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ORIGINAL PAPER

Effect of heat stress mitigations on physiological, behavioural, and hormonal responses of Buffalo calves

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Abstract
This study assessed the effect of heat stress mitigations on the physiological, behavioural, and hormonal responses of buffalo during the hot summer season. Twenty Murrah buffalo male calves were distributed randomly into controlled (C, $n=10$) and treatment groups (T, $n=10$). The buffalo calves in the C group were housed in the existing shed (10–12-ft height and 10-ft width). Buffalo calves of the T group were allocated in the modified shed: 15-ft height and 20-ft width along with time-controlled pressure mist with fans and rubber mats on the floor. Fans were running all days. The cool water was misted on calves at the rate of 1 min in 5 min, from 11:00 to 18:00 h. The water misting system was installed below the roof, but at 3.5 m above the floor. The calves' body weight, rectal temperature, infrared temperature of the eye, blood samples, respiration rate, and pulse rate were recorded fortnightly for two consecutive months. In one-way ANOVA, rectal temperature, eye temperature, cortisol level, and afternoon's respiration and pulse rate were higher in the calves of C group than that of T group ($P<0.05$). Conversely, eating and resting time (min/day) and triiodothyronine were lower in the calves of C group than that of T group ($P<0.05$). Therefore, an increase in shed's height and width, using rubber mats on the floor, and cool water misting to buffaloes during the hot summer seasons positively influence their physiological, hormonal, and behavioural responses.

Keywords Infrared temperature · Rectal temperature · Cortisol · Heat stress

Introduction

India has the world's largest buffalo population, which is 96.9 million, while the world's total buffalo population is 170.4 million (Mishra 2021; Rajesh et al. 2018). Buffalo farming has been considered more convenient to the farmers for their better feed conversion efficiency, sustainable on poor feed and forage quality, rich nutritive value of milk, better adaptability to harsh environment, and higher disease resistance in comparison to crossbred cows (Balhara et al. 2017). Consequently, buffalo farming is contributing immensely to the alleviation of poverty in India through beef and dairy production and exportation. Biologically, buffaloes are homeothermic animals and maintain their internal body temperature in a narrow range along with the adjustment between body temperature and ambient condition in environmental extreme (Das et al. 1999). In buffalo, adjustment of body temperature and high ambient temperature in hot humid summer sometimes needs modification in their inherent physiological, behavioural, and hormonal responses (Ganaie et al. 2013; Gu et al. 2016). For example, heat dissipation from the body at hot humid climatic condition required extensive blood circulation in peripheral body parts which results in lack of blood circulation to gastrointestinal tract and consequent less nutrient digestibility and production. Buffaloes are more

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sensitive to heat stress than other livestock as they have poorly developed sweat glands, thicker and dark-coloured skin, and sparse hairs on their body surfaces (Das et al. 1999; Vo and Wang 2007; Gu et al. 2016). Moreover, continuous increase in ambient temperatures "global warming" has been affected buffaloes by additional heat stress component in hot humid environmental condition identified by higher rectal temperature, respiration and pulse rate, lowering feed intake, growth rate, immunity, milk production, reproduction, and consequent less flow of economy within buffalo farmers (Koga et al. 2004; Marai and Haeeb 2010).

Under tropical climates like India, heat stress effects are unavoidable, but they can be decreased to some extent by different heat stress mitigation strategies (Das et al. 2011). Thus, practical solutions need to be established and verified for the mitigation of heat stress in buffaloes. Literatures showed that the harmful effects of heat stress on bulls' behaviour can be mitigated through ceiling fans (Magrin et al. 2016). Usually, during peak hot hours of the day, young calves are splashed or washed with water to lessen heat load (Das et al. 2011). The evaporative cooling by means of fans, sprinklers, and foggers is being used commonly to ameliorate the heat stress (Singh et al. 2014), and to increase production (Avendano-Reyes et al. 2006) and feed consumption (West 2003; Vijayakumar et al. 2009), and to reduce body temperature, and normalize pulse and respiration rate (Singh et al. 2005; Aggarwal and Singh 2008; Das et al. 2011), thus contributing for the improvement of welfare (Magrin et al. 2016). In addition, it is claimed that use of rubber mats improves behavioural and or physiological performance in cattle (Brscic et al. 2015; Elmore et al. 2015). However, the effects of different heat stress mitigations on the physiological, behavioural, and hormonal responses of buffalo calves are not well known. We hypothesized that increase in height and width of buffalo shelter, use of time-controlled pressure mist with fans, and rubber mats in the floor will positively impact on the physiological, behavioural, and hormonal responses of buffalo calves during the hot summer season.

Materials and methods

This study was carried out on twenty young and healthy Murrah buffalo male calves of Livestock Research Centre, Indian Council of Agricultural Research -National Dairy Research Institute, Karnal, India, during June and July 2018. The geographical location of the experimental area is 29° 42″ 20 s N and 76° 58″ 52.5 s E at an altitude of 834 feet above mean sea level. During experimental months, ambient temperatures and relative humidity were ranges from 25 to 38 °C and 44 to 90 %, respectively (Krishnan 2013; Lakhani et al. 2020). Ambient temperatures range from 45 °C in summer to 2 °C in winter, with a diurnal variation of 15–20 °C. Experimental calves were at 6–8 months old and 123.2 ± 4.10 kg of body weight. Calves were fed individually with a total mixed ration (TMR), as per ICAR (2013) feeding standards. TMR composed of maize grass and grain, wheat bran, rice bran, mustard de-oiled cake, full-fat soya, cottonseed cake, and wheat straw. The farm section of the ICR produces the maize, chopped freshly, and mixed properly with the concentrate feed mixture and wheat straw in a ratio of 60:30:10, before offering it to calves at 08:50 h, daily. Ad libitum clean drinking water was provided in graduated water containers daily at 6:00, 12:00, 16:00, and 20:00 h. The feed and fodder samples were analysed by Association of Official Agricultural Chemists (AOAC 2005) methods and presented in Table 1. Experimental calves were randomly distributed into two groups: control (C) and treatment (T) group, 10 in each group.

Calves of control group were housed in existing shed having 10-feet height and 10-feet width with no additional heat stress ameliorative strategies. The floor was made up of concrete and the roof was of asbestos sheets. In contrast, calves of treatment group were managed with a modified shed: 15-feet height and 20-feet width along with time-controlled pressure mist with fans and rubber mat in the floor. Both sheds had 4 feet high walls, and then the above was open during the study period to ensure air movement. The floor was made up of concrete and the roof was of asbestos sheets in both groups.

Table 1 Proximate composition of feed ad fodder offered to the experimental calves

Particulars (%)	TMR	Maize green	Sorghum green	Wheat straw
Dry matter (g/100g)	52.0	21.3	23.2	90.4
Organic matter (g/100g)	95.3	92.0	89.9	90.1
Crude protein (g/100g)	14.2	9.8	7.8	3.5
Ether extract (g/100g)	2.6	2.1	2.0	0.9
Ash (g/100g)	10.0	9.0	10.1	9.9
Neutral detergent fibre (g/100g)	49.4	63.4	63.0	75.6
Acid detergent fibre (g/100g)	26.0	34.0	37.2	42.5

Fans were running all day during the experimental period with the continuous support of electricity or generator. However, fans were switched off at night time when the recorded THI indicates that animals were in thermo neutral zone. The cool water was misted on calves at the rate of 1 min in every 5 min, from 11:00 to 18:00 h. The water misting system was installed below the roof, but at 3.5 m above the floor. Foggers (NETAFIM, Fogger Cool Pro 7.5 Ltr 20 AD) were used to produce finer droplets of about 65 microns at 60 pounds per square inch (psi), allowing quicker evaporation of cool water, which was made from an engineered thermoplastic material for non-clog and non-corrosive application. Each nozzle was a cluster of the 4-Way Fogger which has a flow rate of 2.0 gallon/h. Specifications were as follows: static mister, 25 L/h per 4 nozzle cluster. Operation pressure of 3.5 to 5 kg/cm^2 (50 psi to 75 psi) was used to get finer water droplets. The circulating fans of 6-m size having 3 blades were used. Each fan motor was 1.1 horsepower at 1000 rpm, placed at a 4-m distance. The rubber mats of 4 × 6 feet and 2-inch thickness were provided as non-slippery bedding for the comfort of calves.

Before commencement of the experiment, all the calves were given 2-week adaptation period in the new sheds. The sheds were cleaned two times daily and the floor was thoroughly washed. The disinfectant solution was applied at weekly intervals to the floor in both groups. All experimental calves were given daily 2-h (07:00 to 09:00 h) exercise by letting them loose in adjoining open paddock beside the covered area.

Measurement of temperature humidity index (THI) of experimental sheds

The ambient temperatures in experimental sheds were recorded by using dry and wet bulb thermometer (Zeal, Merton, London, UK) to calculate THI for each shed. Three dry and three wet bulb thermometers were used in each shed. Thermometers were hung 2 feet above the animal head: one in the centre and one on each side wall (longitudinal) of the shed, to minimize errors. Ambient temperatures and relative humidity were recorded at 08:00 and 14:00 h of Indian Standard Time (IST) considering the findings of Panchbhai (2019); the THI level till 9:00 h are pleasant and similar whereas, while THI between 13:00 h to 14.00 h are extreme with the highest ambient temperature. The THI values of sheds of both groups were calculated using the following formula (McDowell 1972).

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

Measurement of body weight and feed intake of calves

Body weights of all experimental calves were recorded before feeding and watering on two consecutive days at the beginning of the trial, and thereafter fortnightly using an electric scale. Daily body weight gain of each calf was calculated from the average body weights of each calf measured in two consecutive days. The dry matter intake (DMI) of experimental calf was recorded on daily basis during the trial period, by subtracting residual/leftover dry matter from the quantity of DM offered. Water intake was measured by providing water in containers for three times in a day at 7.00, 14.00, and 21.00 h.

Measurement of physiological responses and blood hormones

The physiological parameters of each calf were as follows: rectal temperature (RT), pulse rate (PR), and respiration rate (RR) were recorded fortnightly at 08:00 and 14:00 h. For blood hormone measurements, blood samples were collected in each fortnight at 07:00 to 8:00 h. The rectal temperature (°C) was recorded with a mercury in glass clinical thermometer (Model: LHMED Dr. Equipment-9 M Clinical Oval Thermometer Alfa), inserted 3 inches in the rectum with rectal mucosa in contact with the bulb of the thermometer at least for 2-min period, taking care to avoid faeces. Respiration rate was recorded by observing flank movement, and one inward and outward movement was counted as one respiration and recorded as counts/min by standing 1 m away from the calf. The pulse rate was taken by feeling the pulsation of the middle coccygeal artery at the base of tail with the help of finger in undisturbed calves. The contraction and expansion of the artery is counted as one pulse. The pulse rate per minute was counted twice with a rest of 1–2 min to avoid manual errors.

The temperature variants of inner canthus of the eye were measured at 6-h intervals (07:00 and 13:00 h) using an infrared thermometer (Model: Fluke 59 Max ERTA: Fluke Corporation, USA) from each calf at approximately the same time (07:00 and 13:00 h) of each fortnight. The temperature was taken at 1 m perpendicular to the surface of floor and inner roof, directly in front of the head of calves. Thermometer measures temperature from − 30 to 350 °C.

Blood samples were collected fortnightly from each calf for determination of serum triiodothyronine, thyroxine, and cortisol concentration. Blood was collected through jugular venepuncture using 9 mL plane blood collection tubes for serum separation (Vacuette®, Greiner Bio-one Gmbh, Austria). Immediately after the collection of blood, it was kept for 20 min to separate the serum from the blood. The separated serum samples were stored in cryovials at − 80 °C till the estimation of hormones. Serum triiodothyronine, thyroxine, and cortisol concentrations were estimated by radioimmunoassay (RIA) in National Reference Laboratory, Indian Veterinary Research Institute, Izatnagar. The standard protocols for respective hormones have been previously validated for measuring bovine serum hormones using commercial kits (Immunotech, Czech Republic) on Sr-3000 Stratec Gamma Counter (Stratec Biomed Systems, Birkenfeld, Germany). The samples and calibrators are incubated with ^{125}I-labelled triiodothyronine, as a tracer, in antibody-coated tubes. After incubation, the liquid content of tubes is aspirated and the bound radioactivity is determined in a gamma counter. A standard curve is constructed, and unknown values are obtained from the curve by interpolation.

Behaviour recording

All the calves were simultaneously observed on two consecutive days of each fortnight by video-recording over 24 h. The behaviours of the calves were as follows: eating, ruminating, resting, and standing (Table 2) were observed. All these behaviours were recorded in terms of total time spent per day. The behavioural features were assessed using Closed Circuit Television (CCTV) camera video recording (Model: CP-UNC-TB21ZL6S-VMD; 2 MP Full HD WDR IR Network Bullet Camera, CP PLUS, GmbH & Co. KG. Hans Henny Jahnn, Web 9, Hamburg, D22085, Germany).

Statistical analysis

The effects of different heat stress mitigations on calves were estimated from the data obtained during trial in both sheds. One-way analysis of variance (ANOVA) was carried out on each variable using types of shed as a factor, tested at significance level $P < 0.05$ with means presented with SEM. Residuals of ANOVA were normally distributed except pulse rate and rectal temperature recorded at afternoon session. SPSS version 21.0 software was used to know the effect of treatments, as per the methods described by Snedecor and Cochran (1994). Means were tested for the significant differences by using Duncan's multiple range test. The data of environmental parameter (THI) was analysed by using the Student *t*-test.

Table 2 Definition of behaviours recorded in experimental calves

Behaviours	Definition
Eating	Animals head in the feeder with feed in the mouth
Ruminating	Chewing the cud while standing or lying
Lying	Body in contact with the ground
Standing	Animal inactive and whole-body weight on 4 legs

Results

Temperature humidity index (THI)

THI recorded from the control and treatment shed at each fortnight of June and July of 2018 irrespective of season and time differ significantly in corresponding comparisons, presented in Table 3.

Feed and water intake

The feed and water intake of calves managed in control and treatment groups is presented in Table 4. The DMI of calves reared under routine and modified shed management did not differ significantly, but the DMI of calves reared under modified shed management was numerically higher than control group, whereas the total water intake of calves was significantly higher in control group than calves reared under modified shed management (total water intake by C group: 27.5 ± 0.94, T group: 24.7 ± 0.79 L/day, $P < 0.05$). The morning and afternoon water intake remained unchanged between groups. However, the evening water intake was significantly higher in calves reared under control group than treatment group (evening water intake by C group: 10.3 ± 0.51, T group: 7.1 ± 0.43 L/day, $P < 0.01$).

Body weight

The influence of modified housing management on body weight of calves during summer is presented in Table 4. The body weights of calves reared under routine and modified shed management remained unchanged, though the body weight under modified shed management was numerically higher than that of the control group. Similar trend was observed for average daily gain (ADG) of calves.

Table 3 Temperature humidity index (THI) of experimental sheds, recorded during June and July of 2018

Summer	Month	Fortnight	Control shed		Modified shed		*P*-value
			8:00 h	14:00 h	8:00 h	14:00 h	
Hot dry	June	1	$78.4^{cA} \pm 0.56$	$82.1^{dB} \pm 0.90$	$75.0^{bD} \pm 0.41$	$71.5^{aA} \pm 0.36$	0.00
		2	$79.8^{cA} \pm 0.59$	$83.1^{dB} \pm 0.52$	$73.8^{bB} \pm 0.30$	$72.1^{aA} \pm 0.31$	0.00
Hot humid	July	3	$77.3^{bA} \pm 0.81$	$84.0^{cB} \pm 0.81$	$73.9^{a} \pm 0.52$	$72.5^{a} \pm 0.29$	0.00
		4	$78.9^{cA} \pm 0.54$	$81.3^{dB} \pm 0.47$	$73.9^{bD} \pm 0.30$	$71.9^{aA} \pm 0.26$	0.00

^{a,b} differ significantly between treatments, and ^{A,B} differ ($P < 0.01$) within the treatments at different time intervals.

Table 4 Feeding performance of Murrah buffalo calves managed at different sheds

Parameters	Control shed	Modified shed	*P*-value
Initial weight (kg)	122.0 ± 7.23	124.4 ± 4.29	0.78
Final weight (kg)	163.6 ± 7.47	170.0 ± 4.81	0.58
Weight gain (kg)	41.1	45.9	-
Average daily gain (g/day)	692.2 ± 54.58	759.7 ± 45.12	0.35
Dry matter intake (kg/100 kg BW)	3.5 ± 0.06	3.7 ± 0.05	0.12
Feed conversion efficiency (kgDMI/ kg BW gain)	5.01	4.82	-
Water intake (L)			
Morning	8.2 ± 0.79	7.6 ± 0.56	0.54
Afternoon	9.0 ± 0.57	10.0 ± 0.67	0.28
Evening	10.3 ± 0.51	7.1 ± 0.43	0.00
Water intake (L/day)	27.5 ± 0.94	24.67 ± 0.79	0.02

Physiological responses

The average values of body temperature, respiration rate, pulse rate, and eye temperature of calves recorded in morning and afternoon are presented in Table 5. The body temperature of calves under control group (C) was slightly higher than treatment group (T) during morning, whereas the afternoon body temperature was significantly ($P < 0.01$) lower in calves of T group (38.9 ± 0.25 °C) compared to the calves of C group (40.2 ± 0.14 °C). The respiration rate of calves recorded in the morning (C = 25.3 ± 0.80 No./min, T = 22.5 ± 0.56 No./min, $P < 0.01$) and afternoon (C = 32.5 ± 1.26 No./min, T = 23.5 ± 1.93 No./min, $P < 0.01$) was found to be significantly ($P < 0.01$) lower in T group compared to C group. Similarly, pulse rate recorded in the morning (C = 54.8 ± 1.01 No./min, T = 52.0 ± 0.77 No./min, $P < 0.01$) and afternoon (C = 68.3 ± 1.05 No./min, T = 59.2 ± 1.22 No./min, $P < 0.01$) was found to be significantly ($P < 0.01$) lower in T group compared to C group. The eye temperature was significantly ($P < 0.05$) higher during morning hours in C group (36.4 ± 0.18 °C) compared to T group (36.0 ± 0.12 °C). Also, significantly ($P < 0.01$) higher eye temperature was recorded during afternoon in C group (38.7 ± 0.21 °C) compared to T group (37.5 ± 0.14 °C).

Table 5 Physiological responses of Murrah buffalo calves managed at different sheds

Parameters	Control shed	Modified shed	*P*-value
Rectal temperature (°C)			
Morning	38.5 ± 0.17	38.2 ± 0.08	0.61
Afternoon	40.2 ± 0.14	38.9 ± 0.25	0.00
Respiratory rate (No./min.)			
Morning	25.3 ± 0.80	22.5 ± 0.56	0.00
Afternoon	32.5 ± 1.26	23.5 ± 1.93	0.00
Pulse rate (No./min.)			
Morning	54.8 ± 1.01	52.0 ± 0.77	0.00
Afternoon	68.3 ± 1.05	59.2 ± 1.22	0.00
Eye temperature (°C)			
Morning	36.4 ± 0.18	36.0 ± 0.12	0.05
Afternoon	38.7 ± 0.21	37.5 ± 0.14	0.00

Table 6 Hormonal responses of Murrah buffalo calves managed at different sheds

Parameters	Control shed	Modified shed	*P*-value
Triiodothyronine (nm/L)	1.54 ± 0.27	2.74 ± 0.34	0.00
Thyroxine (nm/L)	38.75 ± 3.57	40.27 ± 2.93	0.51
Cortisol (ng/mL)	7.60 ± 0.28	4.61 ± 0.41	0.00

Triiodothyronine, thyroxine, and cortisol level

The level of serum hormones is shown in Table 6. Triiodothyronine was significantly ($P < 0.01$) higher in the calves of T group (2.74 ± 0.34 nm/L) than C group (1.54 ± 0.27 nm/L), whereas cortisol level was significantly ($P < 0.01$) higher in C group (7.60 ± 0.28 ng/mL) than T group (4.61 ± 0.41 ng/mL).

Behavioural responses

The effect of modified shed management on behaviour is depicted in Table 7. The modified shed management has

Table 7 Behavioural responses of Murrah buffalo calves managed at different sheds

Parameters (min/day)	Control shed	Modified shed	*P*-value
Eating time (min)	364.0 ± 9.12	425.0 ± 8.96	0.00
Ruminating time (min)	609.0 ± 7.39	638.0 ± 9.76	0.65
Lying time (min)	585.5 ± 17.26	677.0 ± 13.89	0.00
Standing time (min)	854.5 ± 17.26	763.0 ± 13.89	0.00

significantly ($P<0.01$) influenced the time spent eating and lying in T group compared to C group ($C=364.0\pm9.12$, $T=425.0\pm8.96$, $P<0.01$; $C=585.5\pm17.26$, $T=677.0\pm13.89$, $P<0.01$, respectively). The rumination time of calves was not changed by summer stress as observed.

Discussion

Physiological and hormonal stress responses were lower in young Murrah buffalo calves while managed in a modified shed (treatment group). Conversely, the eating and resting times were higher in the calves of the treatment group than that of the control group.

Rectal temperature was lower in the calves of the treatment group than in the control group. Buffaloes are more susceptible to heat stress as they possess very few sweat glands than cattle (Kishore et al. 2016). In addition, they absorb a profound quantity of solar radiation due to their dark skin and sparse hair coat (Kapila et al. 2016; Lakhani et al. 2018; Prasad et al. 2020). In our experimental design, calves of the treatment group or modified shed had more opportunities to reduce extra body heats in association with increased height and width of the shed, time-controlled pressure mist fans, and rubber mats in the floor than that of the control group (managed in existing shed). Increased height and width of the shed provide more air space for the animals, which reduces the effect of heat waves emitting from the roof during the summer season. The high-pressure misting fan disperses very fine cool water and reduces relative humidity (Ambulkar et al. 2011; Avendaño-Reyes et al. 2010). Rubber mats reduce conductive heat from the floor to calves as observed in our previous study (Prasad et al. 2020). In agreement with our findings, Ambulkar et al. (2011) and Yadav et al. (2016) reported that sprinkle and misting of cool water on buffaloes and wetting or wallowing of buffaloes during the summer season can effectively reduce body temperature through evaporative cooling. Similarly, Lakhani et al. (2018) observed higher rectal temperature in Murrah buffaloes in hot humid and hot dry conditions.

In the calves of the treatment group, the respiration rate was lower than in the control group. During hot and humid climatic conditions, the environmental temperature is normally higher than the body temperature of calves, and they cannot release extra body heat to the environment. Instead, body temperature tended to increase as our findings of higher rectal temperature in the calves of the control group. In this situation, increased respiration rate may help them to evaporate heat from the body to the environment. The present results align with the values reported by Seerapu et al. (2015), who reported a lower respiration rate among the animals provided with foggers and foggers plus fans during the summer season than the animals of the control group (without foggers and or fans). In addition, Ambulkar et al. (2011) reported that the provision of fogger during summer seasons markedly lowered the respiration rate in Murrah buffaloes.

The pulse rate was also lower in the calves of the treatment group. Heat stress induces hypothalamic–pituitary–adrenal axis to secrete cortisol and adrenaline. Adrenaline constricts blood vessels, thus increasing blood flow within the body system. The eyes have rich capillary beds that are innervated with a sympathetic system and responsible for changes in blood flow (Stewart et al. 2007). Thus, whenever an animal is in a stress situation, the eye temperature rises identified in this study by infrared thermography, and others as well (Uddin et al. 2019, 2020, 2021). It is probably due to the dilation of ocular blood vessels and increased visual alertness or direction (Yarnell et al. 2013). In our previous study, we also observed that the rhythmic changes in eye temperature were influenced by the microclimatic conditions of the sheds (Prasad et al. 2020). Pavlidis et al. (2002) and Cook et al. (2005) claimed that temperature of the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle could be an indicator of stress. Lower pulse rates in Murrah buffaloes (Seerapu et al. 2015) and in Nili Ravi buffalo (Das et al. 2013) were observed under foggers and fans in their shed during the hot summer season. The pulse rate reflects the activity of the sympathetic branch of the autonomic nervous system and it acts as an indicator of the stress response (Sgoifo et al. 1999).

Animal growth is influenced by thyroid hormones as it affects body mass and size primarily by altering skeletal and nitrogen metabolism as well and helps the animal to adapt to the natural changes. Triiodothyronine hormone is a major concern for thermogenesis in farm animals (Rasooli et al. 2004; Pereira et al. 2008; Wankar et al. 2014). Triiodothyronine concentration was reduced with increased temperature humidity index in the current trial probably to reduce body temperature generation (West 2003; Wankar et al. 2017). Therefore, lower levels of thyroid hormones are seen in the control group. Similarly, Wankar et al. (2014) found the uppermost concentration of thyroid hormones in optimal conditions, which has significantly ($P<0.001$) fallen with increased environmental temperature in adult buffaloes. Chaudhary et al. (2015) found lesser Thyroxine concentration in the Surat buffaloes during the hot dry season compared to the hot humid. In contrast, thyroxine did not differ in different seasons in sheep (Yokus et al. 2006) and Murrah buffaloes (Ranjan et al. 2012).

The higher cortisol levels in the calves of control group indicate the effect of heat stress. Excessive nutritional diet can affect heat stress in ruminants by the activation of hypothalamic pituitary adrenal axis (Munksgaard et al. 2006). Ranjan et al. (2012) reported higher cortisol levels in severe heat stress situation making it easier for the body to adapt. The effect of increased cortisol on fat metabolism during heat stress increases cholesterol catabolism to support gluconeogenesis to provide more energy to the animals under thermal stress (Nazifi et al. 2003; Sejian et al. 2010).

Though the final body weight of animals under modified shed is higher, it did not differ significantly. We assume the time of experiment in our study (2 months) may not be enough to have a significant difference in the body weight gain of calves in the compared groups. Similarly, the ADG and DMI were higher in those animals. These results can be attributed to higher DMI of animals under a modified shed which might be due to higher feed intake because of a comfortable environment. Similar to our results, the Murrah's calves reared under regular feeding levels with improved management practices showed better growth (Singh et al. 2012; Panchabhai 2019). Also, the buffalo heifers provided fans with sprinklers had higher daily gain during the summer season (Vijayakumar et al. 2009; Panchabhai 2019).

The increased water intake in the control group's calves was associated with heat stress. Animals under temperatures above the thermoneutral zone increase water losses associated with increased sweat and respiration (McDowell and Weldy 1967). To compensate for water losses through increased sweat and respiration during higher environmental temperature, the water intake must also increase. Similar to the current study, an increase in water intake was observed in dairy cattle by 1.2 kg for an increase of every 1 °C in the barn temperature (Andersson and Lindgren 1987). In addition, Hoffman and Self (1972) observed increased water intake in feedlot steers in the summer than in the winter. Sharma et al. (2018) also reported similar results of water intake among Murrah male buffaloes.

The eating and resting times were higher in young Murrah buffalo calves while managed in a modified shed (treatment group). The eating time was significantly ($P<0.01$) reduced in the calves of the control group than the treatment group. The season can be a critical factor in changing the feeding behaviour of calves (Grant and Albright 2001). Animals facing temperatures above their thermo-neutral zone reduce the voluntary feed intake (Soren 2012). Likewise, Magrin et al. (2016) reported that young bulls exposed to ≥ 74 temperature humidity index (THI) reduced their time eating and ruminating and were less active compared to their counterparts under optimum THI conditions, whereas the provision of ceiling fans increased ruminating time and exploring activities in young calves. The calves in the present study under control shed spent less time ruminating, probably in an attempt to lessen metabolic heat and to maintain their core body temperature (Nardone et al. 2010). Similarly, Sprinkle et al. (2000) reported lower eating time in cattle during the heat stress period compared to the thermal comfort period. The summer management practices significantly influence the average time spent on rumination (Vijayakumar et al. 2009; Kamal et al. 2018).

The lying time was significantly ($P<0.01$) lower in the calves of the control group compared to the treatment group. The higher-lying time observed in calves housed in the modified shed might be due to the providing of rubber mats which gives comfort to the animals and improves welfare too (Brscic et al. 2015; Elmore et al. 2015). The lower time of lying among calves housed in the control shed might be due to concrete flooring and also the animals might have spent most of their time standing for heat dissipation as the body surface area exposed to air is more when they keep standing. Increased standing time is the most common behavioural change observed in animals under heat stress (Widowski 2001; Overton et al. 2002). Tucker et al. (2008) observed 10% increase in standing time when heat load increased by 15% in dairy cattle, might be due to increased heat loss by exposing the more amount of skin to airflow. The evaporation from the body surface increases by standing in calves or there may be a chance of sun-heated floor, which might have increased the standing time in the present study (Kamal et al. 2018).

Provision of wallowing in buffaloes, they have developed the habit to cool themselves for normal physiological functioning particularly in summer. The endocrine response to heat stress is mainly reflected in elevated cortisol in beef cattle (Idris et al. 2021), and also the elevated body temperature, respiration rate, and pulse rate are considered the body's thermoregulatory response to cope up with increasing environmental temperature in buffaloes (Panchabhai 2019; Lakhani et al. 2021).

Conclusion

The results of this study indicate that increased height and width of the shed, time-controlled pressure mist fans, and rubber mats in the floor help buffaloes to release extra body heat during hot summer seasons or heat stress. Therefore, the heat stress ameliorating measures should be provided to Murrah buffalo growing calves for a comfortable environment which influences the growth performance of animals with normal physiological status and helps in normal behavioural expression for optimum weight gain as a sign of welfare.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethics approval All animal studies have been approved by the Institute Animal Ethical Committee dated: 17.05.2018 No:M-20/AG&B/2018/534–537.

Consent for publication All authors have given consent for the publication of this manuscript.

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