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Eye temperature, an indicator for stress levels in young buffalo bulls – A case study of micro-environment modification

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ABSTRACT

The present study was undertaken with an objective to determine diurnal changes of the eye temperature of buffalo young bulls under different shelter management to determine the effect of heat stress. Twenty four buffalo bulls were randomly divided into two groups, each group comprising of 12 animals based on age (16–18 months) and body weight (Control = 301 ± 8.24 kg and Treatment = 311.45 ± 6.24 kg). The control group was housed under normal management practices followed, and the height of the shed was 10 ft. and width was 12 ft. with concrete floor. Whereas, the treatment group was housed in shed having 15 ft. height and 25 ft. width along with rubber mat as flooring and the total area provided for each animal in both the groups was 45.96 sq. feet. They were also provided with dairy fans and mist cooling in day time to ameliorate the heat stress. The thermal humidity index (THI), floor and roof temperature variation of the shed was recorded. The eye temperature of bulls was measured to evaluate the effect of different housing on the animals' thermal status under hot dry summer conditions.Results revealed that the THI of treatment shed was significantly ($P < 0.01$) lower than the control at different times. The floor and roof surface temperature of the treatment shed was significantly ($P<0.05$) lower than the control shed. Diurnal patterns of eye temperature measured in both the groups showed increased eye temperature in control group bulls compared to treatment group. The eye temperature at 7.00 hrs was similar in both groups, whereas it was highly significant ($P<0.01$) at 13.00 and 19.00 hrs. The heat stress in the buffalo bulls was exhibited by increased eye temperature. Thus, the variation in the eye temperature can be effectively used as an indicator of heat stress and the dairy fans and mist cooling along with rubber mat flooring can be utilized to ameliorate the heat stress in the buffalo bulls.

Keywords : Buffalo, dairy fans, eye temperature, heat stress, mist cooling

Heat stress is the state at which the mechanisms in the animal's body activate to maintain body thermal balance, when it is exposed to higher temperature. High ambient temperatures, due to global warming, can increase morbidity and mortality in livestock (Bal and Minhas, 2017; Kovács *et al.,* 2018).The homeotherms can maintain their body temperature within the smaller range to adjust with the existing climatic conditions through behavioural and physiological modifications (Das *et al*., 1999; Singh *et al.,* 2019). The heat stress affects the health of animals when there is an imbalance between heat gain or heat production in the body and the ability to dissipate it (Rawat *et al.,* 2014; Alhussien and Dang 2017; Lakhani *et al.,* 2018).

The heat stress reduces feed intake of animals and changes physiological functions like increase in the rectal temperature, respiration and pulse rate. It also alters the hormonal regulation, sweating and antioxidant status of the animals. Buffaloes are more prone to heat stress due to their poor heat regulation because of less number of sweat glands, hair and dark coloured skin (Gu *et al*., 2016; Lakhani *et al*., 2018). When the buffalo calves were suffered from heat stress, they will have reduced growth rate and thus the delayed puberty (Ganaie *et al.,* 2013 and Gu *et al.,* 2016).

The increased rectal temperature due to high ambient temperature and the subsequent impact on feed and energy intakes are well known. Different methodologies like measurement of cortisol levels, heart rate and respiratory rate have been developed for assessment of stress in the animals (Moberg, 2000). Though, the techniques used to measure them can be stressful to the animals due to handling, and can also influence the stress measures obtained (Stewart *et al.,* 2005). Hence, the use of minimum invasive methodologies for stress assessment can be advisable to assess animal welfare in farm animals.

The studies have already been conducted to assess the effect of heat stress on eye temperature in goats (Bartolomé *et al*., 2019), horse (Johnson *et al*., 2011 and Bartolomé *et al*., 2013), cattle (Church *et al*., 2013; Martello *et al*., 2015) and buffalo (Ahirwar *et al*., 2019). Eye temperature (ET) has been found to be a more consistent measure of temperature changes in response to stress compared to other parts of body (Bartolomé *et al*., 2013).Measuring of eye temperature is rapid, relatively easy, and non-invasive compared to measurement of body temperature using rectal thermometers, tympanic infrared thermometers, thermal microchips, and rumen boluses (Johnson *et al*., 2011; Church *et al*., 2013; Bartolomé *et al*., 2019). All these methods of measurement of temperature are having different limitations (Johnson *et al*., 2011). It has been established that the temperatures of the lacrimal caruncle region of the eye measured by Infra-red thermometer (IRT) may correlate with core body temperature (Ng and Kaw, 2006; Church *et al*., 2013).

Providing shade and water sprinkling may improve thermal comfort inside livestock housing, reduces thermal stress and minimizes productive loss (Davis *et al*., 2003; Gaughan *et al*., 2004;Berman, 2009; Marcillac-embertson *et al*., 2009; Stowell *et al*., 2009). Heat alleviating measures viz. fan, coolers, foggers, mist, showers, wallowing etc. may be used for reducing the thermal stress effects (Singh *et al*., 2014). Specially, sheltering animals from shortwave solar radiation and sprinkling them with water has been shown to be an efficient means of reducing skin temperature (Domingos *et al*., 2013). Therefore, the present study was undertaken with an objective to determine diurnal changes of the eye temperature of buffalo young bulls under different shelter management to determine the effect of heat stress.

MATERIALS AND METHODS

Location of experiment

The study was conducted at Livestock Research Centre (LRC) of National Dairy Research Institute (NDRI), Karnal, India located at 29° 423' 20" N and 76° 583' 52.5" sec E at an altitude of 834 feet above mean sea level. The maximum ambient temperature in summer goes up to 45°C and minimum temperature winter comes down to 2°C with diurnal variation of 15-20°C. The average annual rainfall about 700 mm, most of which is received from early July to mid-September.

Experimental animals

Twenty four Murrah buffalo young bulls were selected for this experiment by considering their bodyweight, age and health status. The animals were randomly divided into two groups of 12 animals each based on age and body weight (Control = 301 ± 8.24 kg and Treatment = 311.45 ± 6.24 kg). The experimental animals used in the study were Murrah buffalo bulls and were aged between 16 to 18 months. These bulls were fed according to ICAR (2013) feeding standards and were in the same shelter management from the age of 6 months old. Vaccination, deworming, regular check-up for communicable diseases, and other herd-health programs were followed as per standard farm practices.

Shelter management of experimental animals

The animals under experiment were housed in well-ventilated shed having the arrangement for individual animal feeding according to BIS (2005) specifications in tied condition (Fig. 1). Before commencement of the experiment all the animals were given two weeks adaptation period. The shed was washed twice daily and thoroughly cleaned to remove faeces and dirt. All the animals were maintained under clean and hygienic conditions, disinfectant solution was applied at regular intervals on the floor of the shed.

Routine shelter management

The animals under control groupwere housed in shelter at LRC (10 ft height and 12 ft width). The total length of the shed was 46 feet and each animal was provided with 3.83 feet. The total area provided for each animal was 45.96 sq. feet.

Modified shelter management

The animals under treatment groupwere provided modified shelter with increased height and width of the shed (15 ft. height and 25 ft. width).Animals under modified management were provided time controlled fogger cooling with dairy fans. The rubber mats were provided as bedding for the comfort of animals. Similar to control group each animal was provided 45.96 sq. feet area.

Feeding of experimental animals

The experimental animals were fed individually with total mixed ration (TMR) to fulfil their nutrient requirement as per ICAR (2013) to gain 600g average daily weight. The

Table 1: Temperature Humidity Index (THI) of control and treatment sheds

Time (Hrs)	Control (°C)	Treatment (°C)	P value
07.00	81.06 ± 0.27^{aC}	76.60 ± 0.23^{bC}	0.00
13.00	88.77 ± 0.21^{aD}	78.62 ± 0.27^{bD}	0.00
19.00	78.69 ± 0.29^{aB}	73.59 ± 0.49^{bB}	0.00
01.00	75.30 ± 0.14^{aA}	72.72 ± 0.23^{bA}	0.00
P value	0.00	0.00	-

Means bearing ^{a,b} differs significantly between treatments and $^{A,B...}$ differs significantly (P<0.01) within the groups

Table 2: Floor temperature variables of control and treatment sheds

Time (Hrs)	Control (°C)	Treatment (°C)	P value
07.00	25.84 ± 1.24^{aA}	22.94 ± 1.02^{aA}	0.11
13.00	34.46 ± 1.13^{aC}	24.68 ± 0.57^{bB}	0.00
19.00	28.84 ± 0.25^{aB}	25.96 ± 0.46^{bC}	0.00
01.00	26.70 ± 0.37^{aA}	24.88 ± 0.29^{bB}	0.01
P value	0.00	0.00	-

Means bearing ^{a,b} differs significantly between treatments and $^{A,B...}$ differs significantly (P<0.01) within the groups

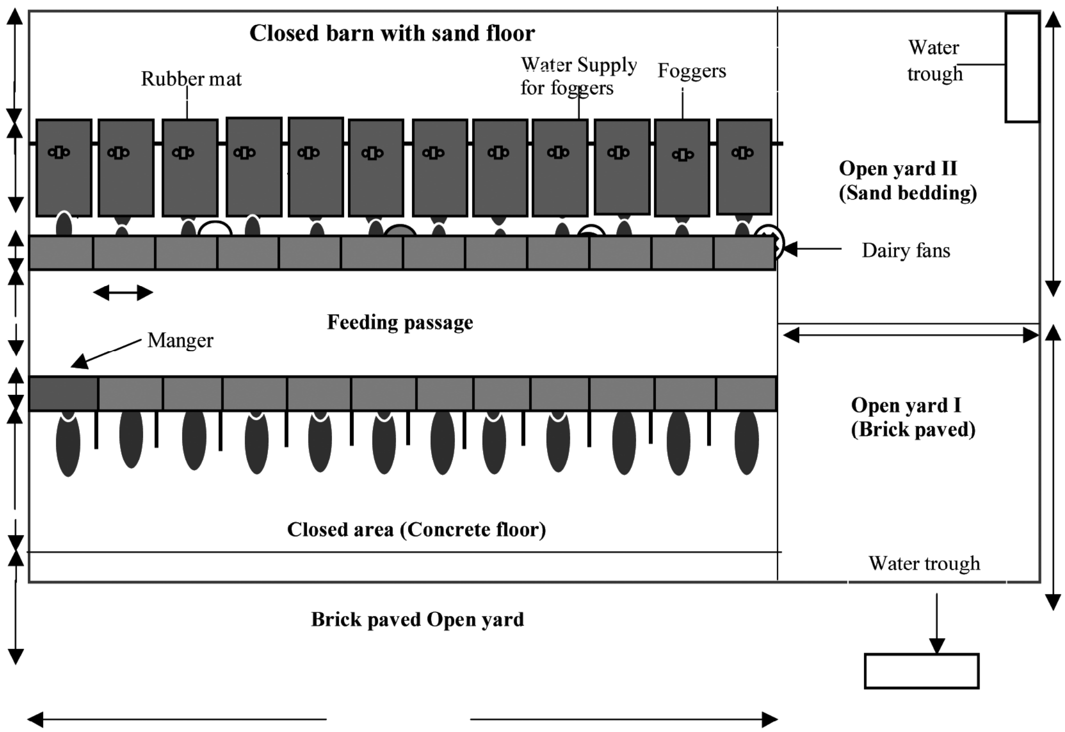

Fig. 1: The arrangements and cross section of buffalo young bull's shelter

maize fodder was supplied by farm section of the institute was chopped freshly and mixed properly with the concentrate and wheat straw in a ratio of 60:30:10 before offering it to animals and it was provided daily at 08:30 hrs All animals were provided with fresh water *ad libitum* at 6:00, 12:00, 16:00 and 20:00 hrs

Measurement of Temperature Humidity Index (THI)

The dry bulb and wet bulb temperature of experimental sheds was recorded using dry and wet bulb thermometer (Zeal, UK), respectively, the recordings were donesix hrs interval at 07:00, 13:00, 19.00 and 1.00 hrs of Indian Standard Time (IST). These thermometers were hanged at equal heights above the animal in each group. The THI values of different groups were calculated by using the following formula(McDowell, 1972).

THI=0.72 (wet bulb temperature + dry bulb temperature) + 40.6

Measurement of floor, roof and eye temperature

The floor and roof surface temperature were measured atsix hrs intervals (07:00, 13:00, 19.00 and 1.00 hrs of Indian Standard Time (IST)) for a period of five daysusing Infrared thermometer (Fluke 59 Max ERTA(Fluke Corporation, U.S.A.) Thermometer, measures temperature from -30^{0}C to 350^{0}C) at a distance of 1 meter perpendicular to the surface of floor and inner roof. Similarly, the temperature variants of eye were measured at sixhrs intervals (07:00,

Table 3: Roof temperature variables of control and treatment sheds

Time (Hrs)	Control (°C)	Treatment (°C)	P value
07.00	31.10 ± 0.81^{aB}	42.70 ± 2.97^{bC}	0.00
13.00	48.34 ± 1.52^{aC}	37.44 ± 0.85^{bB}	0.00
19.00	28.90 ± 0.49^{aAB}	27.38 ± 0.49^{bA}	0.05
01.00	27.36 ± 0.52^{aA}	26.18 ± 0.40^{aA}	0.12
P value	0.00	0.00	-

Means bearing ^{a,b} differs significantly between treatments and $^{A,B...}$ differs significantly ($P<0.01$) within the groups

Table 4: Rhythmicity of eye temperature of control and treatment bulls

Time (Hrs)	Control (°C)	Treatment (°C)	P value
07.00	35.40 ± 0.09^{aC}	35.14 ± 0.11^{bC}	0.05
13.00	37.41 ± 0.09^{aB}	36.46 ± 0.10^{bD}	0.00
19.00	35.36 ± 0.07^{aC}	34.24 ± 0.11^{bB}	0.00
01.00	34.02 ± 0.10^{aA}	33.13 ± 0.12^{bA}	0.00
P value	0.00	0.00	-

Means bearing ^{a,b} differs significantly between treatments and $^{A,B...}$ differs significantly ($P<0.01$) within the groups

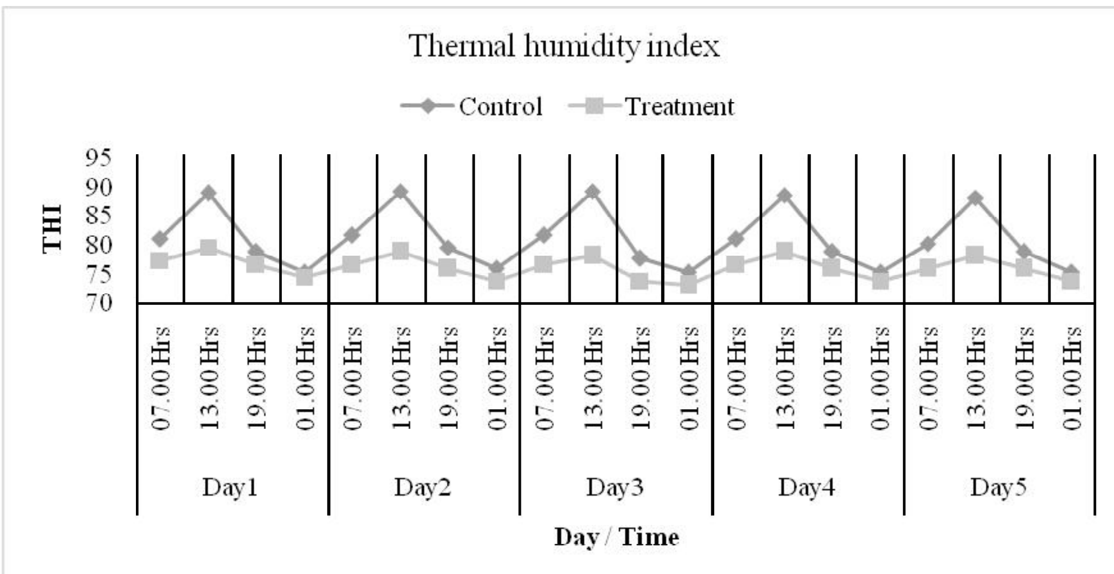

Fig. 2: Thermal humidity index of sheds during experimental period

13:00, 19.00 and 1.00 hrs of Indian Standard Time (IST)) at a distance of 1 meter perpendicular to the surface of animals.

Statistical analysis

The data obtained during present experiment was tabulated and subjected to two way analysis of variance (ANOVA) as per the methods described by Snedecor and Cochran (1994) using the SPSS version 21.0 ® software. The means were tested for the significant difference by using Duncan's multiple range test. The data of environmental parameters was analysed by using student t-test.

RESULTS AND DISCUSSION

Thermal humidity index (THI)

The variation of daily THI of control and treatment shed on all days has been depicted in fig. 2. The THI of treatment group shed was lesser duringwhole day compared to control shed. The average THI of all the five days has been presented in Table 1 and it shows that the THI of treatment shed was significantly ($P< 0.01$) lower than the THI of control shed at all the times. The THI between different time of the day differed significantly ($P<0.01$) in both the sheds. The highest average THI was observed at 13.00 hrs (88.77 ± 0.21)in control shed. Whereas, the lowest average THI was observed at 1.00 hrs (73.72 ± 0.23) in treatment groupshed. It indicates the reduced heat load in the modified shed due to provision of cooling system (Table 1).

The THI values in the control shed was more than 75 during all the five days and influenced the eye temperature of the buffalo bulls. Whereas, the THI of the treatment shed was increased during the afternoon and remained around 75 during morning, evening and night. Anderson *et al*. (2013) also reported lower THI in the sheds provided with flip fans in their study.Similarly, Singh *et al*. (2014) reported theincreased THI under both natural and controlled conditions, but the increase under natural condition was

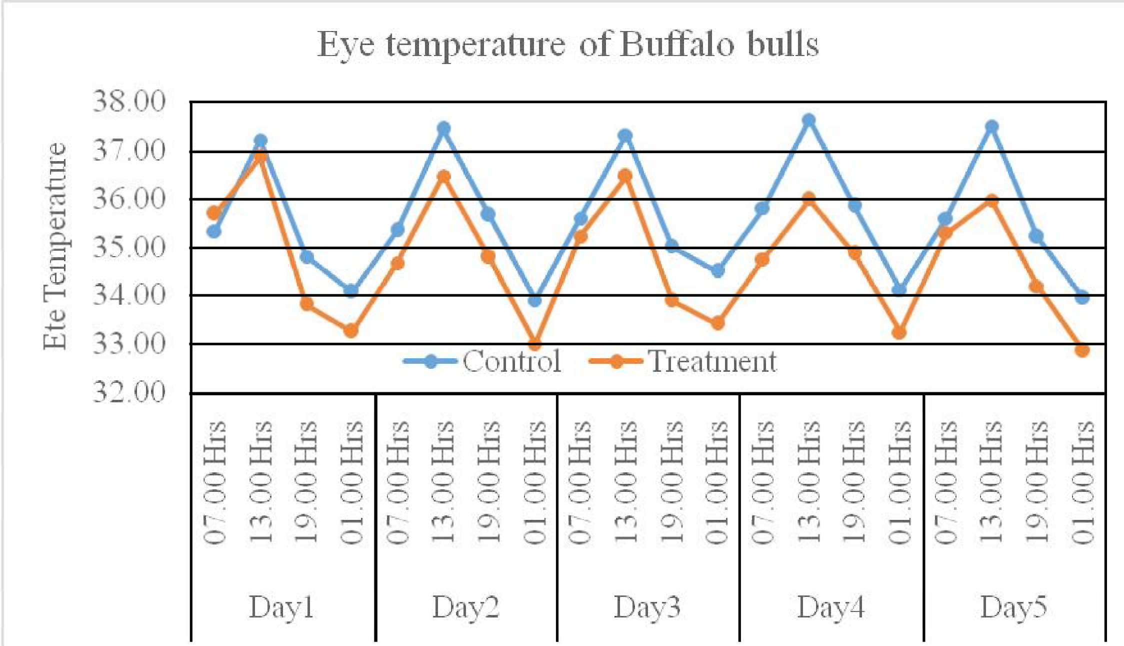

Fig. 3: The diurnal pattern of eye temperature in both the groups

higher (87.62±0.78) compared to fancum- mist system (85.37±0.66) at 16.00h in their study during summer season. Valtorta *et al.* (2002) also suggested spray evaporative cooling (mist, foggers and sprinkling systems) as an effective way of cooling dairy animals.

The floor temperature recordings are presented in Table 2 and it was found that the floor temperature was highest in control shed compared to treatment shed during whole day. It was significantly ($P<0.01$) highest in control shed at 13.00, 19.00 and 01.00 hrs of the day. Highest($P<0.01$) floor temperature was recorded during 13.00 hrs and 19.00 hrs in control and treatment sheds, respectively, whereas lowest floor temperature was recorded at 07.00hrs in both the sheds. The cooler part of night hrs might be the reason for lowest floor temperature during early morning. The lower floor temperature in treatment shed might be due to the cooling effects of dairy fans and foggers.

The roof temperature of both sheds during different hrs of day has been presented in table 3. The roof temperature varies in both the sheds throughout day and was significantly ($P<0.01$) higher in control shed at 07.00 and 13.00 hrs and significantly ($P<0.05$) higher at19.00 hrs. Furthermore, the roof temperature did not vary between the sheds during night hrs i.e. at 01.00 hour. It was significantly ($P<0.01$) highest at 13.00 hrs and lowest during 01.00 hrs in control shed and significantly ($P<0.01$) highest at 7.00 hrs and lowest during 1.00 hrs in treatment shed.

The roof temperature was higher during 7.00 hrs in treatment shed as the long axis of shed was in North – South direction, the morning sun radiations directly fell on the roof of the treatment shed and evening radiations fell on the control shed. The roof temperature seems to be declined gradually from morning to evening due to cooling effect of dairy fans and mist. Whereas, it was highest during 13.00 hrs in control shed due to increased environmental temperature during afternoon. The roof temperature is having effect on the THI of the sheds. Due, to mist and dairy fans the microclimatic conditions of the treatment shed remained cooler throughout the day.

Eye temperature

The diurnal pattern of eye temperature (^{o}C)measured in both the groups throughout the experiment has been depicted in fig. 3 and presented in Table 4. The heat stress in the buffalo bulls has been exhibited by increased eye temperature. The increased eye temperature was observed in control group bulls. The eye temperature was significantly ($P<0.05$) higher at 7.00 and ($P<0.01$) 13.00, 19.00, 1.00hrs in control group compared to treatment group. Significantly ($P<0.01$) highest and lowest ET was recorded at 13.00 and 1.00 hrs, respectively, in control group. Similarly, the significantly ($P<0.01$) highest and lowest ET was recorded at 13.00 and 1.00 hrs, respectively, in treatment group. The eye temperature also varied significantly ($P<0.01$) within the group during different parts of the day in both the groups.

The eye has rich capillary beds innervated by the sympathetic system and responding to changes in blood flow (Stewart *et al.*, 2007). Hence, whenever the animal is inacute stress,the eye temperature tends to increase, possibly due to an increased dilation of the ocular blood vessels and an increased visual attention or orientation(Yarnell *et al.*, 2013).Due to concern of the animal welfare and to avoid the handling stress to the animals the non –invasive tool can be used to assess the eye temperature which indicates the core body temperature.The eye temperature (ET) measured by infrared thermography is asuitable tool for stress and welfare assessment in ruminants (Stewart *et al.*, 2005; Stewart *et al.*, 2007; Stewart *et al.*, 2008., Bartolomé *et al.*, 2019). Even though, it is established in different species and breeds of animals, there are no reported studies in buffaloes with respect to the eye temperature and heat stress correlation. The temperature of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle, may be an indicator of stress (Pavlidis *et al.*, 2002; Cook *et al.*, 2005).

It is evident from the present study that the modified shelter was having comfortable microclimatic conditions for the animals and did not affect the eye temperature of the young bulls as they were not under stress. The bulls under control group were having higher eye temperature indicating the effect of microclimatic conditions on the animal's physiological status. The diurnal fluctuations of the ambient temperatures of the sheds can affect the animals' ability to cope with heat stress. It is also well known that the minimum ambient temperatures during the nighthours can help the animals to dissipate heat load (Silanikove, 2000, Sevi *et al.*, 2001). Anderson *et al.*, (2013) also reported that the cows provided with flip fans had lower core body temperature and were not affected by heat stress. Ortiz *et al.*, (2015) also found lower core body temperature in evaporative cooling system housed cows.To reduce production losses and to provide comfort to the animals there is a need to modify the environmental conditions at micro levels (Singh *et al.*, 2014).

CONCLUSION

The results of the present study shows that the influence of ambient temperature on the microclimatic conditions of the sheds and consequently influenced the thermoregulatory conditions of the young buffalo bulls. The rhythmic changes of eye temperature wereinfluenced by the microclimatic conditions of the sheds. Therefore, the eye temperature can be used as a non-invasive physiological indicator of heat stress in bulls. Also, the dairy fans and fogger cooling influenced the microclimatic conditions of the shelter. Even though there was higher THI in treatment group than thermo neutral zonefor shorter period, it does not affect the physiological condition of the animals. Therefore, the dairy fans and foggers along with rubber mat flooring can be utilized to ameliorate the heat stress in the buffalo bulls.

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