Journal of Dairy Research

cambridge.org/dar

Research Article

Cite this article: Hussain SI, Rashid MA and Shahid MQ. Investigating the relationship between heat load and shade seeking behaviour in dairy buffaloes. *Journal of Dairy Research* https://doi.org/10.1017/S0022029924000256

Received: 2 February 2024
Accepted: 27 March 2024

Keywords:
Body surface temperature; shade-seeking behaviour; water buffaloes

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

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Abstract

The study presented in this Research Communication aimed to investigate the relationship between physiological responses, body surface temperature and shade-seeking behaviour in Nili Ravi dairy buffaloes during summer months. We enrolled 60 buffaloes, and each animal was observed for three consecutive days starting before sunrise until they moved towards the shade structures. A repeated measures ANOVA was employed to assess the changes in physiological parameters and body surface temperature between the early morning and the occurrence of shade-seeking behaviour. The average temperature humidity index and heat load index during the behavioural monitoring period (0400 to 1200 h) were 81.3 ± 6.5 and 92.9 ± 17, respectively (mean ± SD). There was no significant difference in core body temperature between sunrise and the time of shade-seeking event. However, the buffaloes had a slightly higher respiration rate at the time of shade-seeking (19.2 vs. 22.4 breaths/min). In addition, body surface temperature, measured at the flank region, shoulder, base of the ear and forehead was significantly higher at the occurrence of shade-seeking behaviour compared to the early morning. On average, the buffaloes sought shade when the surface temperature was 2°C higher than the temperature recorded before sunrise. Overall, the current findings suggest that body surface temperature, rather than core body temperature was strongly associated with shade-seeking behaviour in dairy buffaloes. These findings could be useful in developing strategies to mitigate the effects of heat stress in dairy buffalo herds and thereby improve animal welfare.

In regions like the Indo-Pakistan subcontinent, where buffaloes are the major dairy animals (Minervino *et al*., 2020), heat stress poses a significant challenge (Bah *et al*., 2021, 2022). Understanding the heat tolerance and behaviour of buffaloes under direct sunlight becomes even more crucial, considering the common practice of smallholder buffalo farmers keeping their animals tied outdoors during the morning hours for daily chores (Shahid MQ, personal observation). This practice significantly increases the heat load experienced by the buffaloes.

Water buffaloes have evolved various morphological, physiological, and behavioural mechanisms to cope with high environmental temperatures and maintain thermal balance. The thick epidermis and dark colour of buffalo skin play a vital role in their thermoregulatory response (Hafez *et al*., 1955; Mason, 1974). The presence of melanin particles in the skin surface gives it a characteristic black colour and helps trap ultraviolet rays, preventing them from penetrating deeper tissue layers (Marai and Haeeb, 2010). However, the dark skin of buffaloes also contributes to the absorption of a significant amount of solar radiation (Mota-Rojas *et al*., 2021). Combined with their sparse coat or hair, buffaloes are more susceptible to heat accumulation than cattle. In response to this heat absorbance, buffaloes have developed adaptive behaviours to cope with high temperatures. One such behaviour is shade-seeking (Marai and Haeeb, 2010), where they actively seek sheltered areas to minimize direct exposure to the sun and reduce heat absorption. Seeking shade provides a cooler environment (Vilela *et al*., 2022), helping to mitigate the negative effects of solar radiation on their body temperature. Additionally, buffaloes utilize wallowing as a thermoregulatory strategy (Marai and Haeeb, 2010). Wallowing involves immersing themselves in water or mud, which helps dissipate heat through convection. One interesting aspect is that the heat load in buffaloes may not be immediately evident due to their less apparent respiration rate compared to other species. While cattle may exhibit more visible signs of heat stress, such as panting, buffaloes may show fewer external indicators. This makes it even more important to delve deeper into their physiological responses and behavioural adaptations to heat stress.

Therefore, the present study aimed to investigate the association between physiological responses, body surface temperature, and shade-seeking behaviour in Nili Ravi dairy buffaloes during the summer months. By examining the changes in physiological parameters and body surface temperature from early morning (before sunrise) until the occurrence of shade-seeking behaviour, we aimed to elucidate the underlying mechanisms involved in the buffaloes' thermoregulatory responses. This knowledge can guide the development of effective strategies to mitigate heat stress and improve the overall welfare and productivity of these animals.

Materials and 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). 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 average herd size comprised approximately 15 adult buffaloes, including dry and lactating animals. All the chosen farms had a dirt floor in the loafing area and lacked trees for natural shade coverage. All experimental procedures were conducted in accordance with the guidelines of the Ethical Review Committee of UVAS (dr/16:19-01-2021).

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 l per day, and were in the mid to late lactation stages. The fresh feeding was done around 1100 h, approximately 5 h after sunrise, under the shade. Additional details about the selected farms and buffaloes are provided in the online Supplementary material and methods section, Supplementary Table S1.

Study plan

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 behaviour as a response to heat stress. Each day, prior to sunrise (approximately 0500 h), a trained observer recorded the respiration rate (RR) and body surface temperature (ST) of the buffaloes while they were in the outdoor loafing area. These same variables, including RR and ST, were recorded during the shade-seeking event. The shade-seeking event was determined as a buffalo's movement towards shade, stopping under the shade structure. Buffaloes were considered to be under shade when approximately more than half of their body, extending up to the flank region, was covered by shade.

Physiological measures and surface temperature recording

The RR was recorded by counting the number of flank movements of a buffalo over a period of 30 s using a stopwatch, which was then converted to breaths per minute. Body surface temperature was measured at different regions, including the flank, shoulder, base of the ear and forehead, using an infrared thermal camera (model: FLIR C3-X compact thermal camera, thermal sensitivity <70 mK; resolution 0.1°C; FLIR Systems, Inc., Wilsonville, OR, USA). The buffaloes were observed without any restraint. The observer recorded temperatures from various body parts while remaining approximately 2–3 m away, ensuring minimal disruption to the buffaloes. There were no metal structures in the vicinity that could potentially interfere with the infrared camera readings. Most of the farms were equipped with cemented water troughs in the loafing area.

The core body temperature (CBT) of each buffalo was continuously monitored for 24 h during each observation period using intravaginal data loggers (Thermochron iButton: model DS1921H-F5, accuracy: ± 1°C, resolution: ± 0.125°C, iButtonLink, Llc., Whitewater, USA) administered with an inert controlled internal drug release insert (Zoetis, Auckland, New Zealand). These data loggers were programmed to record body temperatures at 20-min intervals. For analysis, we utilized the body temperature values recorded within 20 min following a shade-seeking event.

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 min. 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).

Statistical analysis

All the statistical analyses were performed using SAS (SAS for Academics: SAS Institute Inc., Cary, NC, USA). As the buffaloes were monitored in groups of three, which belonged to the same social group within the same farm, the independence of observations could be compromised, potentially leading to pseudo-replication. To resolve this issue, we designated each set of three buffaloes within a farm as a cohort and considered this cohort as the primary observational unit, rather than analysing individual buffaloes separately. To explore the relationship between shade-seeking behaviour 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 behavioural monitoring' (before sunrise and at shade-seeking events) as a fixed effect, farm as a random effect and days for repeated measures statement. The least square means were separated using the PDIFF option with Tukey's adjusted P-values. Significance was considered at $P \leq 0.05$. Further details about statistical analyses are provided in the online Supplementary material and methods section.

Results and discussion

Baseline data

Mean values for animal characteristics and environmental data are reported in online Supplementary Table S1 and environmental changes during the day are in Fig. S1.

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

https://doi.org/10.1017/S0022029924000256 Published online by Cambridge University Press

Table 1. Physiological and body surface temperature variations changes of Nili Ravi buffaloes during shade-seeking behaviour in summer: LS Means ± SE

Items	Event timings		SEM	Change (Δ)	P value
	Early morning	At shade seeking			
Core body temperature, °C	39.24	39.25	0.07	0.01	0.739
Respiration rate, breaths/min	19.2	22.4	0.44	3.2	<0.001
Surface temperature, °C					
Shoulder	35.9	37.6	0.16	1.7	<0.001
Flank	36.4	38.5	0.14	2.1	<0.001
Forehead	35.6	38.2	0.16	2.5	<0.001
Ear base	36.9	38.4	0.12	1.5	<0.001
Udder	36.4	38.6	0.14	2.2	<0.001
Climate measures[4]					
T, °C	26.9	30.3	0.81	3.4	<0.001
THI	77.9	82.1	1.30	4.2	<0.001
HLI	82.1	97.2	2.9	15.1	<0.001
BGT, °C	26.8	34.9	1.13	8.1	<0.001
RH, %	79.2	71.2	2.9	−8.0	0.003
Wind speed, m/s	0.10	0.25	0.03	0.15	0.07

SEM, standard error of mean; Change (Δ), Difference between the values at early morning and at shade seeking; THI, Temperature humidity index; HLI, Heat load index.
Event timings: 'Early morning' refers to the measurements taken before sunrise, while 'At shade seeking' refers to the measurements recorded when buffaloes had moved towards the shade structures.

measurements before sunrise and the measurements taken at the time of shade-seeking event (39.27°C vs. 39.26°C; $P > 0.05$). 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.

In contrast to the stable core body temperature, the study found a significant increase in the respiration rate of the buffaloes from 19.5 breaths per minute before sunrise to 22.4 breaths per minute during the shade-seeking event ($\Delta = 2.9$ breaths per minute, $P < 0.001$). This increase in respiration rate suggests that the buffaloes experienced an elevated respiratory effort and possibly increased heat dissipation during the shade-seeking period. It indicates that shade-seeking behaviour plays a role in the thermoregulatory response of buffaloes to mitigate heat stress and maintain thermal comfort. The respiration rate observed in our study aligns with the reported range documented in the literature (Kumar and Kumar, 2013; Bah *et al.*, 2022) for both dry and humid hot summers. This consistency is particularly notable considering a THI of approximately 80 or above. While our study's RR values are comparable to those reported by Chaudhary *et al.* (2015) for humid hot summers with an average THI of about 80, an additional average increase of 14 breaths per minute in respiration rate during dry hot summers remains insufficiently explained, even with a mere 1-point rise in THI. It's plausible that during the dry hot summer conditions, the buffaloes might have been subjected to extreme heat stress. In our study, the recorded values correspond to a time when the buffaloes first experienced the effects of heat stress. Further research could provide a more comprehensive understanding of the physiological variations observed in buffaloes' responses. The latency time from sunrise to the shade seeking event was about 178 ± 65 min (means ± SD). The sunrise time ranged from approximately 0500 h in June to around 0600 h in August. However, the available data were limited for making comprehensive comparisons regarding these findings.

Body surface temperature measurements showed significant differences across different regions (Table 1). On average, the buffaloes sought shade when the surface temperature was 2.1°C higher than the temperature recorded before sunrise. The shoulder temperature increased from 35.9 to 37.6°C ($\Delta = 1.7$°C), the flank temperature increased from 36.3 to 38.6°C ($\Delta = 2.3$°C), the forehead temperature increased from 35.5 to 38.2°C ($\Delta = 2.7$°C), the ear base temperature increased from 36.9 to 38.4°C ($\Delta = 1.5$°C) and the udder temperature increased from 36.3 to 38.6°C ($\Delta = 2.3$°C, all temperature increases $P < 0.001$). The body surface temperature observed in our study aligns with the range reported in the literature (Kumar and Kumar, 2013; Shenhe *et al.*, 2018; Li *et al.*, 2020; Wang *et al.*, 2022). Specifically, during the summer season, surface temperatures ranged from 33 to 38°C, which closely aligns with our results. It's noteworthy that some other studies that assessed buffalo surface temperatures implemented cooling strategies that may not directly correspond to the specific objectives of our present study. The consistent core body temperature observed before sunrise until the shade-seeking event aligns with findings in previous literature (Jasinski *et al.*, 2023). Studies have indicated that the body temperature of buffaloes remains stable, even in the face of elevated THI levels. The body temperature values fall within the range of temperatures previously reported for buffaloes during the summer season.

The rise in surface temperature and the relatively stable core body temperature as the THI increases emphasize the significant role played by buffalo skin in thermoregulation. The skin acts as a protective barrier (Marai and Haeeb, 2010), absorbing heat from the sun and preventing its immediate transfer to the inner body

https://doi.org/10.1017/S0022029924000256 Published online by Cambridge University Press

parts. This mechanism helps to maintain a stable core body temperature and minimize the impact of external heat on the buffalo's physiological processes. The ability of the skin to absorb heat under direct solar radiation and then dissipate it quickly is crucial for the buffalo's thermoregulatory efficiency (Marai and Haeeb, 2010) and adaptation to varying environmental conditions. The low hair density of buffalo skin facilitates the rapid dissipation of heat through radiation (Napolitano *et al*., 2013). Increased blood volume and flow to the skin could also have played a role in elevating skin surface temperature and maintaining core body temperature in direct solar radiation exposure (Koga *et al*., 1999). This adaptive feature allows buffaloes to withstand and cope with high environmental temperatures more effectively.

Further studies focusing on the skin structure, including skin thickness and its influence on thermal regulation, would provide a deeper understanding of the thermoregulatory adaptations of buffaloes. Such investigations could lead to the development of targeted management strategies and breeding programmes aimed at enhancing the natural thermoregulatory capacity of buffaloes and improving their resilience to heat stress.

In conclusion, the findings of the study indicated that there was no significant difference in core body temperature before sunrise and at the time of shade-seeking behaviour. However, buffaloes exhibited a slightly higher respiration rate during shade-seeking. Furthermore, the body surface temperature, measured at various regions, was significantly higher during shade-seeking compared to before sunrise. On average, the buffaloes sought shade when the surface temperature was 2°C higher than the temperature recorded before sunrise. These results suggest that body surface temperature, rather than core body temperature, was strongly associated with shade-seeking behaviour in dairy buffaloes.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000256

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