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Antioxidants Supplementation on Acid Base Balance during Heat Stress in Goats

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ABSTRACT : The effects of vitamin C and vitamin E with selenium on acid-base balance and some stress hormones were evaluated during heat stress in goats. Goats, 1.5 years of age, were divided into control, heat stress and antioxidant treatment groups 1, 2 and 3. Except for the control, all groups were exposed to a temperature of 40±2°C with a relative humidity of 30% for 5 h/d for 21 days in a psychrometric chamber. Rectal temperature and respiratory rates were recorded daily post exposure. Blood samples were collected on every 3rd day for estimation of plasma vitamins C and E, total antioxidant activity and hormones, and separate blood samples were taken to estimate acid-base status. The rectal temperature and respiratory rates were increased ($p<0.05$) in the heat stress group only. Except for pH and pO_2, which were increased significantly ($p<0.05$) other parameters of acid-base balance such as pCO_2, HCO_3^-, TCO_2, BEb, BEcef, PCV and Hb were significantly decreased ($p<0.05$) in the heat stress group. An improvement in acid-base status was noted in the antioxidant supplemented groups. Prolactin and cortisol levels were significantly ($p<0.05$) higher and free T3 and T4 levels were significantly ($p<0.05$) lower in the heat stress group. Levels of prolactin and cortisol were decreased and free T3 and T4 were increased in antioxidant treatment groups. Different levels of antioxidant supplementation resulted in similar protection against heat stress. (**Key Words :** Antioxidants, Acid Base Status, Stress Hormones, Heat Stress, Goats)

INTRODUCTION

High environmental temperature challenges the animal's ability to maintain energy, thermal, water, hormonal and mineral balance. Heat stress stimulates excessive production of free radicals (superoxide anion radicals, hydroxyl radical, hydrogen peroxide and singlet oxygen) which are continuously produced in the course of normal aerobic metabolism (Bernabucchi et al., 2002) and these free radicals can in turn damage healthy cells if they are not eliminated. Heat production is directly controlled by the nervous and endocrine systems through modifications of appetite, digestive process and, indirectly, by alterations of the activity of respiratory enzymes and protein synthesis (Yousef, 1985). Exposure of animals to heat stress activates the hypothalamo-pituitary-adrenal axis (Abilay et al., 1975) and hence estimation of concentrations of hormones such as thyroxine, cortisol, and prolactin could be one of the important indicators for assessment of stress in animals. The maintenance of blood pH is high on the list of homeostatic priorities as almost all enzyme systems in the body are influenced by H^+ concentrations (Fraser, 1991) and it depends primarily on relative concentrations of carbonic acid and base bicarbonate in blood (Coppock et al., 1982). A major strategy to reduce the effect of heat stress on animals is to alter the environment through the use of sheds, fans or evaporative cooling (Bucklin et al., 1991). Such practices are not possible in semi-intensive systems as goats are grazed in the open during most of the day. This necessitates other strategies to counteract the adverse effects of heat stress such as supplementation of antioxidants. Antioxidants such as Vitamin C and E are free radical scavengers, which protect the body defense system against excessively produced free radicals during heat stress and stabilize health status of the animal. Although ruminants can synthesize vitamin C (McDowell, 1989), a large reduction in plasma vitamin C concentration was reported in calves stressed by housing conditions (Cummins and Brunner, 1991) and in heat- stressed cows (Padilla et al., 2006). Dietary vitamin C is extensively degraded in the rumen (Cappa, 1958). Thus, special preparations for

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ruminants have been developed and some experiments indicated that supplementation with these preparations increased plasma vitamin C concentration in cattle (Hidiroglous, 1999). Ghanem et al. (2008) and Ayo et al. (2006) gave vitamin C to Awassi sheep and goats, respectively, by dissolving ascorbic acid in water and found that this orally supplemented vitamin C was also effective in alleviating stress. Hence we chose the oral route for vitamin C administration.

Careful perusal of the literature revealed an absence of sufficient information regarding the role of antioxidants during heat stress in ruminants. Therefore, the present study was conducted to assess whether supplementation of vitamin C and vitamin E with selenium could alleviate heat stress in goats.

MATERIALS AND METHODS

Healthy, non-pregnant Black Bengal goats (25), 1.5 years of age, were selected from the experimental animal shed of the Physiology and Climatology division IVRI, Izatnagar. The goats were maintained under uniform management and husbandry conditions. The animals were fed individually in troughs with a concentrate mixture which consisted of 40% maize, 37% wheat bran, 20% ground nut cake, 2% mineral mixture and 1% common salt on a dry matter basis. Fresh greens and wheat straw were fed *ad libitum*. The animals were provided with clean drinking water *ad libitum* twice a day at about 9.30 AM and 3.30 PM. This feeding regimen was given to all goats during the entire experiment. Animals were randomly divided into 5 groups, each of 5 animals (Table 1).

In treatment group I, vitamin C was given at 2 mg/animal/d (chewable tablets supplied by Celin, Glaxo SmithKline, India) dissolved in 10 ml of water. In treatment group II, vitamin E was supplemented at 250 mg/animal/d as alpha tocopherol acetate with selenium as sodium selenite, whereas in treatment group III, vitamin C was given at 1 mg/animal/d in 5 ml of water along with 125 mg vitamin E/animal and 0.05 mg selenium. Vitamin C and E were supplemented to the animals orally over and above the NRC recommendations through the stress period.

All groups except the control group were exposed to a temperature of 40±2°C with a relative humidity of 30% for 5 h per day for 21 days in a psychrometric chamber measuring (meters) 7.5 L×7.5 W×2.5 H with facility to increase temperature and humidity, if required. Control group animals were housed adjacent to the psychrometric chamber at a temperature of 25±2°C and a relative humidity of 40%. Body temperature was recorded by inserting a digital clinical thermometer into the rectum for one minute and care was taken to keep the thermometer bulb in close contact with the rectal mucosa. Respiration rates were recorded by counting the flank movements from a distance prior to examining the rectal temperature in order to avoid any disturbance to the animals. Rectal temperatures and respiratory rates were recorded twice daily, i.e. before and after exposure in the psychrometric chamber, through the experimental period.

Blood samples of all groups were collected by venipuncture from the jugular vein into sterile vials containing anti-coagulant heparin every third day post-exposure to heat stress. The plasma samples were separated by centrifugation at 3,000 rpm for 15 min and samples were frozen and stored at -20°C until used. Plasma vitamin E and C concentrations were determined by HPLC (Gaal et al., 1995) and by spectrophotometric method (Raghuramulu et al., 1983), respectively. Plasma total antioxidant activity was measured by ferric-reducing antioxidant power (FRAP) assay (Benzie and Strain, 1999). Blood plasma samples of all the animals were assayed for cortisol, free triiodothyronine (T3) and free thyroxine (T4) by a radio-immunoassay (RIA) technique supplied by Immunotech, Czech Republic. The plasma level of prolactin was estimated by ELISA (hPRL-ELISA Kit No.020123 supplied by Medicorp).

Separate blood samples were collected in a heparinized syringe fitted with a rubber cork for blood acid-base analysis which was performed within 1 h after blood collection. The whole blood pH, pCO_2, pO_2, HCO_3^-, TCO_2 BEb, BEecf, Hb and PCV were analyzed by using stat profile PHOX reagent pack A calibration cartridge-B

Table 1. Physiological responses in group1, 2 and antioxidant treatment groups

Parameter*	Group 1 Control	Group 2 Heat stress	Antioxidant treatment groups		
			Group 1	Group 2	Group 3
Rectal temperature (^oF)	101.80±0.04^a	103.92±0.06^d	102.65±0.06^b	103.06±0.06^c	102.67±0.07^b
Respiratory rate/min	20.30±0.10^a	122.22±0.63^d	73.04±2.13^b	81.82±1.91^c	72.85±2.13^b
Feed Intake (kg)	2.74±0.006^d	2.34±0.01^a	2.58±0.02^c	2.51±0.01^b	2.58±0.01^c

Means with different superscripts in rows differ significantly ($p<0.05$).

* Data represents average of 21 days.

Table 2. Plasma vitamin C and E levels and total antioxidant activity in group 1, 2 and antioxidant treatment groups

Parameter*	Group 1 Control	Group 2 Heat stress	Antioxidant treatment groups		
			Group 1	Group 2	Group 3
Vitamin C mg/dl	0.175±0.0007[b]	0.171±0.0002[a]	0.192±0.001[d]	0.172±0.000[a]	0.187±0.001[c]
Vitamin E μg/ml	3.24±0.01[b]	2.93±0.03[a]	3.03±0.02[a]	3.59±0.05[d]	3.48±0.04[c]
Total antioxidants (μmol/L)	656.57±1.49[d]	545.13±9.32[a]	620.41±3.61[c]	595.63±6.48[b]	627.68±2.69[c]

Means with different superscripts in rows differ significantly ($p<0.05$).
* Data represents average of 21 days.

No.23937 kit by ABG blood gas analyzer (NOVA Biomedicals, Waltham, USA). All the values were corrected to the body temperature. The data were analyzed using one-way ANOVA with the help of SPSS software.

RESULTS

The rectal temperature and respiratory rate in the present experiment are presented in Table 1. There was a significant ($p<0.05$) increase in rectal temperature and respiratory rates in the heat stress group. The rectal temperature and respiratory rates in antioxidant treatment groups were significantly ($p<0.05$) lowered. Daily average rectal temperature and respiratory rates before exposure to the psychrometric chamber were similar to the control group and similar in all treatment groups. Hence, only values of rectal temperature and respiratory rates after 4 h of exposure in the chamber are depicted in Table 1 i.e. the animals were in normothermic condition before exposure on every day.

Plasma vitamin E and C levels were significantly ($p<0.05$) decreased in the heat stress group (Table 2). Vitamin C supplementation increased plasma vitamin C levels in treatment groups 1 and 3; whereas supplementation of vitamin E increased plasma vitamin E in treatment groups 2 and 3. The total plasma antioxidant activity was significantly ($p<0.05$) lower in the heat stress group as compared to either control or treatment groups.

The various parameters of acid-base balance in different groups are set out in Table 3. Except for pH and pO_2 which were increased significantly ($p<0.05$), other parameters of acid-base balance such as pCO_2, HCO_3^-, TCO_2, BEb, BEecf, PCV and Hb were significantly decreased ($p<0.05$) in the heat stress group. An improvement in acid- base status was observed in treatment groups. Except for pH, other parameters of acid-base status did not differ among treatment groups. The various plasma hormonal levels in different groups are presented in Table 4. Prolactin and cortisol levels were significantly ($p<0.05$) higher and free T3 and T4 levels were significantly ($p<0.05$) lower in the heat stress group. The levels of prolactin and cortisol were decreased and free T3 and T4 were increased in treatment groups.

DISCUSSION

Antioxidants are free radical scavengers which protect the body defense system against excessively produced free radicals and stabilize health status of the animal. Vitamin E is a free radical scavenger on the cell membrane and

Table 3. Acid-Base Balance in group 1, 2 and antioxidant treatment groups

Parameter*	Group 1 Control	Group 2 Heat stress	Antioxidant treatment groups		
			Group 1	Group 2	Group 3
pH	7.396±0.003[a]	7.486±0.003[d]	7.433±0.008[b]	7.458±0.008[c]	7.433±0.011[b]
$pCO_{2(mmHg)}$	55.25±0.93[c]	39.48±0.41[a]	48.80±1.6[b]	45.63±1.98[b]	49.49±1.85[b]
$pO_{2(mmHg)}$	40.36±0.54[c]	52.49±0.94[a]	47.01±0.97[b]	48.66±1.52[b]	46.75±1.23[b]
Bicarbonates (mmol/L)	42.86±0.79[c]	28.80±0.23[a]	36.67±1.40[b]	34.01±1.66[b]	37.16±1.78[b]
TCO_2 mmol/L	45.88±1.07[c]	30.13±0.33[a]	40.10±1.69[b]	36.40±1.81[b]	40.02±2.18[b]
BEecf (mmol/L)	9.73±0.18[c]	2.43±0.07[a]	7.29±0.72[b]	5.76±0.84[b]	7.21±0.74[b]
BEb (mmol/L)	18.46±0.86[c]	7.35±0.17[a]	15.92±1.33[b]	13.08±1.49[b]	15.91±1.5[b]
Hemoglobin (g/dl)	11.57±0.13[c]	10.09±0.19[a]	10.67±0.24[b]	10.70±0.24[b]	10.76±0.27[b]
PCV	35.18±0.51[c]	30.87±0.61[a]	32.5±0.75[b]	32.0±0.68[b]	32.87±0.71[b]

Means with different superscripts in rows differ significantly ($p<0.05$).
* Represents average of 21 days.

Table 4. Endocrine responses in group 1, 2 and antioxidant treatment groups

Parameter*	Group 1 Control	Group 2 Heat stress	Antioxidant supplemented groups		
			Group 1	Group 2	Group 3
Prolactin (μg/L)	11.73±0.10[a]	26.37±0.60[d]	16.39±0.57[b]	18.93±0.58[c]	15.81±0.41[b]
Cortisol (nmol/L)	25.27±0.65[a]	40.57±0.92[d]	29.18±1.3[b]	30.84±1.4[c]	29.95±1.8[b]
Free T_3 (Pmol/L)	4.55±0.16[d]	3.21±0.08[a]	3.82±0.08[c]	3.53±0.09[b]	3.91±0.08[c]
Free T_4 (Pmol/L)	21.27±0.51[d]	16.70±0.19[a]	19.96±0.32[c]	18.56±0.36[b]	19.37±0.26[c]

Means with different superscripts in rows differ Significantly ($p<0.05$).
* Represents average of 21 days.

metabolic functions of selenium are closely linked to vitamin E which not only protects biological membranes from oxidative degeneration but also makes up an integral part of the enzyme glutathione peroxidase of the antioxidant system (Hoekstra, 1975). Vitamin C is a water soluble, extra-cellular, natural antioxidant and is involved in a number of oxidation and reduction reactions in the body. It not only scavenges free radicals but also helps to recycle reduced vitamin E *in vivo*.

The rectal temperature and respiratory rates are recognized as important measures of physiological status (Lefcourt et al., 1986) as well as ideal indicators for assessment of stress in animals. Hence, the increased rectal temperature and respiratory rates after exposing goats to 40 ±20°C in the psycrometric chamber suggested that the animals were under a stressful environment in the chamber. The increase in rectal temperature in Black Bengal goats suggested that these animals can store body heat during the periods of heat stress. This can economize the loss of water and increased need for energy associated with increased respiratory rates. In a hot environment, poorly sweating animals like goats attempt to maintain heat balance by increasing their respiratory activity, thereby losing more heat by evaporation from the respiratory tract than under normal circumstances. The decreased rectal temperature and respiratory rates in treatment groups indicated that supplementation of vitamin E with selenium and vitamin C ameliorated the heat stress in goats. Similar decrease in rectal temperature and respiratory rates by vitamin E and C supplementation was reported by Shenglins et al. (2003) in pigs and Kobeisy (1997) in goats under heat stressed conditions. Vitamin E and C directly alters thermal set point by decreasing prostaglandin output, especially of PGE series, whose turnover increases during stress (Hadden et al., 1987) and which has a direct effect on the hypothalamic thermoregulatory zone (Ganong, 2001). Therefore, by affecting the prostaglandin output, these vitamins may have an ameliorating effect upon heat stressed animals.

The vital limits of pH variation for mammals are between 7.3 and 7.44 (Houpt, 1989), and depends primarily on the relative concentration of carbonic acid and base bicarbonate in blood (Coppock et al., 1982). Under normal conditions, acids and bases are added continuously to the body fluids as a result of either ingestion or production during cellular metabolism. To combat any change in acid-base balance, the body utilizes 3 basic mechanisms i.e. chemical buffering, respiratory adjustment of blood carbonic acid and excretion of H^+ or HCO_3^- by kidneys (Houpt, 1989). Under heat stress, four conditions may result; metabolic acidosis and metabolic alkalosis involve bicarbonates and respiratory acidosis and alkalosis are related to partial pressure of CO_2 (pCO_2) (Dale and Brody, 1954).

pH was increased significantly ($p<0.05$) in the heat stress group in the present experiment. Similar findings were also reported during heat stress by Sanchez et al. (1994) in cows and by Srikandakumar et al. (2003) in sheep. This increase in pH may be due to reduced carbonic acid levels created by CO_2 expiration (Benjamin, 1981).

Significant ($p<0.05$) decrease in partial pressure of CO_2 was noticed in the present experiment in the heat stress group. This decrease in pCO_2 is in agreement with the findings of Srikandakumar et al. (2003) in sheep and Elmer and Reimhold (2002) in calves. This reduction in pCO_2 might be due to: i) reduced CO_2-combining capacity with haemoglobin and ii) excessive elimination of CO_2 by hyperventillation. The pO_2 in the present experiment increased significantly ($p<0.05$) in the heat stress group. Singh et al. (1991) and Srikandakumar et al. (2003) also reported similar results in heat stressed animals. This increase in pO_2 during a heat stressed condition might be due to increased alveolar ventilation (Assali, 1968).

Significant ($p<0.05$) decrease in HCO_3^- levels was observed in the heat stress group and this is in agreement with the report of Singh (1991) in cattle. The bicarbonate buffering mechanism maintains HCO_3^- and pCO_2 relatively constant at a ratio of 20:1 under normal conditions. During heat stress, thermally induced hyperventilation causes a decrease in pCO_2 to maintain the ratio of 20:1 and also to counter alkalosis, HCO_3^- is secreted by the kidney resulting in decreased blood HCO_3^- (Masero and Siegel, 1977), and this might be the reason for the decreased HCO_3^- levels in the present study.

Total CO_2 decreased significantly ($p<0.05$) in the heat

stress group. This decrease might be due to decreased CO_2^- combining capacity of blood and also decreased pCO_2 (Dale and Brody, 1954).

The base excess in blood (BEb) and base excess in extra-cellular fluid (BEecf) decreased significantly ($p<0.05$) in the heat stressed group in the present study. Similar findings were also reported by Singh (1991) and Srikandakumar et al. (2003). This reduction in BEb and BEecf might be due to either increased respiratory rate/panting under heat stress, which eliminates CO_2 in excess and causes decreased pCO_2 as well as carbonic acid deficiency, or a decrease in the non-bicarbonate buffer system, particularly hemoglobin which was observed in heat-stressed goats, and this results in decreased levels of buffer in blood (West et al., 1991).

In the present study, both PCV and hemoglobin were decreased in the heat stress group and this is in agreement with the findings of Srikandakumar et al. (2003) in sheep and Abdel Samee et al. (1992) in goats. This reduction of hemoglobin and PCV levels could be due to either increased attack of free radicals on the RBC membrane, which is rich in lipid content, and ultimate lysis of RBC or inadequate nutrient availability for hemoglobin synthesis as the animal consumes less feed (Table 1) or decreases voluntary intake under heat stress (Srikandakumar et al., 2003).

In the treatment groups, the values of the above parameters of acid-base balance were restored towards normal and supplementation of vitamin C and E with selenium was effective in bringing acid-base balance towards normal. Shenglins et al. (2003) also observed a similar improvement in acid-base status in heat-stressed pigs. This improvement in acid-base status by vitamin E with selenium and vitamin C might be due to decreased rectal temperature and respiratory rates in the present study. This decreased respiratory rate might have caused reduction in alveolar ventilation which in turn increased levels of pCO_2, TCO_2, bicarbonates, BEb, BEecf and decreased pO_2 and pH. Increased levels of hemoglobin in the present study may also be a reason for improvement in pCO_2, pO_2 and BEb as it is required for oxygen and CO_2 transportation and itself is a good buffer. The increased levels of hemoglobin and PCV in treatment groups in the present study is in agreement with the report of Yousef et al. (2003). This increase in hemoglobin and PCV levels might be due to either reduction in osmotic fragility/haemolysis and preservation of RBC integrity by vitamin E and C supplementation (Leonart et al., 1989; Kraus et al., 1997), as total antioxidant levels increased in treatment groups (Table 2), or availability of adequate nutrients for synthesis of hemoglobin as feed intake was improved in the present study by vitamin E and C (Table 1).

The prolactin levels in the heat stress group were increased and also differed significantly ($p<0.05$) from either control or treatment groups. Similar increase in prolactin levels during heat stress was also reported by Abdel Samee et al. (1992) in goats. Change in ambient temperature influences serum prolactin concentrations by influencing the hypothalamus via release of prolactin and inhibitory peptides which in turn control prolactin secretion (Smith et al., 1977). Hence increased temperature in the present study might have resulted in increased plasma prolactin levels. Prolactin levels were decreased in treatment groups. This reduction might be due to prolactin levels being influenced by reduction of rectal temperature in treatment groups which caused lowered serum prolactin levels.

Cortisol plays an important role in all types of stress. The stressors induce release of cortisol by activation of the hypothalamic-pituitary-adrenal axis (Minton, 1994). The increased cortisol level in the heat stress group in the present study suggested that the temperature to which animals were exposed in the climatic chamber was stressful. Kaushish et al. (1997) also reported an increased cortisol level during heat stress in Black Bengal and Beetel goats.

Supplementation of vitamin C and vitamin E with selenium decreased plasma cortisol levels, indicating that supplementation may have a negative effect on cortisol levels during heat stress, and showed that supplementation of antioxidants had relieved the severity of heat stress in goats. Similar reduction in cortisol levels by vitamin C in heat stressed goats was reported by Kobisey (1997), by vitamin E by Webel et al. (1998), and in heat-stressed pigs by both vitamin E and C by Shenglins et al. (2003). This reduction in cortisol levels by vitamin E and C is not yet fully understood but may be achieved by reducing the synthesis and/or secretion of cortisol or by breaking it down (Golub and Gershwin, 1985; Orth, 1992; Webel et al., 1998).

Decreased thyroid hormone levels during heat stress are an adaptive response and also might be an attempt to reduce metabolic rate and heat production (West et al., 1999).

Free radical H_2O_2 serves as a substrate for the thyro-peroxidase enzyme which catalyzes the synthesis of thyroid hormone, namely T3 and T4. Production of more H_2O_2 under stress condition might have reduced the levels of thyroid hormone (Usha et al., 2002). Moreover, 5' mono-deiodinase, an enzyme which converts T4 to T3, is affected by free radicals under heat stress (Brzezinska et al., 2001). Increased levels of thyroid hormones by vitamin E and C in the present study might be due to protection of the above-mentioned enzymes from free radicals.

The results from the present study suggest that vitamin E with selenium and vitamin C have an ameliorative effect on physiological parameters, endocrine responses and acid-base status during heat stress. Hence, vitamin C and vitamin E with selenium can be used as anti-heat stressors.

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