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Physiological and thermographic response to heat stress in zebu cattle

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ABSTRACT

The objective of this study was to evaluate the heat tolerance of five zebu breeds using physical, physiological and hematological traits as well as thermographic responses. Forty cows of the Gir, Girolando, Nelore, Sindhi and Indubrasil breeds (eight cows each), approximately three years of age, were evaluated. Body weight, withers and hump heights as well as thoracic circumference were recorded. The density and length of the hair was obtained by collecting one square centimeter in the rump region and skin color using the CIELAB system. Rectal temperature, heart and respiratory rates were evaluated during the morning at 4:30 h, and in the afternoon, at 14:30 h, with six repetitions. Blood samples were collected for hematological evaluation. The surface temperature was obtained using an infrared camera FLIR® T400. Two images were taken from each animal, one laterally of the whole body and the other of the head region. Air temperature, wind speed, relative humidity were obtained from a mobile weather station. The statistics analysis included an analyzes of variance, principal factors, as well as cluster, discriminant and canonical analyzes, logistic regression and calculation of odds ratio. There were significant differences in the rectal temperature, heart and respiratory rates between breeds. Gir and Indubrasil breeds had the highest rectal temperatures. Breed was significant for surface temperatures and showed that physical and physiological factors affected breeds in different ways. Eye and brain surface temperatures were the most affected by environmental parameters. Also, environmental parameters affected packed cell volume and red cell number. Odds ratio test showed that the Gir breed was three times more likely to have higher rectal temperature compared with Sindhi as confirmed by the logistic regression. When the black globe temperature approached 35 °C, the probability of the Gir animals having rectal temperatures above normal was approximately 70%. Gir was the breed least adapted to climate conditions of the experiment while the Sindhi and Girolando breeds showed the best physiological response to thermal stress.

1. Introduction

Bos indicus cattle are native of South and Southeast Asia, regions with a tropical climate. The main body characteristics of these animals include the presence of a hump, abundant and pleated dewlap, pigmented and loose skin and shorter and thinner hair compared to *Bos taurus*. In the early twentieth century these breeds were imported into Brazil in an attempt to increase the productivity of Brazilian locally adapted cattle which were mainly *Bos taurus* (Perez O'Brien et al., 2015). Throughout the twentieth century, these Brazilian local breeds were substituted by breeds of zebu origin.

The choice of *Bos indicus* animals was due to their large frame and adaptation to climatic conditions in production systems similar to that found in the country of origin. Zebu cattle have been shown to have higher thermoregulatory ability compared to cattle of European origin (Pereira et al., 2014) as a result of the reduction in the heat production and increase in the heat loss capacity to the environment (Hansen, 2004). Low metabolic rates resulting from reduced growth rates and milk yields of zebu breeds is a major contributing factor to their thermotolerance compared to European breeds (Hansen, 2004). Nevertheless, Gir cows increased their average annual milk production of 2200–4300 kg between 1987 and 2004 (Rebouças et al., 2008). However, the correlation between the additive genetic effect for milk production and growth and that for heat tolerance are negative (Finocchiaro et al., 2005). Therefore, the heat tolerance of these animals may be reduced with increasing productivity.

Excessive heat causes decreased food intake and disturbances

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in protein and energy metabolism, mineral balance, enzymatic reactions, hormones and metabolites secretion in the blood (Delfino et al., 2012). Metabolic disorders caused by thermal stress lead to reduced milk production, growth, and reproductive rates and increases the susceptibility of animal diseases causing economic loss (Nardone et al., 2010). Climate change causes an increase in average temperature and reduced rainfall, putting the sustainability of the livestock production system in risk (Scholtz et al., 2013), especially in countries such as Brazil, which already has high air temperature averages and grazing systems dependent on the rainy season. Selection has been mainly directed towards productive characteristics, but should include robustness, efficiency, reduced emission intensity and adaptability to heat stress in the future (Hayes et al., 2013).

Adaptability evaluation and heat tolerance of the animals are mainly determined by the physiological parameters such as respiratory rate and body temperature (Costa et al., in press). Restraint and handling procedures are required for the measurement of these parameter that can cause a stress response changing the results (Maziero et al., 2012). The use of new tools such as infrared thermography can be an alternative to assess the impact of environmental factors on thermal stress in animals. Thermographic images may indicate circulatory changes induced by increased body temperature related to environmental heat stress, leading to changes in surface temperatures of the animals (Stewart et al., 2005). The main benefits of this tool are to improve animal welfare during the evaluation and a larger number of evaluations in a shorter time and without animal restraint. The objective of this study was to compare physical, physiological, hematological and thermographic responses to heat stress in five breeds of zebu cattle.

2. Material and methods

Animal care procedures throughout the study followed protocols approved by the Ethics Committee for Animal Use (CEUA) at the University of Brasilia, number 22773/2012.

2.1. Local and animals data

The experiment was carried out in the Zebu Breeds Dairy Technology Transfer Center of the EMBRAPA Cerrados, located in Ponte Alta, at Federal District. The climate is classified as a tropical climate with a defined dry season. Forty, three years old, non-pregnant and non-lactating females were used, eight per breed (Nelore, Gir, Sindhi, Girolando and Indubrasil). The animals were reared as one group with the same environmental and treatment conditions. For the duration of the experiment, animals were maintained in a roofless corral with lateral wooden slating. Animals were adapted to the system for one week before the experiment started.

2.2. Data collection

Physical measurements were taken one week before the start of the experiment. Body weight, withers (the highest point of the interscapular region) and hump height and thoracic circumference were recorded using a measuring tape. With the help of pliers, all the hair was collected from an area of one square centimeter in the rump region for hair count and hair lengths were measured using calipers (Grip 59070 24-inch Jumbo Aluminum Caliper) (Silva, 2000). The skin color of the rump region was measured using a colorimeter, model Minolta Chrome, through the CIELAB, L^*, a^* and b^* system, where: L^* is the brightness, a^* is the red content and b^* is the yellow content. Three measurements were taken and the mean obtained. Skin thickness was measured at the palette region using a digital caliper.

Before physiological collections, the animals were maintained in an open corral for at least 2 h without shade and contained using a cattle crate with thin metal bars. The physiological parameters measured included: respiratory rate, heart rate and rectal temperature. These parameters were taken simultaneously by three different researchers, one for each parameter. Measurements began 5 min after the animals were placed in the crate. Heart rate was measured using a stethoscope, the respiratory frequency was obtained through observation of flank movements for 1 min and the rectal temperature was measured with a digital thermometer with sensitivity to 0.1 °C introduced into the animal's rectum for 3 min and held against the rectum wall at a depth of approximately 10 cm. After collecting the physiological parameters, blood samples were collected using the vacuntainer type tubes with EDTA anticoagulant. Heart and respiratory rates, rectal, body and head region surface temperatures of animals as well as the ground temperature were recorded at 04:30 and 14:30 h. The procedure was repeated on six separate days, over a period of three weeks at the end of the experiment, during the dry season on the Brazilian Cerrado (Savannah).

Animal surface and ground temperatures were measured by infrared Flir thermograph ThermaCAM® model T400 (FLIR Systems Inc, Wilsonville, OR, USA). This camera has infrared resolution 320×240 pixels, with thermal sensitivity of < 0.05 °C at 30 °C (86 °F)/50 mK. Two images were taken from each animal, at a distance of 1.5 m, one laterally of the whole body and the other of the head region. The animals were taken calmly to a covered area immediately after physiological measurements, so no time was allowed for the animals to adapt to the shaded area before recordings were completed. This procedure was adopted because sunlight on the animals alters the conductivity and emissivity of the thermal image (Stewart et al., 2005).

Red and white cell number, platelets and hemoglobin concentration were obtained using an automatic cells counter (CC550 Cellm™). Mean corpuscular volume (MCV) was calculated using the formula hematocrit*10/erythrocytes and the mean corpuscular hemoglobin concentration (MCHC) as hemoglobin*100/Corpuscular Volume. Monocytes, lymphocytes, segmented neutrophils and eosinophils numbers were obtained by manually counting one hundred cells under a microscope (Olympus CX41) with $400\times$ magnification. Total plasma protein concentration was determined by a manual refractometer.

Standard Quickreport® tools (Fig. 1) were used for analysis of the images: tool "line" was used to obtain the average temperature in the brain and neck regions of the animals. The "point" tool was used to obtain the highest temperature in the axilla, groin, rump and eye regions of the animals, as well as the "area" tool was used to measure the average temperature of the whole body, the muzzle and chamfer regions, and the temperature of two distinct areas of the ground near of the animals (Fig. 1).

The wet bulb globe temperature (WBGT) and the black globe temperature (°C) were taken using a mobile globe thermometer ITWTG-2000 (INSTRUTEMP, Measuring Instruments Ltda, SP, BR). The environment temperature (°C), relative humidity (%) and wind speed (km/h) were obtained from a local weather station ITWH1080 (INSTRUTEMP, Measuring Instruments Ltda, SP, BR). The mobile weather station was placed on the fence of the corral.

Temperature and Humidity Index (THI) was calculated according to the following National Research Council (1971) formula: $THI = (1.8 \times Tdb + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26)$, where, Tdb: dry bulb temperature (°C) and RH: relative humidity (%).

The maximum and minimum environment temperatures during the experiment were 35.9 °C and 11.5 °C, respectively. Temperatures above the comfort zone of cattle (27 °C) were recorded on all days, and the air humidity ranged between 25.0–88.0%. The maximum wind speed recorded was 28 km/h, with an average of 5.8 km/h. The

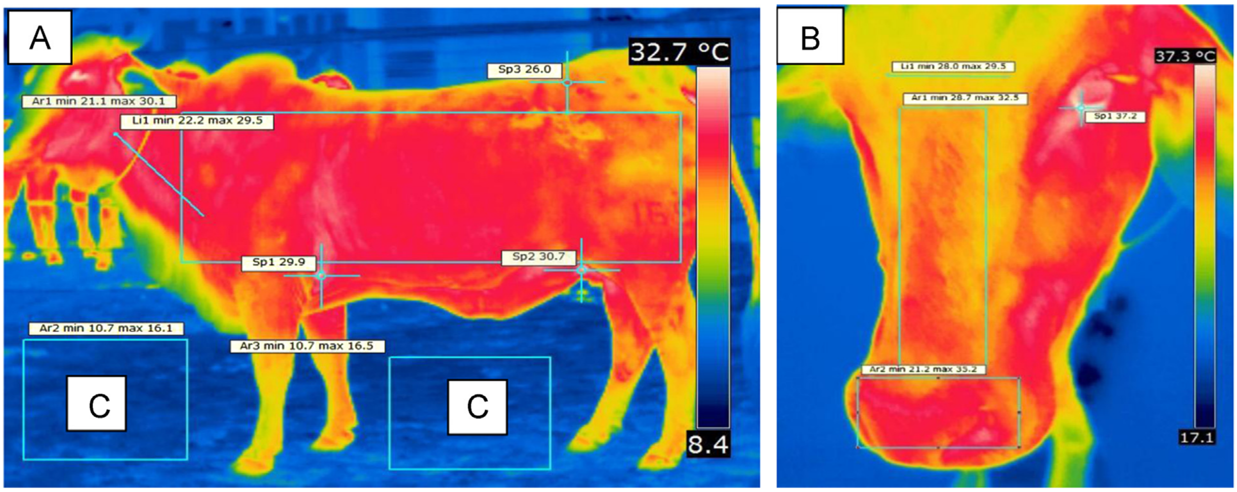

Fig. 1. Analysis of thermographic images of the surface temperature of the body (A), head region temperature (B) and ground temperature (C).

wet bulb globe and the black globe temperatures ranged between 11.9–22.6 and 14.0–36.6 °C, respectively, and the temperature and humidity index average was 69.09. The ground temperature average was 25.2 °C.

2.3. Statistics analyzes

Statistical analyzes were performed using the Statistical Analysis System® package (v.9.3, SAS Inc, Cary, NC, USA). An analysis of variance (PROC GLM) was carried out to see the differences between breeds for physical traits. Two repeated analyzes of variance were carried out. The first looked at the effect (covariance) of physical characteristics (skin thickness, thoracic circumference, weight and hair traits) on physiological parameters, surface temperatures and blood parameters, while the second included temperature and humidity index, wind speed, black globe temperature, wet bulb globe temperature and ground temperature as covariates and breed and period of the day as a fixed effect on physiological parameters, surface temperatures and blood parameters. Covariance structure was tested for each variable and that with the lowest AICC and BICC was considered the most suitable.

After standardization, multivariate analyzes were carried out in accordance with Hair et al. (2010), to place animals in groups in accordance with their degree of similarity and verify discriminatory capacity of the original traits in the formation of these groups. These procedures included principal component analysis to attempt to understand the sources of variation in the data (PRINCOMP), organize information about variables so that relatively homogeneous groups, or “clusters”, were formed (CLUSTER), and observe distances between these groups (TREE), use the groups of traits (physical characteristics; superficial temperatures; physiological and hematological parameters) to determine the percentage of animals correctly classified in their own breed using this groups of traits (DISCRIM) and summarize between-class variation in much the same way that principal components summarize total variation (CANCORR). Logistic regression analysis and odds ratio calculation were carried out to identify which breeds are more tolerant to heat stress considering the maintenance of rectal temperature.

3. Results

Breed influenced all the physical traits ($P < 0.05$, Table 1). Indubrasil and Girolando animals had the highest body weight and thoracic circumference (559.75 and 552.12 kg and 2.05 and 2.05 cm, respectively), however, Girolando and Nelore animals had highest withers height (1.40 and 1.39 m, respectively). Indubrasil and Sindhi breeds had the thickest skin, and for skin coloration, Nelore and Girolando breeds had the lowest brightness values (darker skin). Girolando animals also had the highest hair density and length (1283 cm^2 and 0.99 cm, respectively) (Table 1).

Despite the physical differences between breeds, the weight of the animals as a covariable had no effect on physiological parameters, surface temperatures and blood parameters evaluated and due to this was not presented in the results. Only the skin thickness and brightness affected the temperatures analyzed by the thermographer, and the hair number and b^* value affected the heart rate. The thoracic circumference influenced respiratory rate (data no shown).

Table 1
Least squared means of the physical characteristics in Brazilian Zebu cattle.

Breed	Physical characteristic											Ratio
	WH	ST	TC	Weight	Hair	HL	L^*	a^*	b^*	HH	Dif	
Nelore	1.39^{a}	0.77^{ab}	1.96^{b}	510.12^{b}	1220^{a}	0.76^{b}	19.50^{b}	4.30^{a}	−1.18^{c}	1.47^{a}	0.085^{b}	0.3876^{b}
Indubrasil	1.35^{b}	0.83^{a}	2.05^{a}	559.75^{a}	1201^{ab}	0.75^{b}	22.77^{a}	1.76^{b}	2.27^{ab}	1.46^{a}	0.104^{ab}	0.3706^{b}
Girolando	1.40^{a}	0.73^{b}	2.05^{a}	552.12^{a}	1283^{a}	0.99^{a}	18.96^{b}	2.87^{ab}	1.66^{b}	1.40^{b}	0.005^{c}	0.3720^{b}
Sindhi	1.32^{c}	0.84^{a}	1.93^{b}	514.62^{b}	1185^{ab}	0.67^{b}	20.54^{ab}	2.69^{b}	3.13^{ab}	1.42^{ab}	0.090^{b}	0.3784^{b}
Gir	1.26^{d}	0.75^{b}	1.83^{c}	409.00^{c}	1105^{b}	0.94^{a}	23.01^{a}	3.01^{ab}	4.36^{a}	1.38^{b}	0.120^{a}	0.4582^{a}
SE	0.007	0.015	0.015	8.35	28.57	0.023	0.65	0.38	0.56	0.009	0.004	0.005
Pr > F	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0006	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001	< 0.0001	< 0.0001

WH: withers height (m); ST: skin thickness (cm); TC: thoracic circumference (m); Hair: hair number in a square centimeter; HL: hair length (cm); L^*: brightness; a^*: red content; b^*: yellow content; HH: hump height (m); Dif: difference between withers and hump height (cm); Ratio: [(TC/weight) × 100]; SE: standard error. Means followed by different lower case letters in the column ([a, ab, b, c and d]) differ at 5% by Tukey test ($p < 0.05$).

Table 2
Effect of environment conditions and breed on physiological parameters and surface temperatures (°C) in Brazilian Zebu cattle and least squared means for the breed and period of the day effects.

	Physiological parameter			Surface temperature								
	RT	HR	RR	Body	Neck	Axilla	Groin	Rump	Chamfer	Muzzle	Brain	Eye
Statistical probability												
THI	0.8968	0.4138	0.0265	0.7333	0.5895	0.4542	0.0265	0.0036	0.0071	0.0017	0.0017	< 0.0001
WS	0.4603	0.8263	0.3245	0.1635	0.6777	0.3990	0.9983	0.0206	< 0.0001	0.0951	< 0.0001	< 0.0001
BGT	0.2130	0.5283	0.1289	0.9126	0.6516	0.9571	0.9369	0.5114	0.6846	0.6296	0.6311	0.4080
WBGT	0.3157	0.2179	0.9403	0.6047	0.1705	0.9438	0.5247	0.2372	0.2180	0.5400	0.0532	0.0495
Ground	0.9870	0.6811	0.6826	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.2830	0.5105	0.0013	0.8212
Breed	< 0.0001	0.0003	0.0016	< 0.0001	0.0478	0.0014	0.0234	< 0.0001	0.0025	< 0.0001	0.0001	< 0.0001
Period	0.7640	0.1346	0.0355	0.2040	0.1381	0.7037	0.3347	0.0620	0.0690	0.1895	0.0006	0.2682
Breed*period	0.7810	0.6242	0.2526	0.0006	0.4905	0.1216	0.1133	< 0.0001	0.2988	0.0014	0.0061	0.0425
Breed												
Nelore	38.87^{ab}	64.11^{ab}	41.00^{a}	31.89	32.09^{ab}	33.27^{b}	32.75^{b}	31.73	29.02^{ab}	28.71	30.33	35.51
Indubrasil	39.00^{a}	61.51^{abc}	33.75^{b}	31.57	31.89^{b}	33.35^{b}	33.35^{ab}	32.37	28.32^{b}	27.89	29.46	35.66
Girolando	38.65^{b}	57.91^{bc}	39.15^{a}	32.60	32.40^{ab}	33.58^{ab}	33.15^{ab}	33.91	29.14^{ab}	29.12	29.74	36.32
Sindhi	38.86^{ab}	56.53^{c}	36.53^{ab}	32.08	32.10^{ab}	33.33^{b}	33.17^{ab}	33.14	29.43^{a}	29.18	30.13	36.04
Gir	39.05^{a}	66.82^{a}	36.22^{ab}	32.97	32.89^{a}	34.29^{a}	33.84^{a}	33.44	28.47^{b}	27.14	29.56	35.67
SE	0.060	1.85	1.32	0.21	0.24	0.19	0.22	0.28	0.22	0.27	0.15	0.13
Period of the day												
Morning	38.96	49.34	25.06^{b}	33.39	33.86	33.24	30.62	35.21	30.71	29.99	32.19	35.19
Afternoon	38.81	73.41	49.60^{a}	31.05	30.69	33.89	34.22	30.62	27.04	26.85	27.50	36.49
SE	0.26	8.07	5.85	0.92	1.07	0.85	1.00	1.22	1.00	1.22	0.68	0.58
RV	36.7–39.1	36.0–60.0	26.0–50.0	–	–	–	–	–	–	–	–	–

THI: temperature and humidity index; WS: wind speed (km/h); BGT: black globe temperature (°C); WBGT: wet bulb globe temperature (°C); RT: rectal temperature (°C); HR: heart rate (beats/min); RR: respiratory rate (mov/min). Means followed by different lower case letters in the column ($^{a,\ ab,\ b,\ abc,\ bc\ and\ c}$) differ at 5% by Tukey test ($p < 0.05$). SE: standard error; RV: reference value according to Jain (1993) and Reece et al. (2015).

Breed affected all physiological parameters ($P < 0.05$, Table 2). Girolando, Sindhi and Nelore breeds showed lower rectal temperatures (38.65; 38.86 and 38.87 °C, respectively). Sindhi, Girolando and Indubrasil breeds had the lower heart rate (56.53; 57.91 and 61.51 beats/min, respectively) while Indubrasil animals had the lowest respiratory rate (33.75 mov/min) but not different from the Sindhi and Gir breeds (36.53 and 36.22 mov/min, respectively). There was a significant difference between period of the day for respiratory rate, with the afternoon having the highest value.

There was an interaction between breed and period of the day for body, rump, muzzle, brain and eye surface temperatures as well as for mean corpuscular hemoglobin concentration ($P < 0.05$, Table 3). The Gir breed presented the highest body and rump temperatures (34.24 and 36.11 °C, respectively) as well as red blood cell concentration (34.24) in the morning period. Highest muzzle temperatures were observed in Girolando, Nelore and Sindhi breeds (31.35; 30.71 and 30.94 °C, respectively) in the morning period, while the highest brain temperature was observed in Nelore breed (33.05 °C). Breed also affected the thermographic image points ($P < 0.05$, Table 2). Gir breed obtained the highest neck, axilla and groin surface temperatures (32.89; 34.29 and 33.84 °C, respectively) although Shindhi animals had the highest chamfer surface temperature (29.43 °C) but there was no difference with Girolando and Nelore groups (29.14 and 29.02 °C, respectively). Ground temperature affected body, neck, axilla, groin, rump and brain temperatures ($P < 0.05$). Temperature and humidity index was related to the surface temperature and the main trait influenced was the eye ($P < 0.0001$). Wind speed affected the rump, chamfer, brain and eye surface temperatures.

Blood parameters were affected by breed and environment factors ($P < 0.05$, Table 4). The period of the day affected the packed cell volume and red blood cell number. Packed cell volume was affected by temperature and humidity index, wind speed and black globe temperature. All climatic factors affected the number of red blood cell number and the dry and wet black globes temperatures influenced hemoglobin concentration, mean corpuscular hemoglobin concentration and mean corpuscular volume. Breed affected all blood parameters except for red blood cell number, mean corpuscular volume concentration, monocyte concentration and platelet number. The ground temperature only affected the hemoglobin concentration.

Nelore animals had the lowest packed cell volume (37.46) and Nelore and Indubrasil animals had the lowest value of total plasma

Table 3
Breed and period of the day interactions on surface temperatures (°C) and blood parameters.

Period of the day	Breed				
	Nelore	Indubrasil	Girolando	Sindhi	Gir
Body temperature					
Morning	33.49^{ab}	33.08^{bc}	33.70^{ab}	32.50^{c}	34.24^{a}
Afternoon	30.28^{b}	29.93^{b}	31.38^{a}	31.66^{a}	31.96^{a}
Rump temperature					
Morning	34.44$^{A\ b}$	35.22$^{A\ ab}$	35.71^{a}	34.49^{b}	36.11$^{A\ a}$
Afternoon	29.03$^{B\ c}$	29.68$^{B\ bc}$	32.25^{a}	31.81^{a}	30.45$^{B\ b}$
Muzzle temperature					
Morning	30.71^{a}	28.88^{b}	31.35^{a}	30.94^{a}	28.09^{b}
Afternoon	26.72^{ab}	26.92^{ab}	26.90^{ab}	27.42^{a}	26.16^{b}
Brain temperature					
Morning	33.05$^{A\ a}$	31.92$^{A\ b}$	31.76$^{A\ b}$	32.62$^{A\ a}$	31.60$^{A\ b}$
Afternoon	27.61$^{B\ ab}$	27.09$^{B\ b}$	27.78$^{B\ a}$	27.65$^{B\ ab}$	27.37$^{B\ ab}$
Eye temperature					
Morning	34.95^{b}	34.73^{b}	35.88^{a}	35.53^{a}	34.85^{b}
Afternoon	36.07^{b}	36.59^{ab}	36.77^{a}	36.55^{ab}	36.49^{ab}
MCHC					
Morning	33.49^{ab}	33.08^{bc}	33.70^{ab}	32.50^{c}	34.24^{a}
Afternoon	30.28^{b}	29.93^{b}	31.38^{a}	31.66^{a}	31.96^{a}

MCHC: mean corpuscular hemoglobin concentration. Means followed by different lower case letters in the row ($^{a,\ ab,\ b\ bc,\ and\ c}$) and different capital letters in the column ($^{A\ and\ B}$) differ at 5% by Tukey test ($p < 0.05$).

Table 4
Effect of environment conditions on blood parameters in Brazilian Zebu cattle and least squared means for the breed and period of the day effects.

	Blood parameter											
	PCV	PTP	WBC	RBC	HGB	MCHC	MCV	Mono	Limf	Segm	Eosi	Platelet
Statistical probability												
THI	0.0220	0.1886	0.6157	0.0004	0.8381	0.0259	0.1302	0.8413	0.3779	0.1759	0.2517	0.4895
WS	0.0186	0.0504	0.1336	0.0348	0.2078	0.8115	0.6554	0.2524	0.0750	0.0175	0.4047	0.8975
BGT	0.0099	0.1681	0.2259	<0.0001	<0.0001	0.0035	<0.0001	0.8456	0.1215	0.0984	0.9780	0.4694
WBGT	0.1346	0.6226	0.1583	<0.0001	<0.0001	<0.0001	<0.0001	0.7904	0.2019	0.2430	0.5542	0.5349
Ground	0.1022	0.6854	0.2871	0.8204	0.0191	0.1887	0.3516	0.8030	0.2495	0.2610	0.7319	0.8151
Breed	0.0192	<0.0001	<0.0001	0.7020	0.0332	0.0007	0.2799	0.5648	0.0033	0.0005	0.0001	0.9269
Period	0.0441	0.1226	0.7521	0.0006	0.5390	0.2467	0.0953	0.6454	0.9743	0.7684	0.2388	0.5761
Breed*period	0.6248	0.7366	0.5497	0.3103	0.0974	0.0091	0.6356	0.9698	0.4560	0.5374	0.2195	0.7753
Breed												
Nelore	37.46^{b}	7.58^{b}	10.88^{ab}	7.94	12.98^{ab}	34.72	49.49	2.77	59.72^{ab}	32.13^{ab}	5.38^{abc}	353.48
Indubrasil	38.76^{ab}	7.46^{b}	11.92^{a}	8.24	13.35^{ab}	34.50	48.94	2.54	57.21^{ab}	35.99^{a}	4.22^{c}	377.60
Girolando	40.15^{a}	7.95^{a}	11.03^{a}	8.27	13.41^{a}	33.53	50.37	2.45	63.63^{a}	27.74^{b}	6.16^{ab}	297.44
Sindhi	39.79^{ab}	7.92^{a}	9.33^{b}	8.20	12.24^{b}	30.87	51.97	2.63	56.39^{b}	34.04^{a}	6.89^{a}	365.84
Gir	38.62^{ab}	7.75^{ab}	11.92^{a}	8.46	12.62^{ab}	32.79	47.31	2.98	59.20^{ab}	33.36^{a}	4.41^{bc}	329.39
SE	0.62	0.074	0.39	0.24	0.30	0.69	1.56	0.24	1.41	1.35	0.45	82.29
Period of the day												
Morning	33.45^{b}	8.24	11.57	4.42^{b}	12.10	36.80	61.15	2.18	59.03	30.90	7.85	170.07
Afternoon	44.46^{a}	7.22	10.46	12.02^{a}	13.74	29.76	38.07	3.17	59.43	34.40	2.98	519.43
SE	2.73	0.33	1.76	1.10	1.33	3.04	9.92	1.07	6.25	5.96	2.07	311.34
RV	24.0–46.0	7.0–8.5	4.0–12.0	5.0–10.0	8.0–15.0	30.0–36.0	40.0–60.0	2.0–7.0	45.0–75.0	0–120	0–20	100–800

THI: temperature and humidity index; WS: wind speed (km/h); BGT: black globe temperature (°C); WBGT: wet bulb globe temperature (°C); PCV: packed cell volume; PTP: plasma total protein; WBC: white blood cell; RBC: red blood cell; HGB: hemoglobin concentration; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; Mono: monocytes; Limf: lymphocytes; Segm: segmented neutrophils; Eosi: eosinophils. Means followed by different lowe case letters in the column ($^{a,\ ab\ and\ b}$) differ at 5% by Tukey test ($p<0.05$). SE: standard error; RV: reference value according to Jain (1993).

protein (7.58 and 7.46, respectively) (Table 4). Sindhi animals had the lowest white blood cell, hemoglobin and lymphocytes concentrations (9.33; 12.24 and 56.39, respectively) and the largest eosinophil number (6.89).

In the morning, the canonical correlation showed that the main physical component related to the physiological variables was the skin brightness followed by thoracic circumference. The number of white blood cells was the hematological variable that had the highest correlation with the physical characteristics of the animal. In the afternoon, thoracic circumference maintained a positive canonical correlation with the physiological parameters, but respiratory rate was the physiological component with the highest correlation with the physical parameters, followed by packed cell volume and body surface temperature.

For the canonical correlation of the physiological components, the Nelore animals had the highest respiratory rate together with

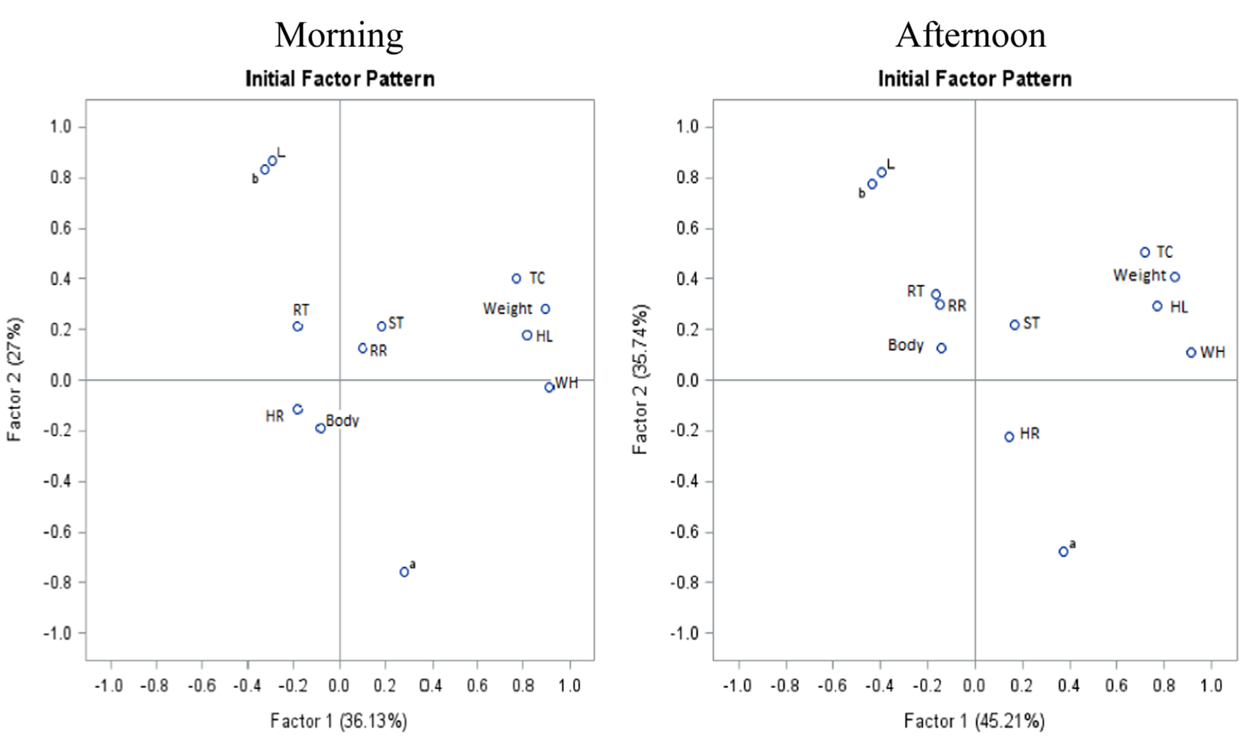

Fig. 2. Principal component analysis for physiological and physical parameters and surface temperature. HR: heart rate; RR: respiratory rate; RT: rectal temperature; Body: body surface temperature; ST: skin thickness; WH: withers height; TC: thoracic circumference; HL: hair length L^*: brightness; a^*: red content; b^*: yellow content.

the Gir, which had the highest heart rate. The Indubrasil and Gir breeds had the highest rectal temperatures and the Girolando breed had the highest body temperature.

The principal component analysis showed that in the morning, 63.13% of the variation was explained by the physiological and physical variables of the animals (Fig. 2). The physical components thoracic circumference, hair length, withers height and weight were grouped. Heart rate was related with the superficial body temperature and the respiratory rate with skin thickness. The higher the values for brightness and b^* lower heart rate and increased respiratory rate. In the afternoon, 80.95% of the variation was explained by the characteristics studied. There was no change in the grouping of the physical components of the animals, but there was a grouping of physiological parameters, rectal temperature, respiratory rate and superficial body temperature, revealing a higher relationship between these parameters in times of increased environmental temperatures.

The dendrograms, produced by the cluster analysis, of the physiological parameters in the morning and in the afternoon showed a separation between breeds in two clusters, one formed by the Sindhi and Girolando and another formed by Gir, Indubrasil and Nelore breeds (Fig. 3). In the morning, the Nelore breed was physiologically closer to Gir breed while in the afternoon the Nelore was grouped with Indubrasil and Gir breeds. Dendrograms of

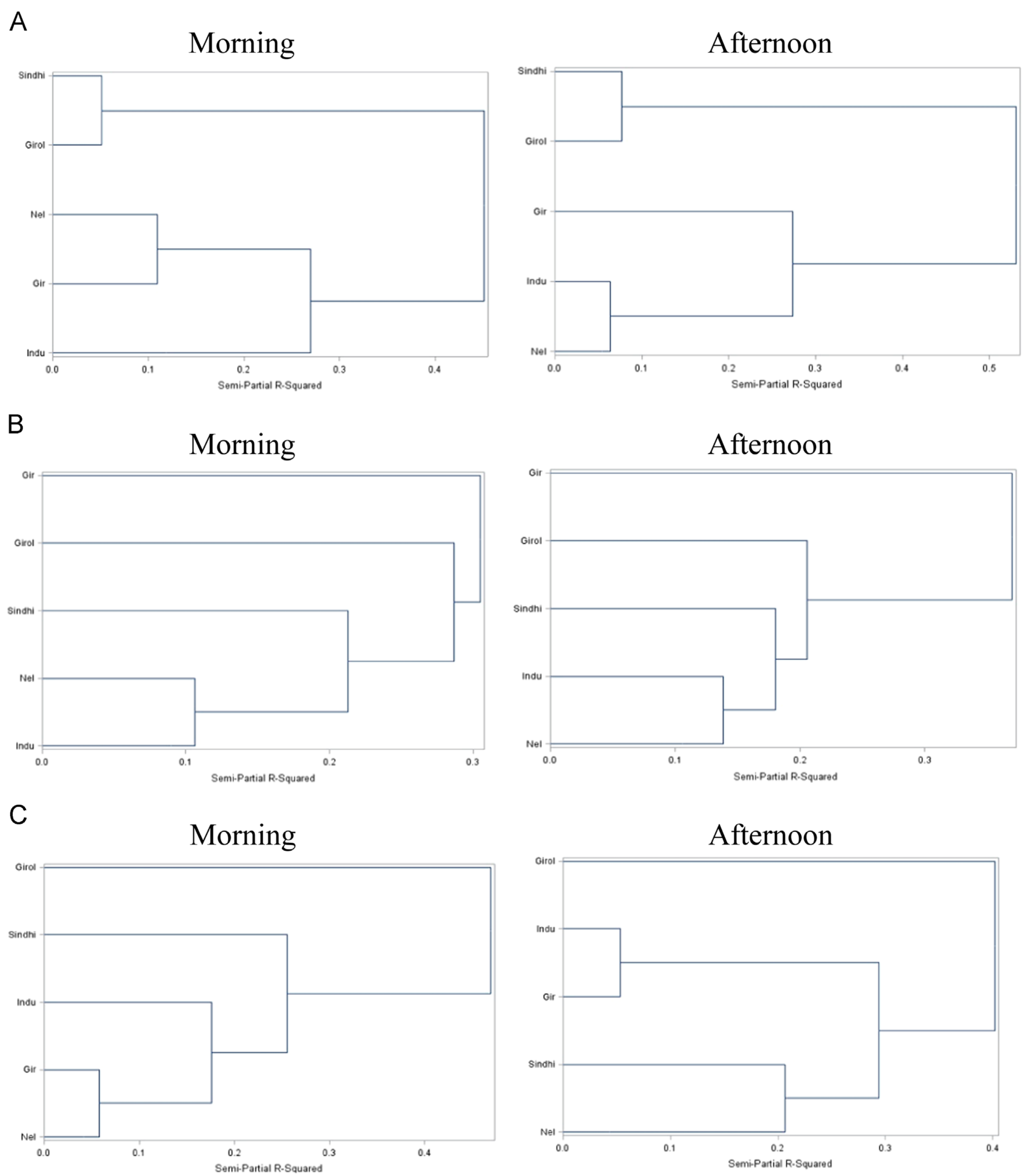

Fig. 3. Cluster analysis in the morning and in the afternoon of physiological parameters (a), surface temperatures (b) and blood parameters (c). (Girol: Girolando; Nel: Nelore; Indu: Indubrasil).

Table 5
Percentage of collections that are above the reference values.

Breed	Rectal temperature (°C)		Respiratory rate (breaths/min)	
	Morning	Afternoon	Morning	Afternoon
Gir	29.2	58.3	4.2	16.7
Girolando	0.00	16.7	8.3	8.3
Indubrasil	16.7	54.2	4.2	0.00
Nelore	12.5	41.7	4.2	20.8
Sindhi	0.00	45.8	4.2	8.3

surface temperatures produced a separation into two clusters, with the Gir breed isolated in one cluster, while the other breeds were divided into three subclusters in both morning and afternoon. There was also a division in two clusters in the blood parameters and Girolando breed formed a group in both morning and afternoon. In the afternoon, the Gir and Indubrasil breeds and Sindi and Nelore breeds formed smaller clusters.

No animals of Girolando and Sindhi breeds had rectal temperature above the reference value (36.7–39.1 °C) in the morning (Table 5). In the afternoon, 58.3% of the Gir animals recorded rectal temperature above the reference value and for the Girolando animals this was only 16.7%. Respiratory rates were generally within the reference values (26–50 mov/min) in the morning. In the afternoon, there was no change in respiratory rate for the Indubrasil breed. The Girolando maintained the same percentage between both periods (8.3) and the Nelore breed had the highest percentage of respiratory rate above of the reference value (20.8).

In the discriminant analysis for both morning and afternoon periods, the Gir animals were classified 100% within their own breed when considering physical characteristics (Table 6). For animals of Indubrasil and Sindhi breeds 62.5% were classified within their own breed while this was 87.5% for Nelore. For the physiological parameters, the Gir breed was ranked as the best within its own group on the morning period (54.2%), but in the afternoon the best breed was the Girolando. Only 12.5% of Sindhi animals were classified in their own breed (Table 6).

For surface temperatures, Gir, Girolando and Nelore breeds had more than 60% of the animals classified within their own breed during the morning (Table 6). In the afternoon, there was a reduction in the classification of animals in their own breeds. The Nelore breed had the best ranking with 54%. This may reflect a similar response between breeds for the traits measured here.

In the morning, the Girolando, Indubrasil and Nelore breed had 100% of the animals classified into their breed for blood parameters (Table 6). In the afternoon, also 100% of the Nelore animals were classified as Nelore breed.

The odds ratio test (Fig. 4) showed that the Gir breed had three times more likely to have higher rectal temperature compared to the Sindhi breed. There was no difference between the Indubrasil and Sindhi breeds and between Nelore and Sindhi breeds. Logistic regression (Fig. 4) showed that when the black globe temperature approached 35 °C, the probability of the Girolando animals having rectal temperatures above normal was less than 25% while the Gir group had approximately 70% of probability. In the Roc Curve, the area under the curve was above 0.78, demonstrating that the model used had high sensitivity for heat tolerance analysis.

4. Discussion

The observed differences were due to breed characteristics. The withers height of Girolando and Nelore breeds were within the average for these breeds and the larger withers height of Girolando animals compared to Gir animals is due to the higher average height of Holstein breed cattle as the Girolando is a cross between Holstein and Gir breeds (Bianchini et al., 2006). The thicker skin of the animals of Sindhi, Indubrasil and Nelore breeds, may be considered a rusticity trait of these Zebu animals for skin protection against parasites (McManus et al., 2014) and ultraviolet rays (Hansen, 2004).

Table 6
Discriminant analysis (%) in morning and afternoon periods for the physical and physiological parameters, surface temperatures (°C) and blood parameters.

Breed	Morning					Afternoon				
	Gir	Girol	Indu	Nelore	Sindhi	Gir	Girol	Indu	Nelore	Sindhi
Physical parameters										
Gir	100.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0
Girol	0.0	87.5	12.5	0.0	0.0	0.0	87.5	12.5	0.0	0.0
Indu	12.5	0.0	62.5	12.5	12.5	12.5	0.0	62.5	12.5	12.5
Nelore	0.0	0.0	25.0	75.0	100.0	0.0	0.0	25.0	75.0	100.0
Sindhi	0.0	0.0	0.0	0.0	22.5	0.0	0.0	0.0	0.0	22.5
Physiological parameters										
Gir	54.2	8.3	12.5	12.5	12.5	20.8	20.8	37.5	20.8	0.0
Girol	4.2	50.0	8.3	16.7	20.8	0.0	54.2	4.2	25.0	16.7
Indu	34.8	13.0	21.7	4.3	26.1	20.8	12.5	45.8	4.2	16.7
Nelore	16.7	25.0	8.3	33.3	16.7	12.5	16.7	8.3	50.0	12.5
Sindhi	8.3	37.5	20.8	0.0	33.3	20.8	37.5	20.8	8.3	12.5
Surface temperatures										
Gir	60.8	17.4	13.0	8.7	0.0	50.0	12.5	16.7	20.8	0.0
Girol	25.0	66.7	0.0	4.2	4.2	12.5	37.5	12.5	20.8	16.7
Indu	20.8	8.3	37.5	20.8	12.5	20.8	20.8	41.7	8.3	8.3
Nelore	4.3	4.3	17.4	65.2	8.7	25.0	0.0	20.8	54.2	0.0
Sindhi	0.00	20.8	8.3	16.7	54.2	8.3	12.5	12.5	16.7	50.0
Blood parameters										
Gir	87.5	12.5	0.0	0.0	0.0	85.7	0.0	0.0	14.3	0.0
Girol	0.0	100.0	0.0	0.0	0.0	0.0	66.7	0.0	16.7	16.7
Indu	0.0	0.0	100.0	0.0	0.0	0.0	25.0	75.0	0.0	0.0
Nelore	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	100.0	0.0
Sindhi	0.0	16.7	0.0	0.0	83.3	0.0	16.7	0.0	0.0	83.3

Girol: Girolando; Indu: Indubrasil.

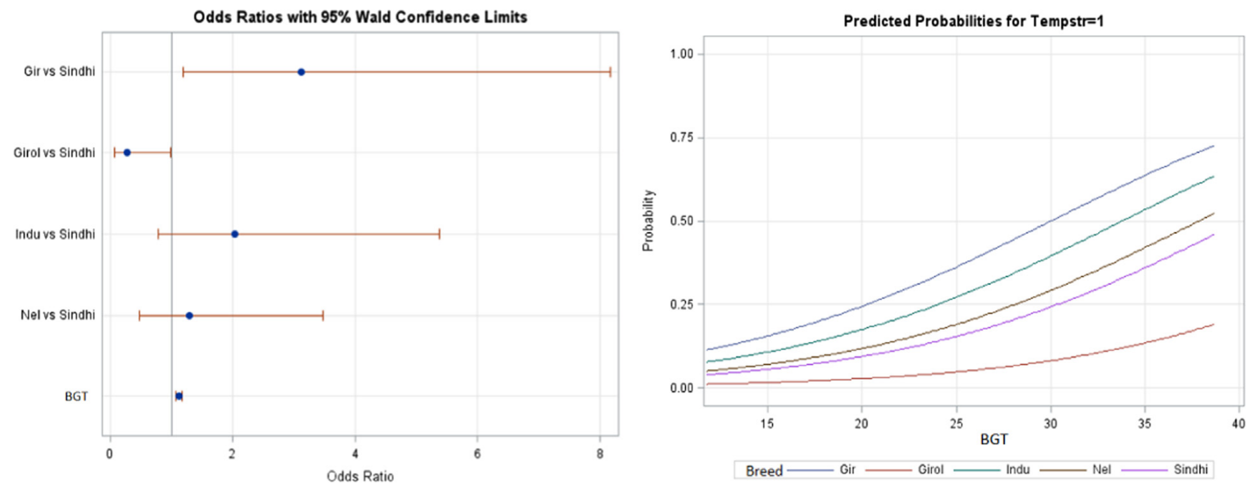

Fig. 4. Odds ratio and logistic regression of the rectal temperature (Girol: Girolando; Indu: Indubrasil; Nel: Nelore; BGT: black globe temperature).

Physical characteristics reflect the manner an animal reacts to stress. Girolando and Gir animals showed lower skin thickness compared to the other genetic groups, but was greater than pure Holsten animals (Bianchini et al., 2006). With an increase in skin thickness, the animal has greater difficulty in dissipating latent heat due to skin evaporation (Holmes, 1981). Girolando and Gir animals had longer hair. Animals of temperate origin have coat features which trap air for cold insulation but can be an obstruction for cooling, slowing down the speed and moisture gradient through the hair layer in hot and humid conditions (Collier and Collier, 2010). However, coats with these higher density and height reduces the radiant energy incidence, thereby absorbing less heat (Maia et al., 2005).

Girolando and Nelore animals had the lowest brightness values, but Girolando animals have some areas with pigmentation and others with no pigmentation. The darker skin of the Nelore may be related to the greater formation of eumelanin compared to pheomelanin. Eumelanin and pheomelanin are two components of melanin. The eumelanin is related to the black to brown color formation and pheomelanin linked to the formation of yellow and red color tones (Hulsman Hanna et al., 2014). A higher proportion of eumelanin forms a darker melanin. This characteristic can be found in Holstein and Angus animals (Robbins et al., 1993). The skin of Nelore animals was uniformly dark in color and the Girolando showed dark and light spots. This gives greater protection to the skin from ultraviolet rays in the Nelore compared to Girolando. Light skins have a higher radiation transmission through a surface that can cause skin lesions, but have lower absorption of thermal radiation (Silva and Pocay, 2001). Nelore, Girolando and Gir breeds had higher a^* values, indicating more red color composition on the skin.

Thoracic circumference may influence the respiratory rate limiting lung expansion at inspiration. Animals with greater thoracic circumference (TC) may exchange larger air volumes and thus more heat lost to the environment through the vapor released in the breath without increasing their respiratory rate (Marai et al., 2007). However, in the Indubrasil breed, which recorded the largest thoracic circumference and lower respiratory rate, this mechanism was not enough to maintain a lower rectal temperature. Also, in times of increased stress and consumption of oxygen, the body releases more red blood cell to increase the oxygen transport to the tissues and thereby increasing the packed cell volume (Correa et al., 2012) as observed in the present study in the afternoon period. Gir had higher TC/weight ratio. This mean that these animals were more compact than the other breeds giving a higher surface area for heat absorption.

Surface temperatures were influenced by breed showing that physical and physiological factors affected animal groups in different ways. The rump is one of the main points of analysis due to the high level of sunlight incidence on this site. Body temperature using thermography gives a larger area for analysis, giving mean values for the whole body, but also gives a larger area to be affected by external factors such as ground temperature, and also physical and physiological factors including differences in the hair color and skin vascularization level. Thus, there is a greater temperature gradient than at specific points. Eye temperature is stated by the literature as the best stress observation point using thermographic images due to the high level of vascularization of the posterior eyelid border and changes in blood flow by the action of the sympathetic system (Stewart et al., 2007). Eye surface temperature was affected by environmental parameters such as temperature and humidity index, wind speed and wet bulb globe temperature. According to Church et al. (2014), environmental variables such as solar radiation incidence and wind speed can influence the body temperature, requiring care in the use of thermographic images in measuring heat tolerance.

Girolando animals had one of the lowest rectal temperatures, but showed one of the highest respiratory rates. This may be due to water evaporation which absorbed heat and was released it through respiration. The animal increased its breathing rate trying to increase the loss of excessive heat and maintaing a low rectal temperature (McManus et al., 2014). This group had one of the highest eye, rump and body surface temperatures. This heat loss would be another mechanism that, through increased skin circulation, increased heat loss occurs by convection and radiation and also by cutaneous vasodilation (Swenson and Reece, 1996). This heat loss through the skin was greater due to the thinner skin of that breed (Hansen, 2004).

Gir animals had high neck and axilla surface temperatures due to skin folds and also the thicker skin in these points. This group also recorded, along with the Indubrasil and Nelore groups, the highest rectal temperature and heart rate, higher than the reference values, reflecting physiological stress. The lower eye temperature and the high respiratory rate suggest that heat loss by cutaneous vasodilation is not the main mechanism for heat loss of the Nelore breed, but the rather an increase in respiratory rate.

Packed cell volume and the red cell number were affected by environment factors such as temperature and humidity index, wind speed and wet bulb globe temperature. This response is related to intense heat stress of the animal, as seen in this study in the afternoon period, which causes the release of catecholamines (epinephrine and norepinephrine), which in turn leads to increased blood pressure and contraction of the spleen, increasing the red blood cell mobilization and placing them in the bloodstream (Swenson and Reece, 1996). The white blood cell and the plasma total protein concentration were affected only by breed, characterizing that the observed variation was due to response to the environment challenge linked to heat stress of each genetic group.

There was an interaction between period of the day and breed for body, rump, muzzle, brain and eye surface temperatures. This was observed because at the hottest times of the day there was a different response of the breeds to heat stress according to their physical characteristics. Although, the mean rectal temperatures were within the reference value for cattle, between 36.7 °C and 39.1 °C (Reece et al., 2015), showing that all the groups were able to maintain their homeothermic condition although air temperatures in the morning and afternoon were considered stressful as seen in changes in the physiological parameters.

There was interaction between period of the day and breed for mean corpuscular hemoglobin concentration. A larger packed cell volume was affected by increased circulating red blood cell number in the afternoon period.

The canonical correlation showed that thoracic circumference was the main physical component that had relationship with the physiological variables in both morning and afternoon periods. In the morning, the white blood cell value had the highest correlation with the physical variables, and was the hematological component that had the greatest variation between time and breed. In the afternoon, the positive canonical correlations between physical parameters and respiratory rate, packed cell volume and surface temperatures showed that the latter was affected by animal size, as larger animals have smaller surface area to lose heat (Santos et al., 2005). The increase in rectal temperature may generate a physiological response, increasing sweating and respiratory rates leading to a loss of body water and increasing cell concentration.

In the principal component analysis, the physical variables related to animal size and body weight were grouped. This was expected due to the correlation of thoracic circumference and animal weight (Pacheco et al., 2008). In the morning, the lower the body surface temperature the lower the heart rate. With lower temperatures, the body surface temperature decreased, probably due to skin vasoconstriction to maintain internal body temperature and reducing the heart rate. In the afternoon, increased heart rate may be to be due to increases in peripheral vasodilation and heat loss. With the increase in heart rate, vasodilation can also increase to maintain blood pressure and adequate blood supply to other tissues as well as the vasoconstriction of the gut tissue to support vasodilation during heat stress. Also in the afternoon, rectal temperature and respiratory rate were closely grouped probably as a response to higher environment temperatures recorded in this period. With an increase in the rectal temperature, the animal increases its respiratory rate trying to reduce the former (Al-Haidary et al., 2001).

The clustering of Indubrasil and Nelore breeds in afternoon showed a similarity between these breeds in terms of physiological parameters and surface temperature. Although there are physical differences between these two groups, the genetic similarity may have contributed in the clustering of the physiological response to thermal challenges. The Gir was not clustered with any other group related to surface temperature due to the inherent physical characteristics such as high brightness and low thickness skin and smaller height and weight. The Girolando was separated from the other groups due to hematological parameters. This difference was related to the different stress level and the greater genetic difference. There was reclustering between period of the day demonstrating that heat stress affected the physiological responses depending on breed.

The physiological response differences among breeds can also be seen by the percentage of samples that were above the reference values for rectal temperature and respiratory rate. Only 16.7% of Girolando animals had, in the afternoon, rectal temperature above of the reference values and maintained the same percentage of respiratory rate above of normal values (8.3%) in both morning and afternoon showing that this group maintained its physiological patterns despite the heat challenge. The Gir had the highest percentage of temperature above the normal range in both morning and afternoon and the second largest respiratory rate. This difference in responses between these groups was related to the physical characteristics and to the heat loss efficiency. Although no animal had the respiratory rate above of the reference values in the afternoon, 54.2% of rectal temperature collection of Indubrasil animals were above the reference values. This high percentage may be related to inefficiency in heat loss through the skin because of its thickness.

The odds ratio test showed that the Gir breed was three times more likely to have the highest rectal temperature compared with Sindhi as confirmed by the logistic regression because when the black globe temperature approached 35 °C, the probability of the Gir animals having rectal temperatures above normal was approximately 70%.

5. Conclusions

Physical characteristics such as height, weight, thoracic circumference, skin thickness and brightness were important in assessing the physiological response when animals were heat challenged, but these characteristics alone are not responsible for conferring tolerance to heat, which also included a set of morphological characteristics and physiological adjustments. Gir animals were the least adapted to the climatic conditions of the experiment and Girolando and Sindhi breeds showed the best physiological indices when heat challenged. Thermography showed a high correlation with the physiological indexes, especially with the rectal temperature and surface temperatures. Axilla, neck and eye were the main points, however, the adaptations in the method for eye surface temperature registration can improve the accuracy of this point.

Conflict of interest

The authors declare that they have no conflict of interest.

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