



## Physiological, hematological and biochemical profile of growing Murrah buffaloes exposed to heat stress in control climatic chamber

S V SINGH\* and CHANDRA BHAN

ICAR-National Dairy Research Institute, Karnal, Haryana 132 001 India

Received: 29 May 2019; Accepted: 31 October 2019

### ABSTRACT

Six growing buffaloes were exposed under natural ambient conditions ( $23\pm 0.54^\circ\text{C}$ ) and in a climatic chamber at  $42\pm 1^\circ\text{C}$  (exposure-I) and  $44\pm 1^\circ\text{C}$  (exposure-II) with a relative humidity of  $40\pm 2\%$  4 h for daily for a period of three days continuously. Blood samples from these animals were collected before exposure and at the end of 4 h of exposure and analyzed for hematological parameters (RBC, WBC, Hb and PCV), enzymatic activity, viz. Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) and Acetylcholinesterase (AChE) and cortisol hormone. The physiological responses, viz. RR, PR, RT and ST were also monitored at the similar interval. RR, PR, RT and ST increased significantly due to exposure at both the exposure conditions, but the magnitude of increase in these parameters were significantly higher at second exposure than first exposure. The hematological parameters decreased significantly with the exposure of animals for four hours at both the climatic chamber conditions. The enzymatic activities in blood plasma were significant higher after 4 h of exposure at both the climatic chamber conditions than natural exposure. The pre-exposure levels of cortisol were lower at natural ( $23\pm 0.54^\circ\text{C}$ ) exposure compared to exposure at both the conditions. After exposure at  $42^\circ\text{C}$  and  $44^\circ\text{C}$  for 4 h, the levels of cortisol increased significantly over pre-exposure values. Exposure to elevated temperatures resulted in significant deviation in physiological, hematological, biochemical and hormone profile of growing buffaloes compared to natural exposure (comfortable temperature) indicating stressful conditions to growing buffaloes. Therefore, growing buffalo needs protection from heat stress conditions for maintaining homeothermy and diverting the available nutrients towards better growth.

**Keywords:** Biochemical, Heat exposure, Hematological, Murrah buffalo, Physiological

India's livestock sector is one of the largest in the world. India has about 108.7 million buffaloes, which represent 56.7% of the world buffalo population (Livestock Census, 2012) and contributing around 50% to the total milk production. Like other mammals, buffaloes are homeotherm and can maintain a constant body temperature within a limited range of ambient temperature. Neurons, which are temperature sensitive, are located throughout the animal's body and send information to the hypothalamus, which invokes numerous, behavioural, physiological and biochemical changes in an attempt to maintain heat balance. When the heat load of an animal is greater than its capacity to lose heat (Wagner, 2001), a portion of the metabolizable energy typically used for production must be diverted to assure thermal balance. The primary factors that cause heat stress in dairy animals are high ambient temperatures and relative humidity (West 2003). In addition, radiant heat from the sun contributes to stress, if animals are not properly shaded. Thermal stress is a unique and complex phenomenon that brings about numerous challenges beyond the animals baseline homeostatic mechanism causing alterations of the normal physiological

\*Corresponding author e-mail: Sohanvir2011@gmail.com

mechanisms thus causing a stressful response. Serum glutamate oxalo-acetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) which are indicators of tissue damage, increased significantly during heat stress in cattle (Bahga *et al.* 2009). High ambient temperature is the major constraint to buffalo productivity due to poor heat tolerance capacity because of low sweat gland density and black coat colour. Under excess thermal loads of heat and work, buffaloes employ moderate levels of sweating and resort to open mouth panting. Earlier studies on buffaloes have been oriented to study the magnitude of changes in some of physiological reactions under natural environmental conditions. However, information on hematological, enzymatic and biochemical parameters during different heat exposures in climatic chamber is scanty on growing buffaloes. Therefore, during the present study, an attempt has been made to describe the changes in physiological, hematological, enzymatic and biochemical parameters due heat exposures. This information is likely to help in suggesting methods for reducing heat stress in buffalo calves by altering shelter system and other managerial practices during expected climate change scenario in future.

## MATERIALS AND METHODS

*Location of study:* Karnal is situated at an altitude of 250 meters above mean sea level and at 29° 42' N latitude and 77° 02' E longitudes. The ambient temperature varied from zero (winter) to >42°C (summer). The average rainfall is about 700 mm mainly during rainy season (July–September).

*Selection and feeding of animals:* Six growing Murrah buffaloes (8–12 months) were selected from the herd of National Dairy Research Institute, Karnal (India). The animals were fed roughages (green and dry fodder) *ad lib.* during different seasons as per Kearn standard (1982). Water was available to the animals round the clock for drinking. The concentrate mixture was fed @ 1–2 kg/animal/day as per the age and body weight for maintenance. Concentrate mixture consisted of mustard cake, maize, wheat bran, rice bran, mineral mixture and salt. The CP of diet was 12% and TDN 60%.

*Experimental protocol:* In order to simulate the conditions expected to be observed in coming years, i.e. increase in extreme events/ ambient temperature due to global warming and climate change, the experimental animals were exposed at two climatic chamber conditions, i.e. 42±1°C (exposure-I) and 44±1°C (exposure-II) with a relative humidity of 40±2% in a controlled climatic chamber (22.6×10.1×8.0 feet) for a period of 4 hrs continuously for three days. These animals were also monitored at natural ambient conditions (23±0.54°C) during spring to compare the deviations in different parameters due to exposure in climatic chamber. Prior to actual experiment the animals were exposed in the climatic chamber for one week for adaptation.

*Recording of physiological parameters:* Respiration rate was recorded by counting inward and outward movement of flank. Pulse rate was recorded by palpation of coccygeal artery and rectal temperature by inserting clinical thermometer into the rectum of animal for at least 2 min. Skin temperature at different body parts were recorded using tele thermometer keeping the instrument two inches away from skin. All these parameters were recorded before and after end of 4 h exposure in climatic chamber at both the exposures and during spring season. The blood samples were also collected from all the animals at the similar interval and analyzed for Hemoglobin (Hb %) and Packed Cell Volume (PCV%) using spectrophotometer and Wintrobe tube method respectively. Red Blood Corpuscles (RBC 10<sup>6</sup>/mm<sup>3</sup>) and White Blood Corpuscles (WBC 10<sup>3</sup>/mm<sup>3</sup>) was counted using hemocytometer. Biochemical parameters, viz. Aspartate aminotransferase (AST-EC 2.6.1.1), Alanine aminotransferase (ALT-EC 2.6.1.2) were carried out using the procedure supplied with Span diagnostic kits. Alkaline phosphatase (ALP-EC 3.1.3.1) by using Kinds and Kings Method. Lactate dehydrogenase (LDH-EC 1.1.1.28) and Acetylcholinesterase (AChE) was carried out by kit supplied by Biovision and Bioassay respectively. Cortisol hormone was estimated in heparinized

plasma samples using EIA kit supplied by Cayman chemical company, USA.

*Statistical analysis:* Statistical analysis of the obtained data was carried out for means, standard errors, analysis of variance and correlation coefficient using Systat software programme.

## RESULTS AND DISCUSSION

*Physiological responses:* Physiological parameters like respiration rate, heart rate, body temperature and skin temperature gives an immediate response to climatic stress and thus to the level of comfort/ discomfort of the animals. So these parameters are important in measuring the comfort/ discomfort level of the animals and thus the production potential of the animals.

The pre-exposure mean respiration rate of growing Murrah buffaloes ranged from 21.33±0.49 to 25.67±0.56/min. After 4 h of exposure the RR increased by 3, 37 and 45 breaths/min over pre exposure values during spring, 1<sup>st</sup> and 2<sup>nd</sup> exposure conditions, respectively (Table 1). The results of the present study are in accordance with those of Chandra Bhan *et al.* (2012, 2013) who observed a linear increase in the respiration rate with the rise in ambient temperature in KF cattle. Similar results of respiration rate were reported by Salam (1980) in buffaloes and crossbred cattle, i.e. higher RR during summer compared to other seasons. Das *et al.* (1999) recommended protection of young buffalo calves from direct solar radiation during extreme summer conditions.

The pre-exposure mean pulse rate ranged from 61.33±0.56 to 64.00±0.49 beats/min. The increase in PR/min was lowest (4 beats) during spring season followed by 1<sup>st</sup> exposure (12 beats) and 2<sup>nd</sup> exposure (13 beats) conditions (Table 1). Lal *et al.* (1987) and Singh and Upadhyay (2009) also reported a significant increase in pulse rate of different breeds of cattle and Murrah buffalo with an increase in ambient temperature and relative humidity. The RR and PR increased linearly with the rise in temperature from 23°C to both climatic chamber exposure conditions (42°C and 44°C) after 4 h of exposure indicating the more stressful conditions at 2<sup>nd</sup> exposure conditions in growing buffaloes. Therefore to fulfill the oxygen demand at tissue levels both RR and PR increased significantly at exposure 2 compared to exposure 1 and natural conditions.

The pre-exposure mean values of rectal temperature of growing buffaloes were 38.50±0.07°C, 37.83±0.07°C and 37.38±0.07°C during natural, 1<sup>st</sup> and 2<sup>nd</sup> exposures respectively (Table 1). The increase in RT was lowest (0.51°C) during spring followed by 1<sup>st</sup> (1.68°C) and 2<sup>nd</sup> (2.63°C) exposure conditions. The results of the present study are in accordance with the findings of Amakiri and Funsho (1979) who observed a diurnal rhythm in core body temperature of domestic animals. Moran (1999) stated that buffaloes are the least heat tolerant (highest rise in rectal temperature) followed by short horn steers and Brahman cross when exposed to sun exposure at 32–37°C.

Table 1. Physiological responses of growing buffaloes during controlled climatic conditions

Parameter	Interval	Temperature		
		23±0.54°C	42±1°C	44±1°C
Respiration rate/min	Before	21.33±0.49 <sup>ax</sup>	25.67±0.56 <sup>ax</sup>	25.67±0.56 <sup>ax</sup>
	After	24.33±0.42 <sup>ay</sup>	63.33±0.92 <sup>ay</sup>	70.67±0.56 <sup>by</sup>
Pulse rate/min	Before	64.00±0.49 <sup>ax</sup>	61.33±0.56 <sup>ax</sup>	61.33±0.56 <sup>ax</sup>
	After	67.50±0.42 <sup>ay</sup>	72.67±0.21 <sup>ay</sup>	74.00±0.37 <sup>by</sup>
Rectal temperature (°C)	Before	38.50±0.07 <sup>a</sup>	37.83±0.07 <sup>ax</sup>	37.38±0.07 <sup>ax</sup>
	After	39.01±0.11 <sup>a</sup>	39.51±0.04 <sup>ay</sup>	40.01±0.04 <sup>by</sup>
Skin temperature (°C)	Before	30.67±0.74 <sup>ax</sup>	36.27±0.20 <sup>ax</sup>	36.27±0.20 <sup>bx</sup>
	After	32.73±0.53 <sup>ay</sup>	38.11±0.13 <sup>ay</sup>	40.19±0.11 <sup>by</sup>
Red Blood Corpuscles (million/mm <sup>3</sup> )	Before	8.29±0.11 <sup>ax</sup>	7.55±0.14 <sup>ax</sup>	7.42±0.12 <sup>bx</sup>
	After	7.86±0.13 <sup>ay</sup>	7.05±0.15 <sup>ay</sup>	6.91±0.17 <sup>by</sup>
White Blood Corpuscles (cells/μl)	Before	13944±547.00 <sup>ax</sup>	16567±241.00 <sup>ax</sup>	15614±566.00 <sup>bx</sup>
	After	11558±523.00 <sup>ay</sup>	14583±253.00 <sup>ay</sup>	13767±700.00 <sup>by</sup>
Hemoglobin (g %)	Before	13.98±0.27 <sup>ax</sup>	15.14±0.78 <sup>ax</sup>	15.14±0.78 <sup>ax</sup>
	After	12.45±0.29 <sup>ay</sup>	13.81±0.72 <sup>ay</sup>	13.50±0.56 <sup>ay</sup>
Packed Cell Volume (%)	Before	35.50±1.09 <sup>ax</sup>	36.33±1.12 <sup>ax</sup>	37.00±0.73 <sup>bx</sup>
	After	34.16±0.60 <sup>ay</sup>	35.00±1.32 <sup>ay</sup>	34.67±0.76 <sup>by</sup>
Mean Corpuscular Volume (fl/cell)	Before	42.78±1.05 <sup>ax</sup>	48.13±1.29 <sup>ax</sup>	49.83±0.81 <sup>bx</sup>
	After	43.56±1.32 <sup>ay</sup>	49.58±1.06 <sup>ay</sup>	50.20±0.65 <sup>by</sup>
Mean Corpuscular Hemoglobin (pg)	Before	16.88±0.44	20.05±0.76 <sup>ax</sup>	20.34±0.79 <sup>bx</sup>
	After	15.88±0.57	19.53±0.61 <sup>ay</sup>	19.61±0.32 <sup>ay</sup>
Mean Corpuscular Hemoglobin Concentration (%)	Before	39.56±1.35	41.90±2.59	40.98±2.15
	After	36.47±0.71	39.37±0.70	39.11±0.98

The values are the mean and SEM of six values on six animals; The values with the different superscript in the same row (a, b, c) and column (x and y) differed significantly (P<0.05). CD value for temperature and treatment = 0.110.

Bernabucci *et al.* (2002) also reported the similar significant (P<0.01) effects of hot season (39.5±0.2°C) on RT in transition dairy cows compared to other season. Significantly (P<0.05) higher rectal temperature was found during afternoon compared to morning in both the groups of crossbred calves maintained inside and outside the shelter (Singh *et al.* 2008). The results of the present study corroborates with Michelle *et al.* (2009) who reported a significant increase in RT of buffaloes at higher temperature (summer) exposure than lower temperature (winter).

The mean pre-exposure skin temperature of growing buffaloes were 30.67±0.74°C, 36.27±0.20°C and 36.27±0.20°C at 23.00±0.54°C (comfortable), 42.00±1.00°C and 44.00±1.00°C (climatic chamber) respectively. The magnitude of increase in skin temperature was linear with the increase in duration of exposure at both the conditions. At 1<sup>st</sup> and 2<sup>nd</sup> exposure conditions, the ST increased by 1.94 and 3.97°C respectively over their pre-exposure values. The increase in ST was slightly higher at natural exposure (2.06) compared to I exposure (1.94) over their pre-exposure values. This may be due to the fact that at exposure 1, the initial ST was higher than that of natural conditions. Koga *et al.* (1999) reported a similar pattern of significant increase in skin temperature of buffaloes as compared to cattle with increased environmental temperature. Ashour (1993) reported an increase in skin temperature due to high ambient temperature that leads to increased heat storage in the body of calves. The linear rise in ST and RT with the increase in ambient temperature under

climatic chamber indicating the higher heat storage at 2<sup>nd</sup> exposure conditions. The increase in RT by 2.63°C over normal body temperature indicating a stressful condition for growing buffaloes.

*Hematological parameters:* Blood fulfills a number of functions in the body and any alteration in the constituents of the blood reflects the functional status of the system.

The mean pre-exposure concentration of RBC in the blood of growing buffaloes were 8.29±0.11, 7.55±0.14 and 7.42±0.12 millions/mm<sup>3</sup> during comfortable, 1<sup>st</sup> and 2<sup>nd</sup> exposure conditions respectively. The mean RBC concentration in the blood of growing buffalo decreased with the increase duration of heat stress exposure in natural and climatic chamber conditions. The lowest values (6.91±0.17 millions/mm<sup>3</sup>) were recorded at 2<sup>nd</sup> exposure conditions (Table 1). Michelle *et al.* (2009) however, reported the normal TEC range of 6.38±0.13 to 7.65±0.04×10<sup>6</sup>/mm<sup>3</sup> in buffaloes. Soly and Singh (2003) reported the range of TEC from 6.39±0.14 to 6.77±0.09×10<sup>6</sup>/mm<sup>3</sup> of blood in blood of crossbred males under different housing conditions. The variations in the concentration of RBCs might be due to the difference in the age, breed, species, temperature and management conditions during present study.

The pre-exposure Hb content of growing buffaloes varied from 13.98±0.27 to 15.14±0.78 g%. The Hb content decreased with the increase in the climatic chamber temperature. The maximum decrease in Hb content (1.64 g %) was recorded at 2<sup>nd</sup> exposure conditions (Table 1).

Mullick and Kehar (1952) found seasonal variation in hemoglobin in Kumaun steers, Sahiwal × Haryana and Deoni × Haryana crossbreds and purebred cows. Chandra (2009) also reported similar results, i.e. lower levels of hemoglobin in the peri-parturient dairy cattle exposed to summer stress. Awodu *et al.* (2002) reported a decline in hemoglobin content with the increase in physiological stress. Similar results have been shown by Abdelatif *et al.* (2009) who reported significantly ( $P < 0.001$ ) lower Hb during summer compared to winter.

The PCV also followed the similar pattern of RBC and Hb, i.e. maximum decrease at higher exposure temperature conditions in a climatic chamber. Significantly ( $P < 0.001$ ) lower values of PCV was reported in dry summer compared to wet summer and winter (Lee *et al.* 1976; Abdelatif *et al.* 2009), whereas contradictory results, i.e. higher PCV and RBC have been reported during summer season (Koubkova *et al.* 2002). The RBC, Hb and PCV decreased due to 4 hours of exposure at all the exposure conditions, but the degree of decrease was significantly higher at exposure 2. The decrease in these parameters may be due to the hemodilution at higher temperature. Upadhyay and Madan (1988) also reported a lower level of all these parameters during treadmill exercise.

The pre exposure mean values of white blood corpuscles in growing Murrah buffaloes varied from  $13944 \pm 547$  to  $16567 \pm 241$  cells/ $\mu$ l (Table 1). The WBC showed the decreasing pattern with increase in the duration of exposure in natural and both the climatic chamber conditions. The decrease in the blood cells per unit of whole blood may be due to plasma volume changes during heat stress. These results are in accordance with those of Lee *et al.* (1976) who reported leucocytosis in cattle with the increase in ambient temperature. Aboulnaga *et al.* (1989) and Abdel-Samee (1992) also reported similar trend in WBC during heat stress.

*Plasma enzymes:* The pre-exposure, mean values of plasma ALP in growing Murrah buffaloes were similar

$25.91 \pm 0.20$  KA unit at  $42^\circ\text{C}$  and  $44^\circ\text{C}$  exposure conditions. The pre-exposure values increased ( $P < 0.05$ ) by 2.61 and 5.72 KA unit after 4 hour exposure at  $42^\circ\text{C}$  and  $44^\circ\text{C}$  respectively (Table 2). Abeni *et al.* (2007) indicated the activity of ALP as quick and reliable blood marker for heat stress in animals. The results obtained during the present study are in accordance with Marai *et al.* (1995, 1997) who observed increase in ALP activity during high ambient summer temperature. ALP was found significantly higher during afternoon as compared to morning.

Before exposure the mean values of ALT of growing Murrah buffaloes were  $25.10 \pm 0.28$ ,  $62.42 \pm 1.80$  and  $48.76 \pm 3.46$  IU/L at natural, I and II exposure conditions. The values decreased and increased significantly ( $P < 0.05$ ) by 8.7 and 1.33 IU/L in growing Murrah buffaloes after 4 hour of exposure at  $42^\circ\text{C}$  and  $44^\circ\text{C}$ , respectively. ALT was found significantly higher during afternoon compared to morning in growing buffaloes. The contrary results have been reported by Okab *et al.* (2008) who found significantly ( $P < 0.01$ ) lower ( $65.7 \pm 2.63$  IU/L) values of ALT during summer compared to spring ( $84.3 \pm 2.94$  IU/L). ALT showed positive correlation ( $P < 0.05$ ) with other physiological responses (RR, PR and ST), biochemical parameters (ALP, AST, LDH, and Cortisol) and THI and negative correlation with hematological parameters (RBC and Hb).

Pre-exposure mean values of AST were  $51.18 \pm 2.40$ ,  $97.77 \pm 11.17$  and  $82.72 \pm 2.27$  IU/L, which increased significantly ( $P < 0.05$ ) by 10.39, 114.01 and 90.35 IU/L in growing Murrah buffaloes after 4 h exposure at  $23^\circ\text{C}$ ,  $42^\circ\text{C}$  and  $44^\circ\text{C}$  respectively (Table 2). Chaiyabutr *et al.* (2011) also stated that changes in aminotransferase activity in the blood can be a consequence of their activity in liver cells and also a reflection of cell structure damage. An increase in AST of cattle due to heat exposure has been observed by Georgie *et al.* (1973) and Shaffer *et al.* (1981). In contrary Srikandakumar *et al.* (2003) observed decreased plasma AST in Merino and Omni sheep. Okab *et al.* (2008) observed no significant difference in AST level during

Table 2. Biochemical and hormonal parameters in growing buffaloes at different exposure temperatures

Parameter	Interval	Temperature		
		Spring ( $23 \pm 0.54^\circ\text{C}$ )	$42 \pm 1^\circ\text{C}$	$44 \pm 1^\circ\text{C}$
Alkaline Phosphatase (KA unit)	Before	$10.56 \pm 0.74^{\text{ax}}$	$25.91 \pm 0.20^{\text{ax}}$	$25.91 \pm 0.20^{\text{ax}}$
	After	$11.85 \pm 0.89^{\text{ay}}$	$28.52 \pm 0.34^{\text{ay}}$	$31.63 \pm 0.49^{\text{by}}$
Alanine amino transferase (IU/L)	Before	$25.10 \pm 0.28^{\text{ax}}$	$62.42 \pm 1.80^{\text{ax}}$	$48.76 \pm 3.46^{\text{bx}}$
	After	$27.97 \pm 0.22^{\text{ay}}$	$53.72 \pm 1.04^{\text{ay}}$	$50.09 \pm 3.72^{\text{by}}$
Aspartate amino transferase (IU/L)	Before	$51.18 \pm 2.40^{\text{ax}}$	$97.77 \pm 11.17^{\text{a}}$	$82.72 \pm 2.27^{\text{b}}$
	After	$61.57 \pm 0.90^{\text{ay}}$	$211.78 \pm 17.90^{\text{a}}$	$173.07 \pm 9.27^{\text{b}}$
Lactate dehydrogenase level (IU/L)	Before	$238.88 \pm 9.16^{\text{ax}}$	$404.58 \pm 14.52^{\text{ax}}$	$404.58 \pm 14.52^{\text{ax}}$
	After	$307.98 \pm 2.56^{\text{ay}}$	$441.31 \pm 12.49^{\text{ay}}$	$460.69 \pm 10.95^{\text{by}}$
Acetylcholinesterase level (IU/L)	Before	$547.37 \pm 35.14^{\text{ax}}$	$405.69 \pm 18.40^{\text{ax}}$	$471.19 \pm 3.90^{\text{bx}}$
	After	$573.43 \pm 57.31^{\text{ay}}$	$473.44 \pm 11.25^{\text{ay}}$	$605.55 \pm 19.44^{\text{by}}$
Cortisol (ng/ml)	Before	$1.42 \pm 0.07^{\text{ax}}$	$10.56 \pm 0.11^{\text{ax}}$	$10.56 \pm 0.11^{\text{ax}}$
	After	$1.78 \pm 0.12^{\text{ay}}$	$11.33 \pm 0.28^{\text{ay}}$	$13.95 \pm 0.12^{\text{by}}$

The values are the mean and SEM of six values on six animals; The values with the different superscript in the same row (a, b, c) and column (x and y) differed significantly ( $P < 0.05$ ). CD value for buffaloes, temperature and treatment = 0.110).

summer and spring season, but AST/ALT ratio was significantly ( $P < 0.01$ ) increased during summer as compared to spring season. The variations in findings during present study compared to earlier reports might be due to the physiological stage, high temperature exposures, management practices followed at the farm etc.

The pre exposure mean values of LDH of growing Murrah buffaloes were  $238.88 \pm 9.16$  during spring and  $404.58 \pm 12.49$  IU/L during 1<sup>st</sup> and 2<sup>nd</sup> exposure conditions. The pre exposure mean values increased significantly ( $P < 0.05$ ) by 69.1, 36.37 and 54.11 (IU/L) in growing Murrah buffaloes after 4 h exposure at 42°C and 44°C, respectively (Table 2). The mean values of AchE in growing Murrah buffaloes were  $547.37 \pm 35.14$ ,  $405.69 \pm 18.40$  and  $395.16 \pm 503.22$  IU/L prior to exposure at 23°C, 42°C and 44°C respectively. The respective values increased ( $P < 0.05$ ) by 26.06, 67.75 and 134.36 IU/L in growing Murrah buffaloes after 4 hour exposure at 23°C, 42°C and 44°C, respectively (Table 2). The increase in the activity of ALT, AST, ALP and LDH in plasma is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream, which reflects liver damage and disruption of normal liver function (Shakoori *et al.* 1994). Similar results have also been reported in sheep by Patel *et al.* (1991) that, lactate dehydrogenase level increased significantly in native Patanwadi sheep and its crosses with Merino and Rambouillet when exposed to direct sunlight from 8.30 (32.3°C) to 14.30 h (38.7°C) for 3 consecutive days in the last week of May. Kachmar and Moss (1976) stated that, LDH was found to be an indicator of anemia, renal stress, muscular dystrophy as well as liver damage and an increase in LDH indicates the deterioration of different tissues suffering from heat stress during the summer.

**Plasma cortisol hormone:** The mean values of cortisol of growing Murrah buffaloes increased by 0.36, 0.77 and 3.39 ng/ml over their respective pre-exposure values of  $1.42 \pm 0.07$  and  $10.56 \pm 0.11$  ng/ml recorded during spring, 1<sup>st</sup> and 2<sup>nd</sup> exposure conditions respectively in climatic chamber after 4 h of exposure (Table 2). The similar results have also been reported by Stott and Wiersma (1971) and Johnson and Uanjonack (1975), i.e. initial increase in cortisol levels due to acute heat stress which was followed by a decline after prolonged exposure to stressor. During heat acclimation there is reduction of plasma cortisol that help the animals in reducing the heat production (Yousef and Johnson, 1967). Chander Bhan *et al.* (2012) reported the similar trend of higher cortisol content during hot dry and hot humid season compared to spring and winter season in growing and adult Sahiwal cattle.

Exposure to elevated ambient temperature during present study causes drastic changes in animal's biological functions, which include disturbances in physiological functions, hematology, enzymatic reactions and hormonal secretions. The information generated regarding the relationship between animals and their environment will provide insight for developing new strategies to ameliorate the adverse impact of heat stress and will improve the

welfare and efficiency of production.

#### ACKNOWLEDGEMENTS

The authors are thankful to the Director, NDRI, Karnal for providing the necessary facilities.

#### REFERENCES

- Abdelatif M A, Ibrahim M Y and Hassan Y Y. 2009. Seasonal Variation in Erythrocytic and Leukocytic Indices and Serum Proteins of Female Nubian Goats Middle-East. *Journal of Science Research* 4(3): 168–74.
- Abdel-Samee A M and Ibrahim H. 1992. Triiodothyronine and blood metabolites in relation to milk yield and spray cooling of heat stressed lactating Friesians and Holsteins in Egypt. *Egyptian Journal of Animal Production* 29: 215–27.
- Abeni F, Calamari L and Stefanini L. 2007. Metabolic condition of lactating dairy cows during the hot season in the Po Valley. 1. Blood indicators of heat stress. *International Journal of Biometeorology* 52: 87–96.
- Aboulnaga A I, Kamal T H, El Masry K A and Marai I F. 1989. Short-term response of spray cooling and drinking cold water for improving milk production of heat stressed Friesian cows. Proceedings of the third Egyptian British Conference, Alexandria, Egypt. pp. 607–612.
- Amakiri S F and Funsho O N. 1979. Studies on rectal temperature, respiratory rate and heat tolerance in cattle in humid tropics. *Animal Production* 28: 329–35.
- Ashour G. 1993. Impact of environmental temperature on heat storage in the body of calf. *Journal of Animal Production* 64: 716.
- Awodu O A, Enosolease M E, Ubaru A G and Famodu A A. 2002. Leucocyte counts in pregnant Nigerian women with sickle cell anemia. *African Journal of Reproduction Health* 6: 112–16.
- Bahga C S, Sikka S S and Saijpal S. 2009. Effect of seasonal stress on growth rate and serum enzyme levels in young crossbred calves. *Indian Journal of Animal Research* 43: 288–90.
- Bernabucci U, Ronchi B, Lacetera N and Nardone A. 2002. Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. *Journal of Dairy Science* 85(9): 2173–79.
- Chandra G. 2009. 'Antioxidative status of high body condition periparturient crossbred cows with and without supplementation of  $\alpha$ -Tocopherol Acetate during summer and winter seasons'. MVSc. Thesis, National Dairy Research Institute (Deemed University), Karnal (Haryana), India.
- Chandra Bhan, Singh S V, Hooda O K, Upadhyay R C, Beenam and Vaidya M. 2012. Influence of temperature variability on physiological, hematological and bio-chemical profile of growing and adult Sahiwal cattle. *Journal of Environmental Research and Development* 7(2A): 986–94.
- Chandra Bhan, Singh S V, Hooda O K, Upadhyay R C and Beenam. 2013. Influence of Temperature Variability on Physiological, Hematological and Biochemical Profile of Growing and Adult Karan Fries Cattle. *Indian Journal of Animal Sciences* 83(10): 109096.
- Chaiyabutr N, Boonsanit D and Chanpongsang S. 2011. Effects of Cooling and Exogenous Bovine Somatotropin on Hematological and Biochemical Parameters at Different Stages of Lactation of Crossbred Holstein Friesian Cow in the Tropics. *Asian-Australasian Journal of Animal Science* 24: 230–38.
- Das S K, Upadhyay R C and Madan M L. 1999. Heat stress in

- Murrah buffalo calves. *Livestock Production Science* **61**: 71–78.
- Georgie G C, Chand D and Razdan M N. 1973. Seasonal changes in plasma cholesterol and serum alkaline phosphatase and transaminases activities in crossbred cattle. *Indian Journal of Experimental Biology* **11**: 448–50.
- Johnson H D and Uanjonack W. 1975. Effects of moderate heat and milk yield on plasma thyroxine in cattle. *Journal of Dairy Science* **58**: 507–11.
- Kachmar J F and Moss D W. 1976. Enzymes, pp. 653–54. *Fundamentals of Clinical Chemistry*. (Ed) Tietz N W. Philadelphia, USA: W.B. Sanders Company.
- Kearl L C. 1982. *Nutrient Requirements of Ruminants in Developing Countries*. International Feed Stuffs Institute. Utah Agriculture Experimental Station. Utah State University, Logon, Utah, USA, pp. 45–58.
- Koga A, Kurata K, Ohata K, Nakajima M, Hirose H, Furukawa R, Kanai Y and Chikamune T. 1999. Internal changes of blood compartment and heat distribution in swamp buffaloes under hot conditions: comparative study of thermo-regulation in buffaloes and Friesian cows. *Asian-Australasian Journal of Animal Science* **12**: 886–90.
- Koubkova M, Knizkova I, Munc P, Hartlova H, Flusser J and Dolezal O. 2002. Influence of high environmental temperatures and evaporative cooling on some physiological, hematological and biochemical parameters in high-yielding dairy cows. *Czech Journal of Animal Science* **47**: 309–18.
- Lal S N, Verma D N and Hussain K. 1987. Effect of air temperature and humidity on the feed consumption, cardio-respiratory responses and milk. Production in Haryana cows. *Indian Veterinary Journal* **64**: 115–21.
- Lee J A, Roussel J D and Beatty J F. 1976. Effect of temperature, season on bovine adrenal cortical function, blood cell profile and milk production. *Journal of Dairy Science* **59**: 104.
- Livestock Census. 2012. DAHDF, Ministry of Agriculture 65–73.
- Marai I F M, Daader A M, Abdel-Samee A M. and Ibrahim H. 1997. *Winter and summer effects and their amelioration on lactating Friesian and Holstein cows maintained under Egyptian conditions*. Proceedings of the International Conference on Animal, Poultry, Rabbits and Fish Production and Health, Cairo, Egypt.
- Marai I F M, Habeeb A A, Daader A H and Yousef H M. 1995. Effect of Egyptian subtropical summer conditions and the heat-stress alleviation technique of water spray and a diaphoretic on the growth and physiological functions of Friesian calves. *Journal of Arid Environment* **30**: 219–25.
- Michelle. 2009. 'Impact of thermal stress and vitamin E supplementation on heat shock protein 72 and antioxidant enzymes in murrah buffaloes'. PhD Thesis, National Dairy Research Institute (Deemed University), Karnal, India.
- Moran J B. 1999. Heat tolerance of Brahman cross, buffalo, banteny and shorthorn steers during exposure to sun and as a result of exercise. *Australian Journal of Agriculture Research* **24**: 775–82.
- Mullick D N and Kehar N D. 1952. Seasonal variations in the pulse rate, respiration, body temperature, body weight and hemoglobin in normal Indian cattle. *Indian Journal of Dairy Science* **22**: 61–65.
- Okab A B, El-Banna S G and Koriem A A. 2008. Influence of environmental temperatures on some physiological and biochemical parameters of New-Zealand Rabbit males. *Slovak Journal of Animal Science* **41**: 12–19.
- Patel J S, Jajane K R, Vadqaria V P, Kullarni V V and Radadia N S. 1991. Effect of temperature on certain blood constituents in Patanwadi and its crosses with Merino and Rambouillet. *Indian Veterinary Journal* **68**: 1134–38.
- Salam I A. 1980. Seasonal variation in some body reactions and blood constituents in lactating buffaloes and Friesian cows with reference to acclimatization. *Journal of Egyptian Veterinary Medicine Association* **40**: 63.
- Shakoori A, Butt G, Riffat R and Aziz F. 1994. Hematological and biochemical effects of danitol administrated for two months on the blood and liver rabbits. *Zeitschrift Fuer Angewandte Zoology* **80**: 156–80.
- Shaffer L, Roussel J D and Koonce K L. 1981. Effects of age, temperature-season, and breed on blood characteristics of dairy cattle. *Journal of Dairy Science* **64**: 62–70.
- Singh, S V, Soly M J and Kundu S S. 2008. Feed intake, growth rate and physiological responses during spring and hot dry season in Karan Fries males. *Indian Journal of Animal Nutrition* **25**: 162–66.
- Singh, S V and Upadhyay R C. 2009. Impact of temperature rise on physiological function, thermal balance and milk production of lactating KF and Sahiwal cows. *Indian Veterinary Journal* **86**: 141–44.
- Soly M J and Singh S V. 2003. Physiological, hematological and growth responses of crossbred male calves under two housing conditions. *Indian Journal of Animal Science* **73**: 202–03.
- Srikandakumar A, Johnson E H and Mahgoub O. 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. *Small Ruminant Research* **49**(2):193–8.
- Stott G H and Wiersma F. 1971. Plasma corticoids as an index of acute and chronic environmental stress. *Proceedings of I Conference on Biometeorology and 10<sup>th</sup> conference on Agricultural Meteorology*, Columbia.
- Upadhyay R C and Madan M L. 1988. Effect of work stress during different seasons on some hematological and biochemical constituent in bullocks. *Indian Journal of Dairy Science* **41**: 29–34.
- Wagner P E. 2001. *Heat stress on dairy cows*. Dairy Franklin Country Publishers.
- West J W. 2003. Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science* **86**: 2131–44.
- Yousef M K and Johnson H D. 1967. Calorigenesis of cattle as induced by hydrocortisone and environmental temperature. *Journal of Dairy Science* **26**: 1087–92.