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ANIMAL PRODUCTION

What is the best temperature-humidity index equation to indicate heat stress in crossbred dairy calves in a tropical environment?

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ABSTRACT: *The aim of this study was to determine among nine temperature-humidity index (THI) equations, the one that best represents the effects of heat stress on crossbred dairy calves reared in a tropical environment. Twelve male and female calves, aged 20 to 60 days, and raised in a tropical pen were evaluated. Respiratory (RR) and heart rates (HR), rectal (RT), body surface (BST), dry bulb (Tdb) and wet bulb (Tbw) temperatures, partial vapor pressure (Pv), relative humidity (RH) and dew point temperature (Tpo) were quantified in the morning and afternoon. Nine THI equations were calculated. The highest correlation between physiological variables and this was used to select the best THI equation. Averages for nine THI equations, Tdb, Twb, Pv, Tdp, RR, HR, RT, and BST were higher in the afternoon than in the morning, whereas that for RH was the opposite. The highest values for RT occurred at temperatures above 26.4°C and when humidity was below 55.5%. The Tdb and Pv correlations with RR (0.697 and 0.707), RT (0.703 and 0.706) and BST (0.818 and 0.817) were significant and positive, whereas the RH correlations with the same physiological variables were significant and negative (–0.622, –0.590 and –0.638, respectively). The best index was the THI sensible heat-based (THI=3.43+1.058xTdb –0.293xRH+0.0164xTdbxRH+35.7), which was significantly correlated with RR (r=0.668 and r^2=0.446), HR (r=0.259 and r^2=0.067), RT (r=0.693 and r^2=0.479) and BST (r=0.807 and r^2=0.650). In conclusion, the THI sensible heat-based equation best represents the effects of heat stress on crossbred dairy calves reared in a tropical environment.*
Key words: *cattle, comfort, dairy, thermal index.*

Qual a melhor equação do índice de temperatura e umidade para indicar estresse por calor em bezerros leiteiros mestiços em ambiente tropical?

RESUMO: *Objetivou-se determinar dentre nove equações do ITU a que melhor representa os efeitos do estresse por calor para bezerros leiteiros mestiços criados em ambiente tropical. Foram avaliados 12 bezerros, machos e fêmeas, com idade de 20 a 60 dias, criados no bezerreiro tropical. As frequências respiratória (FR) e cardíaca (FC), as temperaturas retal (TR), corporal superficial (TCS), de bulbo seco (Tbs) e de bulbo úmido (Tbu) foram quantificadas pela manhã e tarde. Calcularam-se nove equações do ITU. Utilizou-se como critério de determinação da melhor equação do ITU, a maior correlação entre as variáveis fisiológicas com os ITUs. As médias das nove equações de ITU, Tbs, Tbu, Pv, Tpo, FR, FC, TR e TCS foram superiores pela tarde em comparação ao turno da manhã, enquanto que a UR teve comportamento inverso. Os maiores valores de temperatura retal foram observados em temperaturas acima de 26,4 °C e umidade inferior a 55,5%. As correlações entre Tbs e Pv com a FR (0,697 e 0,707), TR (0,703 e 0,706) e TCS (0,818 e 0,817) foram significativas e positivas, enquanto que as correlações da UR, com essas mesmas variáveis fisiológicas, foram significativas e negativas (-0,622, -0,590 e -0,638, respectivamente). O melhor índice foi o ITU calor sensível (ITU=3,43+1,058xTdb–0,293xRH+0,0164xTdbxRH+35,7), que se correlacionou significativamente com a frequência respiratória (r=0,668 e r^2=0,446), frequência cardíaca (r=0,259 e r^2=0,067), temperatura retal (r=0,693 e r^2=0,479) e temperatura corporal superficial (r=0,807 e r^2=0,650). Conclui-se que a equação do ITU calor sensível é a que melhor representa os efeitos do estresse por calor dos bezerros leiteiros mestiços em ambiente tropical.*
Palavras-chave: *bovinos, conforto, gado leiteiro, índice térmico.*

INTRODUCTION

The most commonly used thermal stress index is the temperature-humidity index (THI), which evaluates the combined effects of air temperature and humidity. It was developed by THOM (1959) to evaluate human beings. Later, different equations were proposed that could be used to evaluate production animals (NRC, 1971; YOUSEF, 1985; MADER et al., 2006; BERMAN et al., 2016). The different THI equations can take into account dry and wet bulb temperatures (Tdb and Twb), relative

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humidity (RH) and dew point temperature (Tdp) (BERMAN et al., 2016).

The main advantage of calculating a THI is that the data needed can be easily obtained on the farm or from a nearby meteorological station, whereas the thermal radiation data received from the animal and the wind speed are more difficult to record because they depend on specific equipment and the data needed are often not publicly available (BOHMANOVA et al., 2007).

The THI has previously been investigated as an indicator of milk production losses from lactating cows (BOHMANOVA et al., 2007) or as a way of assessing sperm quality damage to breeding cattle caused by heat stress (MENEGASSI et al., 2016). However, the efficiency of this index is still not clear for dairy crossbred calves, because most of the previous research was conducted using young purebred or adult animals (BROUCEK et al., 2009; BERNABUCCI et al., 2014; MENEGASSI et al., 2016), which means that it is difficult to find results for young and crossbred cattle.

The aim of this study was to determine among nine temperature-humidity index (THI) equations, the one that best represents the effects of heat stress on crossbred dairy calves reared in a tropical environment.

MATERIALS AND METHODS

The experiment was conducted at the Experimental Farm on campus Gloria, at the Federal University of Uberlândia, Uberlândia, MG, Brazil (18°56'56" S and 48°12'47"W, altitude: 925m), where the average temperature ranges from 19°C to 27°C. The winter season is dry and cold with low rainfall intensity, whereas the summer is hot and the rainfall intensity is high. Twelve crossbred male and female dairy calves, aged 20 to 60 days, and raised in a tropical pen were evaluated. They were descendants of crossings between crossbred females (3/4 *Bos taurus* with 1/4 *Bos indicus*) and the semen from Holstein, Girolando 5/8 and Gir bulls.

After birth, calves were separated from their mothers. They received colostrum and were individually identified using numbered earrings. Later, the umbilical structures were submerged in iodine solution. Following these procedures, they were housed in individual tropical pens, where they remained until weaning (approximately 75 days of age), and then they were transferred to another pasture. After they were weaned off colostrum, they were nursed exclusively with milk replacer at a rate of 6L per animal per day. This was divided into two portions: one was given at the beginning of the morning and the other in the beginning of the afternoon. Water and starter mixture were offered *ad libitum*.

Before nursing and on nine consecutive sunny days without clouds and rain in May 2017, measurements were performed on each calve in the morning and the same calves were evaluated in the afternoon, after an interval of five to six hours, totalizing 1944 data values for the physiological variables and the meteorological factor recorded. Respiratory rate (RR) was measured by counting the number of right flank movements per minute. Heart rate (HR) was measured by auscultating the number of heart beats per minute between the third and fifth intercostal space with a stethoscope (Rappaport P.A Med.) and rectal temperature (RT) was measured using a digital clinical thermometer (TH-150 model G-Tech) inserted 5cm into the rectum for 2min. Body surface temperature (BST) was calculated from the mean of the forehead, scapula, groin, and shank temperatures, which were measured using an infrared digital thermometer (Instrutherm TI-550).

The environment Tdb and Twb were measured with an analog hygrometer (Incoterm). Then, the saturation pressure of the wet bulb temperature (Ps(Tw)), partial vapor pressure (Pv), saturation pressure of the dry bulb temperature (Ps(Ta)), relative humidity (RH) and dew point temperature (Tdp) were calculated according to SILVA (2008). Nine THI equations were calculated using temperatures expressed in degrees Celsius.

THI 1=0.4x(Tdb+Twb)x1.8+32+15 (THOM, 1959)
THI 2=(Tdbx0.15+Twbx0.85)x1.8+32 (BIANCA, 1962)
THI 3=(Tdbx0.35+Twbx0.65)x1.8+32 (BIANCA, 1962)
THI 4=0.72x(Tdb+Twb)+40.6 (NRC, 1971)
THI 5=(1.8xTdb+32) – [(0.55–0.0055xRH)x(1.8+Tdb–26.0)] (NRC, 1971)
THI 6=(0.55xTdb+0.2xTdp)x1.8+32+17.5 (NRC, 1971)
THI 7=Tdb+(0.36xTdp)+41.2 (YOUSEF, 1985)
THI 8=(0.8xTdb)+(RH÷100)x(Tdb–14.4)+46.4 (MADER et al., 2006)
THI 9=3.43+1.058xTdb–0.293xRH+0.0164xTdbx RH+35.7 (BERMAN et al., 2016)

The Action® 2.9 tool was used to calculate independent T-test to verify the influence of sex of the crossbred dairy calves on the physiological variables. Calves were divided in two age groups (20 to 40 days of age, and 41 to 60 days of age), and the independent T-test was used to analyze the influence of age on the physiological variables. Paired T-tests were used to analyze the effect of day-time conditions on the environment and the physiological variables. The

Tdb, RH and THI equations (1 to 9) were considered as variables, and the RT classes were considered as treatments when they were used to verify the normality of the residuals for the mathematical model of a random design (Anderson-Darling test) and the homoscedasticity of the variances for the treatments (Levene's test). The differences between the RT classes for each variable were evaluated using ANOVA and Tukey's test. Strength and direction of the relationship between the physiological variables and the thermal environment factors were also analyzed by Pearson's linear coefficient. All tests were performed using the significance level of 5%.

RESULTS

Sex did not influence the physiological variables of crossbred dairy calves (P>0.05). And age also did not influence the physiological variables of crossbred dairy calves (P>0.05).

The mean Tdb, Twb, Pv, Tdp, RR, HR, RT and BST were higher in the afternoon than in the morning. The exception was the RH results, which had an opposite trend to the other variables (Table 1). Results of all nine THI calculated were higher in the afternoon than in the morning (Table 2).

The lowest RT was observed in calves when the air temperature was 19.8°C and RH was 75.8%. The THI values were also lower in comparison to the other RT classes. In contrast, the highest RT values occurred at temperatures above 26.4°C and RH below 55.5% (Table 3).

The Tdb Pearson's linear correlations with RR, RT, and BST were significant and positive (0.697, 0.703 and 0.818, respectively), as were the correlations between Pv and RR, RT, and BST (0.707, 0.706 and 0.817, respectively). Correlations between RH with RR, HR, and BST were significant and negative (–0.622, –0.590 and –0.638, respectively) (Table 4).

All correlations between the THIs and the physiological variables were positive and significant. THI 9 had the highest correlations with the physiological variables compared to the other THI equations (Table 4). In the simple linear regression plot between THI 9 and HR (Figure 1), the points (which represent the individual observations) are far from the line, which suggested that the coefficient value ($r^2 = 0.067$) was lower than the coefficients for the physiological variables evaluated.

DISCUSSION

In the thermal neutral zone, the physiological variables for thermoregulation are within the reference range of the species. If the values for RR and HR rise, but RT remains within the normal range, then it indicates that the thermoregulatory mechanisms were able to maintain thermal equilibrium. However, it is still important to observe the animal because if RT increases, it means that the animal is suffering from heat stress and cannot dissipate the excess heat (SILVA et al., 2015).

Table 1 - Dry bulb temperature (Tdb), wet bulb temperature (Twb), relative humidity (RH), partial vapor pressure (Pv), and dew point temperature (Tdp) of the environment, and the respiratory rate (RR), heart rate (HR), rectal temperature (RT), and body surface temperature (BST) of crossbred dairy calves in the morning and afternoon when they are reared in a tropical environment.

Variables	Morning			Afternoon		
	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum
Tdb, ºC	19.7±1.2b	17.0	21.0	27.2±1.1a	24.0	29.0
Twb, ºC	17.0±1.8b	13.0	20.0	19.9±1.5a	17.0	23.0
RH, %	76.7±8.3a	63.7	91.4	51.7±10.6b	28.5	64.1
Pv, kPa	2.3±0.2b	1.9	2.5	3.6±0.2a	2.9	3.9
Tdp, ºC	17.7±2.8b	11.6	22.5	18.5±3.5a	10.2	19.6
RR, mov.min^{-1}	40.4±9.3b	24.0	88.0	63.8±14.5a	32.0	114.0
HR, beats.min^{-1}	93.9±15.9b	42.0	136.0	102.2±16.4a	72.0	162.0
RT, °C	38.5±0.4b	38.0	39.6	39.3±0.4a	38.4	41.1
BST, °C	28.4±2.9b	22.6	37.4	36.4±3.1a	28.3	47.3

(a,b) mean values followed by the same letters in a line do not differ from each other according to the paired T-test (P<0.05).

Table 2 - Results of nine THI equations calculated in the morning and afternoon to evaluate the effect of heat stress on crossbred dairy calves when they are reared in a tropical environment.

Variables	---------------------------Morning------------------------			---------------------------Afternoon-------------------------		
	Mean±SD	Minimum	Maximum	Mean±SD	Minimum	Maximum
THI 1	73.4±2.1b	68.6	76.5	80.9±1.2a	77.9	84.4
THI 2	67.2±2.1b	62.3	70.2	75.1±1.3a	71.9	78.6
THI 3	66.2±2.1b	61.6	69.2	74.7±1.2a	71.6	78.3
THI 4	63.3±3.0b	56.5	68.3	69.8±2.2a	65.5	75.0
THI 5	64.3±2.7b	57.9	68.6	72.4±1.7a	69.3	77.1
THI 6	67.0±2.1b	62.2	70.1	74.6±1.2a	71.5	78.0
THI 7	66.3±2.0b	61.8	69.2	75.0±1.2a	71.8	78.6
THI 8	75.3±2.1b	70.5	78.3	83.1±1.2a	80.0	86.6
THI 9	62.3±2.8b	56.1	66.0	75.7±1.7a	70.8	80.8

(a,b) mean values followed by the same letters in a line do not differ from each other according to the paired T-test (P<0.05).

The RR of the calves in the afternoon exceeded the limit considered physiologically appropriate for the species, which varies from 20 to 40 movements per minute (SILANIKOVE, 2000). The RT in dairy cattle ranges from 38°C to 39.3°C (COSTA et al., 2015) and can reach up to 39.5°C in younger animals (FEITOSA, 2008). The mean RT in the afternoon did not exceed the reference range for the species. This indicated that the activation of thermoregulatory mechanisms, such as an increased RR were able to eliminate excess heat and maintain a constant deep body temperature (SILVA et al., 2015).

The HR in adult cattle ranges from 60 to 80 beats per minute (FEITOSA, 2008). If the reference range for calves is used, then both the morning and afternoon values were above the physiological limit for the species. However, age may influence the increase in the HR by altering the vagal tonus and intensifying the activity of the cardio accelerator and vasoconstrictor centers (SILVA et al., 2005). In addition; although, the

Table 3 - Mean values and standard deviations for air temperature (Tdb), relative humidity (RH), and the nine THI results, and their relationship with the rectal temperature classes for crossbred dairy calves reared in a tropical environment.

RT (°C)	Tdb (°C)	RH (%)	THI 1	THI 2	THI 3	THI 4
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
38.0–38.5	19.8±1.7c	75.8±9.2a	73.5±2.2c	66.3±2.3c	64.4±2.8c	67.1±2.2c
38.6–39.0	22.1±3.6b	69.1±15.7b	75.8±3.8b	68.9±4.2b	66.9±4.4b	69.4±3.8b
39.1–39.5	26.4±2.5a	54.1±11.9c	80.1±2.6a	73.8±2.9a	71.5±3.1a	73.7±2.6a
39.6–40.0	26.5±2.8a	55.5±12.6c	80.4±3.0a	74.0±3.3a	71.9±3.5a	73.9±3.0a
40.1–41.1	28.0±0.8a	44.1±11.9c	81.0±1.6a	74.8±1.6a	72.2±2.5a	74.6±1.6a
RT (°C)	THI 5	THI 6	THI 7	THI 8	THI 9	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
38.0–38.5	66.5±2.3c	75.5±2.3c	67.4±2.3c	66.3±2.3c	62.5±3.4c	
38.6–39.0	69.1±4.3b	77.8±3.9b	69.7±3.9b	68.9±4.2b	66.6±6.5b	
39.1–39.5	74.1±2.9a	82.2±2.7a	74.2±2.7a	73.8±2.9a	74.3±4.3a	
39.6–40.0	74.3±3.3a	82.5±3.1a	74.5±3.1a	74.0±3.3a	74.6±5.1a	
40.1–41.1	75.1±1.6a	83.1±1.7a	75.1±1.7a	74.8±1.6a	76.0±2.1a	

(a, b, c) Within each THI equation results, mean values followed by the same letters in a column do not differ according to Tukey's test at the 5% significance level.

Table 4 - Analysis of the THI sensible heat-based equation for crossbred dairy calves reared in a tropical environment using Pearson's linear correlation.

	RR	HR	RT	BST
Tdb	0.697*	0.258*	0.703*	0.818*
Twb	0.428*	0.182*	0.483*	0.599*
RH	-0.622*	-0.216*	-0.590*	-0.638*
Pv	0.707*	0.258*	0.706*	0.817*
Tdp	0.003 ns	0.032 ns	0.075**	0.136**
THI 1	0.642*	0.247*	0.668*	0.791*
THI 2	0.519*	0.211*	0.565*	0.687*
THI 3	0.602*	0.236*	0.636*	0.761*
THI 4	0.642*	0.247*	0.668*	0.791*
THI 5	0.656*	0.255*	0.683*	0,800*
THI 6	0.643*	0.247*	0.668*	0.791*
THI 7	0.644*	0.247*	0.669*	0.791*
THI 8	0.652*	0.254*	0.679*	0.797*
THI 9	0.668*	0.259*	0.693*	0.807*

Tdb: dry bulb temperature; Twb: wet bulb temperature; RH: relative humidity; Pv: partial vapor pressure; Tdp: dew point temperature; RR: respiratory rate; HR: heart rate; RT: rectal temperature; BST: body surface temperature. THI: temperature-humidity index.
*P<0.01, **P<0.05, ns: not significant.

correlation values between the thermal environment and HR were significant, they were low, as were the simple linear regression coefficients. This suggested that this variable should not be chosen to evaluate the effects of heat stress on calves.

As the ambient temperature increases, the sensible heat dissipation efficiency decreases because of the lower temperature gradient between the skin and the environment. In this situation, the animal can maintain body temperature by peripheral vasodilatation, which increases surface blood flow and BST (DA SILVA & CAMPOS MAIA, 2013). Therefore, the higher BST in the afternoon observed in this study can be explained, in part, by the higher air temperature. If the ambient temperature continues to rise, the animal will depend on evaporation, respiratory, and cutaneous heat loss, which is one of the main thermoregulation mechanisms in animals (DA SILVA & CAMPOS MAIA, 2013).

The thermal neutral zone for crossbred dairy calves raised in a tropical environment has not yet been fully elucidated. For dairy calves of European origin, this range varies from 5°C to 20°C (NRC, 2001). In this study, the recorded ambient temperature range was 17°C to 29°C. However, the RT values only increased when the temperature was above 26.4°C, which suggested that crossbred dairy calves reared in temperatures between 17°C and 26.4°C can maintain homeothermia throughout the day.

The THI results have previously been categorized into different levels of heat stress (BOHMANOVA et al., 2007). However, several different classifications are currently being used. THOM (1959) categorized THI values from 70 to 74 as uncomfortable, from 75 to 79 as very uncomfortable, and above 80 as seriously uncomfortable. HUHNKE et al. (2001) suggested that from 79 to 83 was a dangerous situation and above 84 was an emergency situation, whereas. COSTA et al. (2015) considered less than 70 as a no stress situation, 70 to 72 to be a state of alert, 72 to 78 to be critical, 78 to 83 to be dangerous, and above 82 as a state of emergency.

There was considerable variation in the results obtained from the different THI equations evaluated. This is due to the fact that the THI equations differ, mainly, in the way of estimating humidity. THI 1, 2, 3 and 4 consider the value of Twb in their equations, which estimates the air temperature reached when the water vapour deficit to saturation in air is vaporized and its latent heat is used to reduce its temperature (BERMAN et al., 2016). THI 6 and 7 use the value of Tdp in their equations that estimates the ambient temperature at which the vapour present in the air would condense faster that it evaporates, leading to the formation of liquid water (BERMAN et

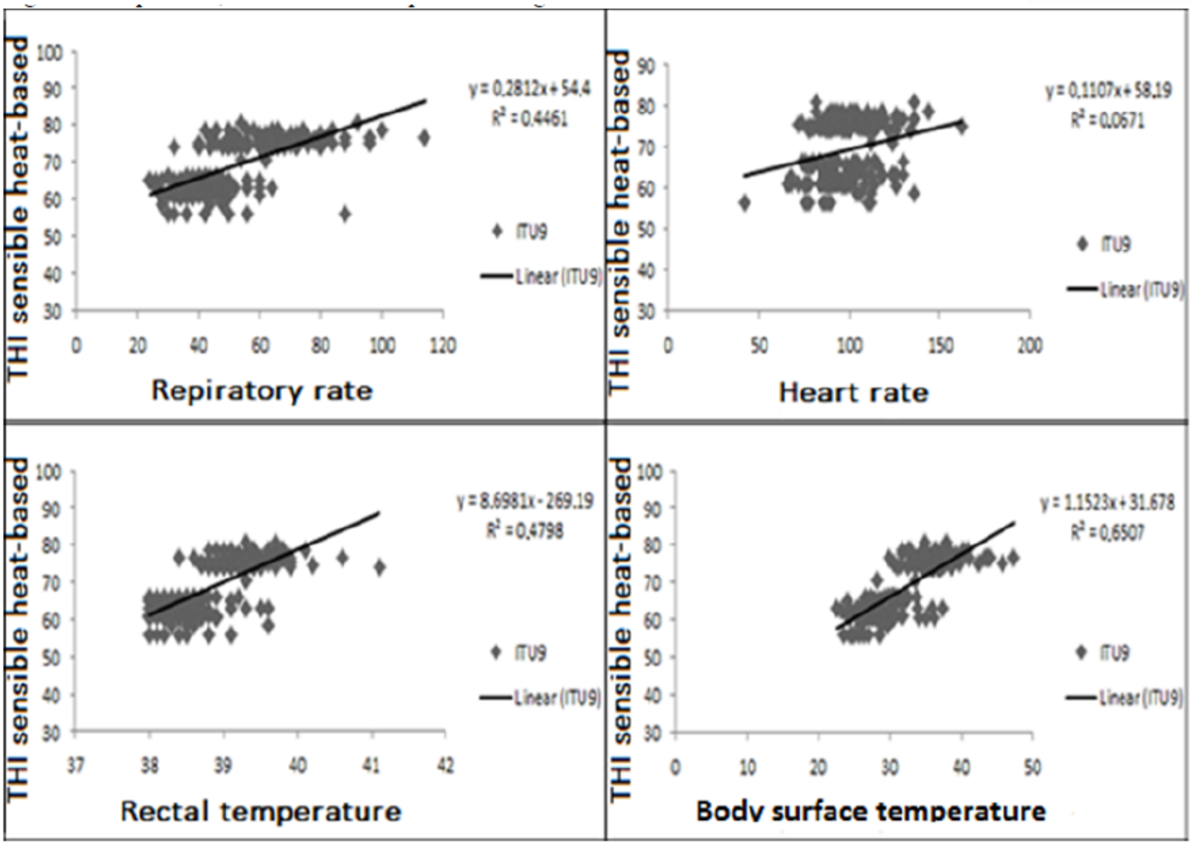

Figure 1 - Relationship between the temperature-humidity sensible heat-based index (THI 9) and the physiological variables. Dots represent the individual observations the lines represent the simple linear regression equations, and R² is the simple linear regression coefficient.

al., 2016). Lastly, THI 5, 8 and 9 consider the value of RH in their equations, and it estimates water vapour present in air as percentage of moisture the air would contain when saturated with moisture at the given air temperature (BERMAN et al., 2016).

Despite the variation in the results obtained from the different THI equations evaluated, all the index values were statistically higher when RTs were above 39.1°C. BERMAN et al. (2016) observed that THI equations differ in the coefficients associated with temperature and humidity estimators, and these coefficients reflect conversion factors between units as well as a possible attribution of different weights to air humidity as a stress factor. BOHMANOVA et al. (2007) evaluated different THI equations for dairy cows and concluded that those that emphasize RH should be used in regions with a humid climate, whereas in those regions where humidity does not reach levels that compromise heat loss by evaporation, the equations of choice are those that emphasize air temperature.

The higher correlation between the temperature-humidity sensible heat-based index (THI 9) and the physiological variables indicated that this index best evaluates heat stress in crossbred dairy calves. At temperatures up to 26.4°C, the results for this index indicated an absence of thermal stress (THI<70). However, when the temperature was above 26.4°C, the THI 9 values rose and entered the discomfort situation category (THI>74), which was confirmed by the increase in RT above the limit considered physiologically appropriate for the species (>39.5°C).

Although, the best temperature-humidity sensible heat-based index equation (THI 9) took into account the RH value, it still produced the most accurate results in this study. In contrast, BOHMANOVA et al. (2007) considered that THI equations that take into account RH should only be used for regions with humid climates. BERMAN et al. (2016) stated that the effect of air temperature is overestimated in the THI equations, while the effect of humidity is underestimated. Therefore, when proposing a temperature-humidity sensible heat-based index, the strong interaction between temperature and humidity (Tdb×RH) should be considered in order to show the potential impact of these variables on bovine thermoregulation.

Understanding the responses and signs that animals present when exposed to heat stress is vital (NIENABER & HAHN, 2004). Results produced by this study suggested that it is possible to make rational decisions regarding animal choice, planning and management of herds and the environments in which cattle are raised.

The temperature-humidity sensible heat-based index calculation (BERMAN et al., 2016) can identify when meteorological conditions negatively affect the crossbred dairy calves reared in a tropical environment. Therefore, the producer will be able to determine whether there is a need to choose crossbred animals that are more adapted to a tropical environment. The producer can also decide whether to upgrade livestock facilities and increase the availability of shade in order to provide thermal comfort and improve animal welfare.

CONCLUSION

In conclusion, the THI sensible heat-based equation best represents the effects of heat stress on crossbred dairy calves reared in a tropical environment.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All procedures involving animals were approved by the Ethics Committee for the Use of Animals of Universidade Federal de Uberlândia, under protocol CEUA/UFU nº031/16.

DECLARATION CONFLICTING OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

FGON, ECG and MRBMN conceived and designed experiments. FGON, HCPA and GMR performed the experiments. FGON and ECG performed statistical analyses of experimental data. All authors prepared the draft of the manuscript. And, all authors critically revised the manuscript and approved of the final version.

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