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Effect of melatonin supplementation on skin surface temperature in buffalo (*Bubalus bubalis*) calves under summer stress

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Abstract
Present study was designed to assess the effect of Melatonin on skin surface temperature (SST) in buffalo calves under summer stress. Twelve healthy Murrah buffalo male calves of 6 month to 1 year age group were taken and divided into control (CG) and treatment (TG) group (n=6). In TG, Melatonin was injected @ 18mg/50 kg BW subcutaneously, twice at 1st and 20th day of experiment. The SST of definite sites was recorded twice daily using infra-red digital thermometer. Result of study showed that there was no significant ($P>0.05$) difference observed in SST of head, neck, foreleg, hindleg, thigh, flank, back and inner canthus of eye of buffalo calves between the control and treatment group. After noon values were significantly higher ($P<0.05$) than the morning, in both group of animals during the experiment. The SST of the head, neck, foreleg, hindleg, thigh, flank, back and inner canthus of eye was significantly ($P<0.05$) increasing in morning to afternoon. Within groups the average SST of head, neck, foreleg, hindleg, thigh, flank, back and temperature of inner canthus of eye observed to vary significantly ($P<0.05$) at some time points. Within and between the control and melatonin treated group water intake of buffalo calves did not differ significantly ($P>0.05$) during the experiment. In general the water intake was at higher side in treated animals as compared to control group. It could be concluded from the result of this study that melatonin injection in murrah buffalo calves during summer did not affect the SST.

Keywords: Melatonin, buffalo calves, summer stress, skin surface temperature

Introduction
Buffalos *(Bubalus bubalis)* have the zones of thermal comfort which are primarily dependent on physiological status of the animals, species, relative humidity and severity of solar radiation. The detrimental effect on the productivity of commercial farm animals is due to high environmental temperature and humidity. Buffaloes exhibit signs of great physical distress when exposed to high environmental temperature because of their body absorbs a great deal of solar radiation and dark skin and sparse coat. Buffalo have less efficient evaporative cooling system due to their poor sweating ability. Buffaloes contribute approximately 96% to total milk production and they are known for their milk production in India. India has been reached to 132.4 million tonnes in milk production in 2012-13 with a growth rate of 3.5% [1]. Heat stress affects the physiological systems and maintenance energy governing thermal regulation of buffalo during extreme summer. High ambient temperature is the major constraint on animal productivity and effect of heat stress is aggravated when heat stress is accompanied with high humidity in case of sheep in tropical and subtropical areas [2]. Exposure of buffaloes to the hot conditions evokes a series of drastic changes in the biological functions that include decrease feed intake, water, disturbances in metabolism of protein, efficiency and utilization, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites. Such changes result in impairment of reproduction and productive performances [3, 4]. The skin of the mammalian is an important pathway for heat exchange between the body surface and the environment. Skin temperature is the result of the adjustment of the skin blood flow that ends with regulation of the heat between the body core and skin [5]. Under the heat stress there is increases in the mean skin surface temperature in case of cattle [6]. It has been observed during one study that skin temperature was lower at 8:00 am than at 12:00 pm and 16:00 pm of which the latter two were similar, during summer conditions [7].
Short term heat acclimation (STHA) is characterized by responses initiated to compensate for the increased heat stress before permanent acclimation can be obtained.

Increased heat dissipation (through evaporative heat loss), reduced feed intake and milk yield and increased water intake are examples of the STHA response [8].

High environmental temperature is the major constraint on animal production in case of tropic and sub tropic regions [9, 10] where extreme low temperature in temperate regions is also have detrimental effect to farm animals. Thermal stress is the perceived discomfort and physiological strain associated with exposure to the uttermost hot or cold environment. Summer stress includes both heat stress, during extreme summer as well as cold stress, during extreme winter. During thermal stress biochemical and physiological changes occurring in the animal body directly or indirectly which affect the animal production [11].

Summer stress is a major control on animal productivity in tropical conditions. In the production and growth of all livestock species, the change in climatic variables like temperature of an environment, humidity and radiations are acknowledged as potential hazards. High environmental temperature accompanied by high air humidity causes an additional discomfort and increases the level of stress which in results in depression of the physiological and metabolic activities of the farm animals. Homeotherms having reaction to moderate climatic changes are compensatory and are directed at restoring thermal balance. However, when environmental temperature becomes near the animal's body temperature, high ambient humidity decreases evaporation, overcomes the animal's cooling capacity and the body temperature rises. The rise in concern of thermal discomfort for animals is disputable not only for countries of tropical zones, but for nations of temperate zones in which environmental temperature is increasing due to climate change [10]. During heat stress, heat increment exceeds heat loss modifying the homeostatic functions. Heat stress elicits an integrative physiological and endocrinalogical modulation changing overall metabolism and helping the animal sustain during the stressful period. Various in-depth studies on heat and nutritional stress on animals, severely compromising thermoregulatory functions which intern affect the reproductive potential of animals [12, 13, 14].

Melatonin is well known for its role in seasonality of reproduction, plays prominent role in relieving heat stress by influencing cardiovascular system and evaporative heat loss. Melatonin has been shown to interact with several other hormones like thyroxine to lessen heat stress and also modify adrenal activity to relieve thermal stress [15]. The functions Melatonin are direct free radical scavenging, stimulation of antioxidative enzymes, enhancing the mitochondrial oxidative phosphorylation efficiency and reducing leakage of electron (thereby lowering free radical generation) and 3) augmenting the efficiency of other antioxidants [16]. Melatonin was a powerful direct free radical scavenger and indirect antioxidant [17, 18, 19]. Melatonin has the high efficacy as a protector against reactive oxygen (ROS) and reactive nitrogen species (RNS). Therefore the present experiment was designed to study the effect of melatonin on skin surface temperature in buffalo calves under summer stress.

Materials and Methods

Animals and experimental design

Healthy Murrah buffalo bull calves between 6 months to 1 year belonging to tropical region were selected for the study purpose. The experiment trial was conducted up to 6 week (42 days) period. Animals were divided into two groups *viz*., control (CG) and treatment (TG) group (n= 6). TG received melatonin @ 18 mg/50 kg body weight, subcutaneously (s/c), on 1st and 20th day of experiment [20]. Animals were reared under strict management and proper hygienic conditions throughout the study. Basal diet of wheat straw *ad libitum* along with the required amount of green and concentrate mixture to meet the maintenance requirement was offered daily at 08:00 am and 2:30 pm. Water was provided in graduated containers and intake was recorded accordingly.

Parameters of study

The skin surface temperatures of a definite site like head, forehead, neck, foreleg, hindleg, thigh, flank, back and inner canthus of eye were taken at every day with the help of infra-red digital thermometer by keeping it 2-3 inches away from the desired skin surface site.

Statistical analyses

Data obtained was analysed statistically by one way ANOVA followed by Tukey's b test [21] within group between the days and independent t test for between groups with the help of SPSS 17.0 software.

Results and Discussion

Table 1: Skin surface temperature (°F) of control and melatonin supplemented animals during summer

Parameters	Group	0 day	7 day	14 day	21 day	28 day	35 day	42 day
Head (Morning)	Control	93.33±0.86^{bB}	94.36±0.84^{abB}	97.01±0.81^{aB}	93.84±1.08^{bB}	96.36±1.07^{aB}	94.38±0.99^{abB}	94.33±0.88^{abB}
	Treatment	94.08±1.56^{abB}	93.54±0.81^{bB}	97.20±0.66^{aB}	94.66±0.99^{abB}	95.63±1.08^{abB}	95.28±0.89^{abB}	93.70±0.94^{bB}
Head (Afternoon)	Control	101.25±1.65^{abA}	102.64±0.83^{aA}	103.98±0.90^{aA}	103.16±0.87^{aA}	101.32±1.07^{abA}	101.07±1.05^{abA}	99.66±0.91^{bA}
	Treatment	100.16±0.42^{bcA}	102.54±0.80^{abA}	104.88±1.15^{aA}	103.22±0.95^{aA}	101.88±1.57^{abA}	100.55±1.43^{bcA}	99.57±1.01^{cA}
Neck (Morning)	Control	95.00±1.16^{abB}	93.15±0.59^{bB}	96.92±0.67^{aB}	94.82±1.04^{abB}	96.35±0.70^{aB}	95.38±0.96^{aB}	94.76±0.96^{abB}
	Treatment	95.16±0.78^{abB}	93.93±0.93^{cB}	96.95±0.89^{aB}	95.38±0.76^{abB}	95.28±1.13^{abB}	96.48±0.89^{aB}	95.09±0.99^{abB}
Neck (Afternoon)	Control	99.16±0.38^{cA}	100.58±0.79^{bcA}	102.38±0.83^{aA}	101.94±0.80^{abA}	101.08±1.08^{abA}	100.52±1.15^{bcA}	99.32±0.97^{cA}
	Treatment	98.41±0.43^{cA}	100.73±0.72^{abA}	101.47±0.71^{aA}	101.55±0.56^{aA}	100.71±1.05^{abA}	99.89±0.98^{bcA}	98.63±1.14^{cA}
Foreleg (Morning)	Control	92.58±0.61^{bcB}	91.83±0.62^{cB}	95.07±0.68^{aB}	92.51±0.95^{bB}	93.97±0.90^{bB}	93.51±0.94^{bB}	93.20±1.07^{bB}
	Treatment	93.5±0.87^{abB}	92.07±0.92^{bB}	94.20±0.97^{aB}	92.80±1.01^{abB}	93.04±1.21^{abB}	93.22±0.87^{abB}	93.44±1.00^{abB}
Foreleg (Afternoon)	Control	97.75±0.52^{cA}	99.70±0.82^{abA}	101.21±0.72^{aA}	100.57±0.84^{abA}	100.09±1.02^{abA}	98.89±1.21^{bcA}	98.27±1.09^{bcA}
	Treatment	99.25±0.51^{bcA}	99.40±0.84^{bcA}	101.77±0.91^{aA}	100.45±0.78^{abA}	100.35±1.33^{abA}	98.83±1.59^{cA}	99.11±1.43^{bcA}
Hindleg (Morning)	Control	86.66±1.70^{bB}	90.83±1.02^{abB}	92.59±0.92^{abB}	91.07±1.45^{abB}	93.15±0.99^{aB}	93.26±1.00^{abB}	90.95±1.26^{abB}
	Treatment	88.41±2.06^{bB}	90.39±1.46^{abB}	92.51±1.34^{aB}	91.66±0.97^{aB}	91.92±1.34^{aB}	92.10±1.02^{aB}	91.82±1.27^{aB}
Hindleg (Afternoon)	Control	98.83±1.44^{abA}	99.35±0.87^{abA}	100.21±0.69^{aA}	99.33±0.60^{abA}	98.58±0.92^{abA}	98.04±1.26^{abA}	97.21±1.20^{bA}
	Treatment	98.75±0.69^{aA}	99.52±0.83^{aA}	100.84±0.92^{aA}	99.90±0.85^{aA}	99.44±1.24^{aA}	98.14±1.26^{aA}	97.03±1.35^{aA}
Thigh (Morning)	Control	84.58±1.01^{dB}	90.61±1.12^{cB}	94.00±0.98^{aB}	91.48±1.55^{abB}	93.67±1.26^{aB}	94.10±1.24^{aB}	93.20±1.46^{aB}
	Treatment	87.5±1.91^{bB}	91.34±1.31^{abB}	93.85±0.92^{aB}	92.03±1.10^{aB}	93.47±1.31^{aB}	93.42±1.02^{aB}	92.64±1.67^{aB}

Thigh (Afternoon)	Control	99.00 ± 1.21^{aA}	99.61 ± 0.91^{aA}	100.27 ± 0.70^{aA}	100.39 ± 0.70^{aA}	99.36 ± 0.74^{aA}	99.08 ± 1.31^{aA}	99.11 ± 1.07^{aA}
	Treatment	99.5 ± 0.83^{abA}	100.38 ± 0.86^{abA}	101.02 ± 0.89^{aA}	101.28 ± 0.58^{aA}	100.23 ± 1.06^{abA}	98.73 ± 1.60^{bA}	99.46 ± 1.16^{abA}
Flank (Morning)	Control	91.5 ± 1.05^{cB}	94.71 ± 0.69^{bB}	97.26 ± 0.80^{aB}	94.78 ± 1.17^{bB}	96.01 ± 0.97^{aB}	96.26 ± 0.92^{aB}	94.13 ± 1.30^{bB}
	Treatment	92.16 ± 0.77^{cB}	94.58 ± 0.83^{bB}	97.65 ± 0.80^{aB}	95.23 ± 1.18^{abB}	95.82 ± 1.17^{abB}	96.05 ± 1.12^{abB}	94.08 ± 1.23^{bB}
Flank (Afternoon)	Control	100.91 ± 1.22^{bcA}	101.23 ± 1.07^{abA}	102.98 ± 0.91^{aA}	102.53 ± 0.84^{aA}	101.98 ± 0.76^{abA}	101.40 ± 1.01^{abA}	99.96 ± 0.89^{cA}
	Treatment	101.08 ± 0.72^{bA}	102.26 ± 0.73^{abA}	103.35 ± 0.93^{aA}	103.27 ± 1.09^{aA}	102.19 ± 1.13^{abA}	101.48 ± 1.26^{abA}	99.82 ± 1.01^{cA}
Back (Morning)	Control	91.08 ± 1.42^{cB}	94.15 ± 0.94^{bB}	97.09 ± 0.67^{aB}	94.76 ± 1.12^{bB}	95.79 ± 0.88^{bB}	96.38 ± 1.59^{abB}	95.27 ± 0.85^{bB}
	Treatment	92.00 ± 1.04^{cB}	93.69 ± 1.10^{cB}	97.32 ± 0.72^{aB}	94.63 ± 0.99^{bcB}	95.19 ± 1.06^{abB}	95.10 ± 1.08^{abB}	95.63 ± 0.89^{abB}
Back (Afternoon)	Control	100.66 ± 1.35^{bA}	101.91 ± 0.92^{bA}	103.36 ± 0.95^{aA}	103.39 ± 0.79^{aA}	101.29 ± 0.98^{bA}	101.55 ± 1.22^{bA}	100.55 ± 1.13^{bA}
	Treatment	101.08 ± 0.85^{bcA}	102.48 ± 0.77^{abA}	103.72 ± 0.88^{aA}	103.30 ± 1.05^{aA}	102.00 ± 1.17^{abA}	100.99 ± 1.65^{cA}	101.09 ± 1.15^{bcA}
Inner canthus of eye (Morning)	Control	93.25 ± 0.38^{cB}	94.79 ± 0.72^{bcB}	97.17 ± 0.63^{aB}	95.60 ± 0.89^{abB}	96.96 ± 0.64^{abB}	97.15 ± 0.62^{aB}	95.98 ± 0.79^{abB}
	Treatment	91.33 ± 1.65^{cB}	94.76 ± 0.81^{bB}	97.26 ± 0.56^{aB}	95.44 ± 0.82^{abB}	96.63 ± 0.79^{aB}	96.55 ± 0.66^{aB}	94.80 ± 0.83^{bB}
Inner canthus of eye (Afternoon)	Control	100.58 ± 0.94^{aA}	100.62 ± 0.91^{aA}	101.14 ± 0.74^{aA}	101.13 ± 0.75^{aA}	100.84 ± 0.64^{aA}	100.23 ± 0.75^{aA}	100.89 ± 0.66^{aA}
	Treatment	99.08 ± 0.67^{bA}	100.36 ± 0.72^{abA}	101.40 ± 0.94^{aA}	101.07 ± 0.90^{aA}	101.33 ± 0.79^{aA}	100.11 ± 0.67^{abA}	100.03 ± 0.90^{abA}

Bars bearing different superscript a, b, c, d differ significantly (P<0.05) in the same group between days and superscript 'A' and 'B' denotes significant (P<0.05) difference between the groups during study period.

Table 2: Water intake (liter) of control and melatonin supplemented animals during summer

Parameter	Group	0 day	7 day	14 day	21 day	28 day	35 day	42 day
Water Intake (Liter)	Control	12.35 ± 0.38^{aA}	13.0 ± 0.60^{aA}	13.62 ± 0.74^{aA}	13.90 ± 0.56^{aA}	15.84 ± 0.68^{aA}	15.59 ± 0.90^{aA}	15.14 ± 0.81^{aA}
	Treatment	12.58 ± 0.76^{aA}	13.24 ± 0.87^{aA}	14.07 ± 1.44^{aA}	13.97 ± 1.22^{aA}	16.17 ± 0.93^{aA}	15.56 ± 1.15^{aA}	15.22 ± 1.08^{aA}

Bars bearing different superscript a, b, c differ significantly (P<0.05) in the same group between days and superscript 'A' and 'B' denotes significant (P<0.05) difference between the groups during study period.

Table 3: Metrological variables during the experiment

Parameter	0 day	7 day	14 day	21 day	28 day	35 day	42 day
Dry bulb Temperature (°C)	33.5	37	36.75	36	37.5	35.5	32
Wet bulb Temperature (°C)	28.5	28	27.75	28	26.5	29	28
Relative Humidity (%)	68.00	63.57	66.42	60.71	56.85	71.42	75.71
Temperature Humidity Index (%)	85.24	87.4	87.04	86.68	86.68	87.04	83.8

Skin is an important pathway for heat exchange between the environment and the body surface. Skin temperature is the result of the adjustment of the skin blood flow that cease with the heat regulation between the body core and skin [5]. Skin surface temperature in case of sheep was observed to be highest during summer and lowest during winter [22, 23]. There are increases in the mean skin surface temperature in case of cattle under the heat stress [6]. Skin surface temperature was lower at 8:00 am than at 12:00 pm and 16:00 pm was observed during summer conditions [7]. Heat stress is the result of environmental forces continuously acting upon livestock which interrupt homeostasis mechanism resulting in new adaptations that can be detrimental or advantageous to the animal [24]. In tropical, subtropical and arid areas heat stress has been of major concern in reducing animal's productivity [25]. Animals having the ability to acclimatize and produce under the specific climate condition signify the adaptation to a particular environmental niche. A highly integrated cascade of physiological and behavioral responses is set in motion in the animal which helps to maintain homeostasis and physiological equilibrium [26]. Animal tries to maintain heat balance during heat stress by increasing heat loss and decreasing heat production. Heat production is closely associated with metabolism of the body which is coordinated by endocrine system which undergoes substantial alteration under thermal stress [27]. Present study revealed that there was no significant (P>0.05) difference observed in SST of head, neck, foreleg, hindleg, thigh, flank, back and inner canthus of eye of buffalo calves between the control and treatment group. After noon values were significantly higher (P<0.05) than the morning, in both group of animals during the experiment. The SST of the head, neck, foreleg, hindleg, thigh, flank, back and inner canthus of eye was significantly (P<0.05) increasing in morning to afternoon. Within groups the average SST of head, neck, foreleg, hindleg, thigh, flank, back and temperature of inner canthus of eye observed to vary significantly (P<0.05) at some time points [Table 1].

Solar radiation is the radiant energy that comes from the sun which is received at the earth surface. The skin temperature increased as the intensity of solar radiation increased. Murrah buffaloes that significant change in temperature pattern at different sites like middle neck, pinna, middle back, rump, foreleg, hindleg and forehead was due to mainly change in skin temperature in relation to the environmental temperature [28]. It has been also stated that the change in temperature at different sites may also be due to water diffusion and evaporation. Our findings are concurrent with this study. Skin temperature is lower at low ambient temperatures due to the fat insulation, while increasing environmental temperature rapidly increases the skin surface temperature, due to the vasocontrolled thermoregulation, supplying more blood to the skin tissue in pigs [29]. The magnitude of changes in skin surface temperature was highest at forehead and lowest at hindleg. The temperature gradient between air temperature and skin surface temperature of forehead, pinna, middle back and rump were very sharp but the temperature gradient of middle neck, udder, foreleg and hindleg were small. The forehead, pinna, middle neck, back, foreleg and hindleg temperature were observed to be minimum when the environmental temperature was less [28]. In case of sheep, it can be stated generally that skin temperature differ according to the season of the year and time of the day and it becomes higher with an elevation in ambient temperature. In short, exposure of sheep to elevated ambient temperature co-occurs with a rise in the dissipation of excess body heat in order to balance the excessive heat load. Dissipation of excess body heat is performed by evaporation of water from the respiratory tract and skin surface via panting and sweating, respectively [30].

When the environmental temperature increases to 36˚C, the ears and legs of sheep dissipate a high proportion of the heat. If the skin surface temperature is below 35˚C, the temperature gradient between the core and skin is large enough for the animals to effectively use all four routes of heat exchange; conduction, convection, radiation and evaporation [30]. Temperature Humidity Index (THI) is commonly used as an indicator of the degree of climatic stress on animals where a THI of below 72 is believe as no heat stress, 73-77 considered as mild heat stress, 78-89 as moderate and above 90 as severe [31]. THI along with increased respiratory rate and rectal temperature with increasing heat exposure confirmed animals are under heat stress that too severe during 40˚C exposure [32]. The mean dry bulb temperature of microclimate during the experiment is 35.27±0.30 and which varied from 31.00 to 39.50°C. The mean wet bulb temperature of microclimate during the experiment was 27.72±0.15, which ranges from 25.25 to 30.50°C. The mean relative humidity of microclimate during the experiment was 66.09±0.13 which ranges from 56.85 to 75.71(%). The mean THI during the experiment was 85.96±0.20 and ranges from 83.08 to 87.04(%) [Table 3].

In our investigation, it has been observed that within and between the control and melatonin treated group water intake of buffalo calves did not differ significantly (P>0.05) during the experiment and water intake was at higher side in treated animals as compared to control group [Table 2]. Similarly, the immediate response to heat load decrease feed intake and increase water intake [33, 34]. Higher water intake/day at 35°C and 40°C of temperature exposure was mainly due to evaporatory heat loss Water (respiration, sweating or panting), resulting in increased osmolarity of the extracellular fluid in the body, ultimately leading to activation of thirst center in the hypothalamus and increase in water intake [35, 36]. Heat stress is known to have a significant effect on the surface temperatures of the ear and eyes [37] and the loin [38] in pigs. Drinking water is not only the most important essential nutrient for dairy cattle [39], but it also has high specific heat which promotes heat dissipation. There are studies that indicate the direct association between the environmental temperature and water intake [40, 41]. Buffalo sweat limitedly and prefer wallowing in water due to specific heat of water and capacity of water due to specific heat of water and capacity of water to absorb far greater heat and readily heat exchanging power. The low hair density on skin helps in readily exchange of heat to water. The thickness of buffalo skin helps in protection against overheating of the body through reduction of thermal conductivity, however, it hinders heat dissipation through convection and radiation [42] but this limitation is overcome while wallowing. Buffaloes are better converter of poor quality fibrous feed and that will convert it into the milk and meat. The increased heat dissipation through the evaporative heat loss which reduced feed intake and milk yield and increased water intake [8].

Conclusion

It could be concluded from the result of this study that melatonin injection in murrah buffalo calves during summer did not affect the skin surface temperature.

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