



Thermoregulatory responses of female buffaloes reared under direct sunlight and shaded areas in the dry season on Marajó Island, Brazil

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ABSTRACT. This research aimed to assess the effects of dry season climate variables on Marajó Island, Brazil, on the thermoregulatory responses of twenty female buffaloes, assigned to shaded (SD), and unshaded groups (US). Data on air temperature (AT), relative air humidity, wind velocity (WV), respiratory rate (RR), rectal temperature (RT), and body surface temperature (BST) were recorded at 6 a.m., 10 a.m., 2 p.m., 6 p.m., and 10 p.m. The temperature and humidity index (THI), practical buffalo comfort climatic conditions index (BCCCIp), and practical buffalo environmental comfort index (BECIp) were calculated. At 10 a.m. and 2 p.m., WV was the highest. The RR of animals in both treatments differed only at 10 a.m. and 2 p.m. A difference in RT between treatments was seen at 10 a.m., 2 a.m., and 6 p.m. Significant changes were seen in the BST of animals in both groups at 10 a.m., 2 p.m., and 10 p.m. BECIp was different between the groups at 10 a.m. and 2 p.m., with higher values for the US group. Buffaloes are prone to thermal stress, particularly between 10 a.m. and 2 p.m., when the highest values of AT, THI, and BCCCIp was observed.

Keywords: heat stress; thermoregulation; *Bubalus bubalis*.

Respostas termorregulatórias de búfalas criadas ao sol e à sombra, no período seco do ano, na ilha do Marajó, Brasil

RESUMO. Este estudo avaliou os efeitos do clima no período seco do ano, na Ilha de Marajó, Brasil, sobre as respostas termorreguladoras de vinte búfalas, divididas entre o grupo com sombra (CS) e o grupo sem sombra (SS). Os dados sobre temperatura do ar (TA), umidade relativa do ar, velocidade do vento (VV), frequência respiratória (FR), temperatura retal (TR) e temperatura da superfície corporal (TSC) foram registradas às 6h, 10h, 14h, 18h e 22h. Foram calculados o índice de temperatura e umidade (ITU), índice de condições climáticas de conforto para búfalos prático (BCCCIp) e índice de conforto ambiental para búfalos prático (BECIp). Às 10h e 14 horas, a VV foi mais elevada. A FR dos animais, em ambos os tratamentos, diferiu apenas às 10h e 14h. Houve diferença na TR entre os tratamentos às 10h, 14h e 18h. Diferenças significativas foram observadas na TSC dos animais, em ambos os grupos, às 10h, 14h e 22h. O BECIp diferiu entre os grupos às 10h e às 14h, com valores maiores para o grupo SS. Os búfalos são propensos ao estresse térmico, particularmente entre 10h e 14h, quando são observados valores mais altos de TA, ITU e BCCCIp.

Palavras-chave: estresse térmico; termorregulação; *Bubalus bubalis*.

Introduction

The buffalo herd was introduced in the Brazilian territory of Marajó Island, Pará by the end of the 19th century (Damé, Riet-Correa, & Schild, 2013). Those animals adapted very well to the environmental conditions in the Amazon due to the similarity between local environmental conditions with those of their countries of origin, characterized by the

predominantly tropical climate, and after introduction continued developing and adapting to the conditions of the island due to the great capacity of adaptation of these buffaloes to different types of environments. These buffaloes most likely were able to attain satisfactory animal indices where cattle would not be able to under the same conditions (Bernardes, 2007).

Nonetheless, despite being able to maintain good body conditions in adverse environments, buffaloes are sensitive to heat and, under high ambient temperatures, show alterations in physiological parameters, decreases in production, and lower reproductive efficiency (Damasceno, Viana, Tinôco, Gomes, & Schiassi, 2010). That occurs because they have specific morphological characteristics, such as a high concentration of melanin in the skin and hair, a small number of sweat glands that have reduced efficiency, and low hair density, which makes them sensitive to solar radiation (Gudev et al., 2007).

Research has shown that tree shade is effective in protecting animals against direct solar radiation, which can improve thermal comfort and, consequently, productivity of meat, milk, and manual labor involved in rearing activities. Therefore, understanding the physiology of buffaloes and the mechanisms involved in their responses to the challenges posed by heat present in the environment is essential for the development of new technologies that are able to increase animal health and well-being (Garcia, 2013). Thus, this research aimed to assess the effects of the dry season climate on Marajó Island, Pará, on the thermoregulatory responses of female Murrah buffaloes reared in systems with and without exposure to direct sunlight.

Material and methods

The experiment was approved by the Ethics Committee on Animal Use of the Federal Rural University of the Amazon under protocols no. 054/2015 (CEUA) and 23084.013102/2015-01 (UFRA), which verified compliance with Federal Law 11,794/08 (Arouca Law) and with COBEA's Ethical Principles in Animal Experimentation.

The experiment was carried out on a rural property in the municipality of Cachoeira do Arari, Marajó Island, Pará, Brazil (00°55'37.8" S and 48°43'48.1" W) in the dry season, between October and November 2015. The study area has Am climate in the Köppen classification, which indicates rainfall in the driest month is below 60 mm, with an annual average between 2,500 mm and 3,000 mm.

Twenty cyclic non-pregnant and non-lactating clinically healthy female Murrah buffaloes with mean age of 24 months and mean weight of 267.92 ± 28 kg were randomly assigned to two groups (shaded group - SD and unshaded group - US). The SD group (n = 10) was kept in a 1 ha pen in a system with native Malay apple (*Syzygium malaccense*) and coite (*Crescentia cujete*) trees, which provided 16% shade. The US group (n = 10) was

kept in a pen with no trees. Both groups were under continuous grazing with *Brachiaria humidicola* and had access to drinking water and mineral salt *ad libitum*.

The meteorological data of air temperature (AT) and relative air humidity (RH) were recorded using a U12-012 HOBO® data logger installed in mini meteorological shelters at the experimental site. A TAD-800 portable digital thermo-anemometer (Instrutherm, São Paulo, Brazil) was used to measure wind velocity (WV). These data were recorded along with physiological variables on the field evaluation days.

They were later used to calculate the temperature and humidity index (THI) proposed by Thom in 1959, i.e.,

$$THI = 0.8Ta + RH(Ta - 14.3)/100 + 46.3$$

where Ta = air temperature (°C) and RH = relative air humidity (%). The practical buffalo comfort climatic conditions index (BCCCIp) was also calculated using the formula:

$$BCCCIp = 0.0571 * RH + 1.0480 + AT$$

developed by Silva et al. (2015) for buffaloes in the Eastern Amazon, Brazil.

The animals remained in the pens and, at 6 a.m., 10 a.m., 2 p.m., 6 p.m., and 10 p.m., they were calmly taken to the holding pens, where the data on the physiological variables respiratory rate (RR), rectal temperature (RT), and body surface temperature (BST) were recorded. RT was obtained using a clinical veterinary thermometer with scale up to 44°C, which was inserted into the animals' rectum. RR was obtained through inspection and by counting the thoracic-abdominal movements for 1 min with the aid of a stopwatch. BST was read using an infrared thermometer (model TD-965 - Instrutemp, São Paulo, Brazil) at a maximum distance of 1 m from the following parts of the animals: forehead, right side of the thorax, and left flank, and the average of those three values was used. The practical buffalo environmental comfort index (BECIp), developed by Silva et al. (2015) for buffaloes in the Eastern Amazon, Brazil, was also calculated using the formula:

$$BECIp = 0.8854 * BST + 0.1695 * RR$$

A completely randomized experimental crossover design was used, in which all animals underwent both treatments so as to eliminate individual influence. The experimental period

covered two consecutive 5-day phases with two days of interval between them for physiological recovery of the animals, and after this recovery period treatment were switched. The data on the physiological (RT, BST, and RR) and climate (AT, RH, and WV) variables are expressed as means and standard deviation. The statistical analysis was performed using the software SAS v.9.2 (Statistical Analysis System [SAS], 2009) with the means compared by the procedure PROC GLM using Tukey's HSD test at 5% probability to separate means.

Results and discussion

THI values up to 70 indicate non-stressful environmental conditions; between 71 and 78, conditions are critical; between 79 and 83 is defined as danger; and above 83, conditions are considered as being in an emergency situation (Du Preez, 2000). According to the THI results, both treatments had unfavorable and emergency thermal comfort conditions at 2 p.m. At 6 p.m. and 10 p.m., the THI indicated danger and, at 6 a.m., the environment was critical (Table 1). A difference ($p < 0.05$) was found between the treatments at 10 a.m., when only the US group was in an emergency condition, with a higher THI value (84.00 ± 1.01). Thus, regardless of the rearing system, the THI indicates all animals were out of their comfort zone and well-being range at those times. Nevertheless, it is interesting to point out that this index was initially created for temperate conditions to assess other types of production animals, which makes it important to assess those data using indices created for buffaloes in the Amazon, such as the BCCCIp, developed by Silva et al. (2015).

Table 1. Temperature and humidity index (THI), means and standard deviations, at different times in the experimental area, Marajó Island, Pará, Brazil.

Time	US Group	SD Group
	Mean	Mean
6 a.m.	75.41 ± 2.81^{Ea}	75.71 ± 1.51^{Ea}
10 a.m.	84.00 ± 1.01^{Ba}	82.65 ± 0.89^{Bb}
2 p.m.	85.13 ± 1.12^{Ba}	84.59 ± 0.89^{Ba}
6 p.m.	80.53 ± 0.99^{Ca}	80.50 ± 0.89^{Ca}
10 p.m.	79.06 ± 0.67^{Da}	78.70 ± 0.42^{Da}

Means followed by the same capital letter, comparing the times within each group, do not differ statistically at 5%. Means followed by the same small letter, comparing the groups at the same time, do not differ statistically at 5%.

BCCCIp values up to 34.65 indicate comfort; from 34.66 to 38.02, danger; from 38.02 to 41.39, thermal stress; and above 41.39, emergency Silva et al. (2015). In this research, the BCCCIp indicated that the female buffaloes were in danger at 10 a.m.

and 6 p.m. (Table 2). However, the index showed thermal stress conditions only at 2 p.m., when the highest values of this index were observed for both experimental groups (39.37 ± 0.88 and 39.48 ± 0.69 for the US and SD groups, respectively).

The highest WV value was observed at the most critical times of the day, i.e., 10 a.m., 2 p.m., and 6 p.m. (Table 3). That may have contributed to animal heat loss through convection and evaporation. At birth, buffaloes have abundant hairs, which naturally rarify over time (Garcia, 2013). That reduced hair density prevents the formation of an isolating air layer over the skin, as is the case in cattle, which favors the dissipation of body heat through convection.

Table 2. Practical buffalo climate comfort conditions index (BCCCIp), means and standard deviations, at different times in the experimental area, Marajó Island, Pará, Brazil.

Time	US Group	SD Group
	Mean	Mean
6 a.m.	31.03 ± 1.71^{Ea}	31.25 ± 0.98^{Ea}
10 a.m.	37.84 ± 0.97^{Ba}	36.92 ± 0.87^{Bb}
2 p.m.	39.37 ± 0.88^{Aa}	39.48 ± 0.69^{Aa}
6 p.m.	34.74 ± 0.89^{Ca}	35.23 ± 0.88^{Ca}
10 p.m.	33.34 ± 0.45^{Da}	33.37 ± 0.29^{Da}

Means followed by the same capital letter, comparing the times within each group, do not differ statistically at 5%. Means followed by the same small letter, comparing the groups at the same time, do not differ statistically at 5%.

That occurs because WV is highly important for convective exchange, with direct involvement in changes in physiological variables since heat can be lost from the body surface by moving air, which causes evaporation of moisture from the animal's skin (Leite & Virgens Filho, 2011). For optimum growth and reproduction, the ideal WV is between 5 km h^{-1} and 8 km h^{-1} (Navarini, Klosowski, Campos, Teixeira, & Almeida, 2009) and, in the present research, WV values at 10 a.m. and 2 p.m. – with the highest temperatures – were 5.61 ± 3.32 and $5.65 \pm 2.90 \text{ m s}^{-1}$.

Table 3. Wind velocity (m s^{-1}), means and standard deviation, at different times in the experimental area, Marajó Island, Pará, Brazil.

Time	Mean (m s^{-1})
6 a.m.	0.23 ± 0.71^B
10 a.m.	5.61 ± 3.32^A
2 p.m.	5.65 ± 2.90^A
6 p.m.	4.68 ± 2.68^A
10 p.m.	1.71 ± 2.16^B

Means followed by the same capital letter, comparing the times, do not differ statistically at 5%.

The present research showed the most likely times for the animals to experience thermal stress in the dry season in Marajó Island, but it also must be established whether or not they undergo physiological alterations in order to confirm whether they are suffering alterations due to the

environment. When RR was analyzed, the highest value among animals in the US group was observed at 2 p.m. (53.49 ± 20.48 mov min^{-1}). In the SD group, the highest RR values were observed at 10 a.m. (32.76 ± 7.96 mov min^{-1}) and 2 p.m. (30.30 ± 7.13 mov min^{-1}) (Table 4). The higher RR values are a result of an attempt by the buffaloes to increase heat loss by evaporation since inhaled air dissipates heat from the organism when heated while traveling through the respiratory system.

Significant differences were seen between the experimental groups at 10 a.m. and 2 p.m., with higher ($p < 0.05$) RR values among the animals in the US group (48.07 ± 14.06 and 53.49 ± 20.48 , respectively). An animal's capacity to withstand thermal stress in tropical conditions can be assessed through the respiratory rate. In buffaloes, heat loss through exhaled air is more important than through transpiration since their heat loss through the skin is inefficient. As air temperature rises from 28.3°C to 34.7°C , the RR of buffaloes increases from 22 to over 48 mov min^{-1} , respectively (Gudev et al., 2007).

The lower RR for the SD group may be related to the fact that tree shade may be efficient in mitigating the effects of climate in tropical regions. Research conducted in Belém, Pará, Brazil, have shown that adult female buffaloes reared in areas with 18 to 21% shade were protected from direct solar radiation (Garcia et al., 2012). Thus, RR is a good thermal load indicator that is easily analyzed in the field (Gaughan & Mader, 2014).

Table 4. Respiratory rate (mov min^{-1}) and body surface temperature ($^\circ\text{C}$), means and standard deviations, for female buffaloes reared under direct sunlight and with shade in Marajó Island, Pará, Brazil.

Time	Respiratory rate (mov min^{-1})		Body surface temperature ($^\circ\text{C}$)	
	US Group	SD Group	US Group	SD Group
6 a.m.	$18.62 \pm 3.20^{\text{Ca}}$	$18.51 \pm 3.26^{\text{Ba}}$	$31.43 \pm 0.74^{\text{Da}}$	$31.07 \pm 0.75^{\text{Ba}}$
10 a.m.	$48.07 \pm 14.06^{\text{Ba}}$	$32.76 \pm 7.96^{\text{Ab}}$	$36.17 \pm 0.97^{\text{Ba}}$	$34.85 \pm 0.83^{\text{Bb}}$
2 p.m.	$53.49 \pm 20.48^{\text{Ab}}$	$30.30 \pm 7.13^{\text{Ab}}$	$37.20 \pm 1.20^{\text{Ba}}$	$35.49 \pm 0.93^{\text{Ab}}$
6 p.m.	$21.23 \pm 4.45^{\text{Ca}}$	$19.88 \pm 3.22^{\text{Ba}}$	$32.43 \pm 0.74^{\text{Ca}}$	$32.16 \pm 0.67^{\text{Ca}}$
10 p.m.	$18.45 \pm 2.76^{\text{Ca}}$	$17.78 \pm 3.02^{\text{Ba}}$	$32.32 \pm 0.55^{\text{Ca}}$	$31.88 \pm 0.67^{\text{Cb}}$

Means followed by the same capital letter, comparing the times within each group, do not differ statistically at 5%. Means followed by the same small letter, comparing the groups at the same time, do not differ statistically at 5%.

Significant differences in BST were seen between the experimental groups at 10 a.m., 2 p.m., and 10 p.m., with higher ($p < 0.05$) values among the animals in the US group (36.17 ± 0.97 ; 37.20 ± 1.20 ; $32.32 \pm 0.55^\circ\text{C}$, respectively). Buffaloes have an anatomic characteristic that differentiates them from other animals, such as a thick epidermis and high concentration of melanin, which makes them susceptible to increases in BST when exposed to direct solar radiation. Hence, shading becomes an essential management technique for the husbandry

of this species in the tropics. Such results are in agreement with previous research (Silva et al., 2012) on the thermal comfort of female Murrah buffaloes in silvipastoral systems wherein the authors found higher BST values in the afternoon (37.00 ± 0.3) compared to the morning (29.4 ± 0.7) in the less rainy season, which featured higher air temperature. Those authors concluded that, under those conditions, blood flow from the body core to the periphery increases in an attempt to eliminate heat, which contributes to a rise in BST.

Changes in RR and BST indicate that the thermoregulatory system of the experimental animals was activated as the THI and BCCCIp values rose since they were out of their thermal comfort range. However, in order to diagnose the thermal stress situation, RT had to be measured, which reflects the body's core temperature.

A significant difference was found in RT between treatments at 10 a.m., 2 p.m., and 6 p.m. (Table 5), with higher ($p < 0.05$) values for animals in the US group (38.63 ± 0.50 , 39.19 ± 0.59 , and $38.65 \pm 0.51^\circ\text{C}$, respectively). Such results show greater body heat storage, with higher energy demand for thermolysis and, consequently, less energy available for production (Garcia et al., 2012). The data are close to those reported by Barros et al. (2016), who observed mean RT value of 38.7°C for buffaloes.

Table 5. Rectal temperature ($^\circ\text{C}$), means and standard deviations, for female buffaloes reared under direct sunlight and with shade in Marajó Island, Pará, Brazil.

Time	US Group	SD Group
	Mean	Mean
6 a.m.	$37.16 \pm 0.59^{\text{Ba}}$	$37.07 \pm 0.57^{\text{Ba}}$
10 a.m.	$38.63 \pm 0.50^{\text{Ba}}$	$38.08 \pm 0.36^{\text{Bb}}$
2 p.m.	$39.19 \pm 0.59^{\text{Ba}}$	$38.14 \pm 0.45^{\text{Bb}}$
6 p.m.	$38.65 \pm 0.51^{\text{Ba}}$	$38.39 \pm 0.42^{\text{Ab}}$
10 p.m.	$37.95 \pm 0.50^{\text{Ca}}$	$37.76 \pm 0.54^{\text{Ca}}$

Means followed by the same capital letter, comparing the times within each group, do not differ statistically at 5%. Means followed by the same small letter, comparing the groups at the same time, do not differ statistically at 5%.

Several studies confirm the effects of ambient temperature on buffalo RT as a function of thermal stress (Barros et al., 2015; Barros et al., 2016; Silva et al., 2015; Silva et al., 2012). In the US group, the highest RT value was seen at 2 p.m., which also had the highest THI and BCCCIp values.

The BECIp has been proposed to diagnose thermal stress in buffaloes and is easily applicable as its formula uses only RR and BST, thus preventing physical contact with the animal that could lead to physiological alterations at the moment of data collection. BECIp values up to 33.55 indicate comfort; from 33.56 to 36.67, danger, from 36.68 to 39.79, thermal stress; and above 39.79, emergency

(Silva et al., 2015). In the present research, the highest BECIp values were seen at 2 p.m. in the US group and at 10 a.m. and 2 p.m. in the SD group ($p < 0.05$) (Table 6). At these times, the animals are more exposed to direct and indirect solar radiation, which leads to unfavorable environmental conditions.

The results show the times that are the most critical where the animals are actually under inappropriate conditions for their well-being. Significant differences were also found between the experimental groups, with higher BECIp values at 10 a.m. and 2 p.m. in the US group (40.17 ± 2.77 and 42.01 ± 4.06), which indicates the animals are under emergency conditions at those times.

Table 6. Practical buffalo environmental comfort index, mean and standard deviation, for female buffaloes reared under direct sunlight and with shade in Marajó Island, Pará, Brazil.

Time	US Group	SD Group
	Mean (°C)	Mean (°C)
6 a.m.	30.98±0.84 ^{Da}	30.65±0.83 ^{Ca}
10 a.m.	40.17±2.77 ^{Ba}	36.41±1.68 ^{Ab}
2 p.m.	42.01±4.06 ^{Aa}	36.56±1.67 ^{Ab}
6 p.m.	32.31±1.08 ^{Ca}	31.84±0.78 ^{Ba}
10 p.m.	31.74±0.70 ^{CDa}	31.24±0.81 ^{BCa}

Means followed by the same capital letter, comparing the times within each group, do not differ statistically at 5%. Means followed by the same small letter, comparing the groups at the same time, do not differ statistically at 5%.

The results for this index showed that, at 6 a.m., 6 p.m., and 10 p.m., the animals of both treatments were in thermal comfort conditions.

Conclusion

It is concluded that, under the climate conditions of the dry season in Marajó Island, buffaloes are prone to show thermal stress, particularly between 10 a.m. and 2 p.m., with the highest values of THI and BCCCIp occurring during this time period. However, thermal comfort can be provided to those animals by making trees available in the pasture so that they are able to take shelter from direct solar radiation at the hottest times of the day.

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