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ORIGINAL ARTICLE

Stress related variations in serum vitamin E and C levels of *Murrah* buffaloes

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The study was designed to determine stress related variations in endogenous vitamin E and C levels of *Murrah* buffaloes. For this purpose, four hundred and fifty healthy adult female *Murrah* buffaloes between 4 and 12 years of age were sampled to harvest the sera during adverse ambiences viz. moderate, extreme hot and cold ambiences. Animals were broadly divided into non-pregnant milch, pregnant milch, pregnant dry, primipara and multipara. The mean values (µmol L^{-1}) of serum vitamin E and C were 4.31±0.03 and 23.34±0.33, respectively during moderate ambience. The mean values of both the vitamin E and C depressed significantly ($p \leq 0.05$) during hot and cold ambiences as compared to moderate ambience. It was observed that decline in each value during hot ambience was greater than that of respective cold ambience. A significant ($p \leq 0.05$) variation was observed in the mean values of vitamin E and C in each ambience in the animals of all physiological states. The mean values of both the vitamins of non pregnant milch animals were highest ($p \leq 0.05$) whereas they were lowest ($p \leq 0.05$) in pregnant dry animals. It could be concluded that extreme ambiences produced oxidative stress in the buffaloes of all physiological states. The depressed levels of endogenous vitamin E and C in the serum showed their depletion in the body probably to combat free radical scavengers. Vitamin E and C should be supplemented to protect the animals from oxidative stress.

Key words: Ambience, vitamin E and C, buffalo, Murrah, serum

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Numerous rapidly evolving methodologies for evaluating oxidative stress are available to researchers and clinicians, each with their own distinct advantages and disadvantages. Differences in models and methodologies make it difficult to provide meaningful comparisons, even for studies that seem quite similar superficially. Clarity of understanding of the pathophysiology of oxidative

stress in ruminants will allow the design of specific antioxidant therapies. Heavy milk yielders like buffaloes have higher metabolic rate to derive energy involving oxidation reactions to meet the demand of production. These processes result in the formation of free radicals. To neutralize these free radicals body employs the system of endogenous antioxidants like vitamin E and vitamin C. This is a part of physiological reactions where both remains in a state of balance. When rate of formation of free radicals overrides the rate of synthesis of endogenous vitamin E and C, problem arises in the form of oxidative stress.

Research in oxidative stress has been implicated in numerous disease processes in animals, however, compared to human medicine, only a limited number of pathological conditions have been investigated in regard to the effects of oxidative stress in milch animals (Kataria *et al.,* 2010a and Kataria *et al.,* 2012). Studies in milch animals have been sporadic and mainly with mastitis, pneumonia, and retained placenta. More recently, studies have been focused on metabolic diseases that affect dairy animals during the peripartum period (Celi and Pietro, 2011).

Future research should focus on the establishment of a reference panel of biomarker of oxidative stress to be used in ruminant medicine. To help accelerate practical applications, researchers have proposed the development of an oxidative stress index as an approach in veterinary medicine. Oxidative stress can result from diminished antioxidant protection as well as increased free radical production. Therefore, investigating antioxidant depletion as a biomarker of oxidative stress may involve assessment of decreases in antioxidant concentrations or increases in their metabolites. However, such changes may reflect a clinically significant or pathogenic event with the indication that the antioxidant defense system is functioning. Timely detection of endogenous antioxidant levels may be helpful in health management of stressed animal (Chirase *et al.,* 2001). This can help in formulating the supplementation of vitamin E and C to heavy producers and protect the animals from various health disorders.

The *Murrah* is the most important premier milking breed of buffalo in India. They contribute greatly in enhancing economical status of farmers. The inevitability of exposure of these animals to extreme temperatures of arid and semiarid tracts results in development of oxidative stress and this makes these animals an easy target of a series of health disorders. Despite of immense quality characteristics of these animals very little scientific savoir faire is there about normal and clinical variations in the values of endogenous vitamin E and C. Therefore the present investigation was planned to establish physiological reference values of the endogenous vitamin E and C to be used in the field of veterinary clinical physiology and to find out the possibility of oxidative stress during extreme ambiences in *Murrah* buffaoes

MATERIALS AND METHODS

The investigation included four hundred and fifty healthy adult female *Murrah* buffaloes between 4 and 12 years of age to determine antioxidant status during moderate, extreme hot and cold ambient temperature periods. All the animals belonged to private dairy farms of Rajasthan state, India which were managed in similar conditions of feeding and watering. Animals were broadly divided into group A included non-pregnant milch (30); pregnant milch (30) and pregnant dry (30) animals and group B which

included primipara (30) and multipara (30) animals. The mean maximum ambient temperatures during moderate and hot periods were 30.33± 0.20 and 45.5 ± 0.08 °C, respectively, whereas mean minimum temperature during cold ambience was 4.88 ± 0.20 °C. In each ambience 150 blood samples were collected to harvest sera. Sampling was carried out in morning hours during moderate, hot and cold ambiences. Blood was collected directly into the clean, dry test tubes without any anticoagulant to harvest sera.

Vitamin E was determined by the basic spectrophotometric method (Nair and Magar,1955) with modifications (Kataria *et al*., 2010b). It was a highly sensitive method based upon the colour reaction between phosphomolybdic acid and vitamin E. Serum vitamin C determination was carried out by a titration of serum ascorbate by 2,6-dichlorophenolindophenol dye (Varley,1988). Calculation was carried out as follows to convert the value in SI units:

$$\text{Serum ascorbic acid } (\mu\text{mol } L^{-1}) = \frac{100}{\text{ml titration}} \times 2 \times 0.008 \times 56.78$$

200µl of the dye solution is equivalent to 0.008 mg ascorbic acid. 56.78 is the conversion factor for umol L^{-1} from mg/dl.

To test the significance, the changes in the means were measured by using multiple mean comparison procedures (Duncan, 1955 and Steel and Torrie, 1980). In each case, the moderate mean value served as the control.

RESULTS AND DISCUSSION

In the present study the mean values of serum vitamin E and vitamin C (Table 1) were significantly ($p \leq 0.05$) lower during hot and cold ambiences as compared to respective moderate mean value. It was observed that decline in each value during hot ambience was greater than that of respective cold ambience. Heat stress in animals reduces antioxidant activity (Calamari *et al.*,1999) through increased production of free (Ishikawa and Kanai, 1998) or due to insufficient endogenous synthesis (McDowell, 2000) resulting into a decrease in the antioxidant defense culminating in oxidative damage of biomolecules (Beckman and Ames, 1998).

Hot ambience associated decrease in serum vitamin E showed its depletion in an attempt to reduce the production of reactive oxygen species and to combat increased oxidative threats (Walwadkar *et al.,* 2006). Insufficient endogenous synthesis of vitamin E under stressful conditions could be another cause for depletion (Kataria *et al.,* 2010c). Decreased levels of vitamin C confirm the presence of oxidative stress because repletion is reported after its supplementation (Weiss, 2001). Kataria *et al.,* (2010c) also recommended the use of vitamin E and C in the disease conditions causing oxidative stress.

In group A, serum mean value of each vitamin in non pregnant milch, pregnant milch and pregnant dry animals differed significantly ($p \leq 0.05$) from each other in all the ambiences and the mean value of each vitamin of non pregnant milch animals was highest whereas it was lowest in pregnant dry animals. Probably in these animals greater stress resulted in depletion of levels of vitamin vitamin E and C. In group B, the mean value of each serum vitamin was significantly ($p \leq 0.05$) lower in multipara animals than primipara in each ambience. Pregnancy associated oxidative stress in reference to vitamin E has been discussed in water buffaloes (Dimri *et al.,* 2010). Age related variation in antioxidant level could be hypothesised on the basis of relationship of free radicals with age (Sastre

et al., 2000). Vitamin E is also important in the management of stressed animals (Chirase *et al.,* 2001), therefore its use is suggested to combat oxidative stress (Bourdel-Marchasson *et al.,* 2001). Probably in these animals greater stress resulted in depletion of levels of vitamin C. Higher vitamin C level in animals reflected towards its higher synthesis to combat free radicals (Long, 1963). Bhadula *et al.* (1970) studied the effect of physiological states on plasma vitamin C level in buffaloes. Nazifi *et al.* (2009) discussed about the influence of age on free radical generation and consequently, the enzyme antioxidant defense. Based on the results, the assumption can be made that the endogenous antioxidant defense systems were altered to adapt and prevent oxidative stress effects (Kataria *et al.,* 2010d).

Table 1. Mean ± SEM values of serum vitamin E and vitamin C in *Murrah* buffalo

Effects	Serum vitamins	
	Vitamin E, µmol L^{-1}	Vitamin C, µmol L^{-1}
Ambience		
Moderate/ Control (150)	4.31±0.03 [a]	23.34±0.33[a]
Group A		
Non-pregnant milch (30)	4.85±0.02 [b]	26.92±0.22 [b]
Pregnant milch (30)	4.30±0.02 [b]	23.00±0.30[b]
Pregnant dry(30)	3.80±0.03 [b]	20.10±0.40 [b]
Group B		
Primipara (30)	4.88±0.02[c]	25.01±0.30[c]
Multipara (30)	3.74±0.01[c]	21.67±0.32[c]
Hot (150)	2.43±0.02[a]	16.00±0.21[a]
Group A		
Non-pregnant milch(30)	2.90±0.02 [b]	18.00±0.30 [b]
Pregnant milch (30)	2.40±0.01[b]	16.20±0.40[b]
Pregnant dry(30)	2.00±0.02 [b]	13.80±0.20 [b]
Group B		
Primipara (30)	3.45±0.02[c]	17.50±0.23 [c]
Multipara (30)	1.41±0.01[c]	14.50±0.25[c]
Cold (150)	3.57±0.02[a]	20.00±0.30[a]
Group A		
Non-pregnant milch (30)	3.67±0.02 [b]	23.50±0.30 [b]
Pregnant milch (30)	3.58±0.02[b]	20.00±0.40[b]
Pregnant dry(30)	3.46±0.01 [b]	16.50±0.30 [b]
Group B		
Primipara (30)	3.69±0.01[c]	22.30±0.30[c]
Multipara (30)	3.45±0.02[c]	17.70±0.40[c]

Figures in the parenthesis indicate number of animals. Means superscribed by same superscripts within a column differ significantly ($p \leq 0.05$) from each other.

The findings clearly reflected the presence of oxidative stress during extreme ambiences in buffaloes of all the physiological states.The depressed levels of endogenous vitamin E and C in the serum showed their depletion in the body probably to combat free radical scavengers. It can be recommended that the vitamin E and should be supplemented during adverse conditions in the animals of all the physiological states to protect them from oxidative stress.

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