

SHORT COMMUNICATION

Effect of Heat Stress on Thermoregulatory Response of Murrah and Nili Ravi Buffaloes in the Subtropical Region of India

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ABSTRACT

Present study was conducted on the effects of thermal stress on adult Murrah and Nili- Ravi buffaloes (n=96). Using the temperature humidity index (THI), the thermal stress was calculated. THI was significantly ($P < 0.01$) different for respiration rate (RR) and rectal temperature (RT). Parity was found to be non-significant. The overall mean values for morning respiration rate (MRR), noon respiration rate (NRR), morning rectal temperature (MRT), and noon rectal temperature (NRT) were 18.86 ± 0.51 breaths/min, 23.55 ± 0.61 breaths/min, 100.29 ± 0.13 °F, and 100.50 ± 0.17 °F, respectively. Both season ($P < 0.01$) and breed ($P < 0.05$) had a significant effect on MRT. In contrast, only season had a significant effect on NRT. Significant effect of both breed and season ($P < 0.01$) was observed on morning respiration rate (MRR), whereas at noon, only season had a significant effect on noon respiration rate (NRR) ($P < 0.01$). Least squares means showed higher MRR (19.37 ± 0.51 breaths/min) and MRT (100.38 ± 0.13 °F) in Nili-Ravi buffaloes compared to Murrah buffaloes (MRR: 18.36 ± 0.52 ; MRT: 100.21 ± 0.13 °F). Murrah buffalo was found to be better thermotolerant than Nili Ravi buffaloes. Further validation in large population is required.

Key words: Buffalo, Heat stress, Murrah, Respiration rate, Temperature Humidity Index

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INTRODUCTION

The growing demand for livestock products in developing countries is being driven by rapid urbanization, rising incomes, and increasing human populations. This surge in demand places additional pressure on animal production systems, which are already challenged by the escalating effects of climate change. One of the most critical consequences of climate change on domesticated animals, particularly in both extensive and intensive production systems, is heat stress. Heat stress is a physiological condition that occurs when the environmental temperature and humidity exceed an animal's capacity to dissipate body heat effectively, thereby disrupting its thermal equilibrium. This condition has profound implications on growth, reproduction, milk production, feed intake, and overall welfare in livestock species.

Buffaloes, which contribute significantly to India's milk economy, are particularly vulnerable to heat stress due to their unique thermoregulatory limitations. Ruminants such as buffaloes are diurnal in nature, being more active during the day and resting at night. As a result, they accumulate more metabolic heat during daylight hours (Veissier *et al.* 2018). The challenge is further elevated when nighttime temperatures remain elevated, preventing effective heat dissipation, leading to chronic thermal stress (Becker *et al.*, 2020). Although several studies have addressed the impact of heat stress on milk yield and reproductive performance in buffaloes (Purohit *et al.* 2020), limited research exists comparing physiological responses such as respiratory

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rate and rectal temperature between Murrah and Nili-Ravi buffaloes under the same environmental conditions. Furthermore, studies assessing intra-day variations, such as early morning and afternoon physiological responses are particularly scarce, especially in the context of Punjab's climatic conditions, which are characterized by hot summers and fluctuating humidity levels. Therefore, the present study was aimed to evaluate and compare key physiological parameters during different seasons and at two distinct times of the day (morning and afternoon). The findings are expected

to provide insights into breed-specific thermotolerance and contribute to improved heat stress mitigation strategies in buffalo management systems.

MATERIALS AND METHODS

Experimental Animals

A total of 96 healthy adult females (46 to 76 months of age) of Murrah and Nili Ravi buffaloes (n=66 and 30, respectively) maintained with free access to feed and water, and standard management at Directorate Livestock Farm (DLF) of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India, were used. Ludhiana is situated between 30.9°N Latitude and 75.85°E longitude. 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. The study protocol was approved by the Institutional Animal Ethics Committee (No. 497/GO/Re/SL/02/CPCSEA/2021).

Measurement of Physiological Traits

Physiological traits, including respiration rate (RR), rectal temperature (RT), and heat tolerance coefficient (HTC) of adaptability, were evaluated as indicators of the heat stress response. Respiration rate and rectal temperature were recorded for all the animals in four seasons, winter, rainy, dry summer and hot humid summer, three times consequently and average was taken as final reading for association analysis. These parameters were recorded at the probable extreme hours of day, 6-8 am and 12-2 pm, respectively. Further, heat tolerance coefficient (HTC) was calculated using heat tolerance index developed by Benezra (1954): $HTC = RR/23 + RT/38.33$, where the denominator 23 and 38.33 are normal RR and RT under ideal conditions.

Temperature-humidity index (THI) were calculated for all days in all four seasons, winter (WS=50.96), rainy (RS=76.51), hot humid summer (HS=83.88) and dry summer (DS=80.67) during which physiological parameters were recorded and used in the association analysis. The THI value was determined based on temperature of wet bulb (Wb) and dry bulb (Db) that was calculated according to formula developed by National Research Council (NRC, 1971): $THI = 0.72 (Wb + Db) + 40.6$.

Statistical Analysis

The General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) software (Version 9.3) was used to conduct the statistical tests in order to identify any significant differences in a population. Effect of heat stress was analyzed using the following GLM (General linear model) in buffaloes:

$$Y_{ijkl} = \mu + T_i + P_j + B_k + A_l + e_{hijkl}$$

Where, Y_{ijkl} = o^{th} observation on RR/RT/HTC of buffaloes with i^{th} THI and k^{th} breed, μ = Overall mean, T_i = Fixed effect of i^{th} THI ($i = 1$ to 4), P_j = Fixed effect of j^{th} parity of animal ($j = H, 1-3$), B_k = Fixed effect of k^{th} breed ($k = 1$ to 2), A_l = Random effect of l^{th}

animal ($l = 1$ to 96) and e_{hijkl} = Random error associated with Y_{ijkl} observation and assumed to be NID ($0, \sigma^2e$)

RESULTS AND DISCUSSION

Seasonal variation had a highly significant ($p < 0.01$) effect on all physiological parameters. The highest values for MRR (22.20 ± 0.51 breaths/min), NRR (27.29 ± 0.61 breaths/min), MRT (100.75 ± 0.13 °F), and NRT (101.09 ± 0.17 °F) were observed during the Dry Summer and Hot Summer seasons, while the lowest values for all traits were recorded in Winter. When morning and noon rectal temperature was compared separately in both the breeds, season ($p < 0.01$) and breed ($p < 0.05$) was found to have significant effect on morning RT. Summers had Least square (LS) means of 100.75 ± 0.132 and 101.09 ± 0.179 , while winters were found to have lowest value (99.50 ± 0.132 and 99.56 ± 0.179) at morning and noon time, respectively (Table 1). Significant ($p < 0.01$) effect of both breed and season in case of respiration rate (RR) was seen at morning time, while at noon only season was found to be significant ($p < 0.01$). LS means of Nili Ravi on morning respiration rate was greater (19.37 ± 0.51) than Murrah buffalo (18.36 ± 0.52). Hot humid summers had highest value of respiration rate (22.20 ± 0.518), while winters were found to have lowest least square mean value (13.97 ± 0.51). On the other hand, LS means of dry summer (27.29 ± 0.61) and winters (18.47 ± 0.612) were found to have highest and lowest value during afternoon time. Non-significant effect of parity was found.

The thermoregulatory response of two breeds when compared, Murrah buffaloes revealed to be better thermotolerant than Nili Ravi buffalo having lowest value of respiration rate and rectal temperature. This may be due to poor thermoregulation mechanism of later. Yadav *et al.* (2016), Kumar *et al.* (2018) and Lakhani *et al.* (2018) in Murrah buffalo, and Ahmad *et al.* (2019) and Li *et al.* (2020) in Nili Ravi buffalo reported similar result of increase in respiration rate with increasing THI in their study. This result could be due to the increase in oxygen demand from the tissues under stressful conditions to maintain homeothermy by dissipating heat. On the other hand, Shenhe *et al.* (2018), Brcko *et al.* (2020) and Liu *et al.* (2019) in Nili-Ravi \times Murrah, Murrah \times Mediterranean and Nili-Ravi \times Murrah buffaloes, respectively, observed a decrease in respiration rate with increasing THI. Shenhe *et al.* (2018) reported that Mediterranean buffaloes exhibited lower heat tolerance compared to crossbred buffaloes under similar environmental conditions. In a related study, Sharma *et al.* (2023) observed that the onset of heat stress occurred earlier in buffaloes than in cattle, with physiological parameters such as rectal temperature (RT) and respiratory rate (RR) showing phasic changes in both species. Supporting these findings, Dayal *et al.* (2025) demonstrated significantly higher expression levels of heat shock protein 70 (HSP70) in Gangatiri cattle compared to buffaloes, indicating a more robust cellular response to thermal stress in cattle.

Table 1: Least square means of subclasses of different fixed effects for RR and RT of *HSPB6* gene in buffaloes for morning and noon respiration rate and rectal temperature

Effect	Subclass	MRR (breaths/min)	NRR (breaths/min)	MRT (°F)	NRT (°F)
	Overall Mean	18.86±0.51	23.55±0.61	100.29±0.13	100.50±0.17
Seasons (THI)	DS (THI=80.67)	21.48 ^b ±0.51	27.29 ^a ±0.61	100.75 ^a ±0.13	101.09 ^a ±0.17
	RS (THI=76.51)	17.80 ^c ±0.51	21.59 ^c ±0.61	100.21 ^c ±0.13	100.60 ^c ±0.17
	WS (THI=50.96)	13.97 ^d ±0.51	18.47 ^d ±0.61	99.50 ^d ±0.13	99.56 ^d ±0.17
	HS (THI=83.88)	22.20 ^a ±0.51	26.86 ^b ±0.61	100.73 ^b ±0.13	100.75 ^b ±0.17
Parity	1 st (n=23)	18.73±0.52	23.51±0.62	100.31±0.13	100.45±0.18
	2 nd (n=30)	19.04±0.50	23.52±0.59	100.27±0.12	100.56±0.17
	3 rd (n=15)	18.70±0.57	23.58±0.68	100.26±0.14	100.56±0.20
	Heifers (n=28)	18.98±0.52	23.60±0.61	100.35±0.13	100.44±0.18
Breed	Murrah (n=66)	18.36 ^b ±0.52	23.39±0.62	100.21 ^b ±0.13	100.51±0.18
	Nili-Ravi (n=30)	19.37 ^a ±0.51	23.72±0.60	100.38 ^a ±0.13	100.49±0.17

Figures with dissimilar superscript differ significantly, WS - winter, RS - rainy, HS - hot humid summer, and DS - dry summer.

Yadav *et al.* (2016), Talukdar *et al.* (2017), Kumar *et al.* (2018), Lakhani *et al.* (2018) in Murrah buffalo; Ahmad *et al.* (2019) in Nili Ravi buffalo and Shenhe *et al.* (2018) and Brcko *et al.* (2020) in Nili-Ravi × Murrah; Murrah × Mediterranean buffalo observed an increase in rectal temperature with increasing THI, while Liu *et al.* (2019) in Nili-Ravi × Murrah buffalo found a decrease in RT. In contrast to this, Li *et al.* (2020) and Wang *et al.* (2022) found no significant change in rectal temperature with increase in THI value in Nili Ravi buffalo. Shenhe *et al.* (2018) also found that crossbred buffalo (Nili-Ravi × Murrah) had a lower RT value compared to the Mediterranean buffalo. Comparing young and adult animals, Chen *et al.* (2025) in Taiwan swamp buffaloes, Osei-Amponsah *et al.* (2023) in Holstein cows and Gupta *et al.* (2022) in dairy cows concluded that younger animals have greater responses to heat stress than adult ones.

The findings in general revealed that the heat stress due to environmental temperature fluctuations concerns water buffaloes more than cattle breeds. Compared to Nili Ravi buffalo, the physiological heat-reduction mechanism of Murrah buffalo likely provides greater protection against heat stress. However, the study's scope is limited to Ludhiana region of Punjab, and future research should include a larger sample size and broader geographic regions to confirm these findings.

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