Investigating the relationship between heat load and shade seeking behavior in dairy buffaloes

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SUPPLEMENTARY FILE

Material & Methods

Study site, animals, housing, and management

A total of 60 Nili Ravi buffaloes were enrolled for the study, sourced from 12 different buffalo farms located near the University of Veterinary and Animal Sciences (UVAS). Among these 60 buffaloes, 24 were enrolled from 8 farms, three from each farm; 12 from 2 farms, 6 from each; 9 from one farm, and 12 from another farm. The average herd size comprised approximately 15 adult buffaloes, including dry and lactating animals. The farm contributed 12 buffaloes, had approximately 40 adult animals. The farms were selected using a convenience sampling approach based on specific criteria, which included farms having a loose housing system with shade structures (sheds) and adjacent loafing areas. The sole available shade structures were the sheds featuring precast concrete roofs.

The study was conducted over a period from June 2021 to August 2021, which represents the summer months when heat stress is a significant concern for dairy animals. All the enrolled buffaloes were lactating, non-pregnant, producing an average milk yield of 5.5 ± 1 liters per day, and were in the mid to late lactation stages (Table 1). Opting for lactating, non-pregnant buffaloes was a deliberate choice to ensure consistency in physiological conditions. This approach helps mitigate the potential impact of variations in physiological states, which can lead to divergent responses to heat stress. Additionally, focusing on lactating animals is of particular interest due to their higher heat load compared to dry animals. This allowed us to explore heat stress susceptibility and its implications more comprehensively.

Study design and behavioral observations

The study plan involved monitoring three buffaloes at a time for a duration of three consecutive days. The buffaloes were kept in the loafing area during the nighttime and had free access to move towards the shed (shaded area) during the daytime. This enabled the monitoring of shade-seeking behavior as a response to heat stress. The behavior was observed in the natural environment (in the field), as defined Bates and Martin (2021), which allowed buffaloes to express their behavior freely in their home environment and interact with each other. Our study employed the 'focused sampling' rule, limiting observations to three buffaloes at a time in a herd at a farm, and utilized the 'continuous recording' method for observing buffalo behavior.

Environmental measures

A portable weather meter (Kestrel 5400 Cattle Heat Stress Tracker: 0854AGLVCHVG) was used to measure the weather conditions during the study. The weather meter was placed in the open areas beyond the animal and was set up to take readings every 10 minutes. The meter recorded various meteorological measures, including atmospheric air temperature (°C), relative humidity (RH%), temperature humidity index (THI), heat load index (HLI), and black globe temperature (°C). The THI and HLI were calculated using the following equations:

THI (Kelly and Bond, 1971):

$$THI = (1.8 * Tdb + 32) - [(.55 - .0055 * RH) * (1.8 * Tdb - 26)]$$

Where Tdb represents the dry bulb temperature in °C and RH represents the relative humidity expressed as a percentage.

HLI (Agriculture Product Line User Guide, Kestrel® Instruments, Boothwyn, PA, USA):

$$HLI = WS(BGT) * HLIHI + (1 - WS(BGT)) * HLILO$$

Where BGT denotes the Black Globe Temperature in °C; RH represents the relative humidity expressed as a percentage; WS signifies the Wind Speed in m/s; HLIHI and HLILO are defined as follows:

If BGT is below 25: $HLILO = 1.3 * BGT + 0.28 * RH - WS + 10.66$

If BGT is 25 or higher: $HLIHI = 1.55 * BGT + 0.38 * RH - 0.5 * WS + \exp(2.4 - WS) + 8.62$

Statistical analysis

To resolve the issue of pseudo-replication, we designated each set of three buffaloes within a farm as a cohort and considered this cohort as the primary observational unit, rather than analyzing individual buffaloes separately. To achieve this, the daily data on buffaloes were averaged to obtain the cohort-level values. We had a total of 20 observational cohorts distributed across 12 farms, resulting in approximately 120 observations (20 cohorts, with two observations per cohort per day, over three days). However, in 8 of these cohorts, we could only obtain data for two days instead of the intended three days, resulting in a total of 112 observations for each studied variable. Normality of the study data was assessed using the Shapiro-Wilk test. To explore the relationship between shade-seeking behavior and other parameters, we employed repeated-measures analysis of variance (ANOVA) using the Mixed Procedure of SAS, as observations were recorded on buffaloes for three days. The model incorporated 'time of behavioral monitoring’ (before sunrise and at shade-seeking events) as a fixed effect, farm as a random effect and days for repeated measures statement. The chosen covariance structure for the analysis was 'compound symmetry,' which was employed to appropriately capture the correlations among repeated measurements within the same cohort. The Kenward-Roger method was applied to estimate the degree of freedom.

Results and Discussion

Shade seeking event and physiological measures

Table 1 presents comparison of various parameters measured during the early morning hours and at the shade-seeking event. Results of the study showed that there was no significant difference in core body temperature between the early morning measurements before sunrise and the measurements taken at the time of shade-seeking event (39.27°C vs. 39.26°C; P = 0.92). This suggests that the buffaloes maintained a relatively consistent core body temperature during these two time points, indicating that they regulated their body temperature by retaining heat within their skin and to some extent through an increased respiratory rate. It is important to note that the data loggers used (iButton, DS1921H) had a relatively low accuracy of ±1°C. Previous studies (Tresoldi et al., 2020) have demonstrated that this model tends to underestimate vaginal temperatures of cows by approximately 0.7°C when compared to higher-accuracy (±0.1°C) models. The relatively low accuracy of our temperature recording device may have contributed to the absence of differences observed in core body temperature at the two time points, and this represents a potential limitation of our study.

The climate measures of THI and HLI were significantly higher during the shade-seeking event compared to before sunrise. The THI increased from 77.6 to 81.7, with a change (Δ) of 4.1 ($p < 0.001$), indicating a rise in thermal discomfort. Similarly, the HLI showed a substantial increase from 81.6 to 96.3, with a Δ of 14.7 ($p < 0.001$), highlighting the increased heat load on the buffaloes. These findings suggest that the buffaloes sought shade in response to the elevated heat stress indicated by the higher THI and HLI values. Shade-seeking behavior can be seen as an adaptive strategy employed by the buffaloes to mitigate the effects of heat stress and seek thermal relief during periods of increased thermal discomfort.

Recommendations

The findings from this study have implications for the development of effective strategies to mitigate heat stress in dairy buffalo herds and improve animal welfare. By understanding the factors influencing shade-seeking behavior, such as body surface temperature, farmers and researchers can implement appropriate cooling and management interventions to create comfortable and thermally conducive environments for buffaloes during periods of high heat stress. Ultimately, this research contributes to enhancing the overall productivity and well-being of dairy buffaloes in hot climatic regions.

References

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Tresoldi, G., K.E. Schütz, and C.B. Tucker. 2020. Sampling strategy and measurement device affect vaginal temperature outcomes in lactating dairy cattle. J. Dairy Sci. 103(6): 5414-5421.

Kelly C.F. and T.E. Bond. 1971. Bioclimatic factors and their measurement. 7–92 in A Guide to Environmental Research on Animals. National Research Council, ed. National Academies Press, Washington, DC.

Table S1. Characteristics of study buffaloes and climate measures during the observation period.

Items	**Means ± SD**	**Minimum**	**Maximum**
Buffalo characteristics			
Parity, n	3 ± 1	1	5
Days in milk, months[1]	6 ± 1	5	8
Milk yield, liters[2]	5.5 ± 1	4	6.5
Climate measures			
Air temperature (T, °C)	30.3 ± 5.2	21	46.9
Temperature humidity index (THI)	81.4 ± 6.4	67.2	94.3
Heat load index (HLI)	93.3 ± 16.9	52	128
Black globe temperature (BGT, °C)	34.2 ± 9.3	18.6	56.5
Relative humidity (RH, %)	70 ± 15	30	100
Wind speed (WS, m/s)	0.19 ± 0.44	0	3.2

[1] Farmers did not maintain data recording; the values were of their best recall.
[2] Milk yield was calculated using one-liter measuring cups, a common tool to measure milk volume at farms.
[3] Climate measures: from sunrise to noon (5:00 AM to 12:00 PM).

Figure S1. Hussain Shahid

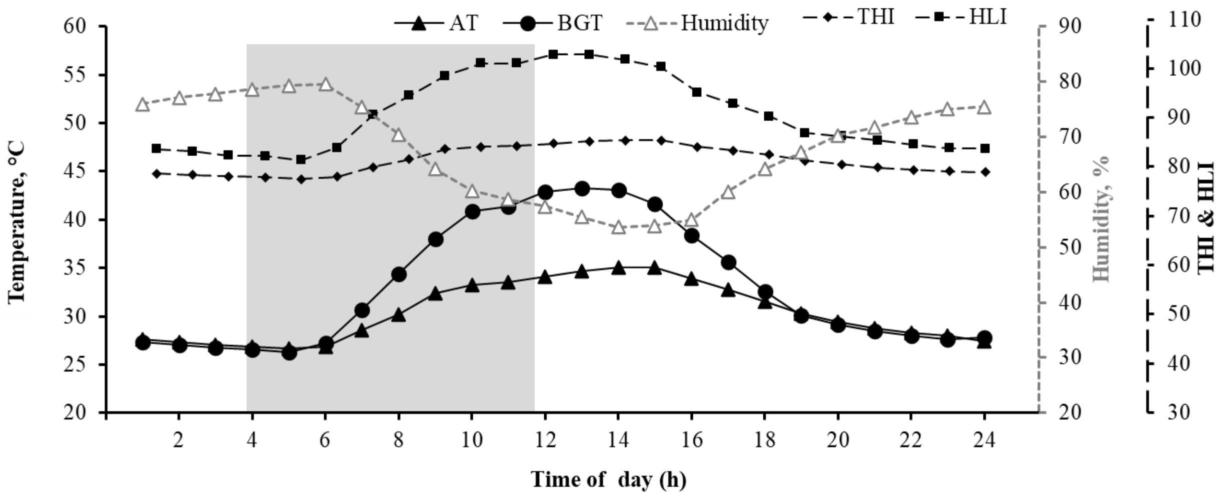

Figure S1 Hourly patterns of climate measures during the study period: air temperature, C (AT), relative humidity (%), black globe temperature (BGT), temperature humidity index (THI), and heat load index (HLI). The shaded area represents the period during which observations were recorded (0400 to 1200 h).