

Research Article

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Evaluating the impact of sprinkler cycle and flow rate on dairy buffalo performance during heat stress

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Abstract

The aim of this study was to evaluate the effect of the sprinkler cycle and flow rate on physiological, behavioural, and productive responses in dairy buffaloes. Nine Nili Ravi lactating buffaloes were subjected to three sprinkler cycles and two flow rates using a double replicated 3 × 3 Latin square design. The flow rates were 1.25 and 2 l/min, and the sprinkler cycles (minutes water on/off, number of cycles/h) were: 3/3, 10 cycles; 3/6, 7 cycles and 3/9, 5 cycles. The showering was applied from 0800 till 1630 h daily. In the first square of 21 d, each of the three sprinkler cycles was applied using a 1.25 l/min flow rate for 7 d per cycle. In the later square, the same treatments (sprinkler cycles) were applied using the 2 l/min flow rate. The average temperature humidity index during the study period was 85.7 ± 3.8 (Mean ± SD). The result showed that the 3/3 treatment group had lower body temperature and respiration rate than the other groups. The buffaloes in the 3/3 group produced 0.5 and 0.7 kg more milk with 1.4 and 2.4% more fat than the 3/6 and the 3/9 treatment groups, respectively. Similarly, the 2 l/min flow rate had a lower core body temperature and respiration rate and higher milk yield than the 1.25 l/min group. The 3/3 showering cycle with a 2 l/min flow rate appeared effective in improving physiological responses and milk yield in dairy buffaloes.

Sprinkler cooling is widely used to mitigate heat stress in dairy cows. However, the use of sprinklers to cool dairy buffaloes is not as prevalent in the Indo-Pak subcontinent, where buffaloes are the primary dairy animal. Typically, dairy buffaloes cool themselves by wallowing in running water or ponds (Napolitano *et al.*, 2013). As the access to surface water sources decreases, sprinkler cooling and water application using handheld hosepipes are now popular ways of cooling dairy buffaloes in the region (Bah *et al.*, 2021a). Since these methods consume significant amounts of groundwater (Bah *et al.*, 2022), it is essential to explore efficient ways of using sprinklers to cool buffaloes.

Sprinklers are commonly used to cool dairy cows during the summer months (Anderson *et al.*, 2013; Tresoldi *et al.*, 2018a), as they positively impact physiological and behavioural responses. Studies have demonstrated that sprinkler cooling can lower body temperature and respiration rate (Chen *et al.*, 2013, 2016; Tresoldi *et al.*, 2019), increase feed intake and boost milk yield (Keister *et al.*, 2002). Additionally, it improves behavioural responses such as eating time, rumination time and resting time (Chen *et al.*, 2016; Tresoldi *et al.*, 2018b). Recently, researchers have been focusing on improving water usage efficiency in cow cooling (Tresoldi *et al.*, 2018a, 2018b, 2019; Bah *et al.*, 2021a, 2021b) as a response to the declining levels of groundwater due to climate change (Taylor *et al.*, 2013). Strategies such as controlling sprinkler flow rate (Chen *et al.*, 2015, 2016; Tresoldi *et al.*, 2019), droplet size (Chen *et al.*, 2015) and sprinkler cycle (Tresoldi *et al.*, 2018b) have been evaluated to optimize the cooling process. Studies have shown that in dry and hot Mediterranean climates, cows cooled with sprinklers with a flow rate of 1.3 and ≥4.5 l/min had similar physiological responses (Chen *et al.*, 2015). Similarly, an earlier study showed that there were no additional benefits of cooling cows with higher flow rates (8.2 and 11.7 l/min) compared to the lowest (5.2 l/min in a free-stall barn; Means *et al.*, 1992). In semi-arid summer, cows cooled with 1.25 and 2.0 l/min flow rates produced similar physiological and productive responses (Bah *et al.*, 2021a; Macavory *et al.*, 2023). While relatively limited research has been conducted on dairy buffaloes, a recent study showed that sprinklers with flow rates of 1.25 and 2.0 l/min resulted in similar physiological and productive responses in buffaloes (Bah *et al.*, 2021b). Further research in this area could lead to a better understanding of the efficiency of sprinkler cooling in buffaloes, particularly in relation to sprinkler cycles and flow rates. The objective of this study was to assess the impact of varying sprinkler cycles and flow rates on the physiological, behavioural, and productive responses of dairy buffaloes during semi-arid summer.

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Materials and methods

Study site

The study was conducted at a commercial dairy farm near the University of Veterinary and Animal Sciences Lahore (UVAS), Ravi Campus, Pattoki, Pakistan during the hot and humid summer months of July and August 2021, spanning 42 d. All experimental procedures were conducted in accordance with the guidelines of the Ethical Review Committee of UVAS (dr/16:19-01-2021).

Study animals, housing and management

Nine Nili Ravi buffaloes with an average daily milk yield of 6.6 ± 0.7 kg, days in milk of 100 ± 14 , and parity of 3.6 ± 0.8 , were enrolled for the study. The buffaloes were housed in a traditional, naturally ventilated shed with a precast concrete roof and brick floor. The daily feeding allowance, on as-fed basis, included 60 kg of fresh sorghum fodder and 3 kg of concentrate per buffalo. Individual water tubs were placed for each buffalo in the shed during the daytime and a water trough, in the loafing area, was available for free access during the nighttime. Hand milking was done twice daily (0500 and 1700 h). Further details regarding housing and management can be found in the online Supplementary File.

Experimental design and treatments

The study employed a double replicated 3×3 Latin square design with the aim of evaluating the effects of two flow rates and three sprinkler cycles. The flow rates were 1.25 and 2 l/min, and the sprinkler cycles (minutes water on/off, number of cycles per hour) were: 3/3, 10 cycles, 3/6, 7 cycles and 3/9, 5 cycles. The study was conducted in two Latin squares, each consisting of three periods with 7 d per period. In the first square, during each period, the three sprinkler cycles were applied to the buffaloes (3 buffaloes/cycle) using a flow rate of 1.25 l/min. The second square had the same design, except that the flow rate was increased to 2.0 l/min. Online Supplementary Table S1 provides a detailed outline of the treatment application. Adaptation was carried out in the first four days of each period, and data were collected for the remaining 3 d.

Environmental measures

The environmental measures of air temperature (T , °C), humidity %, temperature humidity index (THI), black globe temperature, heat load index and wind speed were recorded using a portable weather station (Kestrel 5400 cattle heat stress tracker: 0854AGLVCHVG). The weather station was configured to record climate measures at 20-min intervals, for 24 h. It was placed in the outside loafing area.

Physiological and behavioural measures

The vaginal temperature (VT), as an indicator of core body temperature of buffaloes, was recorded every 20 min for 3 consecutive days in each period using thermochron dataloggers (iButton: model DS1921H-F5, iButtonLink, LLC., Whitewater, USA). The data loggers were placed intravaginally using an inert CIDR device. Respiration rate (RR) of all the enrolled buffaloes was recorded at eight different time points: 0700, 0900, 1100, 1300,

1500, 1700, 1800 and 2100 h. The RR was measured by visually observing flank movements and counting them to determine the number of breaths per minute. The same observer measured the respiration rate of all buffaloes. Body surface temperature (ST) was measured at the same eight different time points of the day as the RR, using a thermal camera (model: FLIR C3-X compact thermal camera, thermal sensitivity <70 mK; FLIR Systems, Inc., Wilsonville, USA). The ST was recorded for four different regions: the shoulder, flank, udder, and lower belly.

The Nedap CowControl™ system (NEDAP, Groenlo, Netherlands) was utilized to record behavioural measures of lying time, eating time, rumination time, standing time and the number of steps.

Milk yield and milk components

The daily milk yield for each buffalo from morning and evening milking was recorded in Kg. Once a week, samples of the milk were taken and analysed for their protein, fat and lactose content using a portable milk analyser (model: Lactoscan Standard; Milkotronic Ltd, Nova Zagora, Bulgaria).

Selected blood metabolites

Blood samples were collected once every week from the jugular vein during the early morning hours (from 4:00 to 5:00 a.m.). The samples were drawn into sterile anticoagulant vacutainer tubes, immediately placed in a cold chain and transported to the laboratory. Once in the laboratory, the samples were centrifuged and the serum was separated and stored at -20°C until analysed. The serum was analysed for glucose (using the Glucose GOD FS kit from DiaSys Diagnostic Systems GmbH, Holzheim, Germany), blood urea nitrogen (using the Randox Urea Kinetic kit from Randox Laboratories Ltd., UK) and cortisol (using the Cortisol Elisa kit from Calbiotech, California, USA) with the help of a spectrophotometer (Epoch2, BioTek, Winooski, VT, USA).

Statistical analysis

All the statistical analyses were performed using SAS software (SAS for Academics: SAS Institute Inc., Cary, NC). To obtain period means, the data collected for individual buffaloes on the recording days of each period were averaged. The normality of the data averages was assessed using the Shapiro-Wilk test, using the Univariate Procedure of SAS. For normally distributed data, ANOVA was performed using the Mixed Procedure of SAS. The following model was used for the ANOVA:

$$Y_{ijkl} = \mu + T_i + F_j + P_{kj} + C_l + e_{ijkl}$$

where Y_{ijkl} = the dependent variable; μ = the overall mean; T_i = the fixed effect of treatment (showering cycle) i , where $i = 3|3, 3|6, \text{ or } 3|9$, three showering cycles; F_j = the fixed effect of flow rate j , where $j = 1$ or 2 , 1.25 or 2.0 l/min; P_{kj} = the fixed effect of period k within flow rate j , where $k = 1, 2, \text{ or } 3$, three periods; C_l = the random effect of buffalo l , where $l = 1 \dots n$; and e_{ijkl} = the random error. The least-square means were separated using Tukey's adjusted P -values. The differences were considered significant at $P \leq 0.05$. Data are presented as mean \pm SEM unless stated otherwise.

Table 1. Effect of showering cycle and flow rate on milk yield and milk components of Nili Ravi buffaloes during summer, presented as LS Means \pm SEM

	Showering cycle min water on/off				Flow rate, l/min			P value		
	3/3	3/6	3/9	SEM	1.25	2.0	SEM	Cycle	Flow	Cycle \times flow
Milk yield, kg/d	7.3 ^a	6.8 ^b	6.6 ^b	0.14	6.5 ^a	7.3 ^b	0.12	0.001	0.001	0.501
Milk components, %										
Fat	7.4 ^a	7.1 ^a	6.4 ^b	0.37	7.0	6.8	0.34	0.001	0.231	0.541
Protein	3.1	3.1	3.2	0.06	3.1	3.2	0.05	0.159	0.252	0.852
Lactose	4.47	4.48	4.64	0.06	4.48	4.57	0.05	0.101	0.284	0.838
Milk components, g/d										
Fat	547 ^a	480 ^b	423 ^c	20.3	466	502	17.8	0.001	0.056	0.938
Protein	231 ^a	212 ^b	213 ^b	6.0	203 ^a	234 ^b	5.0	0.049	0.001	0.812
Lactose	333 ^a	305 ^b	306 ^b	7.9	294 ^a	336 ^b	6.7	0.018	0.001	0.801

Within a row, means with dissimilar superscripts differ, $P < 0.05$ or better

Results

Environmental measures

The environmental measures are summarized in the online Supplementary Table S2. The average T and THI during the daytime treatment application period were $37.6 \pm 2.8^\circ\text{C}$ and 89 ± 2 , respectively (mean \pm SD). The extreme values for these variables were 44.5°C and 94, respectively.

Milk yield and milk components

Data are shown in Table 1. Both the sprinkler cycle and flow rate influenced milk yield. However, no interactions or trends for cycle and flow rate were observed ($P > 0.05$). Buffaloes in the 3/3 group produced 0.7 and 0.5 kg/d more milk per day than those in the 3/9 and 3/6 groups (Table 1). Similarly, buffaloes in the 2.0 l/min flow rate group produced 0.8 kg more milk per day compared to those in the 1.25 l/min group. Buffaloes in the 3/3 group had a 1% higher fat percentage compared to those in the 3/9 group but the 3/3 and 3/6 groups had similar fat percentages (Table 1). The sprinkler cycle and flow rate did not influence the milk protein and lactose percentage. However, the total milk components yield was significantly higher in the 3/3 sprinkler cycle group compared to the other treatment groups. Specifically, buffaloes in the 3/3 group had 124 g more fat yield than the 3/9 group (Table 1). Additionally, the 3/3 group had higher protein and lactose yield than the other groups. Similarly, the milk components yield in the 2.0 l/min flow rate group was higher compared to the 1.25 l/min group (Table 1).

Physiological measures

VT data are presented in Fig. 1. Both the sprinkler cycle and flow rate affected VT. Buffaloes in the 3/3 group had slightly lower VT in comparison to 3/9 group during the daytime (39.0 vs. 39.1°C ; SEM = 0.02; Fig. 1A). Overall, buffaloes in all the groups had higher VT during the early nighttime compared to the daytime and the early morning. On average, the VT during the early nighttime and early morning was 39.5 ± 0.04 and $38.9 \pm 0.03^\circ\text{C}$, respectively. Similarly, buffaloes in the 2 l/min flow rate had 0.2°C lower VT in comparison to the 1.25 l/min group, during the daytime (38.9 vs. 39.1°C ; SEM = 0.02; Fig. 1B). The 24 h VT averages

showed a similar pattern; the 2 l/min flow rate had 0.2°C lower VT than the 1.25 l/min group (39.1 vs. 39.3°C ; SE = 0.02; Fig. 1B).

RR is presented in Fig. 2. Both the sprinkler cycle and flow rate affected RR. There was no interaction observed between cycle and flow rate on RR in our study ($P > 0.05$). Buffaloes in the 3/3 sprinkler cycle group had slightly lower RR in comparison to 3/9 group during the daytime showering period (18 vs. 19 breaths/min; SEM = 0.22; Fig. 2A) whilst the 3/3 and 3/6 groups had similar RR. Overall, buffaloes in all the groups had higher RR in the evening and early nighttime compared to the daytime. The average RR in the early nighttime was 23 breaths/min. Buffaloes in the 2 l/min flow rate group had lower RR in comparison to 1.25 l/min group during the showering period (18 vs. 19 breaths/min; SEM = 0.22; Fig. 2B).

The body surface temperature data for various body parts are given in online Supplementary Figs S1 and S2. Notably, the 3/3 sprinkler group exhibited approximately 1°C lower surface temperatures in the flank, shoulder and udder regions compared to the 3/9 group, particularly during the afternoon hours. Likewise, the 2 l/min flow rate group displayed lower body surface temperatures compared to the 1.25 l/min group, especially in the afternoon.

Behavioral responses

The sprinkler cycle and flow rate did not affect feeding time and rumination activity (Table 2). Buffaloes spent, on average, 155 ± 9 min/d feeding and 783 ± 37 min/d ruminating. The sprinkler cycle and flow rate affected lying time (Table 2). Buffaloes in the 3/3 sprinkler cycle group had 49 min/d more lying than the 3/9 group (560 vs. 51 min/d; SE = 23). Neither sprinkler cycle nor flow rate affected inactive standing time. On average, buffaloes spent 484 ± 30 min/d standing inactive (no walking, rumination or any other activity).

Blood metabolites

Neither sprinkler cycle nor flow rate had a significant impact on blood metabolites, including glucose, BUN, and cortisol levels. However, it's worth noting that blood glucose levels in the 3/3 sprinkler cycle group were numerically (non-significantly) higher compared to the 3/9 group (69.9 vs. 51.4 mg/dl; SEM = 6.6;

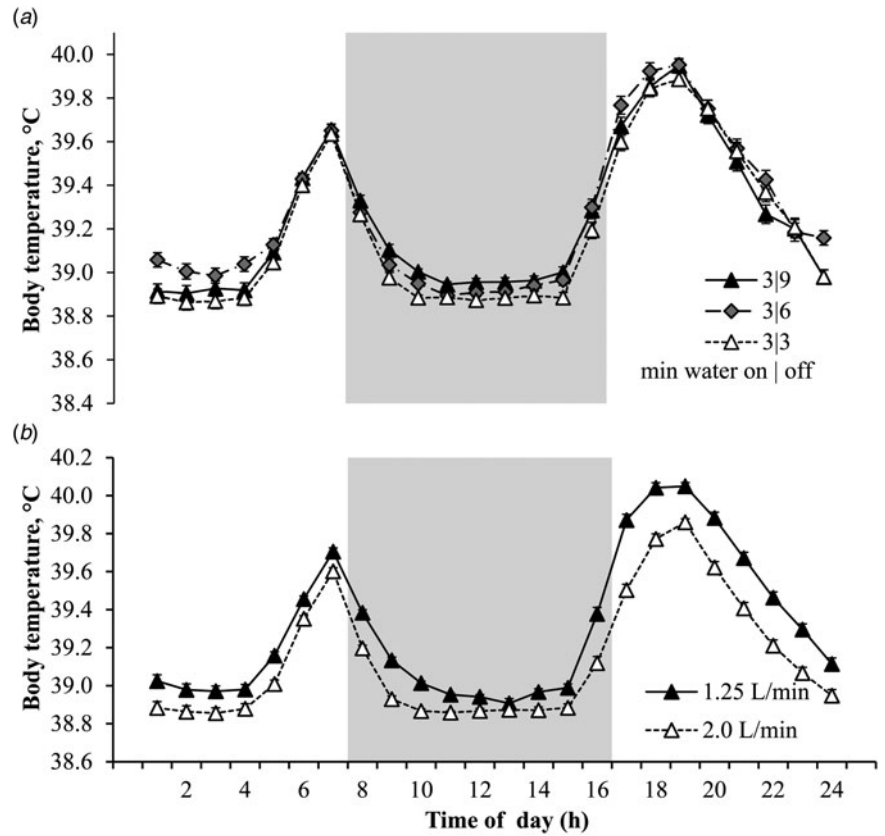


Fig. 1. Mean hourly intravaginal temperature (°C) of Nili Ravi buffaloes in response to sprinkler cycles (A) and sprinkler flow rates (B). Sprinkler cycles (water on/off) were of three categories: 3/3, in which the sprinklers sprayed water for 3 min then stopped for 3 min in a 6 min cycle, 3/6 (3 min water on and 6 min off in a 9 min cycle) and 3/9 (3 min water on and 9 min off in a 12 min cycle); $n = 9$ animals per treatment, 9 d of recording/animal, 24 h/day). Sprinkler flow rates were of two categories 1.25 and 2.0 l/min. Shaded region represents the sprinkler application time. Error bars represent SE.

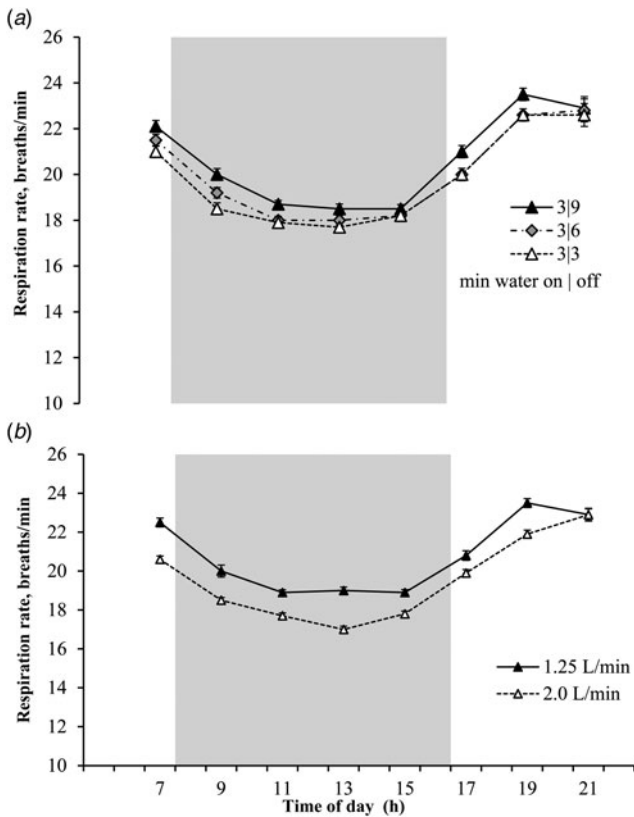


Fig. 2. Mean respiration rate of Nili Ravi buffaloes in response to sprinkler cycles (A) and sprinkler flow rates (B), taken at different time points of a day. Treatment details as for Fig. 1. Shaded region represents the sprinkler application time. Error bars represent SE.

$P = 0.14$; Table 3), with similar levels observed in the 3/3 and 3/6 groups (Table 3).

Discussion

This study found that the sprinkler cycle and flow rate had a significant impact on the productive, physiological, and certain behavioural responses in dairy buffaloes. Cooling buffaloes more frequently with shorter intervals and using more water was much effective in enhancing their performance and well-being. During the study period, the environmental conditions imposed moderate to severe heat stress on the buffaloes. The average daily THI recorded during the study was 85.7, exceeding the threshold of 74 for dairy buffaloes as reported by Choudhary and Sirohi (2019). The relatively high level of heat stress during the study period provided an opportunity to effectively evaluate the impact of sprinkler cycle and flow rate interventions.

Our findings suggest that a higher frequency of cooling, as determined by the sprinkler cycle (water on and off durations), can be more effective in improving milk yield. Buffaloes in the group with the highest frequency of cooling (3/3 group) produced more milk than the other groups, as this provided more consistent and sustained relief from heat stress than the other cycles. This is supported by the observed improvements in core body temperatures and respiration rate, all of which are important indicators of animal welfare and productivity. Whilst the difference in respiratory rate between the treatment groups was not substantial, it closely aligned with rates reported in previous studies conducted under similar conditions (Bah *et al.*, 2021a, 2021b, 2022). Unlike cattle, buffaloes do not primarily rely on respiratory rate as a means to dissipate heat, instead, they employ wallowing

Table 2. Effect of showering cycle and flow rate on behavioural measures of Nili Ravi buffaloes during summer, presented as LS Means \pm SE

Behaviour	Showering cycle min water on/off				Flow rate, l/min			P value		
	3/3	3/6	3/9	SEM	1.25	2.0	SEM	Cycle	Flow	Cycle \times flow
Feeding behaviour										
Total feeding time, min/d	158	144	162	9	155	154	8	0.1149	0.9142	0.8799
Rumination time, min/d	762	805	782	37	785	781	36	0.2517	0.8384	0.0104
Lying behaviour										
Total lying time, min/d	560	549	511	23	533	547	22	0.0479	0.3890	0.2671
Standing behaviour										
Total standing time, min/d	471	494	487	30	506	464	28	0.6759	0.0688	0.6349
Standing bouts, number/d	8	8	9	37	9	8	0.38	0.1979	0.0296	0.9739
Step count/d	158	144	162	9	155	154	8	0.1149	0.9142	0.8799

behaviour, which facilitates heat dissipation through convection. It is important to note that the sprinklers provided convective heat dissipation in all the groups. While the relatively minor difference in RR between the 3/3 and 3/9 groups may not carry significant biological implications, it does suggest that buffaloes in the 3/3 group may experience a somewhat lower heat load compared to those in the 3/9 group. In addition to the frequency of cooling, the flow rate of water also had a significant impact on milk yield in buffaloes. The group that received a higher flow rate of 2 l/min produced more milk than the group that received 1.25 l/min, indicating that increasing water flow can be effective in improving milk production. This finding contrasts with previous research on dairy cows (Chen *et al.*, 2016), which did not observe any additional benefit in milk production with a flow rate higher than 1.3 l/min. The difference in results between buffalo and cows could be attributed to their difference in heat dissipation mechanisms as well as adaptations to heat stress environmental conditions. Cows have a thick coat of hair that can trap water, enabling evaporative cooling, while buffaloes have a sparse coat that allows water to flow directly onto the skin, promoting heat loss through convection. In addition, dairy cows produce much more metabolic heat due to higher milk yield than buffaloes. The presentation of body surface temperature in the Supplementary File as Figs S1 and S2 provides additional insight into the effect of sprinkler flow rate on the cooling of buffaloes. The results show that the surface temperature of various regions of the body was lower in the 2 l/min flow rate group compared to the 1.25 l/min group, indicating that a higher flow rate can improve cooling efficacy. This finding is consistent with the core body temperature and respiration rate results, further supporting the conclusion that a higher sprinkler flow rate may be more effective in cooling buffaloes.

The results indicate that there was a distinct pattern of VT in buffaloes over a 24-h period. The lowest VT occurred in the early morning, which could be attributed to cooler ambient conditions. However, the highest VT ($\geq 39.7^\circ\text{C}$) was observed during the early nighttime. Increased body temperatures during early nighttime (2100 to 2200 h) have been reported previously (Tresoldi *et al.*, 2019). This is likely because after sunset, the environment and surrounding structures release heat absorbed during the day, which is then absorbed by the buffaloes' bodies. The absence of cooling during this time led to the highest VT. Therefore, it is important to implement appropriate heat abatement strategies during this period for dairy buffaloes in semiarid climates. During the daytime, the VT of buffaloes was lower when sprinkler application was applied in all the treatment groups. The association between VT and RR is not quite evident in buffaloes, as they primarily rely on mechanisms other than respiration to cool their bodies, as described earlier. However, in cattle under similar climate conditions, cows with increased RR have been observed to have lower VT (Macavory *et al.*, 2023).

We have shown that the sprinkler cycle and flow rate have a more significant impact on the lying time of buffaloes than on their feeding and rumination activity. Buffaloes in the 3/3 sprinkler cycle group had 49 min more lying time per day compared to the 3/9 group. This suggests that the 3/3 sprinkler cycle may have been more effective in promoting heat dissipation and rest for the animals. Additionally, the study found that neither the sprinkler cycle nor flow rate had a significant impact on inactive standing time. Overall, these results suggest that whilst the sprinkler cycle and flow rate do have some impact on the behaviour of buffaloes, it is not significant enough to affect their overall feeding and rumination activity.

Table 3. Effect of showering cycle and flow rate on blood metabolites of buffaloes during summer, presented as LS Means \pm SE.

	Showering cycle min water on/off				Flow rate, l/min			P value		
	3/3	3/6	3/9	SEM	1.25	2.0	SEM	Cycle	Flow	Cycle \times flow
Glucose, mg/dl	69.9	67.7	51.4	6.6	57.4	68.6	5.4	0.1429	0.1649	0.2852
Blood urea nitrogen, mg/dl	16.6	15.8	16.1	1.8	17.9	14.5	1.5	0.9584	0.1358	0.8536
Cortisol, $\mu\text{g/dl}$	2.6	2.4	2.7	0.37	2.9	2.3	0.30	0.8080	0.1674	0.1556

In conclusion, this study found that the sprinkler cycle and flow rate had a significant impact on the physiological, behavioural, and productive responses in dairy buffaloes. These results suggest that the 3/3 showering cycle with 2 l/min flow rate is an effective strategy for improving the well-being and milk production of dairy buffaloes during hot weather conditions. The study's findings can be useful for farmers who are looking for ways to improve the comfort and productivity of their buffaloes during the hot summer months.

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.1017/S0022029923000687>

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