BIOCHEMICAL PROFILE AND METHANE EMISSION DURING CONTROLLED THERMAL STRESS IN BUFFALOES (*BUBALUS BUBALIS*)

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ABSTRACT

Thermal stress resulted in various physio-biochemical alterations in the exposed buffaloes. The rectal temperature and respiration rates increased concurrently with the rising exposure temperature. There was a significant decrease in the electrolyte, triglyceride, thyroid hormone and aldosterone concentration. On the contrary the reactive oxygen radicals and cortisol hormone increased during thermal stress. The study also revealed high methane production at optimum temperatures as compared during heat stress.

Keywords: physio-biochemical, hormone, methane, reactive oxygen radicals, thermal stress

INTRODUCTION

Homeostasis can be altered by environmental fluctuations like very low or high temperatures and the tropical and sub-tropical regions characterized by high summer temperatures can have deleterious impact on animal health and production. These tropical conditions coupled with the global warming scenario can be devastating to the livestock industry. Several workers have very well documented the negative impact of high temperatures on the livestock (West *et al*., 2003; Marai and Haeeb, 2010; Baumgard, 2011). Animals suffering from heat stress show compromised heat dissipation mechanisms (conduction, convection, vaporation, radiation) leading to excess heat accumulation and disturbances in metabolism. Although, the buffalo is well adapted to the tropical climate but its heat tolerance capacity is poor when compared with cattle making them more vulnerable to thermal stress. Most of the studies have mainly focused on physiological or biochemical aspect during hyperthermia. With this perspective the study was planned to observe the buffaloes' integrated physiological, metabolic, endocrinal acclimation and methane emission patterns so that proper ameliorative measures could be taken during heat stress.

MATERIALS AND METHODS

Four adult dry indigenous Murrah buffaloes (< 3 y, live weight -492.14±9.58 kg) were housed in environmental chamber at Physiology and Climatology Division, Indian Veterinary Research

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Institute. After exposing the animals at 25°C (optimum conditions) they were moved to 30°C (mild stress), 35°C (acute stress) and 40°C (severe stress) respectively, 21 days at each treatment. Daily exposure period was fixed from 1000 to 1500 h. Basal diet of wheat straw *ad libitum* along with required amount of concentrate mixture was offered during entire trial.

The rectal temperature (RT) and respiration rates (RR) were measured daily immediately before and after exposure by standard procedures. Blood collection was done at 5 days interval (before/after exposure) by jugular veni-puncture in sterile glass test tubes. In all serum samples sodium, potassium, chloride, calcium, aspartate aminptransferase (AST), alanine aminptransferase (ALT), triglyceride concentrations were measured on double beam UV-VIS spectrophotometer. Serum superoxide dismutase (SOD) was measured using the method as described by Madesh and Balasubramanian (1998) and reactive oxygen radicals (ROS) were estimated by procedure suggested by Brambilla *et al.* (2001). Thyroxine (T_4), triiodothyronine (T_3), cortisol and aldosterone were determined by radioimmunoassay technique on SR-3000 Stratec Counter (Stratec biomed systems, Germany).Methane was collected by indirect open circuit calorimetry method as standardized by Mc lean and Tobbin (1987) and Derno *et al.* (2009) during last 2 days at each treatment (day 20 to 21). Gas collection lasted for total 12 h and analysis was done on infrared gas analyzer (2RJF4C25, Fuji Electronic Systems, Japan). Methane concentration in the expired air was calculated by using following formula,

$$CH_4 \text{ (L/Day)} = VSTP \times X/1000000,$$

$$VSTP = V \times \frac{273}{273+T} \times \frac{P-Vp}{760}$$

Where,

V=Total volume of air, T=Dry bulb temperature, P=Borometric pressure in mm Hg,

Vp=Partial pressure of water vapor, X=Methane % in ppm (CH_4 out-CH_4 in)

All the data were analyzed by using SPSS 16.0 software package.

RESULTS AND DISCUSSION

Rectal temperature increased significantly at all the treatments except at 35°C (Figure 1). At 25°C it might be due to thermogenic activity of the animals but the subsequent rise in RT reflected heat load on the animals. Respiration rates also paralleled with the RT, except at 25°C and 35°C (Figure 2). This significant increment in RT and RR with thermal stress clearly indicate heat load on the animals and compromised heat loss mechanisms, culminating into panting as only means to lose excess heat quickly. Present results of increased RT and RR are in accordance with those reported by Banerjee and Ashutosh (2011a, b) and Pereira *et al.* (2008) in heat stressed cattle. Methane production was highest at optimum conditions and at 30°C, while it decreased at acute and chronic thermal stress (Figure 3). Total methane produced depends on many factors like dry matter intake, diet digestibility, digesta passage rates, fibre proportion in feed, ruminal pH and temperature and fermentation patterns. Christopherson and Kennedy (1983) attributed low thyroid hormone levels for decreased motility and longer retention of digesta in the gut. Low thyroid activity was also confirmed during thermal stress, possibly increasing digestibility and reducing methane production in present trial. Similarly, Dmytruk *et al.* (1995) and Mc Ginn *et al.* (2008) observed low methane emission during heat stress which supports

our findings.The essential electrolytes sodium and potassium decreased significantly during heat stress conditions which might be due to overall negative mineral balance and increased excretion through urine and skin (Table 1). Similarly, Aboul-Naga (1983) and Banerjee and Ashutosh (2011b) also reported depletion in sodium and potassium concentrations in cattle exposed to high environmental temperatures which support present findings. On the contrary no variation was recorded for serum calcium and chloride indicating optimum metabolism for these minerals even during stressful conditions (Table 1). No variation was seen for free radical during acute thermal stress but a significant rise was observed at chronic conditions (Table 1). This increment in the ROS reflected faster production than that could be possibly neutralized by different antioxidant systems, thus increasing oxidative stress and cellular injury (Zhao *et al*., 2006). Bernabucci *et al.* (2002) also reported increased ROS levels in heat stressed dairy cows (TBARS; 8.8±0.4 vs 7.6±0.4 nmoL/mL). The superoxide dismutase did not varied significantly at any of the treatment (Table 1), possibly due to synergistic action of other antioxidant systems (catalase, glutathione peroxidase, vitamins A, E and C, ubiquinone and flavonoids) in neutralizing free radicals. Similarly, Calamari and co workers found no significant variation for SOD in heat exposed dairy cattle which corroborate present observations (Calamari *et al*., 2011).

Serum triglyceride decreased significantly during acute and chronic heat stress but no variation was seen at optimum or mild stress conditions (Table 1). Cortisol is a known lipolytic agent; mobilizing fatty acids (FA) and triglycerides thus mediating the essential stress adaptations for providing extra energy (Cunningham and Klein, 2007). High levels of cortisol were also noted in present trial during heat stress which possibly increased lipolysis and FA catabolism, resulting into low triglyceride levels. Abeni *et al.* (2007) also

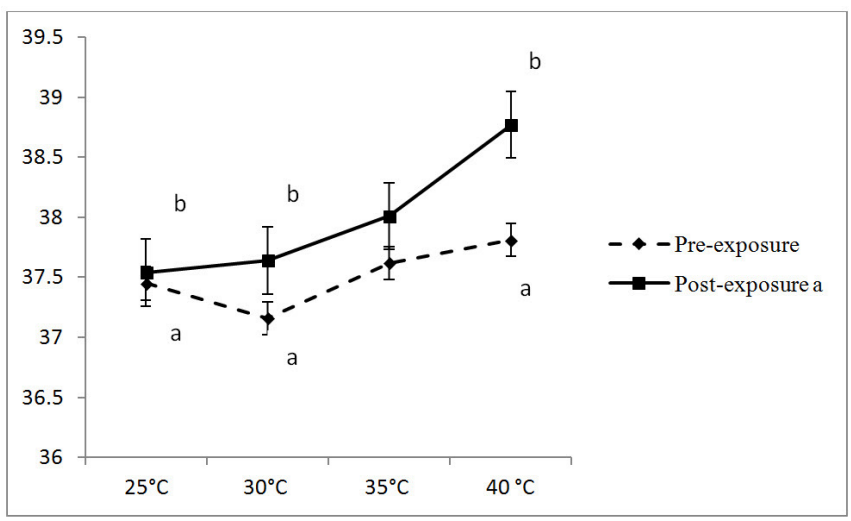

Figure 1. Pooled means±SEM for rectal temperatures (°C) of animals exposed at 25°C, 30°C, 35°C, 40°C.

*Superscript a, b, differ significantly at P<0.01.

[a]The post exposure means are cited from Wankar *et al*. (2014).

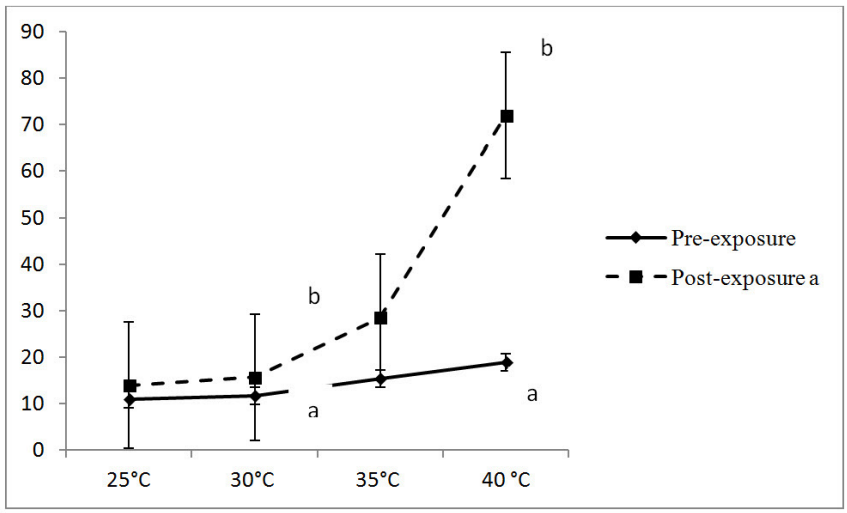

Figure 2. Pooled means±SEM for respiration rates (Breaths/Min) of animals exposed at 25°C, 30°C, 35°C, 40°C.

*Superscript a, b, differ significantly at P<0.01.

[a]The post exposure means are cited from Wankar *et al.* (2014)

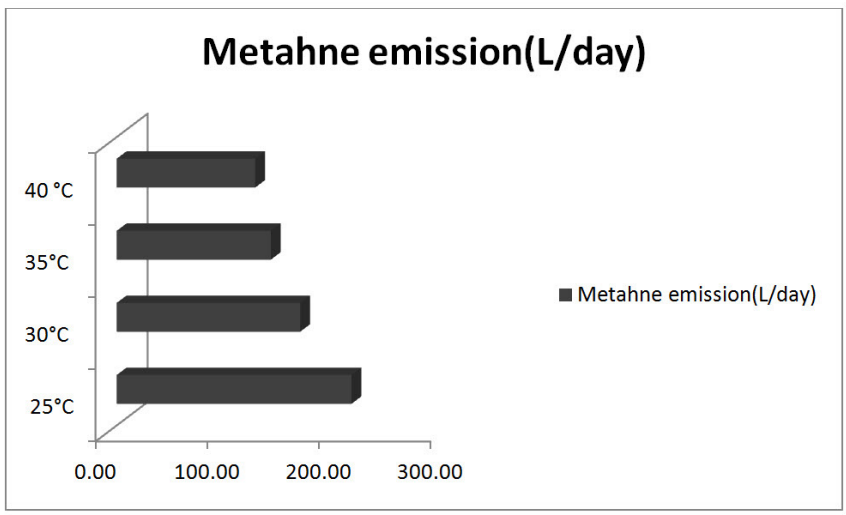

Figure 3. Average methane emission for buffaloes at different treatments.

Table 1. Pooled means±SEM of various biochemical and endocrinal variants at different exposure temperatures. SEM=Standard error of means, ROS=Reactive oxygen radicals, SOD=Superoxide dismutase, AST=Aspartate aminotransferase, ALT=Alanine aminotransferase, T_3=Tri-iodothyronine, T_4=Thyroxine.

Parameter	Period	25°C	30°C	35°C	40°C	SEM
Sodium (mmoL/L)	Pre-exposure	137.28	148.05[a]	143.42[a]	128.69	1.248
	Post-exposure	131.17	120.78[b]	127.94[b]	139.50	
Potassium (mmoL/L)	Pre-exposure	4.60	5.02	5.45	5.66[a]	0.072
	Post-exposure	4.97	4.63	5.08	4.81[b]	
Chloride (mmoL/L)	Pre-exposure	86.72	99.67	91.49	87.03	1.691
	Post-exposure	99.81	101.65	102.84	98.52	
Calcium (mmo/L)	Pre-exposure	2.27	2.24	2.21	2.26	0.039
	Post-exposure	2.42	2.53	2.42	2.34	
ROS (mg H_2O_2/dL)	Pre-exposure	0.05	0.04	0.05	0.05[b]	0.001
	Post-exposure	0.04	0.04	0.05	0.06[a]	
SOD (U/mL)	Pre-exposure	229.34	218.31	154.59	207.96	6.024
	Post-exposure	226.67	211.98	209.52	192.77	
	Post-exposure	24.09	27.42	31.59	32.53	
Triglyceride (mmoL/L)	Pre-exposure	0.93	0.94	0.93[a]	0.85[a]	0.017
	Post-exposure	0.79	0.78	0.69[b]	0.64[b]	
AST (IU/L)	Pre-exposure	162.1[a]	169.31[a]	195.24[a]	166.22	2.892
	Post-exposure	131.57[b]	137.21[b]	158.47[b]	139.46	
ALT (IU/L)	Pre-exposure	72.20	99.34	120.91	105.91	3.769
	Post-exposure	61.52	74.44	105.14	105.21	
T_3 (nmoL/L)	Pre-exposure	1.56	1.50[a]	1.70[a]	1.62[a]	0.026
	Post-exposure	1.50	1.23[b]	1.18[b]	1.12[b]	
T_4 (nmoL/L)	Pre-exposure	38.18	34.64	35.40	30.90	0.694
	Post-exposure	43.80	36.16	38.18	32.20	
Cortisol (nmoL/L)	Pre-exposure	1.32	1.13	2.50[b]	1.73	0.769
	Post-exposure	0.71	1.96	4.56a	3.51	
Aldosterone (nmoL/L)	Pre-exposure	0.02[a]	0.01	0.02[a]	0.02[a]	0.000
	Post-exposure	0.01[b]	0.01	0.01[b]	0.01[b]	

*Superscript a, b, within a column differ significantly at P<0.01 for a respective parameter.

*The post exposure means except that for triglyceride are adopted from Wankar *et al*. (2014).

reported decrease in triglycerides levels during hyperthermia in Friesian cows, due to higher energy demand which confirm present findings. Low Aspartate aminotransferase (AST) activity at optimum condition is quiet surprising but could be due to thermogenic activity, further decrease was probably due increased gluconeogenesis and muscle catabolism during thermal stress (Table 1). Similarly, a significant decrease ($P<0.01$) was also noted in Merino (84.67 to 30.67 IU/l) and Omani (145.00 to 85.83 IU/l) sheep during heat stress, respectively (Srikandakumar *et al*., 2003). However, no variation was seen for alanine aminotransferase (ALT) which might be due to absence of any hepatocellular injury or optimum hepatic metabolism. Our results are in accordance with Yokus and Cakir (2006) observing similar non significant variation for ALT in sheep during summer (119.3±291.97 U/l) than winter (29.9±36.05 U/l) months.

Tri-iodothyronine (T_3) declined with the heat increment in the present trial (Table 1), probably to reduce the metabolic heat generation as is concerned with thermogenesis in domestic animals (West *et al*., 2003). Other reason for this decline could be low thyroid gland activity or low levels of thyroid stimulating hormone or higher glucocorticoid activity during heat stress. Similar, decrease in T_3 was also confirmed by many researchers in heat stressed animals which validate present findings (Pereira *et al*., 2008 and Saber *et al*., 2009). However, thyroxine (T_4) did not vary statistically at any treatment which is in accordance to observations made in sheep during hot and cold seasons (Yokus *et al*., 2006). Cortisol levels were significantly higher at acute thermal stress possibly due to the activation of hypothalamo-pituitary-adrenal axis, facilitating physio-metabolic adaptation (Table 1). Present observations of increased glucocorticoid agree with previous reports in heat stressed ruminants (Meghad *et al*., 2008 and Sejian *et al*., 2010). No notable difference for cortisol at 40°C reflected gradual adaptation and confirm pertinent role of cortisol in both short and long term stress adaptation. Aldosterone decreased after exposure at all the treatments except at 30°C (Table 1). Similarly, Beatty *et al.* (2006) also reported low plasma aldosterone in *Bos taurus* and *Bos indicus* cattle during heat stress. This decrease in aldosterone during acute and chronic heat stress was probably to reduce the potassium losses and to maintain extracellular fluid (ECF) volume. However, the decrease in aldosterone at 25°C is quiet surprising and there are no previous reports in ruminants for confirmation.

Physiological and behavioral responses are activated earliest followed by endocrinal changes, working in unison thus enabling the necessary acclimatization during heat stress. We observed a positive correlation between heat stress and reduction in methane emission which indicate major changes in fermentation and energy metabloism. It can be suggested from the study that heat stress is deleterious for the well being of animals and proper ameliorative measures should be taken to reduce the effect.

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