



## Effect of seasonal variation on physiological parameters in Murrah buffaloes

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### ABSTRACT

Heat stress is a major limiting factor in livestock production under tropical climate. Buffaloes have poor heat tolerance capacity compared to other domestic ruminants and are more prone to heat stress due to scarcely distributed sweat glands, dark body colour and sparse hair on the body surface. Therefore present study was undertaken to study the effect of seasonal stress on Murrah buffalo. Twenty five healthy non-lactating Murrah buffaloes each above two years of age were selected for the experiment. Rectal temperature, respiration rate, haematological parameters and various hormones level were measured in different season to know the effect of heat stress in buffalo. Season had no significant effect on rectal temperature, total leukocyte count (TLC), triiodothyronine (T3) and thyroxine (T4) level. However, it had significant effect on respiration rate, total erythrocyte count (TEC), haemoglobin (Hb) level, lymphocyte and neutrophil counts, and cortisol level in murrah buffalo. They had lowest level of TEC, Hb and lymphocyte, and highest neutrophil and cortisol level in summer season. Therefore, it can be concluded that various season had considerable effect on Murrah buffalo.

**Key words:** Buffalo, Haematological parameter, Heat stress, Hormone, Season

Climate is one of the main factors affecting animal productivity (Marai and Haeb 2010). Heat stress has the greatest negative impact since it challenges the animals' ability to maintain their energy, hormone, and mineral balances (Singh *et al.* 2012). Hence, heat stress is a major limiting factor in livestock production under tropical climate. Heat stress occurs in animal when there is an imbalance between heat production within the body and its dissipation. Under heat stress, a number of physiological and behavioral responses vary in intensity and duration in relation to the animal's genetic makeup and environmental factors (Altan *et al.* 2003). Sweating, high respiration rate, vasodilatation with increased blood flow on skin surface, elevated rectal temperature, reduced metabolic rate, decreased dry matter (DM) intake, efficiency of feed utilization and altered water metabolism are the physiological responses that are associated with negative impacts of heat stress on production and reproduction in dairy animals (West *et al.* 1999). McDowell *et al.* (1976) suggested that temperature humidity index (THI) could be used as an indicator of thermal climatic condition. THI value of 72 is considered comfortable, 72–78 stressful and values greater than 78 causes extreme distress in lactating animal

being unable to maintain thermoregulatory mechanism or normal body temperature. With environmental temperature crossing the zone of thermo neutrality, the milk composition changes and production declines. High producing animals are affected more than the low producing animal due to heat stress because the zone of thermo neutrality shifts to lower temperature as milk production, feed intake and metabolic heat production increases (Coppock *et al.* 1982). Heat stress also have the potential to activate the hypothalamo-pituitary-adrenal cortical axis (HPA) and sympatho-adrenal medullary axis (Minton 1994). This leads to increase in plasma concentration of cortisol in heat stressed animals (Minton 1994). Magdub *et al.* (1982) reported that heat stress leads to decreased concentrations of triiodothyronine (T3) and thyroxine (T4) in plasma and in milk of lactating cows. However, a significant increase in T3 but not in T4 level was observed during heat stress in crossbred cattle (Singh *et al.* 1984). Buffalo is generally reared in hot humid tropical climate where temperature range varied from 46°C in summer to 2–3°C in winter. Moreover, buffaloes have poor heat tolerance capacity compared to other domestic ruminants and are more prone to heat stress due to scarcely distributed sweat glands, dark body colour and sparse hair on the body surface (Das *et al.* 1999). Therefore present study was undertaken to study the effect of seasonal stress on Murrah buffalo.

### MATERIALS AND METHODS

*Experimental animals:* Healthy non-lactating Murrah buffaloes (25) each above 2 years of age were selected for

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the experiment from herd maintained at institute's farm. The animals had *ad lib.* access to feed and water.

**Climatic measurements:** Minimum and maximum temperature, dry bulb and wet bulb temperature was measured daily at buffalo shed. Temperature humidity index (THI) was calculated as per McDowell *et al.* (1976). Experiment period was divided into 3 seasons, viz. summer (May), pre winter (October) and winter (mid December to mid January).

**Measurement of rectal temperature and respiration rate:** Rectal temperature was measured with the help of simple mercury thermometers daily both in the morning and evening for all the animals included in the study. Respiration was also measured daily by silently observing the movement of the thoracoabdominal region for each animal included in the study.

**Collection of blood:** Blood (5 ml) was collected from the jugular vein of each animal in a sterile polypropylene vials containing 2.7% EDTA (0.5 ml/10 ml of blood) as an anti-coagulant under sterile conditions. Blood (2 ml) was collected without adding anticoagulant for the isolation of serum and plasma. Blood collection was done twice at 15 days interval at same time in each season.

**Measurement of haematological parameters:** Total erythrocyte count and total leukocyte count was measured by routine protocol using haemocytometer (Jain 1986). Packed cell volume was determined by the micro-haematocrit method. Haemoglobin level was estimated by the cyano-methaemoglobin method (Van Kampen and Zijlstra 1961). Differential leukocyte count was measured following standard protocol (Kelly 1984).

**Measurement of hormonal parameters:** Cortisol, triiodothyronine (T3) and thyroxine (T4) level in each season was measured by using ELISA kit (Monobind, USA) using manufacturer's protocol.

## RESULTS AND DISCUSSION

**Climatic condition:** The ambient temperature and THI prevailing during experimental period are given in Table 1. THI was highest in summer and lowest in winter. THI level revealed that buffaloes were under marked seasonal variation.

The Livestock Weather Safety Index (LCI 1970) is commonly used as a benchmark to assign heat stress levels to normal, alert, danger and emergency categories. In this index, THI  $\leq 74$  is classified as normal, 75–78 as alert, and

Table 1. Environmental parameters at the time of experiment

Season		Maximum Temp	Minimum Temp	THI
Pre winter	1	34	26	49.6
	2	31	21	52.84
Winter	1	22	18	65.8
	2	28	8	67.96
Summer	1	41	28	86.32
	2	42	30	84.52

79–83 as danger and THI  $\geq 84$  as emergency. Therefore, based on this index buffaloes reared in summer falls under emergency category indicating stressful condition of the animal.

**Effect on rectal temperature and respiration rate:** Rectal temperature and respiration rate measured during experimental period are presented in Table 2. Season had no significant effect on rectal temperature whereas it had significant effect ( $P \leq 0.05$ ) on respiration rate. Das *et al.* (1999) also observed an increase in respiration rate in June in Murrah buffalo calves when exposed to direct sunlight for 6 h.

Table 2. Effect of season on rectal temperature and respiration rate

Season	Rectal temperature		Respiration rate
	Morning	Evening	
Pre winter	100.6 $\pm$ 0.42 $^{\circ}$ F	100.2 $\pm$ 0.36 $^{\circ}$ F	14.3 $\pm$ 0.12 <sup>a</sup>
Winter	100.3 $\pm$ 0.47 $^{\circ}$ F	100.4 $\pm$ 0.49 $^{\circ}$ F	13.2 $\pm$ 0.4 <sup>a</sup>
Summer	100.2 $\pm$ 0.23 $^{\circ}$ F	100.8 $\pm$ 0.63 $^{\circ}$ F	16.9 $\pm$ 0.9 <sup>b</sup>

Different superscripts indicate significance at 5% level.

**Effect on haematological parameters:** Haematological indices measured during experimental period are presented in Table 3. Season had significant effect ( $P \leq 0.05$ ) on total erythrocyte count (TEC) and haemoglobin (Hb). Hb and TEC level were lowest in summer. PCV level also decreased in summer. However, this reduction was statistically nonsignificant. Total leukocyte count (TLC) level was highest in summer but this association was statistically nonsignificant. Differential leukocyte count (DLC) revealed significant ( $P \leq 0.05$ ) difference in lymphocyte and neutrophil count across the season.

TEC was lowest in summer and highest in winter. This reduction in erythrocyte count in summer might be due to destruction of RBC and haemodilution. More water is transported to circulatory system to facilitate evaporative cooling during heat stress resulting in haemodilution (Lee *et al.* 1976). Reduction in TEC and haemoglobin level could also be attributed partly to the reduced feed intake during summer leading to reduced erythropoiesis. Our study revealed higher TLC and neutrophil in summer than the

Table 3. Effect of season on haematological parameters

Parameter	Summer	Pre winter	Winter
Hb (gm/dl)	13.36 $\pm$ 0.96 <sup>a</sup>	14.04 $\pm$ 0.78 <sup>b</sup>	14.57 $\pm$ 1.25 <sup>b</sup>
TEC (millions/ $\mu$ l)	7.03 $\pm$ 0.63 <sup>a</sup>	7.42 $\pm$ 0.57 <sup>b</sup>	8.05 $\pm$ 0.42 <sup>c</sup>
TLC (thousands/ $\mu$ l)	13.55 $\pm$ 1.88	13.06 $\pm$ 1.28	12.33 $\pm$ 1.06
PCV	30.92 $\pm$ 3.31	31.92 $\pm$ 2.81	32.48 $\pm$ 2.65
Lymphocyte (%)	56.28 $\pm$ 1.65 <sup>a</sup>	58.03 $\pm$ 2.12 <sup>b</sup>	59.48 $\pm$ 1.87 <sup>b</sup>
Neutrophil (%)	34.45 $\pm$ 1.23 <sup>a</sup>	32.74 $\pm$ 0.98 <sup>b</sup>	31.76 $\pm$ 1.45 <sup>c</sup>
Monocyte(%)	4.42 $\pm$ 0.45	4.85 $\pm$ 0.62	5.12 $\pm$ 0.46
Eosinophil (%)	2.35 $\pm$ 0.24	2.56 $\pm$ 0.34	2.72 $\pm$ 0.42
Basophil (%)	0.42 $\pm$ 0.12	0.38 $\pm$ 0.16	0.32 $\pm$ 0.09

Different superscripts indicate significance at 5% level.

Table 4. Effect of season on hormone levels

Parameter	Summer	Pre-winter	Winter
Cortisol (ng/ml)	5.34±0.67 <sup>a</sup>	4.17±0.75 <sup>b</sup>	3.72±0.46 <sup>c</sup>
T3 (ng/ml)	1.92±0.20	1.98±0.22	2.19±0.25
T4 (ng/ml)	32.25±1.1	32.86±1.07	35.39±1.07

Different superscripts indicate significance at 5% level.

winter. This could be due to release of corticosteroids due to summer stress which in turn increased leukocyte count and accelerated mobilization of mature neutrophil from bone marrow storage pool (Jain 1986). Similar reports available in literature showed higher TLC in white Fulani cattle (Saror and Coles 1973) and Holstein breeds (Nouty *et al.* 1986) during summer season. Manjari *et al.* (2016) also reported similar results in Tarai buffalo.

*Effect on hormonal assay:* Effect of seasonal stress was measured on cortisol, T3 and T4 levels and data are presented in Table 4. There was seasonal variation in T3 and T4 level. But this variation was statistically nonsignificant. Level of T3 and T4 was higher in winter than the summer. Magdub *et al.* (1982) also reported similar result and observed that during heat stress, T3 and T4 levels in plasma and milk decreases. However, a significant increase in T3 but not in T4 level was reported during heat stress in crossbred cattle (Singh *et al.* 1984).

Our experiment revealed significant effect ( $P \leq 0.05$ ) of season on cortisol level in Murrah buffalo. Cortisol level was much higher in summer (5.34±0.24 ng/ml) than the winter (3.76±0.21 ng/ml). This may be due to heat stress in summer which activates the hypothalmo-pituitary-adrenal cortical Axis (HPA) and sympatho-adrenal medullary axis (Minton 1994). This increases the plasma concentration of cortisol and corticosteron. This result was in agreement with Comin *et al.* (2011) who reported higher cortisol level in dairy cow during heat stress.

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