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Tailoring Dietary Selenium for Breed-Specific Gene Expression Profiles in Rabbits

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Abstract

THE CURRENT study was conducted to assess the effect of different sources of selenium (Se) in growing rabbit diets on gene expression profiles of *IGF-1, PPAR-γ, and COX2* genes. Three rabbit breeds at weaning age were used; Alex line, V-line, and Apri rabbits. The rabbit were divided to control (-Se) and three treatments groups (organic, inorganic, and nano Se). The results indicated that different selenium sources affected the expression of three genes differently across three rabbit breeds. Organic selenium generally led to increased expression of all three genes. Inorganic selenium increased expression of *insulin-like growth factor-1 (*IGF-1) overall breeds. Breed-specific effect was clear in Alex rabbits, as all the three genes were overexpressed when Alex rabbits received inorganic selenium. However, Nano Se unregulated the expression of the three genes in V-line rabbits. Nono.se treatment had mixed effects, depending on the gene and rabbit breed. Sex also affected gene expression. Overall, these results suggest the potential for targeted manipulation of dietary selenium to optimize rabbit health and productivity.

Keywords: Gene expression, Rabbits, Selenium source, Growth traits.

Introduction

Selenium (Se) is a captivating micronutrient that plays a crucial role in animal production and health [1]. It is involved in various biological processes, including antioxidant defense, immune function, and reproduction [2, 3]. Maintaining optimal Se levels, however, is a delicate balancing act, as deficiency can lead to health issues, while excess Se can be toxic [4].

Different Se forms exhibit different absorption rates [5]. For instance, organic forms like selenomethionine generally more bioavailable than inorganic forms [3]. By carefully selecting the appropriate Se source and incorporating it into rabbit feed, researchers aim to optimize Se uptake and utilization, ensuring that rabbits receive the ideal amount of this essential micronutrient [3]. For this, researchers are investigating strategies to enrich rabbit meat with Se. By manipulating the Se content and form in the rabbits' diet, they can increase the Se levels in the final meat products, addressing potential Se deficiencies in human diets [6]. Furthermore, maintaining optimal Se levels in rabbits can have a direct impact on their health and well-being. By precisely regulating Se intake through dietary modifications, researchers can potentially optimize these important biological processes in rabbits [7].

Several studies reported the modulation of the transcription of different gene due to the change in the feed ingredients [8, 9]. Recent research has explored the potential of Se to not only improve the quality and nutritional value of animal products like meat but also to achieve this by precisely regulating gene expression [10, 11]. This is particularly relevant in the context of rabbit diet and the impact of different Se sources on gene expression. Selenium ability to influence gene expression starts where incorporated into selenoproteins, which are essential

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for various cellular functions [12]. By modulating the production of these selenoproteins through targeted manipulation of Se levels and forms in the rabbits' diet, researchers can potentially regulate gene expression and optimize various biological processes in animals [13]. This gene expression regulation opens up possibilities for advancements in animal health, meat quality, and even human health through the consumption of Se-enriched rabbit meat [8, 14].

Therefore, the aim of the current study is to evaluate the changes in the expressions of *insulin like growth factor 1 (IGF-1), Cyclooxygenase-2 (COX2) and peroxisome proliferator activated receptor gamma (PPAR-γ)* genes in three rabbit lines received diets supplemented with different source of selenium.

Material and Methods

Ethical approval

The present study will be carried out in the farm of Poultry Production Department, Faculty of Agriculture, Kafrelsheikh University, Egypt. All the experimental presiders were approved from Institutional Animal Care and Use committee with the approval number of KFS-IACUC/130/2023.

Animals and management

Three rabbit lines differed in their origin and genetic background were used for the current study: V-line rabbits which were developed in Spain, selected for litter size at weaning and imported to Egypt [15]; Alex-line which is a synthetic line developed in Egypt and selected for daily weight gain [16]; and Apri-line which was also a synthetic line developed in Egypt and selected for litter weight [17].

Our experiment was performed in 2022 to investigate the effects of different selenium sources on weaned rabbits. A 96 six-week-old rabbits, averaging 1170 grams in weight, were divided into four groups. All groups received the same pelleted commercial diets. The only difference was the type of selenium supplement: inorganic (0.1 mg Na2SeO3/kg (sodium selenite), organic (0.1 mg, yeast-based), or nano-selenium (0.05 mg/kg). The number of rabbits per group was 9 rabbits of V-line, 9 rabbits of Alex line, and 6 rabbits of Apri line. The differences between the three groups at 6 weeks of age were insignificant. The used nano-Se sphered using milk whey as media along with yoghurt culture (Streptococcus thermophiles and Lactobacillus bulgaricus) and sodium selenite Se (IV) through fermentation process.

Gene expression analysis

For gene expression analysis, tissue samples were collected from liver to liquid nitrogen and then were stored in -80 until use. The total RNA was extracted from three replicates/breed/treatment using GeneJET RNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions. NanoDrop 2000C spectrophotometer (Thermo Scientific, USA) was used to determine the purity and concentration of extracted RNA. The extracted mRNA was then converted to cDNA using RevertAid™ H Minus Reverse Transcriptase using Oligo (dT)18 primer (Thermo Scientific, USA).

The expressions of four genes were assessed using AriaMxe Real-Time PCR machine (Agilent Technologies, USA). The reaction volume was 25 µL containing 15 µL of SYBR® green master mix, 8 µL of cDNA, and 1 µL of each forward and reverse primers. The sequences of the primers are listed in Table (1). The qRT-PCR was completed under the following the cycling conditions include initial activation for 15 min at 95°C, followed by 40 cycles of 15 seconds at 95°C, 15 seconds at 60°C, and 15 seconds at 72°C. Melting curve protocol was applied to ensure the PCR specialty.

Gene expression results were analyzed by calculating the relative mRNA levels using the comparative $2^{-\Delta\Delta Ct}$ method [18], after normalization to the *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) gene as a housekeeping gene. The data was then analyzed according to Yuan et al. [19]. All graphs were developed by ggplot2 package of R [20].

Results

The treatments have significantly ($P<0.037$) affected the expression levels of the *IGF-1* gene within breeds (Fig. 1). However, the effects of breed and breed × treatment interaction were not statistically significant ($P<0.561$ and 0.315, respectively). In Alex rabbits, all treatments led to significant increases in the amount of mRNA, where non-organic selenium had the highest effect, followed by organic selenium and nano selenium treatments compared to the control group. A similar trend was obtained for V-line rabbits, but the organic selenium had the highest effect followed by non-organic selenium and nano selenium treatments. For Apri rabbits, although organic selenium did significantly affect the expression of the *IGF-1* gene compared to the control, the two other treatments (Nono.se- and inorganic) were downregulated the *IGF-1* gene expression.

The treatments were also significantly ($P<0.028$) affected the expression of the *PPAR-γ* gene (Fig. 2). In Alex rabbits, the highest expression was obtained for rabbits fed non-organic selenium. In addition, rabbits fed Nano- or organic-selenium had significantly higher amounts of mRNA compared to the control group, but there were no significant differences between the two groups. For Apri rabbits, significant overexpression was obtained in rabbits fed organic selenium. Nevertheless, the Nono.se and inorganic treatments led to significant down-regulation of the gene expression, this down-

regulation was higher when the rabbits fed non-organic selenium. The effect of inorganic selenium was not significant in comparison to their corresponding control rabbits, however, the effect of Nono-selenium in V-line rabbits was similar to that in APRI rabbits, where it led to down-regulation of the gene expression level. The organic selenium also had a similar trend in V-line and Apri rabbits, where it increased the level of gene expression significantly compared to the control group. Similar to the *IGF-1* profile, the effects of breed and indication between breed and treatment were insignificant ($P < 0.124$ and 0.477, respectively).

For the expression of the *COX2* gene (Fig. 3), there were significant effects for the breed ($P < 0.041$) and treatment ($P<0.019$). However, the treatment × breed interaction was insignificant ($P < 0.309$). A significant overexpression profile was obtained in Alex rabbits fed inorganic selenium. However, the two other treatments did not differ from the control rabbits in the expression level of the *COX2* gene. Significant down-regulation was obtained for Apri rabbits fed Nono- or non-organic selenium. However, organic selenium treatment led to overexpressing in Apri rabbits. For V-line rabbits, the effect of inorganic selenium on the expression of the *COX1* gene was not significant. However, the organic selenium treatment had positively affected the expression level, however, the Nono.se treatment had negatively affected the level of the expression.

When we compared the relative gene expression profiles overall breeds (Fig. 4), the results revealed an insignificant effect for Nono.se treatment on the expression levels of *IGF*-1 and *PPAR-γ* genes, and a significant down-regulation was obtained for the *COX2* gene. The organic selenium treatment led to significant overexpression levels for the three genes. Also, inorganic selenium treatment led to a significant overexpression profile for the *IGF-1* gene but not for *PPAR-γ* or *COX2* genes.

Overall breeds, sex also had affected the expression levels of the different genes (Fig. 5). Although the effect of inorganic selenium treatment on the expression of the *IGF-1* gene was not significant in females, it was significantly increased the expression in males. The organic selenium treatment effect did not differ, as the expressions were up-regulated in both sexes. The big difference in the expression of the *IGF-1* gene between males and females was due to the Nano.se treatment that led to overexpression in the males and decreases expression in the females. A similar trend was also obtained for the *PPAR-γ* and *COX1* genes.

Discussion

Selenium is an essential micronutrient that affects different biological functions through the modulation of the expression of different genes in animals, this is usually done by affecting the activity levels of some selenoproteins by causing changes in the abundance of mRNA of different genes [21]. Selenium supplementation is also reported as a beneficial treatment that improves animal performance and anti-oxidation [22, 23]. However, the chimerical form of Se was found to have a great effect on the expression of different genes including the Selenoprotein gene [24]. In this concern, the use of Se-methyl selenocysteine as an organic selenium supplement for pigs led to an increase in the expression of the liver selenoprotein gene [12]. The *IGF-1* gene is located at chromosome 4 in rabbits and has 4 transcripts. The *IGF-1* gene is one of the main components of the growth-promoter signaling system and is associated with cell proliferation. The *IGF-1* is produced in the liver as a response to the stimulation of growth hormone. Both *IGF-1* and *GH* are considered parts of the somatropic axis, which is responsible for growth and development [25]. The *IGF*-1 gene has a role in the negative feedback of *the GH* level. The *peroxisome proliferator-activated receptor-gamma* gene in rabbits is located at chromosome 9, and its five transcripts [26]. The gene has different biological functions, as it is involved in controlling metabolism, obesity, and reproduction. The *PPARg* gene also regulates the pathway of the expression of the leptin gene. Moreover, it is considered a key regulator of mature adipocyte differentiation and glucose homeostasis. The analysis of the expressions of the *PPARg* and *IGF-1* genes showed a similar trend, where different expression among different breeds was observed although the effect of the breed was not statistically significant. Which may directly be associated with the performance of each breed. Nevertheless, the breed × treatment interaction was also insignificant. The effect of organic selenium was significantly higher than the control in all breeds indicating the high bioavailability of organic selenium compared to other selenium sources. However, the effect of the Nono-selenium treatment seems to be dependent on the performance of rabbits. The effects of selenium source on the expression of growth-related genes may be more visible under harsh conditions. Additionally, selenium effects on growth can be observed when animals are maintained in severe environmental conditions [27].

Selenium attracted the research interest for its role as an antioxidant. Where, antioxidant selenoproteins protect cells against oxidative stress [28], as it helps control the intracellular redox state [29]. The cytochrome c oxidase is a bi-genomic complex that has subunits coded by both nuclear and mitochondrial DNA. The cytochrome c oxidase subunit 2 (*COX2*) gene is a protein-coding gene that has one transcript and is located at the MT chromosome in rabbits. Nano-selenium supplementation was reported to affect the antioxidant mechanisms, where increases in superoxide dismutase and glutathione peroxidase,

and reductions in nitric oxide were reported [30]. In the current study, the analysis of the *COX2* expression revealed a significant breed effect. Where the organic selenium had the highest effect in Apri and V-line breeds but it was not significant in Alex rabbits. Moreover, the differences between the expressions of Nono-selenium treatment between the different breeds need more investigation.

A clear view of the abundance of mRNA of the different genes as a response to the different sources of selenium was obtained when the data were analyzed without the breed effect. The insignificant differences between organic and inorganic selenium in two genes (out of three) may be attributed to the similarity in absorption between organic and non-organic selenium, where both achieve about 80% absorption under normal physiological conditions and normal feed intake [31]. The differences in gene expression between the sexes have also existed, and this was mainly attributed in humans to the differences between males and females in selenium uptake [32]. The insignificance of the interaction between breed and treatment suggests that the breed is unlikely to interact with the different sources of selenium.

Conclusion

In conclusion, this study contributes to the growing body of research on the influence of dietary selenium source on gene expression in rabbits. Our findings demonstrate that different selenium forms (organic, inorganic, and Nono-selenium) have differential effects on the expression of three key genes (IGF-1, PPAR-γ, and COX2) across three rabbit breeds. Notably, organic selenium supplementation consistently upregulated the expression of all three genes. Conversely, inorganic selenium only affected IGF-1 expression, and Nono-selenium exhibited variable effects depending on the gene and breed. These results suggest the potential for targeted manipulation of dietary selenium to optimize rabbit health and productivity. Further research is necessary to explore the downstream impacts of these gene expression changes on meat quality and potential implications for human nutrition.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Sequences of the primers used for gene expression analysis

Gene	Gene bank accession #	Sequence (5' -3')
GAPDH	NM_001082253	F: TGCCACCCACTCCTCTACCTTCG R: CCGGTGGTTTGAGGGCTCTTACT
PPAR-γ	NM_001082148.1	F: GGAGCAGAGCAAAGAAGTCG R: CTCACAAAGCCAGGGATGTT
IGF-1	NM_001082026.1	F: TGTGATCTGAGGAGGCTGGA R: GAAGCAGCACTCATCCACGAT
COX2	NC_001913.1	F: TCCAAGCTGGCCTCACTGATGG R: AGCATGTGTGTGGCCCGACTTG

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; IGF-1: insulin like growth factor 1; COX2: Cyclooxygenase-2; PPAR-γ: peroxisome proliferator activated receptor gamma

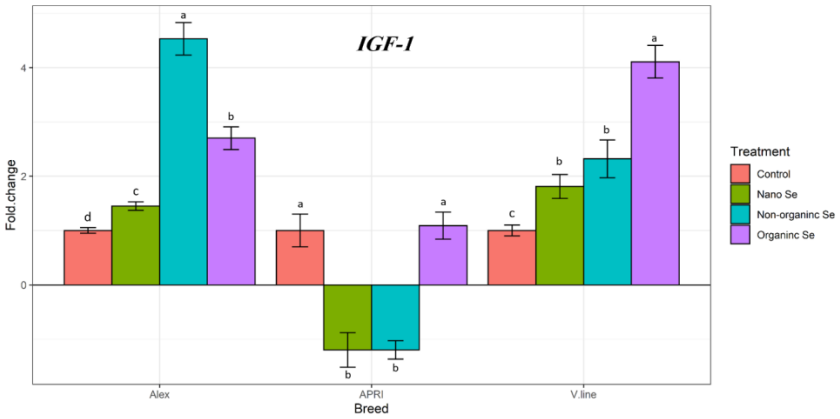

Fig. 1. The effect of different treatments on the expression of *IGF1* gene.

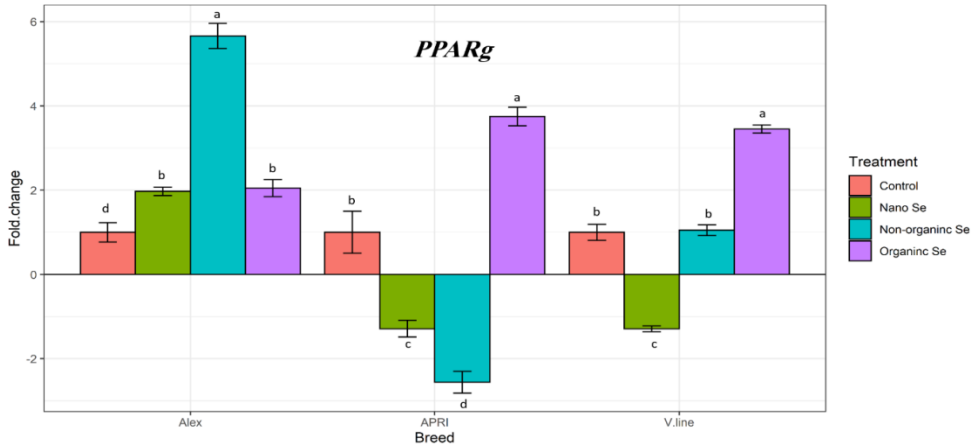

Fig. 2. The effect of different treatments on the expression of *PPARg* gene.

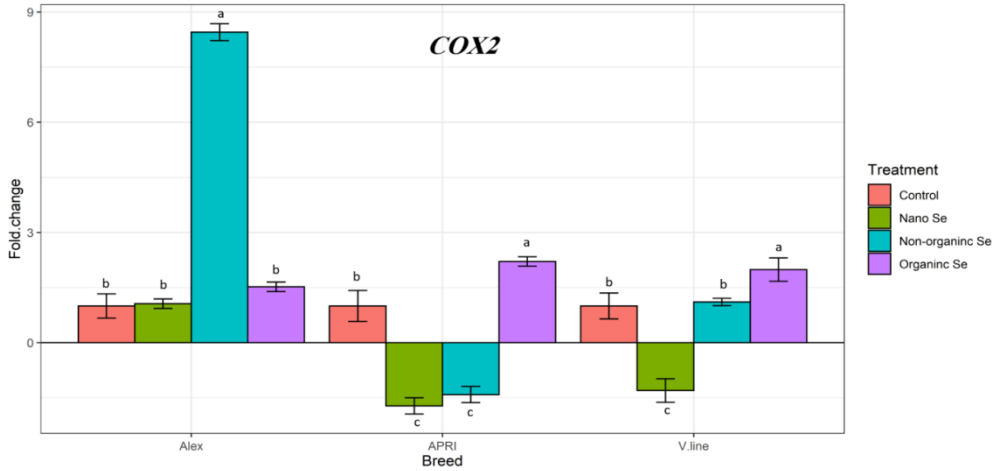

Fig. 3. The effect of different treatments on the expression of *COX2* gene.

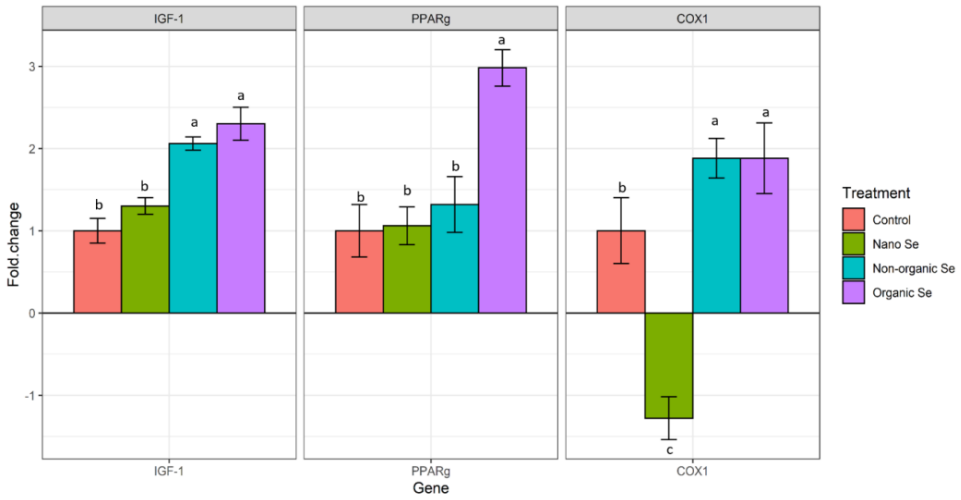

Fig. 4. The effect of different treatments, overall breeds, on the expression of the *IGF1*, PPARg, and COX2 genes.

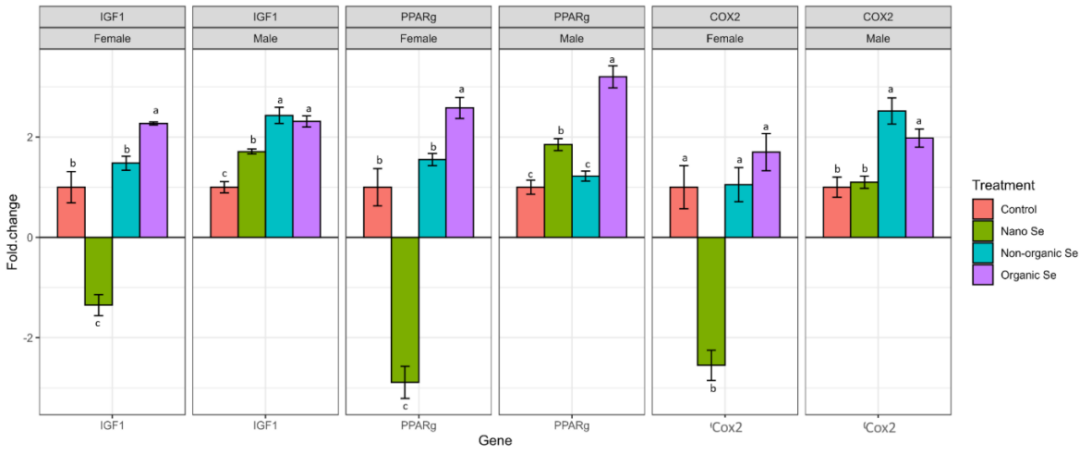

Fig. 5. The effect of different treatments, by sex overall breeds, on the expression of the *IGF1*, *PPARg*, and *COX2* genes.

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تخصيص السيلينيوم الغذائي وفقاً لملامح التعبير الجيني الخاصة بالسلالة في الأرانب

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الملخص

أجريت الدراسة الحالية لتقييم تأثير المصادر المختلفة للسيلينيوم (Se) في عليقة الأرانب النامية على التعبير الجيني لثلاث جينات وهي *IGF-1* و*PPAR-γ* و*COX2*. تم استخدام ثلاث سلالات من الأرانب في سن الفطام؛ خط Alex ، خط V، وأرانب Apri. تم تقسيم الأرانب إلى مجموعة الكنترول وثلاث مجموعات للمعاملات المختلفة (العضوية وغير العضوية والنانوسيلينيوم). وقد أشارت النتائج إلى أن مصادر السيلينيوم المختلفة أثرت على التعبير عن ثلاثة جينات بشكل مختلف عبر ثلاث سلالات من الأرانب. أدى السيلينيوم العضوي عمومًا إلى زيادة التعبير عن الجينات الثلاثة. زاد السيلينيوم غير العضوي من التعبير عن *IGF-1* ولكن ليس الجينين الآخرين. ظهرتأثير السلالة بوضوح في الارانب من سلالة Alex عندما حصلت علي السلينوم من مصدر غير عضوي حيث زاد التعبير الجيني معنوياً في الثلاث جينات ، وكذلك في ارانب V-line والتي حصلت علي السيلنيوم من مصدر عضوي. كان لمعاملة النانوسيلينيوم تأثيرات مختلطة، اعتمادًا على الجين وسلالة الأرانب. يؤثر الجنس أيضًا على التعبير الجيني. بشكل عام، تشير هذه النتائج إلى إمكانية التلاعب المستهدف بالسيلينيوم الغذائي لتحسين صحة الأرانب وإنتاجيتها.

الكلمات الدالة: التعبير الجيني، الأرانب، مصدر السيلينيوم، *IGF1*، *PPARg*، *COX2*.