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HEAT STRESS RESPONSES TO INCREASING TEMPERATURE HUMIDITY INDEX (THI) IN LACTATING MURRAH BUFFALO

Brijesh Yadav[1,*], Sarvajeet Yadav[1], Arun Kumar Madan[1], Mukul Anand[1], Dilip Kumar Swain[1], Vijay Pandey[2] and Rajneesh Sirohi[3]

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ABSTRACT

The present study was conducted to determine the effect of increasing temperature humidity index (THI) on lactating Murrah buffalo. The study was conducted on six lactating buffaloes which were offered the same basal diet and were blocked by days in milk, milk yield and parity. The study was conducted during May, June and July with average THI of 79.88, 80.57 and 85.36, respectively. Milk yield was recorded twice a day at 06:00 and 18:00 h and physiological parameters were recorded and blood was sampled on 30th day of each month at 14:30 h. The results showed a significant decrease (P<0.05) in milk yield in buffaloes only at a THI of 85.36 as compared to THI up to 80.57. The rectal temperature (RT) did not change (P=0.580) with increasing THI up to 85.36, however, the respiration rate (RR) and pulse rate (PR) changed (P<0.05) progressively with increasing THI. Leucocytopnoea, lymphocytopnoea and neutrophilia; and alteration in serum urea, alkaline phosphatase activity, reactive oxygen species and phosphorus concentration were observed only at a THI of 85.36 as compared to THI up to 80.57. Similarly, significant (P<0.05) alteration in serum T_4, cortisol and prolactin levels were observed at a THI of 85.36 as compared to THI up to 80.57. It can be concluded that lactating buffaloes experienced mild heat stress up to a THI of 80.57 (May and June) and a moderate type of heat stress at a THI 85.36 (July).

Keywords: *Bubalus bubalis*, buffaloes, heat stress, milk production, THI

INTRODUCTION

Buffalo (*Bubalus bubalis*) contributes 12.8% of total milk production in the world. Buffalos can survive on poor quality feed and fodder and can convert fiber into milk and meat and, are better acclimatized to hot and humid climates (Marai and Haeeb, 2010). However, scarcely distributed sweat glands resulting in poor

[1]Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Veterinary University, Uttar Pradesh, India, *E-mail: drbrijvet@gmail.com
[2]Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, Veterinary University, Uttar Pradesh, India
[3]Department of Livestock Production Management, College of Veterinary Science and Animal Husbandry, Veterinary University, Uttar Pradesh, India

sweating capacity along with dark body colour of buffaloes make them more susceptible to heat stress (Das *et al*., 1999). Buffaloes are more heat stressed when they are devoid of shelter and prefer wallowing in water. In addition, high milk yield results in increased heat production in lactating buffaloes that make them more susceptible during summer as heat stress and lactation stress act in combination (Das *et al*., 2014). In tropical and subtropical areas, high temperature and humidity reduces the evaporative heat loss from the body, culminating in an escalation in body temperature. The effect of heat stress has been extensively reviewed (Marai and Haeeb 2010; Yadav *et al*., 2013) in livestock.

THI measures the thermal comfort levels which have been applied to understand heat stress states in livestock. THI value have been defined: no stress <72, moderate stress 72 to 78, heavy stress >79 to 83 (Akyuz *et al*., 2010) however, the stratification of THI for measuring the comfort levels in bovine breeds of Indian subcontinent varies (Yadav *et al*., 2015, 2016). A wide variation is seen in environmental temperature and relative humidity during different summer months in the semi-arid regions of India and therefore the levels of heat stress also varies. Kumar *et al.* (2020) reported the threshold THI for different heat stress mediated responses with increasing THI in cattle. Review of literature suggested that many studies have been conducted in cattle but studies are scarce about the effect of differential THI on heat stress mediated responses in buffaloes. Therefore, the study was conducted to explicate the effect of differential THI on physiological, biochemical and endocrine profile in lactating Murrah buffaloes.

MATERIALS AND METHODS

The experiment was conducted at the livestock farm complex of the Veterinary University, Mathura, located in the semi-arid zone of India, with a latitude and longitude of 27.49°N, 77.67°E respectively, and at an altitude of 177 m from mean sea level. The study was conducted during summer months (May to July) when THI ranged between 73 and 90. Daily minimum and maximum temperature and relative humidity was recorded during the study period and THI (Mader *et al*., 2006) was calculated by the formula.

$$THI = (0.8 \times T_{db}) + [(RH/100) \times (T_{db} - 14.4)] + 46.4$$

T_{db}- Dry bulb temperature, RH- Relative humidity

Six lactating Murrah buffalo with similar milk production, stage of lactation, parity and body weight were inducted in the experiment. Balanced diet was offered during whole course of the experiment along with *ad libitum* clean drinking water. Prophylactic deworming and vaccination against infectious diseases were carried out as per farm practices. The animals were kept in loose housing system with provision of shade.

Milking was done twice daily at 06:00 and 18:00 h and milk yield was recorded. Physiological parameters were recorded and blood was sampled on 30th day of each month at 14:30 h. Costal movements per minute was used to record RR and was expressed as breathes per minute (Pereira *et al*., 2008). Pulsation of the middle coccygeal artery at the base of the tail was used to record PR and was expressed as beats per minute while clinical thermometer was used to measure RT and was expressed in °C.

Four millilitres blood, in duplicate, was sampled by jugular vene-puncture with and without anticoagulant. Heparinized blood samples were used for hemogram whereas serum was harvested from non-heparinized blood samples by centrifugation at 3,000 rpm for 20 minutes, after clotting of blood, and stored at -20°C till analysis for biochemical and hormonal profile. The blood samples were analyzed for total packed cell volume (PCV), hemoglobin (Hb), total erythrocyte count (TEC) and leucogram by standard methods.

Alkaline phosphatase (AKP) and aspartate aminotransferase (AST) activity as well asconcentration of urea, glucose, creatinine, chloride, calcium and phosphorous were analyzed using kits (SPAN diagnostic Ltd., India) and flame photometry was used to estimate sodium and potassium concentration in the serum. The reactive oxygen species (ROS) (Brambilla *et al*., 2001) and superoxide dismutase (SOD) activity (Madesh and Balasubramanian, 1998) in serum was estimated to measure redox status. Cortisol, thyroxin (T_4), tri-iodothyronine (T_3) and prolactin was measured by ELISA kits (Thermo Fisher Scientific India, Private Limited).

The effect of THI on monthly lactation yield, physiological, biochemical and endocrine variables was analyzed using repeated measures ANOVA model (SAS, 9.4). Differences among the months were determined using Tukey's test (SAS, 9.4) and indicated by both P-value and superscripts ($P<0.05$). Least squares means and pooled standard errors have been reported. The level of significance was set at $P<0.05$.

RESULTS AND DISCUSSIONS

The average temperature and relative humidity during May, June and July was 36.03, 34.55 and 31.88°C, and 21.67, 33.57 and 77.32% respectively. The THI during May, June and July was 79.88, 80.57 and 85.36 respectively. The magnitude of heat stress depends on combined effect of several abiotic factors such as dry bulb temperature (dbT), relative humidity (RH), solar radiation and wind speed (Marai and Haeeb, 2010). In the present experiment, environmental temperature was high and the relative humidity was low during May and June which facilitated better evaporative cooling whereas in July, comparatively lower temperature but high relative humidity reduced the rate of evaporative cooling. As a result, the ambient THI changed from moderate to high during the course of study.

Milk production declined significantly ($P<0.05$) in the month of July as compared to May and June (Figure 1) indicating that level of stress was higher in July as compared to May and June. The parity, stage of lactation (Lacetera *et al*., 1996), breed and milk yield of the animal (Purwanto *et al*., 1990) influences milk production, however, heat stress is one of the important factors that can affect milk production in buffaloes. The results of present study suggested that milk production did not decline up to a THI of 80 (During June), however, the decrease in milk yield was evident at a THI of 85 (During July). Upadhyay *et al*., 2010 concluded that the milk yield was compromised in buffaloes when the average ambient temperature was increased by more than 4°C. Kumar *et al*. (2020); Ahmad *et al*. (2018) reported a decline in milk yield in Hariana and Sahiwal cattle, respectively only at THI of 81. A decline in milk yield was reported only at a THI above 80 in high producing crossbred cattle (Kohli *et al*., 2014) but minimal change in milk production was observed even at a THI above 80 in low producing crossbred cattle.

The effect of heat stress on physiological parameters in lactating Murrah buffalo is presented in Table 1. The RT did not change (P=0.580) throughout the experiment, however RR increased progressively during June and July while, PR decreased significantly (P<0.01) in the month of July as compared to May and June. In present study, the animals responded to increased heat load due to high THI by escalating heat loss through evaporative mechanism, *viz.* increasing the RR as in previous reports (Das *et al*., 2014; Singh *et al*., 2011; Rahangdale *et al*., 2011). A significant correlation between THI and physiological parameters such as RT, RR and PR was also reported (Bouraoui *et al*., 2002). The results indicated that RR began to escalate at a THI of 80; PR began to alter above a THI of 85 and RT even did not change at a THI above 85. As RR is the first indicator of heat stress (Yadav *et al*., 2017), the results of present study illustrated that buffaloes began to experience an increase in RR at a THI of 80. In present study, a minimal change in RT even at a THI of 85 suggested that physiological mechanisms of heat loss could prevent any increment in RT. Kumar *et al.* (2020) reported that at a THI of 80, RR and PR altered but RT did not change even at a THI of 84 in Hariana cattle.

The TLC and neutrophil percentage increased significantly (P<0.05) whereas the lymphocyte percentage was significantly (P<0.05) decreased during July (THI: 85.36) as compared to May (THI: 79.88). Other hematological parameters did not change during different months of the study (Table 2). The results revealed that only THI at 85 and above resulted in alteration in hematological parameters in buffaloes. Increased neutrophil/lymphocyte ratio (Parmar *et al*., 2013; Aengwanich *et al*., 2011) was also observed during higher THI in cattle which might be attributed to increased glucocorticoid (Silva *et al*., 2014) mediated alterations in the redistribution of lymphocytes from the blood to other body compartments (Dhabhar, 2002) during heat stress. In present study, minimal change in other hematological parameters suggested that at the exposed THI buffaloes were well adapted.

Creatinine, AST, calcium, chloride, superoxide dismutase activity, calcium, sodium and potassium levels did not change (P>0.05) during different summer months (Table 3). The urea and reactive oxygen species concentration in serum was significantly (P<0.05) higher whereas phosphorus concentration and AKP activity was significantly (P<0.05) lower in July (THI: 85.36) as compared to May (THI: 79.88). Serum urea level is highly variable and depends upon rumen ammonia levels, amino acids catabolism and gluconeogenesis (Marai and Haeeb, 2010). In present study, serum urea level increased only at a THI of 85 (Habeeb *et al*., 2007) as the animals experienced more intense heat load during this period. Contrary to findings of present study, serum sodium, potassium and chloride concentration were affected during summer months in buffaloes (Kumar *et al*., 2010; Singh *et al*., 2012). The present study indicated that most of biochemical parameters altered only after the lactating buffaloes were exposed to a THI of 85; and some of the biochemical parameters did not change even at a THI of 85. Kumar *et al.* (2020) reported that total antioxidant capacity increased only at a THI of 85 in Hariana cattle and some of the intermediate energy metabolites even did not change at a THI beyond 85. Redox status of the animal cell is disturbed under heat stress conditions both *in vivo* (Kumar *et al*., 2011; Yadav *et al*., 2015) and *in vitro* (Yadav and Korde, 2011) and results in oxidative stress (Rahal *et al*., 2014). In present study, ROS level increased during July (THI: 85.36) and SOD activity tended to increase

Table 1. Physiologic responses in heat stressed lactating Murrah buffaloes.

Parameter	May	June	July	SEM	P-value
RT	38.25	38.28	38.30	0.02	0.580
RR	31.00 [a]	38.83 [b]	37.00 [b]	0.85	0.000
PR	50.00 [a]	49.00 [a]	46.67 [b]	0.40	0.000

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

RT: Rectal Temperature; RR: Respiratory Rate; PR: Pulse Rate.

Table 2. Hematological parameters of heat stressed lactating Murrah buffaloes.

Parameter	May	June	July	SEM	P value
PCV	26.32	25.61	24.33	0.79	0.808
Hb	9.40	9.23	8.87	0.32	0.808
TEC	4.86	4.63	4.43	0.14	0.561
TLC	7.25 [a]	7.44 [b]	7.52 [b]	0.04	0.001
Neutro	31.00 [a]	32.67 [ab]	35.17 [b]	0.69	0.034
Lympho	60.00 [a]	58.17 [ab]	55.67 [b]	0.60	0.004
Mono	4.00	4.00	4.33	0.14	0.549
Eosino	5.00	5.17	4.83	0.40	0.949
Baso	0	0	0	0	0

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

PCV: Packed Cell Volume; Hb: Hemoglobin; TEC: Total Erythrocyte Count;

TLC: Total Leukocyte Count; Neutro: Neutrophil; Mono: Monocyte; Eosino: Eosinophil; Baso: Basophil.

Table 3. Serum metabolites, electrolytes, enzyme activity and redox status of heat stressed lactating Murrah buffaloes.

Parameter	May	June	July	SEM	P value
Urea	19.53[a]	20.03[a]	21.00[b]	0.18	0.000
Creatinine	1.91	1.87	1.93	0.085	0.075
AST	39.99	39.09	41.51	0.43	0.063
AKP	10.23[a]	10.62[a]	9.18[b]	0.18	0.000
Calcium	10.97	10.87	10.27	0.13	0.053
Phosphorus	5.43[a]	5.41[a]	4.70[b]	0.11	0.002
Chloride	89.13	89.60	89.80	0.23	0.499
SOD	308.80	298.90	306.17	10.16	0.928
ROS	4.56[a]	4.69[a]	5.34[b]	0.10	0.000
Glucose	47.53	48.33	46.60	2.28	0.226
Sodium	151.50	154.50	152.50	1.06	0.530
Potassium	7.17	6.50	7.00	0.24	0.530

Means with different superscripts in a row differ significantly ($P<0.05$).
Urea (mg/100 ml), Creatinine (mg/100 ml), Glucose (mg/100 ml), Sodium (mEq/L), Potassium (mEq/L), Chloride (mg/100 ml), AST Aspartate aminotransferase (IU/L), AKP Alkaline phosphatase (KA units), SOD Superoxide dismutase (U/ml), ROS Reactive oxygen species (mg H_2O_2 equivalents/ml).

Table 4. Endocrine parameters of heat stressed lactating Murrah buffaloes.

Parameter	May	June	July	SEM	P value
Cortisol	2.27[a]	2.79[a]	4.96[b]	0.30	0.000
Prolactin	55.25[a]	45.82[a]	150.43[b]	11.70	0.000
Triiodothyronine	1.62	1.51	1.88	0.09	0.242
Thyroxine	45.98[a]	35.17[b]	35.31[b]	2.08	0.039

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

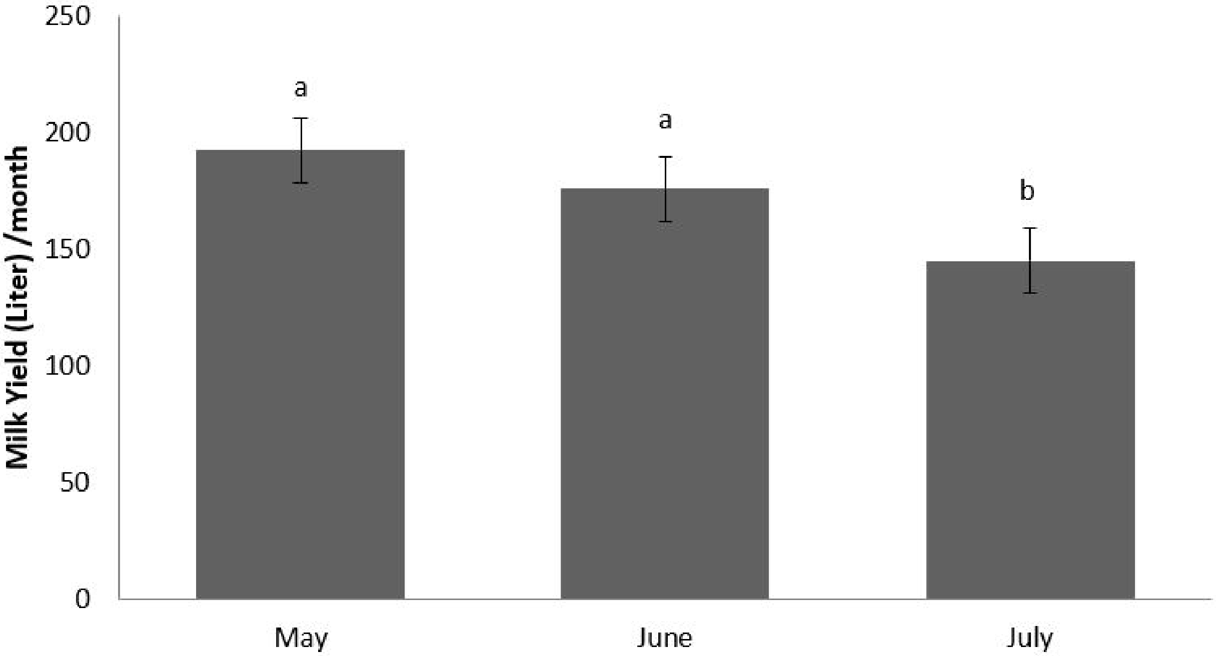

Figure 1. Milk production in heat stressed Murrah buffalo. Bars with different superscript differ significantly ($P<0.05$).

during the same period. In response to increase in ROS level at a THI of 85, SOD activity tended to increase. An increase in SOD activity in response to heat stress was also reported in buffaloes (Kumar *et al*., 2011; Yadav *et al*., 2021a). The results of present study revealed that intrinsic antioxidative defense system could minimize the increased ROS levels at a THI of 85. Kumar *et al.* (2020) reported that redox status did not change even at a THI of 85 in Hariana cattle. Yadav *et al*. (2021a) reported that a THI of 87 to 90 could inflict a moderate level of heat stress in buffalo heifers.

The serum T_3, T_4, cortisol and prolactin during summer stress in lactating buffalo is presented in Table 4. The serum cortisol and prolactin level increased and T_4 level decreased significantly ($P<0.05$) during July (THI: 85.36) as compared to May and June, however, the T_3 level did not change significantly ($P>0.05$). Hormones implicated in the acclamatory response to heat stress primarily include glucocorticoids and thyroid hormones (Silva *et al*., 2014; Yadav *et al*., 2021b), and prolactin (Roy and Prakash, 2007). The results of present study suggested that hormonal acclamatory responses were required only at a THI beyond 85 in lactating buffaloes. Increase in cortisol (Khongdee *et al*., 2011; Silva *et al*., 2014) and prolactin (Roy and Prakash, 2007) has been reported in buffaloes during summer stress. In present study, a decreasing trend in T_4 level was observed at higher THI for decreasing metabolic heat production. A similar decreasing trend in T_4 levels was established in buffalo in response to heat stress (Silva *et al*., 2014; Wankar *et al*., 2014; Yadav *et al*., 2021a).

The physiological, biochemical and hormonal responses during increasing THI suggested that at a THI of 80, lactating buffaloes experienced mild heat stress which was readily compensated by few physiological alterations. The lactating buffaloes experienced only a moderate type of heat stress evident from changes in milk yield and other physio-biochemical parameters at a THI of 85 which the animal was not able to compensate by alterations in its physiological capacities resulting in loss of productivity.

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