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Appraisal of Antioxidative Strategies in the Erythrocytes of *Marwari* Sheep during Extreme Ambiences

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A B S T R A C T

The present investigation was carried out for appraisal of antioxidative strategies in the erythrocytes of *Marwari* sheep during moderate, hot and cold ambiences. Haemolysates were prepared to determine antioxidants. Antioxidants included Superoxide Dismutase (SOD) andGlutathione Reductase (GR).Results revealed mean values of superoxide dismutase (SOD) and glutathione reductase (GR) in erythrocytes were significantly ($p \leq 0.05$) higher during hot and cold ambiences as compared to respective moderate mean value. The mean values were significantly ($p \leq 0.05$) higher in male animals in all the ambiences. Age effect showed a significant ($p \leq 0.05$) increase in the mean values of both indicators being highest in the sheep of 18-24 months of age. On the basis of pattern of observations obtained in the present study, it was appraised that definitive strategies are present in the animals and during adverse conditions modulations occur to combat the stress.

Introduction

Antioxidative strategies are present inside the cells in the form of various endogenous antioxidative enzymes, antioxidants and free radical scavengers. Erythrocytes are constantly subjected to oxidative stress, from their role as an oxygen transporter. Since oxidized erythrocyte components are antigenic in regards to the formation of auto antibodies, a long-term exposure to severe oxidative stress consequently causes an autoimmune response to oxidized erythrocytes that can be regarded as an oxidative modification (Iuchi *et al.,* 2007).

An important consequence of an imbalance between the erythrocytes's components is the poor ability to cope with oxidative stress, which can result in degenerative changes in

haemoglobin, cell membrane and enzymes required for normal erythrocytic function (Edwards and Fuller, 1996).Oxidative stress induces impairment of energy metabolism of erythrocytes (Tavazzi *et al.,* 2001).Heat stress modulates metabolic reactions through free radicals and produces oxidative stress (Kataria *et al.,* 2010).Indicators of oxidative stress allow the assessment of real status of physiological defenses and prevention of the appearance of correlated pathologies (Piccione *et al.,* 2007).

Oxygen can be converted to hydrogen peroxide by superoxide dismutase. Glutathione reductase utilizes NADPH to convert oxidized glutathione to reduced glutathione. Reduced glutathione is then oxidized back to oxidized glutathione. Glutathione peroxidase in a cyclical reaction neutralize hydrogen peroxide into water and oxygen. Reduced glutathione also protects hemoglobin by preventing and reversing oxidation(Iuchi *et al.,* 2007).

Many compounds have the potential to cause oxidative stress in erythrocytes. Oxidative stress is one of the factors proposed to be responsible for damage of erythrocytes during and after exercise or stress. The impact of oxidative stress after acute exhaustion on erythrocyte damage has been extensively investigated in rats and humans but there is paucity of similar type of research in ruminants. Erythrocytes are one of the first cells to be affected by changes in the redox status of the body.

Therefore, alterations in red blood cells are widely used in first step-diagnoses of a number of pathological conditions (Pandey and Rizvi, 2011). Marwari breed of sheep constitutes a major portion of the sheep population in Western part of Rajasthan.Changes in enzymes necessary for physiological adjustments are brought about by great fluctuations in ambient temperatures during extreme ambiences. Timely detection of oxidative stress due to extreme ambiences is an appropriate field of investigation to explore adaptive physiological measures of the animals and their use in health management and clinical diagnosis.

Materials and Methods

Three hundred and sixty blood samples of apparently healthy Marwari sheep of both sexes ageing 6 months to 24 months were collected from private slaughter houses in and around Bikaner district, Rajasthan during moderate, hot and cold ambiences. Blood samples were collected during slaughtering. In each ambience 120 blood samples were collected and the animals were grouped into male (60) and female (60).Moderate ambience was comprise of October-November; hot ambience of May and June and cold ambience of December-January. To assess the effect of hot and cold ambiences on the parameters of antioxidative strategies in the erythrocytes of Marwari sheep, the result of various parameters analysed was compared with those analysed during moderate months serving as control. Following indicators were analysed to appraise the antioxidative strategies in erythrocytes:

1. Superoxide Dismutase (SOD)
2. Glutathione Reductase (GR).

Various computer programmes were used to determine means and standard error (http://www.miniwebtool.com) and analyses of variance (www.danielsoper.com) to test the significance of the effects of ambiences, sex and age groups and correlations (Kaps and Lamberson, 2004).The changes in the means were measured by using multiple mean comparison procedures. For this Duncan's new multiple range test was used (Duncan, 1955).

Results and Discussion

Superoxide dismutase (SOD)

The mean overall value of SOD in erythrocytes of Marwari sheep during moderate ambience (control) was 2.50±0.008 kU gHb-1. It was obtained from 120 Marwari sheep irrespective of sex and age. The range was 2.0-3.0 kU gHb-1. The mean values of superoxide dismutase in erythrocytes were significantly (p≤0.05) higher during hot and cold ambiences as compared to moderate overall mean value. A highly significant (p≤0.01) effect of variation in ambience was also observed by analysis of variance.These results corroborated the earlier findings carried out by Chaturvedi (2011) in goats and Abhimanu (2013) in buffalo calves for SOD status in erythrocytes. The values obtained in erythrocytes were lower than those reported for serum (Kataria *et al.,* 2010 and Joshi, 2012). In present study the higher values of erythrocytic SOD during extreme ambiences were probably to scavenge the free radicals produced, as high ambient temperature is known to stimulate excessive production of free radicals especially super oxide anion radicals and hydrogen peroxide (Siva Kumar *et al.,* 2007). Hot ambience induced oxidative stress was confirmed on the basis of higher activity of SOD during summer or hot environmental temperature by Bernabucci *et al.,* (2002) and Kataria *et al.,* (2010). Reports on cold ambient temperature associated changes are few.

The sex and age effects were significant (p≤0.05) in moderate, extreme hot and cold ambiences. The mean values were significantly (p≤0.05) higher in male animals than female animals in all the ambiences. Age effect showed a significant (p≤0.05) increase in the mean values being highest in the sheep of 18-24 months of age. Highly significant (p≤0.01) sex and age effects were revealed by analysis of variance. In male animals mean

overall value during hot ambience was 344.82 % and during cold ambience was 148.27 % higher in comparison to moderate mean overall value. In female animals mean overall value during hot ambience was 342.85 % and during cold ambience was 185.71 % higher in comparison to moderate mean overall value.Chaturvedi (2011) and Abhimanu (2013) also observed sex and age related changes in SOD status of erythrocytes. Sex and age related changes in serum SOD values have been also reported in several animal species by earlier researchers (Joshi, 2012; Kataria *et al.,* 2012 and Pandey *et al.,* 2012).Higher activity in male animals suggested higher rate of formation of free radicals. However, Nazifi *et al.,* (2009) did not observe sex effect on the activity of SOD in goats. Influence of age on serum SOD activity was also observed by earlier workers (Nazifi *et al.,* 2009), who suggested that age influenced the level of enzyme antioxidant defense due to generation of free radicals.

Glutathione reductase (GR)

The mean overall value of GR in erythrocytes of Marwari sheep during moderate ambience (control) was 1.50±0.03 kU gHb-1. It was obtained from 120 Marwari sheep irrespective of sex and age. The range was 1.42-1.60 kU gHb-1. The mean values of glutathione reductase in erythrocytes were significantly (p≤0.05) higher during hot and cold ambiences as compared to moderate overall mean value.

A highly significant (p≤0.01) effect of variation in ambience was also observed by analysis of variance. It was observed that increase in the values during hot ambience was greater than that of cold ambience. The mean overall value during hot ambience was 42.22 % higher in comparison to cold ambience mean overall value. Mean overall value during hot ambience was 113.3% higher whereas during cold ambience 50 % higher in

comparison to moderate mean overall value.These results corroborated the earlier findings carried out by Chaturvedi (2011) in goats and Abhimanu (2013) in buffalo calves for SOD status in erythrocytes. The values obtained in erythrocytes were lower than those reported for serum (Kataria *et al.,* 2010; Joshi, 2012 and Pandey, 2012).Glutathione reductase helps the body to counteract oxidative stress generated due to hot and cold ambient temperatures.
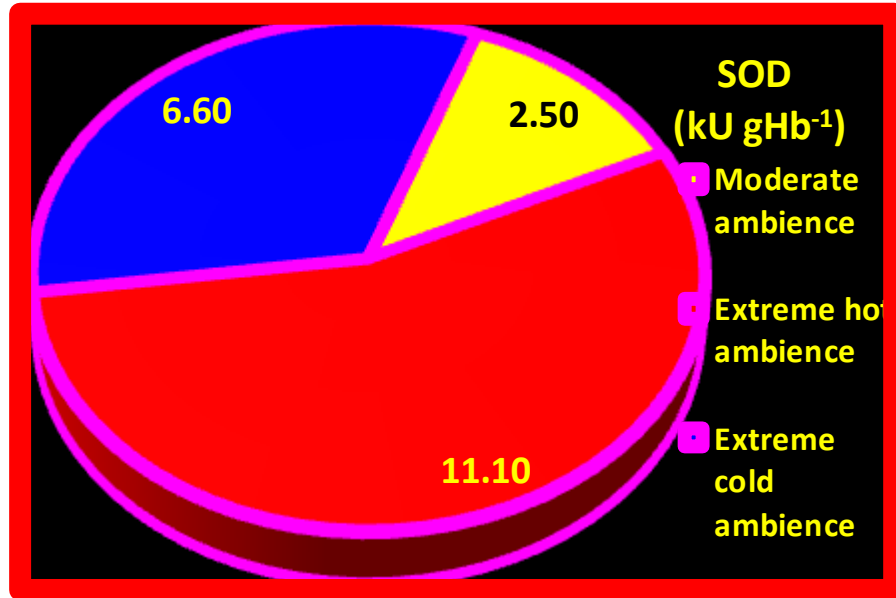

Fig.1 Mean changes in superoxide dismutase (kU gHb-1, SOD) overall values in the erythrocytes of Marwari sheep during extreme ambiences

Fig.2 Mean changes in superoxide dismutase (kU gHb-1, SOD) values according to sex and age groups in the erythrocytes of Marwari sheep during extreme ambiences
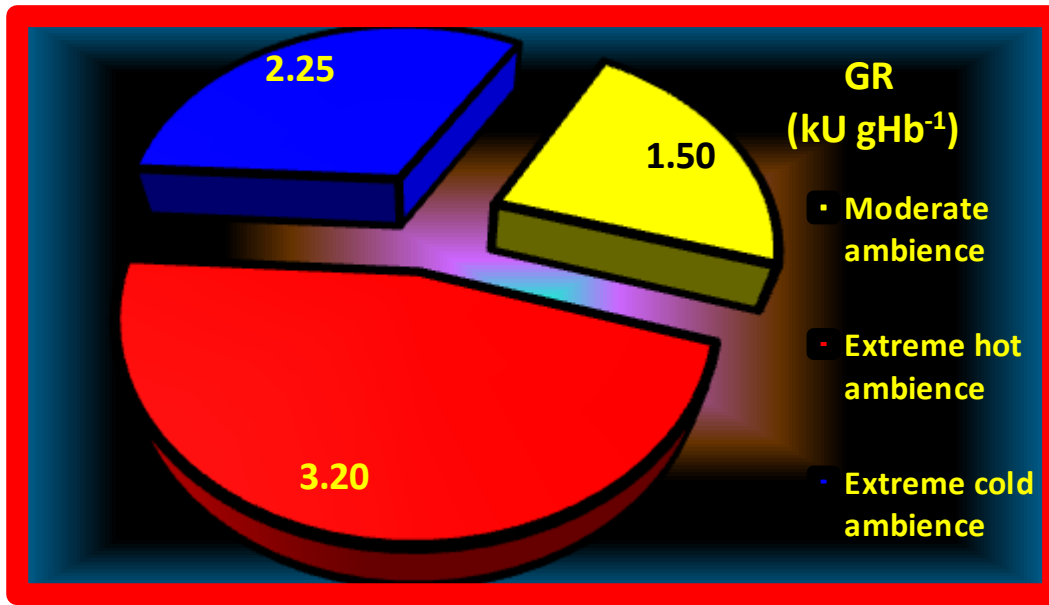
Fig.3 Mean changes in glutathione reductase (kU gHb-1, GR) overallvalues in the erythrocytes of Marwari sheep during extreme ambiences
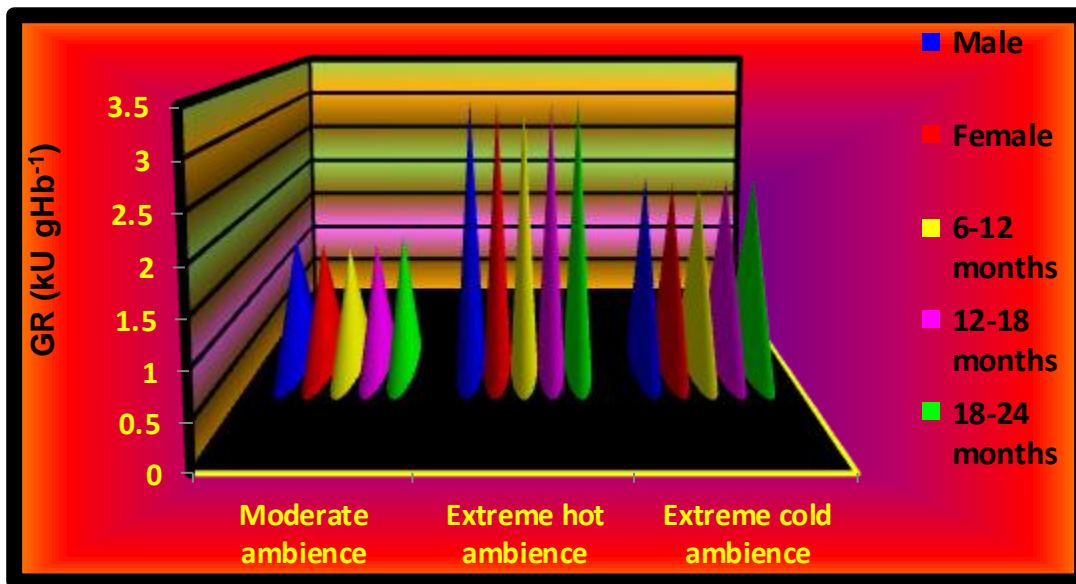

Fig.4 Mean changes in glutathione reductase (kU gHb-1, GR) values according to sex and age groups in the erythrocytes of Marwari sheep during extreme ambiences

The role of glutathione reductase as erythrocyte antioxidant systems to overcome peroxide challenge is well understood (Walsh *et al.,* 1993 and Kurata *et al.,* 1993). Kataria *et al.,* (2010) also observed significantly ($p \leq 0.05$) higher serum GR activity during hot environmental condition as compared to moderate condition in Marwari goats and concluded that hot environmental condition can produce oxidative stress by modulation of physiological mechanisms in response to heat stress. The sex and age effects were significant ($p \leq 0.05$) in moderate, extreme hot and cold ambiences. The mean values were significantly ($p \leq 0.05$) higher in male animals than female animals in all the ambiences. Age

effect showed a significant (p≤0.05) increase in the mean values being highest in the sheep of 18-24 months of age. In male animals mean overall value during hot ambience was 110.45 % and during cold ambience was 49.01 % higher in comparison to moderate mean overall value. In female animals mean overall value during hot ambience was 116.32 % and during cold ambience was 51.02 % higher in comparison to moderate mean overall value. Chaturvedi (2011) and Abhimanu (2013) also observed sex and age related changes in GR status of erythrocytes.

Sex and age related changes in serum GR values have been also reported in several animal species by earlier researchers (Joshi, 2012; Kataria *et al.,* 2012 and Pandey *et al.,* 2012). Higher activity in male animals suggested higher rate of formation of free radicals. Pinto and Bartley (1969) reported influence of sex and age on GR activity in rats, the values being higher in adults and in females whereas Braven *et al.,* (1989) did not find effect of sex and age on GR activity in humans.

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