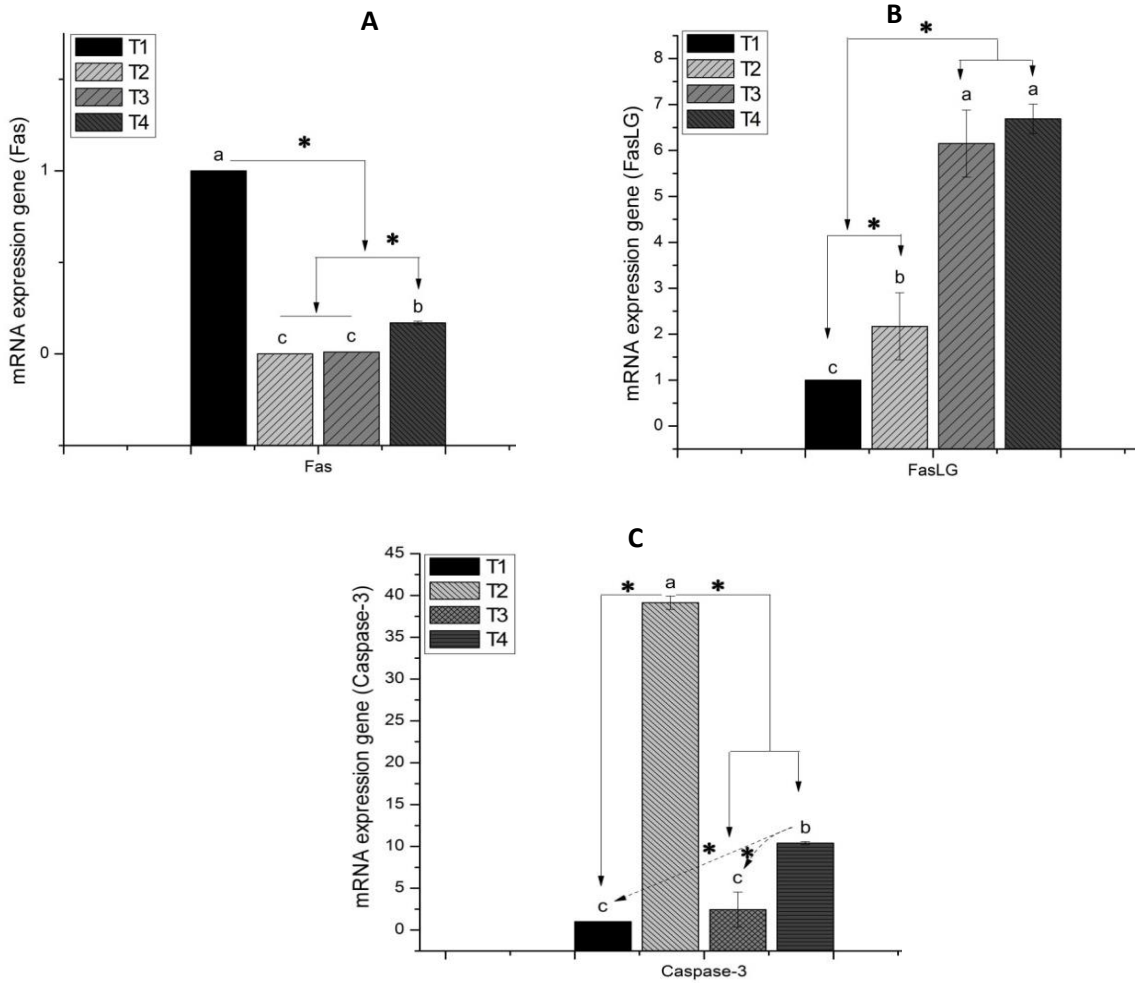

Fig. 3: Effect of dietary selenium supplementation on mRNA levels of apoptosis genes in the testicular tissues of poultry under different treatments.

(A) Fas, (B) FaslG, and (C) caspase-3 gene expressions. Values are means ± standard error (SD). Different lowercase letters indicate significant differences between treatments. *P ≤ 0.05.

Figure 4 demonstrates the effect of increased selenium inputs on antioxidants in chicken testicles. As seen in Figure 4A, increasing selenium levels in water or both feed and water (T3 + T4), and in limited amounts, led to a significant increase in SOD 3 gene expression compared to the control group, However, it declined markedly in the feed-only treatment (T2).

As for its effect on the NOX5 and AGT genes, it was highly statistically significant (P≤0.05) when added to the feed and water (T2 + T3) but had no significant impact on gene expression compared to the control treatment (Fig. 4 B and C). In contrast, the gene expression of the antioxidant Gpx3 increased significantly (P ≤ 0.05) in the feed

treatment (T2); it also increased in the other selenium treatments (T3 + T4) but without statistical significance compared to the control treatment (Fig. 4D).

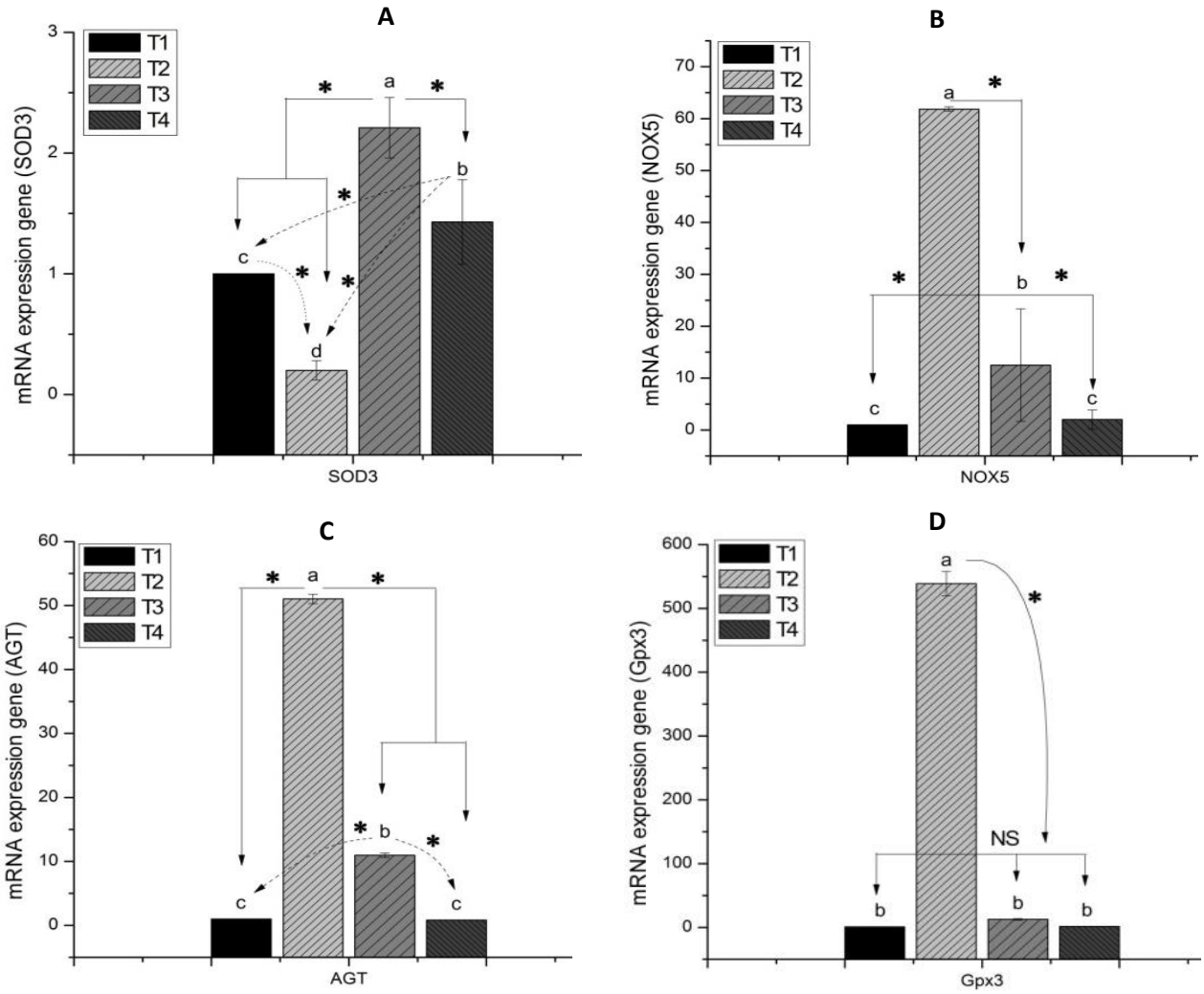

Fig. 4: Effect of dietary selenium supplementation on mRNA levels of antioxidant genes in the testicular tissues of poultry under different treatments.

(A) SOD3, (B) NOX5, (C) AGT, and (D) Gpx3 gene expressions. Values are means ± standard error (SD). Different lowercase letters indicate significant differences between treatments. *P ≤ 0.05.

The results in Figure 5 show the effect of selenium dosages on metabolic genes. Figure 5A indicates a significant decrease in POR gene expression compared to the control treatment. Increasing the availability of selenium through water or both food and water did not affect POR gene expression. AKT gene expression increased significantly in the selenium treatments (T4, T3, T2) compared to the control treatment, suggesting a positive relationship between the two factors (Fig. 5B).

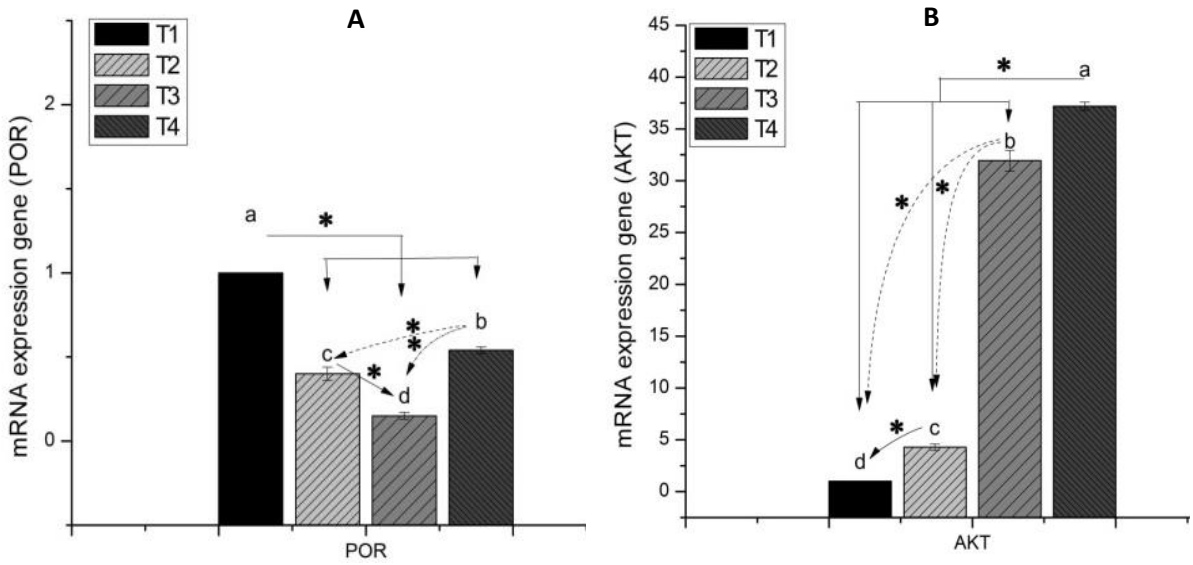

Fig. 5: Effect of dietary selenium supplementation on mRNA levels of metabolic genes in the testicular tissues of poultry under different treatments.

(A) POR and (B) AKT gene expressions. Values are means ± standard error (SD). Different lowercase letters indicate significant differences between treatments. *P≤0.05.

Histological study: In comparing gene expressions, several distinctive features and changes can be observed from studying the testicular tissue of selenium-treated poultry using a TEM (Figure 6). The irregular shapes in T1 within the nucleus may indicate structural changes resulting from oxidative stress caused by decreased SOD3, increased free radicals, and ineffective apoptosis. These irregular shapes may be the result of changes in the chromatin structure or components of the nucleus due to accumulated stresses. Also, the increase in IL-1B may reflect an inflammatory response that interacts with oxidative stress, leading to overlapping effects on the structure of cells and the nucleus.

In general, these changes reflect a state of cellular stress with an inflammatory response, which can impair the stability and structure of the nucleus, and increase the likelihood of the appearance of abnormal cells. The increased expressions of NOX, Gpx3, caspase-3 and AGT genes in the selenium diet treatment (T2) indicate the cellular response to high oxidative stress, which lead to DNA damage and high activity in the nucleus to repair the damage, appearing as dense granules inside the nucleus.

The increased FasL and lower Fas appears to enhance apoptosis mechanisms in neighboring damaged cells without affecting the cells themselves. This leads to chromatin condensation inside the nucleus and promotes the appearance of granules as the cell prepares for death. Thus, these changes reflect an adaptive response of the cell to oxidative damage and increased cell death activity, which leads to chromatin reorganization and the appearance of dense granules as a sign of this adaptation. In the T3 selenium-water treatment, the changes indicate a state of readiness for cell death, but due to the increase in SOD3 and AKT, the cells were able to protect themselves from potential damage and not enter the full programmed cell death pathway. The absence of granules in the nucleus indicates that the defense mechanisms succeeded in maintaining chromatin stability, despite the presence of warning signals.

The results from increasing selenium in the feed and water (T4) revealed a state of inflammatory response with a decrease in cell death pathways. The decrease in caspase-3, SOD3, AKT and AGT suggests a deficiency in protective mechanisms, but the absence of granules in the nucleus indicates that oxidative damage is not yet sufficient to induce cell death or affect chromatin stability.

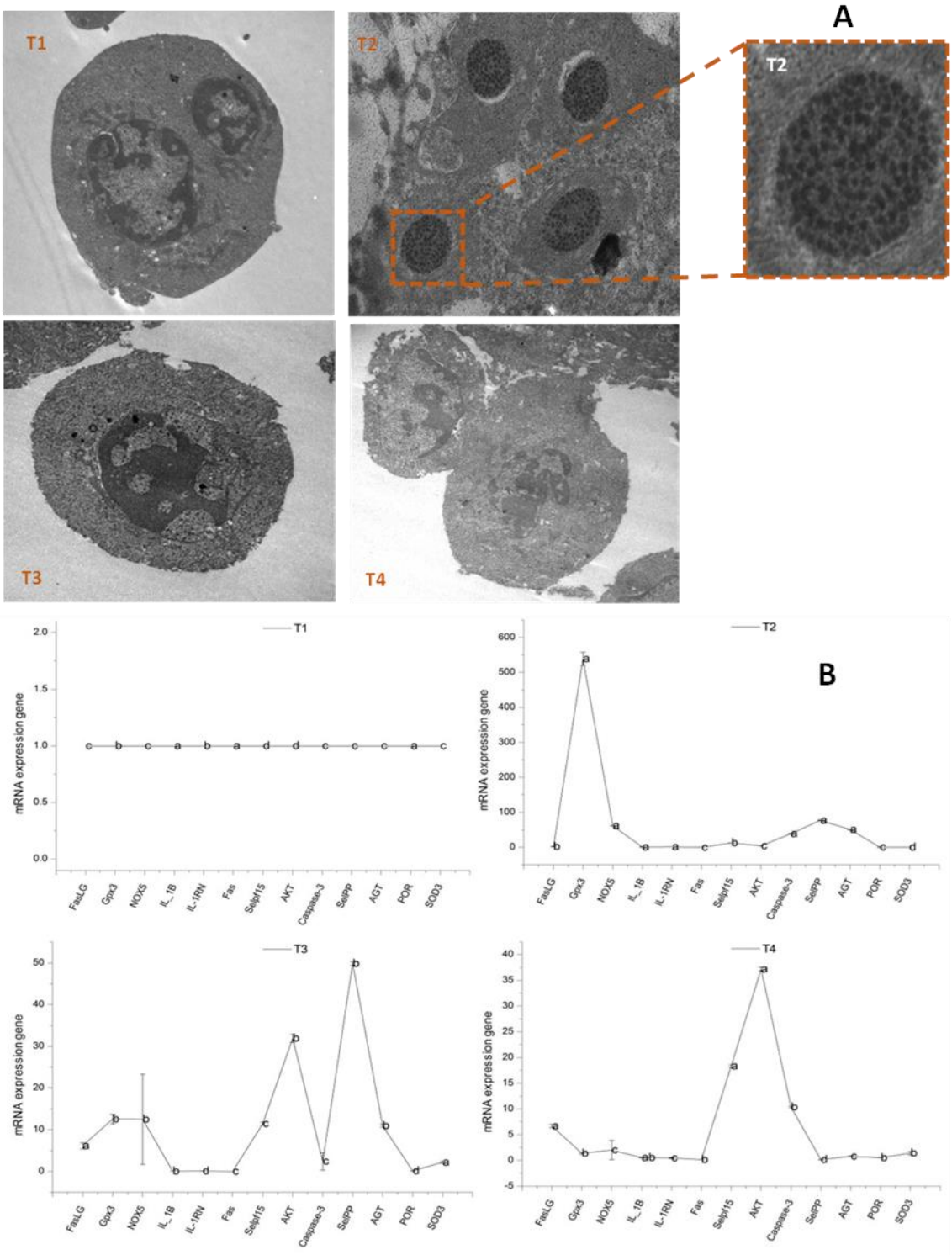

Fig. 6: TEM images of a cross-section of testicular tissue showing the effect of selenium supplementation on spermatids in chicken testes.

(A) Compared with the control group, the TEM image of selenium treatments shows a difference in the distribution and density of chromatin granules within the nucleus. (B) Shows the gene expression profile of all studied genes for each treatment group: T1 - control group; T2 - basal diet containing +0.3 mg

inorganic selenium/kg and untreated water; T3 - standard feed and treated water (250 ppm selenium solution); and T4 - represented by adding selenium to the water (250 ppm selenium solution) and the experimental feed (basic feed containing + 0.3 mg inorganic selenium Se/kg).

Discussion: Selenium (Se) is a crucial trace element in the diet that has a key role in regulating various physiological functions in humans, livestock, and poultry by being incorporated into a variety of selenoproteins. A global issue is the low selenium content in primary feed ingredients, which has led to the common practice of supplementing poultry and livestock diets with the element. Recent studies have shown that sodium selenite, which has been used as a feed supplement for the past 40 years, is not the most effective form of selenium. In contrast, incorporating organic selenium into animal and poultry diets can better meet its requirements while supporting high levels of immune competence and enhancing both productive and reproductive performance (6 and 27).

The obtained results reveal significant improvement in the testicular structure of the selenium-supplemented group when compared to the control. The TEM images show reduced inflammatory responses induced by IL-1β in the testicular tissues and higher IL-1RN activity, which suppresses inflammation. This is evidenced by improvements in the mitochondria structures, cell membranes, and nuclei in testicular cells (4).

SelP15 and SelPP proteins are associated with increased antioxidant protection in testicular tissue. TEM images show these proteins, in conjunction with increased selenium levels, playing a vital role in improving the structure of mitochondria, cell membranes, and nuclei. Testicular cells are shown to be in excellent condition with completely intact mitochondria. Cell membranes appear intact, and the nucleus contains compact chromatin with no signs of damage. Cell vacuoles are almost absent, indicating the optimal health of the cells. Higher selenium levels enhance the ability of SelP15 and SelPP to protect testicular tissues from oxidative stress while lowering the activation of the Fas/FasL apoptosis pathway. This improvement is shown in the TEM images as a restoration of the structure of mitochondria, cell membranes, and nuclei, and reduced signs of damage or apoptosis (Fig. 6).

Increased selenium levels also enhance POR and AKT activity in testicular tissue, leading to improved metabolic performance and protection from oxidative stress. The TEM images show significant improvements in mitochondria structures, cell membranes, and nuclei from selenium supplementation reflecting positive effects on testicular tissue in each treatment group.

Previous studies have reported that sodium selenite protects testicular gametogenic and spermatogenic disorders against carbimazole and prevents testicular oxidative stress by increasing the antioxidant status (12). Khalid et al. (16) reported that supplementing selenium to chicken testis reduced testicular germ cell apoptosis activity (8). Another study observed a notable decrease in the apoptotic index of spermatogenic cells after scrotal hyperthermia in selenium-supplemented mice (7).

Furthermore, a recent study by Zaki et al. (26) showed that selenium led to a decrease in MDA and an increase in the activities of SOD and GPx (26). Also, Ahmad et al. reported that changes in antioxidant enzyme activities (CAT, SOD) are related to the overexpression of the selenoprotein iodothyronine deiodinase, thus leading to the high-level production of reactive oxygen species (1). Some studies suggest that the stimulation of antioxidant activity by selenium may be related to the effects of GH and

IGF in vitro (16), which would promote oxidation and diminish GPx activity when they act as somatic growth inductors. Thus, selenium and GPx, among other selenoproteins, may play a crucial role in biological development by preventing concomitant oxidation.

Conclusions

This study showed that increased levels of selenium in the diets of male poultry boosted antioxidant enzyme activity, enhanced immunity and apoptosis, as well as improved metabolism. These findings provide further evidence of the positive effects of increased selenium levels on the testicular growth of poultry.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Ahmed Khalid: methodology, writing—original draft preparation; Nagam Khudhair, Sherif Melak, Zheng Peng writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding:

This research received no external funding.

Institutional Review Board Statement:

The study was conducted using the protocol authorized by the Department of Animal Genetics, Breeding, and Reproduction, College of Animal Science and Technology, Northeast Agricultural University, Harbin, China and the Department of Animal Production, College of Agriculture, Tikrit University, Iraq.

Informed Consent Statement:

No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

Acknowledgments:

This work was supported by the Department of Animal Genetics, Breeding, and Reproduction, College of Animal Science and Technology, Northeast Agricultural University, Harbin, China and the Department of Animal Production, College of Agriculture, Tikrit University, Iraq.

Disclaimer/Journal's Note:

The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of AJAS and/or the editor(s). AJAS and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

References

1. Ahmad, H., Tian, J., Wang, J., Khan, M. A., Wang, Y., Zhang, L., and Wang, T. (2012). Effects of dietary sodium selenite and selenium yeast on antioxidant enzyme activities and oxidative stability of chicken breast meat. Journal of

Agricultural and Food Chemistry, 60(29): 7111-7120. https://doi.org/10.1021/jf3017207.

2. Cao, Z., Huang, W., Sun, Y., and Li, Y. (2020). Deoxynivalenol induced spermatogenesis disorder by blood-testis barrier disruption associated with testosterone deficiency and inflammation in mice. Environmental Pollution, 264: 114748. https://doi.org/10.1016/j.envpol.2020.114748.
3. Cordeiro, L., Lin, H. L. H., Carvalho, A. V., Grasseau, I., Uzbekov, R., and Blesbois, E. (2021). First insights on seminal extracellular vesicles in chickens of contrasted fertility. Reproduction, 161(5): 489-498. https://doi.org/10.1530/REP-20-0462.
4. Fan, R., Yao, H., Cao, C., Zhao, X., Khalid, A., Zhao, J., Zhang, Z., and Xu, S. (2017). Gene silencing of selenoprotein K induces inflammatory response and activates heat shock proteins expression in chicken myoblasts. Biological Trace Element Research, 180: 135-145. https://doi.org/10.1007/s12011-017-0979-1.
5. Goldman, R. H., Racowsky, C., Farland, L. V., Munné, S., Ribustello, L., and Fox, J. H. (2017). Predicting the likelihood of live birth for elective oocyte cryopreservation: a counseling tool for physicians and patients. Human Reproduction, 32(4): 853-859. https://doi.org/10.1093/humrep/dex008.
6. Ibrahim, D., Kishawy, A. T., Khater, S. I., Hamed Arisha, A., Mohammed, H. A., Abdelaziz, A. S., El-Rahman, G. I., and Elabbasy, M. T. (2019). Effect of dietary modulation of selenium form and level on performance, tissue retention, quality of frozen stored meat and gene expression of antioxidant status in ross broiler chickens. Animals, 9(6): 342. https://doi.org/10.3390/ani9060342.
7. Kaur, S., and Bansal, M. P. (2015). Protective role of dietary-supplemented selenium and vitamin E in heat-induced apoptosis and oxidative stress in mice testes. Andrologia, 47(10): 1109-1119. https://doi.org/10.1111/and.12390.
8. Khalid, A., Khudhair, N., He, H., Peng, Z., Yaguang, T., and Guixue, Z. (2016). Effects of dietary selenium supplementation on seminiferous tubules and SelW, GPx4, LHCGR, and ACE expression in chicken testis. Biological Trace Element Research, 173: 202-209. https://doi.org/10.1007/s12011-016-0646-y.
9. Li, M., Zhang, Y., and Li, S. (2020). Effects of selenium deficiency on testis development and autophagy in chicks. Italian Journal of Animal Science, 19(1): 753-761. https://doi.org/10.1080/1828051X.2020.1786739.
10. Liu, L., Yang, B., Cheng, Y., and Lin, H. (2015). Ameliorative effects of selenium on cadmium-induced oxidative stress and endoplasmic reticulum stress in the chicken kidney. Biological Trace Element Research, 167: 308-319. https://doi.org/10.1007/s12011-015-0314-7.
11. Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods, 25(4): 402-408. https://doi.org/10.1006/meth.2001.1262.
12. Long, M., Yang, S., Wang, Y., Li, P., Zhang, Y., Dong, S., Chen, X., Guo, J., He, J., Gao, Z., and Wang, J. (2016). The protective effect of selenium on chronic zearalenone-induced reproductive system damage in male mice. Molecules, 21(12): 1687. https://doi.org/10.3390/molecules21121687.

13. Mao, B., Yan, M., Li, L., and Yan Cheng, C. (2018). Blood-testis Barrier, in Encyclopedia of Reproduction, eds M. K. Skinner, C. Simon, and C. Niederberger (Amsterdam: Elsevier), 152-160. https://doi.org/10.1016/B978-0-12-801238-3.64572-5.
14. Mohammed, T. T., and Hamad, E. H. (2024). Effect of adding natural zeolite and vitamin e to laying hans diets on some productive traits during the summer season. Anbar Journal of Agricultural Sciences, 22(1): 501-516. https://doi.org/10.32649/ajas.2024.183746.
15. Nafea, H., H., and Ahmed, M. Th. (2024). Individual and synergistic effect of the addition of astaxanthin and magnolol compared with vitamin e to the diet of laying hens on some physiological traits and oxidation indicators of stored eggs. Anbar Journal of Agricultural Sciences, 22(1): 719-734. https://doi.org/10.32649/ajas.2023.141634.1057.
16. Ojeda, M. L., and Nogales, F. (2022). Dietary selenium and its antioxidant properties related to growth, lipid and energy metabolism. Antioxidants, 11(7): 1402. https://doi.org/10.3390/antiox11071402.
17. Shi, L., Zhao, H., Ren, Y., Yao, X., Song, R., and Yue, W. (2014). Effects of different levels of dietary selenium on the proliferation of spermatogonial stem cells and antioxidant status in testis of roosters. Animal Reproduction Science, 149(3-4): 266-272. https://doi.org/10.1016/j.anireprosci.2014.07.011.
18. Stanton, P. G. (2016). Regulation of the blood-testis barrier. In Seminars in Cell and Developmental Biology, 59: 166-173. https://doi.org/10.1016/j.semcdb.2016.06.018.
19. Suchý, P., Straková, E., and Herzig, I. (2014). Selenium in poultry nutrition: a Review. Czech Journal of Animal Science, 59(11): 495-503. DOI: 10.17221/7730-CJAS.
20. Surai, P. F., and Earle-Payne, K. (2022). Antioxidant defences and redox homeostasis in animals. Antioxidants, 11(5): 1012. https://doi.org/10.3390/antiox11051012.
21. Surai, P. F., and Speake, B. K. (1998). Distribution of carotenoids from the yolk to the tissues of the chick embryo. The Journal of Nutritional Biochemistry, 9(11): 645-651. https://doi.org/10.1016/S0955-2863(98)00068-0.
22. Watson, M. L. (1958). Staining of tissue sections for electron microscopy with heavy metals: II. Application of solutions containing lead and barium. The Journal of Cell Biology, 4(6): 727-730. https://doi.org/10.1083/jcb.4.6.727.
23. Yang, Z., Liu, C., Zheng, W., Teng, X., and Li, S. (2016). The functions of antioxidants and heat shock proteins are altered in the immune organs of selenium-deficient broiler chickens. Biological Trace Element Research, 169: 341-351. https://doi.org/10.1007/s12011-015-0407-3.
24. Yao, H. D., Wu, Q., Zhang, Z. W., Li, S., Wang, X. L., Lei, X. G., and Xu, S. W. (2013). Selenoprotein W serves as an antioxidant in chicken myoblasts. Biochimica et Biophysica Acta (BBA)-General Subjects, 1830(4): 3112-3120. https://doi.org/10.1016/j.bbagen.2013.01.007.
25. Yao, H. D., Wu, Q., Zhang, Z. W., Zhang, J. L., Li, S., Huang, J. Q., Ren, F. Z., Xu, S. W., Wang, X. L., and Lei, X. G. (2013). Gene expression of endoplasmic

reticulum resident selenoproteins correlates with apoptosis in various muscles of se-deficient chicks. The Journal of Nutrition, 143(5): 613-619. https://doi.org/10.3945/jn.112.172395.

26. Zaki, M. S. A., Haidara, M. A., Heitham, M., Asim, A., Massoud, E. E. S., and Eid, R. A. (2020). Antioxidant activity of selenium on bisphenol-induced apoptosis and testicular toxicity of Albino rats. International Journal of Morphology, 38(6): 1786-1796. http://dx.doi.org/10.4067/S0717-95022020000601786
27. Zheng, S., Zhao, J., Xing, H., and Xu, S. (2019). Oxidative stress, inflammation, and glycometabolism disorder-induced erythrocyte hemolysis in selenium-deficient exudative diathesis broilers. Journal of Cellular Physiology, 234(9): 16328-16337. https://doi.org/10.1002/jcp.28298.