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Effect of selenium-yeast feeding on amelioration of simulated heat stress and reproductive performance in Malpura ewe under semi-arid tropical environment

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ABSTRACT

The present study was carried out for 35 days to assess the effect of selenium (Se)-yeast feeding on amelioration of simulated heat stress and reproductive performance of sheep. Twelve adult Malpura ewes were divided into two groups viz., G1 (n=6, control) and G2 (n=6, Se-yeast supplemented). The ewes of G2 were provided with Se at 0.3 mg/kg feed dry matter (DM) through Se-enriched yeast (*Saccharomyces cerevesiea*). The animals were stall fed individually with a diet consisting of 70% roughage (*Cenchrus ciliaris*) and 30% concentrate. All the ewes were estrus synchronized using intra-vaginal progesterone sponges+eCG protocol. To simulate heat stress, the ewes were kept inside the psychometric chamber and were exposed to different temperature at different hours of the day. Supplementation of Se-yeast had no significant effect on physiological response. Higher glucose and a lower protein level was found in the Se-yeast supplemented group under heat stress. Plasma metabolic hormone and cortisol level was higher in G1 as compared to Se-yeast supplemented ewes. Plasma glutathione peroxidase (GPx) was 10.62% higher in G2 as compared to the G1. Estrus percentage and estradiol level was higher in G2 as compared to the G1. From the findings of the present experiment, it is pertinent to conclude that the level of Se enriched yeast fed in this study have limited potential to ameliorate heat stress. However, different doses of Se could be tried in sheep to improve resilience against heat stress.

Key words: Heat stress, Reproduction, Selenium, Sheep

In semi-arid region, since immemorial time, sheep husbandry was serving as a sustainable livelihood resource option for poor marginal farmers. In present time as well as in near future, sheep industry will grow further and will play a vital socio-economic role in arid and semi-arid region (Ben Salem and Smith 2008). Major constraints in sheep production of the semi-arid region arise during the extreme summer. Exposure of sheep to high ambient temperature leads to disturbance of the animal's normal physiological balances. Feed and water intake, blood metabolite, plasma hormonal and enzyme levels are also disturbed as a result of heat stress. These all together have a negative impact on production, immunity and welfare. Therefore, it is essential to explore new initiatives that could be simple, affordable or at least allow to reduce the heat stress of sheep in this region.

Heat load reduces the blood antioxidant micronutrient concentration through oxidative (zinc, selenium (Se) and vitamin E) concentrations in ruminants. The micronutrient mobilization and excretion increases under hot climate (Alhidary *et al*. 2012). Selenium is an essential antioxidant having an important role in growth, health and defence system. Kumar *et al*. (2009) showed that Se supplementation improves the growth rate, humoral immune response and antioxidant status of lamb. Studies suggested that Se enriched yeast reduces the impact of heat stress (Sejian *et al*. 2012). The effects of Se are mediated in antioxidant metabolism by glutathione peroxidase (GPx) in which Se is incorporated in the core of selenocysteine (Chung *et al*. 2007). Reproductive performances of ewes may be improved by Se supplementation (Gabryszuk and Klewiec 2002).

Selenium is essential for thyroid function and homeostasis (Kohrle *et al*. 2005), that controls the metabolism and may affect different blood biochemicals. But, there is a paucity of literature on the effect of Se feeding on blood biochemical, endocrine parameters and reproduction during heat stress period especially in sheep in semi-arid environment. It is expected that Se supplementation during heat stress may ameliorate the adverse effect of thermal stress and restore normal biological process. Therefore, the study was carried out to assess the effect of Se feeding on the sheep during heat stress.

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MATERIALS AND METHODS

The study was carried out at the sheep farm of Central Sheep and Wool Research Institute, Avikanagar, Rajasthan. The institute is located at 26°26'00.0"N and 75°28'00.0"E at an altitude of 320 m, under semi-arid tropical environment. The average temperature and relative humidity ranges from 4°C (minimum) to 46°C (maximum) and 20% (minimum) to 85% (maximum), respectively. The rainfall of this area is erratically distributed throughout the year, which ranges from 200 to 500 mm. The experiment was carried out during May and June months.

The experiment was carried out on Malpura ewes for 35 days covering two estrus cycles under controlled conditions. The mean body weight of the selected ewes was 42.08±0.82 kg. The selected 12 ewes were randomly divided into two equal groups of 6 animals in each viz. G1 (n=6, control) and G2 (n=6, Se-yeast supplemented). Both the groups were kept in same environmental condition inside the climatic chamber (Table 1). The dimension (length × width × height) of the climatic chamber was 3.7 × 2.4 × 2.1 m, respectively. The chamber has a programmable temperature and humidity regulator to maintain the desired temperature and humidity as per the requirement of the experiment. The temperature can be altered from 5° to 60° C and humidity from 1 to 100%. All sides of the climatic chamber were made of stainless steel. The simulated heat stress model followed was based on the existing climatic conditions under semi-arid environment in India. All the ewes were exposed to heat stress in the climatic chamber for 35 days by exposing them to simulate temperature variance at different hours of the day, i.e. 38°C at 10:00 – 11:00 h; 40°C at 11:00 – 12:00 h; 42°C at 12:00 – 13:00 h; 43°C at 13:00 – 14:00 h; 44°C at 14:00 – 15:00 h and 42°C at 15:00 – 16:00 h. The ewes of G2 were supplemented with Se (0.3 mg/kg DMI) in the form of Se enriched yeast (Se-yeast). Similar amount of yeast was also fed to the ewes of the control group. The animals were stall fed individually with the diet consisting of 70% roughage (*Cenchrus ciliaris*) and 30% concentrate (maize 45%, barley 45%, groundnut cake 4%, mustard cake 3%, mineral mixture 2%, common salt 1%). The animals had free access to feed and water during 06:00 to 10:00 h and 16:00 to 19:00 h in the conventional shed and the observations on daily offer and leftover of feed and water were recorded. Physiological responses such as respiration rate (RR), pulse rate (PR) and rectal temperature (RT) were recorded twice daily (08:00 and 14:00 h) at weekly interval. The blood samples were collected at weekly intervals in heparinised tubes from the external jugular vein. Immediately after blood collection, haemoglobin (Hb) and packed cell volume (PCV) were estimated. Then, plasma was separated by centrifugation at 3,500 g at room temperature for 15 min and stored at –20°C for subsequent analysis of biochemical and endocrine parameters. The plasma glucose, plasma protein, albumin, plasma urea, cholesterol, glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were estimated by standard spectrophotometric (UV-160A) method using diagnostic kits. The glutathione reductase (GR) and glutathione peroxidase (GPx) was also estimated by standard spectrophotometric (UV-160A) method. The hormonal parameters like thyroxine (T_4) (analytical sensitivity, 13 nM; intra-assay and inter-assay coefficient of variations, 5.1 and 8.6%, respectively), triiodothyronine (T_3) (analytical sensitivity, 0.1 nM; intra-assay and inter-assay coefficient of variations, 3.3 and 8.6%, respectively), cortisol (analytical sensitivity, 10 nM; the intra- and inter-assay coefficient of variations, 5.8 and 9.2%, respectively), estradiol (analytical sensitivity, 6 pg/ml; intra- and inter-assay coefficient of variations were 5.8 and 9.0% respectively) and progesterone (analytical sensitivity, 0.05 ng/ml; intraassay and inter-assay coefficient of variations, 12.1 and 11.2%, respectively) were estimated through validated process of Sejian *et al*. (2011) and were estimated by using radio immunoassay (RIA) kits in a gamma counter.

Table 1. Meteorological data during heat exposure

Time	DBT (°C)	WBT (°C)	RH (%)	THI
10:00 h	38.00	24.44±0.37	25.56±1.93	32.63±0.14
11:00 h	40.00	27.70±0.28	31.74±1.32	34.58±0.10
12:00 h	42.00	30.66±0.60	33.91±1.21	35.30±0.99
13:00 h	43.00	30.75±0.53	35.50±1.18	37.28±0.10
14:00 h	44.00	31.86±0.24	36.47±1.07	38.17±0.10
15:00 h	42.00	31.22±0.49	36.97±0.95	36.61±0.06
16:00 h	42.00	31.38±0.35	38.92±1.18	36.77±0.10

DBT, Dry bulb temperature; WBT, Wet bulb temperature; RH, Relative humidity; THI, Temperature humidity Index.THI was calculated on the basis of DBT and RH of each hour inside the psychometric chamber. Temperature humidity index were calculated with the formula of THI = DB {(0.55–0.55 RH) (DB - 58)} given by Marai *et al*. (2007).

All the ewes were synchronized for estrus on the first day of the experiment using intra-vaginal progesterone sponges. After insertion of sponges inside vagina, it was kept *in situ* for a period of 12 days. A single dose (200 IU) of equine chorionic gonadotrophin was injected intramuscularly at the time of sponge removal to each ewe. Estrus detection was started from the next day of sponge removal by parading aproned rams of proven vigour at every 8 h interval in morning (06:00 h), noon (13:00 h) and evening (20:00 h). The oestrus duration was calculated from the first time the ewes allowed the aproned ram to mount them until the end period by which the ewes did not allow the ram to mount. Estrus cycle length was calculated from the day of sponge insertion until the day of end of the oestrus period. The study was conducted after obtaining approval for undertaking the above experimental protocol from the Institute's Animal Ethics Committee.

The data were analyzed by general linear models (SPSS 14.0). Feed intake, water intake, respiration rate, pulse rate, rectal temperature, haemoglobin, packed cell volume, plasma glucose, total protein, albumin, cholesterol, urea, triiodothyronine, thyroxine, estrogen, progesterone were studied through linear model using least squares analysis of variance. In this experiment, the effect of treatment (G1 and G2) was taken into the consideration. Estrus duration and estrus length was calculated using independent samples T test. Statistical significance levels were set at P<0.05 for each parameter.

RESULTS AND DISCUSSION

Thermal stress has been reported to cause severe economic losses in sheep husbandry. Therefore, any approach that could alleviate or reduce the impact of stress will definitely benefit the sheep producer, and also will contribute to the economy of sheep husbandry. Under heat stress, free radical production increased than their utilisation rate that cause oxidative damage to the cell. Selenium, the structural component of glutathione peroxidase protects against oxidative damage by catalysing the destruction of H_2O_2 or decomposition of lipid hydroperoxides. The main function of both Se is to prevent the oxidative damage of biological membranes by neutralising reactive oxygen species (Chauhan *et al.* 2016). The present study was therefore intended at harnessing the benefits of Se supplementation vide feeding of Se-yeast aimed at reducing the effect of induced heat stress on native Malpura sheep.

The effect of Se-yeast supplementation on feed and water intake of heat stressed Malpura ewes was non-significant. The feed intake of G1 and G2 was 894.63 and 892.25 g/ day, respectively. The water intake was 3.68 and 3.62 l/day in G1 and G2, respectively. Sheep in both groups consumed more feed during 06:00 to 10:00 h and drank more water during 16:00 to 19:00 h.

Table 2. Effect of Se-yeast on physiological response of heat stressed Malpura ewes

Attribute	Respiration rate (breaths/min) Morning	Respiration rate (breaths/min) Afternoon	Pulse rate (beats/min) Morning	Pulse rate (beats/min) Afternoon	Rectal temperature (ºC) Morning	Rectal temperature (ºC) Afternoon
µ±SE	33.94± 1.20	96.74± 1.63	63.17± 0.71	74.93± 0.64	38.31± 0.03	39.15± 0.04
G1	35.57^a	97.40^b	61.39^a	74.13^b	38.28^a	39.19^b
G2	32.32^a	97.07^b	61.94^a	75.74^b	38.33^a	39.11^b
Pooled SE	1.69	2.30	1.01	0.90	0.04	0.06

Se-yeast, Selenium enriched *Sachharomyces cerevisiae;* µ, overall mean; G1, Control group (heat stress group, n=6); G2, treatment group (heat stress + Se-yeast, n=6). Means with different superscript letter with in a row of same attributes differ significantly (P>0.05) from morning to afternoon.

Feed intake in both the groups was lower than normal as evidenced from the values depicted. Koyuncu and Yerlikaya (2007) explained that appetite centre gets a suppressive nerve impulse from the peripheral thermal receptor which leads to reduced feed intake. Animal tries to prevent extra rumen fermentation to reduce heat generation. Therefore, feed intake was reduced. Supplementation of Se-yeast did not significantly effect on FI and WI. In the same line, Alhidary *et al.* (2012) observed no significant effect of Se supplementation to steers on dry matter intake. It is further consistent with the previous reports that dietary source and amount of Se supplementation in sheep feeding do not affect DMI under thermo-neutral conditions (Kumar *et al.* 2009).

The effect of Se-yeast feeding on the physiological response of heat stressed Malpura ewes is described in Table 2. The RR and RT did not differ significantly between the groups during morning and afternoon. The PR during the morning was higher in heat stressed control group. There was significant (P<0.05) increase in RR, PR and RT values during afternoon compared to the morning in both groups.

The numerical difference showing lower RR in Se-yeast supplemented group may be indicative of countering heat stress or showing resilience to withstand stressful conditions better than the non-supplemented control. This observation was supported well with the findings that sheep on Se-yeast had significantly higher plasma glucose concentration. Both Hb and PCV are considered to be important markers of dehydration in sheep (Sejian *et al.* 2011) and the observation in this study show the severity of thermal stress to affect these haematological parameters. The observation on total protein, albumin, cholesterol and urea remained similar in both the groups. Supplementation of Se-yeast could not therefore exert any beneficial effect. Our observations (Table 3) on Hb, PCV, albumin, cholesterol urea are in agreement with Alhidary *et al.* (2012) and Kumar *et al.* (2009). The observed values on haemato-biochemical parameters were within the reference ranges.

The effect of Se-yeast feeding on endocrine profiles and plasma enzyme levels of heat stressed Malpura ewes is

Table 3. Effect of Se-yeast supplementation blood on biochemical parameters of heat stressed Malpura ewes.

Attribute	Glucose (mg/d)	Hb (g/dl)	PCV (%)	Total protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Urea (mg/dl)
µ±SE	52.58±0.78	9.85±0.16	33.49±0.57	11.55±0.24	5.04±0.07	59.33±1.41	39.14±1.43
G1	50.99^a	10.02	33.68	12.13^a	5.00	58.09	40.81
G2	54.16^b	9.68	33.30	10.96^b	5.08	60.57	37.47
Pooled SE	1.10	0.22	0.81	0.34	0.10	1.20	2.02

Se-yeast, Selenium enriched *Sachharomyces cerevisiae;* µ, overall mean; G1, control group (heat stress group, n=6); G2, treatment group (heat stress + Se-yeast, n=6); Hb, haemoglobin; PCV, packed cell volume. Means values with different superscript within a column differ significantly at P<0.05.

Table 4. Effect of Se-yeast on plasma hormone and enzymes of heat stressed Malpura ewes.

Attribute	T_3 (nmol/l)	T_4 (nmol/l)	Cortisol (nmol/l)	GOT (IU/l)	GPT (IU/l)	GR (IU/l)	GPx (IU/l)
μ±SE	2.28±0.07	59.58±3.06	35.05±3.38	39.56±1.95	26.09±1.87	0.025±0.001	0.119±0.01
G1	2.32	63.15	37.96	39.46	27.37	0.26	0.113
G2	2.23	56.00	32.14	39.66	24.80	0.26	0.125
Pooled SE	0.10	2.33	1.75	2.77	2.64	0.002	0.014

Se-yeast, Selenium enriched *Sachharomyces cerevisiae;* μ, overall mean; G1, control group (heat stress group, n=6); G2, treatment group (heat stress + Se-yeast, n=6); T_3, tri-idothyronine; T_4, thyroxine, GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; GR, glutathione reductase; GPx, glutathione peroxidase.

described in Table 4. The treatment had no significant effect on any of the hormonal or enzyme parameters. However, plasma T4 and cortisol concentrations were lower in G2.The plasma glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), glutathione reductase (GR) and glutathione peroxidase (GPx) did not differ significantly between the groups. Plasma GPT level was 10.36% higher in G1 as compared to G2. Plasma GPT and GR level did not vary among the groups. Plasma GPx was 10.62% higher in G2 as compared to G1.

Ewes in Se-yeast supplemented group showed decline in T4 and cortisol level, which would be seen as a means of better adaptability of animals to thermal stress by controlling metabolic rate and stress response. The non-significant difference in T3, GOT, GPT, GR and GPx levels between the groups may also establish the adaptability of native Malpura ewes to experimental heat stress. There was a relative decline in GPT and increase in GPx activity in G2. In agreement with our result, serum GPT levels increased during heat stress in goats (Sharma and Kataria 2011) while no change was observed in SGOT concentration in heat stressed goats (Sharma and Kataria 2011). The findings of lower GPT level in Se-yeast supplemented group (G2) could be attributed to lower tissue damages during heat stress. Similarly, an increase in GPx activity in G2 may be correlated to less oxidative stress in ewes. These findings were also in agreement with other studies (Yue *et al*. 2009). It may thus be inferred that ewes in Se-yeast supplemented group with lower cortisol, T4, GPT and higher GPx would be considered to show better resilience against heat stress.

Heat stress had a negative effect on reproduction of ewes. The estrous percentages in G1 and G2 were 83.3 and 100%, respectively, in both the cycles (Table 5). Estrus duration and estrous length were non-significantly higher in G2.There were relatively higher values of plasma estradiol and lower values of progesterone in G2, but the difference between the groups was non-significant.

Jean-François and Marc-André (2001) reported that antioxidant had a role in female fertility. In the present study, the higher estrus percentage was recorded in Se-yeast supplemented ewes. Additionally, an increase in estrus duration in G2 may have reflected on the importance of antioxidant for normal reproduction in sheep during heat stress. In agreement with our findings, Koyuncu and Yerlikaya (2007) also reported significant increase in estrus incidences in sheep with Vitamin E–selenium injections. The previous reports established similar findings of decrease in plasma estradiol and an increase in plasma progesterone concentration during heat stress in sheep (Sejian *et al*. 2011). The suppression of follicular recruitment, growth to ovulation along with decreased LH receptor levels during heat stress and reduced estradiol synthesis in follicles may be the possible reason of reduced estradiol level during heat stress (Roth 2008). The plasma progesterone concentration was higher in ewes subjected to heat stress probably due to less metabolic clearance by the liver.

Table 5. Effect of Se-yeast on oestrus incidences and reproductive hormone status of heat stressed Malpura ewes

Parameter	G1	G2	SE
1^{st} oestrus (%)	83.3% (5/6)	100% (6/6)	
2^{nd} oestrus (%)	83.3% (5/6)	100% (6/6)	
Oestrus duration (hr)	37.20	44	2.82
Estrous length (days)	15.77	16.50	0.93
Estradiol (pg/ml)	17.58	19.56	2.60
Progesterone (ng/ml)	9.98	7.46	1.33

Se-yeast, Selenium enriched *Sachharomyces cerevisiae;* G1, control group (heat stress group, n=6); G2, treatment group (heat stress + Se-yeast, n=6).

From the findings, it is pertinent to conclude that the level of Se enriched yeast fed in this study has limited potential to ameliorate heat stress. Still the trend indicated that the Se enriched yeast can be fed to ameliorate the heat stress and improve reproductive performance of ewes. Nevertheless, variable doses of Se and other source of antioxidants could be tried in sheep and other livestock species to improve resilience against heat stress during extreme summer.

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REFERENCES

Alhidary I A, Shini S, Al Jassim R A and Gaughan J B. 2012. Effect of various doses of injected selenium on performance and physiological responses of sheep to heat load. *Journal of Animal Science* **90**: 2988–94.

Ben Salem H and Smith T. 2008. Feeding strategies to increase small ruminant production in dry environments. *Small Ruminant Research* **77**:174–94.

Chauhan S S, Liu F, Leury B J, Cottrell J J, Celi P and Dunshea F R. 2016. Functionality and genomics of selenium and vitamin E supplementation in ruminants. *Animal Production Science* http://dx.doi.org/10.1071/AN15263.

Chung J Y, Kim J H, Ko Y H and Jang I S. 2007. Effect of dietary supplemented inorganic and organic selenium on antioxidant defence system in the intestine, serum, liver and muscle of Korean native goats. *Asian Australian Journal of Animal Science* **20**: 52–57.

Gabryszuk M and Klewiec J. 2002. Effect of injecting 2- and 3-year-old ewes with selenium and selenim-vitamin E on reproduction and rearing of lambs. *Small Ruminant Research* **43**: 127–32.

Jean-François B and Marc-André S. 2001. Levels of enzymatic antioxidant defenses are modulated in the bovine oviductal fluid during the estrous cycle. In proceedings of 34th annual meeting of society for the study of reproduction. Canada,University of Ottawa.

Kaneko J J, Harvey J W and Michael L B. 1997. *Clinical Biochemistry of Domestic Animals.* pp. 857–79. San Diego California, USA Academic Press.

Kohrle J, Jakob F, Contempre B and Dumont J E. 2005. Selenium, the thyroid, and the endocrine system. *Endocrine Reviews* **26**: 944–84.

Koyuncu M and Yerlikaya H. 2007. Effect of selenium-vitamin E injections of ewes on reproduction and growth of their lambs. *South African Journal of Animal Science* **37**: 233–36.

Kumar N, Garg A K, Dass R S, Chaturvedi V K, Mudgal V and Varshney V P. 2009. Selenium supplementation influences growth performance, antioxidant status and immune response in lambs. *Animal Feed Science and Technology* **153**: 77–87.

Marai I F M, El-Darawany A A, Fadiel A and Abdel-Hafez M A M. 2007. Physiological traits as affected by heat stress in sheep-A review. *Small Ruminant Research* **71**:1–12.

Roth Z. 2008. Heat stress, the follicle, and its enclosed oocyte: mechanisms and potential strategies to improve fertility in dairy cows. *Reproduction* in *Domestic Animals* **43**: 238–44.

Sejian V, Maurya V P and Naqvi S M K. 2011. Effect of thermal, nutritional and combined (thermal and nutritional) stresses on growth and reproductive performance of Malpura ewes under semi-arid tropical environment. *Journal of Animal Physiology and Animal Nutrition* **95**: 252–58.

Sejian V, Naqvi S M K, Ezeji T, Lakriz J and Lal R. 2012. *Environmental Stress and Amelioration in Livestock Production.* Springer-Verlag Berlin Heidelberg.

Sharma A K and Kataria N. 2011. Effects of extreme hot climate on liver and serum enzymes in Marwari goats. *Indian Journal of Animal Sciences* **81**: 293–95

Yue W, Zhang C, Shi L, Ren Y, Jiang Y and Kleemann D O. 2009. Effect of supplemental selenomethionine on growth performance and serum antioxidant status in Taihang Black goats. *Asian Australasian Journal of Animal Sciences* **22**: 365–70.