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Extreme ambiences *vis-à-vis* endogenous antioxidants of Marwari goat from arid tracts in India

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Abstract. To investigate the ambience associated variations in endogenous antioxidants status of Marwari goat of arid tracts in India, blood samples were collected to harvest sera from male and female Marwari goats of varying age groups during moderate, extreme hot and cold ambiences. The endogenous antioxidants *viz*. glutathione, vitamin E, vitamin C, vitamin A and β carotene were analysed in the serum samples. The moderate mean values (µmol L^{-1}) were 5.30±0.01, 4.67±0.03, 26.42±0.31, 2.12±0.003 and 2.46±0.002, respectively. The levels of all the antioxidants decreased significantly ($P<0.05$) during hot and cold ambiences as compared to moderate ambience. The extent of decrease was greater during hot ambience than cold ambience. In each ambience the effect of sex and age was significant ($P<0.05$) on each antioxidant. It was concluded that extreme ambiences produced oxidative stress in the animals which resulted in depletion of the level of each of the antioxidant.
Key Words: Ambience, antioxidants, cold, hot, Marwari goat.

Introduction. Oxidative stress is an active field of research in veterinary medicine and has been implicated in numerous disease processes. The understanding of the role of oxidants and antioxidants in physiological and pathological conditions is rapidly increasing. Clarity of understanding of the pathophysiology of oxidative stress in animals will allow the design of specific antioxidant therapies to be used in veterinary medicine. To help accelerate practical applications, the development of an oxidative stress index is gaining importance as an approach in veterinary medicine by the scientific community (Celi 2011).

Biochemical processes naturally lead to the formation of free radicals, which in excess can alter the way in which the cells code genetic material. When free radicals are produced at a faster rate, antioxidant mechanism is highly activated to neutralise them, however, an oxidative stress results. All animal cells protect themselves against free radical damage by endogenous antioxidants such as ascorbic acid, tocopherol and glutathione. Sometimes these protective mechanisms are disrupted by various pathological processes, and antioxidant supplementation becomes vital to combat oxidative damage. Recently, much attention has been directed towards the development of ethno medicines with strong antioxidant properties but low cytotoxicities (Hazra et al 2008).

Ambient stress is one of the factors causing oxidative stress by modulating metabolic reactions. Inactivation or transformation of these oxidants requires implications of antioxidants like vitamin E, an effective lipophilic antioxidant, which protects lipid membranes against lipid peroxidation through the action of seleno proteins. Therefore stressed animals may have lower concentration of serum alpha-tocopherol (Sconberg et al 1993), making it a useful bio marker of oxidative stress. Ambient stress can reduce antioxidant activity of blood (Harmon et al 1997) resulting in oxidative stress.

Glutathione, vitamin E, vitamin C, vitamin A and β-carotene are considered as good free radical scavengers of oxidative stress. Despite of immense quality characteristics of Marwari goat very little scientific *savoir faire* is there about normal and clinical variations in the values of endogenous antioxidants 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 planned to determine serum levels of endogenous antioxidants during extreme ambiences in Marwari breed of goat and to set their physiological reference values for future research in veterinary medicine.

Materials and Methods. To carry out the study six hundred and thirty apparently healthy Marwari goats of either sex, between 6 months to 4.5 years of age were screened to determine antioxidants in the serum during extreme ambiences. All the animals were kept in similar conditions of feeding and watering. In each ambience 210 blood samples were collected and the animals were grouped into male (105) and non pregnant female (105). 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 2-4.5 years (35 male and 35 female). Blood samples were collected from jugular vein during slaughtering from private slaughter houses (Bikaner, Rajasthan, India). Sampling was carried out in morning hours during moderate (Mean maximum ambient temperature 28.60± 0.32 ^{o}C), hot (Mean maximum ambient temperature 45.5 ± 0.08 ^{o}C) and cold (Mean minimum ambient temperature 2.08 ± 0.10 ^{o}C) ambiences.

Blood was collected directly into the clean, dry test tubes without any anticoagulant to harvest serum. Supernatant non-haemolysed sera were stored into sterilised vials at 0^{o}-4^{o}C for a very short period of time until analysis. A rapid colorimetric micro method (Owens & Belcher 1965) with modifications (Kataria et al 2010) was employed for glutathione. Vitamin E was determined by the spectrophotometric method (Nair & Magar 1955) with modification (Kataria et al 2010). Titrimetric method (Varley 1988) was used for Vitamin C determination. Vitamin A and β carotene were determined by the methods as described by Varley (1988). To test the significance of the effects of ambiences, sex and age, the changes in the means were compared by using multiple mean comparison procedures keeping moderate mean as control for each antioxidant (Steel & Torrie 1980).

Results and Discussion. The mean values of serum glutathione, vitamin E, vitamin C (Table 1), vitamin A and β carotene (Table 2) were significantly ($P<0.05$) lower during hot and cold ambiences as compared to moderate mean value. The decrease was comparatively more in hot than in cold ambience for each antioxidant. The sex and age effects on the mean values of glutathione, vitamin E, vitamin C, vitamin A and β carotene were significant ($P<0.05$) in all ambiences. For each antioxidant, the mean value was significantly ($P<0.05$) higher in male animals than females. Age effect showed a significant ($P<0.05$) increase in the mean values being highest in the 2-4.5 years of animals for each antioxidant.

Low serum glutathione level during hot and cold ambiences indicated its depletion in the process to prevent oxidative stress (Kataria et al 2010). Glutathione is a major endogenous antioxidant which protects cells from reactive oxygen species such as free radicals and peroxides and spares ascorbate and improves antioxidant capacity of blood (Gropper et al 2004). Dehghan et al (2010) unequivocally suggested that glutathione levels changed during different environmental conditions. Decreased vitamin E level has been well correlated with increased oxidative threats (Walwadkar et al 2006) because it works by removing the free radical intermediates and thus preventing the oxidation reaction from continuing. Low serum vitamin E level during hot ambience showed its depletion in an attempt to reduce the production of reactive oxygen species (Kataria et al 2010). Ambient stress stimulates the production of free radicals with concomitant decrease in antioxidant capacity (Lovel 1988). McDowell (2000) opined that reduction in vitamin E during heat stress might be due to either depletion of endogenous reserves to combat free radicals produced excessively in the body or insufficient endogenous synthesis under stressful conditions. Vitamin E is also important in the management of stressed animals (Chirase et al 2001). Cortisol mediated increase in antioxidant levels lead to higher rate of reaction between oxidants and antioxidants, ultimately leading to depletion of antioxidants. These discussions suggest the use of these antioxidant vitamins to prevent the development or continuation of oxidative stress (Bourdel-Marchasson et al 2001).

Table 1

Mean ± SEM values of serum glutathione, vitamin E and vitamin C in Marwari goat

Effects	Antioxidants, μmol L^{-1}		
	Glutathione	Vitamin E	Vitamin C
Moderate (210)	5.30±0.01^{a}	4.67±0.03^{a}	26.42±0.31^{a}
Sex			
Male (105)	5.59±0.02^{b}	4.91±0.05^{b}	32.55±0.30^{b}
Female (105)	5.01±0.01^{b}	4.43±0.04^{b}	19.31±0.36^{b}
Age			
Below 1 Year (70)	4.28±0.01^{c}	3.95±0.02^{c}	21.32±0.31^{c}
1-2 Years (70)	5.82±0.02^{c}	4.64±0.02^{c}	23.15±0.30^{c}
2 -4.5 Years (70)	5.81±0.01^{c}	5.44±0.01^{c}	34.80±0.32^{c}
Hot (210)	4.31±0.01^{a}	2.73±0.03^{a}	13.80±0.30^{a}
Sex			
Male (105)	4.93±0.01^{b}	3.00±0.04^{b}	15.64±0.30^{b}
Female (105)	3.70±0.02^{b}	2.47±0.05^{b}	11.97±0.31^{b}
Age			
Below 1 Year (70)	3.45±0.01^{c}	2.06±0.02^{c}	10.68±0.32^{c}
1-2 Years (70)	3.78±0.01^{c}	2.48±0.04^{c}	13.88±0.33^{c}
2 -4.5 Years (70)	5.73±0.02^{c}	3.66±0.03^{c}	16.91±0.30^{c}
Cold (210)	4.65±0.01^{a}	3.57±0.04^{a}	18.74±0.33^{a}
Sex			
Male (105)	5.07±0.01^{b}	4.00±0.05^{b}	22.12±0.31^{b}
Female (105)	4.24±0.01^{b}	3.14±0.04^{b}	14.35±0.30^{b}
Age			
Below 1 Year (70)	4.03±0.01^{c}	3.15±0.05^{c}	14.20±0.32^{c}
1-2 Years (70)	4.62±0.01^{c}	3.55±0.05^{c}	19.00±0.30^{c}
2 -4.5 Years (70)	5.30±0.01^{c}	4.02±0.06^{c}	23.05±0.31^{c}

Figures in the parenthesis indicate number of animals and same superscripts within a column differ significantly (P<0.05) from each other.

Table 2

Mean ± SEM values of serum vitamin A and β carotene in Marwari goat

Effects	Antioxidants , μmol L^{-1}	
	Vitamin A	β Carotene
Moderate (210)	2.12±0.003^{a}	2.46±0.002^{a}
Sex		
Male (105)	2.37±0.002^{b}	2.61±0.002^{b}
Female (105)	1.87±0.001^{b}	2.31±0.001^{b}
Age		
Below 1 Year (70)	1.71±0.003^{c}	2.01±0.003^{c}
1-2 Years (70)	2.27±0.006^{c}	2.54±0.001^{c}
2 -4.5 Years (70)	2.39±0.001^{c}	2.82±0.001^{c}
Hot (210)	1.81±0.002^{a}	1.90±0.003^{a}
Sex		
Male (105)	1.86±0.001^{b}	2.12±0.003^{b}
Female (105)	1.77±0.002^{b}	1.71±0.001^{b}
Age		
Below 1 Year (70)	1.73±0.001^{c}	1.63±0.002^{c}
1-2 Years (70)	1.81±0.002^{c}	2.13±0.003^{c}
2 -4.5 Years (70)	1.91±0.002^{c}	2.61±0.001^{c}
Cold (210)	1.90±0.003^{a}	2.22±0.003^{a}
Sex		
Male (105)	2.00±0.001^{b}	2.44±0.002^{b}
Female (105)	1.76±0.006^{b}	2.10±0.002^{b}
Age		
Below 1 Year (70)	1.75±0.003^{c}	2.05±0.003^{c}
1-2 Years (70)	1.95±0.001^{c}	2.23±0.003^{c}
2 -4.5 Years (70)	2.01±0.003^{c}	2.41±0.001^{c}

Figures in the parenthesis indicate number of animals and same superscripts within a column differ significantly (P<0.05) from each other.

The observations of present study showed the ambient stress related decrease in serum vitamin C to combat free radicals (Kataria et al 2010). Depletion of vitamin C confirms the presence of oxidative stress because repletion is reported after supplementation of vitamin C (Hidiroglou 1999; Weiss 2001). Kataria et al (2010) also recommended the use of antioxidants in the disease conditions causing oxidative stress. Antioxidant effect of vitamin A is well documented (Wang et al 1999). The decreased levels of vitamin A and β carotene indicated towards the presence of oxidative stress in goat. The decrease in the values of vitamin A and β carotene during hot ambience was greater than in cold ambience. A decrease in antioxidant defense leads to oxidative damage of biomolecules (Beckman & Ames 1998).

Effect of sex and age on antioxidant levels has been associated to free radical formation. Low serum vitamin E levels in females has been implicated to the presence of oestrogen as an antioxidant (Sastre et al 2000). Higher vitamin C level in male animals reflected towards its higher synthesis to combat free radicals (Long et al 1963). The low vitamin A level in the females could be due to higher mobilization of this vitamin for various metabolic purposes (Knig 1961). Taylor et al (1968) reported significant effects of age in both sexes on plasma vitamin A. In present study the antioxidant defense system was changed to adapt and prevent the continuing of oxidative stress effects.

Conclusion. It was concluded that extreme ambiences produced the oxidative stress in the Marwari goat of both the sexes and all age groups, which was reflected in the form of altered status of the antioxidants in the serum. The paucity of literature over this aspect in Marwari goat marked the importance of establishment of appropriate physiological reference values for this native breed, which could help in assessment of the health and management practices including antioxidant supplementation and diagnosis of diseases and stress.

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