

# Thermal comfort indices of female Murrah buffaloes reared in the Eastern Amazon

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**Abstract** The study aimed to develop new and more specific thermal comfort indices for buffaloes reared in the Amazon region. Twenty female Murrah buffaloes were studied for a year. The animals were fed in pasture with drinking water and mineral supplementation ad libitum. The following parameters were measured twice a week in the morning (7 AM) and afternoon (1 PM): air temperature (AT), relative air humidity (RH), dew point temperature (DPT), wet bulb temperature (WBT), black globe temperature (BGT), rectal temperature (RT), respiratory rate (RR), and body surface temperature (BST). The temperature and humidity index (THI), globe temperature and humidity index (GTHI), Benezra's comfort index (BTCI), and Ibéria's heat tolerance index (IHTI) were calculated so they could be compared to the new indices. Multivariate regression analyses were carried out using the canonical correlation model, and all indices were correlated

with the physiological and climatic variables. Three pairs of indices (general, effective, and practical) were determined comprising the buffalo comfort climatic condition index (BCCCI) and the buffalo environmental comfort index (BECI). The indices were validated and a great agreement was found among the BCCCI (general, effective, and practical), with 98.3 % between general and effective and 92.6 % between general and practical. A significant correlation ( $P < 0.01$ ) was found between the new indices and the physiological and climatic variables, which indicated that these may be used in pairs to diagnose thermal stress in buffaloes reared in the Amazon.

**Keywords** Equations · Thermal stress · Thermoregulation · Buffaloes · Animal welfare

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## Introduction

Buffaloes (*Bubalus bubalis*) have been known in India since 60,000 years BC and were domesticated in the Indus Valley and in the Ur region 3,000 years BC (Zava 1984 cited by Bernardes 2007). They are sturdy animals and are more resistant to illnesses and parasites than bovines, which makes them a viable option for milk, meat, and draught purposes (Oliveira 2005).

In Brazil, buffalo farming began in 1890 when Carabao animals were introduced in the island of Marajó, state of Pará, from French Guiana (Miranda 1986). The current buffalo farming is spread throughout Brazil, and the largest herd is located in the North region of the country (IBGE 2013). The Brazilian buffalo herd is made up of about 3.5 million animals and grows between 3 and 3.5 % every year (Bernardes 2007).

Approximately 63 % of the domestic buffaloes in Brazil are reared in the Amazon (IBGE 2008), where they face high air temperature, relative air humidity, and solar radiation levels,

which decrease zootechnical performance by reducing productive and reproductive indices (Garcia 2013). Although buffaloes can adapt to a wide range of environmental conditions, these animals have specific structural features, such as high melanin concentration in their skin and fur (Shafie 1985), low number of sweat glands (Nagarcenkar and Sethi 1981), low fur density, and well-developed sebaceous glands with greater secretion activity than in bovine cattle (Shafie and Abou El-Khair 1970), which makes them susceptible to solar radiation and endocrinal imbalances when exposed to thermal stress situations (Silva et al. 2014). Despite having an efficient thermoregulation system, they show signs of heat stress when subjected to air temperatures of 36 °C or more (Guimarães et al. 2001), which hinders their individual productivity.

In order to achieve successful production in tropical and subtropical regions, such climate effects must be diminished (Yanagi Junior 2006). The thermoregulatory response of buffaloes in tropical climates has been studied by several research groups (Ablas et al. 2007; Megahed et al. 2008; Marai and Haebe 2010; Mukherjee et al. 2011; Haque et al. 2012; Khongdee et al. 2012). Several researches have been carried out in an attempt to identify animals and production systems more appropriate to the climate conditions in the Amazon while considering the productive and reproductive responses of buffaloes reared in pasture under such conditions (Garcia et al. 2010; Moraes Júnior et al. 2010; Garcia et al. 2011a; Garcia et al. 2011b; Silva et al. 2011a, b, Silva et al. 2014). Nevertheless, the thermal comfort indices used in these researches are classic indices from the scientific literature which were originally validated in conditions different from those found in the Amazon.

Since no specific thermal comfort index is available for buffaloes reared in Eastern Amazon conditions, the aim of the present research was to determine such indices to increase the herd's productive and reproductive performances in order to optimize the economic viability of these animal production systems.

## Material and methods

The trial was carried out from January to December 2009 at the Animal Research Facility Senador Álvaro Adolpho (01° 26' 02" S and 48° 26' 21" W; altitude 8 m) belonging to Embrapa Eastern Amazon, in Belém, PA, Brazil. Experimental procedures were conducted according to the Brazilian bioethics standards for animal research, and protocols were previously approved by the Internal Technical Committee of Embrapa Eastern Amazon.

The local climate is listed as Afi in the Köppen classification, with average yearly rainfall of 3,001.3 mm evenly distributed throughout the year. The rainy season is between January and June and the less rainy, from July to December.

The site has yearly average temperature of 26.8 °C, monthly average maximum temperatures between 30.3 and 33.8 °C, relative air humidity (RH) around 83 %, and receives 2,279 h of sunlight annually (Pachêco et al. 2009). The soil is Yellow Latosol, gravel phase I, of clay texture.

Twenty female Murrah buffaloes between 4 and 5 years old were used, weighing  $479.6 \pm 57.8$  kg on average, which were chosen for their sanity and body condition score to ensure statistical uniformity. Before data collection, the animals remained in the experimental area for 14 days to adapt to the management and feeding conditions. Endo- and ectoparasites were controlled prophylactically (Láu 1999). The experimental area comprised approximately 13 ha and was divided into six pens. The animals remained in rotational grazing with 14 days of occupation and 28 days of rest. They fed exclusively on *Urochloa humidicola* grass with drinking water and mineral supplementation ad libitum. The animals had free access to the shade of *Racosperma mangium* trees in a silvopastoral system. This leguminous forest species was planted in 2005, with trees 4 m apart, between the perimeter and division fences, which were electrified with two wires.

The meteorological data were recorded every minute by a portable automated meteorological station (TGD 300, Instrutherm, São Paulo, Brazil). The station had the routine sensors plus a black globe thermometer and was installed in the microclimate of each pen at the same height as the animals' back so as to follow their rotation in the pasture. 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 between 6 and 7 AM and 12 and 1 PM, when the physiological variables were collected.

Rectal temperature (RT, °C), body surface temperature (BST, °C), and respiratory rate (RR, movements/min) were assessed on Wednesdays and Fridays between 6 and 7 AM and 12 and 1 PM. In order to assess the physiological data, the animals were handled and maintained in a corral. Between data collection sessions, the animals remained in pens in the experimental area. A veterinary clinical thermometer graded up to 44 °C was used to measure RT, and the results were expressed as degree Celsius. BST was obtained from the average of readings from the forehead, left side of the thorax, and left flank taken with an infrared thermometer (TD 965 - Instrutemp, São Paulo, Brazil) at a maximum distance of 1 m. RR was obtained by inspection and by counting the thoracic-abdominal movements for 1 min. These clinical observations and the animals' behavior enable assessing their clinical status and classifying them into Comfort, Danger, Stress, and Emergency conditions.

This clinical assessment involved interpreting the set of physiological variables RT, BST, and RR. RT is the variable considered the most indicative of thermal stress since its values increase only when the physiological adjustments were

not enough to maintain homeothermy (Hansen 2005). On the other hand, the values of BST and RR also increase as a way of dissipating heat to maintain homeothermy in animals subjected to high-temperature environments (Perissinotto et al. 2009). The reference values used for the clinical assessment were the following: RT 37.4 to 37.9 °C, BST 25.6 to 35.5 °C, and RR 18 to 30 movements/min (Shafie 1985). Thus, when all physiological variables were above the normal values for buffaloes, the animals were in an emergency state. When RT values were above normal, but the other variables, or only one of them, had normal values, the animals were in a thermal stress state. On the other hand, if all variables had normal values, the animal was in thermal comfort state. Finally, if RT was normal, but the values of one or two of the other physiological variables were high, the animal was in a danger state, indicating that the thermoregulatory mechanisms had been activated and were efficient in maintaining normal body temperature.

Three thousand six hundred forty measurements were collected in total. Those collected on Wednesdays (1,880 measurements) were used to determine the new buffalo comfort indices, while the ones collected on Fridays (1,760 measurements) were used to validate the equations calculated.

Multivariate regression analyses were performed using the canonical correlation model of the statistical software BioEstat 5.0 (Ayres et al. 2007) to adjust the effect of climate variables (AT, RH, DPT, WBT, and BGT) on the physiological variables (BST, RR, and RT). The climate variables are represented by Equation U, which yields the buffalo comfort climatic condition index (BCCCI), while the physiological variables are represented by Equation V, which yields the buffalo environmental comfort index (BECI), with the highest canonical correlation coefficient ( $R_c$ ) between U and V.

Initially, the first pair of general indices was developed involving all climate variables (RH, AT, DPT, WBT, and BGT), which yielded BCCCIg, and all the animals' physiological variables (RT, BST, and RR), which yielded BECIg. The pair of so-called effective indices (BCCCIe and BECIe) was calculated involving only the variables

RH, AT, BGT, RT, BST, and RR. Finally, given the need to facilitate measuring indicative variables of buffalo thermal stress and that it is not always possible to obtain data from meteorological stations close to the farms, the pair of more practical indices (BCCCIp and BECIp) was developed using the climate variables RH and AT—which can be measured just with a low-cost thermohygrometer—as well as the physiological variables BST and RR—which can be assessed approximately 1 m away from the animal with no need for confinement or handling, which could affect the thermal stress response results.

The chi-squared test at 5 % probability ( $P < 0.05$ ) was used to verify the model's effectiveness. Animal comfort status was determined by ranges defined as a function of the mean (M) and standard deviation (SD) of the indices calculated as follows:

Clinical status	Range
Comfort	Up to M
Danger	From M to (M+SD)
Stress	From (M+SD) to (M+2*SD)
Emergency	Above (M+2*SD)

After the new indices were developed, they were compared with some of the thermal comfort indices most widely used to assess thermal stress of production animals. Two indices related to climate factors were considered: temperature and humidity index (THI), proposed by Thom (1959), whose formula is  $THI = AT + 0.36 DPT + 41.5$ , and the black globe temperature and humidity index (BGTHI) (Buffington et al. 1981), whose formula is  $GTHI = BGT + 0.36 DPT + 41.5$ .

Two other indices that use physiological variables in their formulas were also considered: Benezra's thermal comfort index (Benezra 1954), whose formula is  $BTCI = (RT/38.8) + (RR/23)$ , and Ibéria's heat tolerance index, developed by Rhoad in 1944, whose formula is  $IHTI = 100 - 18(RT - 38.33)$ .

The correlation among all indices (THI, GTHI, BICT, ITC, and the newly determined indices) and the physiological (RT,

**Table 1** Response scale of the new comfort indices of buffaloes reared in the Eastern Amazon

Indices	Mean±SD	Comfort	Danger	Stress	Emergency
BCCCIg	23.78±4.48	$X \leq 23.78$	23.79–28.26	28.27–32.75	$X \geq 32.76$
BECIg	32.44±3.25	$X \leq 32.44$	32.45–35.68	35.69–38.93	$X \geq 38.94$
BCCCIe	25.66±4.45	$X \leq 25.66$	25.67–30.11	30.12–34.56	$X \geq 34.57$
BECIe	32.46±3.25	$X \leq 32.46$	32.47–35.71	35.72–38.96	$X \geq 38.97$
BCCCIp	34.65±3.37	$X \leq 34.65$	34.66–38.02	38.03–41.39	$X \geq 41.40$
BECIp	33.55±3.12	$X \leq 33.55$	33.56–36.67	36.68–39.79	$X \geq 39.80$

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

**Table 2** Relation among the classification of the general, effective, and practical buffalo comfort climatic condition indices

General	Effective				Practical				Total
	Comf	Dan	Str	Emer	Comf	Dan	Str	Emer	
Comf	960	20			960	20			980
Dan		350			10	280	60		350
Str		10	410			40	380		420
Emer				10				10	10
Total	960	380	410	10	970	340	440	10	1,760

*Comf* comfort, *Dan* danger, *Str* stress, *Emer* emergency

BST, and RR) and climate variables (AT, RH, DPT, WBT, and BGT) were calculated using Pearson's method with significance level of 5 % probability. The software NTIA 4.2.1 was used for the correlation analyses (Embrapa 1995).

## Results

For the general index pair BCCCIg and BECIg, the canonical correlation coefficient was  $R_c=0.9200$ , with chi-squared of 411.68 ( $df=15$ ,  $P<0.001$ ), indicating a strong correlation and a highly significant ratio between the indices. The mean BCCCIg was  $23.78\pm 4.48$ . The formula established to calculate BCCCIg is  $BCCCIg=-0.0470*RH+0.6052*AT-0.0534*DPT+0.0946*WBT+0.3225*BGT$ . The equation to determine BECIg is  $BECIg=-0.0656*RT+0.9173*BST+0.1822*RR$ .

The index pairs considered effective can be calculated through the following equations: The equation established for BCCCIe is  $BCCCIe=-0.0309*RH+0.6493*AT+0.3330*BGT$ . The canonical correlation coefficient was  $R_c=0.9198$ , with chi-squared of 399.58 ( $df=9$ ,  $P<0.0001$ ), a result similar to those obtained in the general equations. The mean BCCCIe was  $25.66\pm 4.45$ . The BECIe equation found was  $BECIe=-0.0660*RT+0.9144*BST+0.1865*RR$ . The mean was  $32.46\pm 3.25$ .

For the BCCCIp and BECIp equations, the canonical correlation coefficient was  $R_c=0.9158$ , with chi-squared of

338.76 ( $df=4$ ,  $P<0.0001$ ), a result similar to those obtained in the general equations. The BCCCIp formula is  $BCCCIp=0.0571*RH+1.0480*AT$ . The mean was  $34.65\pm 3.37$ . The practical equation based on the physiological data is  $BECI_p=0.8854*BST+0.1695*RR$ . The mean was  $33.55\pm 3.12$ . Table 1 presents the interpretation of the results for thermal stress diagnosis in buffaloes by using the pairs of proposed index equations.

The indices were validated with the experimental data, and a great fit was found among the BCCCI (general, effective, and practical), with 98.3 % (1,730/1,760) between general and effective and 92.6 % (1,630/1,760) between general and practical (Table 2). Table 3 shows the relations among the classifications yielded by the general, effective, and practical BECI. The fit among them is 99.7 % (1,755/1,760) and 99.4 % (1,750/1,760) for the general vs. effective and general vs. practical indices, respectively.

Table 4 shows that the fits among the classifications given by the effective, general, and practical BCCCI and BECI index pairs were around 70 %, being higher for the general pair (71.7 %) and lower for the practical pair (68.8 %).

The ratio between the classifications given by the different BECI and the animals' clinical status classification provided by a veterinary physician are shown in Table 5. The fit ranged between 79 and 80 %.

Table 6 presents the correlations of the indices related to the climate data (THI and GTHI) and the new climate condition indices (BCCCIg, BCCCIe, and BCCCIp) with the

**Table 3** Relations among the classifications yielded by the general, effective, and practical buffalo environmental comfort indices

General	Effective				Practical				Total
	Comf	Dan	Str	Emer	Comf	Dan	Str	Emer	
Comf	905				904	1			905
Dan		438	3		1	436	4		441
Str			324	2		1	324	1	326
Emer				88				86	88
Overall Total	905	438	327	90	905	438	330	87	1,760

*Comf* comfort, *Dan* danger, *Str* stress, *Emer* emergency

**Table 4** Agreements among the classifications given by the effective, general, and practical buffalo comfort climatic condition indices and buffalo environmental comfort indices (BECI) index pairs

Classifications	General	Effective	Practical
Comf	862	849	853
Dan	194	206	163
Str	203	200	191
Emer	3	3	3
Total of agreements	1,262	1,258	1,210
% of agreements	71.70	71.48	68.75

*Comf* comfort, *Dan* danger, *Str* stress, *Emer* emergency

physiological variables (RT, BST, and RR) of the animals analyzed. The correlations among the indices related to the physiological data (BTCl, IHTI, and the new BECIg, BECIe, and BECIp) and the physiological (RT, BST, and RR) variables are presented in Table 7.

## Discussion

Among the climatic variables studied, the most important were RH and AT, which can be measured with a simple portable thermohygrometer, and BGT, which indicated the effects of combining dry bulb temperature, RH, solar radiation, and air movement (Marcheto et al. 2002), besides providing an indirect measurement of the radiant heat in the environment. Therefore, the so-called effective indices (BCCCIe and BECIe) are more easily applied when compared with the general indices (BCCCIg and BECIg) determined in this research.

However, in order to diagnose thermal stress cases in buffalo herds, the easier reading of indicative variables must be considered. It is also not always possible to obtain data from meteorological stations close to the farms. Hence, the pair of more practical indices (BCCCIp and BECIp) is

recommended, which can be measured just with a low-cost thermohygrometer, as well as the physiological variables BST and RR, which can be assessed approximately 1 m away from the animal with no need for confinement or handling, which could affect the thermal stress response results.

The strong fit among the general, effective, and practical BCCCI allows any of the index pairs (BCCCI and BECI) to be used to study the thermal comfort status of female buffaloes given the climate conditions to which they are subjected. The relations among the classifications given by the different BECIs and the animals' clinical status classification provided by a veterinary physician reflect the strong fits (79 and 80 %), which shows that the indices proposed are reliable when used to assess the thermal stress of buffaloes in the Eastern Amazon.

According to Silva et al. (2007), the correlation values with the physiological variables show the efficiency of each index as indicators of the animals' responses to the environment. In the present research, all climate condition index studies were positively correlated with the physiological variables of the female buffaloes in the experiment, which shows that the values of these indices increase as the values of RT, BST, and RR increase. However, BST was the physiological variable most strongly correlated with the indices assessed. According to Silva et al. (2011a), BST can be considered a good indicator of thermal stress in buffaloes; hence, it can be part of formulas to assess thermal comfort in these animals.

Azevedo et al. (2005) found results similar to the ones in the present research when estimating the critically high THI levels for dairy cows in tropical environment, when RT, BST, and RR were positively correlated with THI ( $P < 0.01$ ), which indicates that THI increases as these physiological parameters increase. The positive correlation of GHTI with RT, BST, and RR found in the research matches the results reported by Silva et al. (2011a), who found highly significant correlations of GHTI with RT, BST, RR, and HR both in the rainier and the less rainy seasons in Belém, PA, Brazil. In the semi-arid Agreste region of the Brazilian state of Pernambuco, in an experiment with buffalo heifers, Costa (2007) found a positive

**Table 5** Relations among the classifications yielded by the general, effective, and practical buffalo environmental comfort indices and the classifications yielded by the animals' clinical status as observed by the veterinary physician at the moment of the observations

Clinical Status	General classification				Effective classification				Practical classification				Total
	Comf	Dan	Str	Emer	Comf	Dan	Str	Emer	Comf	Dan	Str	Emer	
Comf	870	68	25	3	870	68	25	3	871	67	25	3	966
Dan	32	300	99	5	32	298	101	5	31	301	98	6	436
Str	3	69	178	34	3	68	179	34	3	66	182	33	284
Emer		4	24	46		4	22	48		4	25	45	74
Total	905	441	326	88	905	438	327	90	905	438	330	87	1,760

*Comf* comfort, *Dan* danger, *Str* stress, *Emer* emergency

**Table 6** Correlation of the climatic condition indices vs. physiological variables for female Murrah buffaloes reared in the Eastern Amazon

Variables	THI	GTHI	BCCCIg	BCCCIe	BCCCIp
RT	0.7925 <sup>a</sup>	0.7375 <sup>a</sup>	0.7002 <sup>a</sup>	0.7057 <sup>a</sup>	0.7561 <sup>a</sup>
BST	0.9294 <sup>a</sup>	0.9578 <sup>a</sup>	0.9615 <sup>a</sup>	0.9611 <sup>a</sup>	0.9445 <sup>a</sup>
RR	0.8320 <sup>a</sup>	0.8312 <sup>a</sup>	0.8410 <sup>a</sup>	0.8424 <sup>a</sup>	0.8407 <sup>a</sup>

RT rectal temperature, BST body surface temperature, RR respiratory rate, THI temperature and humidity index, GTHI globe temperature and humidity index, BCCCIg general buffalo comfort climatic condition index, BCCCIe effective buffalo comfort climatic condition index, BCCCIp practical buffalo comfort climatic condition index

<sup>a</sup> Significant at 1 %

correlation between RT ( $r=0.486$ ), BST ( $r=0.440$ ), RR ( $r=0.496$ ), and GTHI.

All thermal comfort indices assessed were correlated with the physiological variables of buffaloes reared in the Eastern Amazon. However, the new indices had very strong positive correlations with BST. According to Avila et al. (2013), if AT is higher than the critical limit, the skin also warms up and this heat sensation is conducted to the hypothalamus, which is responsible for activating surface blood vessel dilation to lead to a greater blood flow along the body surface to dissipate heat. This phenomenon causes thermal exchange to the skin by conduction and, next, to the environment by radiation, which increases BST values.

The new indices were also strongly positively correlated with RR, which suggests that the higher the index values, the higher the RR values are in order to increase heat loss by evaporation since the inhaled air dissipates heat from the organism when heated while traveling through the respiratory system (Avila et al. 2013).

The correlations between IHTI and the physiological variables found in the present research match the results by Rocha et al. (2012), who found similar results in tropical conditions when researching dairy cows, with a strong negative correlation between IHTI and RT ( $P<0.01$ ) for both seasons.

**Table 7** Correlation of the thermal comfort indices vs. physiological variables for female Murrah buffaloes reared in the Eastern Amazon

Variables <sup>a</sup>	IHTI	BTCI	BECIg	BECIe	BECIp
RT	-1.0000 <sup>a</sup>	0.7259 <sup>a</sup>	0.7536 <sup>a</sup>	0.7538 <sup>a</sup>	0.7554 <sup>a</sup>
BST	-0.7460 <sup>a</sup>	0.8543 <sup>a</sup>	0.9954 <sup>a</sup>	0.9952 <sup>a</sup>	0.9956 <sup>a</sup>
RR	-0.7055 <sup>a</sup>	0.9996 <sup>a</sup>	0.8955 <sup>a</sup>	0.8965 <sup>a</sup>	0.8943 <sup>a</sup>

RT rectal temperature, BST body surface temperature, RR respiratory rate, IHTI Iberia's heat tolerance index, BTCI Benezra's thermal comfort index, BECIg general buffalo environmental comfort index, BECIe effective buffalo environmental comfort index, BECIp practical buffalo environmental comfort index

<sup>a</sup> Significant at 1 %

When researching buffaloes reared in silvopastoral systems in Belém, PA, Brazil, Castro et al. (2008) found results similar to the ones of present research when they observed that RR and BTCI are correlated ( $r=0.99$ ), which suggests that the higher the BTCI, the higher the RR is. According to those authors, this result highlights the need for a rational management of buffalo production systems so as to improve the animals' environment.

The results of the highly significant correlations with the physiological variables of buffaloes reared in the Eastern Amazon confirm that the new indices can be safely used to indicate environments likely to cause thermal stress to the animals. In tropical regions, AT values are high virtually all year long, being above the thermal comfort range for buffaloes (Garcia 2013), which can lead to thermal stress. Thus, these new indices can help assess the thermal comfort of animals reared in this region.

## Conclusions

The new indices show a significant correlation with the physiological and climate variables. They are, therefore, recommended in determining the physiological adaptability of buffaloes reared in the Eastern Amazon conditions.

The use of the practical indices BECIp and BCCCIp, given their easy applicability, is indicated as the simplest way to diagnose the thermal stress status in buffaloes in that region, which allows for interventions in the management in order to improve thermal comfort and, consequently, the productivity of these animals.

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