



Assessment of indices of thermal stress indicators among male buffaloes reared in the Eastern Brazilian Amazon

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ABSTRACT. This research aimed to determine the most appropriate thermal comfort index for buffaloes reared in the Eastern Amazon, Pará, Brazil. Twenty male Murrah buffaloes were used, and data on climate variables, rectal temperature (RT), body surface temperature (BST), and respiratory rate (RR) were recorded. Subsequently, the following indices were calculated: Temperature and Humidity Index (THI), Global Temperature and Humidity Index (GTHI), Benezra's Thermal Comfort Index (BTCl), Ibéria's Heat Tolerance Index (IHTI), the general, effective, and practical Buffalo Comfort Climatic Conditions Indices (BCCCIg, BCCCIe, and BCCCIp, respectively) and the general, effective, and practical Buffalo Environmental Comfort Indices (BECIg, BECIe, and BECIp, respectively) in the morning and afternoon. Higher values for THI, GTHI, RT, BST, RR, BTCl, BCCCIg, BCCCIe, BCCCIp, BECIg, BECIe, and BECIp were observed in the afternoon, and the highest IHTI values were found in the morning. A positive correlation was found among the physiological variables and THI, GTHI, BTCl, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, BECIp, while a negative correlation was found for RT and BST with IHTI. The indices THI, GTHI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, and BECIp were determined to be very efficient to assess the thermal comfort status of buffaloes under the conditions of the present research.

Keywords: bioclimatology; *Bubalus bubalis*; heat, stress.

Variáveis de clima e fisiologia para explicar índices indicadores de estresse térmico de búfalos criados na Amazônia Oriental

RESUMO. A pesquisa objetivou determinar o índice de conforto térmico mais adequado para búfalos criados na Amazônia Oriental, Pará, Brasil. Foram utilizados vinte búfalos Murrah. Foram registrados dados de variáveis climáticas e temperatura retal (TR), temperatura da superfície corporal (TSC) e frequência respiratória (FR), e foram calculados os índices: Índice de Temperatura e Umidade (ITU), Índice de Temperatura de Globo e Umidade (ITGU), Índice de Conforto Térmico de Benezra (ICB), Índice de Tolerância ao Calor de Ibéria (ITCI), Índice de Condições Climáticas de Conforto para búfalos geral (BCCCIg), efetivo (BCCCIe) e prático (BCCCIp) e Índice de Conforto Ambiental para búfalos geral (BECIg), efetivo (BECIe) e prático (BECIp), nos períodos da manhã e da tarde. Foram observados valores de ITU, ITGU, TR, TSC, FR, ICB, BCCCIg, BCCCIe, BCCCIp, BECIg, BECIe e BECIp mais elevados à tarde. Os maiores valores de ITCI foram encontrados de manhã. Houve correlação positiva entre as variáveis fisiológicas e ITU, ITGU, ITCI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, BECIp, enquanto uma correlação negativa foi encontrada para TR e TSC com ITCI. Os índices THI, GTHI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp e BECIp são eficientes para avaliar o estado de conforto térmico dos búfalos na Amazônia Oriental.

Palavras-chave: bioclimatologia; *Bubalus bubalis*; calor, estresse.

Introduction

The Brazilian buffalo herd increased by 7.8% in 2011, and the State of Pará stood out with 38% of the Brazilian herd, with 1.3 million animals (Anuário da Pecuária Brasileira [ANUALPEC], 2017).

The State of Pará is a region where adverse climate conditions have the potential to cause significant production losses due to poorly adapted animals. Buffaloes, although seen as animals adapted to a wide range environmental conditions, have specific structural features, such as melanin

concentration in the skin and fur, a low number of sweat glands, and a low fur density, which makes them sensitive to solar radiation (Gudev et al., 2007; Marai & Habeeb, 2010; Silva et al., 2014).

In order to assess the animals' ability to adapt to the environmental conditions prevalent in regions with a hot, tropical climate, several indices have been developed (Souza et al., 2007) that consider the physiological variables pertaining to thermal regulation, such as rectal temperature, respiratory rate, and body surface temperature. Among these indices, Ibéria's Heat Tolerance Index and Benezra's Comfort Index stand out in their importance to farmers and researchers since they quantify the thermal stress to which the animal is subjected at a given moment and place (Moraes Júnior et al., 2010; Rocha, Salles, Moura, & Araújo, 2012). Silva et al., 2015 have developed, in Brazil, new indices that are recommended to be applied to tropical regions, such as the general, effective, and practical Buffalo Comfort Climatic Conditions Index (ICCCB) and the Buffalo Environmental Comfort Index (BECI).

However, buffaloes reared in the Eastern Amazon, Brazil, require specific practical indices to be used in the field to assess the animals' clinical comfort status. Therefore, this research aimed to evaluate and validate thermal comfort indices for buffaloes and determine the most appropriate ones for animals reared in the Eastern Brazilian Amazon.

Material and methods

The research was carried out on a buffalo ranch in the city of Nova Timboteua, PA, Brazil (Santa Rita farm, 01°12'28" S and 47°23'33" W) over ten days in October. This is considered the hottest month of the year, with mean temperature of 26.7°C. Before data collection, the animals remained in the experimental area for 15 days to adapt to the management and feeding conditions. After these 15 days the animals were transitioned from the adaptation period to the management regime used in this research, and then shortly thereafter data collection was initiated for each experimental unit. The local climate is Am in the Köppen and Geiger (1928) classification with a dry season between September and November and a rainy season between December and August. The mean annual temperature is 26.1°C and the mean annual rainfall is 2,467 mm (Prata, Miranda, Alves, & Jardim, 2010).

The study used 20 clinically healthy male Murrah buffaloes with an average weight of 333.5 kg and about 18 months of age. The animals were kept without access to shade and remained in grazing

with *Panicum maximum* cv Mombaça and *Brachiaria humidicola* grass with drinking water and mineral supplementation *ad libitum*. The experiment was approved by the Animal Ethics Committee of Embrapa Eastern Amazon, Protocol nº 002/2015 (CEUA) - in which it was verified that all the requirements of the Federal Law 11,794/08 (Lei Arouca) were met respecting The Ethical Principles of Animal Experimentation of COBEA.

The meteorological data were recorded every minute by a portable automated meteorological station (TGD-300, Instrutherm, São Paulo, Brazil) and stored in data loggers. Air temperature (AT, °C), relative air humidity (RH, %), dew point temperature (DPT, °C), wet bulb temperature (WBT, °C), and black globe temperature (BGT, °C) data were recorded. The environmental variables were read in the morning (5 to 6 A.M.) and afternoon (4 to 5 P.M.) while the physiological variables were collected. In order to assess the physiological variables, the animals were calmly conducted to the corral, which was located near the picket where they remained in the pasture between data collection sessions.

The physiological variables studied were: rectal temperature (RT, °C) body surface temperature (BST, °C), and respiratory rate (RR, movements min⁻¹). A clinical veterinary thermometer with a scale up to 44°C was used to measure RT and the readings were expressed in degrees Celsius. 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, left side of the thorax, and left flank. The final value was considered the mean of these measurements. RR was obtained through inspection and by counting the abdominal movements of the thorax for 1 min. The reference values were evaluated according to Shafie (1985): RT 37.4 to 37.9°C, BST 25.6 to 35.5°C, and RR 18 to 30 movements min⁻¹.

Initially, two indices were calculated regarding classic climatic factors from the literature: temperature and humidity index (THI), proposed by Thom in 1959, whose formula is $THI = AT + 0.36 DPT + 41.5$, and the black globe and humidity index, proposed by Buffington et al. (1981), whose formula is $GTHI = TG + 0.36 DPT + 41.5$.

Two indices whose formulas contain physiological variables were also calculated: Benezra's Thermal Comfort Index, determined by Benezra (1954) and calculated through the equation $BTCI = (RT/38.8) + (RR/23)$ and Ibéria's Heat Tolerance Index, proposed by Rhoad (1944) and calculated as $IHTI = 100 - 18 (RT - 38.33)$.

Finally, the new indices – to be used in pairs according to the recommendations of Silva et al. (2015) were applied. These indices were developed to assess the thermal comfort status of buffaloes reared in tropical environments, as is usual in the Eastern Amazon. The general pair is made up of the following indices: general Buffalo Comfort Climatic Conditions Index (BCCCIg), obtained through the equation $BCCCIg = -0.0470 \cdot RH + 0.6052 \cdot AT - 0.0534 \cdot DPT + 0.0946 \cdot WBT + 0.3225 \cdot BGT$ (values up to 23.78 = comfort, from 23.79 to 28.26 = danger, from 28.27 to 32.75 = thermal stress, above 32.75 = emergency) and the general Buffalo Environmental Comfort Index (BECIg), obtained through the equation $BECIg = -0.0656 \cdot RT + 0.9173 \cdot BST + 0.1822 \cdot RR$ (values up to 31.44 = comfort, from 32.45 to 35.68 = danger, from 35.69 to 38.93 = thermal stress, above 38.93 = emergency).

The effective index pair (BCCCIe and BECIe) was obtained using the following equations: $BCCCIe = -0.0309 \cdot RH + 0.6493 \cdot AT + 0.3330 \cdot BGT$ (values up to 25.66 = comfort, from 25.67 to 30.11 = danger, from 30.12 to 34.56 = thermal stress, above 34.56 = emergency) and $BECIe = -0.0660 \cdot RT + 0.9144 \cdot BST + 0.1865 \cdot RR$ (values up to 32.46 = comfort, from 32.47 to 35.71 = danger, from 35.72 to 38.97 = thermal stress, above 38.97 = emergency).

The pair of practical indices (BCCCIp and BECIp) was obtained using the following formulas: $BCCCIp = 0.0571 \cdot RH + 1.0480 \cdot AT$ (values up to 34.65 = comfort, from 34.66 to 38.02 = danger, from 38.03 to 41.39 = thermal stress, above 41.39 = emergency) and $BECIp = 0.8854 \cdot RT + 0.1695 \cdot RR$ (values up to 33.55 = comfort, from 33.56 to 36.67 = danger, from 36.68 to 39.79 = thermal stress, above 39.79 = emergency).

The physiological variables data (RT, BST, and RR) and the indices (THI, GTHI, BTCl, IHTI, Baccari HTI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, and BECIp) were expressed as means and standard deviations. The analyses of variance were carried out using the statistical software SAS (Statistical Analysis System [SAS], 1997), version 6.08, to verify the effect of the periods of the day (morning and afternoon) on the physiological variance and indices, whose means were compared using Tukey's test. The correlations between the physiological variables and the thermal comfort indices were calculated using Pearson's method. All statistical analyses used a 5% probability level.

Results and discussion

Differences ($p < 0.05$) were found for THI and GTHI between the periods of the day, with higher

means in the afternoon (Table 1). The significant differences of THI and GTHI between the periods of the day suggest that the animals are experiencing thermal stress given that the thermal condition well above the comfort zone of up to 70 for THI and up to 74 for GTHI (Souza et al., 2007).

Differences ($p < 0.05$) were also found for RT between the periods of the day, with values of 39.03 ± 0.27 in the afternoon. Several studies have shown the effects of ambient temperature on buffalo RT as a function of thermal stress (Barros et al., 2015; Moraes Júnior et al., 2010; Silva, Araújo, Lourenço Júnior, Santos et al., 2011; Silva, Araújo, Lourenço Júnior, Viana et al., 2011). The higher RT means ($p < 0.05$) in the afternoon are in agreement with the results from Silva, Araújo, Lourenço Júnior, Santos et al. (2011) and Silva, Araújo, Lourenço Júnior, Viana et al. (2011), who reported higher RT for female Murrah buffaloes in the afternoon than in the morning throughout the year, regardless of access to shade. In the afternoon, the animals are more exposed to direct and indirect solar radiation, which leads to unfavorable environmental conditions. The higher RT in a hot climate suggests that the heat-release mechanisms of these buffaloes are insufficient to maintain homeothermy (Bernabucci et al., 2010; Gudev et al., 2007).

BST values were also higher ($p < 0.05$) in the afternoon than in the morning. In the present research, BST values are similar to those reported by Silva, Araújo, Lourenço Júnior, Viana et al. (2011) and are higher in the afternoon (33.12 ± 0.78) than in the morning (29.17 ± 1.16) in the less rainy season of the year, when air temperature is higher. In these conditions, blood flow from the body core to the periphery increases in an attempt to eliminate heat, which contributes to raising BST (Silva, Araújo, Lourenço Júnior, Santos et al., 2011). The RR behavior was similar to BST, with higher values in the afternoon. It must be pointed out that, in buffaloes, heat loss through exhaled air is more important than through transpiration since these animals are inefficient in losing heat through the skin (Gudev et al., 2007; Marai & Habeeb, 2010).

The similar behavior was found for BTCl, with the values in the afternoon being significantly higher ($p < 0.05$) (2.03 ± 0.15). Nevertheless, both the values in the afternoon and in the morning (1.90 ± 0.15) are close to 2.0, i.e., the ideal value, which suggests that the animals are in thermal comfort in both periods of the day (Diniz et al., 2017). Moraes Júnior et al. (2010) reported values close to 2.0 for Murrah buffalo calves at 6 A.M., in Belém, PA, Brazil.

Difference was also found for IHTI between the periods of the day ($p < 0.05$), with the higher values found in the morning (93.97 ± 4.24).

Table 1. Mean values and standard deviation of the thermal comfort indices and physiological variables of Murrah buffaloes measured in the morning and afternoon at Nova Timboteua, PA, Brazil.

Variable	Period	
	Morning	Afternoon
THI	70.35±1.11 ^b	81.72±0.31 ^a
GTHI	70.46±1.00 ^b	83.35±1.16 ^a
RT (°C)	38.66±0.23 ^b	39.06±0.26 ^a
BST (°C)	29.17±1.16 ^b	33.12±0.78 ^a
RR (mov min ⁻¹)	20.51±3.38 ^b	23.30±3.47 ^a
BTCI	1.90±0.15 ^b	2.03±0.15 ^a
IHTI	93.97±4.24 ^a	87.40±4.79 ^b
BCCCIg	16.86±0.64 ^b	27.67±0.52 ^a
BECIg	27.96±1.38 ^b	32.07±1.11 ^a
BCCCIe	18.57±0.66 ^b	29.17±0.49 ^a
BECIe	27.94±1.39 ^b	32.06±1.12 ^a
BCCCIp	27.88±0.69 ^b	36.31±0.38 ^a
BECIp	29.30±1.32 ^b	33.28±1.05 ^a

THI: temperature and humidity index; GTHI: globe temperature and humidity index; RT: Rectal temperature; BST: body surface temperature; RR: Respiratory rate; BTCI: Benezra's thermal comfort index; IHTI: Ibéria's heat tolerance index; BCCCIg: general buffalo comfort climatic conditions index; BECIg: general buffalo environmental comfort index; BCCCIe: effective buffalo comfort climatic conditions index; BECIe: effective buffalo environmental comfort index; BCCCIp: practical buffalo comfort climatic conditions index; BECIp: practical buffalo environmental comfort index. ^{a,b} Means of the variables within each period of the day followed by different small letters in the same rows are different ($p < 0.01$).

The higher values of IHTI in the morning suggest that the animals are in the zone of thermal comfort. In this index, the closer the value is to 100, the more adapted the animal is to the conditions where the test was performed (Silva, Souza Junior, Santos, Marques, & Torreão, 2013). Thus, the animals were more adapted to the environmental conditions to which they were exposed in the morning.

The indices BCCCIg, BCCCIe, and BCCCIp differed between the periods of the day ($p < 0.05$), with higher values in the afternoon (27.67 ± 0.52 , 29.17 ± 0.49 , and 36.31 ± 0.38 , respectively). According to the interpretation of these indices proposed by Silva et al. (2015), the climate conditions in the morning favored the animals' thermal comfort, while in the afternoon the values suggested that the animals were at or near the danger zone.

The indices BECIg, BECIe, and BECIp followed the same pattern, with values in the afternoon of 32.07 ± 1.11 , 32.06 ± 1.12 , and 33.28 ± 1.05 , respectively, differing ($p < 0.05$) from those in the morning. However, according to these indices, the animals experienced thermal comfort even in the afternoon.

A moderate positive correlation ($p < 0.01$) was found between rectal temperature and respiratory rate and the indices THI, GTHI, BCCCIg, BECIg,

BCCCIe, BECIe, BCCCIp, and BECIp (Table 2). The correlations between BST and THI, GTHI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, and BECIp were very high and significant ($p < 0.01$), as seen in Table 2. BTCI had a weak correlation with RT, a moderate correlation with BST, and a strong correlation with RR, all of which were positive and highly significant ($p < 0.01$). IHTI had a strong negative correlation with RT ($p < 0.01$), a moderate negative correlation with BST ($p < 0.01$), and was not correlated with RR.

The correlations found among the tested indices and the physiological variables allowed the efficiency of these formulas to be assessed so that the actual thermal comfort conditions of the buffaloes reared in the Eastern Brazilian Amazon could be determined. According to Silva, Morais, & Guilhermino (2007), the correlations between the indices and the physiological variables are an indication of the efficiency of each index and reflect the animals' response to the environment.

Table 2. Correlation among climate and physiological variables and adaptability indices of Murrah buffaloes in Nova Timboteua, PA, Brazil.

Index	RT (°C)	BST (°C)	RR (mov min ⁻¹)
Temperature and humidity index	0.569**	0.918**	0.430**
Global temperature and humidity index	0.581**	0.909**	0.401**
Benezra's thermal comfort index	0.168**	0.487**	0.999**
Ibéria's heat tolerance index	-1.000**	-0.503**	-0.119
General buffalo comfort climatic conditions index	0.581**	0.914**	0.412**
General buffalo environmental comfort index	0.449**	0.969**	0.670**
Effective buffalo comfort climatic conditions index	0.580**	0.915**	0.414**
Effective buffalo environmental comfort index	0.448**	0.967**	0.675**
Practical buffalo comfort climatic conditions index	0.575**	0.918**	0.427**
Practical buffalo environmental comfort index	0.458**	0.971**	0.663**

RT – Rectal temperature; BST – Body surface temperature; RR – Respiratory rate. **significance at the probability level $\alpha \leq 1\%$.

The highly significant moderate positive correlation ($p < 0.01$) between RT and RR and the indices THI, GTHI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, and BECIp show that the values of RT, BST, and RR rise along with the values of these indices.

Except for BTCI and IHTI, all indices were very strongly correlated with BST, which shows that this physiological variable is the most precise to indicate thermal stress in buffaloes. Silva, Araújo, Lourenço Júnior, Viana et al. (2011) report that BST is strongly correlated with the climatic variables in buffaloes, which shows that this variable can be considered a good indicator to make up index formulas aiming to assess thermal stress in this

species. On the other hand, the strong correlations of the new indices with BST ($p < 0.01$) and the moderate correlation with RT and RR ($p < 0.01$) show that these indices are efficient and can indicate the clinical status of comfort, stress, danger, or emergency.

The weak positive correlation of BTCI with RT shows that this index, when compared to the others tested, is less efficient to show the thermal comfort status in buffaloes despite its moderate correlation ($p < 0.01$) with BST and strong correlation ($p < 0.01$) with RR.

Likewise, IHTI, despite having a strong negative correlation ($p < 0.01$) with RT and moderate negative correlation ($p < 0.01$) with BST, was not correlated with RR, which is an important physiological variable to indicate thermal comfort status (Barros et al., 2015; Moraes Júnior et al., 2010). Silva, Araújo, Lourenço Júnior, Santos et al. (2011) found similar results when assessing the thermal comfort of buffalo heifers. Rocha et al. (2012) reported that, for dairy cows, IHTI is the only index with a strong negative correlation with RT ($r = 0.99$; $p < 0.05$). This index also has the best correlation compared to Baccari's HTI and Rauschenbach-Yerokhin's HTI.

The correlation results suggest that THI, GTHI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, and BECIp indices are quite efficient to assess the thermal comfort status of buffaloes under the conditions of the present research. Nonetheless, it is worth highlighting that, among all indices assessed, the new indices BCCCIp and BECIp are the most appropriate ones given how easy obtaining the data is to apply in the formulas. For instance, BCCCIp can be calculated using only RH and AT, which can be measured by a low-cost thermohygrometer, and BECIp requires only BST and RR, measured about a meter away from the animal with no need for confining or handling, which are management activities that could influence the responses to thermal stress.

Conclusion

The indices THI, GTHI, BCCCIg, BECIg, BCCCIe, BECIe, BCCCIp, and BECIp demonstrated a high level of efficiency for assessment of the thermal comfort status of buffaloes under the conditions of the present research.

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