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## Fluctuations of serum glutathione reductase activities due to changes in extreme ambient temperatures in Marwari sheep from arid tracts

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Abstract. An endeavor was carried out to find out the fluctuations of serum glutathione reductase activity due to changes in ambient temperatures in Marwari sheep from arid tracts in India. This target was achieved by harvesting serum samples from apparently healthy Marwari sheep of both sex and varying age groups during moderate, extreme hot and extreme cold ambiences. The mean value during moderate ambience was  $9.60\pm0.35$  kUL<sup>-1</sup>. A significant (p≤0.05) increase was observed in the mean values during hot and cold ambiences as compared to moderate mean value. The mean values were 1.62 times and 1.2 times higher during hot and cold ambiences, respectively. The impact of hot ambience was greater. Gender and age effects were significant (p≤0.05) in all the ambiences. The mean values were significantly ( $p \le 0.05$ ) higher in male animals than the female ones. In each ambience the effect of age showed a significant ( $p \le 0.05$ ) increase in the mean values which was highest in the animals of 2.5-4.5 years of age. The enormity of increase in the values was greater in hot than cold ambience suggesting higher degree of oxidative stress. It was concluded that changes in ambient temperatures during extreme ambiences produced oxidative stress in Marwari sheep of both genders and all age groups which was reflected in terms of fluctuations in serum glutathione reductase activities. The evaluation of the magnitude of oxidative stress can be useful to redefine its role in abiotic stress. Generated data can be used as reference values to help in laboratory diagnosis. Variations in the values due to extreme hot and cold ambiences could help in practical assessment of the management practices including antioxidant supplementation and diagnosis of pathologies.

Key Words: Extreme ambient conditions, cold, glutathione reductase, hot, Marwari sheep, serum.

**Introduction**. High ambient temperature is one of the major constraints on sheep productivity in tropical and subtropical areas. Heat stress and oxidative stress are two types of damaging agents which have been reported to induce an adaptive response. The main physiological benefit of adaptive response is protection of cells from high doses of toxic agents (Dehghan et al 2010).

Reactive oxygen species (ROS) are formed under physiological conditions. Metabolic activities of living cells result in continuous pouring of free radicals. However, efficient antioxidant system of the body keeps them regulated. Imbalance in the formation of free radicals and their subsequent scavenging may lead to generation of oxidative stress. A wide range of antioxidants are involved in cellular defense mechanisms against ROS, which can be generated in excess during stressful conditions, these include enzymes and non-enzymatic antioxidants (Caetano et al 2013). Free radical scavenging enzymes are important to maintain health status of the animals. Glutathione reductase (GR) is one such enzyme (Kataria et al 2010a). Powerful scavenger works as an antioxidant. An effective antioxidant possesses important

chemical and biological properties and present in sufficient amounts in the body (Rose & Bode 1993).

Heat stress modulates metabolic reactions through free radicals and produces oxidative stress (Kataria et al 2010a). GR is an enzyme that reduces glutathione disulfide to the sulfhydryl form, which is an important cellular antioxidant. The activity of GR is used as an indicator for oxidative stress (Kataria et al 2012).

Marwari breed of sheep plays an important role in the economy of arid and semi arid tract. Abiotic stressors like extreme hot and cold ambient temperatures put their health status at stake and make them vulnerable to biotic stressors. Initiation of adverse health conditions start with the modulation in physiological mechanisms. Prolonged exposure results in the variable pathologies to these animals and one of the problems is oxidative stress (Kataria et al 2010b). Scientific community is paying attention to select some important parameters to assess onset of oxidative stress at an early stage. Therefore, the present investigation was planned to determine variations in the activities of GR during extreme hot and cold ambiences in Marwari sheep and to set reference values for the use in veterinary clinical physiology and future research.

**Material and Method**. The study was carried out in 630 apparently healthy Marwari sheep of both gender, between 6 months to 4.5 years of age to determine GR enzyme in the serum during moderate (mean maximum ambient temperature  $30.34\pm0.20^{\circ}$ C), hot (mean maximum ambient temperature  $45.10\pm0.09^{\circ}$ C) and cold (mean minimum ambient temperature  $4.83\pm0.03^{\circ}$ C) ambiences. Blood samples were collected through jugular vein during slaughtering from private slaughter houses (Bikaner, Rajasthan, India) where all the animals were kept in similar conditions of management. In each ambience, 210 blood samples were obtained to harvest the serum samples and the animals were categorized gender wise as male (105) and non-pregnant female (105) and age wise as below 1 year (35 male and 35 female); 1-2 years (35 male and 35 female) and 2.5-4.5 years (35 male and 35 female).

Blood samples were collected in sterilized and dried test tubes and sera were harvested by centrifugation (10 minutes at 2700 rpm). Serum GR was determined by the colorimetric method (King 1965) with little modification. Serum was treated with coenzyme solution for reduction of endogenous substrates. Then substrate is added and enzyme activity is determined by change in extinction.

In a spectrophotometer cuvette 2.4 ml phosphate buffer (0.2 M, pH 7.5), 0.5 ml serum and 0.1 ml of coenzyme solution (5 mg NADPH<sub>2</sub> per ml distilled water) were added and kept for 2 minutes. After this 0.1 ml of substrate solution (5 mg of glutathione (oxidized) per 0.1 ml of distilled water) was added and optical density (OD) was determined at 340 mµ at every one minute interval. Five readings were taken. The activity was determined as follows:

Activity (kU  $L^{-1}$ ) = Change in OD per minute at 340 mµ x 1000 x 0.5 x 2

Were: 1000 is the dilution factor; 0.5 is the quantity of serum and 2 is the time in minutes for first reaction.

The changes in the means were measured by using multiple mean comparison procedures (Duncan 1955; Steel & Torrie 1980).

**Results and Dscussion**. Mean  $\pm$  SEM values of serum GR during different ambiences, genders and age groups are presented in Table 1. The mean value of serum GR was significantly (p≤0.05) higher during hot and cold ambiences as compared to moderate mean value. The mean value was 1.62 times higher in hot ambience and 1.2 times higher in cold ambience showing greater impact of hot ambient temperature.

Fluctuations in serum GR activities due to changes in ambient temperatures were also observed by earlier researchers in different species (Kataria et al 2010b; Kataria et al 2010c). Antioxidant defense system is affected by higher temperature. Higher activities of GR have been correlated with potent oxidative stress (Kataria et al 2012). The magnitude of increase in the values was higher in hot than in cold ambience which suggested higher degree of oxidative stress during hot ambience than in cold ambience. Supplementation of antioxidants can change the activities of enzyme GR which is involved in removal of ROS (Caetano et al 2013). Significantly higher GR activity in sheep may suggest a significant role of GSH metabolism in the mechanism of oxidative stress development. Changes in ambient temperatures are known to pose stress on the animals. GR probably provided a protective effect against ambience stress (Kataria et al 2010b). The presence of oxidative stress can be speculated in the present study based on the result and surmise of the earlier reports.

Table 1

Effects	Ambience		
	Moderate	Extreme hot	Extreme cold
Ambience	9.60±0.35 b	15.59±0.61b	11.61±0.32 b
Gender			
Male (105)	10.99±0.40 d	16.47±0.80 d	13.0±0.31 d
Female (105)	8.30±0.30d	14.7±0.40d	10.21±0.34 d
Age			
Below 1 Year (70)	7.9±0.15 f	13.48±0.70 f	10.18±0.33 f
1-2 Years (70)	9.0±0.43f	15.40±0.83 f	10.90±0.31 f
2.5-4.5 Years (70)	11.40±0.45f	17.88±0.43 f	13.77±0.34 f

Mean ± SEM values of glutathione reductase (GR, kUL<sup>-1</sup>) in the serum of Marwari sheep during moderate, extreme hot and extreme cold ambiences

Figures in the parenthesis indicate number of sheeps, <sup>b</sup> marks significant ( $p \le 0.05$ ) differences among overall mean values of a parameter, <sup>d</sup> marks significant ( $p \le 0.05$ ) differences between male and female mean values of a parameter within an ambience, <sup>f</sup> marks significant ( $p \le 0.05$ ) differences among mean values of different age groups of a parameter within an ambience.

The effect of gender and age was significant ( $p \le 0.05$ ) in all ambiences. The mean values were significantly ( $p \le 0.05$ ) higher in male animals than in females. In each ambience the age showed a significant ( $p \le 0.05$ ) increase in the mean values which was highest in the sheep of 2.5-4.5 years of age. Pinto & Bartley (1969) reported influence of gender and age on GR activity in rats. Kamper et al (2009) reported lower GR activity in females. Probably oestrogen and testosterone hormones influenced the activities of GR (Mestre-Alfaro 2011). Earlier workers (Maris et al 2010) have elaborated the gender effect on GR activities at cortex and hippocampus levels in rats. The gender differences could be due to sex difference in stress responsiveness. Researchers have suggested that for most species newborns and senescent organisms are more sensitive than other ages to environmental stressors. Nevertheless, it must be considered that there are several biochemical and physiological compensatory processes which are not expressed with the same magnitude during the whole life cycle (Alberto et al 2011). This helps to understand the age effect on the GR activity.

**Conclusions**. It was concluded that vast changes in ambient temperatures during extreme hot and cold ambiences produced the oxidative stress in the Marwari sheep of both genders and all age groups, which was reflected in the form of fluctuations in the serum GR activities. Determination of GR activity during stress period could help in monitoring the progression of oxidative stress in order to protect the sheep in early stages. Variations in the values due to extreme hot and cold ambiences could help in practical assessment of the management practices including antioxidant supplementation and diagnosis of pathologies.

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