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Status of antioxidants and free radical scavenging enzymes in heat stressed buffalo calves

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Abstract
A study was carried out in male and female buffalo calves of *Murrah* breed belonging to arid tracts from India to find out status of antioxidants and free radical scavenging enzymes during hot ambience. Blood sample were collected during moderate and hot ambiences. Maximum ambient temperature ranged from 44.5-46.5 degree centigrade. All calves were managed in similar condition and were apparently healthy. The antioxidants selected were Vitamin C and Glutathione and free radical scavenging enzymes was Glutathione reductase. The moderate (control) mean values of vitamin C and Glutathione in erythrocytes were 2.50±0.02 µmol gHb-1 and 1.51±0.03µmol gHb-1, respectively. The mean values of vitamin C and Glutathione in erythrocytes were 1.54 and 1.23 times lower, respectively during extreme hot ambience than respective moderate ambience mean values. The moderate (control) mean values of Glutathione reductase in erythrocytes were 0.82±0.01kU gHb-1. The erythrocytes mean values was 1.97 times higher during extreme hot ambience than respective moderate ambience mean values. The sex and age effects were significant ($p \leq 0.05$) in moderate and hot ambiences for all parameter studied. The mean values were significantly ($p \leq 0.05$) higher in male buffalo calves than female buffalo calves. In each ambience the age effect showed a significant ($p \leq 0.05$) increase in the mean values being highest in the buffalo calves of 6 months- one year of age. Results clearly indicated that status of antioxidants and free radical scavenging enzymes changed in heat stressed calves probably to scavenge higher number of free radicals formed during hot ambience. All above three parameter used as important biomarker of oxidative stress. Present study provided data which can be used as reference values to for future studies and for diagnostic purposes.

Keywords: antioxidants, free radical scavenging enzymes, vitamin C, glutathione, glutathione reductase

Introduction
Oxidative stress, caused by increased formation of free radicals and reactive oxygen species through different metabolic processes and in response to exogenous stimuli, is controlled by various antioxidant defence mechanisms including antioxidant enzymes [1]. Therefore it's very much essential to understand the role of oxidant in physiological conditions. Buffaloes represent an integral part of the animal production sector. Free radicals are normally present in the body in small numbers. An imbalance between the production of reactive oxygen and biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage can cause oxidative stress. Heat stress is one of the wide varieties of factors which causes oxidative stress *in-vivo*. Excessive generation of reactive oxygen species can induce oxidative damage to vital cellular molecules and structures including DNA, lipids, proteins, and membranes. Uncontrolled generation of reactive oxygen metabolites can impair performance of dairy animals. To optimize performance, oxidative stress in high producing animals must be controlled by supplying all known antioxidant nutrients and by minimizing effects of substances that stimulate reactive oxygen metabolites [2]
Heat stress is one of the wide varieties of factors which causes oxidative stress in-vivo. The erythrocyte is the preferable target of oxidative modification in the blood and is prone to degradation leading to anaemia. Oxidative stress can disturb biochemical and physiological functions of red blood cells thereby affecting membrane integrity. . Vitamin E can decrease the effect of stress and prevent membrane destruction and haemolysis [3]. The present investigation was planned to determine effect of extreme hot ambience on antioxidants and free radical scavengers in erythrocytes of male and female buffalo calves. It is essential to observe physiological consequences versus scaling of environmental stress in buffaloes from arid tracts [4]. Since there is paucity of study of physiological effects of oxidative stress in buffalo calves from arid tracts [5] and their importance as future stock led the foundation of this investigation

with the aim to find out the status of antioxidants and free radical scavenging enzymes during hot ambience in heat stressed calves.

Materials and Methods

Animals

Two hundred and forty blood samples of apparently healthy buffalo calves of both sexes ageing one month to one year were collected from private dairies during moderate and hot ambiences. In each ambience 120 blood samples were collected and the animals were grouped in to male (60) and female (60). Further each group was divided according to age as below 6 month (30 male and 30 female) and 6 month to one year (30 male and 30 female).

Analysis

The experiment was designed to determine the free radical scavengers in the erythrocytes of buffalo calves during moderate and hot ambiences. To assess the effect of hot ambience on the free radical scavengers in the erythrocytes, the result of various parameters analysed were compared with those analysed during moderate months serving as control.

Vitamin C was determined by the method as described by Varley (1988) [6] with little modification [7]. This method was based upon the titration of haemolysate ascorbate by 2, 6-dichlorophenolindophenol dye.

Glutathione was determined by the rapid colorimetric micro method of Owens and Belcher (1965) [8] with modifications [9]. Sample is treated with phosphate buffer, 5, 5'-dithiobis (2-nitrobenzoic acid), EDTA and glutathione reductase. Addition of NADPH2 to the system initiates a progressive reduction of 5, 5'-dithiobis (2-nitrobenzoic acid) by glutathione which causes a colour increase at 412 mμ.

Glutathione reductase was determined by the colorimetric method as described by King (1965) [10]. Serum is treated with coenzyme solution for reduction of endogenous substrates. Then substrate is added and enzyme activity is determined by change in extinction.

Results and Discussion

Mean ± SEM value of erythrocytes vitamin C, Glutathione and Glutathione reductase during moderate and hot ambiences, gender and age groups are shown in table 1.

Vitamin C

The moderate (control) mean value of erythrocytic vitamin C was 2.50±0.02 µmol gHb-1. The range obtained from all animals irrespective of ambience, sex and age was 1.20-3.09µmol gHb-1. Vitamin C as an anti-oxidant protects the body against oxidative stress [11]. Research conducted by the earlier workers emphasized upon the role of vitamin C in maintaining integrity of erythrocytes by preventing the oxidative stress and suicidal death [12].

The mean values of erythrocytic vitamin C were significantly ($p \leq 0.05$) lower during hot ambience as compared to respective moderate mean values. The erythrocytic mean value was 1.54 times lower during extreme hot ambience than respective moderate ambience mean values. Decreased total antioxidant levels during heat stress [13] might be due to increased production of free radicals (Lovel, 1988), depletion of endogenous reserves to combat free radicals produced excessively or insufficient endogenous synthesis under stressful conditions. Vitamin C in erythrocytes is suggested to be essential in the detoxification of superoxide radicals and hydrogen peroxide formed during red cell metabolism [14]. Oxidative stress in erythrocytes can cause haemolytic episodes with resultant anaemia because the normal functionality of the erythrocyte is based on its ability to maintain its membrane integrity. The compromised erythrocyte membrane integrity arises from the increased lipoperoxidative changes which lead to the destruction of erythrocytes being more in the animals during hot-dry season [15].

Table 1: Mean ± SEM values of Vitamin C, Glutathione and Glutathione reductase (µmol L^{-1}) in the erythrocytes of buffalo calves during moderate and extreme hot ambiences

		Vitamin C		Glutathione (GSH)		Glutathione Reductase (GR)	
		Mean ±SEM values		Mean ±SEM values		Mean ±SEM values	
S.N.	Effects	Moderate	Extreme Hot	Moderate	Extreme Hot	Moderate	Extreme Hot
1.	Ambience	$2.50^{b} \pm 0.02$ (100)	$1.62^{b} \pm 0.02$ (100)	$1.51^{b} \pm 0.03$ (100)	$1.22^{b} \pm 0.02$ (100)	$0.82^{b} \pm 0.01$ (100)	$1.61^{b} \pm 0.05$ (100)
2.	Sex						
(i)	Male (50)	$2.94^{bd} \pm 0.02$	$1.91^{bd} \pm 0.03$	$1.61^{bd} \pm 0.03$	$1.31^{bd} \pm 0.03$	$0.94^{bd} \pm 0.01$	$1.76^{bd} \pm 0.02$
(ii)	Female (50)	$2.07^{bd} \pm 0.02$	$1.32^{bd} \pm 0.03$	$1.41^{bd} \pm 0.03$	$1.13^{bd} \pm 0.02$	$0.71^{bd} \pm 0.01$	$1.46^{bd} \pm 0.02$
3.	Age						
(i)	Below 6 months (50)	$2.01^{bf} \pm 0.02$	$1.31^{bf} \pm 0.02$	$1.31^{bf} \pm 0.03$	$1.11^{bf} \pm 0.02$	$0.62^{bf} \pm 0.01$	$1.25^{bf} \pm 0.02$
(ii)	6 months – one year (50)	$3.00^{bf} \pm 0.02$	$1.92^{bf} \pm 0.02$	$1.71^{bf} \pm 0.03$	$1.33^{bf} \pm 0.02$	$1.02^{bf} \pm 0.02$	$1.97^{bf} \pm 0.03$

Kataria *et al*. (2010d) [16] also recommended the use of antioxidants in the conditions causing oxidative stress in animals because repletion is reported after supplementation of vitamin C [17, 18].

In present study the decreased level of vitamin C in erythrocytes indicated towards the presence of oxidative stress in buffalo calves as well as in heifer calves. Singhal *et al.* (2016) [19] reported importance of antioxidants in animals. Extreme hot ambience can influence antioxidant status of animals [20].

Glutathione (GSH)

The moderate (control) mean value of erythrocytic glutathione was 1.51±0.03µmol gHb-1. The range obtained from all animals irrespective of ambience, sex and age was 0.99-1.80µmol gHb-1. Glutathione is an endogenous antioxidant which protects the cells from reactive oxygen species such as free radicals and peroxides [21, 22]. Glutathione spares ascorbate and improves antioxidant capacity of blood [23] and without it dehydroxy ascorbate could not be converted back to ascorbate. The ratio of reduced glutathione to oxidised glutathione within cells is used as a measure of cellular toxicity [24].

The mean values of erythrocytic glutathione were significantly ($p \leq 0.05$) lower during hot ambience as compared to respective moderate mean values. The erythrocytic mean value was 1.23 times lower during extreme hot ambience than respective moderate ambience mean values. In present study erythrocytic glutathione level was lower during hot ambience which indicated its depletion in the process to prevent oxidative stress. Depletion of glutathione was correlated to oxidative stress by various earlier workers [25, 26]. Effect of ambient temperature on serum glutathione levels was recorded by many earlier researchers in animals (Dehghan *et al*., 2010) [27] in rams; (Kataria *et al*., 2010b) [26] in goats; (Kataria *et al*., 2010d) [16] in camels and (Maan, 2010) [28] in sheep.

The findings clearly reflected the presence of oxidative stress during extreme hot ambience in erythrocytes and plasma. Extreme ambiences can disturb fluid status of buffaloes thereby affecting overall physiological profile [29].

Glutathione reductase (GR)

The moderate (control) mean value of eryhrocytic GR was 0.82±0.01kU gHb-1. The range obtained from all animals irrespective of ambience, sex and age was 0.42-2.01kU gHb-1. Various earlier workers have used GR activity as an indicator to assess oxidative stress in animals [26, 30, 31].

The mean values of eryhrocytic GR were significantly ($p \leq 0.05$) higher during hot ambience as compared to respective moderate mean values. The erythrocytic mean value was 1.97 times higher during extreme hot ambience than respective moderate ambience mean values. Earlier researchers have also showed higher activities of GR in various animals during hot ambience in erythrocytes [22] and in serum (Kataria *et al*., 2010b) [26] in goats; (Kataria *et al*., 2010d) [16] in dromedary camel; (Maan, 2010) [28] in sheep; (Joshi, 2012) [30] in buffaloes and (Pandey, 2012) [31] in goats).Scientists have discussed the role of glutathione reductase as erythrocyte antioxidant systems to overcome peroxide challenge [32, 33] and in anaemia [34].

Glutathione reductase probably provided a protective effect against ambience stress. In conclusion, it can be said that the high ambient temperature increases neuroendocrine stress and lipid peroxidation which in turn contributes to the reduced erythrocyte antioxidant response [35]. On the basis of earlier reportings, the results of present study theorise the presence of oxidative stress during extreme ambiences in buffalo bull and heifer calves. Extreme environments produce effect on physiology of animals [36].

Conclusion

Results clearly indicated that status of antioxidants and free radical scavenging enzymes changed in heat stressed calves probably to scavenge higher number of free radicals formed during hot ambience. Further sex effect was more pronounced in female buffalo calves than in male buffalo calves. Likewise buffalo calves of below 6 months of age were more affected. All the three parameter used are important biomarker 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. The study will signify the role of antioxidant supplementation in dams prior to parturition for proper growth and immunity development in buffalo calves.

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