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Physiological parameters for thermal stress in dairy cattle

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ABSTRACT - The objective of this study was to investigate changes in physiological parameters of dairy cows and understand which physiological parameters show greater reliability for verification of heat stress. Blood samples were collected for analysis and included hematocrit (Ht), erythrocyte count (ERY), and hemoglobin count (HEMO). In addition, physiological variables, including rectal temperature (RT), heart rate (HR), respiratory rate (RR), and panting score (PS) were recorded in 38 lactating cows. These varied according to genetic group (½, ¾, and pure bred Holstein (HO)). Analysis of variance considering the effects of genetic group, days, and their interaction as well as linear and quadratic effect of the black globe humidity index (BGHI) was performed, as well as broken-line regression. These values were higher in pure HO than in ¾ and ½ groups. The average BGHI during the morning was 74, when 70, 43, and 13% of pure HO, ¾, and ½, respectively, presented RR above reference value. The RR was the best indicator of heat stress and its critical value was 116 breaths/min for ½, 140 for ¾, and 168 breaths/min for pure HO cows. In the HO group, physiological variables increased linearly with BGHI, without presenting inflection in the regression. The inflection point occurred at a higher BGHI for the ½ group compared with the other groups. Hematocrit and HEMO were different among genetic groups and did not vary with BGHI, showing that stress was not sufficient to alter these hematological parameters. The ½ HO group was capable of maintaining normal physiological parameters for at least 3 BGHI units above that of HO and 1 to 3 units higher than ¾ HO for RR and RT, respectively. Respiratory rate is the physiological parameter that best predicts heat stress in dairy cattle, and the 1/2 Holstein group is the best adapted to heat stress.

Key Words: broken line, critical values, thermal comfort, thermoregulation

Introduction

Productivity in dairy cows depends on the use of specialized animals as well as on their reproductive health, nutritional characteristics, and environment in which they are raised. Selection for milk production reduces the ability of the cow to withstand the stress caused by heat, thereby increasing susceptibility to heat stress and decreasing production and reproductive efficiency during the hotter months of the year (Vasconcelos and Demetrio, 2011).

Breeds of European origin suffer more from heat stress, in part due to their higher productivity, reducing their threshold of thermal comfort (Silva et al., 2002). Therefore, Brazilian breeders have sought to combine the desirable characteristics of European and zebu breeds through the production of crossbred animals, like the Girolando, which is the result of crossbreeding Holstein (European) and Gyr (zebu). The Brazilian national herd is composed of approximately 80% of this crossbred population (Lopes et al., 2012), accounting for about 70% of milk production (Alvim et al., 2005).

Heat stress causes changes in homeostasis and has been quantified by the measurement of physiological variables such as body temperature, respiratory rate, and hormone concentrations. Despite the cited and well-known differences in breeds and reaction to heat stress (McManus et al., 2009a), there is still little information regarding the critical levels of these traits for crossbred cows.

Cattle in subtropical and tropical environments are subjected to numerous stress factors (Prayaga et al., 2006), including parasites (tick and tick borne diseases, internal parasites, flies); seasonally poor nutrition; high temperatures or high daily temperature variation; and high and/or

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low humidity and temperature which is exaggerated by extensive production systems. In these cases, management interventions may be possible, but they are difficult and expensive to implement, particularly in poorly adapted cattle. The best method of ameliorating the effects of these environmental stress factors to improve productivity and animal welfare is to select and breed cattle that are adapted and productive, without the need for managerial interventions (Scholtz et al., 2011).

The objective of this study was to investigate changes in the physiological parameters of dairy cows, determine critical threshold values, and identify the physiological parameters that show higher reliability for verification of heat stress in dairy cows.

Material and Methods

The experiment was approved by the Ethics Committee on Animal Use of Universidade Federal do Rio Grande do Sul (CEUA), no. 22773/2012 with number of repetitions calculated in accordance with Kaps and Lamberson (2009). The experiment was conducted in Coronel Pacheco, Minas Gerais, Brazil (21°33'23" S latitude, 43°6'15" W longitude, and 430 m altitude). The climate is classified according to Köppen as Cwa (mesothermal), alternating between dry (May-October) and rainy (November-April), with average temperatures of 22 °C in the summer and 16.8 °C in the winter.

Thirty-eight lactating cows were used: 19 purebred Holstein (HO) and 19 Girolando ½ HO (Holstein × Gir; n = 08) and ¾ HO (n = 11). At the start of their respective period of analysis, purebred Holsteins cows presented an average 249.15±68.19 days in milk (DIM) and 14.80±2.59 L day^{-1} milk production. Girolando cows averages were 95±72.33 DIM and 12.4±3.7 L day^{-1} milk production for ½, and 169.3±95.85 DIM and 15.5±3.8 L day^{-1} milk production for ¾ HO. There were no significant differences between genetic groups for mean milk yield.

Data collection for each breed was performed on three consecutive days, with experimental procedures being the same for the different breeds. Holstein and Girolando (½ and ¾ HO) cows were analyzed in separate locations and periods but within the same experiment station with a distance of about 1.5 km.

The study consisted of inducing heat stress by exposing cows to a non-shaded environment — with water and fresh feed *ad libitum* — between morning and evening milkings.

During experimental procedures, temperature varied from 21 to 34 °C (average of 26.61 °C) and relative humidity ranged from 56 to 95% (average of 77.55%) for ¾ HO and ½ HO. For HO cows, the same parameters ranged from 22 to 35 °C (average of 28.3 °C) and from 52 to 95% (average of 76.68%), respectively.

Holstein cows were housed in a free stall, receiving a total mixed ration of maize silage and concentrate (59% corn, 35% soybean, 3.5% protein-mineral-vitamin mix, 0.5% mineral salt, 1% urea, and 1% bicarbonate); between milkings, cows were conducted to a *Brachiaria brizantha* pasture. The ½ HO and ¾ HO were conducted to a *Pennisetum purpureum* pasture and fed concentrate before each milking (70% corn, 25% soybean, 3.5% protein-mineral vitamin mix, 0.5% mineral salt, and 1% urea) in quantities according to milk production. Animals used belong to Embrapa; thus, housing and feeding techniques were not altered or established by authors, with the exception of heat stress induction.

The physiological parameters — rectal temperature (RT), respiratory rate (RR), heart rate (HR), and panting score (PS) — were monitored before morning and afternoon milkings, with animals individually held in a shaded holding pen. Rectal temperature (RT) was measured using a clinical veterinary thermometer inserted at the rectum wall of the animal at a depth of approximately 30 cm during 3 min. Heart rate (HR), expressed in number of beats per minute, was measured using a stethoscope and a stopwatch for 30 s and multiplying the result by two to obtain this variable in minutes. The respiratory rate (RR), expressed in number of breaths per minute, was measured using a stethoscope and stopwatch upon auscultation of respiratory movements for 30 s and the value obtained multiplied by two to obtain this variable in minutes. The panting score (Table 1) was assigned at the time of collecting physiological data, according to the methodology suggested by Mader et al. (2006).

The frequency of the number of animals presenting parameter values outside the physiological reference standards as defined by Pires and Campos (2008) were calculated and simple linear regressions analysis was performed, which considered THI (Temperature-Humidity Index) as the independent variable and RT, RR, HR, and

Table 1 - Panting score in cattle

Score	Description
0	Normal breathing
1	Respiratory rate increased slightly
2	Moderate panting and/or presence of small amount of drool or saliva
3	Saliva usually present, panting hard with mouth open
4	Severe panting with open mouth, protruding tongue, excessive drooling, and generally, extended neck

Source: Mader et al. (2006).

PS, as dependent variables, to check which of dependent variables were more suited to identify heat stress.

After physiological data collection, blood samples were taken to analyze number of erythrocytes (ERY), hemoglobin (HEMO), and hematocrit (Ht), which were compared with reference values. Blood samples were obtained by caudal venipuncture, collecting 4 mL in Vacutainer® tubes containing EDTA. Immediately after collection, a blood smear was taken. Red blood cells were counted using a hemotocitometer. The hemoglobin content was determined by acid hematin and hematocrit by the microhematocrit method (Matos and Matos, 1995).

During the period of measurement of animal physiological parameters and blood samples collection, the meteorological variables relative humidity (RH, %) and dry bulb temperature (DBT, ºC) were measured. From these data, several thermal indices were calculated as summarized in Collier and Collier (2012), including: DI_{Thom}, RT_{Bianca}, $THI_{Johnston1962}$, $THI_{Johnston1965}$, and $THI_{JohnstonVanjonack}$. As all correlations between these indices were greater than 0.90, the following were used in the present study: Temperature-humidity index (THI), calculated according to Johnson et al. (1962):

$$THI = (1.8 \times DBT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times DBT - 26.8)]$$

and the black globe humidity index (BGHI), calculated according to Buffington et al. (1981), in which BGT is black globe temperature. To calculate the Tdp (dew point temperature, °C) GRAPSI software 6.0 (Melo et al., 2004) was used:

$$BGHI = BGT + 0.36\,(Tdp) + 41.5$$

As the correlation between these indices were above 0.86 and between THI and BGHI was 0.96, the latter will be used in this study.

All statistical procedures were performed using SAS software (Statistical Analysis System, version 9.3). The experimental design was completely randomized with repeated measures. Statistical analysis included procedures PROC MIXED, considering the effects of genetic group day and their interaction as fixed effects and BGHI and $BGHI^2$ with tests of means (PROC LSMEANS) for significant variables. Linear and quadratic regressions were calculated for the effect of BGHI on the traits.

The mathematical model used for analysis of variance was:

$$y_{ijk} = \mu + G_i + D_j + GD_{ij} + b_1(BGHI - BGHIm) + b_2(BGHI - BGHIm)^2 + e_{ijk},$$

in which μ = overall mean; G_i = effect of genetic group (n = 3); D_j = effect of the day of measurement (n = 3); GD_{ij} = effect of the interaction between genetic group and day; BGHI = black globe humidity index; BGHIm = mean black globe humidity index; b_1 and b_2 = regression coefficients for BGHI and $BGHI^2$, respectively; and e_{ijk} = error

Logistic and broken line regressions (PROC LOGISTIC and PROC NLIN) as well as chi square test (PROC FREQ) were calculated to determine the limiting environmental conditions when the animals are at different levels of stress. We assumed the following values as the maximum values for non-heat stressed cows: RR = 40 breaths/min; RT = 39.1 °C; and HR = 60 beats/min. The model used for the broken line regression was:

$$y_i = \beta_o + \beta_1 x_{i1} + \beta_2 (x_{i1} - x)\delta_i + \varepsilon_i,$$

in which: δ_i = 1 if $x_{i1} > x$ and 0 if $x_{i1} < x$, and in which y is the dependent variable; x is the independent variable; and β is the regression coefficient.

The model used for the logistic regression was:

$$y_i = \beta_o + \beta_1 x_{i1},$$

in which y is the dependent variable; x is the independent variable; and β is the regression coefficient.

Results

The ambient temperature in this study ranged from 21.90 to 37.90 °C; relative humidity from 55% to 95%; THI from 72.60 to 87.00; and BGHI from 71.27 to 86.23%, for Holstein cows. For Girolando cows, ambient temperature ranged from 20.70 to 34.20 °C; relative humidity from 52 to 95%; THI from 69.20 to 87.70; and BGHI from 69.91 to 88.53. The measurements taken in the morning and in the afternoon were above the upper threshold for the thermal neutral zone (Table 2).

Averages for RR, HR, PS, and RT varied significantly ($P<0.05$) between the genetic groups (Table 3). The ½ HO cattle usually had the lowest physiological parameters, while no differences were seen between genetic groups for HR, RT, and PS. The ½ HO group had a significantly lower proportion of observations outside reference values for RR

Table 2 - Environmental data at time of measurement of physiological parameters

	Morning				Afternoon			
	AT	RH	THI	BGHI	AT	RH	THI	BGHI
				Girolando				
Minimum	20.70	67.00	69.20	69.91	28.10	52.00	82.00	78.16
Maximum	31.60	95.00	85.30	87.66	34.20	93.00	87.70	88.53
Average	26.15	81.00	77.25	78.78	31.15	72.50	84.85	83.34
				Holstein				
Minimum	22.00	84.00	72.60	71.27	21.90	55.00	82.10	82.82
Maximum	25.60	95.00	75.70	76.29	37.90	95.00	87.00	83.64
Average	23.80	89.50	74.15	73.78	29.90	75.00	84.55	86.23

AT - ambient temperature; RH - relative humidity; THI - temperature-humidity index; BGHI - black globe humidity index.

Table 3 - Mean values of physiological parameters by genetic group for dairy cattle under heat stress

	HR (beats/min)	RT (°C)	RR (breaths/min)	PS	ERY ($x10^6$ mL)	HEMO (g/dL)	Ht (%)
R^2	0.46	0.62	0.65	0.53	0.2	0.22	0.27
CV	23.08	2.03	23.26	20.17	11.63	13.16	9.16
GG	***	***	***	***	***	***	***
BGHI	***	***	***	***	ns	***	ns
$BGHI^2$	ns	ns	ns	ns	ns	ns	ns
HO	78.44a	39.86a	81.74a	1.57a	5.07a	23.09a	9.05a
¾ HO	62.24a	39.39b	77.47b	1.12b	5.24a	23.97a	9.00a
½ HO	46.62b	39.01c	52.86c	0.38c	5.92b	27.52b	10.35b
Reference[1]	40 to 60	38.3 to 39.3	23 to 40		4.5 to 10.0	24 to 46	8 to 15

HR - heart rate; RR - respiratory rate; RT - rectal temperature; PS - panting score; ERY - erythrocytes; HEMO - hemoglobin; Ht - hematocrit.
R^2 - coefficient of determination; CV - coefficient of variation; GG - genetic group; BGHI - black globe humidity index; HO - Holstein; ns - not significant.
Means in each variable and evaluation day followed by different letters are significantly different according to Tukey's test (P<0.05).
[1] Source: Silanikove (2000) and Dukes (1996).

than the other two groups. The average BGTI during the morning was 74, when 70, 43, and 13% of pure HO, ¾, and ½, respectively, presented RR above reference value.

In group HO, no inflection point was seen in the curve (Table 4 and Figure 1). During the days of the experiment, the BGHI was equal or higher than 72.

For all groups, considering the logistic regressions, the probability of non-critical RR, RT, and HR values decreased with BGHI, but values were lower for the HO group compared with the ½ group, mainly for the lower BGHI, confirming the results of the broken line regression.

The ½ HO cows showed higher ERY, HEMO, and Ht values compared with genetic groups ¾ and HO.

Regression analyses (Table 5) showed that, in general, the physiological traits in ½ HO cattle responded linearly to BGHI, whereas ¾ HO and HO had quadratic relations showing a limiting point for the increase in these parameters. Blood parameters in general did not show significant regressions.

The changes in hematological parameters ERY, HEMO, and Ht as BGHI increased also showed no inflection point for the Holstein breed, while for Girolando cows, the point of inflection occurred with a BGHI below 72, except for ERY (Table 6 and Figure 2). For the group of pure HO cows, the BGHI was never less than 72.

Table 4 - Inflection point of the curve (I) of physiological parameters according to the THI

	½ HO		¾ HO		HO	
	I	CI95%	I	CI95%	I	CI95%
RR	73.61	68.5 to 78.7	72.29	67.8 to 76.7	72	-
RT	76.44	71.8 to 81.1	73.51	73.4 to 81.6	72	-
HR	76.43	61.8 to 91.1	71.78	64.5 to 79.1	72	-

THI - temperature-humidity index; CI - confidence interval; RR - respiratory rate; RT - rectal temperature; HR - heart rate.

Table 5 - Regression equations for effect of black globe humidity index (BGHI) on physiological and blood parameters in Holstein (HO) and Girolando cattle

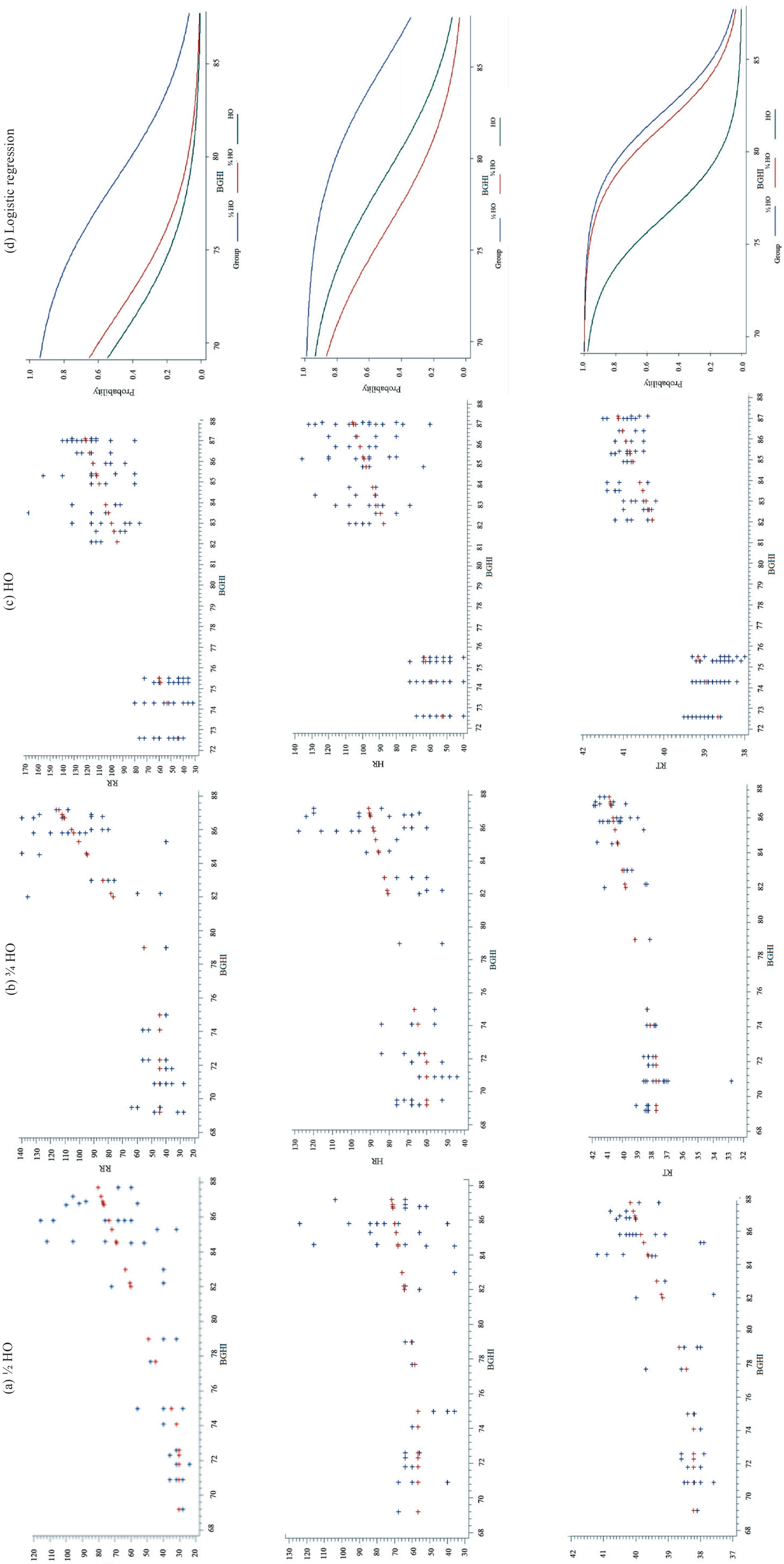
	GG	Equation
RT	½ HO	Y = 28.829 + 0.130*BGHI
	¾ HO	Y = −57.312 + 2.279*BGHI − 0.0133*BGHI²
	HO	Y = 27.923 + 0.150*BGHI
RR	½ HO	Y = −198.420 + 3.210*BGHI
	¾ HO	Y = −2395.174 + 59.088BGHI − 0.349*BGHI²
	HO	Y = 282.841 + 4.57*BGHI
HR	½ HO	Y = −13.036 + 0.973*BGHI
	¾ HO	Y = −57.234 + 1.689*BGHI
	HO	Y = −168.843 + 3.105*BGHI
PS	½ HO	Y = −5.330 + 0.074*BGHI
	¾ HO	Y = −94.556 + 2.322*BGHI − 0.014*BGHI²
	HO	Y = −77.297 + 1.768*BGHI − 0.010*BGHI²
ERY	½ HO	ns
	¾ HO	Y = 192.313 − 4.409*BGHI + 0.029*BGHI²
	HO	ns
Ht	½ HO	ns
	¾ HO	ns
	HO	ns
Hemo	½ HO	ns
	¾ HO	ns
	HO	ns

GG - genetic group; RT - rectal temperature; RR - respiratory rate; HR - heart rate; PS - panting score; ERY - erythrocytes; Ht - hematocrit; HEMO - hemoglobin; ns - not significant.

Table 6 - Inflection point of the curve (I) of physiological parameters according to the BGHI

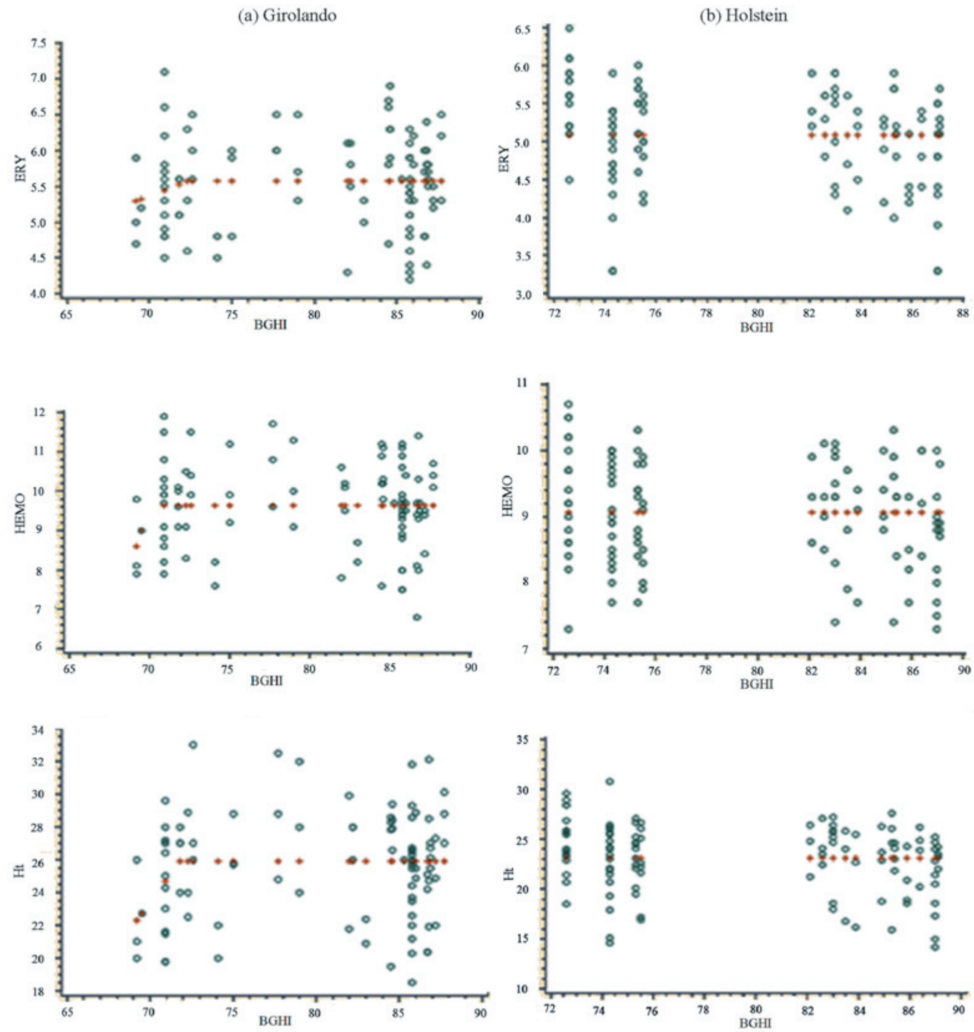
	Girolando		Holstein	
	I	CI95%	I	CI95%
Erythrocytes	72.27	65.38 to 79.16	5.08	-
Hemoglobin	70.00	65.48 to 74.47	9.06	-
Hematocrit	71.80	69.40 to 74.19	23.10	-

CI - confidence interval; BGHI - black globe humidity index.



(a) Red dotted line represents the broken line. Each blue point represents an observation.
(b) Probability of animals with respiratory rate below 40 breaths/mov (not stressed), with heart rate below 60 beats/min (not stressed), and with rectal temperatures below 39.1 °C (not stressed), with respect to BGHI (black globe humidity index).

Figure 1 - Broken-line (a, b, c) and logistic regressions (d) for respiratory rate (RR), heart rate (HR), and rectal temperature (RT) values in relation to BGHI for groups ½, ¾, and HO.



ERY - erythrocytes; HEMO - hemoglobin; Ht - hematocrit; BGHI - black globe humidity index.
Red dotted line represents the broken line regression. Each blue circle represents an observation.

Figure 2 - Hematological parameters relative to BGHI.

Discussion

In relation to BGHI, Baêta (1985) points out that values in the range of 74-78 characterize a state of alert and values in the range of 79-84 characterize that animals are in danger and may lead to low yield. Akyus et al. (2010) reported that mild, moderate, and severe heat stress for cattle are related to THI values of 72, 79, and 89. Ferreira et al. (2006) reported that THI values in the range of 69-70 were considered non-stressful, while values above 83 indicate severe heat stress for ½ × ½ Holstein cattle. Azevedo et al. (2005) estimated upper critical values of THI as 80, 77, and 75 based on rectal temperature in Girolando cattle (½, ¾, and 7/8, respectively). The BGHI is a more accurate indicator of comfort than THI, when animals are exposed to indirect or direct solar radiation (Buffington et al., 1981). In our study, the correlation between these was 0.96, probably due to the high inverse correlation between air temperature or black globe temperature and humidity, as is characteristic of this region of Brazil.

The mean RR and RT values for the HO and ¾ HO groups (Table 3) tended to be above the reference values, indicating that animals were suffering heat stress. These results are in agreement with those of Collier et al. (2009), who reported that high-producing cows got heat-stressed with THI below 72, but in disagreement with other authors (Johnson et al., 1962; Armstrong, 1994; Silanikove, 2000; Martello et al., 2004).

To further investigate the influence of BGHI on these parameters (HR, RR, RT, and PS), logistic equations and broken line regressions were used (Table 4 and Figure 1) and showed different inflexion points for each parameter and each genetic group. This shows that animals which had the highest values were in thermal discomfort. This was expected, since *Bos indicus* animals have a superior ability to cope with heat stress relative to the tropical *Bos taurus* (Santos et al., 2005). The values of RR, RT, and HR increased linearly from BGHI 72, indicating that the response of physiological parameters increased with the BGHI. One can assume that the inflexion point might have occurred at a value of BGHI below 72. The inflection point for RT and HR occurred at higher BGHI values for ½ HO than ¾ HO, except for RR, and it indicates that ½ HO cows are more heat-tolerant, as reported by Azevedo et al. (2005). The ½ HO cattle began to use RR to control their body temperature with a BGHI of approximately 73.6, while this was 72.5 for ¾ HO, and HO cattle were already using their RR at 72, the lowest recorded in this study (Table 4). Rectal temperature began to rise in ¾ HO approximately 3 BGHI units before the ½ HO.

The values found for ERY, HEMO, and Ht (Table 3) were within the normal range according to reference values (Jain, 1993). The ½ HO cows showed higher values of ERY, HEMO, and Ht compared with genetic groups ¾ and HO, probably related to genetic differences, handing and feeding, which may result in variations in the complete blood count (Souza et al., 2011). This may have also been due to a better response to heat stress in this genetic group. Some authors showed that hematological parameters increased during heat stress (Silva et al., 2006; Ferreira et al., 2009), unlike those found in this study. However, the period of analysis for these studies lasted longer and compared mostly warmer seasons with cooler seasons, not within a season with high BGHI. The change in erythropoiesis is one of the adjustments of the body to heat stress. The process usually takes a minimum of four mitoses and their maturation time, approximately 4-5 days in cattle (Trhall, 2007), which could explain the lack of significant variation in the present work, since our experimental procedures lasted only three days. The differences between genetic groups (Table 6 and Figure 2) may have led to a greater variation in the hematological variables evaluated. Therefore, the upper and lower limits of BGHI should be calculated considering the conditions of each situation including management, genetic group, and feeding, among others.

As a result of global warming, livestock in the developing countries of the southern hemisphere will need to adapt to higher ambient temperatures, lower nutritional value of the grass in some cases, and spread of diseases and environmental challenges such as ticks and tick borne diseases in Africa (Scholtz and Theunissen, 2010). With such challenges, matching genotypes with production environments will become crucial, requiring the utilization of diverse genetic resources with the appropriate genetic potential for growth, milk production, resistance to disease, and fertility (Blackburn and Mezzadra, 2006). The question is how to measure adaptation and how to select for it. McManus et al. (2009a,b) discussed the consequences of decisions made in animal breeding and their consequences for genetic variability, adaptability and conservation and concluded that a production system approach should be taken when deciding on which breed should be used and decisions taken should be in the context of cost-benefit and practical applications. This will allow a more pragmatic look at the use of technology and the evaluation of different production systems (Scholtz et al., 2013). The determination of the limits of thermal comfort may require an evaluation with a larger number of animals, covering all stages of lactation and productivity levels, and other environmental conditions.

Conclusions

The ½ Holstein animals are more tolerant to heat stress than the other genetic groups evaluated in this study, because they do not have any increase in rectal temperature or heart rate as the black globe humidity index is increased. The black globe humidity index is the most suitable environmental indicator of comfort, and respiratory frequency is the best physiological indicator of thermal stress in dairy cattle.

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