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Multivariate analysis for characteristics of heat tolerance in horses in Brazil

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Abstract The environment in which the horse is reared affects its ability to maintain thermal balance which is in turn related to thermal characteristics and regulatory physiological mechanisms. In this study a multivariate analysis of physiological traits in relation to heat tolerance in horses was carried out in the Federal District, Brazil. The aim was to test the ability of these analyses to separate groups of animals and determine which physiological traits are most important in the adaptation to heat stress. Forty adult horses (4 to 13 years) were used, ten from each of four different genetic groups (English thoroughbred, Brazilian show-jumper, crossbred and Breton). The traits examined included heart and breathing rate, rectal temperature as well as blood parameters. The data underwent multivariate statistical analysis including cluster, discriminate and canonical using Statistical Analysis System - SAS ® procedures CLUSTER, STEPDISC, CANCORR and DISCRIM. The tree diagram showed clear distances between groups studied and canonical analysis was able to separate individuals in groups. The discriminate analysis identified the variables which were most important in separating these groups. The multivariate analysis was able to separate the animals into groups with RR, HR and RT being important in this separation.

Keywords Adaptation · Equine · Statistical methods · Temperature · Thermolysis

Introduction

Since the Portuguese colonization, the Brazilian army has used horses for both traction and riding, as seen

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with armed forces in other countries. The Brazilian army uses horses for patrolling the frontier and teaching camps spread over the country, for military ceremonies, show jumping competitions, polo and dressage. The production of horses for such a wide range of activities and territory is a challenge (Campos et al. 2007).

Environmental adaptation is necessary to achieve equilibrium between production, storage and dissipation of heat. The adaptability of horses to new environments has been evaluated by the ability of the animal to adjust to mean environmental conditions (Paludo et al. 2002).

Thermoregulation in domestic animals occurs due to physical and physiological adaptation of the animal to the environment. Thermal stress is caused by environmental factors such as air temperature, radiation, humidity and wind speed, altering physiological variables so that the animal can adjust its body temperature. Thermoregulatory alterations are controlled by the hypothalamic centers of the central nervous system (Müller 1989). The thermal comfort zone is defined as environmental temperature in which the metabolic rate is constant and ranges from 5°C to 25°C (Morgan 1996). Endothermic animals such as horses regulate their rectal body temperature between 37.5°C and 38.5°C (Cunnigham 2004).

The capacity of an animal to resist caloric stress has been evaluated physiologically by alterations in rectal temperature and respiratory rate (Pereira 2005; Mota 1997). An increase in rectal temperature shows that heat releasing mechanisms are insufficient to maintain homeothermia. As body temperature increases, metabolic processes are more and more affected until the temperature reaches 42°C, when death may occur. The difference in rectal temperature between "normal" and "fatal" is only 3°C. According to Cheung and McLellan (1998), animals adapted to hot temperatures have a higher sweating rate, normal heart rate and lower core and skin temperatures indicating less stress suffered by these animals.

While studies have been carried out with production animals such as cattle, goats and sheep (Silanikove 2000), little information is available on horses in tropical climates where exercise can apply additional stress on thermoregulatory mechanisms. Regulatory mechanisms in horses are seen to be different from other animals (Geor et al. 2000) and greater thermoregulatory limitations to exercise imposed on horses are accentuated in environmental conditions of high temperature and relative humidity. The same authors state that physical limitations are primarily imposed by the extensive muscle mass, high mass-specific rate of metabolic heat production, and the low mass-specific surface area for heat dissipation. Dissipation of two-thirds of this heat is achieved via sweating and sweat losses of up to 10 L/hr have been reported (Hodgson et al. 1994) despite the small surface area relative to body mass when compared to other species.

Many traits can be measured to determine adaptability, so to aid in the interpretation of these a multivariate analysis was carried out looking at physiological traits which affect heat tolerance in horses in the Federal District, Brazil, to determine if it is possible to separate the animals into their genetic groups and which traits are important in this separation.

Material and methods

Forty adult horses were used (4 to 13 years old) from the 1st Mounted Guard Regiment and 32nd Gun Campaign of the Ministry of Defense in Brasília, DF, with 10 animals from each of the following groups: English Thoroughbred (ETB), Brazilian Showjumper (BSJ), Breton (B) and crossbred BSJ with ETB. Data collection is described in Paludo et al. (2002) and was repeated on three separate days, but only the data collected when the animals were under stress were used here.

Blood was collected by caudal venopuncture using vacutainer with EDTA when the physiological traits were measured. The number of erythrocytes ($\times 10^6/mm^3$), leukocytes ($x\ 10^3/mm^3$) and the concentration of hemoglobin (g/100 mL) were carried out in an automatic cell counter (BL550, Cellm™). The hematimetric parameters (Mean Corpuscular Volume (fl) – MCV and Mean Corpuscular Hemoglobin Concentration (%) – MCHC) were determined by calculation. Packed red cell volume - PCV (%) was obtained using a micro centrifuge and Total plasma proteins – TPP (g/100 mL) using a refractometer.

After standardization, multivariate analyses were carried out using CLUSTER, TREE, DISCRIM, STEPDISC and CANCORR procedures of *Statistical Analysis System* - SAS ® (SAS 1999), according to Sneath and Sokal (1973), to place animals in groups in accordance with their degree of similarity and

Table 1 Means, variation coefficients (CV) and standard deviations (SD) of heat tolerance traits in horses in the Federal District, Brazil

Trait		Mean	SD	CV
Weight (kg)	Wt	427.15	64.56	15.11
Heart Rate (beats/minute)	HR	40.81	9.10	22.30
Respiratory Rate (breaths/minute)	RR	23.03	11.79	51.19
Rectal temperature (ºC)	RT	37.86	0.43	1.15
Leukocytes (x10 ^{3}/mm ^{3})	LEUC	7.33	1.77	24.15
Erythrocytes (x10^6/mm ^{3})	HEM	8.13	1.52	18.67
Haemoglobin (mg/100 mL)	HB	12.87	2.02	15.67
Packed red cell volume (%)	PCV	36.58	6.46	17.66
Mean Corpuscular Volume (fl)	MCV	45.56	7.52	16.50
Mean Corpuscular Haemoglobin	MCH	16.02	2.11	13.17
Mean Corpuscular Hemoglobin Concentration %	MCHC	38.70	43.56	112.53
Total plasma protein (g/100 mL)	TPP	6.59	0.75	11.44

verify discriminatory capacity of the original traits in the formation of these groups.

Results and discussion

Table 1 shows means, variation coefficients and standard deviations of the parameters studied. These data are in general agreement with other studies in Brazil (Lacerda et al. 2006). These traits are interrelated. According to Feldman et al. (2000), MCV represents the mean size or volume of the red blood cells and HCM is the hemoglobin content per red cell and a reflection of the hemoglobin mass. PCV measures the percentage of red cells present in the blood and higher PCV leads to a lower MCV.

Figure 1 shows the tree diagram of distances between breeds with two distinct groups, one with the Breton animals and the other with the other breeds. The Breton is considered a “cold blooded” horse (Kingsley and Lerner 1998), grouped with traction animals, much larger and heavier than the other breeds and a more lethargic temperament. Erickson and Poole (2006) noted that horses trained for racing have more red blood cells per unit volume of blood than traction animals, in agreement with data used here. According to Morris (1998) and Tyler et al. (1987), hot blooded breeds are characterized by higher metabolic requirements, and consequently higher levels of HEM, PCV and HB, compared to coldblooded horses such as the Breton breed. Levels of HEM, hematocrit and HB can be important when better physical condition is necessary, such as when the horse is used for sports (Vaz et al. 2000). Paludo et al. (2002) found that the Breton showed higher thermoregulatory capacity than the other breeds studied here and were therefore less stressed. Blood component differences between BSJ and Breton mares during pregnancy were found by Orozco et al. (2007).

The crossbred animals were also separated from the two purebreds as they were lighter than the other breeds and close to ETB in terms of RT, HR and RR, being characterized as least adapted to the environment studied. The BSJ and ETB were closest as they were of approximately the same weight (403 kg for BSJ and 404 kg for ETB) as well as being selected for sporting activities and being of a more agitated temperament (Torres et al. 1982).

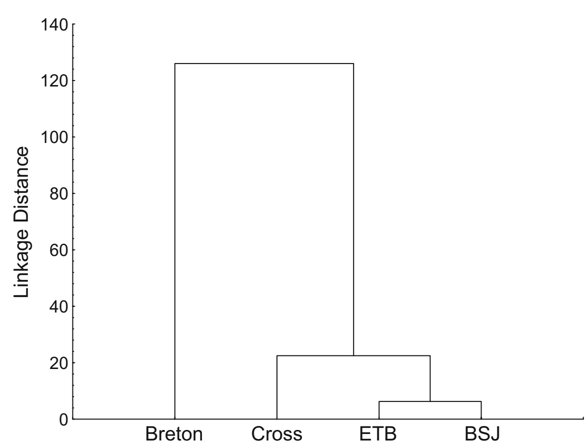

Fig. 1 Tree diagram of distances between breeds of horses in Brazil. BSJ – Brazilian Showjumper, ETB – English Thoroughbred

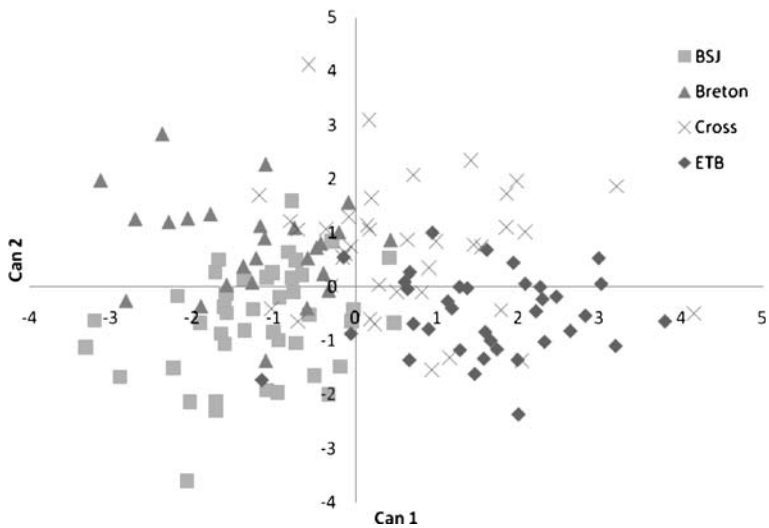

Fig. 2 Canonical analysis of individuals within horse breeds, BSJ – Brazilian Showjumper, ETB – English Thoroughbred

The discriminant canonical analysis is shown in Fig. 2. The BSJ and ETB showed the closest distance between animals with little variation for the traits evaluated. The BSJ was formed using many breeds (Dias et al. 2000), but of the known breeds the ETB is the main contributor, especially in the initial years of breed formation (1977–1988). The ETB originated from a cross between Arab and the extinct Berbere breed with native English mares (Torres et al. 1982), this being the most popular breed for production of half-breeds for military and sporting purposes. Various authors (Zechner et al. 2001; Pinto et al. 2005; Santos 2006) have used multivariate techniques to analyze distances between breeds using morphological traits in horses. Prado and Alberdi (1994) used these techniques for paleontology studies of horses in America.

The Breton and crossbreds were more disperse with overlaying of the crosses on all other breeds. The Breton was more difficult to handle and make and maintain a gallop during the experiment. This breed was formed in 1830 in France, Bretanha region (Torres et al. 1982), with the animals used taken from army farms and some non-registered crossbreeding may have occurred which may account for they overlay which occurred here.

Table 2 shows the variation of the canonical variables measured. The first variable explained 73.87% of the variation and the two presented explained 95.02%. Total variation showed that the crossbreds showed highest variation followed by BSJ and Breton. The ETB showed the lowest variation.

Figure 3 shows mean canonical variables for the breeds studied, with all breeds well separated. Figure 4 shows the canonical variables for the traits studied. MCHC did not show any discriminatory capacity, maybe due to the fact it is used to classify anemia and no animals were anemic (Feldman et al. 2000). On the other hand, the red blood series may be used to differentiate between breeds. This may be due to spleen contraction during exercise and exposure to heat. This is in agreement with Garcia-Navarro and Pachaly (1994), Brandi (2004) and Erickson and Poole (2006) who state that an increase in hemoglobin is a result of physiological adaptations. Erickson and Poole (2006) also state that in animals adapted to exercise the spleen is larger and therefore ETB and BSJ have more red cells in the blood.

RT and RR were more important that HR in explaining differences in blood parameters in the horses using a canonical correlation redundancy analysis. In a simulation study, Nardone and Valentini (2000) concluded that selection based on RT of a high-yielding cattle breed was the more efficient as heat tolerance could be improved in a few gener-

Table 2 Variance of canonical variables per breed

Genetic group	Can 1	Can2	Total
English Thoroughbred (ETB)	1.01	0.75	1.76
Brazilian Showjumper (BSJ)	0.85	1.02	1.87
Breton	0.92	0.90	1.82
Cross	1.16	1.21	2.37

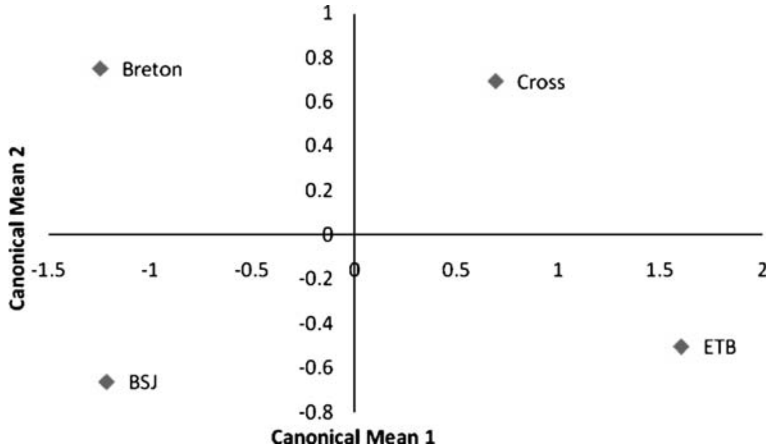

Fig. 3 Canonical means per breed from morphological and physiological traits in Brazilian horse breeds. BSJ – Brazilian Show-jumper, ETB – English Thoroughbred

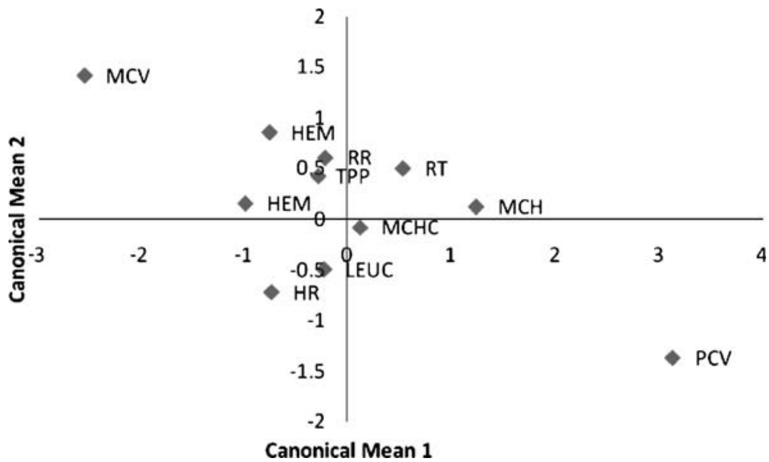

Fig. 4 Standardized canonical means for morphological and physiological traits in horse breeds in Brazil (RT – rectal temperature, RR – respiratory rate, HR – heart rate, PCV – packed cell volume, MCV – mean corpuscular volume, HEM – red blood cells, HB - hemoglobin, MCH – Mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, TPP – total plasmatic protein, LEUC - leuckocytes

Table 3 Discriminant analysis for breed comparison in Brazilian horses

Genetic groups	English Thoroughbred	Cross	Breton
Brazilian Showjumper	**HR, RT**, RR, PCV, MCHC	**PCV, HR, RT**, **MCHC**, LEUC	**RT, HR, RR**, HB, HCM
English Thoroughbred		**TPP**, RR, LEUC, HB, HCM	**HB, RR, RT**, TPP, MCHC
Cross			**PCV**, HR

RT – rectal temperature, RR – respiratory rate, HR – heart rate, PCV – packed cell volume, MCV – mean corpuscular volume, HEM - hemacias, HB - hemoglobin, MCHC – mean corpuscular hemoglobin concentration, TPP – total plasmatic protein, LEUC – leuckocytes.

Table 4 Percentage of animals classified in each breed of horse

Breed	Brazilian Showjumper	Breton	English Thoroughbred	Cross
Brazilian Showjumper	76.74	18.60	2.33	2.33
Breton	11.54	73.08	0.00	15.38
English Thoroughbred	5.56	0.00	83.33	11.11
Cross	7.69	12.82	23.08	56.41
Error	0.23	0.27	0.16	0.45
Prior	0.25	0.25	0.25	0.25

ations, whereas a local breed would need several generations (more than 30) to reach comparable milk production levels.

The results of the discriminant analysis between all breeds showed that the traits explaining more than 10% of breed variation were HB, HR, RT and PPT, while Table 3 shows a breed by breed discriminant comparison with variables explaining more than 10% of variation in bold type. HR and RT were significant in all comparisons with BSJ. In four of the six comparisons studied HR, RT and RR were discriminant variables.

In general more than 70% of the animals studied were correctly classified in their breeds (Table 4) with the exception of the crossbreds (56.41%). ETB was less classified in other groups, showing a better breed definition.

Studies comparing heat tolerance in other species (Ravagnolo and Misztal 2002) concluded that genetic variation for heat tolerance was as important as the additive variance to produce milk independently of heat stress when animals were exposed to heat stress. Ability to produce under stressful conditions is therefore an important trait in tropical countries and blood parameters can be useful in separating breeds and animals within breeds.

Conclusion

The multivariate analysis was able to separate the animals into groups with RR, HR and RT being important in this separation.

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