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Effects of Addition of Electrolyte and Ascorbic Acid in Feed during Heat Stress in Buffaloes

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ABSTRACT : The ameliorative effect of salts and ascorbic acid polyphosphate supplementation on heat stress was studied in buffaloes. Adult buffaloes of either sex were randomly divided into 2 groups of 4 animals each. Group I served as control and Group II was supplemented with sodium bicarbonate, potassium carbonate and ascorbic acid polyphosphate. All the animals were exposed to two conditions of temperature and humidity: hot-dry and hot-humid in a psychrometric chamber for 4 h daily for 10 days. Blood was collected on day 1, 5 and 10 of treatment. The activities of catalase and superoxide dismutase (SOD), concentrations of serum glutathione (GSH), cortisol, sodium, potassium, and chloride and lipid peroxidation were estimated in serum. Lymphocyte proliferation was assessed in blood. The activities of catalase and SOD, serum concentration of GSH, sodium, potassium and chloride decreased while lipid peroxidation and serum cortisol increased in both groups when subjected to heat stress. Dietary supplementation resulted in further decreasing of the enzyme activities but increasing of the serum concentrations of GSH, sodium, potassium and chloride. Lipid peroxidation and serum cortisol increased in the supplemented group in both types of stress. Dietary supplementation caused an increase in lymphoproliferative response to con A. Thus, supplementation of ascorbate in addition to electrolytes relieves the animals of oxidative stress and boosts cell mediated immunity. (**Key Words :** Heat Stress, Electrolytes, Ascorbic Acid, Buffalo)

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 costs 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 *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, body employs antioxidants to quench these free radicals. Antioxidants can fit into two broad categories-enzymatic and non-enzymatic (Agarwal and Prabhakaran, 2005). The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) 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).

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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). Excessive amounts of ROS are harmful for the immune cells as they can attack cellular components and lead to cell death by oxidizing membrane lipids and proteins (De La Fuente and Victor, 2000). Supplementation of antioxidants may quench these free radicals and thus act as immunomodulators. Considering these facts, the present study was conducted in buffaloes to determine the levels of antioxidants, electrolytes and cortisol after providing some dietary factors during heat stress in buffaloes and to find out the change in immunological status of the animals after heat stress due to dietary manipulations.

MATERIALS AND METHODS

Experimental animals

The experiments were performed on 8 buffaloes of either sex, divided into 2 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 Physiology and Climatology Division of the Institute. All the animals were reared under uniform management and proper hygienic condition throughout the period of study.

One group was termed as control and the other as treated. Both the groups were maintained on standard ration as per Kearl (1982). Animals were offered Maize 40%, Wheat bran 37%, Groundnut cake 20%, Mineral Mixture 2% and Salt 1%. In addition, the treated group was fed sodium bicarbonate at 15 g/animal/d, potassium carbonate at 12.5 g/animal/d and ascorbic acid polyphosphate at 10 g/animal/d. Water was given *ad libitum*.

Schedule for induced heat stress

Animals 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.

Biochemical and immunological analysis

Blood samples were collected from jugular vein on day 1^{st}, 5^{th} and 10^{th} in the morning before feeding. Subsequently, the animals were offered concentrate mixture, wheat straw and water. Then they were exposed in the psychrometric chamber and blood samples were again collected after the exposure to heat stress. Serum was harvested for biochemical parameters. For lymphocyte proliferation assay blood was collected separately with 2.7% EDTA under sterile conditions.

Catalase activitiy was estimated by the method described by Cohen 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 1 mM pyragallol solution. 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 ELISA reader at 570 nm. SOD activity was estimated using the following formula.

$$\text{SOD activity (units/ml)} = 2\times100\times\text{Absorbance}_{\text{Test}}/\text{Absorbance}_{\text{Blank}}$$

Concentration of serum GSH was estimated by the method 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. Sodium and potassium concentration were estimated by flame photometry, described by Oser (1965) and chloride concentration by semi auto analyzer using a kit supplied by Span Diagnostic Ltd, Surat. Lymphocyte culture was done as per the method described by Boyum (1968) and lymphocyte proliferation was assessed by the method of Mosmann (1983).

Statistical analysis

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

RESULTS AND DISCUSSION

In hot-dry condition, serum catalase activity was lower ($p<0.05$) on day 5 and day 10 in both control and treated groups (Figure 1). The post exposure value was lower ($p<0.05$) than pre exposure value only on day 1 in both groups. Serum superoxide dismutase (SOD) activity was lower ($p<0.05$) in both the groups both before and after exposure on all the three days. The enzyme activity decreased ($p<0.05$) on day 5 but not thereafter in control. Supplemented group also showed similar trend in pre exposure samples, however the activity increased ($p<0.05$) on day 10 in post exposure samples (Figure 1).

In hot-humid condition, serum catalase activity was lower in treated group on day 1 and 10 in post exposure samples (Figure 5). The enzyme activity increased ($p<0.05$) from day 1 to 5 and later decreased on day 10 in post exposure samples in treated group. Post exposure value was higher than pre exposure value on day 1 and 10 in control group alone. Serum superoxide dismutase (SOD) activity

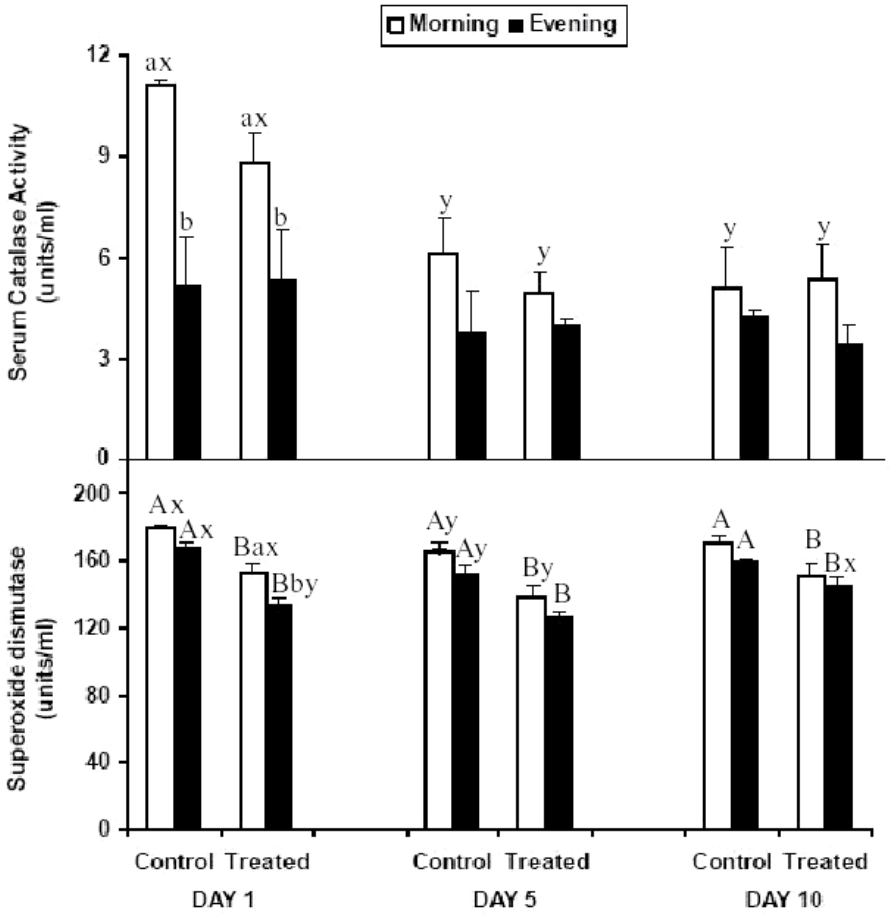

Figure 1. Serum catalase and superoxide dismutase activity on different days of exposure (Hot-dry condition). [A, B] Means with different letters differ significantly ($p<0.05$) among groups. [a, b] Means with different letters differ significantly ($p<0.05$) between morning and evening samples. [x, y] Means with different letters differ significantly ($p<0.05$) between days of exposure.

was lower ($p<0.05$) before exposure on day 1 and 10 and both before and after exposure on day 5 as compared to control (Figure 5). The enzyme activity declined ($p<0.05$) from day 1 to day 10 in both the groups. Post exposure values were lower than pre exposure ones on all the 3 days in control but in group II, this trend followed only on days 5 and 10.

Both enzymatic (catalase and superoxide dismutase) and non-enzymatic (glutathione) antioxidants play important roles in combating oxidative stress. Catalase detoxifies H_2O_2 produced during different metabolic processes and also in stressful conditions by reducing it to H_2O and O_2. Superoxide dismutase (SOD) which exists in three different forms viz. SOD1 (copper-zinc SOD) found in cytosol, SOD2 (manganese SOD) found in mitochondria and SOD3 (extracellular SOD) found extracellularly, is known to catalyze dismutation of superoxide radicals into H_2O 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). Glutathione (γ Glutamyl cysteinyl glycine) annihilates oxygen toxicity by interrupting the reaction leading to O_2^- formation (Yoda et al., 1986). In its reduced form it metabolizes H_2O_2 and hydroxyl radicals.

Earlier it has been reported that 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 our treated groups was more than the control group since the supplemented groups had ascorbic acid polyphosphate in addition to salts. There are possibilities that ascorbate might have interfered in the actions of catalase and SOD. Ascorbate is known to scavenge reactive oxygen species (ROS) during oxidative stress (Prasad, 1979; Bisla et al., 2004) and spare other antioxidants in relieving oxidative stress (Frey, 1991). Thus, it is likely that in supplemented group, ascorbate has played major role as an antioxidant and the activities of catalase and SOD remained low.

In hot-dry condition, the treated group had less ($p<0.05$) GSH concentration as compared to the control before

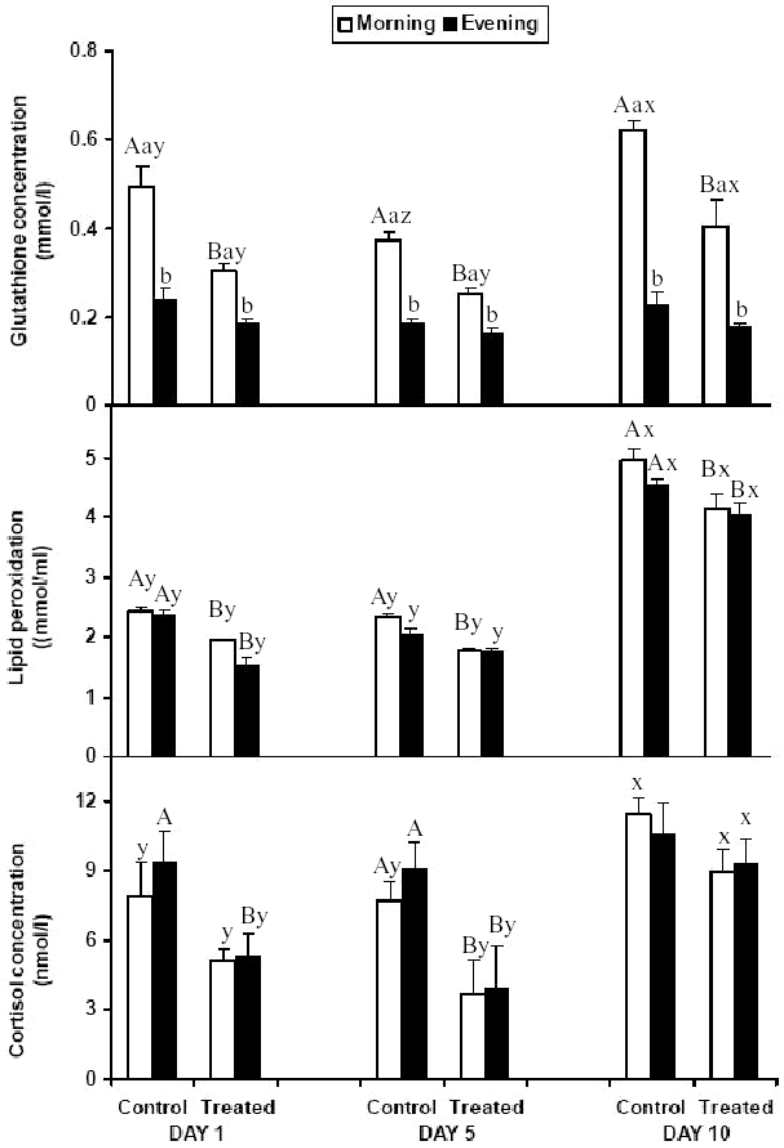

Figure 2. Serum glutathione, cortisol and lipid peroxidation on different days of exposure (Hot-dry condition). [A, B] Means with different letters differ significantly ($p<0.05$) among groups. [a, b] Means with different letters differ significantly ($p<0.05$) between morning and evening samples. [x, y, z] Means with different letters differ significantly ($p<0.05$) between days of exposure.

exposure on all the three days (Figure 2). Serum GSH concentration fall ($p<0.05$) on day 5 but rose again on day 10 in control in pre exposure samples, while the supplemented group showed a rise ($p<0.05$) on day 10 in pre exposure ones. The post exposure value was lower ($p<0.05$) than pre exposure value on all the three days in both groups. Lipid peroxidation was found to be lower in treated group as compared to the control on all the three days. A significant rise in lipid peroxidation was observed from day 1 to day 10 in both control and treated groups (Figure 2).

Serum cortisol concentration was lower ($p<0.05$) in treated group as compared to control on day1 and day 5 in hot-dry condition (Figure 2). Heat stress caused a significant increase in serum cortisol from day 1 to day 10 in both groups. The concentrations of serum GSH, cortisol and lipid peroxidation under hot-humid condition are depicted in Figure 6. Serum GSH concentration was lower ($p<0.05$) before exposure on days 1 and 5 and both before and after exposure on day 10 in treated group as compared to control. GSH concentration increased ($p<0.05$) from day1 to day 5 in pre exposure samples in control. Post exposure values were significantly lower than pre exposure ones on days 1 and 5 in control group alone. Lipid peroxidation was lower ($p<0.05$) on day 1 and day 5 in treated group as compared to control. It increased ($p<0.05$) from day 1 to day 10 in both groups. None of the groups showed variations in pre and post exposure values. The

variations between control and supplemented groups as well as pre to post exposure in cortisol concentration were insignificant (Figure 6).

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, body synthesizes more GSH, a considerable amount of which becomes protein bound (Sies, 1991). However, the less GSH in treated group than control observed in this study may again be due to ascorbate as in case of catalase and SOD. 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. Shim et al. (2006) demonstrated peroxidizability in broiler chicks under chronic heat stress could be reduced by feeding taurine. 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 anterior pituitary, which in turn is regulated by corticotrophin releasing hormone (CRH) from hypothalamus. Various stressors (thermal, transportation weaning, etc.) are reported to activate

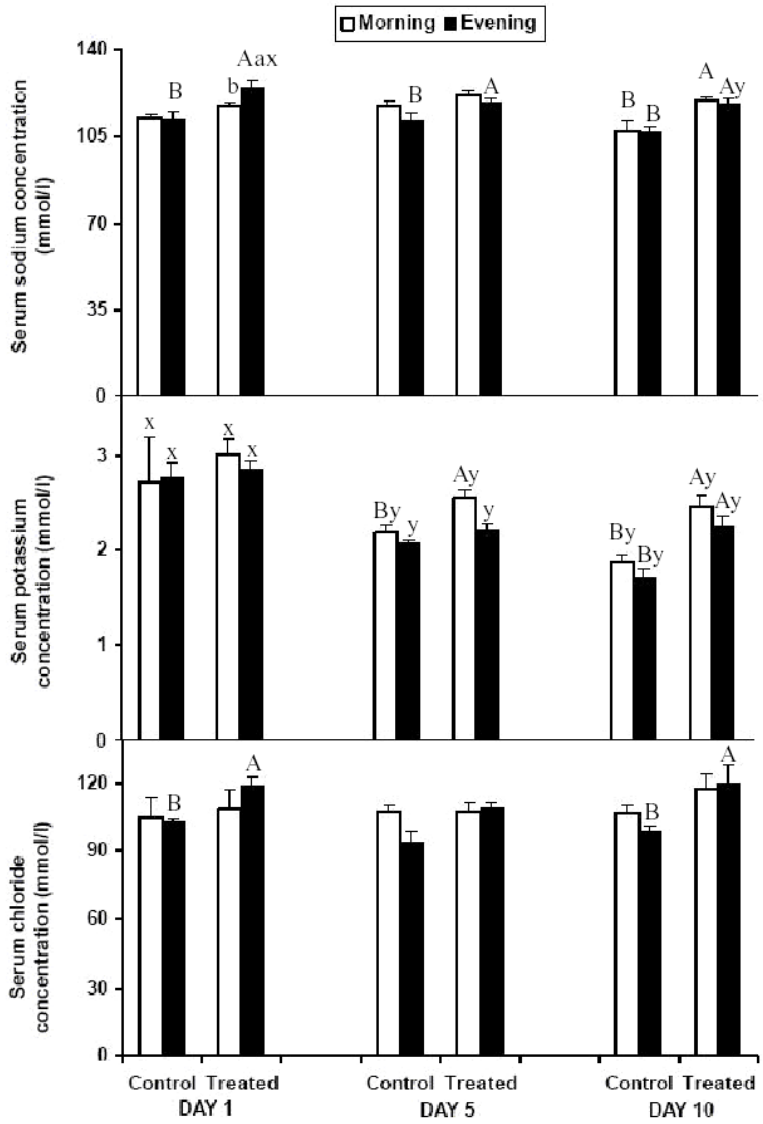

Figure 3. Serum sodium, potassium and chloride concentration on different days of exposure (Hot-dry condition). [A, B] Means with different letters differ significantly ($p<0.05$) among groups. [a, b] Means with different letters differ significantly ($p<0.05$) between morning and evening samples. [x, y] Means with different letters differ significantly ($p<0.05$) between days of exposure.

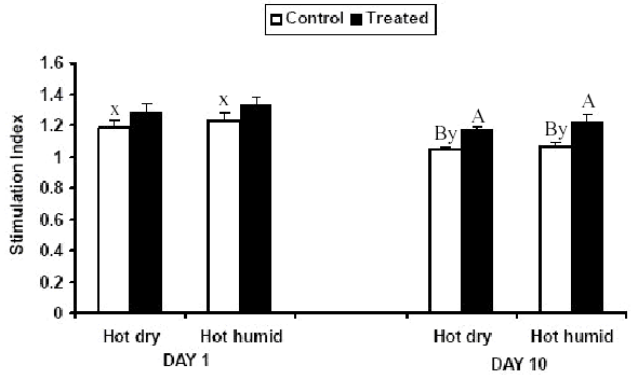

Figure 4. Lymphocyte proliferation assay on different days of exposure (Hot-dry and hot-humid condition). [A, B] Means with different letters differ significantly ($p<0.05$) among groups. [x, y] Means with different letters differ significantly ($p<0.05$) between days of exposure.

hypothalamo-pituitary-adrenal axis in domestic farm animals resulting in increased production of cortisol (Minton, 1994). In hot-dry condition, significant increase in cortisol concentration had taken place in both groups. But the increase was less in treated group than control. This indicates supplementation of ascorbate reduced the stress in animals.

Under hot-dry condition, the electrolyte profile depicted in Figure 3 shows, that serum sodium concentration was higher in supplemented group as compared to control. In the treated group, a significant fall in serum sodium concentration was observed from day 1 to 10 in post exposure samples. In general, there was no significant difference between pre and post exposure values. Supplemented group showed significantly higher values of serum potassium concentration on day 5 before exposure and day 10 both before and after exposure. There was a decline ($p<0.05$) in its concentration from day 1 to day 5 but not there after in control and treated groups. Pre and post exposure values revealed no significant differences. Serum chloride concentration increased ($p<0.05$) in treated group on day 1 and 10 post exposure. None of the groups showed variation upon days of exposure. Post exposure value was found to be lower than pre exposure value on day 5 in control group alone.

The electrolyte profile showed similar trend in hot-humid condition as was observed in hot-dry condition (Figure 7). The supplemented group had higher ($p<0.05$) concentration of serum sodium on all the three days both before and after exposure. No variation was observed upon days of exposure. Potassium concentration was higher in

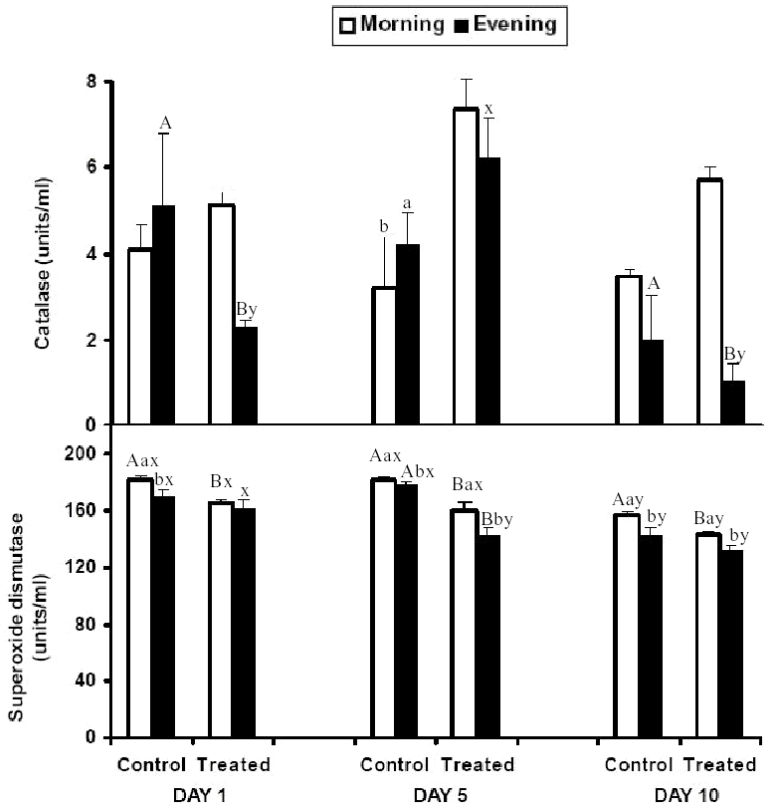

Figure 5. Serum catalase and superoxide dismutase activity on different days of exposure (Hot-humid condition). [A, B] Means with different letters differ significantly ($p<0.05$) among groups. [a, b] Means with different letters differ significantly ($p<0.05$) between morning and evening samples. [x, y] Means with different letters differ significantly ($p<0.05$) between days of exposure.

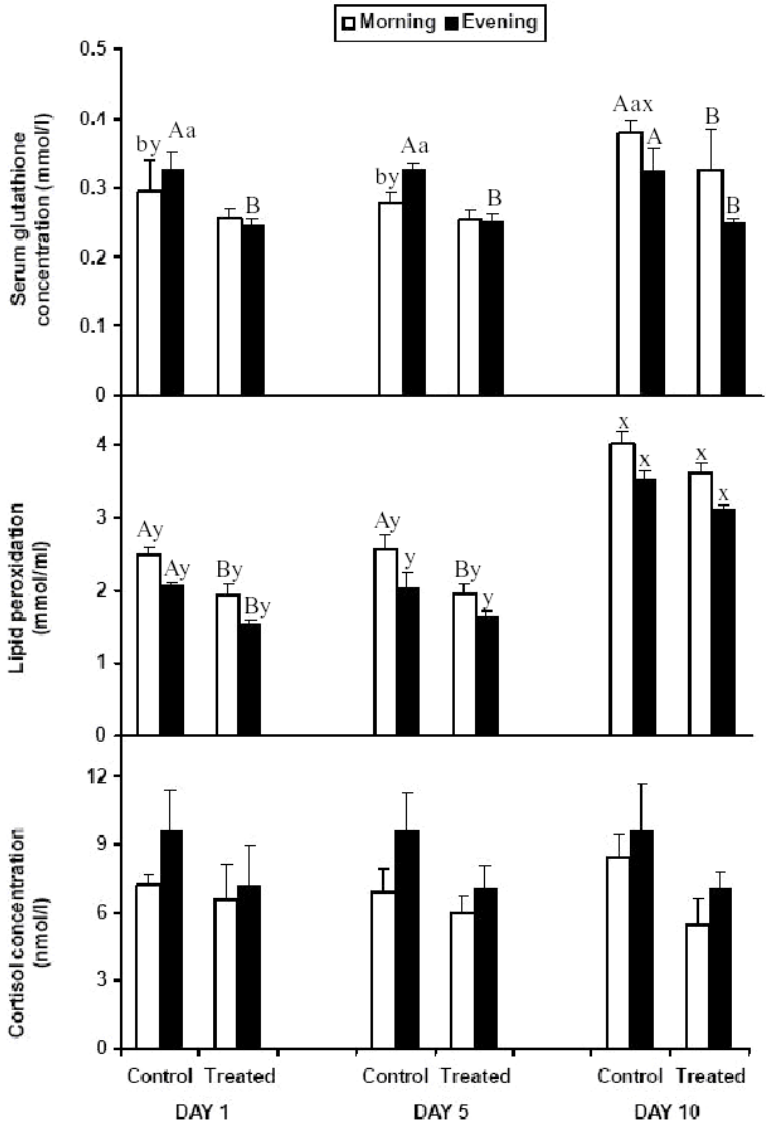

Figure 6. Serum glutathione, cortisol and lipid peroxidation on different days of exposure (Hot-humid condition). [A, B] Means with different letters differ significantly ($p<0.05$) among groups. [a, b] Means with different letters differ significantly ($p<0.05$) between morning and evening samples. [x, y] Means with different letters differ significantly ($p<0.05$) between days of exposure.

treated group on day 10. Its level declined ($p<0.05$) from day 1 to 10 in control both before & after exposure and in treated group only before exposure. There were no differences between pre and post exposure values in both groups. Serum chloride concentration increased ($p<0.05$) in treated group both before and after exposure on days 1 and 5 and after exposure on day 10. Loss of electrolytes during heat stress has been reported by many workers (Collier et al., 1982; Shalit et al., 1991). This decrease occurs mainly due to increased excretion of electrolytes in urine, sweat and other secretions to alleviate heat stress. Addition of sodium bicarbonate, potassium carbonate and ascorbate in feed decreased loss of electrolytes. Sodium and potassium status of the body has been reported to remain stable during heat stress when supplemented with electrolytes (West et al., 1999). It appears from the present study that in addition to electrolytes, supplementation with ascorbate may help in better retention of electrolytes. Supplementation of sodium and potassium in the form of bicarbonate/carbonate also help in better regulation of acid-base balance in the blood (Sanchez et al., 1994).

Stimulation index (S.I) was observed to rise ($p<0.05$) in supplemented group on day 10 (Figure 4). There was a decrease in S.I. from day 1 to day 10 in both groups. Trends similar to those observed during hot-dry condition were also observed in hot-humid condition in case of lymphocyte proliferation assay (Figure 4). One common way of assessing the influence of various factors on the immune

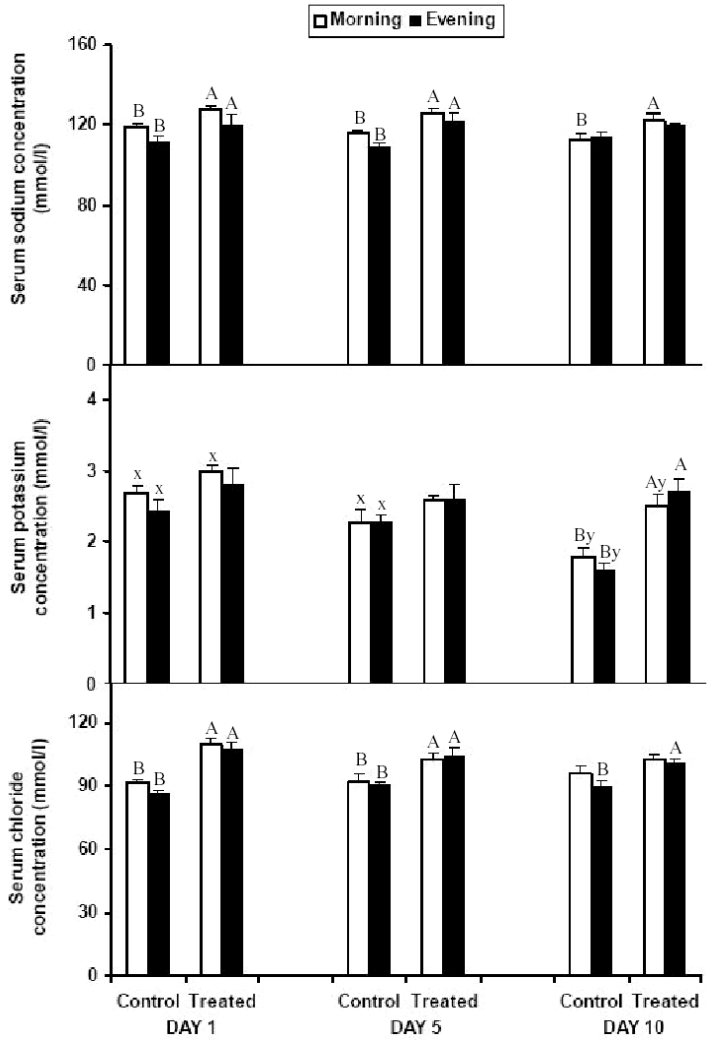

Figure 7. Serum sodium, potassium and chloride concentration on different days of exposure (Hot-humid condition). [A, B] Means with different letters differ significantly ($p<0.05$) among groups. [a, b] Means with different letters differ significantly ($p<0.05$) between morning and evening samples. [x, y] Means with different letters differ significantly ($p<0.05$) between days of exposure.

response is to measure the response of peripheral blood lymphocytes to *in-vitro* mitogen stimulation. In the present work, Con A (T-cell mitogen) was used to evaluate the influence of heat stress on cell-mediated immunity. There was a significant ($p<0.05$) fall in the stimulation index (SI) in both groups upon exposure to heat stress in both hot-dry and hot-humid conditions. This is in accordance with earlier reports which stated that lymphocyte proliferative response to Con A was reduced in cattle subjected to transportation stress (Blecha et al., 1984). The decrease in lympho proliferative response might be due to the fact that excessive production of ROS due to thermal stress renders harmful effect on the cell membrane by causing lipid peroxidation and cells of immune system are no exception (Goldstone and Hunt, 1997). Dietary supplementation caused an increase in lymphoproliferative response to Con A. Similar findings have been reported in people given with vitamin E (De La Fuente and Victor, 2000) and in domestic pigs fed with zinc and vitamin C (Bhar et al., 2003).

It is concluded that heat stress decreases serum catalase and superoxide dismutase activities, electrolyte concentration but increases cortisol concentration and lipid peroxidation. Cell mediated immune response is also decreased due to heat stress. These changes can be partly modulated by addition of salts (sodium bicarbonate, potassium carbonate) and vitamin C (ascorbic acid polyphosphate) in the feed.

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