Derna Academy Journal for Applied Sciences

E-ISSN: 3006-3159

DAJAS

Epidemiological Assessment of Environmental and Nutritional Determinants of Selenium Status and Serum Muscle Enzyme Activities in Lambs from Al-Jabal Al-Akhdar, Libya

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Volume : 6 **Issue: 2** **Page Number: 1 - 8**

Keywords:
Selenium; Epidemiology; Lambs; Soil; Barley; Creatine kinase; Aspartate aminotransferase; Al-Jabal Al-Akhdar; Libya.

Received: 09\05\2026
Accepted: 21\05\2026
Published: 22\05\2026

https://doi.org/10.71147/b7933h87

ABSTRACT

Selenium is an essential trace element involved in several biological functions, particularly antioxidant defense, immune response, growth, reproduction, and muscle integrity. Selenium deficiency in young ruminants is commonly associated with nutritional muscular disorders, including white muscle disease. This epidemiological study was conducted to evaluate environmental and nutritional determinants of selenium status and their association with serum muscle enzyme activities in lambs from Al-Jabal Al-Akhdar, Libya. Soil selenium concentrations from the mountainous and South Mountain areas, as well as selenium concentrations in national and imported barley used as a major dietary source, were determined using Atomic Absorption Spectrophotometry. Serum creatine kinase (CK) and aspartate aminotransferase (AST) activities were measured in 100 apparently healthy local lambs using a Roche Cobas C311 analyzer. The selenium concentration in soil was higher in the South Mountain area (15.979 ppm) than in the mountainous area (0.302 ppm). Imported barley showed a higher selenium concentration (7.28 ppm) than national barley (5.529 ppm). Serum AST activity remained within the normal reference range across selenium-status groups. In contrast, CK activity was higher than the normal reference value in all selenium-status groups; however, no statistically significant differences were detected among low, marginal, and adequate selenium groups. These findings indicate marked environmental and nutritional variation in selenium exposure within the study area. The elevation of CK activity may reflect subclinical muscle stress or non-specific muscle damage rather than selenium deficiency alone. Further studies with larger sample size, replicated soil and feed sampling, and integrated assessment of selenium bioavailability are recommended to clarify the epidemiological relationship between environmental selenium, dietary intake, and lamb health in this region.

1. INTRODUCTION

Selenium is an essential trace element that contributes to animal health by supporting growth, reproductive performance, immune function, and antioxidant defense (Jamal Rajab et al., 2023). Its biological role is primarily mediated through its incorporation into selenoproteins, particularly glutathione peroxidase, which helps protect cellular membranes from oxidative injury by reducing hydrogen peroxide and lipid hydroperoxides (Sobiech & Kuleta, 2002). In grazing ruminants, selenium status is largely determined by dietary intake, which in turn depends on selenium concentration and bioavailability in soils and plants (Gupta & Gupta, 2000). Selenium deficiency, often associated with vitamin E deficiency, is an important nutritional disorder in sheep, goats, and calves. It is commonly linked to nutritional muscular dystrophy, also known as white muscle disease, a degenerative condition that primarily affects skeletal muscles and, in severe cases, may involve cardiac muscles (Bostedt & Schramel, 1990; Ekinci et al., 2025; Gupta & Gupta, 2000). Muscle degeneration and necrosis associated with this condition may result in clinical signs such as weakness, stiffness, poor growth, respiratory distress, or sudden death. Several biochemical indicators have been used to assess muscle damage in suspected selenium-related disorders, particularly creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and glutathione peroxidase (GSH-Px) (Ataollahi, Mohri, & Seifi, 2013; Sobiech & Kuleta, 2002). However, the relationship between selenium status and serum enzyme activities is not always consistent, as enzyme responses may also be influenced by age, stress, management, nutrition, and subclinical muscle injury (Faixová et al., 2007; Kumar et al., 2008; Teke et al., 2014). From an epidemiological perspective, selenium status in animals reflects a complex interaction between environmental, nutritional, and animal-related determinants. Soil selenium concentration, soil type, rainfall, pH, organic matter, and plant species can influence selenium uptake by forage and grains, thereby affecting dietary selenium exposure in grazing animals (Cuvardic, 2003; Humann-Ziehank, Tegtmeyer, Seelig, Roehrig, & Ganter, 2013; Mehdi & Dufrasne, 2016). Regional variation in selenium status has been reported in ruminants, and such variation may be associated with differences in local geology, soil selenium availability, feed resources, and feeding practices (Ademi et al., 2015; Hailu et al., 2022). Therefore, evaluating selenium status requires an integrated approach that considers environmental sources, dietary exposure, and animal-level biochemical indicators. In Al-Jabal Al-Akhdar region of Libya, previous local findings indicated that a proportion of lambs had low or marginal serum selenium concentrations, particularly in mountainous areas (Abdulmawli, Ismaael, Mustufa, Eissa, & A, 2023). However, the contribution of environmental and nutritional factors, particularly soil and barley selenium concentrations, to selenium status and related muscle enzyme activities in lambs has not been sufficiently clarified. Therefore, the present epidemiological study was conducted to evaluate selenium concentrations in soils from mountainous and South Mountain areas, estimate selenium levels in national and imported barley used as a major dietary source, and assess serum CK and AST activities across selenium-status groups in local lambs from Al-Jabal Al-Akhdar, Libya.

2. METHOD

2.1 Study design and area:

A cross-sectional epidemiological observational study was conducted in two geographically distinct areas of Al-Jabal Al-Akhdar region, Libya: the Mountainous area and the South Mountain area. These areas were included to represent different ecological and geographical conditions within the region. During the same study period, animal, soil, and barley samples were collected to evaluate environmental and nutritional determinants of selenium exposure and to assess serum CK and AST activities across selenium-status groups in local lambs.

2.2 Animals:

The study included 100 apparently healthy local lambs randomly selected from different flocks in the study areas. Lambs of both sexes were included, and their ages ranged from 1 to 4 months, with body weights ranging from 3 to 10 kg. A clinical examination was performed before sampling to assess the general health condition of the animals. All examined flocks were managed under an open grazing system.

2.3 Serum biochemical and selenium analysis

Blood samples were collected from the jugular vein and then centrifuged at 3000 rpm for 15 minutes to obtain serum. Serum samples were transported to Al-Taraham Laboratory for biochemical and selenium analysis. Serum creatine kinase (CK) and aspartate aminotransferase (AST) activities were measured using a Roche Cobas C311 analyzer according to the procedure described by Pathipati, Jayasri, Supriya, and Siva Kumar (2020). The reference values used for interpretation were CK < 208 U/L and AST < 126 U/L, according to Laboklin (2022).

Serum selenium concentrations were determined from the same blood samples collected from the examined lambs using Atomic Absorption Spectrophotometry. Based on serum selenium concentrations, lambs were classified into three selenium-status groups: low selenium status (< 0.03 mg/L), marginal selenium status (0.03–0.06 mg/L), and adequate selenium status (0.06–0.40 mg/L). These categories were used to compare serum CK and AST activities among selenium-status groups.

2.4 Soil and grain samples

In each study area, three soil samples were collected and pooled to obtain one representative composite sample for each area. Soil samples were randomly collected from two depths, 0–15 cm and 15–30 cm, placed in clean plastic bags, labeled, and transported to the laboratory for analysis. Grain samples included national and imported barley. The national barley sample was obtained from the same barley source used for feeding the examined flock, whereas the imported barley sample was obtained from local commercial feed markets to represent a commonly available alternative feed source in the study region. Selenium concentrations in soil and barley samples were determined using Atomic Absorption Spectrophotometry according to the procedure described by Mangan et al. (2016). Soil and barley selenium concentrations were expressed as parts per million (ppm).

2.5. Statistical analysis

Data were analyzed using SPSS software, version 23. Serum CK and AST activities were presented as mean ± standard deviation (SD) according to selenium-status groups. One-way analysis of variance (ANOVA) was used to determine differences in CK and AST activities among low, marginal, and adequate selenium-status groups, with statistical significance set at $P < 0.05$. Selenium concentrations in soil and barley were presented descriptively in ppm and compared with relevant reference values because the soil samples were analyzed as composite representative samples.

3. ETHIC APPROVAL

The study protocol was reviewed and approved by the Al-Mukhtar Committee for Biosafety and Bioethics (MCBB), Omar Al-Mukhtar University, Libya, under approval reference number NBC: 007. A.26.81. Blood sampling was performed by qualified veterinarians following standard animal welfare procedures, and consent was obtained from flock owners before sample collection.

4. RESULT

1. Selenium concentration in soil:

Selenium concentration varied between the two studied areas. The South Mountain soil sample showed a higher selenium concentration, with a value of 15.979 ppm, compared with 0.302 ppm in the Mountainous soil sample (Table 1).

2. Selenium concentration in barley:

Selenium concentration also differed between the two barley sources. The imported barley sample showed a higher selenium concentration, with a value of 7.28 ppm, compared with 5.529 ppm in the national barley sample (Table 2).

3. Serum AST and CK activities according to selenium-status groups

Serum AST activity remained within the normal reference range across all selenium-status groups. The mean AST activity was 124.44 ± 41.716 U/L in the low selenium group, 120.29 ± 44.429 U/L in the marginal selenium group, and 121.56 ± 7.209 U/L in the adequate selenium group. No statistically significant difference in AST activity was detected among the selenium-status groups ($P = 0.938$).

In contrast, serum CK activity was higher than the normal reference value (< 208 U/L) in all selenium-status groups. The mean CK activity was 676.69 ± 142.59 U/L in the low selenium group, 627.56 ± 180.23 U/L in the marginal selenium group, and 687.71 ± 163.77 U/L in the adequate selenium group. However, CK activity did not differ significantly among the selenium-status groups ($P = 0.661$) (Table 3).

5. DISCUSSION

The present study showed a marked descriptive difference in soil selenium concentration between the two studied areas. Selenium concentration was higher in the South Mountain soil sample (15.979 ppm) than in the Mountainous soil sample (0.302 ppm) (Table 1). This difference supports the importance of local environmental and geochemical conditions in determining selenium availability in grazing areas. In general, low soil selenium concentrations may contribute to insufficient selenium intake in grazing ruminants, particularly when forage and feed resources are also selenium-deficient. Soil selenium concentrations below approximately 0.1 mg/kg have been considered inadequate for ruminants (Huo, Wu, Song, & Shen, 2020); while critical soil selenium values have also been reported within the range of 0.2–0.4 mg/kg (Cuvardic, 2003). Therefore, the selenium concentration recorded in the Mountainous area in the present study may indicate a relatively low selenium environment compared with the South Mountain area.

The observed difference between the two areas may be related to variations in soil parent material, local geology, climate, rainfall, relief, organic matter, and selenium chemical forms. These factors are known to influence total selenium concentration and selenium bioavailability in soils (Cuvardic, 2003). Wide geographical variation in soil selenium concentrations has been reported, with low values in some regions and elevated values in others, largely reflecting differences in parent material, local geology, soil chemistry, and environmental conditions (Cuvardic, 2003; Fordyce, 2013). For example, regional differences in soil selenium concentrations have been reported in Serbian soils, where selenium levels varied considerably among locations, further supporting the influence of local geochemical conditions on soil selenium distribution (Cuvardic, 2003). The selenium concentration recorded in the South Mountain soil sample was markedly elevated compared with the commonly reported average range for soils and may indicate a potentially seleniferous environment. However, soil selenium concentration alone is not sufficient to confirm toxicity risk, because selenium bioavailability and plant uptake depend on selenium chemical form, soil pH, organic matter, redox conditions, competing ions, plant species, and rainfall patterns (Cuvardic, 2003; Fordyce, 2013; Schiavon & Pilon-Smits, 2017).Rainfall may also influence selenium distribution in soils; in semi-arid areas, limited leaching can contribute to selenium accumulation, whereas higher rainfall may increase selenium leaching from upper soil layers (Cuvardic, 2003; Fordyce, 2013). Therefore, further studies including replicated soil sampling, forage analysis, and assessment of selenium bioavailability are required to determine whether the elevated soil selenium concentration recorded in the South Mountain area is reflected in plant selenium accumulation and represents a true toxicological risk for grazing animals.

The present study also showed variation in selenium concentration between the two barley sources. Selenium concentration was higher in imported barley (7.28 ppm) than in national barley (5.529 ppm) (Table 2). This finding differs from Abobaker, Mahara, and Al-Rmalli (2017), who reported higher selenium levels in barley grain of Libyan origin. Such variation may be attributed to differences in grain origin, soil selenium availability, agronomic practices, and environmental conditions in the production areas. Selenium concentration in cereal grains is influenced by several soil and geochemical factors, including soil pH, redox conditions, selenium speciation, organic matter, mineral composition, and the presence of competing ions such as sulfate and phosphate. Plant-related factors, including species, cultivar, growth stage, and maturity, may also affect selenium uptake and accumulation in grains (Broadley et al., 2010; Hawkesford & Zhao, 2007; Mangan et al., 2016; Schiavon & Pilon-Smits, 2017).

The relatively high selenium concentration observed in imported barley may reflect differences in selenium availability in the soils of the exporting country, the use of selenium-enriched fertilizers, or other agronomic and environmental factors. Selenium fertilization has been shown to increase selenium concentrations in cereal crops, demonstrating that agronomic practices can substantially influence grain selenium content (Broadley et al., 2010). However, because the imported barley sample in the present study was obtained from local commercial feed markets and was not necessarily the same feed consumed by the examined lambs, its result should be interpreted as an indicator of a commonly available feed source rather than direct evidence of dietary exposure in the sampled animals.

Both national and imported barley samples showed selenium concentrations above the levels generally required in sheep diets. The selenium requirement for growing lambs is commonly reported within the range of 0.2–0.3 mg/kg dry matter, depending on diet type and animal performance level (National Research Council, 2007). In contrast, the maximum tolerable dietary selenium concentration for livestock has been reported at approximately 5 mg/kg dry matter (EFSA FEEDAP Panel, 2016; National Research Council, 2005). Therefore, continuous consumption of barley with high selenium concentration as a major dietary component may increase the risk of selenium overexposure, particularly in areas where soil selenium is also elevated, such as the South Mountain area. Nevertheless, the actual toxicological risk depends on the amount of barley consumed, total diet composition, selenium chemical form, bioavailability, duration of exposure, and the selenium contribution from other feed resources. Further feed-based studies are required to evaluate total dietary selenium intake and its relationship with serum selenium status and clinical outcomes in lambs.

Regarding serum enzyme activities, AST levels remained within the normal reference range across selenium-status groups, with no statistically significant difference among groups ($P = 0.938$). This finding is consistent with Kumar et al. (2008), who reported that selenium supplementation had no significant effect on AST activity in lambs. In contrast, CK activity was higher than the normal reference value (< 208 U/L) in all selenium-status groups; however, CK activity did not differ significantly among low, marginal, and adequate selenium groups ($P = 0.661$). Increased CK activity has been reported in lambs affected by selenium-related muscular disorders and subclinical muscle damage (Ataollahi et al., 2013; Sobiech & Kuleta, 2002).

Although CK elevation may indicate muscle membrane damage or subclinical muscular degeneration, it is not specific to selenium deficiency. Previous studies have reported increased CK activity in cases of nutritional muscular dystrophy and white muscle disease; however, CK activity can also be influenced by non-specific factors such as handling, transportation, stocking density, physical stress, and subclinical muscle injury (Sobiech & Kuleta, 2002; Teke et al., 2014). Therefore, the elevated CK activity observed in the present study should be interpreted cautiously. The absence of significant differences among selenium-status groups suggests that CK elevation cannot be attributed to selenium status alone and may reflect non-specific muscle stress or other management-related factors. Further studies with larger sample sizes, repeated enzyme measurements, and additional biomarkers such as glutathione peroxidase, lactate dehydrogenase, vitamin E, and cardiac troponin I are recommended to better clarify the relationship between selenium status and muscle enzyme activity in lambs.

Limitations of the study

This study has some limitations that should be considered when interpreting the findings. First, soil and barley selenium concentrations were assessed using representative samples rather than multiple independent replicate samples; therefore, these results should be interpreted descriptively and cannot be used to infer statistically significant differences between areas or barley sources. Second, the imported barley sample was obtained from local commercial feed markets and was not necessarily the same feed consumed by the examined lambs, which limits direct interpretation of its contribution to animal selenium status. Third, the study did not include forage selenium analysis, total dietary selenium intake estimation, or selenium speciation and bioavailability assessment. Finally, CK and AST activities were measured at a single time point, and additional biomarkers such as glutathione peroxidase, lactate dehydrogenase, vitamin E, and cardiac troponin I were not assessed. These limitations indicate the need for further longitudinal and feed-based studies using replicated environmental sampling and broader biochemical evaluation.

Table 1. Selenium concentration in soil samples from the studied areas.

Soil sample	Selenium concentration (ppm)
Mountainous area soil	0.302
South Mountain area soil	15.979

Table 2. Selenium concentration in national and imported barley samples.

Barley sample	Selenium concentration (ppm)
National barley	5.529
Imported barley	7.280

Table 3. Serum AST and CK activities according to selenium-status groups in lambs.

Selenium status	Serum selenium level (mg/L)	No. of animals	AST mean ± SD (U/L)	CK mean ± SD (U/L)
Low	< 0.03	12	124.44 ± 41.716	676.69 ± 142.59
Marginal	0.03–0.06	33	120.29 ± 44.429	627.56 ± 180.23
Adequate	0.06–0.40	55	121.56 ± 7.209	687.71 ± 163.77

P-value: AST = 0.938; CK = 0.661.

Reference values: AST < 126 U/L; CK < 208 U/L.

6. CONCLUSION

The present study demonstrated marked descriptive variation in selenium concentrations between soil samples from the Mountainous and South Mountain areas of Al-Jabal Al-Akhdar, Libya, suggesting the influence of local environmental and geographical conditions on selenium distribution. Selenium concentration was higher in imported barley than in national barley; however, this difference should be interpreted cautiously because the imported barley sample represented a commercially available feed source rather than the confirmed diet of the examined lambs.

Serum AST activity remained within the normal reference range across selenium-status groups, whereas CK activity was elevated in all groups without significant differences among low, marginal, and adequate selenium groups. Therefore, CK elevation in this study may reflect non-specific muscle stress or subclinical muscle damage rather than selenium deficiency alone. The elevated selenium concentration recorded in the South Mountain soil sample and the relatively high selenium concentrations in barley highlight the need for further studies using replicated soil and feed sampling, forage analysis, total dietary selenium assessment, and broader biochemical indicators to clarify selenium exposure, bioavailability, and potential health risks in lambs in this region.

ACKNOWLEDGMENT

The authors would like to thank all individuals who assisted during field sampling and laboratory analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed to the conception and design of the study. Field sampling and data collection were performed by the research team. Laboratory analyses and data interpretation were carried out by the authors. The first draft of the manuscript was prepared by the authors, and all authors critically reviewed, revised, and approved the final version of the manuscript.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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