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Selenium with feed and/or water upregulates gene expression in Liver tissues related to immune response, programmed cell death, antioxidant and metabolism in broilers

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ABSTRACT

Selenium is a vital nutrient for poultry, crucial for immune system regulation and function. We investigated the effects of dietary selenium (Se) supplementation on the expression genes of an immune response, selenoprotein P, programmed cell death, Antioxidants, and metabolism genes in the development of chicken liver. 400 chicks (broiler) of males were used and birds were divided equally across 4 diet treatments as 100 birds for each treatment. The control first group (T1) was fed a standard diet, the second experiment group (T2) was fed the experimental diet (a basic diet containing + 0.**4** mg inorganic selenium Se/kg) and nontreated water, the third experiment group (T3) added selenium to water (standard diet and treated water (**300**ppm) Solution selenium), and the fourth experiment group (T4) added selenium to water (Solution selenium **300**ppm) and to fed the experimental diet (basic diet containing + 0.**4**mg inorganic selenium Se/kg). The liver was collected individually after 6 weeks of feeding. The results indicated that IL-1β gene expression increased in T4 and the SePP1 gene increased in T3, as a significant increase in the Fas and FaslG genes in T4 and T3 respectively. Antioxidants and metabolic genes also increased in the T4 and T3 respectively. Therefore, these results indicate that nutritional supplements containing selenium especially when given with water or with water and feed, improve the immune response, apoptosis, antioxidants, and metabolic genes in chicken liver tissue.

دور السيلينيوم المضاف مع العلف/ او الماء في تنظيم التعبير الجيني في أنسجة الكبد واثرها التنظيمي على الاستجابة المناعية وموت الخلايا المبرمج ومضادات للأكسدة والتمثيل الغذائي في فروج اللحم

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الخلاصة

السيلينيوم هو عنصر غذائي حيوي للدواجن، ضروري لتنظيم الجهاز المناعي ووظيفته. ، قمنا بالتحقيق في آثار مكملات السيلينيوم الغذائية (Se) على جينات التعبير عن الاستجابة المناعية، والسيلينوبروتين P، والموت الخلوي المبرمج، ومضادات الأكسدة، وجينات التمثيل الغذائي في نمو كبد الدجاج. تم استخدام 400 فرخ (دجاج لاحم) من الذكور وتم تقسيم الطيور بالتساوي على 4 معاملات غذائية بواقع 100 طائر لكل معاملة. تم تغذية المجموعة الضابطة الأولى (T1) على غذاء قياسي، وتم تغذية المجموعة التجريبية الثانية (T2) على الغذاء التجريبي (غذاء أساسي يحتوي على + 0.4 ملغ سيلينيوم غير عضوي Se/كجم) وماء غير معالج، وتم اضافة السيلينيوم إلى الماء للمجموعة التجريبية الثالثة (T3) (غذاء قياسي وماء معالج (300 جزء في المليون) محلول سيلينيوم)، كذلك تم اضافة السيلينيوم إلى الماء للمجموعة الرابعة (T4) (محلول سيلينيوم 300 جزء في المليون) وتم تغذيتها على الغذاء التجريبي (غذاء أساسي يحتوي على + 0.4 ملغ سيلينيوم غير عضوي Se/كجم). وتم جمع الكبد بشكل فردي بعد 6 أسابيع من التغذية. وأشارت النتائج إلى أن التعبير الجيني IL-1β زاد في T4 وزاد تعبير جين SePP1 في T3، كما زادت بشكل ملحوظ في تعبير جينات Fas وFaslG في T4 وT3 على التوالي. كما زادت مضادات الأكسدة والجينات الأيضية في T4 وT3 على التوالي. لذلك، تشير هذه النتائج إلى أن المكملات الغذائية المحتوية على السيلينيوم وخاصة عند إعطائها مع الماء أو مع الماء والعلف، تعمل على تحسين الاستجابة المناعية، وموت الخلايا المبرمج، ومضادات الأكسدة، والجينات الأيضية في أنسجة كبد الدجاج.

الكلمات المفتاحية: السيلينيوم، الدجاج، الاستجابة المناعية، موت الخلايا المبرمج، مضادات الأكسدة، جينات التمثيل الغذائي

INTRODUCTION

Oxidative stress is defined as an imbalance between antioxidants and pro-oxidants. The production of reactive oxygen species (ROS) exceeds the body's ability to remove them. When ROS are present in excess of what animal cells can take up as antioxidants, oxidative stress results. As part of normal metabolism, the body constantly produces ROS (Mishra & Jha, 2019). Selenium's role in antioxidants, its distinct redox characteristics, and its utilisation in enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPX) all contribute to its importance (Brenneisen, Steinbrenner, and Sies 2005). According to Reddi and Bollineni (2001) and Sakiyama *et al*. (2016), fatty liver can occur when selenium deficiency reduces the expression of intracellular antioxidant enzymes such Superoxide dismutase^{-1} (SOD1). In

addition to its anti-inflammatory and redox-balancing functions, the trace element selenium (Se) is vital to the immune system (Guillin *et al*. 2019). An important pro-inflammatory cytokine involved in this inflammatory process is interleukin (IL1). In addition to IL1A and IL1B, the IL1 receptor antagonist (IL1RA) is the only cytokine in the IL1 family that acts as an antagonist (Briens *et al*., 2013). Inflammatory factors IL1A and IL1B are generated by distinct cell types in reaction to diverse stimuli. They have impacts on endothelial cells, such as prothrombotic effects and adhesion molecule activation, and naturally occurring competitive IL1RA may counteract the immunological response.

In addition, Apoptosis is one of the most common ways to remove unwanted cells, which occurs in the body of multicellular and even monocellular organisms. In this process, the cell is responsible for its own death; thereby, it is also called cell suicide. In the review of the effects of selenium on apoptosis, researchers concluded that selenium is effective in reducing apoptosis (Fang *et al.*, 2018). Early documents have shown that selenium could have a protective effect on the decreased expression of death receptors pathway-related molecules such as FAS, FASL, TNF-α, and CASPASE-3 (Miao *et al.* 2013; Valadbeygi *et al*., 2016; Wan *et al.* 2018).

Selenium is a vital nutrient for poultry, crucial for immune system regulation and function. It controls oxidative stress, redox mechanisms, and key cellular processes in immune responses via selenoproteins (Zhang *et al*., 2011; Zubair *et al*., 2023) among other key roles.

Selenium proteins such as the glutathione peroxidase (GPx) family (including GPx1, GPx2, GPx3, and GPx4), selenophosphate synthetase 2 (SPS2), selenoprotein (Sep) n1, Sepw1, Sepx1, and Sep15) are selenium-containing proteins that are closely related to the biological functions of selenium (Yang *et al*., 2016). Several investigations have demonstrated that hens' organs and tissues were damaged due to Se deprivation. The primary processes were oxidative stress, inflammation, and apoptosis.

Selenoprotein synthesis is affected by dietary selenium supplementation amounts and its nutritional type (Dalia *et al*., 2017; Zhang *et al*., 2013). Some researchers think that selenium's biological effects are due to the amino acid selenocysteine, which selenium incorporates into selenoproteins. One of these rare proteins is selenoprotein P (SEPP1), which stands out from the others because it has more than one selenocysteine residue per molecule. Many tissues express seleniprotein P; however, hepatocytes produce and release the most SEPP1 into the bloodstream (Schweizer *et al*., 2005). The fact that SEPP1 contains almost half of the selenium

in plasma raises the possibility that this protein is involved in the transfer of selenium (Motsenbocker and Tappel 1982). Selenoprotein synthesis is affected by dietary selenium supplementation amounts and its nutritional type (Dalia *et al*., 2017; Zhang *et al*., 2013). Selenoprotein expression in animal tissues has been linked to dietary selenium supplementation in a large number of studies. The expression of GPX4 Mrna was discovered to be down-regulated in chicken liver when exposed to high Se (Zoidis *et al*., 2010). According to Sun *et al*. (2011), broilers that were given a diet enriched with sodium selenite for 90 days demonstrated an increase in SELENOW1 liver mRNA .

Therefore, the study aimed to find out the effect of an increase in selenium in the diet and water on the gene expression of some genes affecting the liver in chickens.

MATERIALS AND METHODS

Experimental Birds, Diets, and Tissue Sampling

Ross 308 hens Roosters, which were deemed healthy for this investigation, were procured from the Experimental Chicken. From the time they were six weeks old; four hundred hens were housed in cages and randomly assigned to one of four groups. Each group received five treatments, with twenty chicks each replicate. First, the control group (T1) received a standard diet. Second, the experimental diet (basic diet with + 0.4 mg inorganic selenium Se/kg) and non-treated water were given to the second group (T2). Third, a solution of 300 ppm selenium was added to the water in the third group (T3), which also received the experimental diet (basic diet with + 0.4 mg inorganic selenium Se/kg). Fourth, a solution of 300 ppm selenium was added to the water in the fourth group (T4). The experimental diet and water treatment were continued as before. After six weeks of feeding, ten roosters were chosen at random from each group. After euthanasia and liver removal, the birds were weighed, frozen in liquid nitrogen, and stored at -80 °C for the purpose of real-time polymerase chain reaction (RT-PCR) study. This study followed all protocols established by Northeast Agricultural University's Institutional Animal Care and Use Committee, including the collection of samples and execution of experiments.

RNA Extraction and Real-Time PCR

Isolation of RNA from frozen liver tissues was accomplished using the Trizol/chloroform/isopropanol method in accordance with the instructions provided by the manufacturer (Invitrogen Corporation, Carlsbad, CA, USA). For the following PCR reactions,

which were conducted with 0.24 μmol/l of each sense and antisense primers, 0.06 mmol/l of rTaq polymerase, 0.8 mmol/l of deoxynucleotide mixes, and 10× PCR buffer, RT-PCR and PCR were utilised to produce cDNA from this process. The following steps were taken to conduct the PCR reaction: 5 minutes of predenaturation at 94 °C, 30 seconds of denaturation at 94 °C, 30 seconds of annealing at 61.5 °C, 40 seconds of extension at 72 °C, 30 cycles of extension at 72 °C and 4 °C. A 1.5% agarose electrophoresis gel was used for the analysis of the data. Primers and β-actin were designed using the Primer Premier Software (Oligo, China) according to known sequences (Table 1). Applied Biosystems's PRISM 7500 real-time PCR machine was used to conduct the quantitative polymerase chain reaction (q-PCR).

An ABI PRISM 7500 Detection System (Applied Biosystems, USA) was used to conduct the quantitative real-time PCR. The reactions were carried out in a 20-milliliter reaction mixture that included 10 milliliters of a 2× SYBR Green I PCR Master Mix (TaKaRa, China), 2 milliliters of diluted cDNA, 0.4 milliliters of each primer (10 mM), 0.4 milliliters of 50× ROX reference dye II, and 6.8 milliliters of PCR-grade water. The mixture was heated to 95 °C for 30 seconds, followed by 40 cycles of a 2-temperature program, with 5 seconds at 95 °C and 20 seconds at 60 °C. Reference gene β-actin was utilized, and Table 1 displays the oligonucleotide sequences of the forward and reverse primers for the target genes. Livak and Schmittgen (2001) used the delta-delta Ct ($2^{\Delta\Delta}$Ct) approach to express the relative quantification as a ratio of the target gene to the control gene.

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	TCTCGGTGTGAACATTGCG AGTGTCTGAAGTTGAAGTAC	NM_001199487	81
AGT	CCAAAAGCAAAGGCCAAAGC CGAACATCCACTGCAACCA	XM_419584.6	79
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	NM_001031609.2	91

AKT (B)	CCCTCATTCTCTAACTTCACTC ACAGAACTCACGGCATCCA CCCGGTCTTCAGAAAATACACGC	NM_205055.1	143
β-actin	CCGAGAGAGAAATTGTGCGTGAC TCGGGGCACCTGAACCTCTC	L08165	166

RESULTS AND DISCUSSION

The results of Figures (1A and B) showed the effect of increasing selenium on the immune response of chickens. Figure 1A shows that increasing the use of selenium added through feed or water and feed (T2+T4) led to an increase in the gene expression of the IL-1β interleukin ($P \leqslant 0.05$, $P \leqslant 0.01$), while the gene expression of the IL-1RN gene decreased significantly ($P \leqslant 0.05$) when it was increased added through feed or water or water and feed.

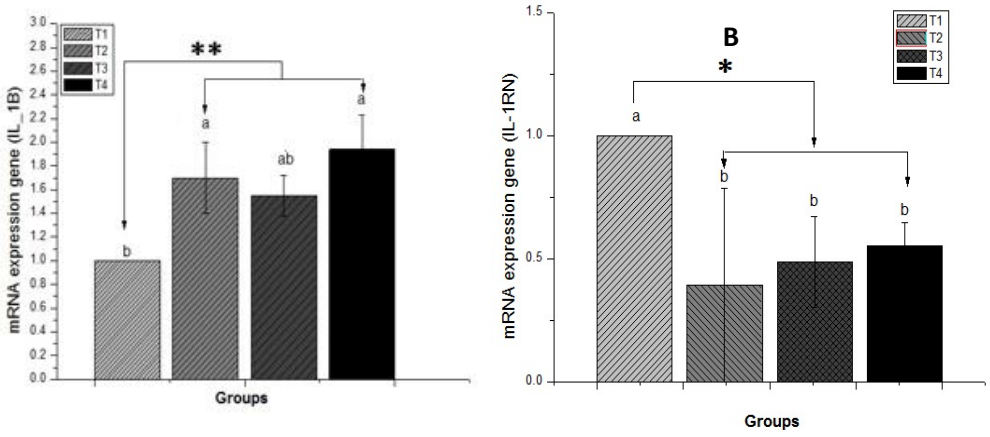

Figure 1: (A and B): Effect of increased selenium supplementation on gene expression of immune responses in Liver tissues in chickens. (**A**) the gene expression of IL-1β. (**B**) the gene expression of the IL-1RN gene. Different lowercase letters indicate significant differences between treatments. (∗$P \leqslant 0.05$, ∗∗$P \leqslant 0.01$)

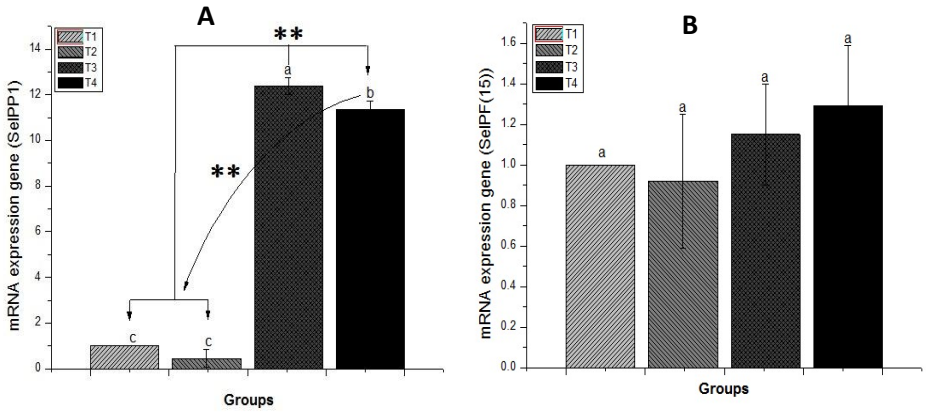

Figure 2: (A and B)**:** Effect of increased selenium supplementation on gene expression of selenoprotein P in Liver tissues in chickens. (A) the gene expression of SelPP1. (B) the gene expression of the SelPF15 gene. Different lowercase letters indicate significant differences between treatments. (**P ⩽ 0.01)

Figures (2 A and B) indicate a highly significant difference (P ⩽ 0.01) in the readiness of selenium through adding water and increasing the gene expression of the SelPP1 gene, while there were no significant differences in measuring the expression of the SelPF15 gene in all treatments as showed in figure (2 B). The results of Figure 3 (A and B) show the effect of increased selenium on Apoptosis, where Increasing the availability of selenium by adding it to drinking water leads to an increase in the gene expression of the Fas, FaslG genes, while there was no significant effect of increasing selenium through adding it to the diet on the gene expression of the Fas, FaslG genes.

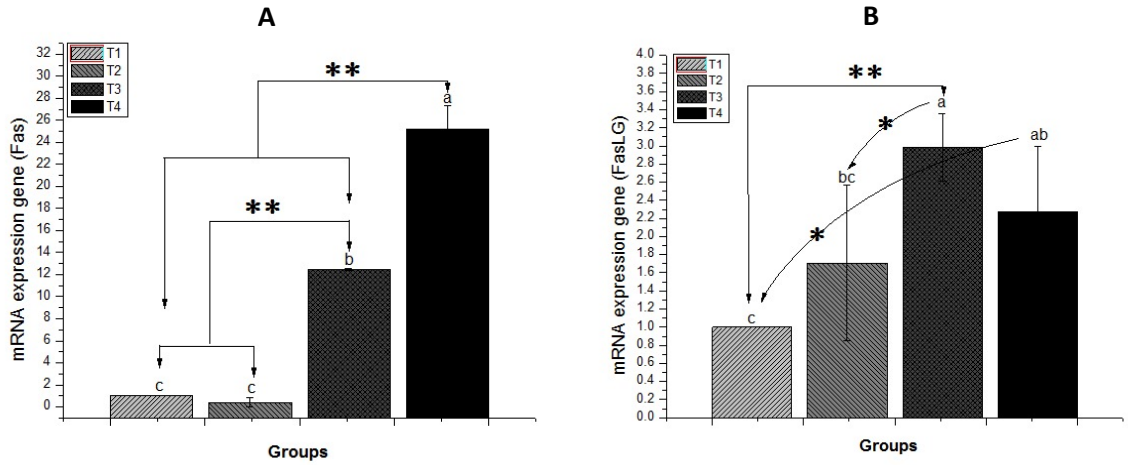

Figure 3 (A and B)**:** Effect of increased selenium supplementation on gene expression of Apoptosis in Liver tissues in chickens. (**A**) the gene expression of Fas. (**B**) the expression of the FaslG gene. Different lowercase letters indicate significant differences between treatments. (*P ⩽ 0.05, **P ⩽ 0.01)

The results of Antioxidants are shown in Figure 4 (A, B, and C). The result of the figure (Fig 4 A) showed that increasing the preparation of selenium through feed or water only (T2+ T3) and in a limited amount led to a reduction in the gene expression of the gene SOD, while this effect was lost when the amount of preparation was increased by adding it to the feed and water (T4). As for its effect on the NOX5 gene, it was highly significant when added to feed or feed and water (T2+T4) and selenium did not have a significant effect on gene expression when added to water compared to the control treatment (Fig 4 B) . Also, in Figure 4 C the result of the AGT gene was non-significant in the control treatment compared with the rest treatments but significantly decreased in the diet treatment.

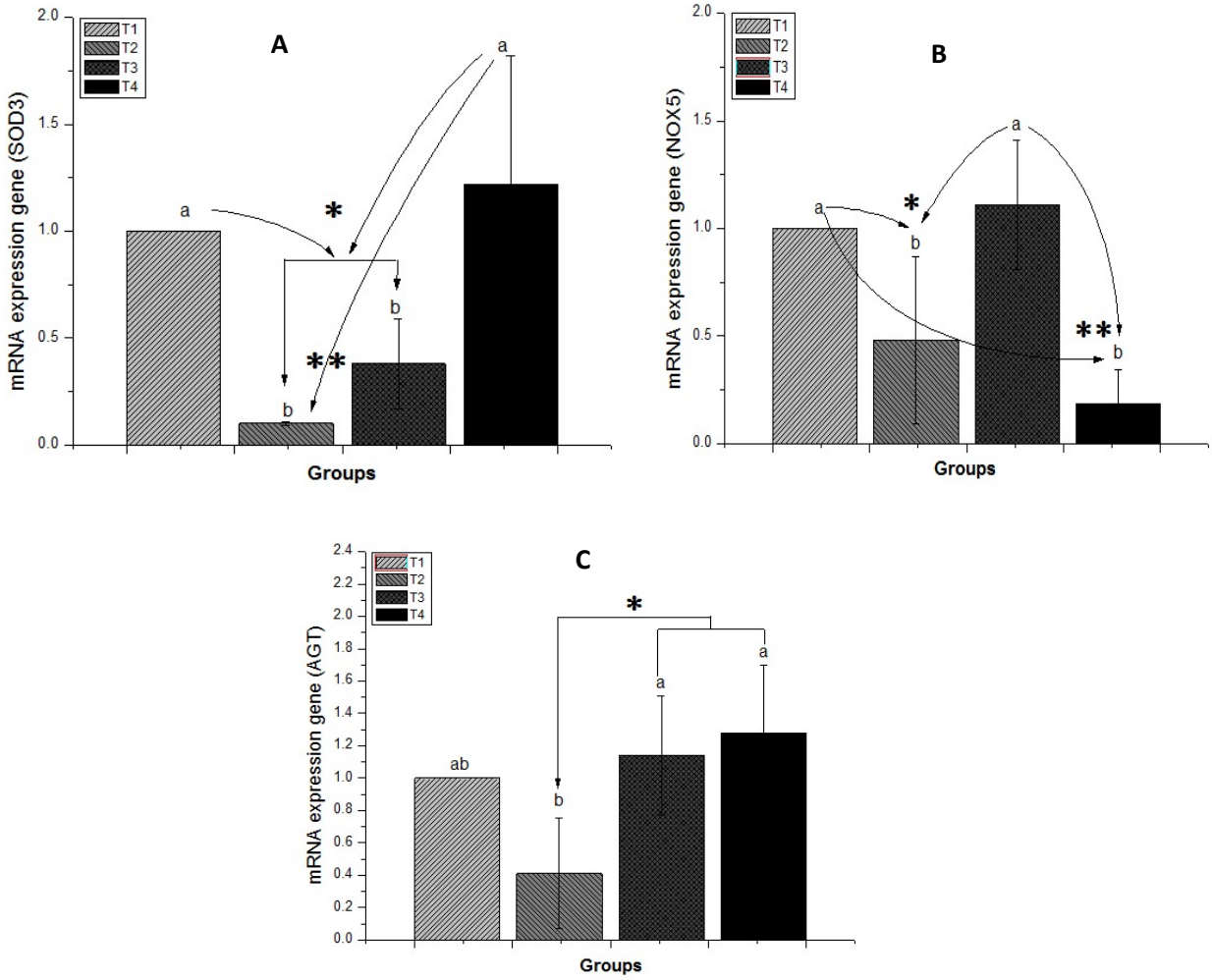

Figure 4 (A, B, and C): Effect of increased selenium supplementation on gene expression of Antioxidants in Liver tissues in chickens. (**A**) the gene expression of SOD3. (**B**) the expression of the NOX5 gene. (**C**) the gene expression of AGT. Different lowercase letters indicates significant differences between treatments. (∗P ≤ 0.05, ∗∗P ≤ 0.01).

The results of Figure 5 (A and B) showed an increase in metabolism genes. Where (Fig 5 A) shows that increasing the availability of selenium through feed (T2) led to a significant reduction in the gene expression of the POR gene while increasing the availability of selenium through water or diet and water did not affect the gene expression of the POR gene . while the gene expression decreased significantly in gene AKT when adding selenium through feed (T2) and increased significantly when adding it in water (T3) compared to the control treatment (T1), and it also increased significantly when selenium was increased with water and feed (T4) compared to the treatment treated through diet only (T2).

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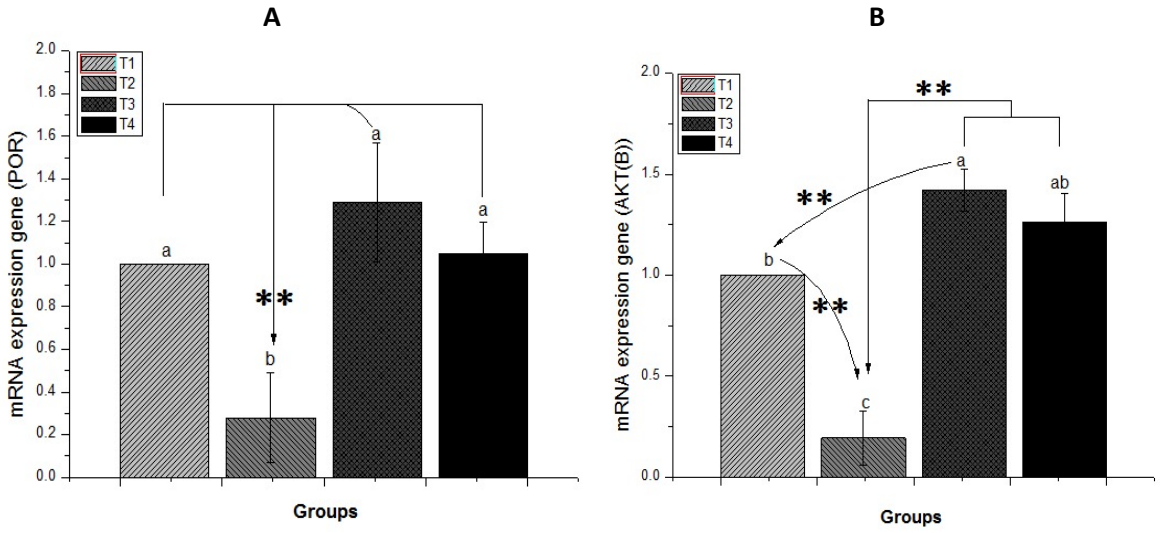

Figure 5 (A and B): Effect of increased selenium supplementation on gene expression of metabolism genes in Liver tissues in chickens. (**A**) the gene expression of POR. (**B**) the expression of the AKT gene. Different lowercase letters indicate significant differences between treatments. (∗P ⩽ 0.05, ∗∗P ⩽ 0.01).

Correlation matrix based on coefficients of Spearman rank correlation were performed using gene expression data (figure 6.A). The correlation coefficients between genes ranged between low to unity. The highest positive correlation coefficients were found between AKT (B) and POR (0.99), SeIPF (15) and Fas (0.98) and AKT (B) and AGT (0.96), whereas the highest negative correlation was found between IL_1B and IL-1RN (-0.83). The lowest positive correlation coefficients were found between FasLG and NOX5 (0.03), and AGT and IL_1B (0.05), whereas the lowest negative correlation was found between AKT (B) and IL_1B (-0.02), and between SelPP1 and NOX5 (0.05-).

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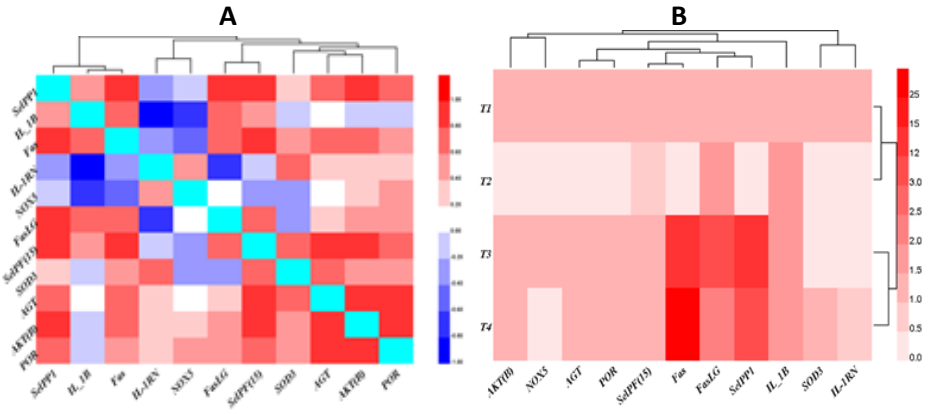

Figure 6. (A) Heat map with clustering analyses showing the pairwise Spearman correlations between genes, (B) Heat map with clustering analyses showing the pairwise gene expression between genes and treatments

For gene expression, the heat map of all pairwise gene expression between genes and treatments were constructed and visualized in Figure 6.B with their clustering analyses. The highest gene expression was found for SelPP1 (12.38 and 11.34) and Fas (12.46 and 25.22) genes in treatment three and four, respectively, whereas the lowest gene expression was found for SOD3 gene (0.1) in treatment two. The clustering analysis showed that treatment two is so close to control and treatment three is so closed to treatment four.

In this study, we assessed the effects of adding Se to diets and water on levels of selected liver genes to better define the potential roles of each in chicken immune response, antioxidants, and apoptosis. We found that increasing selenium in the water and diet affected the studied genes. Selenium is absorbed from drinking water faster because it dissolves in water, making it directly available for absorption in the digestive system. The selenium concentration in drinking water can also be more easily adjusted to ensure accurate dosing, reducing the risk of over- or under-dosing .While selenium added to the feed ensures that poultry ingest the element with every meal, providing a continuous and consistent level of supplementation. Therefore, combining the two methods can ensure permanent and continuous availability of selenium in water and food, enhancing overall absorption and ensuring optimal levels.

These findings corroborate previous research showing that Se administration promotes better skeletal and muscular development (Ruan *et al*., 2012). According to Huang, Rose, and Hoffmann (2012), seleniproteins impact immunity in numerous ways. Additionally, selenium supplementation controls selenoprotein gene expression (Zoidis *et al*., 2010). Additionally, the study sheds light on selenium's function and how it works by coordinating with natural killer cells. The impact of excessive selenium dosage is controversial, however adequate and moderate doses are widely believed to sustain a normal, fully functional immune system.

Se may raise IL-2R expression on T cells and improve T cell responses, leading some writers to speculate that supplementation may "boost" cellular immunity (McKenzie *et al*., 1998). Reducing soluble IgA quantities in the duodenal mucosa and increasing levels of pro-inflammatory cytokines interleukin-1β (IL-1β), IL-17A, and interferon-gamma (IFN-γ) were seen in commercial broilers with low Se content, which may also impact intestinal mucosal immunity. Alternatively, according to Liu *et al*. (2016), there was a considerable suppression of anti-inflammatory cytokines such TGF-β1 and IL-10. Researchers Mahmoud *et al*. (2016) found that at both high ambient and thermos neutral temperatures, Nano-Se feeding enhanced the mRNA expression of cytokine genes (interleukins 2 and 6). Nutritional supplements with

0.4 mg/kg of food of Nano-Se, on the other hand, may improve the antioxidant or immunological qualities of broilers maintained in hot environments, therefore enhancing their growth performance.

Antioxidant enzymes control the immune system, and selenium controls them. When antioxidants and selenium are lacking in immune cells, inflammatory responses and factors can be generated. A number of other factors are critical for immune system and inflammatory response expression, including the nuclear transcription factor NF-kB and inflammatory factors such as IL-1b, IL-6, IL-11, and TNFα. The activation of the NF-kB signalling pathway was decreased by the addition of SY. According to Fang *et al* (2022), these factors may be associated with the anti-inflammatory effects of selenium. Oxidative stress is caused by an imbalance between the body's antioxidant system and intracellular oxygen production, which results in high levels of reactive oxygen species (ROS). As an example, the levels of hydrogen peroxide (H_2O_2) and superoxide dismutase (SOD) are important markers of the body's antioxidant capacity. Research into the antioxidant capabilities of selenium has a lengthy history.

Our research also shows that selenium helps activate the immune system by making the Fas and Fasl pathways more active, confirming the discovery of Huang *et al*. (2015) that testicular apoptosis occurs in the presence of Se deprivation.

CONCLUSION

This study concluded that increasing selenium supplements in drinking water and feed can positively affect gene expression in poultry, enhancing multiple functions such as immunity, oxidative stress, and programmed death. These genetic benefits can translate into tangible improvements in the health and productivity of poultry.

Acknowledgment

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Conflict of interest

The researchers declare no conflicts of interest

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