

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

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SUPPLEMENTARY FILE

11 **Material & Methods**

12 *Study animals, housing, and management*

13 The buffaloes were housed in a traditional, naturally ventilated shed with a precast concrete
14 roof and brick floor. The shed measured 21 meters in length (east-west) and 10 meters in width,
15 with the south side closed and the north side fully open, and an adjacent loafing area with a
16 brick floor. The feed manger was located alongside the south wall of the shed. A polyvinyl
17 water pipe fitted with sprinkler nozzles was installed about 2.1 meters above the floor along
18 the manger and approximately 50 cm away from it. Each nozzle was 2 meters apart. The
19 buffaloes were tied at the manger with neck chains during the daytime (8:00 to 16:00 h) and
20 released in the adjacent outdoor loafing area during the nighttime. The buffaloes were tied in a
21 way that one sprinkler nozzle was directed towards each buffalo. The sprinkler nozzles had an
22 angle of 180 degrees, directed towards the back of the buffalo, spreading water on the withers,
23 back, and the main trunk. The daily buffalo ration consisted of fresh sorghum fodder and
24 concentrate @ 60 kg, and 3 kg per buffalo, respectively. Individual water tubs were placed for
25 each buffalo in the shed during the daytime and a water trough, in the loafing area, was
26 available for free access during the nighttime. Hand milking was done twice daily (0500 and
27 1700 h). The experimental buffaloes were milked by the same person throughout the study,
28 and concentrate was provided during milking to stimulate milk letdown (Shahid et al. 2013).
29 This was consistent with the prevailing practice at the farm.

30 *Experimental design and treatments*

31 The present study was conducted on nine lactating Nili Ravi buffaloes. The buffaloes were
32 subjected to three sprinkler cycles and two flow rates using a double replicated 3 x 3 Latin

33 square design. The flow rates were 1.25 and 2 L/min, and the sprinkler cycles (minutes water
34 on | off, number of cycles per hour) were: 3 on | 3 off, 10 cycles; 3 on | 6 off, 7 cycles; and 3
35 on | 9 off, 5 cycles per hour. The study was divided into two squares, each consisting of 21
36 days with 7 days per period. In the first square, each of the three sprinkler cycles was applied
37 to 3 buffaloes in each period using 1.25 L/min flow. The second square was identical to the
38 first one, except the flow rate was 2.0 L/min. The treatment application is outlined in Table S1.
39 The first four days of each period were used for adaptation, and data were collected for the
40 remaining 3 days. The showering cycles were regulated using an automated valve installed in
41 the showering line for each group and powered by a programmable logical control panel
42 (Wecam Technology; Model: Levi 2070D; Version: VI.2.4.1.7.2.0).

43 *Water use characteristics*

44 The average area that the water from the sprinkler nozzle covered on a single buffalo was about
45 3.24 ± 0.1 square meters (Mean \pm Standard Deviation; Table 1). The flow rate did not affect
46 the spread of water; however, the flow rate of 2 L/min used more water than the 1.25 L/min.
47 Additionally, the 3|3 sprinkler cycle used more water than the other cycles. The amount of
48 water used for cooling during 8.5 hours per day was highest in the 3|3 cycle group (319 liters
49 and 510 liters for 1.25 L/min and 2.0 L/min flow rate, respectively), followed by the 3|6 cycle
50 group (212.5 liters and 340 liters) and the 3|9 cycle group (159 liters and 255 liters).

51 *Environmental measures*

52 The metrological measures are summarized in Table 2. The average T, THI and LHI during
53 the daytime treatment application period were $37.6 \pm 2.8^{\circ}\text{C}$, 89 ± 2 , and 111 ± 6 , respectively
54 (Mean \pm SD). The extreme values for these variables were 44.5°C , 94, and 124, respectively.
55 The daytime T, THI and LHI averages were 4°C , 0.3, and 10 points higher than the 24 h
56 averages, respectively. The daytime average BGT was 9.5°C higher than the 24 h average BGT
57 (46.6 ± 5.0 vs $37.1 \pm 8.8^{\circ}\text{C}$, respectively; Means \pm SD).

58 **Table S1.** Treatment application arrangement in a double replicated crossover design.

Flow rate, L/min	Period ¹	Showering cycle treatment (min on off) ²		
1.25	1	3 3	3 9	3 6
1.25	2	3 9	3 6	3 3
1.25	3	3 6	3 3	3 9
2	1	3 3	3 9	3 6
2	2	3 9	3 6	3 3
2	3	3 6	3 3	3 9

59 ¹ Each period lasted for 7 days.

60 ² The sprinkler cycles (minutes water on | off, number of cycles per hour) were of three categories: 3 on | 3 off, 10 cycles; 3 on | 6 off, 7 cycles;
 61 and 3 on | 9 off, 5 cycles per hour. Each sprinkler cycle was applied to 3 buffaloes individually in each period, resulting in 9 buffaloes per
 62 sprinkler cycle across three periods.

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65 **Table S2.** Water spread characteristics of different showering cycle

Water spread	Showering cycle treatments (min on off)		
	3 3	3 9	3 6
Along the feed bunk, m	1.8	1.8	1.8
Away from the feed bunk, m	1.8	1.8	1.8
Area covered area, m ²	3.24	3.24	3.24
Height of nozzles, m	3.24	3.24	2.3
Water use ¹ , L/9 h (1.25 2 L/min)	338 540	69 270	203 324

66 ¹Calculation are based on showering on duration per day

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70 **Table S3.** Summary of daily meteorological measures on experimental days during July and August 2021.

	24 h			Treatment period (0800 to 1700 h)		
	Mean \pm SD	Minimum	Maximum	Mean \pm SD	Minimum	Maximum
Air temperature (T, °C)	33.3 \pm 4.3	27.1	44.5	37.6 \pm 2.8	31.5	44.5
Temperature-humidity index (THI)	85.7 \pm 3.8	79.5	94	89 \pm 2	84	94
Heat load index (HLI)	101 \pm 10	85	124	111 \pm 6	87	124
Black globe temperature (BGT, °C)	37.1 \pm 8.8	26.6	55.5	46.6 \pm 5.0	34.1	55.5
Relative humidity (RH, %)	69 \pm 15	37	97	55 \pm 9	37	82
Wind speed (WS, m/s)	0.12 \pm 0.3	0	1.6	0.2 \pm 0.4	0	1.5

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73 **Figure legends:**

74 **Figure S1:**

75 The mean surface temperature of various body parts of Nili Ravi Buffaloes subjected to different sprinkler cycle treatments (n = 9 animals per
76 treatment, 9 d of recording/animal, 8 times/day). The sprinkler cycles (water on|off) were of three categories: 3|3, in which the sprinklers
77 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
78 on and 9 min off in a 12 min cycle). The shaded region represents the sprinkler application period during a day. The four panels (labeled
79 A, B, C, and D) in the figure the show the average surface temperature of the flank, shoulder, udder, and lower belly, respectively.

80 **Figure S2:**

81 The mean surface temperature of various body parts of Nili Ravi Buffaloes subjected to different sprinkler flow rates (n = 9 animals per treatment,
82 9 d of recording/animal, 8 times/day). The sprinkler flow rates were 1.25 and 2 L/min. The shaded region represents the sprinkler
83 application period during a day. The four panels (labeled A, B, C, and D) in the figure the show the average surface temperature of the
84 flank, shoulder, udder, and lower belly, respectively.

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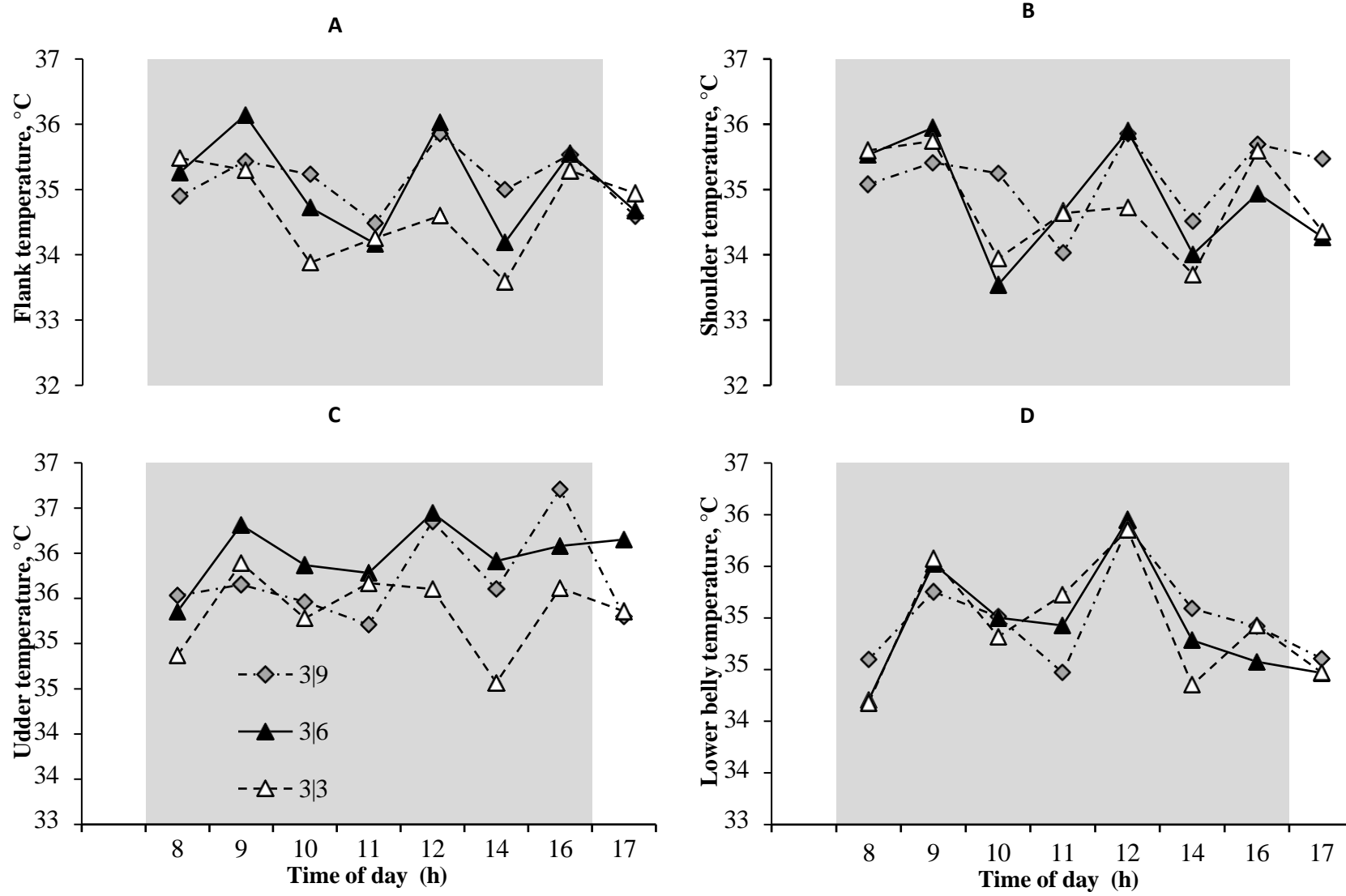
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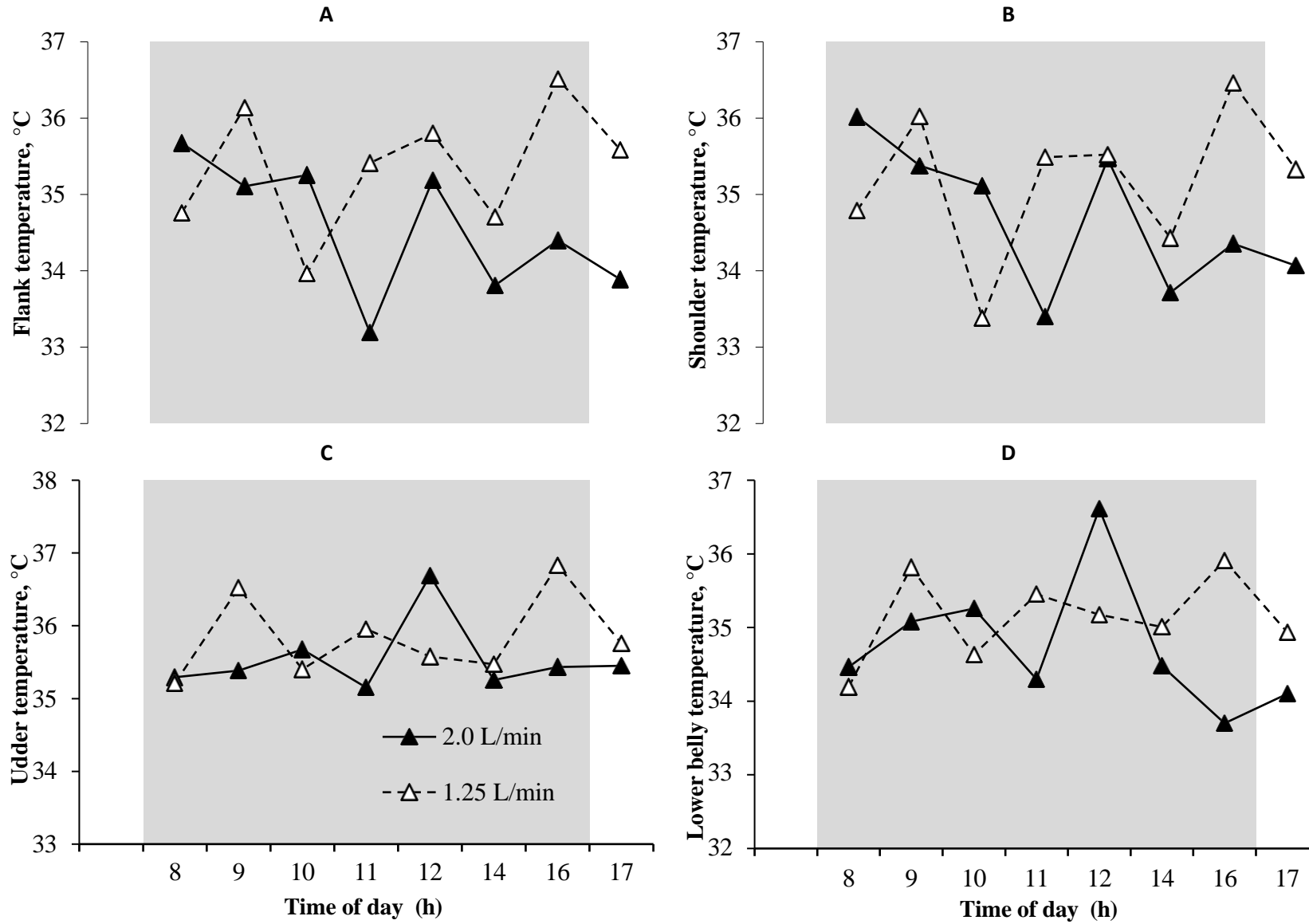
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Figure S1:



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100 **References:**

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