

The Effects of Dietary Supranutritional Selenium on the Selenium Concentration and Oxidative Status in Serum and Muscles of Goat

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ABSTRACT

The study examined the effects of supranutritional selenium (Se) supplementation on selenium levels, oxidative status, and biochemical parameters in goats. Sixteen goats were divided into two groups: control (0.3 mg Se/kg) and treatment (0.65 mg Se/kg). After 10 weeks of trial, the results revealed several significant findings. Selenium concentrations in serum were markedly higher in group B (0.37 ppm) compared to group A (0.068 ppm). Similarly, longissimus dorsi (LD) muscle Se levels were greater in group B (0.2467 ppm) than in group A (0.051 ppm), and semimembranosus (SM) muscle concentrations were also elevated in group B (0.1533 ppm vs. 0.0373 ppm). Liver enzyme activities reflected significant increases in group B: AST was higher in serum (74.25 U/L) compared to group A (60.5 U/L), while LD muscle levels were 38.37 U/g (B) vs. 31.62 U/g (A), and SM muscle levels were 34.25 U/g (B) vs. 29.75 U/g (A). ALT levels in serum were also elevated in group B (19.87 U/L) compared to group A (12.5 U/L). GGT levels followed a similar trend: serum levels in group B were 78.5 U/L compared to 66 U/L in group A. Total protein levels were significantly higher in group B for serum (6.37 g/dL) and muscle tissues (LD: 3.13 g/g; SM: 3.01 g/g) compared to group A (serum: 5.35 g/dL; LD: 2.67 g/g; SM: 2.22 g/g). Antioxidant enzyme activities, specifically glutathione peroxidase (GSH-Px) and catalase (CAT), were also significantly increased in group B, indicating enhanced oxidative status. In conclusion, dietary supranutritional selenium supplementation notably improved selenium concentrations, enhanced antioxidant enzyme activities, and increased liver enzyme levels in goats, suggesting its potential for improving goat health and productivity.

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Authors' Contribution

MM and ABK formulated and designed the study, MAM conducted the experimental work, GSB, SAS and JS analyzed and interpreted the data, and FHM, DHK and TAK reviewed and revised the manuscript.

Key words

Goat, Muscles, Oxidative status, Selenium, Serum

INTRODUCTION

Selenium (Se), the word is derived from Greek language Selene means Moon. It is nonmetallic in nature possesses atomic number of 34 and atomic weight of 78.96 (Garcia *et al.*, 2013). It is an important component of the

body in most of multi-cellular eukaryotic species as well as few prokaryotic species (Kim and Gadd, 2019). It is vital trace element for animal and human from nutraceutical point of view. The positive effects of Se in animal nutritional science started in 1957. Schwarz and Foltz (1957) discovered one of the factors in yeast, which involved in the prevention of liver necrosis in rats, therefore, Se popularity increased as an important trace mineral in the animals diet. Se is involved in controlling diverse range of physiological processes including regulation of antioxidants (Zoidis *et al.*, 2020), immune-associated mechanisms, thyroid hormones biotransformation, and involvement in the reproduction (Surai *et al.*, 2019).

Oxidative stress (OS) is the consequence of disruption of antioxidants (AOXs) to prooxidants (PROX) ratio, either due to enhanced prooxidants generation or

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lowered antioxidants production or both (Ma *et al.*, 2022). However, the development of OS to some extent is quite essential to regulate many physiological processes in the cells but the severe increase in OS for prolonged period causes modifications of structural macromolecules and leads to cellular damages (Bhattacharyya and Saha, 2015). PROX are generally oxygen (O_2) and nitric oxide (NO) derived strongly reactive free radicals or non-radical compounds termed as reactive oxygen (ROS) and nitrogen (RNS) species, normally produced during metabolism (Bhattacharyya and Saha, 2015). Disruptive redox status causes either generation and/ or accumulation of large concentrations of ROS such as O_2^- (superoxide radical), OH (hydroxyl radical) and H_2O_2 (hydrogen peroxide) and other peroxides which through chemical reactions alter the biochemical composition and functions of the structural macromolecules including proteins, lipids, and DNA and lead to abnormal cellular signaling mechanisms (Schieber and Chandel, 2014). However, the cellular machinery is well-equipped with AOXs defense system which constantly neutralize the excessive reactive species and overcome or otherwise minimize their hazardous effects through antioxidants production. This mechanism includes both enzymatic and non-enzymatic AOX. The principal antioxidant enzymes (AOX-E) including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) together constitutes first-line AOX-defense system.

It is believed that HC diets induce OS in liver through GIT-derived LPS by disrupting the AOX and PROX ratio in hepatic tissue (Abaker *et al.*, 2017). Liver is a principal detoxification center and excretory organ which constantly receives harmful chemicals, pathogens, microbial toxins including LPS from body via circulation, specially via portal vein from GIT. Reticuloendothelial system (RES) in liver possesses phagocytes and many other types of immune cells which kill pathogens and neutralize toxins, other harmful chemicals and wastes and eventually remove them through their respective excretory pathways, while retaining the purified and cleansed blood in circulation (Guo *et al.*, 2023). Therefore, normally produced endotoxins in gut and other parts of body are well-tolerated by liver. However, once HC diet induced elevating LPS levels exceeds hepatic removal rate, the LPS escaping detoxification interact with phagocytic like Kupffer cells and initiate acute phase response (APR) with simultaneous development of oxidative stress.

MDA and T-AOC are commonly used methods to assess oxidative stress (OS) in tissues. Lactating goats fed a diet with 65% concentrates for 12 weeks showed a 28% decrease in T-AOC in liver tissue compared to those on a low (35%) concentrate diet (Huang *et al.*, 2022). In lactating cows with a dietary concentrate-to-forage ratio of 60:40

for 18 weeks, MDA content increased by approximately 67% and 130%, accompanied by a simultaneous decrease in T-AOC by about 52% and 66% in plasma and liver, respectively (Abaker *et al.*, 2017). Nitric oxide synthases (NOS) play a role in reactive nitrogen species (RNS) production. High-concentrate (HC) diets exhibited variable effects on NOS activities in ruminants. Plasma and hepatic levels of total (tNOS) and inducible nitric oxide synthase (iNOS) were unaffected by feeding 60% concentrates in cows. Conversely, Wang *et al.* (2021) noted no significant difference in MDA content but observed a 52% increase in hepatic NOS activity in goats fed 90% concentrates compared to those on a 55% concentrate diet, suggesting species and diet-dependent effects on NOS activity. HC diets were associated with reduced levels of antioxidant markers, including non-enzymatic antioxidants (e.g., GSH) and enzymatic antioxidants (SOD, CAT, GSH-Px, GR, GST) in liver and plasma of ruminants (Ma *et al.*, 2022). Changes in OS markers and antioxidants were linked to alterations in gene expressions of SOD, GSH-Px, and CAT in hepatic tissue of goats and cattle fed HC diets (Wang *et al.*, 2021). Nuclear factor E2-related factor-2 (Nrf2) is crucial in the transcriptional regulation of antioxidant genes, preventing cells and tissues from OS (Huang *et al.*, 2022). Molecular studies revealed decreased expressions of Nrf2 and total glucocorticoid receptor (GR), along with higher GR nuclear translocation, suggesting that HC diet-induced OS in the liver involves LPS-mediated downregulation of the Nrf2-dependent antioxidant signaling pathway (Alam *et al.*, 2017).

The issue at hand involves understanding the consequences of incorporating excessive selenium into the diet of goats, particularly its impact on the Se concentration and oxidative status observed in both serum and muscles. This study aims to address the potential challenges and implications associated with dietary supranutritional selenium intake in goats, shedding light on its effects on oxidative processes in these specific physiological aspects. Keeping in view the facts stated above, therefore this study was designed to observe the effects of dietary supranutritional Se on the Se concentration and oxidative status in serum and muscles of goat.

MATERIALS AND METHODS

Animal selection and adaptation

A total of sixteen male goats (non-descriptive) weighing between 10 and 13 kg body weight were randomly chosen for the study and brought to the Livestock Experimental Station (LES), Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. The goats were purchased from the local market at the age of three to four months. During a

two-week period of adjustment, the animals were given a concentrate diet at 2% of body weight and free access to forage. All of the animals received ear tags for identification throughout the adaption phase, as well as ivermectin injections and drenching's to prevent helminthes and other parasite infestations, as well as vaccinations against many prevalent infectious illnesses.

Experiment design and feeding management

Following the adaptation phase, sixteen male goats were housed in individual steel cages and randomly divided into two dietary groups: group A (n = 8) and group B (n = 8). For ten weeks of the trial, goats were given the equivalent concentrate meals twice a day at 2% of BW and allowed to graze as they pleased (Table I). On the first and last days of the trial, body weight was recorded.

Selenium source and dosage

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 (Table II). Se was fed from organic source (i.e., selenium yeast (SY); Sel-Plex®, Alltech®, USA).

Determination of selenium levels

To ascertain the selenium content, samples of skeletal muscle tissues and blood serum were obtained. As shown by Hseu *et al.* (2002), samples were digested using the nitric acid digestion procedure in order to prepare them for the quantitative analysis of selenium.

Blood sample collection

The pre-slaughter 20 mL blood samples were drawn from the jugular vein on the last day of the study and placed into two 10 mL glass tubes, one containing anticoagulant (heparin) and the other containing serum and plasma, respectively. After centrifuging both samples for 15 min at 3000 g at 4 °C, plasma and serum were extracted and frozen for further examination. The indicators associated with oxidative stress (OS) and liver function test (LFT) were identified using serum samples.

Slaughtering and skeletal muscle collection

The animals were slaughtered at the Small Ruminant Research Unit, Livestock Experimental Station, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam, by severing their jugular veins (the Halal procedure) at the conclusion of the feeding experiment. As soon as the goats were killed, their muscles were carefully removed, gathered in a clean

tub, and cleaned in cold phosphate buffer solution.

The method used to prepare the homogenate for LD and semi membranous muscle (thigh muscle) was to chop tissue and add phosphate buffered saline (PBS) to a glass homogenizer (1:9). The homogenate was then broken up using ultrasonography using a Bransonic R ultrasonic cleaner (Branson ultrasonics corporation, USA). After centrifugation at 4,000 × g for 10 min at 4°C, the supernatant was then transferred to a 1.5 mL tube and kept at -80°C until further analysis.

Analysis of biochemical tests

Using commercially available kits and closely according to the manufacturer's instructions, the quantities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma glutamyl transferase (GGT) in muscle were determined. A succinct explanation of the components and each kit's reaction principle.

Analysis of oxidative stress (OS) markers

Analyzing the amounts of prooxidants (POX) and antioxidants (AOX), as well as any associated products, may help determine the OS. Using a spectrophotometer, the activities of GSH-Px and CAT in serum and skeletal muscles were measured. The producers of the kit, Nanjing Jiancheng Bioengineering Institute Nanjing Jiancheng Technology Co. Ltd., properly followed the procedure for all the measurements.

Statistical analysis

The study used statistical analysis, employing ANOVA to compare means across multiple groups. A post-hoc LSD test was then applied to identify significant differences between individual group pairs.

RESULTS

Selenium concentration

The effects of dietary supranutritional Se treatment on Se concentration in serum and skeletal muscles of goat are shown in Figure 1A. Se concentration of serum, longissimus dorsi (LD) muscle and semimembranosus (SM) muscle (thigh muscle) was significant ($P < 0.05$) between A and B group. Se concentration of serum was higher in B group (0.37 ppm) compared to A group (0.068 ppm). Similarly, Se concentration of longissimus dorsi (LD) muscle was higher in B group (0.2467 ppm) compared to A group (0.051 ppm). Se concentration of semimembranosus (SM) muscle was higher in B group (0.1533 ppm) compared to A group (0.0373 ppm).

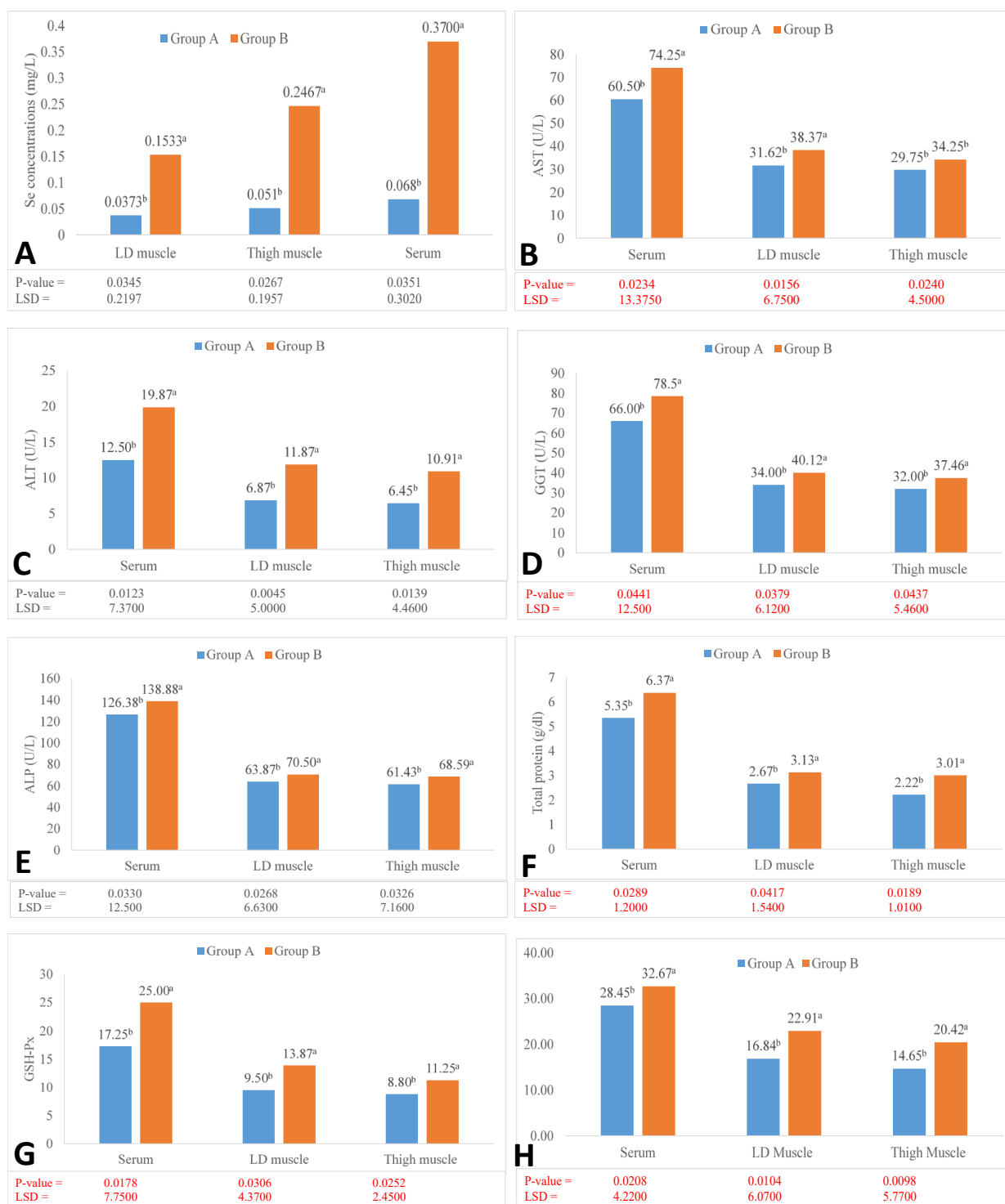


Fig. 1. Effect of dietary supranutritional selenium on selenium (Se) concentration in ppm (A), aspartate aminotransferase (AST) (B), alanine aminotransferase (ALT) (C), gamma glutamyl transferase (GGT) (D), alkaline phosphatase (ALP) (E), total protein (F), glutathione peroxidase (GSH-Px) (G), catalase (CAT) (H) level in serum and skeletal muscles of goat. Group A: Control; goats were fed selenium @ 0.3 mg kg⁻¹ diet. Group B: Treatment; goats were fed supranutritional selenium @ 0.650 mg kg⁻¹ diet for 10 weeks. Values are means $\pm$ SEM and ^{a, b} values with different superscripts were considered significant at $P < 0.05$.

AST

The effects of dietary supranutritional Se treatment on AST level in serum and skeletal muscles of goat are shown in [Figure 1B](#). AST level of serum, LD muscle and SM muscle (thigh muscle) was significant ($P < 0.05$) between A and B group. AST level of serum was higher in B group (74.25 U/L) compared to A group (60.5 U/L). Similarly, AST level of LD muscle was higher in B group (38.37 U/g) compared to A group (31.62 U/g). AST level of SM muscle was higher in B group (34.25 U/g) compared to A group (29.75 U/g). Moreover, the AST level was determined higher in serum than LD muscle and SM muscle for both A and B group.

Table I. Composition of diet fed to goats.

Chemical composition	Concentrate	Roughage
DM (%)	87.75	89.81
Crude protein (% DM)	20.89	7.32
Crude fat (% DM)	3.64	2.02
Crude fiber (% DM)	6.67	28.25
Crude ash (% DM)	7.73	6.4
ME (MJ/kg DM)	10.85	6.96

Table II. Background Se in diet.

Se treatment (mg kg ⁻¹ diet)	A (Control) group	B (Treatment) group
Roughage	0.035	0.035
Concentrate	0.150	0.150
Se* Added in diet	0.125	0.465
Final Se level	0.3	0.650

* Source: Synthetic organic form, selenium yeast (SY); Sel-Plex®, Alltech®, USA.

ALT

The effects of dietary supranutritional Se treatment on ALT level in serum and skeletal muscles of goat are shown in [Figure 1C](#). ALT level of serum, LD muscle and SM muscle (thigh muscle) was significant ($P < 0.05$) between A and B group. ALT level of serum was higher in B group (19.87 U/L) compared to A group (12.5 U/L). Similarly, ALT level of LD muscle was higher in B group (11.87 U/g) compared to A group (6.87 U/g). ALT level of SM muscle was higher in B group (10.91 U/g) compared to A group (6.45 U/g). Moreover, the ALT level was determined higher in serum than LD muscle and SM muscle for both A and B group.

GGT

The effects of dietary supranutritional Se treatment on GGT level in serum and skeletal muscles of goat are shown in [Figure 1D](#). GGT level of serum, LD muscle and SM muscle (thigh muscle) was significant ($P < 0.05$) between

A and B group. GGT level of serum was higher in B group (78.5 U/L) compared to A group (66 U/L). Similarly, GGT level of LD muscle was higher in B group (40.12 U/L) compared to A group (34 U/L). GGT level of SM muscle was higher in B group (37.46 U/L) compared to A group (32 U/L). Moreover, the GGT level was determined higher in serum than LD muscle and SM muscle for both A and B group.

ALP

The effects of dietary supranutritional Se treatment on ALP level in serum and skeletal muscles of goat are shown in [Figure 1E](#). ALP level of serum, LD muscle and SM muscle (thigh muscle) was significant ($P < 0.05$) between A and B group. ALP level of serum was higher in B group (138.88 U/L) compared to A group (126.38 U/L). Similarly, ALP level of LD muscle was higher in B group (70.5 U/g) compared to A group (63.87 U/g). ALP level of SM muscle was higher in B group (68.59 U/g) compared to A group (61.43 U/g). Moreover, the ALP level was determined higher in serum than LD muscle and SM muscle for both A and B group.

Total protein

The effects of dietary supranutritional Se treatment on total protein level in serum and skeletal muscles of goat are shown in [Figure 1F](#). Total protein level of serum, LD muscle and SM muscle (thigh muscle) was significant ($P < 0.05$) between A and B group. Total protein level of serum was higher in B group (6.37 g/dl) compared to A group (5.35 g/dl). Similarly, total protein level of LD muscle was higher in B group (3.13 g/g) compared to A group (2.67 g/g). Total protein level of SM muscle was higher in B group (3.01 g/g) compared to A group (2.22 g/g). Moreover, the total protein level was determined higher in serum than LD muscle and SM muscle for both A and B group.

GSH-Px

The effects of dietary supranutritional Se treatment on GSH-Px level in serum and skeletal muscles of goat are shown in [Figure 1G](#). GSH-Px level of serum, LD muscle and SM muscle (thigh muscle) was significant ($P < 0.05$) between A and B group. GSH-Px level of serum was higher in B group (25 U/ml) compared to A group (17.25 U/ml). Similarly, GSH-Px level of LD muscle was higher in B group (13.87 U/g) compared to A group (9.5 U/g). GSH-Px level of SM muscle was higher in B group (11.25 U/g) compared to A group (8.80 U/g). Moreover, the GSH-Px level was determined higher in serum than LD muscle and SM muscle for both A and B group.

CAT

The effects of dietary supranutritional Se treatment

on CAT level in serum and skeletal muscles of goat are shown in [Figure 1H](#). CAT level of serum, LD muscle and SM muscle (thigh muscle) was significant ($P < 0.05$) between A and B group. CAT level of serum was higher in B group (32.45 U/ml) compared to A group (28.45 U/ml). Similarly, CAT level of LD muscle was higher in B group (22.91 U/g) compared to A group (16.84 U/g). CAT level of SM muscle was higher in B group (20.42 U/g) compared to A group (14.65 U/g). Moreover, the CAT level was determined higher in serum than LD muscle and SM muscle for both A and B group.

DISCUSSION

The present study investigated the effects of dietary supranutritional selenium (Se) treatment on various biochemical parameters in serum and skeletal muscles of goats. The results demonstrate significant differences between the control (Group A) and se-supplemented (Group B) groups in terms of se concentration, liver function enzymes (AST, ALT, GGT, ALP), and antioxidant enzymes (GSH-Px, CAT). These findings suggest that dietary supplementation with selenium at supranutritional levels can influence oxidative status and liver function in goats.

The higher se concentrations observed in both serum and skeletal muscles of goats in the se-supplemented group (Group B) compared to the control group (Group A) align with findings from previous research. Numerous studies have demonstrated that dietary supplementation with selenium leads to increased selenium levels in serum and various tissues of goats ([Ahmad *et al.*, 2012](#)). The organic selenium source utilized in this study, selenium yeast (Sel-Plex®), has been widely recognized for its superior bioavailability and effectiveness in enhancing selenium concentrations in animal tissues compared to inorganic sources such as sodium selenite ([Surai, 2006](#)). Se yeast contains selenomethionine, a naturally occurring organic form of selenium that is readily absorbed and incorporated into body tissues ([Van-Metre and Callan, 2001](#)). This form of Se is more efficiently utilized by animals, leading to higher tissue selenium levels compared to inorganic Se sources ([Zhang *et al.*, 2017](#)). Furthermore, the enhanced Se concentrations observed in serum and skeletal muscles of goats supplemented with Se are indicative of successful Se absorption and tissue deposition. Se plays essential roles in various physiological processes, including antioxidant defense, immune function, and thyroid hormone metabolism ([Rayman, 2000](#)). Adequate Se levels are crucial for maintaining optimal health and productivity in livestock species, making selenium supplementation a common practice in animal nutrition.

The liver associated enzymes, such as AST, ALT, ALP

and GGT are indicative of liver homeostasis. The elevated levels of liver function enzymes (AST, ALT, GGT, ALP) observed in both serum and skeletal muscles of goats supplemented with selenium indicate potential alterations in liver function. Furthermore, ALT, AST and GGT reflect hepatic function status and their disproportionate leakage into blood is linked with disintegration of hepatic cell membrane and impaired hepato-cellular function ([Abdel-Daim and Abdou, 2015](#); [Abdou *et al.*, 2019](#)). In present study, increased concentration of LPS was observed in goats fed with HC diet simultaneously with an elevated concentration of liver associated enzymes (ALT, AST, ALP and GGT). LPS induce hepato cellular damage, alters structure and function of liver cells ultimately results in a significant elevation in both serum and tissue ALT and AST levels ([Latha *et al.*, 2017](#)). Parallel with higher concentration of LPS, the activity of ALT and AST in blood circulation were significantly higher in cows fed HC diet in comparison to LC fed diet cows, that may suggest a damage to hepatocytes and inhibition in their due to HC feeding ([Guo *et al.*, 2017](#)). Besides with the findings of ([Wang *et al.*, 2023](#)), the concentrations of ALT and ALP in peripheral blood were higher in the goats fed HG diet in comparison with goats fed LG diet. Interestingly, HG diet feeding to goats did not affect plasma concentration of AST in comparison to the LG group. Moreover, effect of interactions of diet with time was significant on ALT concentration in plasma. However, a significant increase in AST activity was only observed after feeding HC diet, whilst GGT values did not differ significantly between these two groups (i.e. before feeding and after feeding) ([Zhang *et al.*, 2021](#)). In another report, GGT activity was not affected statistically with carbon tetrachloride-injection, but, AST, ALT and ALP activities were significantly increased ([Bitiren *et al.*, 2004](#)). Furthermore, various studies report increased concentration of liver function/damage markers in hepatotoxicity caused by diverse agents reported increased levels of ALT, AST and ALP in hepatic toxicity in goat challenged with arsenic against the control group. Furthermore, they reported that gradual increase was observed in the concentration of ALT, AST and ALP in goats treated with arsenic on the 0, 45, 90, 135 and 180 days of experiment ([Radfar *et al.*, 2014](#)).

The observed elevation in the activities of antioxidant enzymes, including GSH-Px and CAT, in both serum and skeletal muscles of goats supplemented with Se suggests a reinforcement of antioxidant defense mechanisms. Se, as an essential component of selenoproteins, particularly GSH-Px, plays a pivotal role in scavenging ROS and mitigating oxidative stress-induced cellular damage ([Rayman, 2000](#)). GSH-Px catalyzes the reduction of hydrogen peroxide and organic hydroperoxides, thus protecting cells from oxidative damage and maintaining cellular homeostasis.

Similarly, CAT serves as another critical antioxidant enzyme involved in the breakdown of hydrogen peroxide into water and oxygen molecules, thereby averting the accumulation of this potent oxidant within cells (Zhang *et al.*, 2023). The increased activities of GSH-Px and CAT in response to Se supplementation reflect an enhancement of the antioxidant defense system, aimed at neutralizing ROS and preserving cellular integrity. Moreover, the augmentation of total protein levels in both serum and skeletal muscles of goats in the Se-supplemented group further supports the notion of Se-mediated improvements in antioxidant status. Total protein serves as a crucial indicator of overall health and nutritional status, and its elevation suggests enhanced protein synthesis and metabolic function. Se role in modulating protein metabolism and synthesis may contribute to the observed increase in total protein levels, indicating a positive impact on cellular repair and regeneration processes (Tang *et al.*, 2020). Overall, the higher activities of antioxidant enzymes and total protein levels in goats supplemented with selenium underscore the beneficial effects of selenium in bolstering antioxidant defense mechanisms and promoting overall health and well-being.

CONCLUSION

It is concluded that this study demonstrated that dietary supranutritional Se supplementation significantly influenced various biochemical parameters in the serum and skeletal muscles of goats. Notable differences in markers associated with OS and LFT including AST, ALT, GGT, ALP, total protein, GSH-Px, and CAT were observed between the control group (Group A) and the Se-supplemented group (Group B). Group B consistently exhibited higher enzyme levels, indicating the substantial impact of supra-nutritional Se on Se concentration and oxidative status in serum and muscles of goat.

DECLARATIONS

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IRB approval

The study proposal received approval from Board of Advanced Studies and Research (BASR) under reference number (DAS/1666) in the year 2023.

Ethical statement

Ethical approval for this study was obtained from

the departmental board of studies followed by Directorate of Advanced Studies (DAS) meeting through reference number DAS/1666, Sindh Agriculture University Tandojam.

Statement of conflict of interest

The authors have declared no conflict of interest.

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