AMELIORATION OF HEAT STRESS BY FEEDING ELECTROLYTES, ASCORBIC ACID AND ZINC IN BUFFALOES

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ABSTRACT

The ameliorative effect of salts, ascorbic acid polyphosphate and zinc oxide supplementation on heat stress was studied in buffaloes. Adult buffaloes of either sex were randomly divided into two groups of four animals each. Group 1 served as control and Group 2 was supplemented with sodium bicarbonate, potassium carbonate ascorbic acid polyphosphate and zinc oxide. All the animals were exposed to two conditions of temperature and humidity: hot-dry and hot-humid in psychrometric chamber for 4 h daily for 10 days. Blood was collected on days 1, 5 and 10 of treatment. The activities of catalase and superoxide dismutase (SOD), concentrations of serum glutathione (GSH) and cortisol and lipid peroxidation were estimated in serum. Significant decrease in activities of serum catalase and SOD, and serum concentration of reduced GSH and significant increases in lipid peroxidation and serum cortisol in both groups was observed when subjected to heat stress. Dietary supplementation of ascorbate and zinc in addition to electrolytes resulted in further decrease in the enzymes’ activities whereas the serum GSH increased at par to normal value. Lipid peroxidation and serum cortisol concentration were comparatively lower in the supplemented group in both types of stress. Thus, supplementation of ascorbate and zinc in addition to electrolytes relieves the animals of oxidative stress.

Keywords: electrolytes, ascorbic acid, zinc, buffalo, heat stress

INTRODUCTION

Stress occurs when an animal suddenly faces a change in its environment. Stress is a broad term, generally used in negative connotation and is described as the cumulative detrimental effect of a variety of factors on the health and performance of animals (Rosales, 1994). Heat stress during summer and post summer months in tropical countries is a problem of great concern among farmers and livestock producers as it increases costs in both production and reproduction of animals. Heat stress occurs in animals when there is an imbalance between heat production within the body and its dissipation. Buffaloes are more prone to physical distress when exposed to heat stress as compared to other farm animals. The scarcely distributed sweat glands and dark body color render buffaloes with poor heat tolerance capacity (Das et al., 1999). This may greatly depreciate their value as a source of milk, meat and draught power.

Heat stress is one of the wide varieties of factors which causes oxidative stress in-vivo. Reactive oxygen species (ROS), the major
molecules for causing oxidative stress, are constantly generated \textit{in vivo} as an integral part of metabolism. Despite acting as the first line of defense in combating infection, ROS may cause oxidative stress when their level exceeds the threshold value. They trigger progressive destruction of polyunsaturated fatty acids (PUFA), ultimately leading to membrane destruction (Halliwell, 1990). As a part of defense against the menace of ROS, the body employs antioxidants to quench these free radicals. The enzymatic antioxidants include superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). In general, these enzymatic antioxidants act by scavenging both intracellular and extracellular superoxide radical and preventing lipid peroxidation of plasma membrane. Non-enzymatic antioxidants include vitamins like vitamins C, A and E, proteins like albumin, transferrin, glutathione (GSH) etc. Studies have shown that antioxidant nutrient supplementation, especially vitamins C, A and E, zinc and chromium, can be used to attenuate the negative effects of environmental stress in poultry (Nojoku, 1986), smaller ruminants and rats (Garg and Bansal, 2000).

The activities of antioxidant enzymes and lipid peroxidation alter significantly during oxidative stress. So, they can be used as markers of oxidative stress (Agarwal and Prabhakaran, 2005). Considering these facts, the present study was conducted in buffaloes to determine the levels of antioxidants and cortisol after providing some dietary factors during heat stress in buffaloes.

**MATERIALS AND METHODS**

**Experimental animals**

The experiments were performed on eight buffaloes of either sex, divided into two groups. The mean body weight of animals was 550 kg. Deworming was done for both ecto and endoparasites before the start of experiment. The animals were maintained in the experimental shed of the Physiology and Climatology Division of the Indian Veterinary Research Institute. All the animals were reared under uniform management and proper hygienic condition throughout the period of study.

One group was termed the control and the other the experimental. Both the groups were maintained on a standard ration. In addition, the experimental group was fed sodium bicarbonate 15 g/animal/day, potassium carbonate 12.5 g/animal/day ascorbic acid polyphosphate 10 g/animal/day and zinc oxide 160 mg/animal/day. Water was available \textit{ad libitum}.

**Schedule for induced heat stress**

Animals of both groups were exposed to two different conditions of temperatures and relative humidities, viz. hot-dry (40°C and ambient relative humidity i.e. 30% approx) and hot-humid (35°C and a relative humidity of 70%) conditions in psychrometric chamber for four hours daily for 10 days. In between the two types of exposure, a 15 days rest was given to the animals.

**Biochemical analysis**

Blood samples were collected on 1\textsuperscript{st}, 5\textsuperscript{th} and 10\textsuperscript{th} day (both before and after exposure to heat stress) from the jugular vein. Serum was separated for biochemical analysis. Catalase activity was estimated by the method described by Cohen \textit{et al.} (1970). Superoxide dismutase (SOD) activity was estimated as described by Madesh and Balasubramanian (1998), with some modifications. In brief, the assay mixture in a total volume of 300
μl per well consisted of 120 μl PBS, 10 μl serum sample, 5 μl of 1.25 mM MTT and 15 μl of freshly prepared 1mM pyragallol solution. The sample was replaced with PBS in the blank. After an incubation period of 15 minutes, 150 μl DMSO was added and absorbance was taken in a microplate at 570 nm. SOD activity was estimated using the formula

\[
\text{SOD activity (units/ml)} = 2 \times 100 \times \frac{\text{Absorbance}_{\text{Test}}}{\text{Absorbance}_{\text{Blank}}}
\]

Concentration of serum GSH was estimated as described by Lin Hu et al. (1988). Lipid peroxidation was determined as per Beuge and Aust (1978) modified by Suleiman et al. (1996). Cortisol concentration was estimated by RIA using the diagnostic I^{125} kit supplied by Immunotech, Czech Republic.

Statistical analysis

The data obtained were analyzed using analysis of variance technique as described by George (1994).

RESULTS

Serum catalase activity decreased significantly (P<0.05) on exposure to hot dry heat stress in both control (Group 1) and experimental groups (Group 2) with days. The post exposure values were significantly (P<0.05) lower than pre-exposure values (Figure 1a). The experimental group showed significantly (P<0.05) less enzyme activity as compared to the control in pre-exposure samples (Figure 1a). In the hot-humid condition, the catalase activity significantly (P<0.05) decreased with time in the control group but no effect was observed in the experimental group (Figure 1b).

The post-exposure value was significantly lower than the pre-exposure value on the 10th day in both the groups. The experimental group showed less (P<0.05) enzyme activity with respect to the control both before and after exposure on all the 3 days (Figure 1b).

Serum superoxide dismutase (SOD) activity was lower (P<0.05) in the experimental as compared to the control group both before and after exposure on all the three days in the hot dry condition but there was no change in SOD activity upon days of exposure (Figure 2a). The post-exposure values were significantly lower (P<0.05) than the pre-exposure values in both the groups on all three days (Figure 2a). In the hot-humid condition, the experimental group showed significantly (P<0.05) lower SOD activity on day 1 and day 5 only (Figure 2b). There was no significant variation in SOD activity upon days of exposure.

In the hot-dry condition, the experimental group had significantly (P<0.05) less GSH concentration only on all three days both before and after exposure (Figure 3a). Serum GSH concentration rose significantly (P<0.05) on day 10 in both the pre- and post-exposure samples of both the groups. The pre-exposure values were significantly lower than the post-exposure values in both the groups. The variations between control and supplemented groups as well as pre- to post-exposure in GSH concentration were insignificant in the hot humid condition (Figure 3b). Lipid peroxidation was found to be lower (P<0.05) in the experimental group as compared to the control in the hot-dry condition (Figure 4a). A significant rise in lipid peroxidation was observed from day 1 to day 10 in both groups. A pre- to post-exposure decline in lipid peroxidation was significant only in the control group on day 1 and day 5. In the hot-humid condition lipid peroxidation was lower (P<0.05)
Figure 1a. Serum catalase activity on different days of exposure (Hot-dry condition).

A, B Means with different letters differ significantly (p<0.05) among groups.

a, b Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.

x, y, z Means with different letters differ significantly (p<0.05) between days of exposure.

Figure 1b. Serum catalase activity on different days of exposure (Hot-humid condition).

A, B Means with different letters differ significantly (p<0.05) among groups.

a, b Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.

x, y Means with different letters differ significantly (p<0.05) between days of exposure.
Figure 2a. Serum superoxide dismutase activity on different days of exposure (Hot-dry condition).

A,B  Means with different letters differ significantly (p<0.05) among groups.

a,b  Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.

x,y  Means with different letters differ significantly (p<0.05) between days of exposure.

Figure 2b. Serum superoxide dismutase activity on different days of exposure (Hot-humid condition).

A,B  Means with different letters differ significantly (p<0.05) among groups.

a,b  Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.

x,y  Means with different letters differ significantly (p<0.05) between days of exposure.
Figure 3a. Serum glutathione concentration on different days of exposure (Hot-dry condition).

A,B Means with different letters differ significantly (p<0.05) among groups.

a,b Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.

x,y Means with different letters differ significantly (p<0.05) between days of exposure.

Figure 3b. Serum glutathione concentration on different days of exposure (Hot-humid condition).
Figure 4a. Serum lipid peroxidation on different days of exposure (Hot-dry condition).

A,B Means with different letters differ significantly (p<0.05) among groups.
a,b Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.
x,y Means with different letters differ significantly (p<0.05) between days of exposure.

Figure 4b. Serum lipid peroxidation on different days of exposure (Hot-humid condition).

A,B Means with different letters differ significantly (p<0.05) among groups.
a,b Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.
x,y Means with different letters differ significantly (p<0.05) between days of exposure.
Figure 5a. Serum cortisol concentration on different days of exposure (Hot-dry condition).
A,B Means with different letters differ significantly (p<0.05) among groups.
A,b Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.
x,y Means with different letters differ significantly (p<0.05) between days of exposure.

Figure 5b. Serum cortisol concentration on different days of exposure (Hot-humid condition).
A,B Means with different letters differ significantly (p<0.05) among groups.
a,b Means with different letters differ significantly (p<0.05) between pre and post-exposure samples.
x,y Means with different letters differ significantly (p<0.05) between days of exposure.
on all the three days in experimental group as compared to control. Lipid peroxidation increased (P<0.05) from day 1 to day 10 in both groups. However, in the experimental group, this variation was not observed in the post-exposure samples. A pre- to post-exposure decline was significant only on day 10 in both the groups (Figure 4b).

Serum cortisol concentration was significantly lower (P<0.05) in the experimental group as compared to the control on all the three days in both the hot-dry and hot-humid conditions (Figures 5a and b). Heat stress caused a significant (P<0.05) increase in serum cortisol from day 1 to day 10 in both groups in both conditions. The post-exposure values were significantly (P<0.05) higher than the pre-exposure values in both groups on day 1 alone during the hot-dry exposure while the in hot-humid condition such variation was evident on day 1 and day 10 (Figures 5a and b).

**DISCUSSION**

Both enzymatic (catalase and superoxide dismutase) and non-enzymatic (reduced glutathione) antioxidants play important roles in combating oxidative stress. Catalase detoxifies H$_2$O$_2$ produced during different metabolic processes and also in stressful conditions by reducing it to H$_2$O and O$_2$. Superoxide dismutase (SOD) which exists in three different forms viz. SOD1 (copper-zinc dependent SOD) found in cytosol, SOD2 (manganese dependent SOD) present in mitochondria and SOD3 (extracellular SOD), is known to catalyze dismutation of superoxide radicals into H$_2$O and O$_2$ (Fridovich, 1978). It scavenges both intra and extracellular superoxide radicals by acting in conjugation with catalase and glutathione peroxidase (Agarwal and Prabhakaran, 2005). In the present study, both catalase and SOD activities decreased significantly due to heat stress (either the hot-dry or the hot-humid condition). Similar findings have been reported in oxydementon-methyl induced oxidative stress in buffaloes (Rampal et al., 2002), ammonium acetate induced oxidative stress in rats (Dakshayani et al., 2002) and molybdenum induced oxidative stress in crossbred calves (Sharma et al., 2004). However, the decrease in the experimental group was more than the control group since the experimental group had ascorbic acid polyphosphate and zinc oxide in addition to salts. Ascorbate and zinc interferes in the actions of catalase and SOD. Ascorbate is known to scavenge reactive oxygen species (ROS) during oxidative stress (Prasad, 1979 and Bisla et al., 2004) and spare other antioxidants in relieving oxidative stress (Frey, 1991). Thus, it is likely that in supplemented group, ascorbate and zinc played major role as an antioxidant and thus the activities of catalase and SOD remained low. Reduced glutathione (GSH) concentration has been reported to increase upon long term oxidative stress caused by diaphragmatic herniorrhaphy in buffaloes (Bisla et al., 2000). During chronic oxidative stress, the body synthesizes more GSH, a considerable amount of which becomes protein bound due to which the post-exposure GSH concentration was lower than pre-exposure one (Sies, 1991). However, upon long term exposure to heat stress, the body synthesizes more GSH to cope up with oxidative stress. The lower GSH in the experimental group than in the control observed in the present study may again be due to ascorbate and zinc as in case of catalase and SOD. The net effect of oxidative stress is lipid peroxidation, which is a complex biochemical reaction involving free radicals, oxygen, metal ions and a host of other factors in biological system (Ramchandran et al., 2002) and
a potent marker of oxidative stress. In the present study, dietary supplementation caused decrease in lipid peroxidation because ascorbic acid acts as an inhibitor/chain blocker of lipid peroxidation (Tanaka et al., 2007).

Cortisol is the major glucocorticoid produced and secreted from adrenal cortex having profound effects on carbohydrate, fat and protein metabolism. Production and secretion of cortisol is dependent upon (adrenocorticotrophic hormone) ACTH secreted from the anterior pituitary, which in turn is regulated by corticotrophin releasing hormone (CRH) from hypothalamus. Various stressors (thermal, transportation weaning, etc.) are reported to activate hypothalamo-pituitary-adrenal axis in domestic farm animals resulting in increased production of cortisol (Minton, 1994). In the hot-dry condition, significant increase in cortisol concentration took place in both groups. But the increase was less in the experimental group than in the control. This indicates supplementation of ascorbate and zinc reduced the stress in the animals. The ameliorative effect of ascorbate alone on heat stress has also been documented in buffaloes (Kumar et al., 2010).

It is concluded that heat stress decreases serum catalase and superoxide dismutase activities, but increases glutathione and cortisol concentration and lipid peroxidation. These changes can be partly modulated by addition of salts (sodium bicarbonate, potassium carbonate), vitamin C (ascorbic acid polyphosphate) and zinc (zinc oxide) in the feed.

REFERENCES


