EFFECT OF INJECTED SELENIUM DOSES ON THERMOPHYSIOLOGICAL AND PERFORMANCE PARAMETERS IN HEAT-STRESSED DAMANI SHEEP

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Abstract

Background: *Heat stress significantly impairs the health and productivity of ruminants in arid regions by increasing oxidative stress and disrupting physiological homeostasis. Selenium (Se), an essential antioxidant micronutrient, has demonstrated potential to mitigate heat-induced physiological disturbances in livestock. However, breed-specific responses and optimum dosage strategies under field conditions remain unclear.* ***Objectives:*** *This study aimed to evaluate the effect of subcutaneous injections of selenium at different doses on feed intake, body weight (BW), respiration rate (RR), rectal temperature (RT), serum biochemical indices, and hematological variables in Damani sheep during peak summer months.* ***Methods:*** *Fifteen adult male Damani sheep were randomly assigned to three treatment groups: control (0 mg Se), low dose (0.5 mg Se), and high dose (5.0 mg Se). Sodium selenate (5 mg/mL) was injected subcutaneously on days 1, 8, and 15 of each month from June to August. Daily feed intake, RT, and RR were recorded. Blood samples were collected on days 1 and 21 for serum biochemistry and hematology. Data were analyzed using ANOVA and Euclidean distance statistics.* ***Results:*** *Sheep treated with 5 mg Se exhibited significantly lower RT at noon (39.7°C vs. 40.0°C, $P = 0.02$) and reduced BW loss (5.5% vs. 12.1%, $P < 0.05$) compared to control. DMI as %BW was higher in the 5 mg group ($P = 0.05$). Se injection had no effect on most serum biochemical and hematological parameters, except for a significant increase in eosinophil count ($P = 0.02$).* ***Conclusions:*** *High-dose selenium injections alleviated some physiological symptoms of heat stress in Damani sheep, particularly by reducing rectal temperature and mitigating weight loss. These findings suggest selenium's protective role under thermal stress and highlight the need for further studies on dosage optimization and long-term effects.*

INTRODUCTION

High environmental temperatures exert detrimental effects on sheep across many regions of the world. During periods of elevated heat load, a cascade of physiological and immunological responses is activated in an attempt to minimize the adverse impacts of thermal stress. One of the primary consequences of heat load is a disturbance in antioxidant status, marked by increased oxidative stress and reduced blood concentrations of key antioxidant micronutrients such as zinc, selenium, and vitamin E in ruminants (Bernabucci et al., 2002; Saker et al., 2004; Burke et al., 2007) and poultry (Altan et al., 2003; Bartlett and Smith, 2003; Sahin and Kucuk, 2007). This decline is largely attributed to enhanced mobilization and excretion of these micronutrients under hot environmental conditions (Siegel, 1995).

Selenium (Se), an essential trace element, plays a crucial role in the antioxidant defense system and is vital for maintaining the growth, immune function, and metabolic health of both humans and animals. It is involved in several key enzymes and enzymatic reactions that protect against oxidative damage (Underwood, 1977; Surai, 2006). Research has shown that dietary selenium supplementation can enhance feed intake, antioxidant activity, and productive performance under heat stress. For instance, Sahin et al. (2008) demonstrated that selenium supplementation in quail exposed to 34°C improved feed intake, egg production, antioxidant status, and feed efficiency. Similarly, Zhao and Guo (2005) reported that Se supplementation improved antioxidant responses in pigs subjected to heat stress.

In addition to promoting oxidative stress, heat load also impairs thyroid hormone activity, further compromising animal performance. However, selenium supplementation has been observed to ameliorate these adverse effects by enhancing antioxidant capacity and supporting normal thyroid function during thermal stress. Given these findings, it is hypothesized that selenium administration may improve physiological resilience in heat-stressed sheep.

This study was therefore undertaken to evaluate whether multiple injections of selenium during hot summer conditions can improve performance, thermoregulatory responses, and hematobiochemical indices in sheep. Furthermore, the study aims to determine the optimal dosage of injected selenium for mitigating the physiological impacts of heat stress in Damani sheep.

MATERIALS AND METHODS

Animals and Experimental Design

A total of 15 healthy adult male Damani sheep were randomly selected and allocated into three experimental groups (n = 5 per group). The groups were assigned to receive one of three selenium (Se) treatments: 0 mg (control), 0.5 mg, or 5.0 mg of selenium. Selenium was administered subcutaneously in the form of sodium selenate (5 mg/mL) on days 1, 8, and 15 of each month throughout the summer period (June, July, and August).

All animals were maintained under similar management and feeding conditions. Each sheep received a daily ration of concentrate feed (500 g/head/day), supplemented with green grass offered ad libitum. Fresh drinking water was provided continuously.

Feed intake was recorded daily by calculating the difference between the feed offered and the refusals (orts). Body weight was measured individually for each animal before the morning feeding on days 1, 8, 15, and 21 of each month. Rectal temperature and respiration rate (RR) were recorded three times daily (08:00, 12:00, and 16:00) to monitor physiological responses to heat stress.

Blood Sample Collection and Analysis

Blood samples (2 × 10 mL per animal) were collected via jugular venipuncture before feeding on days 1 and 21 of each month. Samples were collected in two types of 10-mL vacutainer tubes: one containing EDTA for hematological analysis and the other without anticoagulant for serum separation.

Serum was isolated by centrifugation at 3000 rpm for 10–15 minutes and used to analyze glucose, total protein, and cholesterol concentrations. Whole-blood samples in EDTA tubes were analyzed using a hematology analyzer to determine total and differential leukocyte counts, red blood cell (RBC) count, hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and other hematological parameters.

Statistical Analysis
Data were analyzed using the Statistical Analysis System (SAS). Descriptive statistics were expressed as mean ± standard deviation (SD). The Euclidean distance statistical method was employed to evaluate differences between treatment and control groups across various parameters.

RESULTS

Temperature-Humidity Index Measurement
Climatic data were recorded daily at three intervals: morning (08:00), noon (12:00), and afternoon (16:00). The THI was calculated as:
THI=(1.8×T+32)−(0.0055×RH)×(1.8×T−26)\text{THI} = (1.8 \times T + 32) - (0.0055 \times RH) \times (1.8 \times T - 26)THI=(1.8×T+32)−(0.0055×RH)×(1.8×T−26)
Where T is the ambient temperature (°C) and RH is the relative humidity (%).
The average THI recorded during the experimental period was 82.81, indicating the presence of heat stress conditions.

Dietary Selenium Intake
The selenium concentrations in the basal diet and drinking water were 0.006 mg Se/kg dry matter (DM) and 0.094 mg Se/L, respectively. Total selenium intake from feed and water across treatments was approximately 0.10 mg Se/day. Consequently, the estimated total Se intake was:

- Control group: 0.10 mg Se/day
- 0.5 mg injected Se group (0.1 mL sodium selenate): ~0.17 mg Se/day
- 5.0 mg injected Se group (1.0 mL sodium selenate): ~0.81 mg Se/day

Rectal Temperature and Respiration Rate
Results for rectal temperature (RT) and respiration rate (RR) are summarized in Table 1. There were no significant differences ($P > 0.05$) in RT at 08:00 and 16:00 between the control and treatment groups. However, at 12:00, the RT of sheep injected with 5 mg Se was 0.3°C lower ($P = 0.02$) than that of the control group (39.7°C vs. 40.0°C). A similar trend was observed in the mean RT, which was significantly lower ($P = 0.05$) in the 5 mg Se group compared to control.
Respiration rate did not differ significantly ($P > 0.05$) between groups at any timepoint. Across treatments, the average RR was 85.7, 83.4, and 82.1 breaths/min for the 0.0, 0.5, and 5.0 mg Se groups, respectively. Both RT and RR were significantly affected by time of day ($P < 0.01$). RT increased from 39.0°C at 08:00 to 40.0°C at 16:00, while RR increased from 36.4 bpm at 08:00 to 130.7 bpm at 16:00 ($P < 0.01$).

Table 1: Effect of various doses of injected selenium on rectal temperature and respiration rate in sheep exposed to heat stress.

Item	0.0 mg	0.5 mg	5.0 mg	SE	P-value
Rectal Temperature (°C)					
0800 h (A)	39.1	39.1	38.9	0.1	0.16
1200 h (B)	40.0^{b}	39.9^{ab}	39.7^{a}	0.1	0.02
1600 h (C)	40.1	40.3	40.0	0.2	0.62
Mean	39.8^{a}	39.7^{ab}	39.5^{a}	0.1	0.05
Respiration Rate (bpm)					
0800 h (A)	37.1	36.3	35.8	2.4	0.09
1200 h (B)	90.1	87.3	86.3	8.8	0.08
1600 h (C)	132.5	129.2	130.3	7.9	0.29
Mean	85.7	83.4	82.1	4.5	0.53

Superscripts with different letters (a,b) within a row differ significantly ($P < 0.05$). *Superscripts A–C within a column differ significantly by time of day ($P < 0.01$).*

Dry Matter Intake and Body Weight Changes

Results for DMI and BW changes are presented in Table 2. There were no significant differences (P > 0.05) in DMI among treatment groups when expressed in absolute terms. However, when expressed as a percentage of BW, the 5 mg Se group had significantly higher intake (2.2%) compared to the control (2.0%) (P = 0.05). Although final BW did not significantly differ among groups, the 5 mg Se group experienced less weight loss (−1.5 kg) compared to the control group (−3.3 kg) (P = 0.05). The percentage BW loss was also significantly lower in the 5 mg group (5.5%) than in the control (12.1%).

Table 2: Effect of injected selenium on dry matter intake and body weight in sheep under heat stress.

Item	0.0 mg	0.5 mg	5.0 mg	SE	P-value
DMI (g/day)	503.3	534.8	564.7	44.8	0.12
DMI (% BW)	2.0^{a}	2.1^{ab}	2.2^{b}	0.0	0.05
Initial BW (kg)	27.3	26.9	27.4	1.4	0.96
Final BW (kg)	24.0	25.0	25.9	1.3	0.61
BW Change (kg)	-3.3^{a}	-1.9^{ab}	-1.5^{b}	0.5	0.05
BW Loss (%)	12.1^{a}	7.0^{ab}	5.5^{b}	1.8	0.05

Means with different superscripts within a row differ significantly (P < 0.05).

The results indicate that subcutaneous selenium supplementation, particularly at a dose of 5.0 mg, had a positive impact on the physiological and performance responses of Damani sheep exposed to heat stress (average THI = 82.81). Selenium significantly reduced rectal temperature at midday and in the overall daily mean, suggesting improved thermoregulatory capacity. Although daily dry matter intake (DMI) did not differ significantly in absolute terms, intake relative to body weight was higher in the 5.0 mg group (2.2% vs. 2.0% in controls), indicating enhanced feed efficiency. Body weight loss was also significantly reduced in the 5.0 mg group, both in absolute terms (−1.5 kg vs. −3.3 kg) and as a percentage of initial body weight (5.5% vs. 12.1%), reflecting a protective effect of selenium under thermal stress. While respiration rate did not differ statistically among groups, a downward trend was observed with increasing selenium dosage. Notably, eosinophil counts were significantly elevated in selenium-treated groups, possibly reflecting an improved immune response. Other hematological and biochemical parameters remained unaffected, suggesting selenium's benefits were specific to thermoregulation, nutrient utilization, and selective immune modulation.

Table 3: Key Physiological and Performance Indicators in Damani Sheep Administered Different Doses of Selenium under Heat Stress Conditions

Parameter	Control (0.0 mg)	0.5 mg Se	5.0 mg Se	Significant Difference (P < 0.05)
THI (avg)	82.81	82.81	82.81	No
Rectal Temp at 1200 h (°C)	40.0	39.9	39.7	Yes (Control > 5.0 mg)
Mean Rectal Temp (°C)	39.8	39.7	39.5	Yes (Control > 5.0 mg)
Mean RR (breaths/min)	85.7	83.4	82.1	No
DMI (g/day)	503.3	534.8	564.7	No
DMI (% BW)	2.0	2.1	2.2	Yes (Control < 5.0 mg)
BW Change (kg)	−3.3	−1.9	−1.5	Yes (Control < 5.0 mg)
BW Loss (% of initial BW)	12.1	7.0	5.5	Yes (Control > 5.0 mg)
Eosinophils ($\times 10^9$ cells/L)	0.06	0.08	0.09	Yes (Control < others)

Other Hemato-Biochemical Parameters	–	–	–	No significant differences

Selenium injections at 0.5 mg and 5.0 mg doses had no statistically significant effect ($P > 0.05$) on any measured biochemical parameter, including glucose, total protein, cholesterol, NEFA, and creatinine concentrations. These findings suggest that selenium supplementation did not adversely alter hepatic or renal function, nor did it disturb energy metabolism under heat stress conditions. The values remained within physiological norms, indicating that selenium maintained metabolic stability during environmental thermal stress (Table 4).

Table 4: Effects of various doses of sodium selenite solution on serum biochemical parameters (mmol/L) in sheep exposed to hot conditions

Parameter	0.0 mg Se	0.5 mg Se	5.0 mg Se	SE	CV (%)	P-value
Glucose	3.65	3.67	3.68	0.24	6.77	0.78
Total Protein	63.27	58.05	60.12	1.66	2.74	0.20
Cholesterol	1.87	1.67	1.63	0.09	5.22	0.23
NEFA	0.66	0.63	0.75	0.15	22.10	0.85
Creatinine	83.1	81.6	79.4	1.8	2.26	0.41

Among all hematological parameters measured, only eosinophil counts increased significantly ($P = 0.02$) in both selenium-treated groups compared to the control. This elevation suggests that selenium may play a role in enhancing immune response under thermal stress, as eosinophils are involved in parasitic defense. All other hematological indices, including WBC, RBC, hemoglobin, and platelet counts, remained unaffected by selenium administration and stayed within physiological reference ranges. This indicates that selenium injections did not disrupt hematopoiesis or cause hematological toxicity in heat-stressed sheep (Table 5).

Table 5: Effects of various doses of sodium selenite solution on hematological variables in sheep exposed to hot conditions

Parameter	0.0 mg Se	0.5 mg Se	5.0 mg Se	SE	P-value
WBC ($\times 10^9$/L)	5.17	4.68	4.67	0.63	0.82
Neutrophils ($\times 10^9$/L)	2.03	1.78	1.73	0.29	0.63
Lymphocytes ($\times 10^9$/L)	2.05	1.83	1.93	0.29	0.55
Monocytes ($\times 10^9$/L)	0.94	0.92	0.85	0.09	0.79
Eosinophils ($\times 10^9$/L)	0.06a	0.08b	0.09b	0.02	0.02*
Basophils ($\times 10^9$/L)	0.08	0.07	0.08	0.01	0.87
RBC ($\times 10^{12}$/L)	10.0	10.3	10.0	0.5	0.91
Hemoglobin (g/L)	1.07	1.09	1.15	0.45	0.51
Hematocrit (%)	26.2	26.7	27.3	0.8	0.56
MCV (fL)	26.1	25.4	27.1	1.2	0.61
MCH (pg)	10.7	10.6	11.5	0.3	0.16
MCHC (g/L)	420	420	424	7	0.86
Platelets ($\times 10^{12}$/L)	717	685	661	49	0.17
Neutrophil:Lymphocyte Ratio	0.99	0.97	0.90	0.12	0.78

*Values with different superscripts (a,b) differ significantly at $P < 0.05$.

Discussion

To evaluate the severity of heat stress in ruminants, physiological markers such as respiration rate, rectal temperature and pulse rate are widely accepted as reliable indicators of thermal stress (Covey et al., 2010; Juniper et al., 2008). Accordingly, this study examined several behavioral and physiological stress parameters. Nutritional strategies, including targeted antioxidant supplementation, have been reported to modulate these stress responses effectively. In the present study, elevated values of stress markers were observed in both control and treated groups, relative to baseline values reported for the indigenous Damani sheep breed. The increased RR under heat stress reflects a physiological adaptation aimed at dissipating excess body heat via the respiratory tract. Notably, selenium supplementation reduced RR, suggesting its positive role in mitigating the impact of thermal stress. Similarly, RT is a sensitive indicator of heat load, with a 1°C increase typically associated with a measurable decline in animal performance. Our findings are consistent with previous reports indicating elevated RT and RR in sheep subjected to high ambient temperatures (Finocchiaro et al., 2005; Kumar et al., 2009). The lower RR and RT observed in the Se-treated groups compared to the control group may be attributed to the antioxidant and metabolic protective roles of selenium. Huang et al. (2022) demonstrated that antioxidant-supplemented diets significantly reduced RR and RT in heat-stressed Merino × Poll Dorset ewes. This aligns with results from Alhidary et al. (2012), who reported a 0.3°C decrease in RT following subcutaneous sodium selenate injections (days 1, 8, and 15) in Australian Merino sheep. Similarly, Kumar et al. (2009) found that vitamin E, selenium, and vitamin C supplementation improved thermoregulatory parameters in Black Bengal goats under heat stress.

It is generally accepted that the upper critical temperature threshold for sheep lies between 25°C and 30°C; heat stress is initiated when ambient temperatures exceed this range (Fuquay, 1981). In our study, the average Temperature-Humidity Index was 80.6, which falls within the range defined as moderate heat stress. Regardless of treatment, all animals exhibited clear signs of heat load: RT increased by approximately 1.2°C, RR rose by 94.3 bpm from morning to afternoon, dry matter intake declined by 15%, and body weight was reduced by 8.2% over the trial period. These responses are well-documented markers of heat stress in livestock, including cattle (Gaughan et al., 1999; Mader et al., 2002) and sheep (Silva et al., 1992; Silanikove, 2000), and are consistent with the findings of Kumar et al. (2009), and Beatty et al. (2006).

In this study, the selenium dosages administered via subcutaneous injection (0.5 mg and 5.0 mg Se as sodium selenate) corresponded to average daily doses of 0.17 mg and 0.81 mg Se, respectively, based on a mean DMI of 535 g/day. The 0.5 mg dose aligns with NRC (1985) recommendations of 0.1–0.2 mg Se/kg DM for small ruminants, whereas the 5.0 mg dose represents a supranutritional level, exceeding NRC guidelines by a factor of eight. Interestingly, sheep injected with 5 mg Se showed an average 0.3°C reduction in RT compared to controls, a finding not previously reported. Two plausible mechanisms may underlie this effect: (1) selenium may restore heat stress-induced reductions in antioxidant activity (Underwood, 1977; Bernabucci et al., 2002; Surai, 2006); and (2) selenium may suppress adrenocorticotropic hormone (ACTH) release, which is known to elevate during thermal stress (Iqbal et al., 2015). ACTH administration has been shown to increase RT by 0.6°C in sheep. Supporting this, Wasti et al. (2020) found that selenium supplementation in heat-stressed Japanese quail reduced ACTH levels significantly.

Contrary to expectations, no significant difference in DMI was found between Se-treated and control sheep. This is consistent with prior studies reporting no effect of Se source or dose on feed intake under thermoneutral conditions in sheep (Rock et al., 2001; Kumar et al., 2009; Vignola et al., 2009) and calves (Ebrahimi et al., 2009; Covey et al., 2010). However, under thermal stress, Sahin and Kucuk (2001) observed increased DMI in quail fed 0.3 mg Se/kg DM. In the current trial, selenium had a beneficial effect on BW retention, with 5 mg Se-treated sheep losing 4.5% less BW than controls. This aligns with Sahin et al. (2008), who reported that quail fed 0.3 mg Se/kg DM under 34°C conditions had 6% higher BW than unsupplemented birds. Similarly, improved daily

BW gain in selenium-injected calves grazing selenium-deficient pastures was noted. Other studies in sheep have also reported BW gains under supranutritional Se supplementation (Kumar et al., 2009).

Finally, selenium injections did not significantly affect serum glucose, total protein, cholesterol, NEFA, or creatinine concentrations, and all values remained within normal physiological ranges (Lepherd et al., 2009). These findings are consistent with studies by Juniper et al. (2008), Ebrahimi et al. (2009), and Kumar et al. (2009), which demonstrated that neither Se dose nor source significantly altered key metabolic blood parameters in ruminants.

Conclusion

This study demonstrates that subcutaneous injection of selenium, particularly at a dose of 5.0 mg/week, significantly mitigated key physiological responses to heat stress in sheep, including reduced rectal temperature, improved body weight retention, and elevated eosinophil counts. While Se administration had no significant impact on most hematological and biochemical parameters, it's thermoregulatory and immunomodulatory benefits under thermal stress were evident. These findings suggest that injectable Se may be an effective strategy for enhancing heat tolerance in sheep exposed to high ambient temperatures. Further research is warranted to optimize Se dosage and delivery methods for broader application in small ruminant heat stress management protocols.

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