Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2021; 9(1): 711-714

Received: 19-11-2020
Accepted: 21-12-2020

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Modulations in plasma antioxidants and free radical scavenging enzymes of buffalo calves from arid tracts during hot ambience

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Abstract
Present investigation was envisaged to find out extreme ambient temperature association variation in plasma antioxidants and free radical scavenging enzymes in buffalo calves from arid tracts. The antioxidants selected were Vitamin A and Vitamin E and free radical scavenging enzymes was Catalase. The moderate (control) mean values of vitamin A and Vitamin E in plasma were 2.08±0.01µmolL-1, 4.69±0.02 µmol L-1, respectively. The mean values of vitamin A and vitamin E were 1.18 and 1.7 times lower in plasma, respectively during extreme hot ambience than respective moderate ambience mean values. The moderate (control) mean values of catalase in plasma were 71.81±1.11kU L -1. The plasma mean values was 1.43 times higher during extreme hot ambience than respective moderate ambience mean values. It could be concluded that variation in plasma antioxidants and free radical scavenging enzymes were associated with changes in ambient temperatures. On the basis of result it can be recommended that buffalo calves should be protected from the heat stress to maintain integrity of erythrocytes and the body as a whole.

Keywords: ambient temperature, buffalo calves, vitamin A, vitamin E, catalase

Introduction
Free radicals are atoms or groups of atoms that can cause damage to cells, impairing the immune system and leading to infections and various degenerative diseases. A group of vitamins, minerals and enzymes is named as antioxidants or free radical scavengers that help to protect the body from the formation of free radicals. 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. Certain enzymes serve this vital function namely superoxide dismutase, glutathione reductase, catalase, peroxidase etc. There are also a number of nutrients that act as antioxidants, including vitamin A, C, E and glutathione [1]. 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 [2].
Oxidative stress leads to damage of biological macromolecules and disruption of normal metabolism and physiology. It contributes in initializing various processes in the body resulting in health disorders [3]. Oxidative stress is extremely dangerous as it does not exhibit any symptom and is recognizable with great difficulty by means of laboratory methods [4]. Development of oxidative stress can be evaluated by measuring steady state concentration of these free radical scavengers in the blood as a whole [5]. Which is important in monitoring health and the nutritional status of the calves Extreme environments produce effect on physiology of animals [6]. Extreme ambiences can disturb fluid status of buffaloes thereby affecting overall physiological profile [7]. It is essential to observe physiological consequences versus scaling of environmental stress in buffaloes from arid tracts [8]. Since there is paucity of study of physiological effects of oxidative stress in buffalo calves from arid tracts [9] therefore the present study was launched.

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 antioxidants and free radical scavengers in the plasma of buffalo calves during moderate and hot ambiences. To assess the effect of hot ambience on the antioxidants and free radical scavengers in the plasma, the result of various parameters analyzed were compared with those analyzed during moderate months serving as control.

Procedure of Goth (1991) [10] was employed for determination of plasma catalase which incorporated a blend of spectrophotometric method of hydrogen peroxide with plasma catalase measurement. This involved the incubation of plasma was incubated with hydrogen peroxide and sodium-potassium phosphate buffer. It is reported in the method that one unit catalase decomposes 1pmol of hydrogen peroxide per one minute. After finishing of the reaction, hydrogen peroxide is analyzed by spectrophotometer.

Process given by Varley (1988) [11] with little modification (Joshi, 2012) [12] was employed for determination of plasma vitamin A in which plasma was blended with alcohol in a tube and the retinol was extracted into the layer of light petroleum. Then evaporation of light petroleum was done and the remainder was blended in chloroform.

Spectrophotometric procedure of (Nair and Magar) with little modification (Joshi, 2012) [12] was employed for determination of plasma vitamin E. Being a sensitive method, it is able to detect the minute amount of plasma vitamin E. Basis of the method is a colour reaction happening between phosphomolybdic acid and vitamin E.

Results and Discussion

Mean ± SEM value of plasma vitamin A, E and Catalase during moderate and hot ambiences, gender and age groups are shown in table 1.

Table 1: Mean ± SEM values of vitamin A, E and Catalase (µmol L^{-1}) in the plasma of buffalo calves during moderate and extreme hot ambiences

		Vitamin A		Vitamin E		Catalase	
		Mean ± SEM values		Mean ± SEM values		Mean ± SEM values	
S.N.	Effect	Moderate	Extreme Hot	Moderate	Extreme Hot	Moderate	Extreme Hot
1.	Ambience	2.08^{b} ± 0.01 (100)	1.76^{b} ± 0.04 (100)	4.69^{b} ± 0.02 (100)	2.75^{b} ± 0.01 (100)	71.81^{b} ± 1.11 (100)	103.21^{b} ± 1.10 (100)
2.	Sex						
(i)	Male (60)	2.19^{bd}± 0.01	1.82^{bd} ± 0.04	5.74^{bd} ± 0.02	3.59^{bd} ± 0.02	82.91^{bd} ± 1.11	115.31^{bd} ± 1.12
(ii)	Female (60)	1.97^{bd}± 0.01	1.71^{bd} ± 0.04	3.62^{bd} ± 0.02	1.91^{bd} ± 0.02	60.71^{bd} ± 1.12	91.11^{bd} ± 1.12
3.	Age						
(i)	Below 6 months (60)	1.95^{bf}± 0.01	1.69^{bf} ± 0.04	3.64^{bf} ± 0.02	1.89^{bf} ± 0.02	64.91^{bf} ± 1.13	90.11^{bf} ± 1.10
(ii)	6 months – one year (60)	2.21^{bf}± 0.04	1.84^{bf} ± 0.01	5.72^{bf} ± 0.02	3.61^{bf} ± 0.01	79.71^{bf} ± 1.11	116.31^{bf} ± 1.11

Vitamin A: The moderate (control) mean value of plasma vitamin A was 2.07±0.01µmolL-1. The range obtained from all animals irrespective of ambience, sex and age was1.50-2.30µmolL-1. The mean value of plasma vitamin A were significantly (p≤0.05) lower during hot ambience as compared to respective moderate mean value. The plasma mean value was 1.18 times lower during extreme hot ambience then respective moderate ambience mean value. Vitamin A is considered as a powerful antioxidants along with other vitamins like C and E with its role in healthy maintenance [13, 14]. A decrease in antioxidant defence leads to oxidative damage of biomolecules [15]. Antioxidant effect of vitamin A is also used to recycle back the oxidised α-tocopheroxyl radicals to the active reduced form [16]. Antioxidants terminate the cell-damaging chain reactions caused by oxidation by removing free-radical intermediates. When free radical production exceeds the capacity of natural tissue antioxidants enzymes or as a result of depletion of endogenous antioxidant enzymes due to increased lipoperoxidative changes, oxidative stress develops [2]. The oxidative modification of the erythrocyte membrane has been shown to increase the fragility of the erythrocytes [17]. Decreased levels of erythrocytic vitamin A and E have been correlated to considerable peroxidation damage [18].

In present study the decreased level of vitamin A in plasma 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].

Vitamin E: The moderate (control) mean value of plasma vitamin E was 4.68±0.02 µmol L-1. The range obtained from all animals irrespective of ambience, sex and age was 1.50-5.99 µmol L-1. The mean value of plasma vitamin E was significantly (p≤0.05) lower during hot ambience as compared to respective moderate mean value. The plasma mean value was 1.70 times lower during extreme hot ambience then respective moderate ambience mean value. Vitamin E is the most important lipid-soluble antioxidant, protecting cell membranes from oxidation by reacting with lipid radicals during lipid peroxidation chain reaction [21]. Vitamin E is essential for proper health, immunity and reproductive functions of animals. Vitamin E is a non enzymatic scavenger of free radicals. It improves immune responses [22]. Determination is considered important in disease diagnosis and as a marker of oxidative stress in animals [3]. Decreased vitamin E levels have been well correlated with increased

oxidative threats [23]. In present study vitamin E levels in erythrocytes and plasma were lower during hot ambience which showed its depletion in an attempt to reduce the production of reactive oxygen species [2].

Scientists have suggested the depletion of vitamin E in heat stressed animals, as its supplementation increased plasma vitamin E levels [24]. 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. Extreme hot ambience can influence antioxidant status of animals [20].

Discussions and conclusions drawn by earlier workers [25, 26] along with the pattern of observations of present investigation helped in processing the surmise that extreme hot ambience depleted the vitamin E levels in erythrocytes and plasma thereby produced oxidative stress in the buffalo calves. Singhal *et al.* (2016) [19] reported importance of antioxidants in animals.

Catalase: The moderate (control) mean value of plasma CAT was 71.80±1.11kU L -1. The range obtained from all animals irrespective of ambience, sex and age was 55.00- 120.55kU L -1. Catalase activities in erythrocytes and plasma are considered important to assess oxidative stress [27, 1, 28]. 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) [29] and to different diets [27]. These reactions are crucial to life [1]. The mean value of plasma catalase was significantly ($p \leq 0.05$) higher during hot ambience as compared to respective moderate mean value. The plasma mean value was 1.43 times higher during extreme hot ambience then respective moderate ambience mean value.

Earlier researchers have recommended the use of catalase in the situations where free radicals are formed [30]. The increased activity of erythrocytic catalase during hot ambience suggested the ability of the animals to provide defence against free radicals. It was the body's response to combat the oxidative stress [4], as it is an enzyme of antioxidant defence system that eliminates and controls the toxic oxygen species. Phua (2004) [31] observed higher catalase activity in sheep where reactive oxygen species were generated.

Extreme hot ambience can influence antioxidant status of animals [20].

Kataria *et al*. (2010b) [2] suggested that higher serum CAT was due to higher rate of formation of hydrogen peroxide. From the result, it can be hypothesized that hot ambience caused a stressful condition in comparison to moderate, leading to excessive production of free radicals, which resulted in oxidative stress and an imbalance between oxidant and antioxidants systems [32, 33]. During stress or exercise, oxidative stress can be provoked [27, 2]. Singhal *et al.* (2016) [19] reported importance of antioxidants in animals.

Conclusion

It was concluded that extreme ambiences produced the oxidative stress in buffalo calves. This was based on the altered status of the free radical scavengers in the plasma. Further sex effect was more pronounced in female buffalo calves than in male buffalo calves. Likewise buffalo calves of below 6 month of age were more affected. The evaluation of the extent of oxidative stress in the form of values, can be useful to redefine the role of oxidative stress in different pathologies and can be used for clinical diagnosis and in health management of calves. It can be recommended that buffalo calves should be protected from the heat stress to maintain integrity of erythrocytes and the body as a whole.

Acknowledgement

I would like to extend my gratitude to Prof. (Mrs.) Nalini Kataria, Former head and professor in Department of Veterinary Physiology, College of Veterinary and Animal Science, Bikaner whose guidance and supervision made me able to write this research paper. A great thanks to my wife Mrs. Rekha Choudhary and my loving daughter Ms. Dronika Choudhary for their dedicated support with a congenial environment to write the paper. I would like to thanks Dr. Saurabh Singh Singhal and Dr. Sunita for helping me to write my research paper and a special thanks to Almighty who bestowed me with all this.

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