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IMPACT OF DIETARY SUPRA NUTRITIONAL SELENIUM SUPPLEMENTATION ON FAT METABOLISM IN THE SKELETAL MUSCLES OF GOATS

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ABSTRACT

This study aimed to evaluate the effects of dietary supranutritional selenium (Se) supplementation on gene expression related to fat metabolism in the skeletal muscles of male goats. Sixteen male goats, aged 3-4 months and weighing 10-13 kg, were randomly divided into two groups (n=8 each) and housed individually at the Livestock Experimental Station, Sindh Agriculture University, Tandojam. The goats underwent a 2-week adaptation period, during which they were offered a diet containing concentrate and forage. Afterward, they were fed a basal diet with two different Se levels: Group A (control) received 0.3 mg/kg diet Se, while Group B (treatment) received 0.65 mg/kg diet Se, with selenium yeast (Sel-Plex®, Alltech®, USA) as the organic Se source. Over the 10-week experimental period, mRNA expression levels of genes involved in fat metabolism, including peroxisome proliferator-activated receptor alpha (PPARα), lipoprotein lipase (LPL), carnitine palmitoyltransferase 1 (CPT-1), cytosolic phospholipase A2 (cPLA2), and heart-type fatty acid-binding protein (H-FABP), were analyzed via quantitative real-time PCR. Results indicated a significant increase ($P < 0.05$) in the expression of these genes in Group B compared to Group A. In conclusion, this study demonstrates that dietary supranutritional selenium supplementation significantly enhances the expression of key genes involved in fat metabolism, such as PPARα, LPL, CPT-1, cPLA2, and H-FABP, in the skeletal muscles of goats. The upregulation of these genes suggests improved lipid utilization and oxidative capacity, which may contribute to better energy production and meat quality.

Keywords: Selenium, Supplementation, Fat metabolism, Skeletal muscles, Goat

INTRODUCTION

Selenium (Se) is a vital micronutrient required in trace amounts for maintaining numerous critical biological functions. It plays a central role in the proper functioning of antioxidant enzymes such as glutathione peroxidases and thioredoxin reductases, which protect cells from oxidative damage caused by reactive oxygen species (ROS) (Zoidis et al., 2018). In addition to its role in protecting cells, selenium is crucial for the thyroid gland, where it helps synthesize and activate thyroid hormones that regulate energy metabolism in the body (Schomburg, 2012). Furthermore, selenium is essential for immune health, as it supports the growth and function of immune cells and modulates immune responses, helping the body respond effectively to infections and other health threats (Huang et al., 2012).

Recent studies have focused on the potential health benefits of supranutritional selenium intake, which refers to consuming selenium beyond the levels required to avoid deficiency. Research indicates that such increased intake may offer additional benefits, particularly in enhancing the body's metabolic processes. Selenium's antioxidant properties can aid in reducing oxidative stress, especially in tissues involved in energy metabolism, such as muscles and the liver, thereby improving glucose and fat metabolism (Zhao et al., 2022). Moreover, selenium's influence extends to regulating gene expression related to fat metabolism.

This includes affecting enzymes that govern the synthesis, breakdown, and storage of fatty acids, such as those involved in lipid metabolism pathways (Zhang et al., 2014). Selenium's synergistic effect with nutrients like vitamin E further amplifies its positive impact on metabolic health, making it a key player in maintaining energy balance (Liao et al., 2022).

Skeletal muscles, which account for a significant portion of the body's resting energy expenditure, are crucial in managing glucose and fat metabolism (Egan & Zierath, 2013). As such, muscle tissue is a primary focus in studies examining the metabolic effects of selenium supplementation. Selenium contributes to muscle health by supporting the production of selenoproteins, which guard muscle cells against oxidative damage and help maintain the balance of reactive oxygen species (Rayman, 2012). Selenium's ability to modulate gene expression, particularly in genes related to fat metabolism such as PPARα, CPT, and SREBPs, underscores its role in regulating fat storage and utilization in muscle tissue (Zhang et al., 2014). Additionally, selenium enhances insulin sensitivity, enabling more efficient glucose and fat uptake by muscle cells, which helps prevent fat accumulation and inflammation (Park et al., 2020). Selenium's anti-inflammatory properties, including its ability to reduce inflammatory markers like TNF-α and IL-6, further support muscle health and overall metabolic balance (Avery & Hoffmann, 2018).

Despite significant advances in understanding of the role of selenium (Se) supplementation in metabolic health, there remains a gap in knowledge regarding its specific effects on lipid metabolism in skeletal muscle, a key determinant of overall metabolic health. Therefore, the primary objective of this study is to investigate the effects of supranutritional selenium supplementation on lipid metabolism in skeletal muscle, with a focus on elucidating the underlying molecular mechanisms, including antioxidant defense, gene expression regulation, and modulation of inflammatory pathways.

MATERIALS AND METHODS

Animal selection and adaptation

A total of 16 male goats (Non-descriptive), obtained from local market approximately 3-4 months of age, weighing 10-13 kg body weight were randomly selected for study and brought at Livestock Experimental Station, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. Animals were offered a concentrate diet at 2% of BW and forage ad libitum for a 2 weeks-adjustment period. During adaptation period, all the animals were ear tagged for identification, drenched, and injected with ivermectin against helminthes and other parasitic infestations, and vaccinated against some common infectious diseases.

Experiment design and feeding management

Immediately after completion of adaptation period, 16 male goats were housed in steel cages individually and randomly allotted to two dietary groups: viz. A & B (n= 08 each). Goats were fed the corresponding concentrate diets twice a day at 2% of BW and forage ad libitum during a 10 weeks' experimental period. Body weight was measured on the initial and final days of the experiment.

The Se source and dosage

The Se levels of basal feed was analyzed. Then animals in control (A) group was fed diet containing Se levels up to 0.3 mg kg^{-1} diet. In treatment (B) group goats were received a diet supplemented with increased dose of Se levels up to 0.65 mg kg^{-1} diet. Se was fed from organic source (i.e., selenium yeast (SY); Sel-Plex®, Alltech®, USA.

Quantitative real time PCR

Muscle tissues were quickly collected and immediately preserved in RNA latter and stored at -80 °C for RNA isolation. Determination of mRNA expression level was performed with real time PCR by using SuperScript™ III One-Step RT-PCR System with Platinum™ Taq DNA Polymerase.

Total RNA isolation

For total RNA isolation, the Invitrogen RNA Extraction kit was used following the manufacturer's instructions. A 30 mg tissue sample was homogenized in 350 µl of FARB buffer with 3.5 µl of β-Mercaptoethanol, using a micropestle and passing it through a 20-G needle syringe. After 5 minutes of room temperature incubation, the mixture was filtered and centrifuged. The filtrate was mixed with 70% RNase-free ethanol and transferred to a FARB mini column, followed by washing with buffer 1 and buffer 2. Residual ethanol was removed by additional centrifugation. RNA was eluted with 100 µl of RNase-free water, and RNA concentration and purity were measured using a Nano drop spectrometer. All samples showed purity values between 1.72 and 1.84, and the RNA was stored at -70°C for further use.

Real-time Rt-PCR protocol

The mRNA expression levels were determined using real-time PCR with the SuperScript™ III One-Step RT-PCR System and Platinum™ Taq DNA Polymerase. The cDNA synthesis was carried out in a three-step cycling process, incubating the reaction at 25-60°C for 15-30 minutes (as detailed in Table 2). A master mix was prepared in nuclease-free, thin-walled PCR tubes to minimize reagent loss and ensure precise pipetting (Table 3). After gently mixing and centrifuging, the reaction was placed in a preheated thermal cycler. The data analysis was performed using the PCR array data analysis approach based on the ΔCt method, normalizing the raw data to GAPDH (ΔCt = Ct target – Ct GAPDH). The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ formula (Livak & Schmittgen, 2001). All analyses were done in triplicate, and the target genes, their sources, and primer sequences are provided in Table 3.

Table-01Cycles of cDNA synthesis

cDNA synthesis and pre-denaturation		**Denature**	**Anneal**	**Extend**	**Final extension (optional)**
1 CYCLE			40 CYCLES		1 CYCLE
45–60°C	94°C	94°C	55–66°C	68°C	68°C
15–30 minutes	2 Minutes	15 seconds	30 seconds	1 minute/kb	5 minutes

Table-02Concentration and volume of reagents/ solutions used in total mixed reaction

Component	Volume
2X Reaction Mix	25 µL
Template RNA (.01 pg to 1 µg)	x µL
Forward primers (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
SuperScript™ III RT/Platinum™Taq $_{Mix}$[1]	2 µL
Autoclaved distilled water	20 µL

Table 03 Gene name, primer sequence, and NCBI accession number for quantitative real time RT-PCR analysis

Gene	Primer sequence 5' to 3'	Source	Size(bp)
PPARα	ATGGCTTCATAACCCGTGAG AATCCCCTCCTGCATTTTCT	XM_004007050.1	206
LPL	TTCAACCACAGCAGCAAAAC AAACTTGGCCACATCCTGTC	NM_001009394.1	210
CPT-1	TCGCGATGGACTTGCTGTATA CGGTCCAGTTTGCGTCTGTA	FJ415874.1	100
cPLA$_2$	TTGTGCTACAGAGAGGAGAGGA GTGCCACGTAGCACCACTAC	XM_018060732.1	119
H-FABP	AGACCACGGCAGATGACA AACTATTTCCCGCACAAG	Long et al., 2022	113
GAPDH	GGGTCATCATCTCTGCACCT GGTCATAAGTCCCTCCACGA	NM_001034034	176

†PPARα=peroxisome proliferator-activated receptor α; LPL=lipoprotein lipase; CPT-1= Carnitine palmitoyltransferase I; cPLA$_2$= cytosolic phospholipase A2; H-FABP= Heart-Type Fatty Acid-Binding Protein.
‡The first primer listed for each gene is the forward primer and the second primer is reverse primer.
§The reference sequence number
¶The base pair of primers
Aliquots of cDNA samples were subjected to electrophoresis through a 1.4% agarose-formaldehyde gel to verify integrity. The RT products (cDNA) were stored at - 20℃.

RESULTS

Gene expression

Expression of genes related to fat metabolism in skeletal muscles of goat Peroxisome Proliferator-Activated Receptor Alpha (PPARα), Lipoprotein Lipase (LPL), Carnitine Palmitoyl transferase 1(CPT-1:), Cytosolic Phospholipase A2(cPLA2), Heart-Type Fatty Acid Binding Protein (H-FABP) The Peroxisome Proliferator-Activated Receptor Alpha (PPARα) controls how the body uses fats by encouraging the breakdown of fatty acids and the production of ketones, which are important for maintaining energy levels and keeping fats balanced in the body. Lipoprotein Lipase (LPL) helps break down fats in blood vessels into simpler parts, free fatty acids and glycerol, which can then be taken up by body tissues for energy or stored. Carnitine Palmitoyl transferase 1 (CPT-1) is essential for moving long-chain fatty acids into the energy-producing parts of cells, called mitochondria.
Cytosolic Phospholipase A2 (cPLA2) releases a substance called arachidonic acid from cell membranes, which affects how cells respond to inflammation and communicate with each other. Heart-Type Fatty Acid Binding Protein (H-FABP) helps carry fatty acids inside muscle cells, aiding

in energy production and metabolism, and can also indicate if the heart is under stress. Figure. 5.1, shows the effects of Selenium supplemented diets i.e. 0.3 mg kg-1 diet (Group A i.e. control group) and 0.65 mg kg-1 diet (Group b) on PARα, LPL, CPT-1, cPLA2, and H-FABP mRNA expression in fat metabolism in skeletal muscles of goat. The mRNA expression levels of PARα, LPL, CPT-1, cPLA2, and H-FABP were increased significantly ($P > 0.05$) in group B (higher selenium dose i.e. 0.65 mg kg-1 diet) as compare to control group A (low selenium dose i.e. 0.3 mg kg-1 diet)

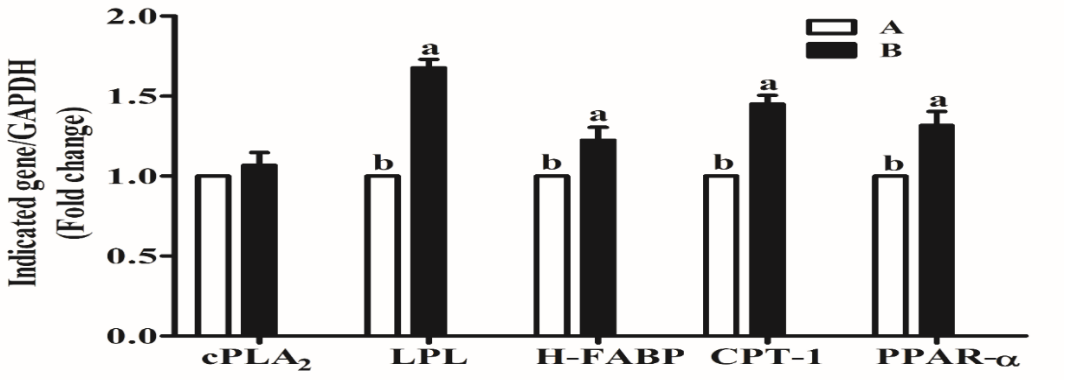

Figure – 01 Cyclins genes and Kinases mRNA expressions

Effects of selenium supplemented diets on PARα, LPL, CPT-1, cPLA2, and H-FABP mRNA expression in fat metabolism in skeletal muscles of goat. Then animals in control (A) group was fed diet containing Se levels up to 0.3 mg kg^{-1} diet. In treatment (B) group goats were received a diet supplemented with increased dose of Se levels up to 0.65 mg kg^{-1} diet. The level of gene expression was calculated with real-time PCR in comparison with GAPDH RNA. Values are mean ± S.E and [a, b] different letters on the bars exhibit the differences between groups with $P < 0.05$.

DISCUSSION

The research looked at how adding selenium to the diet affects the activity of genes that control fat breakdown in the muscles of goats. Results showed that when goats ate more selenium (0.65 mg per kg of food), important genes for fat processing, like PPARα, LPL, CPT-1, cPLA2, and H-FABP, were more active than when they ate less selenium (0.3 mg per kg of food). Selenium is important because it helps control these genes, which in turn affects how cells use fat for energy. We looked at how much of certain important genes related to fat processing were active in the muscles of goats when they were given extra selenium in their food. These genes included peroxisome proliferator-activated receptor alpha (PPARα), lipoprotein lipase (LPL), carnitine palmitoy ltransferase I (CPT-1), cytosolic phospholipase A2 (cPLA2), and heart-type fatty acid-binding protein (H-FABP).

We found that giving goats more selenium made the PPARα gene more active. This is important because PPARα is a key player in controlling how the body uses fat and makes energy in the mitochondria (Kersten et al., 1999). Increasing its activity might help speed up these metabolic processes, which could lead to better energy use and more efficient use of stored fats. Past studies have shown that selenium can adjust how active PPARα is and how much of it is made (Tinkov et al., 2020). This study's results match those findings, suggesting that adding selenium could help improve the pathways controlled by PPARα, which in turn could boost the breakdown of fats and energy production in muscle. Higher levels of PPARα have been linked to more mitochondria being made and working better, which results in better ability to produce energy and do it more efficiently (Kersten et al., 1999). This indicates that providing more selenium could improve the health and function of mitochondria in goat muscles, which could lead to better physical performance and more efficient metabolism. Additionally, activating a protein called PPARα has been associated with increasing the activity of genes that help break down fats, like the one for carnitine palmitoyltransferase I (CPT-1) (Kersten et al., 1999). So, the increase in PPARα gene activity might

boost the activity of other genes that help with fat metabolism, which would further support using fats for energy.

The study found that when goats received more selenium, the amount of a specific gene (LPL) increased in their muscle tissue. This gene is important because it helps break down fats from the blood, allowing muscles to use these fats for energy. This process is part of how the body manages its fat levels (Pfaehler et al., 2012). The increase in this gene's activity suggests that higher selenium levels might help the body take in and use more fats. This finding supports earlier research showing that selenium can affect how the body handles fats. Another study by Jing et al. (2013) showed that selenium supplements increased a gene related to fat breakdown in chicken liver, hinting at selenium's role in controlling fat metabolism. In research by Liu and colleagues (2023), it was shown that adding selenium to the diet boosted the activity of genes important for fat processing in the liver of chickens. This suggests that selenium helps control how fats are managed in the body. Additionally, higher levels of a protein called LPL, which helps in taking up and using fats, can lead to better energy production and overall efficiency (Pfaehler et al., 2012). The rise in LPL activity seen with more selenium suggests that fat processing might improve, potentially leading to better physical performance and efficiency in goats.

The rise in the amount of a specific gene (CPT-1) in the muscles of goats that received more selenium shows that selenium might help improve how the body uses and stores fat for energy. CPT-1 is an important enzyme that helps move fats into the part of the cell where they are broken down to make energy (McGarry & Brown, 1997). The increase in CPT-1 gene activity suggests that more selenium leads to better fat breakdown and energy production. This effect of selenium on CPT-1 is consistent with other studies that found selenium can control how fats are processed. For example, a study by Wang et al. (2023) found that giving selenium to chickens increased the activity of genes that are important for fat processing, including CPT-1, in their liver. The rise in CPT-1 mRNA levels indicates that more selenium in the diet could boost the breakdown of fats and energy generation in goat muscles. This could mean better mitochondrial activity and ability to use oxygen, resulting in more efficient metabolism and better physical abilities. Also, higher CPT-1 levels are linked to better use of fats for energy, which helps maintain good metabolic health (McGarry & Brown, 1997).

The rise in the amount of cytosolic phospholipase A2 (cPLA2) mRNA in goat muscle cells with more selenium added to their diet hints at selenium's possible role in helping to move lipids and activate signaling pathways. Cytosolic phospholipase A2 is a type of enzyme that helps release arachidonic acid from the fats in cell membranes. Arachidonic acid is important for making different signaling molecules and substances that cause inflammation (Lee et al., 2003). The increase in cPLA2 mRNA suggests that more fatty acids are being moved around inside muscle cells when there's more selenium. This could mean that adding selenium might make more arachidonic acid available for important processes that control inflammation, cell communication, and how fats are used in the body. Selenium's effect on cPLA2 expression is consistent with earlier research showing its role in controlling lipid metabolism and inflammation. For instance, a study by Shi et al. (2021) found that adding selenium reduced inflammation by adjusting the levels of important enzymes that affect lipid metabolism and inflammatory signals, including cPLA2, in rats with colitis. Additionally, higher cPLA2 expression has been linked to better lipid signaling and metabolic responses, which can lead to better overall metabolic health (Lee et al., 2003). The increase in cPLA2 mRNA expression seen with more selenium supplementation suggests it might adjust lipid signaling pathways, potentially affecting metabolic processes and inflammatory responses in goat muscles.

Higher levels of selenium in goats seem to boost the activity of a protein called heart-type fatty acid-binding protein (H-FABP) in their muscles. This protein helps move fatty acids inside muscle cells, where they can be used for energy and other processes. When there's more selenium, there's more of this protein's message (mRNA) being made, which means the muscle cells can handle and use fatty acids better. This could help goats use their energy more efficiently and perform better physically. Selenium's role in controlling this protein fits with what we know about how it affects how the body uses fats and energy. Additionally, higher levels of H-FABP have been linked to better absorption and use of fatty acids, which can lead to more efficient energy production and better overall

metabolism (Glatz et al., 1995). The rise in H-FABP gene activity seen with more selenium added to the diet indicates a boost in how fatty acids are moved around and used, possibly leading to better energy use and improved physical abilities in goats.

CONCLUSION

The conclusion of the study on the gene expression related to fat metabolism in the skeletal muscles of goats, focusing on PPARα, LPL, CPT-1, cPLA2, and H-FABP, reveals that selenium supplementation at a higher dose (0.65 mg kg-1 diet) significantly upregulated the expression of these key genes involved in fat metabolism compared to the control group (0.3 mg kg-1 diet). This suggests that increasing selenium intake enhances the breakdown and utilization of fats in skeletal muscles, as evidenced by the elevated mRNA expression of enzymes and proteins involved in fatty acid transport, oxidation, and energy production. These findings underscore the potential role of selenium in improving fat metabolism, which could have implications for optimizing energy use and overall metabolic health in livestock.

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STATEMENT OF CONFLICT OF INTEREST

The authors declared that there is no conflict of interest regarding the publication of this article.

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