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Heat tolerance in Pantaneiro horses subjected to different exercise regimes

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Abstract – The objective of this work was to determine how different types of gait affect the physiological and thermographic responses of Pantaneiro horses (*Equus ferus caballus*) subjected to field conditions under high environmental temperatures. Ten horses were evaluated in a double 5x5 Latin square experimental design, with five gait types: walk, trot, extended trot, gallop, and extended gallop. The following physiological measures were determined immediately after exercise: heart and respiratory rates, blood lactate content, and rectal temperature. Body surface temperatures were evaluated using an infrared camera. The walk, trot, and extended trot were the most adequate gaits for Pantaneiro horses to maintain thermoregulation within the physiological norms under conditions with a high air temperature. The temperatures obtained in the eye and elbow regions show the best predictive capacity for physiological parameters.

Index terms: *Equus ferus caballus*, animal welfare, body temperature, gait, lactate, thermal balance, thermal stress.

Tolerância ao calor em cavalos Pantaneiros sob diferentes regimes de exercício

Resumo – O objetivo deste trabalho foi determinar como os diferentes tipos de andamento afetam as respostas fisiológicas e termográficas de cavalos Pantaneiro (*Equus ferus caballus*) submetidos a condições ambientais com alta temperatura. Dez cavalos foram avaliados em delineamento experimental de quadrado latino 5x5 duplo, com cinco andamentos: passo, trote, trote estendido, galope e galope estendido. As seguintes medidas fisiológicas foram determinadas logo após os exercícios: taxas cardíaca e respiratória, teor de lactato sanguíneo e temperatura retal. As imagens termográficas foram avaliadas por meio de câmera de infravermelho. O passo, o trote e o trote estendido foram os andamentos mais adequados para os cavalos Pantaneiros manterem a termorregulação dentro das normas fisiológicas em condições de alta temperatura do ar. As temperaturas obtidas nas regiões da área do olho e da axila mostram maior capacidade preditiva para os parâmetros fisiológicos.

Termos para indexação: *Equus ferus caballus*, bem-estar animal, temperatura corporal, andamento, lactato, balanço térmico, estresse térmico.

Introduction

The locomotion process of horses can be described as the type of gait (natural and artificial) and stride characteristics. According to the symmetry of the limb movement, gaits can be distinguished into two types: symmetric (walk, trot, "tölt", and pace) and asymmetric (canter, transverse, and rotary gallop) (Barrey, 2014), with variations of the increasing order of speed as collected, working, medium, and extended (Clayton et al., 2014). Horses generally change gait and select speed within a gait to minimize energy consumption (Toeda et al., 2020). Much information has been published on the biomechanics of locomotion and applications of gait analysis (Barrey, 2014), mainly in laboratory conditions. Still, little information is available about the effect of gait on the physiological responses of horses in extreme environments, such as at high temperatures.

The thermoneutral zone is the range of ambient temperatures where homeothermic animals balance heat loss and production, so the body maintains the core body temperature (Renaudeau et al., 2012). When environmental conditions reach values beyond the limit of those of the thermoneutral zone, thermal stress occurs, prompting behavioral and physiological responses to preserve homeostasis (Bernabucci et al., 2010). Commonly, the capacity of an animal to resist to thermal stress has been evaluated through alterations in the rectal temperature and respiratory rate, and, more recently, thermographic cameras have been used to facilitate this evaluation (McManus et al., 2016b). Studies have shown several regions of interest (ROIs) for studying thermographic responses to exercise in horses (Soroko et al., 2019; Teixeira Neto et al., 2020; Witkowska-Pilaszewicz et al., 2020), with varying results.

The Brazilian Pantanal is the world's largest freshwater wetland, characterized by habitat flooding dynamics (Alho & Sabino, 2012). The primary economic activity in this region is cattle ranching. Horses should be highly adaptable to long treks to accompany the cattle and need spurts of faster movements when animal capture is necessary (Santos et al., 2016a). Horses work 6 to 8 hours per day, on alternate days, and troops are rotated every 15 or 20 days (Juliano et al., 2016), undergoing significant variations of temperature, which requires body temperature homeostasis maintenance (Yarnell et al., 2014). Pantaneiro horses have experienced natural selection in this environment for more than two centuries, with little or no human interference. This breed has, therefore, become well adapted to the environment (Santos et al., 2016b). According to Brownlow et al. (2016), when the heat accumulated during exercise in hot and humid climates exceeds a critical thermal maximum (~42°C), horses can manifest exertional heat illness (EHI). Still, little information is available, especially on native breeds under work conditions (Witkowka-Pilaszewicz et al., 2020).

The objective of this work was to determine how different types of gait affect the physiological and thermographic responses of Pantaneiro horses subjected to field conditions with a high temperature.

Materials and Methods

The experiment took place in the Brazilian Pantanal, at Nhumirim ranch, Nhecolândia subregion, Pantanal, in the municipality of Corumbá, in the state of Mato Grosso do Sul, Brazil (18º59'S, 56º39'W, at 90 m altitude). The climate of the region is Aw (tropical savanna with dry winters), according to the Köppen-Geiger's classification (Souza et al. 2022). Air temperature (Ta) and relative humidity (RH) were collected from an automatic weather station installed at the ranch. A thermal comfort index (TCI) was calculated according to Jones (2009) as: TCI = Ta + RH, where Ta is the air temperature (ºF) and RH is the relative humidity (%).

Ten castrated, 5-to-8-year-old male Pantaneiro horses were used. They were clinically healthy, with similar fitness levels and similar work histories, with mean 397.0 kg (range: 363.0 to 428.0 kg) body weight, and with body condition scores between 3 and 4 (scale from 0 to 5) according to Carroll & Huntington (1988). All horses in the ranch were used for working cattle maintained exclusively on natural pastures.

The horses were randomly assigned to five treatments (exercise type/gaits) in a 5x5 Latin-square design replicated twice. Speed treatments were applied as follows: walk (4 to 6 km h^{-1}); trot (8 to 12 km h^{-1}); extended trot (12 to 15 km h^{-1}); gallop (20 to 25 km h^{-1}); and extended gallop (above 40 km h^{-1}); as well as at rest (control).

The tests were performed in the morning (between 9:00 a.m. and 11:00 a.m.), on a 1,000 m long circular outdoor grass track, where they ran twice (a total of 2,000 m) in four consecutive days. Care was taken to not overload the horses. Each horse had a rest period of at least 24 hours between the exercise tests. The trial period lasted five days, and, in each test, two horses performed at least one type of gait, so that, at the end, each horse performed all gaits twice. Two riders of similar weights (about 70 kg) rode the horses. Riders rode two horses each (n=10), keeping the same gait.

Heart rate (HR, beats per minute) was measured using a stethoscope, and respiratory rates (RR, breaths per minute) were determined by counting the flank movements. Rectal temperature (RT) was taken using a mercury thermometer placed against the rectal wall of the animal for 2 min. Blood lactate was measured by a portable lactate analyzer (Accusport, Roche Diagnostics, Indianapolis, IN, USA); and the measurements were taken both at rest and from 1 to 3 min after the exercise. Velocity (m s^{-1}) was determined by dividing the stopwatch clocked by the distance covered in each test.

Superficial temperatures (ST) were measured using a thermographic camera (FLIRT420, FLIR Systems Brazil, Sorocaba, SP, Brazil), and emissivity (e) was 0.97, after environmental temperature and relative humidity adjustments at each data collection. Images were taken from direct sunlight without airflow, with animals on a flat hardened dirt surface. Environmental conditions were the same as outdoors. Before each measurement, all mud and dirt were brushed away from the animal's coat.

All horses were photographed from the left-hand side at a 90° angle and 3.0 m distance before and after the test, immediately after saddle removal (Bartolomé et al., 2013). Images were analyzed in QuickReport software (FLIR QuickReport 1.2). The same observer took all thermal pictures. Average superficial temperatures were taken at ten regions of interest (ROIs), considering those that did not have contact with the saddle: croup, buttock, stifle, elbow, gaskin, eye region (ET), muzzle, ventral neck at the jugular groove, shoulder, and forelimb based on the body conformation described by McManus et al. (2016a). Each point in each thermography photo was evaluated 15 times, and the mean was calculated. For ET, the maximum temperature (°C) was measured by tracing an oval area around the eye, including the eyeball, and ~1 cm around the outside of the eyelids.

Radiated heat (W m^{-2}) was calculated for each body region of the horse using the equation by Autio et al. (2006) derived from the Stefan-Boltzmann's law (Dereniak & Boreman, 1996), as follows: $Qr = \epsilon\sigma\ (Ts^4 - AT^4)$, where Qr is the heat loss by radiation (W m^{-2}); ϵ is the emissivity of 0.95; s is the Stefan-Boltzmann's constant (5.67×10^{-8} W m^{-2} K^{-4}); Ts is the horse surface temperature (°K); and AT is the air temperature (°K). The body surface area was calculated using the horse chest girth and body length presuming the surface area of a cylinder.

The analyses were carried out in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Several factor analyses were carried out to examine the relationships between the traits (Proc Factor) because lactate was not measured in all days, nor at rest. ST was evaluated for the effect of the measured body region and type of exercise, their interactions, and air temperature as a covariate. An analysis of variance was carried out to examine the effect of the exercises on the response of the animals (Proc Mixed), using a repeated measure analysis and covariance of ambient temperature. Means were compared using the Tukey's test, at 5% probability. A cluster analysis was carried out using the physiological variables to compare the stress response, depending on the type of exercise. Stepwise multiple regression models (Proc PHREG) were fitted to see the effects of TCI, velocity, thermographic measures and weight on HR, RR, lactate and RT. Heat loss at the different points of the animal body was analysed using GLM with the type of exercise, TCI, and animal fitted as a random effect. The heat loss by radiation was classified as "normal" and "high" by calculating the standard deviation (SD) and indicating animals that were higher than 1.96 SD above the mean (Miot, 2017). A logistic regression (Proc Logistic) was then carried out, to determine if heat loss differed by type of exercise. The experimental protocol was approved by the ethics committee for animal use of the Universidade Federal do Rio Grande do Sul, number 22773.

Results and Discussion

The mean air temperature during the experiment was 35.1ºC (CV 8.43%) and varied from 31.1 to 39.0°C; the relative humidity was 58.4% (CV 4.6%) and varied from 54.3 to 60.5. These conditions led to a TCI of 174.96 (CV 3.14%) which ranged from 167.8 to 182.2. According to Jones (2009), values for TCI should be under 130. Therefore, the TCI values of the present experiment suggest that, even at rest, the animals were under thermal stress. This fact may explain some of the results in the present study, as the animals would have difficulty to liberate heat to the environment due

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to the already stressful environmental conditions (high air temperature and relative humidity).

The Qr mean values varied significantly for ROIs (Table 1). Heat loss by radiation showed mean values from 7.03 kcal h^{-1} m^2 for the eye to 17.07 kcal h^{-1} m^2 for the croup. Autio et al. (2006) also saw a range for heat loss by ROI, but these authors measured fewer regions and saw differences when air temperatures were ≤ 2°C. In the present study, as air temperature was high, there was little difference between the environmental temperature and that of the animal's body, thereby limiting the heat loss. Only the eye region differed ($p<0.05$) from the croup temperature. A high-amplitude heat loss (Qr) was observed between rest (5.54 W m^{-2}) and exercises (19.78 W m^{-2}), but there is no difference between the types of exercises (Table 2).

The mean surface temperature of the horses ranged from 35.9ºC, at rest, to 38.2ºC, at an extended gallop (Table 1). There were significant differences after the exercises among ROIs for all gait types. Among the types of gait, extended gallop showed a greater surface temperature, which indicates a higher exercise intensity, as expected. In the present study, under field conditions, the highest mean body surface temperature occurred on the forelimb (38.1ºC) and elbow (38.1ºC) of the animals (Table 1). Still, this latter did not differ from the hind limb (stifle and gaskin), which agrees with the findings by Soroko et al. (2019) in controlled conditions on a treadmill.

Thermography has been used to study the physical efforts of muscles through their relation with surface temperatures, as 70–80% of the energy produced by working muscles is liberated as heat (Soroko et al.,

Table 1. Effect of body region of interest (ROI) and type of exercise on the infrared thermography surface temperatures (°C) and heat loss by radiation (Qr) in Pantaneiro horses[(1)].

ROI	Rest	Walk	Trot	Extended trot	Gallop	Extended gallop	Qr W m^{-2}	Qr (kcal h^{-1} m^2)
Buttock	35.37aA	37.71abB	37.27bB	37.34bB	37.38bB	37.88bB	13.49abc	11.60
Croup	35.25aA	37.93abB	37.74bB	37.60bB	38.03bcB	37.90bB	19.85a	17.07
Elbow	35.75aA	38.75bcB	38.30cB	38.37cB	38.35cB	39.33cC	17.60abc	15.13
Eyes	36.65b	36.14a	36.22a	36.04a	36.52a	36.47a	8.18c	7.03
Forelimb	35.65aA	38.65bcB	38.18cB	38.42cB	38.31cB	39.07cC	19.25abc	16.55
Gaskin	35.43aA	38.60bB	37.96bB	37.89bB	37.98bcB	38.76cB	16.84abc	14.48
Muzzle	36.86b	36.99a	37.39b	37.32b	36.36a	37.00a	12.34abc	10.61
Neck	35.45aA	36.94abB	36.88abB	36.79abB	36.78abB	37.92bB	11.10bc	9.54
Shoulder	35.78aA	38.02bB	37.74bB	37.70bB	38.00bcB	38.85cB	15.15abc	13.03
Stifle	35.40aA	38.39bBC	38.07bB	38.15bB	37.90bcB	38.86cC	17.43abc	14.99

[(1)]Means followed by different letters, lowercase in the columns and uppercase (between gaits) in the lines, differ by Tukey's test, at 5% probability. Body regions of interest (ROI) according body conformation are described by McManus et al. (2016a).

Table 2. Comparison of gaits for the mean values of physiological variables and percentage of animals with high heat loss by radiation, depending on the type of exercise of Pantaneiro horses[(1)].

Exercise	Qr	RT	RR	HR	Velocity	Lactate	Animals (%)	
	W m^{-2}	(°C)	(mov per min)	(beats per min)	(m s^{-1})	(mmol L^{-1})	Normal	High
Rest	5.54a	38.12cd	39.26d	-	-	-	100a	0
Walk	18.10b	38.17d	44.30c	49.64ab	1.85e	2.54b	94a	6
Trot	17.02b	38.31bc	46.80bc	52.74a	2.20d	2.72b	78b	22
Extended trot	16.37b	38.44a	47.19b	52.33a	3.51c	3.13b	85ab	15
Gallop	16.41b	38.41ab	51.99a	47.52b	6.17b	3.2b	77b	23
Extended gallop	19.78b	38.48a	49.09b	47.76b	11.66a	13.5a	74b	26

[(1)]Means followed by different letters, in the same column, differ by Tukey's test, at 5% probability. Qr, heat loss by radiation; RT, rectal temperature; RR, respiratory rate; and HR, heart rate.

2019). According to Roy et al. (2020), the surface temperature varied significantly among ROIs, when horses were evaluated under outdoor conditions. Under controlled conditions, surface temperature was similar among ROIs. Thus, the results obtained in the present study showed that the surface temperature patterns depend on the intensity of exercises and field conditions, corroborating the results obtained by Esteves Trindade et al. (2019).

Resting temperatures were lower than surface temperatures for gaits in all body regions, except for eyes and muzzle (Table 1). Eye region temperature did not differ among exercises, but at rest. Gloster et al. (2011) found that eye temperature was not affected by the environment temperature, and it is a valuable indicator of body (rectal) temperature. However, Janson et al. (2021) observed that the maximal eye temperature was affected by exogenous and environmental factors unrelated to rectal temperature.

Different ROIs show varying responses due to their involvement in the limb contraction and to increased local blood flow caused by the greater exercise intensity which results in the increase of temperatures of working muscles (Santos et al., 2016b). Other studies showed hind limb muscle surfaces with higher temperature increases after exercises (Yarnell et al., 2014), which may reflect their use; these latter authors used a treadmill which requires different muscle groups. In the report by Kawai et al. (2009), the forelimb muscles showed a higher percentage of type IIa fibers and a lower percentage of type IIx fibers than the hind limb muscles, which is similar to the fiber population of the thoracic and trunk portions. Type IIx fibers are thought to be involved in dynamic action, fast contraction, and propulsion (Santos et al., 2016a), which are not requirements for the Pantaneiro horse which needs to deal with long treks and endurance, often in water-logged fields. According to Rivero & Hill (2016), forelimbs are highly specialized for doing active work, and hind limbs are specialized for generating force.

Different responses were seen between different exercise (gaits) types for RT, RR, HR, velocity, and lactate (Table 2). In the present study, the highest rectal temperature was 38.5ºC and remained within the reference parameters (Feitosa, 2004). Although RR at rest was above the reference values of 10 to 20 breaths per minute (Feitosa, 2004), the results of the present work are in agreement with those by Santos et al. (2016b) for Pantaneiro horses, suggesting that these higher values possibly indicate adaptation to high temperatures in the region.

There was an increase of lactate concentration in the extended gallop, with rapid elevation of lactate concentration at speeds of 11.66 m s^{-1} (40 km h^{-1}) which are close to values of 7.0–9.0 m s^{-1} described by Neil (2008), as velocity was associated with the increase of lactate concentration. Lactate concentrations were below the anaerobic threshold (V4) of 4.0 mmol L^{-1} (Lindner, 2000) for all gaits, which suggests a predominance of aerobic metabolism, except for the extended gallop (13.5 mmol L^{-1}) which suggests anaerobic metabolism.

The stepwise logistic regression identified variables with the best predictive value for RT, RR, HR, and lactate (Table 3). For multiple regression models, TCI, velocity, eye region temperature (ET), and elbow region temperature (ELBOW) affected ($p<0.05$) the physiological responses; however, the other thermographic temperatures and weight were not significant. The coefficient of determination was greater for lactate ($R^2 = 0.75$), when the independent variables were velocity and eye region temperature. The other models displayed lower R^2, with RT showing better predictive capacity than RR or HR. Johnson et al. (2011) verified that eye temperature could be a primary tool for determining RT in ponies, while Kim & Cho (2021) validated a protocol to assess the welfare of horses using ET. Concerning elbow temperature, our results were similar those obtained by Jodkowska et al. (2001) for forelimbs (elbow, shoulder) after training. Soroko et al. (2019), in turn, found higher temperatures for both forelimbs and hind limbs (gluteous and quarter). Several authors also found

Table 3. Multiple regression equations for the prediction of physiological variables of Pantaneiro horses[(1)].

Model	R^2
RT = 27.343 + 0.014×VEL + 7.485×ET + 0.061×TCI	0.45
RR = -26.147 + 0.211×VEL + 13.579×ELBOW	0.27
HR = 46.898 + 22.6811×ELBOW	0.22
Lactate = 38.553 + 0.310×VEL + 9.328×ET	0.75

[(1)]RT, rectal temperature (ºC); RR, respiratory rate (mov per minute); HR, heart rate (beats per minute); VEL, velocity (km h^{-1}); TCI, thermal comfort index; and thermograhic temperatures (ELBOW = elbow region, and ET = eye region).

thermography temperatures useful for the prediction of physiological measures, such as lactate (Witkowska-Pilaszewicz et al., 2020), rectal temperature (McManus et al., 2016b), and muscle activity (Yarnell et al., 2014). However, Teixeira Neto et al. (2020), who studied animals at constant and not at different, did not find this relationship. For being a rapid and noninvasive method, further studies are necessary to validate these relationships and suggest the physiological limits.

In the principal component analysis without thermographic data (Figure 1 A), velocity and air temperature are associated with the increase of RR, RT, HR, and ST, as expected, and a decrease of Qr. Horses control body temperature by the following heat loss mechanisms: conduction, convection, radiation, and evaporation (Santos et al., 2016a). As Qr represents the sensible heat loss by radiation dependent on the thermal gradient between the skin and environment temperature, the horse can gain heat in high-temperature conditions. Maia et al. (2005) evaluated the latent and sensible heat loss from the body surface of Holstein cows, under natural conditions, in a tropical environment. They verified that when the air temperature is near or greater than the body temperature, Qr is a mean to gain heat. In these conditions, the animals dissipate heat mainly by evaporation and respiration. The respiratory rate increased with an increasing velocity (Figure 1 B) that depended on the gait type (Table 2). In factor 1, the increase of a temperature was accompanied by the increase of the others. In turn, the heat loss (Qr) by radiation increased with the type of gait (exercise intensity) (Table 1), and the extended gallop showed Qr values 26% higher than the mean values. The second component (Figure 1 A) separated the HR responses from the other variables. HR was measured 1–3 min after the exercise, as opposed to the other measures, and decreased with the velocity (Table 2). According to Borresen & Lambert (2008), the recovery of HR is influenced by the coordinated interaction between parasympathetic and sympathetic nervous activity. A slower HR recovery after the high-intensity exercises is attributed to a more intense withdrawal of the sympathetic nervous system, and to the reactivation of parasympathetic effects that occur rapidly in the first minute. Thus, the recovery heart rate provides helpful information on how horses respond to exercises.

The second factor (Figure 1 C) showed that the increase of velocity was accompanied by the

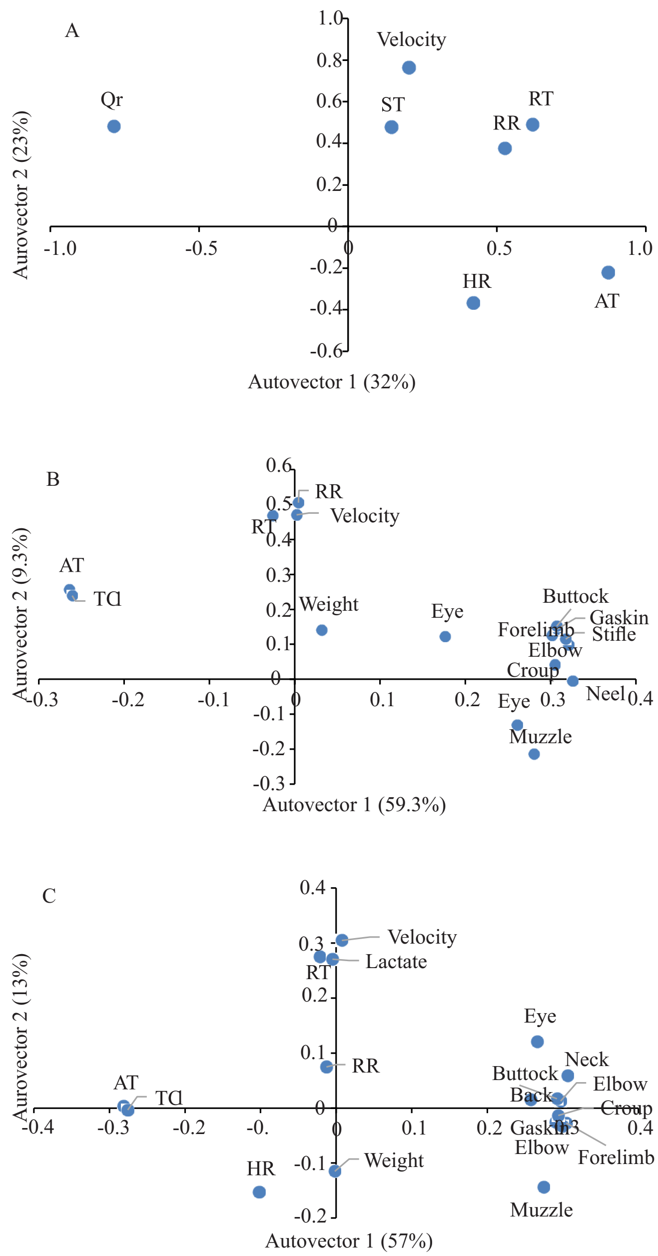

Figure 1. Principal component analysis of physiological and environmental factors during the exercises of Pantaneiro horses: A, without thermographic data; B, with thermographic data; and C, including lactate. Qr, heat loss by radiation (W m^{-2}); RT, rectal temperature (°C); FR, respiratory rate (mov per minute); HR, heart rate (beats per minute); velocity (km h^{-1}); lactate (mmol L^{-1}); AT, air temperature (°C); ST, surface temperature (°C); and TCI, thermal comfort index.

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increase of lactate, RT, RR, and hind thermographic temperatures. The velocity (exercise intensity) increases lactate slowly until at a certain point, when it begins to rise exponentially (anaerobic threshold). The increase of blood lactate concentration and surface temperature measures for the ROIs, immediately after exercises, are in agreement with the findings by Witkowska-Piłaszewicz et al. (2020), who found a correlation between these variables. Still, these authors measured responses 30 min after exercises. Further studies should also take into account the time after the exercises. High surface temperature is due to blood flow to the skin, to help dissipate heat (Santos et al., 2016b). Nevertheless, a decrease of the muzzle temperature (Figure 1 C) was observed. Horses use respiration to lose heat, which may cause a reduction of the muzzle temperature (Pirrone et al., 2007).

The walk, trot, and extended trot gaits were grouped in the cluster analysis (Figure 2) indicating a better thermal balance than gallop and extended gallop. Therefore, maintaining the horses within these gaits help these animals to maintain the normal body temperature in stressful environments, such as the Pantanal. Considering that all gaits are below the anaerobic threshold (Lindner, 2000), except for the extended gallop, these gaits represent a submaximal exercise regime.

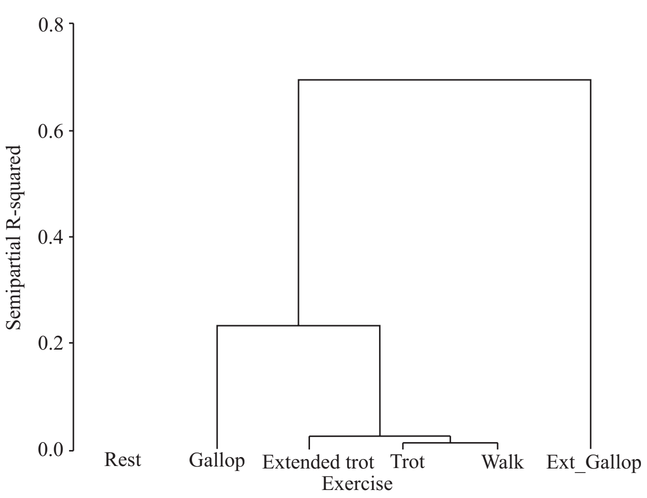

Figure 2. Cluster for types of exercise of Pantaneiro horses. Ext_Gallop, extended gallop.

Conclusions

1. The walk, trot, and extended trot are the most adequate gaits for Pantaneiro horses to maintain physiological parameters at high-temperature conditions in the field.

2. Pantaneiro horses increase the respiratory rate to help with heat dissipation and to decrease the sensible heat loss by radiation to avoid gain heat.

3. Recovery heart rate immediately after the exercises decreases with the velocity (type of gait); however, it remains within the physiological ranges.

4. Eye temperatures are adequate for predicting changes in physiological parameters in Pantaneiro horses.

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