

## Status of Free Radical Scavenging Enzymes in Heat Stressed Rathi Calves

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**Abstract:** A study was carried out in male and female calves of Rathi breed belonging to arid tracts from India to find out status of free radical scavenging enzymes during hot ambience. Blood samples were collected during moderate and hot ambiances to harvest sera. Maximum ambient temperature ranged from 44.5-46.5°C during hot ambience. All calves were managed in similar conditions and were apparently healthy. Free radical scavenging enzymes included Superoxide Dismutase (SOD), Glutathione Reductase (GR) and Catalase (CAT). The moderate (control) mean values of serum SOD, GR and CAT were 173.00±1.43, 11.00±0.09 and 74.80±1.00 kU L<sup>-1</sup>, respectively irrespective of gender and age. Similar pattern of significant change ( $p < 0.05$ ) was observed by all the three enzymes in heat stressed calves being higher during hot ambience. The mean values were 2.67, 2 and 1.41 times higher, respectively in heat stressed calves during hot ambience. The sex and age effects were significant ( $p < 0.05$ ) in moderate and hot ambiances for all the three enzymes. The mean values were significantly ( $p < 0.05$ ) higher in male animals than female animals. In each ambience the age effect showed a significant ( $p < 0.05$ ) increase in the mean values being highest in the calves of 6 months to 1 year of age. Results clearly indicated that status of free radical enzymes changed in heat stressed calves probably to scavenge higher number of free radicals formed during hot ambience. All the three enzymes used are important biomarkers of oxidative stress. Present study provided data which can be used as reference values to assess the extent of oxidative stress. This will help in timely protection of calves from ensuing health disorders.

**Key words:** Female, Rathi breed, temperature, male, animals

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### INTRODUCTION

Free radicals are normally present in the body in small numbers. Each free radical may exist for only a tiny fraction of a second but the damage it leaves behind can be irreversible. Biochemical processes naturally lead to the formation of free radicals and under normal circumstances the body can keep them in check by the action of free radical scavengers that occur naturally in the body. These scavengers neutralize the free radicals. Certain enzymes serve this vital function namely superoxide dismutase, glutathione reductase, catalase, etc. (Kataria *et al.*, 2010a). An imbalance between the production of reactive oxygen and a biological systems' ability to readily detoxify the reactive intermediates or easily repair the resulting damage can cause oxidative stress. Oxidative stress can influence the metabolism of cells in vital organs of the body (Kataria *et al.*, 2010b).

Proper evaluation of oxidative stress is the requirement in making the health strategies for animals. Many a times differences in models and methodologies make it difficult to deduce significant judgement. Perspicuity in comprehension of the pathophysiology of oxidative stress in animals may help in developing explicit antioxidant therapies. This will also help to understand the role of oxidative stress in biotic stressors (Kataria *et al.*, 2010c).

Normally, the body is protected by a wide range of antioxidant systems working in concert. Superoxide dismutases, glutathione reductase and catalase within cells remove free radicals before they react with metal catalysis to form more reactive species. Heat stress is one of the wide varieties of abiotic factors producing oxidative stress. Free radicals can persuade oxidative damage to vital cellular molecules and structures thereby stunting

the growth of calves. Unrestrained generation can overpower the antioxidant system and can alter metabolic functions. Timely assessment of oxidative stress can help in making health stratagem. Paucity of research work in calves of Rathi breed and their importance as future stock led the foundation of this investigation with the aim to find out the status of free radical scavenging enzymes during hot ambience in heat stressed calves.

**MATERIALS AND METHODS**

**Animals:** Two hundred blood samples of apparently healthy Rathi calves of both genders ageing 1 month to 1 year were collected from private dairies during moderate (maximum ambient temperature ranged from 27-29°C) and hot ambiances (maximum ambient temperature ranged from 44.5-46.5°C). Blood samples were collected in the morning hours from dairies to harvest sera. In each ambience 100 blood samples were collected and the animals were grouped into male (50) and female (50). Further each group was divided according to age as below 6 months (25 male and 25 female) and 6 months to 1 year (25 male and 25 female).

**Analysis:** The samples were analysed to determine free radical scavengers in the serum. Serum superoxide dismutase, glutathione reductase and catalase were determined following the methods described by Kataria *et al.* (2012). The mean value of each biomarker of the animals during extreme hot and cold ambiances was compared to the corresponding mean value of the moderate ambience to find out the levels of significance (Kaps and Lamberson, 2004).

**RESULTS AND DISCUSSION**

Mean±SEM values of serum SOD, GR and CAT during moderate and hot ambiances, gender and age groups are shown in Table 1. The moderate (control)

mean values of serum SOD, GR and CAT were 173.00±1.43, 11.00±0.09 and 74.80±1.00 kU L<sup>-1</sup>, respectively irrespective of gender and age. Similar pattern of significant change (p≤0.05) was observed by all the three enzymes in heat stressed calves being higher during hot ambience. The mean values were 2.67, 2 and 1.41 times higher, respectively in heat stressed calves during hot ambience. The gender and age effects were significant (p≤0.05) in moderate and hot ambiances for all the three enzymes. The mean values were significantly (p≤0.05) higher in male animals than female animals. In each ambience the age effect showed a significant (p≤0.05) increase in the mean values being highest in the calves of 6 months to 1 year of age.

**Superoxide Dismutase (SOD):** Earlier researchers have also showed increased activities of SOD in various animals during hot ambience (Kataria *et al.*, 2010b) in goats and (Kataria *et al.*, 2010d) in dromedary camel. In present study the higher values of SOD were probably to scavenge the free radicals produced as high ambient temperature is known to stimulate excessive production of free radicals (Kataria *et al.*, 2010b). Hot ambience induced oxidative stress was confirmed on the basis of higher activity of SOD by earlier researchers Kataria *et al.* (2010b) and Kataria *et al.* (2010d). Maral *et al.* (1977) indicated a primary role for this enzyme in protection of the cell against uncontrolled oxidative processes. High ambient temperature could increase oxidative stress by increasing lipid peroxidation and decreasing antioxidant defense (Bhat *et al.*, 2008). It can be theorised that hot ambiances generated free radicals to produce oxidative stress in calves suggesting an adaptive response of Rathi calves to oxidative stress in an attempt to improve the antioxidant status. Higher activity in male animals suggested higher rate of formation of free radicals. Oxidative stress affects immune responses. Disturbed immunity in calves can result in a disease process. Therefore, determination of blood SOD activities in calves

Table 1: Mean±SEM values of Superoxide Dismutase (SOD), Glutathione Reductase (GR) and Catalase (CAT) in the serum of Rathi calves during moderate and extreme hot ambiances

| Effects                 | Mean±SEM values (kU/L)         |                                |                               |                               |                               |                                |
|-------------------------|--------------------------------|--------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------------|
|                         | SOD                            |                                | GR                            |                               | CAT                           |                                |
|                         | Moderate                       | Extreme hot                    | Moderate                      | Extreme hot                   | Moderate                      | Extreme hot                    |
| Ambience                | 173.00±1.43 <sup>b</sup> (100) | 462.00±1.20 <sup>b</sup> (100) | 11.00±0.09 <sup>b</sup> (100) | 22.00±0.10 <sup>b</sup> (100) | 74.80±1.00 <sup>b</sup> (100) | 106.20±1.10 <sup>b</sup> (100) |
| <b>Gender</b>           |                                |                                |                               |                               |                               |                                |
| Male (50)               | 238.00±1.14 <sup>bd</sup>      | 538.00±1.16 <sup>bd</sup>      | 18.00±0.11 <sup>bd</sup>      | 27.40±0.12 <sup>bd</sup>      | 87.80±1.11 <sup>bd</sup>      | 122.40±1.12 <sup>bd</sup>      |
| Female (50)             | 108.00±1.00 <sup>bd</sup>      | 386.00±1.00 <sup>bd</sup>      | 4.00±0.10 <sup>bd</sup>       | 16.60±0.10 <sup>bd</sup>      | 61.80±1.00 <sup>bd</sup>      | 90.00±1.00 <sup>bd</sup>       |
| <b>Age</b>              |                                |                                |                               |                               |                               |                                |
| >6 months (50)          | 121.00±0.90 <sup>bf</sup>      | 352.00±1.00 <sup>bf</sup>      | 9.30±0.09 <sup>bf</sup>       | 20.00±0.10 <sup>bf</sup>      | 68.80±1.10 <sup>bf</sup>      | 94.00±1.00 <sup>bf</sup>       |
| 6 months to 1 year (50) | 225.00±1.00 <sup>bf</sup>      | 572.00±1.00 <sup>bf</sup>      | 12.70±0.10 <sup>bf</sup>      | 24.00±0.10 <sup>bf</sup>      | 81.80±1.10 <sup>bf</sup>      | 118.40±1.00 <sup>bf</sup>      |

Figures in the parenthesis indicate number of animals; <sup>b</sup>Marks significant (p≤0.05) differences between moderate and hot ambience for a row; <sup>bd</sup>Marks significant (p≤0.05) differences between male and female mean values within an ambience; <sup>bf</sup>Marks significant (p≤0.05) differences between mean values of both the age groups within an ambience

of various age groups and both genders will help in providing reference values as susceptibility of calves to oxidative stress during the neonatal period may be explained by the immature defense system against superoxide radicals (Inanami *et al.*, 1999).

**Glutathione Reductase (GR):** Various earlier researchers have used GR activity as an indicator to assess oxidative stress in animals (Kataria *et al.*, 2010b). Earlier researchers have also showed higher activities of GR in various animals during hot ambience (Kataria *et al.*, 2010b) in goats and (Kataria *et al.*, 2010d) in dromedary camel. Scientists have discussed the role of glutathione reductase as an antioxidant systems to overcome peroxide challenge (Kurata *et al.*, 1993; Walsh *et al.*, 1993) and in anaemia (Agar *et al.*, 1983).

Glutathione reductase probably provided a protective effect in heat stressed calves. Neuroendocrine stress and lipid peroxidation are observed in heat stressed animals which in turn contribute to the reduced antioxidant response (Bhat *et al.*, 2008). The presence of oxidative stress during hot ambiances in calves was evident based upon the altered status of free radical scavenging enzymes. Effects of sex and age on the activities of GR emphasized the importance of age and sex matched controls in experimental studies of antioxidant enzyme alterations in disease and stress processes.

**Catalase (CAT):** Catalase activities in serum are considered important to assess oxidative stress (Kataria *et al.*, 2010a). Variation in the values of catalase in different species could be related to free radical formation and decomposition of hydrogen peroxide (Chelikani *et al.*, 2004). Earlier researchers have also showed higher activities of CAT in various animals during hot ambience (Marti *et al.*, 2007) in rams (Kataria *et al.*, 2010b) in goats (Kataria *et al.*, 2010d) in dromedary camel and (Maan and Kataria, 2012) in sheep. Higher serum CAT also indicates higher rate of formation of hydrogen peroxide (Kataria *et al.*, 2010b) and considered as body's response to combat the oxidative stress (Kataria *et al.*, 2010d). Researchers have recommended the use of catalase in the situations where free radicals are formed (Seekamp *et al.*, 1988).

Consequence of heat stress is oxidative stress resulting in higher CAT activity making the latter a potent biomarker of oxidative stress (Maan and Kataria, 2012). Higher concentration of catalase in males could be due to higher formation of free radicals as in females oestrogen provides strong antioxidant property than testosterone (Tiidus, 2000). Earlier research workers have also observed the effect of sex on the oxidative status and

they opined that lower generation of free radicals in the females was the reason that they lived longer than males (Sastre *et al.*, 2002). Low catalase activity in young animals can be related with low rate of formation of free radicals (Maan and Kataria, 2012).

## CONCLUSION

Results clearly indicated that status of free radical enzymes changed in heat stressed calves probably to scavenge higher number of free radicals formed during hot ambience. All the three enzymes used are important biomarkers of oxidative stress. It can be concluded that hot ambience produced heat stress to the calves and led to development of oxidative stress. Present study provided data which can be used as reference values to assess the extent of oxidative stress. This will help in timely protection of calves from ensuing health disorders.

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