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Original Research Article

Seasonal Variation of Physiological Responses, Plasma Hormones, Electrolytes and Hematological Parameters in Buffaloes Physiological and Biochemical Changes in Buffaloes

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A B S T R A C T

The present study was conducted on buffaloes (n=12) to investigate the various adaptive physiological mechanisms employed by buffaloes during different seasons. The physiological responses, hematological parameters, electrolyte, biochemical and hormonal concentrations of the animals were evaluated to ascertain their role in the thermoregulation of buffaloes. The physiological responses viz. respiratory rate, heart rate, rectal temperature and skin temperature of buffaloes increased with increase in ambient temperature. The hematological components varied seasonally with PCV being lowest during summer, Hb and TEC count being highest during spring, and TLC being highest during winter. The high concentration of plasma anti diuretic hormone accompanied by low packed cell volume during summer is indicative of its role to conserve body water content in buffaloes. The hematological and physiological parameters of buffaloes are modulated during different seasons as mode of adaption to varied environmental conditions.

Introduction

Buffalo is an important livestock in tropical countries with hot and humid climate. Due to its dark coat colour and low density of sweat glands, buffaloes are prone to heat stress when exposed to direct solar radiation. As water homeostasis and energy balance are essential for survival and adaptation of living organisms, buffaloes employ various adaptive mechanisms to maintain their body thermal balance and homeostasis under different climatic conditions. The thermo neutral zone in buffaloes ranges from 10°C to 36°C (Aggarwal and Upadhyay, 2013).

When the ambient temperature deviates from the thermo neutral zone, animals become prone to environmental stress. Under extreme hot weather, animals may undergo dehydration leading to altered plasma volume and increased plasma osmolality. The animal responds to dehydartion by activation of the renin-angiotensin-aldosterone system and also the secretion of antidiuretic hormone from posterior pituitary gland as a measure of maintaining body fluid balance (Reece, 2015). Majority of acute endocrine responses to thermal stress are associated with altering water and electrolyte turnover.

This response is necessary to comply with the increase in evaporative water loss which is the major route of heat exchange when ambient temperature approaches body temperature (Collier *et al.*, 1982); and accordingly, thermal stress imposes a need to conserve electrolytes. The present study was undertaken to understand the underlying physiological and biochemical changes taking place in buffaloes as thermoregulatory responses during different seasons.

Materials and Methods

Twelve male Murrah buffaloes with age group between 1 to 2 years were selected from the herd of Livestock Research Centre, National Dairy Research Institute, Karnal. The experiment was conducted during three seasons viz. winter (January), spring (mid February to first week of March) and summer (mid May to early June). The Temperature Humidity Index (THI) was calculated by using the following equation: THI= 0.72 x (T_{db} + T_{wb}) + 40.6 (Mc. Dowel *et al.*, 1976)**.** Feed and water intake was recorded during the experimental period. The physiological responses were recorded and then blood sample collection was done between 1:30 P.M.to 2:30 P.M when the THI values were highest during the day. Blood samples were collected twice per season at fortnightly interval and the plasma were separated and stored at -20°C for estimation of hormones, electrolytes and biochemical parameters. The plasma and urine osmolality were determined by using cryoscopic osmometer (Model: OSMOMAT 030, Manufactured by Gonotec, Germany). Erythrocyte and total leukocyte count was done by using routine hemacytometer method. Packed cell volume was determined by micro hematocrit method. Hemoglobin estimation was done by Sahli's method. Plasma calcium, chloride, glucose and total protein estimation was done by using diagnostic kits supplied by Recombigen Laboratories Pvt. Ltd., New Delhi. Plasma sodium and potassium estimation was done by using kits supplied by Avecon Healthcare Pvt. Ltd., Parwanoo. Plasma urea was estimated by using kits supplied by Beacon Diagnostics pvt. Ltd., Navsari. Plasma hormone concentrations were estimated by using bovine specific ELISA kits supplied by Bioassay Technology Laboratory, Sanghai. The statistical analysis was done by ANOVA using SPSS version 16.0 software (IBM corp., USA).

Results and Discussion

Water and feed intake: The average THI values recorded during the experimental period were 59.27, 70.97 and 84 during winter, spring and summer, respectively. The water intake per 100 kg of body weight per day during winter, spring and summer were 1.71 ± 0.20, 4.10 ± 0.17 and 6.91 ± 0.29 liters, respectively. The intake of water was significantly ($P<0.05$) different between the seasons, with highest being recorded during summer, and the lowest intake was during winter. In male buffalo calves, water intake is highly correlated with ambient temperature (Singh *et al.*, 2001). The average dry matter intake (kg) per 100 kg body weight per day were 1.53 ± 0.04, 1.69 ± 0.05 and 0.89 ± 0.14 during winter, spring and summer, respectively. The average dry matter intake of the animals during summer was significantly ($P<0.05$) lower than spring and winter seasons. During acute heat stress, cattle initially activate their heat dissipation mechanism followed by reduced feed consumption and reduction of metabolic rate (Scharf *et al.*, 2014). The dry matter intake per 100 kg body weight per day observed in the present study was lowest during summer and may be attributed to the thermoregulatory mechanism to combat heat stress.

Physiological responses: The physiological responses recorded during different seasons are presented in Table 1. A significant ($P<0.05$) increase in respiration rate, heart rate and rectal temperature was observed during summer when compared with winter. The skin temperature (°C) recorded from forehead varied significantly ($P<0.05$) between the seasons. For the neck region, the skin temperature (°C) varied significantly ($P<0.05$) between the values recorded during winter and summer as well as between summer and spring season, however it did not vary significantly between winter and spring season. In sacral region, the skin temperature showed a significant ($P<0.05$) increase in summer when compared to winter season.

In the findings of the current study, during summer, the mean rectal temperature and respiration rate were lower whereas the heart rate was higher than those reported by Aggarwal and Upadhyay (1998) in Murrah buffalo calves recorded at a higher ambient temperature and relative humidity. Buffaloes can withstand moderate heat and increases body heat storage when exposed to higher temperature (Das *et al.,* 1997). When buffaloes are exposed to heat stress, the rectal temperature is increased (Chaiyabutr *et al.*, 1997, Koga *et al.*, 2004). The increase in average ambient temperature induce rise in frequency of respiration rate, rectal temperature, heat storage and skin temperature in Murrah buffaloes (Aggarwal and Upadhyay, 1998, Das *et al.*, 1999, Gudev *et al.,* 2007, Verma *et. al*, 2000). Chaudhary *et al.*, (2015) reported significant increase in respiration rate during hot season than comfort, however no significant seasonal differences were observed in rectal temperature and heartbeat. Dayal *et al.*, (2017) in their study on the seasonal effect on physiological parameters of non lactating Murrah buffaloes, did not find any significant variation in rectal temperature during different seasons, whereas the respiration rate was significantly higher during summer than winter season. In the present study, the rectal temperature, respiration rate and heart rate increased significantly during summer when compared with values recorded during winter; however the values of summer were non significantly higher than spring.

The skin temperature in the forehead, neck and sacral region recorded during summer were lower than the values recorded by Das *et al.*, (1999) on Murrah buffaloes at a higher ambient temperature. Das *et al.*, (1999) observed the highest and lowest changes in skin temperature of heat exposed Murrah buffaloes in forehead and attributed the magnitude of changes in skin temperature to the duration of sunlight exposure and the ability of the exposed surface to undergo diffusion and evaporation; and the magnitude of skin temperature changes in buffaloes was lowest in middle neck. In buffaloes, there is high correlation between skin temperature and ambient temperature (Koga *et al.*, 2004). In the present findings, the skin temperature was highest in forehead region during summer; whereas during winter and spring, the skin temperature was highest in sacral region.

Hematological parameters

The hematological parameters recorded during different seasons are presented in Table 2. The packed cell volume recorded during summer was significantly ($P<0.05$) lower than those recorded during winter and spring. The mean value of packed cell volume (%) did not show any significant difference ($P<0.05$) between winter and spring values. The hemoglobin content was significantly ($P<0.05$) higher during spring than the estimated values of winter and summer seasons. Total erythrocyte count obtained during spring was significantly ($P<0.05$) higher than other seasons, and the lowest was recorded during summer. The total WBC count obtained during summer was significantly ($P<0.05$) lower than winter and spring values. The blood pH increased during summer.

Blood pH is directly proportional to respiration (Korde, 2007). The packed cell volume, in the present study, decreased during summer when there is heat stress which is similar with the findings of Chaiyabutr *et al.,* (1997), Korde *et al.,* (2007), Jabbar *et al.,* (2012) and Yadav *et al.,* (2016). During heat stress, the blood and plasma volume is increased and total body water content is increased with resultant decrease in packed cell volume in buffaloes (Chaiyabutr *et al.,* 1997, Korde *et al.,* 2007). However, a rise packed cell volume during heat stress was reported by Verma *et al.,* (2000) and Parmer *et al.,* (2013). The hemoglobin concentration and erythrocyte count was lowest during heat summer which was similar with the findings of Verma *et al.,* (2000) and Patel *et al.,* (2016) in heat stressed animals. The lowest value of hemoglobin and total erythrocyte count during summer may be attributed to the destruction of red blood cells, hemodilution and reduced feed intake driven decreased erythropoiesis (Dayal *et al.,* 2017). The white blood cell count, in the present study, was significantly lower during summer than other seasons. However, Verma *et al.,* (2000) and Jabbar *et al.,* (2012) reported that the highest counts of total leukocyte during summer and spring. Higher total leukocyte and neutrophil during summer is attributed to summer stress influenced increased glucocorticoid release and increased mobilization of neutrophils from bone marrow (Dayal *et al.,* 2017).

Plasma and urine osmolality

The mean plasma and urine osmolality estimated during different seasons are presented in Table 3. The mean osmolality of plasma estimated during winter was significantly ($P<0.05$) higher than summer. The urine osmolality during spring was significantly ($P<0.05$) higher than the values of summer and winter seasons.

The plasma osmolality estimated in the current study during spring season was similar to the values reported by Koga *et al.,* (2002) during hydration in buffaloes. During heat stress, the tendency to increase total body water content accompanied by increased drinking water, sodium excretion and decreased plasma sodium leads to reduced serum osmolality in cattle (El-Nouty *et al.,* 1980). The increased water intake in buffaloes during summer may have caused hemodilution in the animals leading to lower plasma osmolality values.

Plasma electrolytes and biochemical parameters

The mean plasma electrolytes and biochemical values during different seasons are presented in Table 4. The concentrations of chloride varied significantly ($P<0.05$) between the seasons with the highest and lowest value being recorded during winter and spring respectively. The potassium concentration recorded during winter was significantly ($P<0.05$) higher than the concentrations during spring and summer. The plasma sodium concentration during winter was significantly ($P<0.05$) higher than the concentrations recorded during spring and summer. No significant ($P<0.05$) variation in glucose concentration was observed between the seasons. The concentration of total protein during winter was significantly ($P<0.05$) higher than that of spring and summer values. The average blood urea concentration during winter was significantly ($P<0.05$) lower than spring and summer values.

The plasma chloride, sodium and potassium concentration, in the present study, decreased significantly from winter to summer, which is similar to findings of Hooda and Singh (2009) in buffalo heifers after heat exposure. In contrary, Singh *et al.*, (2001) reported higher plasma chloride concentration in Murrah buffalo calves during summer than winter. Chaudhary *et al.*, (2015) reported an increased plasma sodium concentration during hot season in Surti buffaloes and attributed to dehydration. In the current finding, the plasma potassium concentration during summer with hot ambience was significantly lower than winter concentrations, which are similar to the findings of Chaiyabutr *et al.*, (1997) and Singh *et al.*, (2012) in buffaloes. Acute heat stress induce shift of sodium and potassium ions between intracellular and extracellular components, while chronic heat stress induces external and internal electrolyte balance primarily involving urinary potassium excretion (Chaiyabutr *et al.*, 1997). The decreased potassium level in heat stressed Murrah baffaloes was on account of loss of potassium through sweating (Singh *et al.*, 2012). There is a shift in blood electrolyte balance during heat stress (Hooda and Singh, 2009). Singh *et al.*, (2001) reported increased plasma sodium concentration in buffalo calves kept in open area than those kept in shaded area both during summer and winter season. However, Jabbar *et al.*, (2012) found the serum sodium, potassium and chloride were highest during spring and lowest during summer, and the winter values were significantly higher than summer values. The decreased aldosterone concentration during heat stress in Murrah heifers result in urinary loss of sodium leading to decreased plasma sodium concentration. Singh *et al.*, (2012) found a decreased calcium level in heat stressed buffaloes was due to less secretion of parathyroid hormone. However in the present study, no significant variation in plasma calcium and glucose concentration was observed between the seasons. Verma *et al.*, (2000) reported lower blood glucose, protein and urea during summer season in lactating Murrah buffaloes. The plasma total protein concentration, observed in the current study, was significantly lower in summer than the winter values, and the trend is similar to the findings of Joshi *et al.*, (2012) in Murrah buffaloes. The concentration of total plasma protein is decreased during high ambient temperature due to reduced feed intake (Srikandakumar and Johnson, 2004) and hemodilution condition induced by heat stress (Gudev *et al.*, 2007). However, Farouk (2012) did not find any significant differences in plasma total protein between summer and winter season. In the present study, plasma urea was significantly higher during summer than winter season. The negative effect of increased body temperature during heat stress on rumen micro flora leads to increase in plasma urea concentration (Gudev *et al.*, 2007). Joshi *et al.*, (2012) reported highest value of serum urea during hot followed by cold and moderate ambience. Chaudhary *et al.*, (2015) attributed the increased plasma urea during hot season to the increased catabolism of protein and dehydration. Yadav *et al.*, (2016) in their study, however, did not find any significant difference in serum urea, glucose, potassium and chloride between the exposed and cooled buffaloes.

Plasma hormones

The average plasma hormone concentrations of the animals during different seasons are presented in Table 5. The plasma anti diuretic hormone during summer was significantly ($P<0.05$) higher than the concentrations during spring and winter. The anti diuretic hormone concentration during winter was significantly lower than spring values. Aldosterone concentration during summer was significantly ($P<0.05$) lower than winter. No significant ($P<0.05$) difference was observed between the plasma angiotensin II concentrations of different seasons.

In the present study, the concentration of anti diuretic hormone during winter season is similar to the values reported by Koga *et al.,* (2002). The trend of increase of anti diuretic hormone during summer with hot ambience is similar to the findings of El-Nouty *et al.,* (1980) in cattle. In heat exposed cattle, plasma ADH is increased due to direct effect of heat on hypothalamus (El-Nouty *et al.,* 1980). When buffaloes were deprived of water, plasma ADH increased; whereas aldosterone level fluctuated with no significant trend (Koga *et al.,* 2002). In human, the plasma ADH increases during summer to compensate the water loss through sweating (Kanikowska *et al.,* 2010). In heat exposed cattle, the indirect inhibitory effect of resultant high plasma ADH, and low plasma potassium concentration leads to reduced concentration of plasma aldosterone (El-Nouty *et al.,* 1980). Singh *et al.,* (2001) reported higher values of plasma aldosterone concentration in buffaloes kept in open area than those kept in shed during both summer and winter seasons. Joshi *et al.,* (2017) observed in Murrah buffaloes, during hot environment, serum aldosterone increased significantly and found a positive correlation with glucose, sodium and chloride; whereas negative correlation was found with serum potassium, calcium and phosphorus. The decreased potassium concentration observed during summer, in the present study, may lead to decrease in plasma aldosterone concentration. During high ambient temperature, the increase in insensible water and sodium loss cause contraction of intravascular volume which stimulates the release of angiotensin-II (Kruse *et al.,* 2002). However, there was no significant difference of angiotensin II concentrations in buffaloes between the seasons which was similar to of the findings of Kanikowska *et al.,* (2010) in human.

In conclusion, the variations in hematological values and plasma parameters including osmolality, electrolytes and hormones during different seasons indicate the adaptive mechanisms employed by buffaloes to regulate their physiological status to maintain homeostasis during different ambient conditions.

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