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Adaptive Capability as Indicated by Redox Status and Endocrine Responses in Crossbred Cattle Exposed to Thermal Stress

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ABSTRACT

A series of studies were conducted to assess the effect of increasing environmental temperatures on redox status and endocrine responses in crossbred cattle under simulated conditions. In present study four non-lactating crossbred cattle were exposed to 25, 30, 35 and 40°C temperature with relative humidity of 40-50% in psychrometric chamber for 5 hours/day from 10:00 to 15:00 hours for 21 days. The physiological responses, feed and water intake were recorded and blood samples were collected at 15:00 hours on day 1, 6, 11, 16 and 21 of the experiment. Blood serum was harvested to estimate superoxide dismutase activity (SOD), reactive oxygen species (ROS), tri-iodothyronine (T₃), thyroxine (T₄) and cortisol concentration. The temperature exposures of 25 and 30°C did not affect physiological responses, feed intake, water intake, T₃, T₄, cortisol concentration and SOD activity in serum. The dry matter intake (DMI)/day decreased significantly (p<0.05) whereas water intake, respiratory rate and rectal temperature increased progressively at 35 and 40°C as compared to 25 and 30°C temperature exposure. The serum ROS level increased progressively with increase in exposure temperature and a corresponding increase in serum SOD activity was also observed up to a temperature exposure of 35°C however, SOD activity declined at 40°C exposure. Serum T₄ concentration decreased whereas cortisol level increased significantly at 35 and 40°C exposure as compared to 25 and 30°C temperature exposure. It can be concluded from the present study that the crossbred animals can readily adapt to a temperature exposure of 25 and 30°C and can acclimatize up to 35°C with compensatory physiological, biochemical and endocrine changes but fail to do so at the temperature exposure of 40°C.

Keywords: heat stress; oxidative stress; endocrine responses; crossbred cattle

The crossbreeding program has been extensively adopted in Indian subcontinent to accelerate the milk productivity however; the crossbred animals are reported to be susceptible to diseases and production losses as compared to their indigenous counterparts especially under increased environmental temperature (Banerjee and Ashutosh, 2011a; Banerjee and Ashutosh, 2011b; Deb *et al.*, 2014).

Thermal exposure to temperate breeds of cattle more than 30°C resulted in increased rectal temperature and respiratory rate in lactating cows (Rhoads *et al.*, 2009; Chaiyabutr *et al.*, 2008; Wheelock *et al.*, 2010) and heifers (Nonaka *et al.*, 2008). The crossbred animals

showed lower rectal temperatures and respiratory rate than the pure exotic breeds (Johnston *et al.*, 1963), but had higher rectal temperatures, respiratory rate and pulse rate than the *Bos indicus* breeds both at thermoneutral and higher temperatures (Banerjee and Ashutosh, 2011b). Heat stress has shown to induce the production of reactive oxygen species, resulting in some of the deleterious effects (Loven, 1988; Tanaka *et al.*, 2008) such as reduced metabolic activity of several tissues, resulting in many disease condition and consequent decrease in the performance of domestic animals (Tanaka *et al.*, 2007). Higher temperature humidity index (THI) was found to have a positive correlation with superoxide dismutase

activity (Banerjee and Ashutosh, 2011a; Bernabucci *et al.*, 2002; Kumar *et al.*, 2007; Kumar *et al.*, 2011) in different livestock animals. The thyroid gland is highly sensitive to the environment heat variation (Rasooli *et al.*, 2004). The levels of T₃ and T₄ have been reported to decline in cows under high ambient temperatures (Sejian *et al.*, 2010) and serum cortisol levels have been used extensively as a marker of heat stress.

In the scenario of climate change and considering the economical perspective of crossbred cattle in Indian subcontinent, it is pertinent to study the biological responses and adaptability of crossbred animals to different temperature exposures simulating global warming conditions which will be helpful in formulation of heat stress amelioration strategies. Therefore, the present study was undertaken to assess the effect of increasing environmental temperatures on redox status and endocrine responses, and adaptability in crossbred cattle.

MATERIALS AND METHODS

Animal, Feeding and Management

The present study was conducted at the climatology and nuclear research laboratory, Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar, India. Experiment was conducted on four dry crossbred cattle (exotic [50 to 75%]: Brown Swiss, Holstein and Jersey and indigenous [25 to 50%] Haryana) (Age, 3.78±0.22 years and weight, 386.75±14.75Kg). The animals were selected randomly from the herd of the Dairy Farm of Indian Veterinary Research Institute, Izatnagar, India. The animals were housed in well-ventilated pucca shed under uniform managerial condition having facilities for individual feeding and watering. The animals were offered basal diet of wheat straw *ad libitum* along with required amount of concentrate mixture to meet the maintenance requirement (NRC, 2001). The concentrate mixture consisted of 60% ground maize, 17% soyabean meal, 20% wheat bran, 2% mineral mixture and 1% common salt and 20 g Vitablend AD3 per 100 Kg concentrate mixture was added which contained Vitamin – A 50,000 IU, and Vitamin D3 – 5,000 IU/g. The chemical composition of the wheat straw and concentrate mixture is given in table 2. All experimental procedures were reviewed and approved by the animal ethics committee

at Indian Veterinary Research Institute, Izatnagar, India. The details of meteorological data are given in table 1. The temperature-humidity index (THI) was calculated according to formula given by Ravagnolo *et al.* (2000).

Table 1. Meteorological variables of psychrometric chamber and shed during the experiment

Chamber		Shed		
Exp. Temp. (°C)	THI	Average Temp. (°C)	Average Relative Humidity (%)	THI
25	71.25	14.57	67.79	56.90
30	77.53	20.08	70.27	62.83
35	83.81	25.07	68.82	68.92
40	90.09	29.60	70.35	74.57

Exp. Temp. (Exposure Temperature), THI (Temperature Humidity Index)

Psychrometric Chamber Detail

The size of the psychrometric chamber is 8, 8 and 3.5 meter of length, width and height respectively. All sides of the climatic chamber are made up of stainless steel. The chamber has the programmable temperature and humidity regulator to maintain the ideal temperature and humidity as per the requirement of the experiment. The psychrometric chamber has the provision to hold eight animals at a time. The climatic chamber has a trevice to restrain each animal for blood collection. The chamber has individual tie stall, feeders and waterers for each animal. The animals were acclimated for a period of 15 days to the climatic chamber and restraining inside the chamber.

Experimental design

In the four phases of the study, the animals were exposed to 25, 30, 35 and 40°C temperature respectively, with a relative humidity of 40-50% in the climatic chamber for 5 hours/day from 10:00 to 15:00 hour for 21 days. The time duration between two phases was at least 10 days in order to adapt the animal to prevailing environmental conditions. The animals were kept in the shed attached to the psychrometric chamber before and after the thermal exposure. Feed and water intake, and physiological

responses were recorded at 15:00 hours on day 1, 6, 11, 16 and 21. The blood samples were collected at 15:00 hours in the climatic chamber and serum was harvested for biochemical and hormone analysis.

Table 2: Chemical composition of the (% DM basis) feeds offered to experimental animals.

Attributes	Concentrate Mixture	Wheat Straw
Organic Matter	90.01	91.68
Crude Protein	21.97	3.27
Ether Extract	2.23	1.09
Neutral Detergent Fiber	35.54	85.81
Acid Detergent Fiber	17.03	17.03
Hemicellulose	16.51	21.94
Total Carbohydrates	55.77	87.33
Calcium	1.11	0.78
Phosphorus	1.06	0.23

Physiological observations

The rectal temperature (RT) was recorded by a clinical thermometer and presented in °C. The respiration rate (RR) was recorded based on the flank movements at the paralumbar fossa of the animals using a stop watch and was expressed by number of breaths per minute. The pulse rate (PR) of the animals was recorded based on the pulsation noted in the middle coccygeal artery at the base of the tail and expressed as beats per minute.

Blood sampling

Six millilitre of blood was collected at five days intervals during all the temperature exposures at 15:00 hours using 18 gauge sterilized needles and plastic syringe from external jugular vein in tubes with Ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Serum was harvested from blood by centrifugation at 1870 g at room temperature for 15 minutes. The serum was then divided into two aliquots in microcentrifuge tubes and kept frozen at -20°C till further analysis.

Biochemical estimations in serum

Serum SOD activity was measured using the method as described by Madesh and Balasubramanian (1998). In

the microtitre plate method, 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 pyrogallol solution to be added at the end. Sample was replaced with PBS in the blank. After an incubation period of 15 minutes, 150 µl DMSO was added and absorbance was measured in ELISA reader at 570 nm. The percent inhibition by the presence of SOD was calculated from the reduction of the MTT colour formation as compared to the MTT formazan formed in the absence of SOD which was measured as 100%. The reactive oxygen species (ROS) level was estimated in terms of hydroxyl radical in serum samples as suggested by Brambilla *et al.* (2001).

Hormone Assay

Serum cortisol, tri-iodothyronine (T₃) and thyroxin (T₄) concentrations were estimated by ¹²⁵I-RIA kit supplied by Immunotech, Czech Republic using SR 300 Stratec gamma counter (Stratec Biomed systems AG, Brikenfeld, Germany). The coefficient of variation for intra and inter assays and sensitivity for cortisol, T₃ and T₄ was 5.8 %, 9.2% and 1.02 nM/l; 6.3%, 7.7% and 0.10 nM/l; 6.2%, 8.6% and 9.56 nM/l respectively.

Statistical Analysis

Data were analyzed by GLM (SPSS 16.0, Chicago, IL, USA). The linear model was used for all the respondent variables using least squares analysis of variance. Effect of fixed factors, namely exposure temperatures (25, 30, 35 and 40°C) and days over which experiment was carried out i.e. day 1, 6, 11, 16 and day 21 and also interaction of exposure temperature and days was analyzed on the different parameters studied. Differences among treatments were determined using Tukey's test (SPSS 16.0, Chicago, IL, USA) and indicated by the superscripts (P<0.05).

RESULTS AND DISCUSSION

The effect of temperature exposure at 25, 30, 35 and 40°C on rectal temperature (RT), respiratory rate (RR) and pulse rate (PR) are presented in table 3. After thermal exposure, the RT, RR and PR were significantly (P<0.05) higher at 35 and 40°C as compared to 25 and 30°C, and

also RT and RR was significantly ($P < 0.05$) higher at 40°C thermal exposure as compared to 35°C whereas PR did not change significantly ($P \geq 0.05$). In addition, the interaction between temperature exposure and experimental days influenced the PR ($P < 0.05$). At 35 and 40°C temperature exposure resulted in heat stress because of an increment in total heat load (internal production and environment) which exceeded the heat dissipation capacity. In response to heat stress physiological, biochemical and behavioral changes occurred in cattle to reduce the stress. During heat stress cattle increased the avenues of heat loss and reduced heat production in an attempt to maintain eutheria. The immediate responses to heat load are increased respiration rates, decreased feed intake and increased water intake (Bernabucci *et al.*, 2010). In present study, respiratory rate of the crossbred animals increased steeply at 35 and 40°C temperature exposure in order to expedite the evaporatory heat loss through respiratory system to decrease heat load of the body (Hansen, 2004; Brown-Brandl *et al.*, 2005; Beatty *et al.*, 2006). The increase in heat load of the body also culminated in an increment in rectal temperature at 35 and 40°C temperature exposure (Banerjee and Ashutosh, 2011b and Vaidya *et al.*, 2011). The findings also confirmed that heat load produced by temperature exposure of 25 and

30°C was dissipated by normal physiological heat loss mechanisms without any alteration in respiratory rate and rectal temperature. In present study, the increase in PR at 35 and 40°C temperature exposure may be attributed to increased catecholamine secretions at higher environmental temperature (Janzekovic *et al.*, 2006). However, unlike steep rise in RR and RT at 35 and 40°C temperature exposure, PR increased after exposure at 35°C but remained same at 40°C exposure. It may be either due to diminished catecholamine secretion or down regulation of their receptors during high chronic temperature exposure. Wankar *et al.* (2014) found that the pulse rate decreased in buffalo subjected to chronic, moderate thermal stress.

The DMI/day reduced significantly ($P < 0.05$) at 40°C thermal exposure than at 25, 30 and 35°C thermal exposure while DMI/day at 35°C was significantly ($P < 0.05$) lower than DMI/day at 30°C (table 3). Dry matter intake is one of the most important factors which affect the productivity of the animals. In present study, the DMI/day decreased progressively at 35 and 40°C temperature exposure which may be attributed to reduced the gut motility, rumination, ruminal contractions (Attebery and Johnson, 1969) and depressed appetite (Warren *et al.*, 1974) by having a direct negative effect on appetite centre of the hypothalamus (Baile and Forbes, 1974) due to increased temperature. It is important to note that feed intake in temperate breeds of cattle began to decline at ambient temperatures of 25–26°C and reduced more rapidly above 30°C and dietary intake might decline by as much as 40% at 40°C (NRC, 1989) however, in our study, we noted that feed intake in crossbred animals started declining only after temperature exposure at 35°C which was compensated by increase in digestibility (Yadav *et al.*, 2013). The water intake/day was significantly ($P < 0.05$) higher at 35 and 40°C temperature exposure than at 25 and 30°C. Further, interaction between the temperature exposure and experimental days also influenced ($P < 0.05$) the water intake (table 3). Higher water intake/day at 35 and 40°C of temperature exposure in present study was mainly due to evaporatory heat loss (respiration, sweating or panting), resulting in increased osmolarity of the extracellular fluid in the body, ultimately leading to activation of thirst center in the hypothalamus and increase in water intake (Pereira *et al.*, 2008 and Banerjee and Ashutosh, 2011a).

The effect of temperature exposure at 25, 30, 35 and 40°C on SOD activity and ROS concentration in serum

Table 3: Effect of different temperature exposures on physiological parameters (Mean and SE)

Exp. Temp. (°C)	25	30	35	40	SE	P Value		
						Temp.	Day	Temp.*Day
RT	38.12 c	38.17c	38.41 b	39.17 a	0.07	<0001	0.31	0.27
RR	21.70 c	24.10 c	35.90 b	71.20 a	2.07	<0001	0.11	0.47
PR	53.30b	52.55 b	60.65 a	60.60 a	1.17	<0001	0.42	0.04
DMI (Kg)/Day	6.18a	6.37 a	5.85 a	4.99 b	0.22	<0003	0.83	0.49
Water Intake (L)	17.79bc	14.85c	21.85 ab	23.83a	1.12	<0001	0.99	0.04

Means bearing different superscripts in a row differ significantly ($P < 0.05$).

The standard error uses pooled estimate of error variance. RT (Rectal Temperature), PR (Pulse Rate)

are presented in table 4. Serum SOD activity increased significantly ($P<0.05$) at 35°C temperature exposure as compared to 25 and 30°C, and further serum SOD activity decreased significantly ($P<0.05$) to lowest level at 40°C temperature exposure as compared to 25, 30 and 35°C exposure. The serum ROS level increased significantly ($P<0.05$) at each higher temperature exposure as compared to its corresponding lower temperature exposure. In addition, the interaction between exposure temperature and experimental days also influenced ($P<0.05$) SOD activity and ROS concentration in serum. Cells continuously produce free radicals and ROS as part of metabolic processes. Under normal conditions, the physiologically important intracellular levels of ROS are maintained at low levels by various enzyme systems participating in the *in vivo* redox homeostasis (Rahal *et al.*, 2014). In present study, the serum ROS level increased progressively at 35 and 40°C due to induced production of more free radicals in response to increased heat stress. Our findings are in agreement with the reports of Tanaka *et al.* (2008) who suggested an increase in serum ROS levels during heat stress. Heat stress has been reported to induce the production of oxygen derived free radicals, resulting in the deleterious effects (Loven, 1988). Superoxide dismutase decomposes the superoxide free radical into hydrogen peroxide (H_2O_2). In present study, increase in serum SOD activity was more prominent at 35°C and reached to its peak in attempt to neutralize excess ROS. But at 40°C temperature exposure, SOD activity decreased along with a simultaneous increase in ROS level in serum which indicated that consistent increase in serum ROS. Due to higher heat stress at 40°C resulted in decreased SOD activity. SOD could

neutralize the increased ROS levels in the serum up to certain limit and then began to decline thereafter which resulted in oxidative stress. Our findings are similar to the results reported by Banerjee and Ashutosh (2011a); Bernabucci *et al.* (2002); Kumar *et al.* (2007), Kumar *et al.* (2011) and Yadav and Korde (2011). Further, influence of experimental days on these parameters suggests that the crossbred animals were adapting to heat stress over the period of time as after the initial increase in serum ROS levels and SOD activity, it subsequently decreased to its normal levels.

The serum T_3 , T_4 and cortisol level after temperature exposure at 25, 30, 35 and 40°C are presented in table 4. The serum T_3 did not show significant ($P\geq 0.05$) increase on different temperature exposures whereas T_4 decreased significantly ($P<0.05$) after 35 and 40°C exposure, however the serum cortisol increased significantly ($P<0.05$) at 35 and 40°C temperature exposure as compared to 25 and 30°C. Further, the effect of experimental days and interaction of temperature exposure and experimental days was not observed. The thyroid gland is highly sensitive to the environmental heat variation and also to nutritional status of the animal. In fact, the major exogenous regulator of thyroid gland activity is the environmental temperature (Dickson, 1993). Heat stress in general is associated with significant depression in thyroid gland activity resulting in lowering of thyroid hormones level (Nazifi *et al.* 2003; Rasooli *et al.* 2004; Sejian *et al.*, 2014). When the animals start suffering due to heat, food ingestion is reduced and metabolism slows down, causing a hypo-function of the thyroid gland (McManus *et al.*, 2009) which leads to decrease in T_4 level. In our study, the serum thyroid

Table 4. Effect of different temperature exposures on dry matter and water intake (Mean and SE).

Exp. Temp. (°C)	25	30	35	40	SE	P Value		
						Temp.	Day	Temp.*Day
SOD activity	280.32 ^b	263.26 ^b	341.78 ^a	205.6 ^c	5.02	<.0001	<.0001	<.0001
ROS	4.48 ^d	4.69 ^c	5.39 ^b	5.69 ^a	0.06	<.0001	<.0001	<.0001
T3 (nM/L)	1.64 ^b	1.42 ^b	1.48 ^b	1.76 ^{ab}	0.09	0.61	<.0001	0.02
T4 (nM/L)	69.38 ^a	61.17 ^a	55.63 ^b	56.94 ^b	3.29	<.0001	.238	0.002
Cortisol (nM/L)	15.70 ^d	15.75 ^d	29.23 ^{ab}	32.23 ^a	0.83	<.0001	<.0001	<.0001

Means bearing different superscripts in a row differ significantly ($P<0.05$). The standard error uses pooled estimate of error variance. SOD- Superoxide dismutase (U/ml), ROS- Reactive oxygen species (mg H_2O_2 equivalents/ml), T_3 - Triiodothyronine, T_4 - Thyroxin



hormone levels decreased which lead to reduction in the metabolic heat production, in an attempt to optimize the process of thermoregulation. In present study, the serum cortisol was higher after thermal stress at 35 and 40°C which might be attributed to increased heat load. The glucocorticoids work as vasodilators to help heat loss and have stimulatory effect on proteolysis and lipolysis, hence, providing energy to the animal helps offset the reduction of intake (Cunningham and Klein 2007). The association between heat stress and increased secretion of cortisol, the principal glucocorticoid hormone in ruminants, is well documented (Wise *et al.*, 1988; Ali and Hayder, 2008; Cunningham and Klein, 2007).

The data obtained from the study indicated that crossbred animals have the ability to alter their adaptive capability on exposure to different environmental temperatures. This is evident from the significant alteration in physiological responses, redox status and endocrine response during different temperature exposures. The crossbred cattle tend to maintain redox homeostasis even at 35°C temperature exposure but undergo oxidative stress at 40°C temperature exposure. It can be concluded from present study that the crossbred animals can readily adapt to a thermal exposure of 25 and 30°C and may acclimatize at 35°C with sustainable biochemical and physiological changes but fail to do so at the thermal exposure of 40°C.

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