EFFECT OF DIETARY SUPPLEMENTATION ON ELECTROLYTE PROFILE AND LYMPHOCYTE PROLIFERATION DURING HEAT STRESS IN BUFFALOES

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Received: July 26, 2011; Accepted: December 12, 2011

Abstract: Heat stress in large farm ruminants is a problem of great concern among livestock farmers during summer and post-summer months. Serum electrolyte concentrations, in particular Na⁺, K⁺ and Cl⁻ are found to be reduced during heat stress. Heat stress also causes a decline in immunological response in animals. In this study, effect of supplementing salts, ascorbic acid polyphosphate and zinc oxide on heat stress was studied in buffaloes. Adult Murrah buffaloes of either sex were randomly divided into 2 groups viz. control and experimental. The latter was supplemented with sodium bicarbonate, potassium carbonate, ascorbic acid polyphosphate and zinc oxide. All the animals were exposed to induced heat stress with two experimental treatments: hot-dry and hot-humid, in psychrometric chamber, 4 hours daily for 10 days. Blood was collected on day 1 and 10 of treatment. The concentration of sodium, potassium, and chloride was estimated in serum. Lymphocyte proliferation was assessed in blood. Heat stress caused a decrease in serum electrolyte level while dietary supplementation resulted in moderation of the serum electrolyte profile. Dietary supplementation also caused an increase in lymphoproliferative response to con A. results from this study indicated that supplementation of ascorbate and zinc in addition to electrolytes relieves the animals of stress caused by electrolyte deficit and boosts cell mediated immunity.

Key words: Heat stress, Buffalo, Electrolytes

INTRODUCTION

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 [1]. 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. Buffalo in particular are adversely affected by heat stress due to poor heat tolerance and reduced immune function [2-4]. 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, the cells associated with immunological functions being no exception [5]. 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 [6], smaller ruminants and rats [7]. Zinc is a catalytic cofactor for cu/zn superoxide dismutase [SOD] and catalyzes dismutation of superoxide anion. Supplementation of electrolytes is one among the nutritional strategies to combat heat stress in animals. 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 [8]. Supplementation of antioxidants may quench these free radicals and thus act as immunomodulators. Considering these facts, the present study was conducted in adult Murrah buffaloes to determine the levels of electrolytes after providing some dietary factors during heat stress and also to find out the change in immunological status of the animals.

MATERIALS AND METHODS

All the animal procedures and protocols were approved in advance by the Institutional Animal Care Committee of Indian Veterinary Research Institute, India. The experiments were performed on two groups [n=6 each] of adult Murrah buffaloes [Bubalus bubalis] of either sex [males and non-lactating females], weighing 550 ± 20 Kg at 4 years of age. 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 Indian Veterinary Research Institute. All the animals were reared under uniform management and proper hygienic condition throughout the period of study. Both the groups were maintained on standard ration as per Kearl [9]. In addition, the experimental group was supplemented with sodium bicarbonate @ 15g/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 given ad libitum. The experimental feeding was started 15 days before the start of heat exposure.

Both the groups were taken to psychrometric chamber [temperature and humidity controlled] for inducing heat stress. The treatments were given separately in the chamber [i] hot-dry treatment: animals were exposed to 40°C with existing relative humidity [30% approx] 4 h daily for 10 days and [ii] hot-humid treatment: animals were exposed to 35°C with relative humidity of 70%, 4 h daily for 10 days. A rest of 15 days was provided in between the two exposures.

Blood samples were collected before and after exposure to the prescribed treatment on 1st and 10th day from jugular vein. Serum was harvested for biochemical parameters. For lymphocyte proliferation assay blood was collected in 2.7% EDTA under sterile conditions. Sodium and potassium concentration were estimated by flame photometry [10] and chloride concentration by semi auto analyzer using a kit supplied by Span Diagnostic Ltd, Surat. Lymphocytes were separated by density gradient centrifugation using histopaque as described by Boyum [11]. Cell counting and viability were determined by Trypan blue dye exclusion. Lymphocyte proliferation was assessed using colorimetric MTT assay and the intensity of formazan crystals was measured at 492 nm with reference correction at 650 nm in an ELISA plate reader [12]. Stimulation index [SI] was calculated as mean OD from stimulated lymphocytes cultured over the unstimulated.

The data obtained were analyzed using analysis of variance technique as described by Snedecor and Cochran [13]. P dŠ 0.05 was considered to indicate statistical significance.

RESULTS

Periodic exposure of hot-dry and hot-humid stress regimen showed profound impact on electrolyte profile of Murrah buffaloes. In hot-dry condition, there was a significant [P<0.05] fall in serum sodium concentration from day 1 to 10 in both the groups. In general, there was no significant difference between pre- and post-exposure values. Supplemented...
The experimental group had higher serum sodium compared to control on both day 1 and day 10 (Table 1). Similar trend followed in hot-humid condition as well (Table 2). Supplemented group showed significantly (P<0.05) higher values of serum potassium concentration as compared to control both in hot dry as well as hot humid conditions (Tables 1 and 2). The decline (P<0.05) serum K⁺ concentration from day 1 to day 10 was significant in control group alone only in hot-dry exposure. Pre- and post-exposure values revealed no significant differences.

Serum chloride concentration was significantly higher (P<0.05) in experimental group as compared to control during both types of stress. However, no significant change in serum Cl⁻ concentration was observed upon days of heat exposure in both the groups of buffaloes (Table 1). The electrolyte profile showed similar trends in hot-humid condition as was observed in hot-dry condition (Table 2).

Stimulation index (S.I) was observed to rise (p<0.05) in supplemented group as compared to control both on day 1 & 10 (Fig. 1). There was a decrease in S.I from day 1 to day 10 in both groups during hot-dry as well as hot-humid exposures (Fig. 1).

### DISCUSSION

The effect of heat stress on electrolyte depletion has been studied since long. This decrease occurs mainly due to increased excretion of electrolytes in urine, sweat and other secretions to alleviate heat stress [14]. Dietary supplementation of electrolytes is one among the nutritional strategies to combat heat stress in animals. Vitamins C, E and trace elements like zinc, chromium etc. act by helping the antioxidant enzymes like catalase, SOD etc in neutralizing the
free radicals generated during various metabolic processes in vivo. These antioxidants might play thermo adaptive functions to maintain homeostasis. Therefore, this study was undertaken to observe the effect of dietary supplementation on electrolyte and immunological status of buffaloes during heat stress.

The different conditions of temperatures and relative humidity used in the experiment were sufficient to induce heat stress in Murrah buffaloes [3,4,14]. Loss of electrolytes during heat stress has been reported by many workers in buffaloes [3,15,16] and goats [17,18]. It appears from the present study that in addition to electrolytes, supplementation with ascorbate and zinc may help in better retention of electrolytes. It has been reported that supplementation of ascorbic acid alone along with electrolytes had some ameliorative effect on heat stress in buffaloes [3]. Supplementation of sodium and potassium in the form of bicarbonate/carbonate also help in better regulation of acid-base balance in the blood [15].

One common way of assessing the influence of various factors on body immune 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 finding corroborates with earlier reports which stated that lymphocyte proliferative response to Con A was reduced in cattle subjected to transportation stress [19]. The decrease in lympho proliferative response might be due to excessive production of ROS due to thermal stress, which initiates the cascade of lipid peroxidation of biological membranes [20] there by reducing the cell mediated immune response. In the present study, dietary supplementation caused an increase in lymphoproliferative response to Con A. This might be due to the fact that supplementation of antioxidants prevents lipid peroxidation thereby preventing cell membrane disruption. Similar findings have been reported in human beings given with vitamin E [8], domestic pigs fed with zinc and vitamin C [21] and buffaloes fed with vitamin C along with electrolytes [3]. From the above findings, it may be concluded that changes in electrolyte profile and immunological status of Murrah buffaloes, induced by heat stress can be partly modulated by addition of salts (sodium bicarbonate, potassium carbonate), vitamin C (ascorbic acid polyphosphate) and zinc (zinc oxide) in the feed.

ACKNOWLEDGEMENTS

The authors thank the Director, Indian Veterinary Research Institute for providing all the necessary financial assistance for conducting this experiment.

REFERENCES