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ARTICLE

The effect of vitamin E and selenium on physiological, hormonal and antioxidant status of Damani and Balkhi sheep submitted to heat stress

Shakirullah[1] · Muhammad Subhan Qureshi[1] · Sohail Akhtar[1] · Rifat Ullah Khan[2]

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Abstract The present study was conducted to find the effect of vitamin E and selenium (Se) on physiological, hormonal and antioxidant status of Damani and Balkhi sheep under high ambient temperature. Forty Damani and Balkhi healthy multiparous, non-pregnant sheep having similar initial body weight were selected. The sheep in each breed were further equally divided into control and treated groups. The sheep were fed a dietary plan recommended by the National Research Council (Nutrient requirements of small ruminants: sheep, goats, cervids, and new world camelids, National Academies Press, Washington, DC, 2007). In addition, the sheep in the treated groups were supplemented with Se (0.3 mg) and vitamin E (50 mg) per kg of diet for 4 weeks. The results indicated that respiration and pulse rate decreased significantly ($P < 0.05$) on day 28 compared to the first day of the study in the treatment groups. The concentration of T3 and T4 was significantly ($P < 0.01$) high in Damani sheep compared to Balkhi. In addition, except follicle-stimulating hormone (FSH) and progesterone, T3 and T4 were significantly ($P < 0.01$) high in the treated sheep compared to the control. Malondialdehyde (MDA), cortisol and heat-shock protein (HSP-70) increased significantly ($P < 0.05$) in Balkhi sheep compared to Damani. In the treated sheep, MDA, cortisol and HSP-70 were significantly ($P < 0.01$) low, while superoxide dismutase (SOD) and glutathione peroxidase (GPx) were significantly ($P < 0.01$) high in the treatment sheep. It was concluded that vitamin E and Se at the present doses improved the physiological, hormonal and antioxidant status in Damani and Balkhi sheep. In addition, Damani sheep were more tolerant to heat stress than Balkhi sheep.

Keywords Antioxidant · Balkhi · Damani · Heat stress · Hormone · Sheep

Introduction

Thermal comfort in livestock has a long-term consequence which largely depends upon species, breed and health. Heat stress is one of the major concerns of the livestock industry, which adversely affects their health and productivity [2, 3]. Usually the tolerance level of temperature ranges from −12 to 32 °C for the adult sheep [4]. The National Research Council [1] has suggested the nutrient requirements for the optimum performance for the adult sheep. However, during heat stress, the production and reproduction of sheep may be adversely affected. Therefore, under such environmental condition, the nutrient requirements of sheep are usually below their need, and animal productivity may be adversary affected [5]. Therefore, for the optimum performance and health, the sheep diet must be supplemented with advanced nutritional formulation and identification of heat-tolerant breeds.

Antioxidants are the substances which are necessary to neutralize the reactive oxygen species (ROS) produced during the heat stress [2]. In livestock, the dietary supplementation with vitamin E and selenium (Se) may improve the negative effect of heat stress and restore the redox

✉ Rifat Ullah Khan
rifatullahkhhan@gmail.com

[1] Department of Livestock, Breeding and Genetics, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

[2] Department of Animal Health, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

homeostasis in different breeds of sheep [6, 7] showing the synergistic effect of vitamin E and Se than alone [7]. The sheep farmers in the hot climate should consider the higher solar radiation and temperature fluctuations and poor quality of feed resources. Damani and Balkhi sheep are the famous local breeds. The aim of the present study was to find the effect of vitamin E and Se supplementation on the antioxidant status, metabolic and reproductive hormonal profile in the two breeds under heat stress condition.

Materials and methods

This experiment was approved by the departmental committee on ethics and animal welfare approved by the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan

Selection of animals and feeding management

In this experiment, 40 Damani and Balkhi (3–4 years old) healthy multiparous, non-pregnant sheep having similar initial body weight (Damani, 36 ± 2.3 kg, Balkhi, 43 ± 1.2 kg) and body condition score (3.5 ± 0.1) were selected. The sheep in each breed were further equally divided into control ($n = 10$) and treated groups ($n = 10$ each group). The sheep were fed a dietary plan recommended by the National Research Council [1] having 16% crude protein. The sheep in the control and treated groups were fed a concentrate (500 mg/day/sheep) diet and green grass (ad libitum). The concentrates were composed of 25% cotton seed cake, 14% mustard, 12% rice polish, 16% wheat bran, 10% maize oil cake, 10% corn gluten, 11% molasses and 1% dicalcium phosphate. In addition, the sheep in the treated groups were supplemented with Se (0.3 mg) and vitamin E (50 mg) per kg of diet. The treatment was continued for 4 weeks.

Measurement of temperature and humidity index (THI)

The climatic data were compiled on a daily basis by measuring the temperature and humidity at morning (08:00), noon (12:00 AM) and afternoon (04:00) using the formula described by Ihsanaullah et al. [8].

$$\text{THI}: (1.8 \times \text{T} \pm 32) - (0.0055 \times \text{RH}) \times (1.8 \times \text{T} - 26),$$

where T is the temperature (°C), RH is the relative humidity (%)

The average THI during the experimental period was 82.81.

Blood collection and analysis

Blood samples (10 ml) were collected from the jugular vein of each ewe in the beginning of the study (day 0), after 14 days and at the end (28 days) of the study. The blood sample was centrifuged at 3000 RPM for 10 min. Then, serum was stored at −20 °C till further analysis. Hormonal profile of follicle-stimulating hormone (FSH), progesterone and cortisol were assayed with the help of ELISA reader using commercially available kits (Calbiotech, USA). Malondialdehyde (MDA), superoxide dismutase (SOD), heat-shock protein (HSP-70) and glutathione peroxidase (GPx) were determined with the help of spectrophotometer using commercial kits (BioVision, USA). Triiodothyronine (T3) and thyroxin (T4) were determined with help of commercial kits (BioCheck, USA).

Statistical analysis

Data were analyzed with the help of statistical software using Statistical Analysis System [9]. Data were analyzed with the help of analysis of variance (ANOVA) to detect the statistical difference at days 0, 14 and 28 taking breed, group and day as dependant factors.

Results

The effect of treatments on Balkhi and Damani breeds at various days on respiration, rectal temperature and pulse rate during heat stress is given in Table 1. No significant ($P > 0.05$) difference was found between these parameters in the two breeds and groups. Respiration (45.40 ± 1.12/min) and pulse rate (58.95 ± 1.30/min) decreased significantly ($P < 0.05$) on day 28 compared to the first day of the study in response to the treatment of vitamin E and Se.

The effect of treatments on Balkhi and Damani breeds at various days on progesterone, FSH, T3 and T4 during heat stress is given in Table 2. The results indicated that progesterone and FSH did not vary between the breeds, treatment and days of the experimental period. The blood concentration of T3 (1.75 ± 0.03; nmol/l) and T4 (69.84 ± 0.91; nmol/l) was significantly ($P < 0.01$) high in Damani sheep compared to Balkhi. In addition, except FSH and progesterone, T3 and T4 were significantly ($P < 0.01$) high in the treated sheep compared to the control. On day 28, progesterone, T3 (1.67 ± 0.07, nmol/l) and T4 (66.24 ± 1.82 nmol/l) were significantly ($P < 0.01$) high in the treated sheep compared to day 1, while blood FSH decreased significantly ($P < 0.01$) on day 28 compared to day 1.

Table 1 Means (±SE) of various hormonal and physiological stress parameters of Balkhi and Damani sheep in treated and control group during heat stress

Independent variable	Respiration/min	Rectal temperature/min	Pulse/min
Breed			
Balkhi	50.46 ± 0.59	102.72 ± 0.09	63.30 ± 1.11
Damani	48.96 ± 0.82	102.43 ± 0.08	59.66 ± 0.95
P value	0.07	0.32	0.56
Group			
Treated	47.70 ± 1.00	102.52 ± 0.08	57.13 ± 0.83
Control	45.73 ± 0.95	102.63 ± 0.09	65.83 ± 0.62
P value	0.07	0.30	0.11
Day			
Day 1	47.90^{a} ± 1.35	102.85^{a} ± 0.13	64.25^{a} ± 1.28
Day 14	46.85^{ab} ± 1.15	102.50^{b} ± 0.09	61.25^{b} ± 1.16
Day 28	45.40^{b} ± 1.12	102.37^{b} ± 0.08	58.95^{c} ± 1.30
P value	0.01	0.01	0.01
Breed × group	0.14	0.97	0.18
Group × day	0.14	0.97	0.18
Breed × group × day	0.43	0.21	0.68

Mean values bearing different superscripts differ significantly ($P < 0.05$)

Table 2 Means (±SE) of various reproductive and metabolic hormones of Balkhi and Damani sheep in treated and control group during heat stress

	Progesterone (ng/ml)	FSH (IU/l)	T3 (nmol/l)	T4 (nmol/l)
Breed				
Balkhi	2.38 ± 0.31	0.37 ± 0.02	1.46 ± 0.05	60.66 ± 1.38
Damani	3.18 ± 0.41	0.34 ± 0.02	1.75 ± 0.03	69.84 ± 0.91
P value	<0.11	<0.11	<0.01	<0.01
Group				
Treated	4.62 ± 0.19	0.36 ± 0.03	1.84 ± 0.02^{a}	70.83 ± 0.80^{a}
Control	3.95 ± 0.07	0.35 ± 9.53	1.38 ± 0.03^{b}	59.67 ± 1.19^{b}
P value	<0.01	0.45	<0.01	<0.01
Day				
Day 1	3.03 ± 0.37	0.48 ± 0.03	1.55^{c} ± 0.05	63.50^{b} ± 1.77
Day 14	3.02 ± 0.43	0.32 ± 0.01	1.60^{b} ± 0.06	66.02^{a} ± 1.70
Day 28	3.30 ± 0.51	0.27 ± 0.01	1.67^{a} ± 0.07	66.24^{a} ± 1.82
P value	<0.11	<0.31	<0.01	<0.01
Breed × group	<0.23	<0.64	<0.01	<0.01
Group × day	<0.34	<0.92	<0.01	<0.90
Breed × group × day	0.25	0.53	0.05	0.32

Mean values bearing different superscripts in a column differ significantly ($P < 0.05$)

The effect of treatments on Balkhi and Damani breeds at various days on MDA, SOD, GPx, HSP-70 and cortisol during heat stress is given in Table 3. The results revealed that MDA (5.11 ± 0.13, nmol/l), cortisol (2.26 ± 0.05, nmol/l) and HSP-70 (48.22 ± 1.07 ng/ml) increased significantly ($P < 0.05$) in Balkhi sheep compared to Damani, while SOD (88.45 ± 3.21, U/ml) and GPx (448.26 ± 15.12 mU/l) decreased ($P < 0.01$) in the same breed of sheep. In the treated sheep, MDA (3.93 ± 0.09, nmol/l), cortisol (1.86 ± 0.04, (ng/ml) and HSP-70 (39.08 ± 0.96, nmol/l) were significantly ($P < 0.01$) low, while SOD (109.43 ± 2.22 U/ml) and GPx (575.00 ± 10.01 mU/l) were significantly ($P < 0.01$) high in the treatment sheep. No significant ($P > 0.05$) difference was found in MDA and SOD concentration between days 1, 14 and 28. The GPx (521.58 ± 20.65 mU/l) increased significantly ($P < 0.01$) on day 28 compared to the days 1 and 14, while HSP-70 (42.81 ± 1.81 ng/ml) and cortisol (2.09 ± 0.08 nmol/l) decreased significantly ($P < 0.05$) on day 28.

Table 3 Means (±SE) of hormonal and molecular stress indicators of Balkhi and Damani sheep in treated and control groups during heat stress

Independent variable	MDA (nmol/l)	SOD (U/ml)	GPx (mU/l)	HSP-70 (ng/ml)	Cortisol (nmol/l)
Breed					
Balkhi	5.11 ± 0.13	88.45 ± 3.21	448.26 ± 15.12	48.22 ± 1.07	2.26 ± 0.05
Damani	4.02 ± 0.11	101.74 ± 3.41	547.27 ± 14.91	39.28 ± 0.88	2.01 ± 0.06
P value	<0.01	<0.01	<0.01	<0.01	<0.01
Group					
Treated	3.93 ± 0.09	109.43 ± 2.22	575.00 ± 10.01	39.08 ± 0.96	1.86 ± 0.04
Control	5.20 ± 0.11	80.76 ± 2.43	420.53 ± 10.41	48.41 ± 0.93	2.41 ± 0.02
P value	<0.01	<0.01	<0.01	<0.01	<0.01
Day					
Day 1	4.53 ± 0.16	97.02 ± 2.63	469.08^{c} ± 21.76	45.41^{a} ± 1.17	2.19^{a} ± 0.06
Day 14	4.59 ± 0.19	94.95 ± 3.57	502.63^{b} ± 21.10	43.01^{b} ± 1.63	2.12^{ab} ± 0.07
Day 28	4.58 ± 0.22	93.30 ± 6.10	521.58^{a} ± 20.65	42.81^{b} ± 1.81	2.09^{b} ± 0.08
P value	0.32	0.93	<0.01	<0.01	<0.05
Breed × group	0.01	0.78	0.73	0.07	<0.01
Group × day	<0.01	<0.01	<0.01	<0.01	<0.01
Breed × group × day	0.98	0.45	0.47	0.63	<0.28

Mean values bearing different superscripts in a column differ significantly ($P < 0.05$)

Discussion

To evaluate the danger of heat stress, respiration rate, rectal temperature and pulse rate are the fine markers of thermal stress [10–12]. Therefore, in the current study, different behavioral stress parameters were determined. Feeding plan along with antioxidant supplementation has a beneficial effect on behavioral stress parameters during heat stress. Higher mean values of behavioral stress parameters were observed in Balkhi ewes in both groups (treated and control) as compared to local Damani breed during stress. It means that Balkhi breed is the least susceptible to heat stress as compared to Damani breed. The higher respiratory rate during heat stress is a mechanism to dissipate heat through the respiratory tract. Interestingly, the combined effect of vitamin E and Se reduced the respiratory rate indicating the positive effect in alleviating the severity of the heat stress. Rectal temperature is an important indicator of heat stress, and a rise of temperature by 1 °C in rectum reduces the performance of the animal. The increased rectal temperature and respiratory rate during heat stress were also reported in different breeds of sheep during heat stress [13, 14].

Lower values of all behavioral stress markers in the treated groups compared to control may be due to the dietary supplementation of vitamin E plus Se. Chauhan et al. [6] reported that diet supplemented with antioxidants decreased respiratory rate and rectal temperature in heat-stressed Merino × Poll Dorset crossbred ewes. Our results are in line with Alhidary et al. [13], who reported that subcutaneous injection of sodium selenate on days 1, 8, and 15 decreased rectal temperature by 0.3 °C in Australian Merino sheep uncovered to harsh circumstances. Similar results were also reported in goats by Sivakumar et al. [14] who suggested that supplementation of vitamin E, Se and vitamin C may have beneficial consequences on the physiological parameters of Black Bengal goats during heat stress.

Higher plasma cortisol was reported and reduced by the supplementation of different antioxidants (vitamin E, C and Se) in Black Bengal and Malpura goats [14, 15]. However, the mechanism through which they exert their beneficial effects is still not clear. Generally, the release of cortisol is the major indication of the activation of hypothalamic–pituitary–adrenal axis during stressful condition [8]. Cortisol is the general stress hormone and plays a critical role in many physiological, especially thermal regulation, energy productions, lactogenesis and regulation of milk production [16]. The mean values of cortisol in Balkhi breed of sheep were higher in both groups (treated and control) of animals as compared to a local Damani breed of sheep. It means that Balkhi breed is relatively more prone to stress as compared to the local Damani breed of this area.

Under thermoneutral conditions, the recommended level of vitamin E and Se is sufficient to keep the balance

of production of ROS and the level of antioxidants; however, during heat stress, the level of production of free radicals is higher and beyond the body scope to neutralize them leading to oxidative damage [17]. Chauhan et al. [6] reported that vitamin E + Se stimulated/activated and upregulated SOD and GPx but downregulated ROS. Chung et al. [18] reported that organic Se in feed influences the capability of antioxidant increasing GSH-Px and GST functions in Korean native goats which support our study. Yue et al. [19] recorded that supplementation of vitamin E at the rate of 200 IU in diets may have a positive effect in decreasing MDA and higher concentration of SOD and GSH-Px in testicular cell membrane and mitochondria.

Heat-shock protein is generally present in the cytosol and nucleus. Cytoprotection is the primary function of HSP-70 during heat stress [20]. Our observations are in accordance with the results of Romero et al. [21], who reported that at 43 °C, heat stress significantly increased HSP-70 concentration in sheep. Similar findings were also reported by other authors that increased HSP-70 production is due to the high temperature as common cellular action which protects cells from injury and might take place in the acclimatization process [22–24].

The thyroid gland is the most important and sensitive organ to the thermal stress. It has been reported that the thyroid hormones are the critical modulators of general metabolism and developmental processes [25]. Therefore, the effect of improved feeding plan and management along with antioxidant supplementation on metabolic hormones was determined in the current study. Improved practices of feeding along with antioxidant supplementation have a beneficial effect on metabolic hormones during heat stress. The mean values of both metabolic hormones T3 and T4 in different breeds of sheep were significantly higher in the supplemented group as compared to control group of sheep. It shows that improved feeding along with vitamin E plus Se supplementation and the levels of both metabolic hormones (T3 and T4) were higher as compared to control group during stress. In the current study, we expected that the oxidative stress resulted from the heat stress may have a negative effect on the availability of Se leading to reduced levels of thyroid hormones. Moreover, In the current study, sheep in the treated group were benefited from the combined supplementation of vitamin E and Se resulting in increased production of thyroid hormones concentration during heat stress.

From the results of the present study, it was concluded that vitamin E and Se at the present doses improved the physiological, hormonal and antioxidant status in Damani and Balkhi sheep. In addition, Damani sheep were more tolerant to heat stress than Balkhi sheep.

References

1. National Research Council (2007) Nutrient requirements of small ruminants: Sheep, goats, cervids, and New World camelids. National Academies Press, Washington, DC
2. Khan R, Naz S, Nikousefat Z, Tufarelli V, Javadani M, Rana N, Laudadio V (2011) Effect of vitamin E in heat-stressed poultry. World's Poultry Sci J 67(3):469–478
3. Alhidary IA, Abdelrahman MM, Khan RU (2016) Comparative effects of direct fed microbial alone or with a traces mineral supplement on the productive performance, blood metabolites and antioxidant status of grazing Awassi lambs. Environ Sci Poll Res 23:25218–25223
4. Cwynar P, Kolacz R, Czerski A (2014) Effect of heat stress on physiological parameters and blood composition in Polish Merino rams. Berliner und Münchener Tierärztliche Wochenschrift 127:177–182
5. Alhidary IA, Abdelrahman Khan RU MM, Haroon RM (2016) Antioxidant status and immune responses of growing camels supplemented a long-acting multi-trace minerals rumen bolus. Ital J Anim Sci 15:343–349
6. Chauhan S, Celi P, Leury B, Clarke I, Dunshea F (2014) Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. J Anim Sci 92(8):3364–3374
7. Shah AA, Khan MS, Khan S, Ahmad N, Alhidary IA, Khan RU (2016) Effect of different levels of alpha tocopherol on performance traits, serum antioxidant enzymes, and trace elements in Japanese quail (Coturnix coturnix japonica) under low ambient temperature. Rev Bras Zootec 45:622–626
8. Ihsanullah, Qureshi MS, Suhail SM, Akhtar S, Khan RU (2017) Postpartum ovarian activities, blood metabolites and milk yield are influenced by changing levels of thermal stress in crossbred dairy cows. Intern J Biometerol (**in press**)
9. SAS (2004) Statistical Analysis Systems, 9th edn. SAS Institute, Raleigh
10. Al-Haidary A (2000) Effect of heat stress on some thermoregulatory responses of cattle, sheep and goat. Zagazig Vet J 28:101–110
11. Sunagawa K, Arikawa Y, Higashi M, Matsuda H, Takahashi H, Kuriwaki Z, Hongo F (2002) Direct effect of a hot environment on ruminal motility in sheep. Asian Aust J Anim Sci 15(6):859–865
12. Srikandakumar A, Johnson E, Mahgoub O (2003) Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. Small Rumin Res 49(2):193–198
13. Alhidary I, Shini S, Al Jassim R, Gaughan J (2012) Effect of various doses of injected selenium on performance and physiological responses of sheep to heat load. J Anim Sci 90(9):2988–2994
14. Sivakumar A, Singh G, Varshney V (2010) Antioxidants supplementation on acid base ba lance during heat stress in goats. Asian-Aust J Anim Sci 23(11):1462–1468
15. Sejian V, Maurya V, Kumar K, Naqvi S (2012) Effect of multiple stresses (thermal, nutritional and walking stress) on growth, physiological response, blood biochemical and endocrine responses in Malpura ewes under semi-arid tropical environment. Trop Anim Health Prod 10:1007
16. Marai I, Bahgat L, Shalaby T, Hafez A (2000) Response of male lambs to concentrate mixtures given with or without natural clay under Egypt conditions. Ann Arid Zone 39(4):449–460
17. Majid A, Qureshi MS, Khan RU (2015) *In vivo* adverse effects of alpha-tocopherol on the semen quality of male bucks. J Anim Physiol Anim Nutr 99:841–846

18. Chung J, Kim J, Ko Y, Jang I (2007) Effects of dietary supplemented inorganic and organic selenium on antioxidant defense systems in the intestine, serum, liver and muscle of Korean native goats. Asian Aust J Anim Sci 20(1):52
19. Yue D, Yan L, Luo H, Xu X, Jin X (2010) Effect of Vitamin E supplementation on semen quality and the testicular cell membranal and mitochondrial antioxidant abilities in Aohan fine-wool sheep. Anim Rep Sci 118:217–222
20. Gupta A, Cooper ZA, Tulapurkar ME, Potla R, Maity T, Hasday JD, Singh IS (2013) Toll-like receptor agonists and febrile range hyperthermia synergize to induce heat shock protein 70 expression and extracellular release. J Biol Chem 288:2756–2766
21. Romero RD, Pardo AM, Montaldo HH, Rodríguez AD, Cerón JH (2013) Differences in body temperature, cell viability, and HSP-70 concentrations between Pelibuey and Suffolk sheep under heat stress. Trop Anim Health Prod 45:1691–1696
22. Hansen PJ (1999) Possible roles for heat shock protein 70 and glutathione in protection of the mammalian preimplantation embryo from heat shock. Ann Rev Biomed Sci 1:5–29
23. Horowitz M (2002) From molecular and cellular to integrative heat defense during exposure to chronic heat. Comp Biochem Physiol Part A: Mol Integ Physiol 131:475–483
24. Meza-Herrera C, Martínez L, Aréchiga C, Bañuelos R, Rincón R, Urrutia J, Mellado M (2006) Circannual identification and quantification of constitutive heat shock proteins (HSP 70) in goats. J Appl Anim Res 29(1):9–12
25. Rasooli A, Nouri M, Khadjeh G, Rasekh A (2004) The influences of seasonal variations on thyroid activity and some biochemical parameters of cattle. Iran J Vet Res 5(2):1383–1391