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ORIGINAL RESEARCH

Use of multivariate analyses for determining heat tolerance in Brazilian cattle

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Abstract Adaptability can be evaluated by the ability of an animal to adjust to environmental conditions and is especially important in extreme weather conditions such as that found in tropical Brazil. A multivariate analysis using physical and physiological traits in exotic (Nellore and Holstein) and naturalized (Junqueira, Curraleira, Mocho Nacional, Crioula Lageana, and Pantaneira) cattle breeds was carried out in the Federal District of Brazil to test and determine which traits are important in the adaptation of animal to heat stress as well as the ability of these traits and statistical techniques to separate the breeds studied. Both physical and physiological traits were measured on three occasions and included body measurements, skin and hair thickness, hair number and length, pigmentation, sweat gland area as well as heart and breathing rates, rectal temperature, sweating rate, and blood parameters. The data underwent multivariate statistical analyses, including cluster, discriminate, and canonical procedures. The tree diagram showed clear distances between the groups studied, and canonical analysis was able to separate individuals in groups. Coat traits explained little variation in physiological parameters. The traits which had higher discriminatory power included packed cell volume, shoulder height, mean corpuscular volume, body length, and heart girth. Morphological and physiological traits were able to discriminate between the breeds tested, with blood and size traits being the most important. More than 80% of animals of all breeds were correctly classified in their genetic group.

Keywords Adaptation · *Bovidae* · Naturalized breeds · Temperature · Thermolysis

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Introduction

Animal production systems have been undergoing radical transformations in recent years due to the need to increase productivity and incorporate new technologies for the improvement of the efficiency, profit, animal welfare, and product quality. This trend involves not only animal breeding and nutrition but also animal comfort based on its relationship with the environment as well as costs and facilities for the farmer in terms of animal management, installation cleaning, and labor usage (Perissinotto et al. 2007).

In recent years, many farmers in tropical regions have opted for specialized breeds from temperate countries which have poorly adapted to the natural environment. The exposure to higher temperatures, feed alterations, and humidity changes caused these genetically more productive animals to undergo behavioral, endocrine, and physiological changes, which could affect their production. These animals are more demanding in terms of management and nutrition as well as environmental factors (Pereira 2005). On the other hand, some naturalized Brazilian breeds are at risk of extinction, which may cause irreparable loss as little is known about these animals in terms of production and adaptation. Local adapted breeds could be important for a national production scheme because they may contain alleles which confer resistance to diseases or survival in harsh conditions (Woolliams et al. 1986).

The climate in a certain locality or region, in particular air temperature and relative humidity, directly influence the production potential of the animals. Heat stress is one of the main factors involved in the reduction of productivity and animal development, as the lack of thermal comfort means that the animal looks for ways of losing heat. This involves a series of adaptations and for cattle production in hot regions, and respiratory, circulatory, excretory, nervous, endocrine, and enzymatic traits have been shown to be important in determining the tolerance of each breed to their environment (Perez 2000; McManus et al. 2009). The coordination of all these systems to maintain the productive potential under thermal stress is variable between species, breeds, and individuals within a breed (Marai and Haeeb 2009).

As many characteristics are involved in heat tolerance and their measurement is often time consuming and expensive, especially in developing countries where laboratory facilities and a trained on-farm labor force are lacking, there is a need to evaluate the usefulness of these traits in determining differences between breeds and animals. The aim of this study was to associate physical traits with physiological responses and determine which physical or physiological traits linked to heat tolerance were capable of separating breeds and individuals through multivariate analyses of exotic and naturalized cattle in Brazil.

Material and methods

This study was carried out in EMBRAPA Sucupira Experimental Research Farm in Brasilia DF. Animals in this study are part of the Brazilian Program for Conservation of Animal Genetic Resources and came from research and commercial herds around the country. Animals were chosen based on their breed and genetic characteristics using markers described in Egito et al. (2007).

Physiological and physical information collected on seven breeds of cattle (five naturalized: Curraleira, Pantaneira, Junqueira, Mocho Nacional, and Crioula Lageana and two commercial: Holstein and Nellore) was examined in this study (Table 1), with ten animals per genetic group. Each animal was measured and blood and physiological traits determined on six occasions (McManus et al. 2009; Bianchini et al. 2006).

Body measurements included shoulder height (SH), heart girth (HG), body length (BL), and cannon bone girth (CBC) using a metric tape and hipometer. Skin thickness was taken with an adipometer while coat thickness, and hair length (HL) was measured with a paquimeter (Udo 1978). Hairs were collected for counting (number of hair, NH) using adapted pliers according to Lee (1953). Samples were collected in light and dark areas in speckled animals (Crioulo Lageano and Holstein). Pigmentation in the skin (skin reflectance, SR) and coat (coat reflectance, CR) was measured using a reflectometer (Silva 1998). The skin was biopsied (Carter and Dowling 1954) and samples stained with hematoxilin and eosin to measure secretor gland size (area).

Physiological traits included rectal temperature (RT), respiratory rate (RR), heart rate (HR), packed cell volume (PCV), total plasma protein, leukocytes (LEUK), red blood cells (HEM), hemoglobin concentration (HB), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC). These data were collected when the animals were under heat stress after 2 h of exposure to the sun (mean temperature, 27°C and mean humidity, 15%).

After standardization, multivariate analyses were carried out using the Statistical Analysis System, SAS® (SAS 1999), in accordance with Sneath and Sokal (1973), 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.

Table 1 Experimental herd used to test heat stress in cattle breeds in Brazil

Breeds	Purpose	Number of animals	
		Morphological	Physiological
Curraleira	Meat	15	19
Mocho Nacional	Meat	07	05
Crioulo Lageano	Meat/traction	17	12
Pantaneira	Meat	14	10
Junqueira	Meat/traction	11	06
Nellore	Meat	15	10
Holstein	Dairy	11	08
Total		90	70

These procedures included correlations between physical and physiological traits, principal component analysis to attempt to understand the sources of variation in the data, organize information about variables so that relatively homogenenous groups or "clusters," were formed, and see distances between these groups, use the characteristics to predict the group to which a given unit belongs, select a subset of the quantitative variables for use in discriminating among the groups, and summarize between-class variation in much the same way that principal components summarize total variation.

Results

In general, all measured characters had a lower to medium coefficient of variation and could be used appropriately on the analysis (Table 2). While air temperature caused an increase in RT and RR and a decrease on PCV and MCV, humidity acted in the opposite direction (Table 3). Radiation had little effect on physiological traits, possibly because it did not vary much. Larger animals had lower PCV, HB, and HEM but also lower HR and little effect on RR and RT. These relationships can be seen graphically using principal components where the first two components explained 55% of the total variation (Fig. 1).

Table 2 Means, CV, and SD for morphological and physiological traits in cattle breeds in Brazil

Traits	Mean	SD	CV
BL (cm)	140.76	10.97	7.79
CBC (cm)	20.40	2.12	10.41
SH (cm)	128.89	9.65	7.48
HG (cm)	181.22	12.93	7.14
HL (cm)	4.28	3.79	88.42
NH (number per cm^2)	659.18	394.30	59.82
RT (°C)	38.73	0.93	2.40
RR (respirations per minute)	31.08	9.58	30.85
HR (beats per minute)	67.44	13.08	19.40
PCV (%)	33.06	5.52	16.69
MCV (fl)	49.74	5.91	11.89
TPP (g/100 mL)	7.32	0.57	7.85
HEM ($\times 10^6$/mm^3)	6.62	0.99	15.00
HB (g/100 mL)	12.58	2.09	16.68
MCHC (%)	37.88	3.39	8.97
ST (mm)	0.94	0.36	38.90
CT (mm)	0.89	0.47	52.53
SR (Å)	17.15	5.63	32.81
CR (Å)	17.55	5.34	30.41
Area (%)	29.92	7.14	23.87

CV coefficients of variation, *SD* standard deviations, *TPP* total plasma protein, *ST* skin thickness, *CT* coat thickness, *SR* skin reflectance, *Area* sweat gland area

In the tree diagram (Fig. 2) obtained from the analysis of the distances between breeds, three distinct groups were seen, one with Holstein animals, the other with Mocho Nacional, and the third with the rest of the breeds in the study, including Crioulo Lageano, Junqueira, Pantaneira, Curraleira, and Nellore.

With the canonical analysis of the seven breeds studied, the naturalized breeds are grouped together (Fig. 3). The first two canonical variables explained 84.7% of the total variation with the first explaining 67.6%. The estimated variance of canonical variables within breed showed that Crioulo Lageano, Holstein, and Pantaneira had highest variance while Mocho Nacional and Curraleira varied least (not shown). The canonical correlation showed that the most important physical traits affecting physiological characteristics were cannon bone circumference, shoulder height, and coat reflectance followed by heart girth and body length.

Table 4 shows a comparison of traits used in the separation of breed pairs. Those with R^2 greater than 10% are in bold type. HG, SH, and BL were important in all comparisons with Nellore cattle while for Crioula Lageano BL and SH were important. Holstein animals differed due to CR, SR, and Area. In 19 of the 21 comparisons, SH appeared as a discriminatory variable followed by HG (18), CBC and HL (15), NH (14), and BL which appeared in 13 of the 21 breed comparisons. For blood traits, HB and HEM were significant in 12 and ten comparisons, respectively. Skin and coat thickness were not generally important.

Standardized canonical means for morphological and physiological traits show the traits which had higher discriminatory power included PCV, SH, MCV, BL, and HG (Fig. 4). In general, the size traits had a higher discriminatory value than the physical traits of the coat and skin, with SH (R^2=64%), HG (59%), cannon bone circumference (38%), number of hairs (28%), hair length (26%), BL (23%), LEUK (23%), and HB (18%) being the most important. Other traits showed an R^2 lower than 10%.

The morphological and physiological traits were employed to classify the analyzed animal to your own breed (Table 5). In general, more than 80% of the animals were correctly classified in their genetic group with Nellore 100%, followed by Curraleira in more than 92% of the cases, and Holstein and Pantaneira in more than 90%. Crioula Lageano were classified as Curraleira (15%) and Mocho Nacional as Junqueira (14%).

Discussion

Several authors have used multivariate analyses to calculate distances between cattle breeds. Leotta (2004) used

Table 3 Correlations between physical and physiological parameters in cattle

	RT	RR	HR	PCV	MCV	TPP	HEM	HB	MCHC
Air temperature	0.49	0.23	0.11	−0.32	−0.30	0.10	−0.13	−0.17	0.28
Humidity	−0.36	−0.15	0.04	0.29	0.25	−0.17	0.13	0.14	−0.28
Radiation	−0.02	0.01	0.02	−0.04	0.11	0.20	−0.08	−0.03	0.01
BL	−0.19	0.00	−0.27	−0.12	0.00	0.09	−0.14	−0.16	−0.11
CBC	0.01	0.06	−0.21	−0.43	−0.31	−0.10	−0.22	−0.50	−0.08
SH	−0.06	−0.09	−0.29	−0.30	−0.21	−0.13	−0.12	−0.37	−0.11
HG	−0.03	0.13	−0.14	−0.09	0.06	0.13	−0.13	−0.09	−0.02
NH	−0.03	0.09	−0.02	−0.07	0.12	0.06	−0.19	−0.08	0.01
HL	0.00	0.06	−0.04	0.04	−0.24	−0.06	0.24	0.08	−0.02
ST	−0.04	0.02	0.02	0.17	−0.02	0.02	0.16	0.20	0.00
CT	0.07	0.16	−0.06	0.08	0.03	0.03	0.10	0.12	0.03
SR	−0.07	−0.13	−0.12	−0.14	0.01	0.06	−0.14	−0.11	0.02
CR	−0.10	0.02	−0.19	−0.22	−0.21	−0.04	−0.05	−0.19	−0.02
Area	−0.04	−0.01	−0.01	0.04	0.05	0.09	0.08	0.08	0.07

ST skin thickness, *CT* coat thickness, *SR* skin reflectance, *Area* sweat gland area, *TPP* total plasma protein

multivariate analyses to compare milk traits in three cattle breeds in Italy while Insausti et al. (2008) used them to differentiate meat quality traits in native breeds in Spain. In the present study, morphological and physiological characteristics related to heat tolerance were used to test their ability in placing animals in their genetic groups and to determine which traits were most appropriate in differencing between animals and groups.

While air temperature and humidity showed medium correlations with most physiological traits as expected, correlations between physical and physiological traits were generally low. Some size traits such as BL and CBC showed medium correlations with HR, HB, MCV, and PCV while hair and coat traits generally showed low and non-significant correlations with physiological traits. Highest (negative) correlations were generally found with coat reflectance, showing that coat color is the first important means of defense of the animal against radiation.

The first principal component (Fig. 1) showed that higher air temperature and radiation led to higher RT, RR and lower PCV and HB, as expected. For this first component also, larger animals had higher RR and RT. Size has been shown to be an important factor in regulating heat with smaller animals having a relatively larger surface area to facilitate heat loss. The second principal component separated a subgroup of large animals with lower physiological parameters. This is possibly due to the presence of Crioula Lageano and Junqueira breeds which are relatively large (Bianchini et al. 2006) but have high heat tolerance (McManus et al. 2009). A redundancy canonical correlation analysis (not shown) showed that shoulder height and cannon bone circumference were the most useful of the

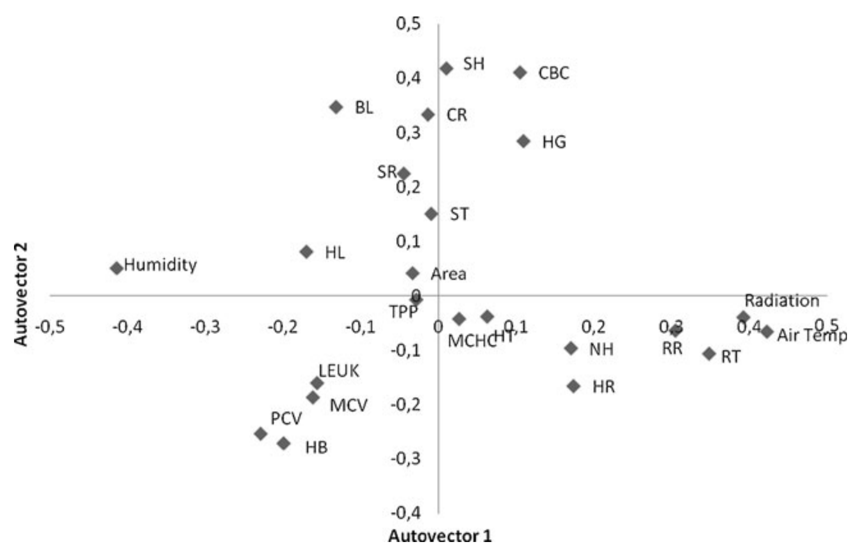

Fig. 1 Graphical representation of the first two principal components for physiological and physical traits in Brazilian cattle. (*SH*) shoulder height, (*RR*) respiratory rate, (*TPP*) total plasma protein, (*HT*) hair thickness, (*CR*) coat reflectance, (*SR*) skin reflectance, (*ST*) skin thickness

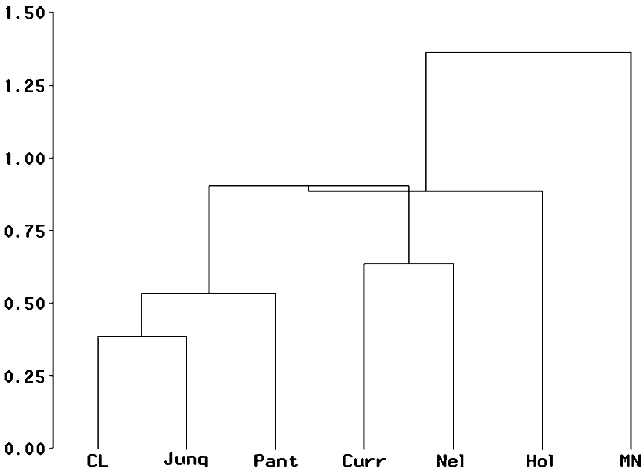

Fig. 2 Tree diagram of distances between breeds of cattle in Brazil. (*CL*) Crioula Lageano, (*Junq*) Junqueira, (*Pant*) Pantaneira, (*Curr*) Curraleira, (*Nel*) Nellore, (*Hol*) Holstein, (*MN*) Mocho Nacional

physical traits in explaining physiological reactions to heat. Coat traits explained little variation in physiological parameters, except for CR.

The Holstein and Mocho Nacional breeds were closest using the distance analysis (Fig. 2) which is in agreement with McManus et al. (2009). McManus et al. (2005), studying physical traits affecting heat adaptation in cattle, also found that the Mocho Nacional and Holstein were separated from other breeds analyzed. The Mocho Nacional is the largest naturalized breed in this study. Serrano et al. (2004) and Egito et al. (2007), looking at genetic diversity and population structure in naturalized Brazilian breeds, found that the Mocho National was genetically diluted with other breeds. According to Santiago (1984), this breed arose from the crossing of Creole animals with bulls from English breeds such as Red Polled and Lincoln Red, imported at the beginning of the twentieth century, creating this larger polled breed. In the early 1980s, this breed was almost extinct but has been useful in cross-breeding as it is the only naturalized polled (mocho) breed. The separation of the Holstein breed was expected as they are from a highly selected milk breed adapted to cooler climates.

The third group is divided in two, one with Crioulo Lageano and Junqueira, along with the Pantaneira and the other with Curraleira and Nellore. Crioulo Lageano and Junqueira were probably closer due to their size, as the breed was formed in regions of higher quality feed. Spritze et al. (1999) state that the Crioulo Lageano was formed in the region of Campos da Serra in Rio Grande do Sul State and Santa Catarina Highlands while Cotrim (1913) showed that the Junqueira found in Minas Gerais State is a product of crossing Caracu breed with other national breeds. The breed was formed in the Jequetinhonha Valley, with lower natural challenges than some other breeds present here. The Pantaneira, according to Mazza et al. (1994), became adapted to the hot and humid Pantanal regions in Center West Brazil over 300 years and used immersion in water to cool its body temperature, different from the conditions where the experiment took place which was in the dry center-west of the country.

The Curraleira and Nellore breeds are better adapted to high environmental temperatures according to Bianchini et al. (2006). Azevêdo et al. (2008) found that Curraleira animals maintained rectal temperatures and respiratory rates within normal limits independent of age, sex, time of day or year indicating good adaptation to hot semiarid conditions. The Nellore was grouped with the naturalized cattle, in particular the Curraleira, because of their adaptive characteristics. This breed is of tropical origin (India) with good heat tolerance.

Fig. 3 Canonical representation of individuals within cattle breeds

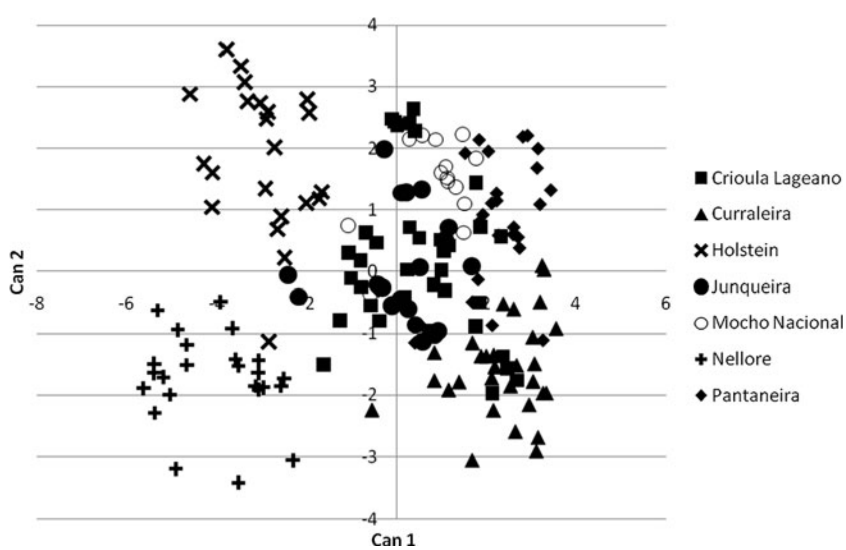

Table 4 Value of variables in discrimination between breeds from gradual discriminant analysis in Brazilian cattle

	Nellore	Crioula Lageano	Mocho Nacional	Junqueira	Curraleira	Holstein
Pantaneira	**HG, CBC, SH, BL, RT, Area, SR,** NH, HB, HEM	**HB, HG, CBC**, **Area, SR,**SH, HL, RT	**CBC, HL, NH, SH, HB, HEM**, **Area, SR, CR,** RT	**CBC, HG, SH, HL, NH**, TPP, MCHC, BL, HR	**SH, HG**, RR, HL, HB, HEM, NH	**CBC, HG, HB, HL, HEM, Area, SR,** MCV, RT, NH
Nellore		**HG, SH, BL, NH, CBC**, **Area, SR,** HEM, HL, HB	**HL, NH, HG, SH, Area, SR, CR,** BL, HR, CBC	**HL, BL, HEM, HG, Area, SR,** MCHC, CBC, SH, HR	**SH, Area, SR,** CBC, NH, BL, RR, HL, HG, TPP, HB	**HG, BL, SH, HB**, **Area, SR,** RT
Crioula Lageano			**HL, NH, HB, HG, CBC, BL, CR,** RR, SH, RT	**HL, NH, SH, HB**, HG, HR, RR, BL, TPP	**SH, BL, Area, SR,** RR, TPP, HEM, MCHC	**MCV, MCHC, SH, NH, Area, SR,** BL, HG, CBC,TPP
Mocho Nacional				**RR, HG, SH, TPP, CR,** HL, MCV, MCHC	**HG, SH, NH, RR, CR,** HL, TPP	**HB, CBC, HG, SH, BL, Area, SR, CR,** HEM, HR
Junqueira					**SH, HG, HL, BL, CBC, MCV, MCHC**, TPP, NH, HR, RT	**MCV, MCHC, CBC, HL, Area, SR,** NH, HEM
Curraleira						**SH, MCV, HB, MCHC, HG, CBC, Area, SR,** HEM, HR

Data in bold are the most significant variables in discrimination between breeds

TPP total plasma protein, *SR* skin reflectance, *Area* sweat gland area

The Nellore and Holstein breeds appear separated from each other and the rest of the animals in the canonical analysis. This is probably due to their history as Nellore is a *Bos taurus indicus* breed and Holstein *Bos taurus taurus* adapted to cooler climates (Torres et al. 1982). Santiago (1984) stated that the first Nellore were imported to Brazil in 1826 today make up 85% of the natural bovine herd of over 200 million cattle mainly due to their ability to adapt to diverse climates found in the country.

The canonical analysis (Fig. 4) is in agreement with Müller (1989) who showed that, in general, larger animals had greater trouble in dissipating heat to the environment. Maia et al. (2003), evaluating coat traits in Holsteins in a tropical environment, observed that skin thickness, hair length, diameter, and number are modifiers of environmental factors and vary with coat color. These authors found that thinner skin with fewer and shorter hairs had greater inclination angle and diameter in black hairs compared to white hairs. These traits facilitate convective thermolysis and skin evaporation thereby are more advantageous in terms of adaptation to tropical regions. Silva (1999) also stated the importance of coat length in cattle. Better adapted animals for rearing in open pasture in tropical regions should have short pale colored hairs with a well-pigmented skin to protect against ultraviolet radiation.

Silva et al. (2003) found that light-colored hair coats have greater reflectance values than dark-colored hair coats for the wavelengths from 300 to 850 nm and that Nelore cattle, which have a mixture of white and dark hairs, had a higher reflectance than white cattle for the wavelengths from 300 to 600 nm. The same authors also showed that gray coats reflect better than red coats. In the present study, Curraleira, Crioula Lageana, and Pantaneira had a significant proportion of red in their coats. Mariante and Cavalcante (2006) stated that most naturalized breeds in Brazil descended from bay-reddish Portuguese breeds: Barrosã, Mirandesa, Minhota, Alentejana, and Moura. Primo (1993) showed that the Curraleira, Crioulo Lageano, and Pantaneira breeds probably had a common ancestor (*Bos taurus ibericus*) while Caracu, Junqueira, and Mocho Nacional probably descended from *Bos taurus aquitanicus*.

Skin color is also important for heat tolerance in cattle, as non-pigmented skin has a reflectance level that is lower than light gray skin, especially for the wavelengths from 300 to 600 nm. Red, brown, black, and gray skin have similar reflectance levels in this range, but above 600 nm the values increase for red/brown skin but remain constant for darker skin (Silva et al. 2003). As little difference was found here between skin and coat traits for naturalized breeds, these were not good discriminatory variables.

Errors in classifying animals within their correct breed group can be explained by breed history and traits measured. Crioula Lageano may have been classified as

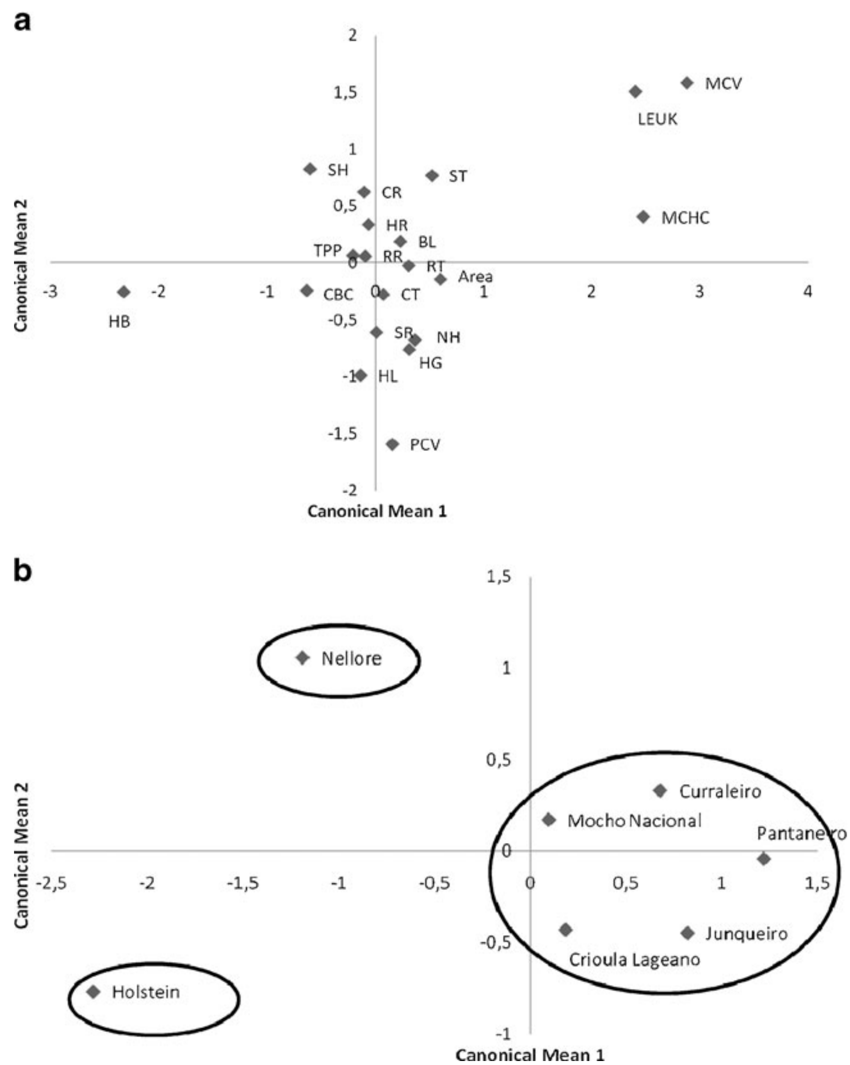

Fig. 4 Standardized canonical means for morphological and physiological traits (**a**) and breeds (**b**) in Brazil. (*BL*) body length, (*HG*) heart girth, (*CBC*) Cannon bone circumference, (*SH*) shoulder height, (*NH*) number of hairs, (*HL*) hair length, (*RT*) rectal temperature, (*RR*) respiratory rate, (*HR*) heart rate, (*PCV*) packed cell volume, (*TPP*) total plasma protein, (*LEUK*) leukocytes, (*HB*) hemoglobin, (*MCV*) mean corpuscular volume, (*MCHC*) mean corpuscular hemoglobin concentration, (*ST*) skin thickness, (*CR*) coat reflectance, (*CT*) coat thickness, (*MN*) Mocho Nacional, (*CL*) Crioula Lageano

Curraleira by the fact that these breeds have similar coats (brownish red), which may have influenced their heat tolerance characteristics while errors in classifying Mocho Nacional as Junqueira may be due to the fact these breeds originated in the same region of the country (central west–southwest) thereby facilitating crosses between these breeds in the past and the fact that both are large naturalized breeds (Bianchini et al. 2006).

Table 5 Percentage of animals classified in each breed of cattle

From breed	Crioula Lageano	Curraleira	Holstein	Junqueira	Mocho Nacional	Nellore	Pantaneira
Crioula Lageano	74.67	14.67	1.33	5.33	2.67	1.33	0.00
Curraleira	1.54	92.31	0.00	0.00	1.54	0.00	4.62
Holstein	0.00	0.00	90.20	3.92	1.96	3.92	0.00
Junqueira	8.51	0.00	4.26	85.11	2.13	0.00	0.00
Mocho Nacional	0.00	0.00	0.00	13.79	82.76	0.00	3.45
Nellore	0.00	0.00	0.00	0.00	0.00	100.00	0.00
Pantaneira	1.82	0.00	0.00	5.45	1.82	0.00	90.91
Error	0.25	0.07	0.09	0.14	0.17	0.00	0.09
Prior	0.14	0.14	0.14	0.14	0.14	0.14	0.14

Conclusion

Morphological and physiological traits associated with heat resistance were able to discriminate between the breeds tested, with blood and size traits being the most important.

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