

Evaluation of oxidative stress during adverse environmental conditions in Marwari sheep from arid tracts in India

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Abstract. The purpose of the study was to evaluate oxidative stress during adverse environmental conditions in Marwari sheep breed, to monitor the health status. Six hundred and thirty animals were sampled belonging to arid tracts of Rajasthan, India during adverse environmental conditions including extreme hot and extreme cold. Oxidative stress was evaluated by determining serum catalase activities by basic colorimetric method in which serum was reacted with hydrogen peroxide for specific period of time. The control mean value of serum catalase was 66.90 ± 2.01 kU L⁻¹. Hot environmental condition resulted in a significant ($p \leq 0.05$) increase, whereas cold environmental condition caused a significant ($p \leq 0.05$) decrease in the mean value as compared to moderate or control. Rise in catalase level was maximum at the individuals of 2.5-4.5 years age group irrespective of gender, followed by male animals irrespective of age during hot environmental condition. As catalase is considered one of the endogenous antioxidant enzymes, it was concluded that its levels increased tremendously during extreme hot environmental condition probably to combat formed free radicals. Results pointed out towards the development of oxidative stress in extreme hot environmental condition as compared to cold environmental condition. Supplementation of antioxidants must be there during adverse environmental conditions to maintain proper health of these animals as oxidative stress is a prelude to many pathological conditions.

Key Words: Environmental condition, catalase, Marwari, serum, sheep.

Introduction. Living in an oxygenated environment has required the evolution of effective cellular strategies to detect and detoxify metabolites of molecular oxygen known as reactive oxygen species. The appropriate and inappropriate production of oxidants, together with the ability of organisms to respond to oxidative stress, is intricately connected to ageing and life span of animals (Finkel & Holbrook 2000). Oxidative stress is extremely dangerous as it does not exhibit any symptom and is recognisable with great difficulty by means of laboratory methods only (Kataria et al 2010a). The exposure to different kind of stress impacts on the reactive oxygen species production with potential risk to the integrity of the tissues. Environmental stress functions as one of the stressors encountered by the animals of arid tracts. Heat stress tempers metabolic reactions through reactive oxygen species and generates oxidative stress (Kataria et al 2010b).

Catalase is considered as an antioxidant enzyme found in nearly all the cells that are exposed to oxygen, where it catalyses decomposition of hydrogen peroxide to water and oxygen. Hydrogen peroxide is a harmful by product of many normal metabolic processes. To prevent damage, it must be quickly converted into other, less dangerous substances. Higher serum catalase activity in stressed animals indicate the higher rate of hydrogen peroxide formation (Kataria et al 2010c), therefore it is considered as one of the markers to assess oxidative stress in animals (Kataria et al 2010a; Kataria et al 2012).

The inevitability of exposure of sheep to extreme hot and cold environmental conditions of arid and semiarid tracts, makes oxidative stress associated with extreme environmental conditions an appropriate field of investigation to explore adaptive

physiological measures of the body and their use in health management and clinical diagnosis. Despite of immense quality characteristics of Marwari sheep very little scientific *savoir faire* is there about normal and clinical variations in the values of antioxidant enzymes present in the blood. To understand the real worth of these animals and to explore the productive potential, establishment of their own norms becomes very important in the field of veterinary clinical medicine. Therefore, the present investigation was aimed to determine serum catalase activity during extreme hot and cold environmental conditions in Marwari sheep and to set its normal values for the use in future research.

Material and Method. The present investigation was carried out in six hundred and thirty apparently healthy Marwari sheep of either gender, between 6 months to 4.5 years of age, belonging to arid tracts of Rajasthan, India. Blood samples were collected during slaughtering (jugular vein) from private slaughter houses (Bikaner, Rajasthan) to harvest sera in morning hours during moderate (Mean maximum temperature $30.34 \pm 0.20^\circ\text{C}$), hot (Mean maximum temperature $45.1 \pm 0.09^\circ\text{C}$) and cold environmental (Mean minimum temperature $4.83 \pm 0.30^\circ\text{C}$) conditions. In each environmental condition, 210 blood samples were collected (105 male and 105 non pregnant female). Further each group was divided according to age 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). Mean values of moderate environmental condition were considered as control values.

Serum catalase was determined by basic colorimetric method as described by Goldblith and Proctor (1950) with little modifications by Kataria et al (2010b). The serum was allowed to react with H_2O_2 for a specific period of time. The reaction was then stopped by using H_2SO_4 . Then excess of KMnO_4 solution was added to allow reacting with the peroxide not decomposed by the catalase. Within one minute, the excess in KMnO_4 was determined photometrically. Mean changes in serum catalase levels during adverse environmental conditions were compared from those of moderate environmental condition by using statistical significance (Steel & Torrie 1980).

Results and Discussion. The range ($39\text{-}126 \text{ kUL}^{-1}$) and mean value of serum CAT (Table 1) in the present study was close to the earlier reports (Kataria et al 2010a, 2010b). Studies pertaining to serum CAT in sheep are few in the literature and work has been done on erythrocytic catalase. 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) and to different diets (Nazifi et al 2009b).

The mean value of serum CAT was significantly higher ($p \leq 0.05$) during hot and significantly lower ($p \leq 0.05$) during cold environmental condition in comparison to moderate mean value (control). Environmental condition associated variations in the catalase activities have been reported by many researchers (Marti et al 2007; Kataria et al 2010b, 2010c). The increased activity of serum catalase during hot environmental condition suggested the ability of the animals to provide defense against free radicals (Kataria et al 2010c). In a study, Carpenter et al (2001) observed that lambs, who did not receive antioxidant enzymes, had low catalase activities. Earlier researchers have recommended the use of catalase in the situations where free radicals are formed (Seekamp et al 1988). The increased activity of serum catalase during hot environmental condition suggested the ability of the animals to provide defense against free radicals. It was the body's response to combat the oxidative stress (Kataria et al 2010b), as it is an enzyme of antioxidant defense system that eliminates and controls the toxic oxygen species. Phua (2004) observed higher catalase activity in sheep where reactive oxygen species were generated. However, Kozat et al (2007) found lower catalase activity in diseased lambs and Cam et al (2009) in pox affected ewes and lamb.

From the results, it can be hypothesized that hot environmental condition caused a stressful condition in comparison to cold, leading to excessive production of free radicals, which resulted in oxidative stress and an imbalance between oxidant and antioxidant system (Nazifi et al 2009a). During stress or exercise, oxidative stress can be provoked (Nazifi et al 2009b). Halliwell & Gutteridge (1999) described several lines of

defense against reactive oxygen species in animals and antioxidant enzyme catalase is one of them, which can be used effectively as marker of oxidative stress. Further it can be inferred that higher catalase activities showed activation of defense system, whereas its lowered activities could have a negative impact on cellular resistance against the oxidant induced damage of the cell (Cam et al 2009).

Table 1

Mean \pm SEM values of serum catalase (CAT) in Marwari sheep

<i>Parameter</i>	<i>Environmental conditions</i>	<i>CAT, kU L⁻¹</i>
	Moderate (210)	66.90 \pm 2.01 ^b
Gender	Male (105)	74.86 \pm 2.01 ^d
	Female (105)	58.94 \pm 3.02 ^d
Age	Below 1 year (70)	53.81 \pm 3.41 ^f
	1-2 years (70)	66.95 \pm 3.01 ^f
	2.5-4.5 years (70)	79.94 \pm 2.02 ^f
	Hot (210)	96.92 \pm 2.72 ^b
Gender	Male (105)	103.28 \pm 2.51 ^d
	Female (105)	90.56 \pm 2.80 ^d
Age	Below 1 year (70)	71.41 \pm 2.20 ^f
	1-2 years (70)	100.9 \pm 2.10 ^f
	2.5-4.5 years (70)	118.45 \pm 2.10 ^f
	Cold (210)	59.79 \pm 2.00 ^b
Gender	Male (105)	70.51 \pm 2.50 ^d
	Female (105)	49.06 \pm 2.50 ^d
Age	Below 1 year (70)	37.34 \pm 2.50 ^f
	1-2 years (70)	65.03 \pm 2.02 ^f
	2.5-4.5 years (70)	76.95 \pm 2.41 ^f

Figures in the parenthesis indicate number of animals and means superscripted by same superscript differ significantly ($p \leq 0.05$) from each other.

The gender and age effects were significant ($p \leq 0.05$) in all the environmental conditions. The mean values were significantly higher ($p \leq 0.05$) in males than females. Age effect showed a significant increase ($p \leq 0.05$) in the mean values being highest in the animals of 2.5-4.5 years. The interactions between environmental condition X gender and environmental condition X age were highly significant ($p \leq 0.01$), which showed the effect of environmental condition on the animals of both sexes and all age groups. The interaction between age X gender was significant ($p \leq 0.05$), which showed that in each age group gender effect was there. Higher concentration of serum catalase in males could be due to higher formation of free radicals as in females, because oestrogen provides stronger antioxidant property than testosterone (Tudus 2000). Earlier research workers have also observe the effect of gender 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). However, Nazifi et al (2009b) did not observe significant changes in the activity of catalase between male and female goats in the erythrocytes. Earlier workers have suggested that age influenced the free radical generation greatly and consequently the level of enzyme antioxidant defense (Nazifi et al 2009a). De & Durad (1991) conducted a study in rats and observed that catalase activity increased with the advancement in age. Low catalase activity in young animals can be related with low rate of formation of free radicals.

Catalase is considered as one of the endogenous antioxidant enzymes. It was concluded that antioxidant enzyme activity increased tremendously during extreme hot environmental condition probably to combat free radicals formed.

Conclusions. Results pointed out towards the development of oxidative stress in extreme hot environmental condition as compared to cold condition. This study has tried to provide comprehensive values of catalase in serum of Marwari breed of sheep, probably serving as the first report. The silence of literature over this aspect in Marwari sheep underlined the significance of establishment of appropriate physiological reference values for native breeds, which could help in realistic evaluation of the health management practices including antioxidant supplementation. The data obtained can also serve as a baseline for future research in the field of veterinary clinical medicine.

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