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RESEARCH ARTICLE

Physiological responses of two equine breeds to a tropical winter climate

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ABSTRACT

In tropical regions of Mexico, breed horses introduced from European countries are used for recreational activities under hot and humid conditions without considering their well-being. In order to evaluate the effect of winter conditions on physiological responses of two equine breeds, twelve horses (six Spanish and six Friesian breeds) were evaluated in the western Pacific coast of Mexico. The winter season was divided in 3 periods so that the statistical model included the factors breed and period. The physiological responses evaluated were: heart rate (HR), respiratory rate (RR), capillary refill time (CRT), and rectal temperature (RT). The climatic variables ambient temperature (AT) and relative humidity (RH) were used to estimate the temperature-humidity index (THI). Averages for THI, AT and RH were 71.4 units, 24.3 °C, and 53.4%, respectively. The CRT was similar ($P > 0.05$) among breeds and periods. The interaction $B \times P$ was significant ($P < 0.05$) for HR, RR, and RT in the second and third periods, but not in the first one. As a result, Spanish horses experimented higher ($P < 0.05$) HR (38.1 vs 37.6 and 38.0 vs 37.0 beats/min), RR (18.8 vs 18.5 and 18.9 vs 17.4 bpm), and RT (38.1 vs 38.0 °C and 38.0 vs 37.8 °C) than Friesian horses during the second and third periods, respectively. Both breeds of horses were exposed to a mild to moderate heat stress during winter tropical conditions; however, Spanish breed horses showed better tolerance to heat stress than Friesian horses based on their physiological measures evaluated.

Keywords: Spanish horses; Friesian horses; Heat stress; Temperature-humidity index; Tropical weather.

INTRODUCTION

Cavalcades with horses in Mexico are very popular outdoor recreational activities. A cavalcade in Mexico is a kind of parade where horses are ridden and driven in the field for variable time (5 – 8 hours; Nájera-González et al., 2021). Therefore, it is important to consider the weather as a preponderant factor to achieve efficiency of this equestrian activity, and ensuring that this activity is performing in a comfortable environment for the well-being of horses (Domínguez et al., 2014; Nájera-González et al., 2021). For these activities, Spanish and Friesian breed horses are preferred not only for their great fame but also for their appearance and beauty. Although these breeds were brought from Europe on a discretionary basis, they have shown certain adaptation to temperate and tropical climates. The Spanish is a horse breed from the Iberian Peninsula, where its ancestors have lived for thousands of years (IALHA, 2020). The Friesian horse is the only breed native in Netherlands, where it has been known since the 13th century (KFPS, 2020).

Environmental temperature has intensified generally in arid and tropical areas due to global warming, resulting in hotter summers (Avendaño et al., 2012). In addition, climate change can also contribute to the presence of droughts, fires, floods and storms, which can affect the livestock/agriculture relationship in four important aspects: (i) production, availability and price of forage (ii) forage quality (iii) welfare and health, as well as production and reproduction of livestock, (iv) and finally the increased distribution of diseases (Francis and Vavrus, 2012). In the USA, economic losses have been attributed to heat stress (HS) in the livestock industry and estimated in $2.4 billion/year (St-Pierre et al., 2003; Sejian et al., 2018). The HS is described by increased body temperature due to nutrient catabolism and animal

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inefficiency for losing body heat under high environmental conditions such as relative humidity, temperature ambient, wind speed, and solar radiation (McManus et al., 2011; Holcomb et al., 2015). In fact, solar radiation is the main factor for gaining heat directly or indirectly from the environment (Krishnan et al., 2017; Holcomb, 2017).

Hence, these climatic conditions can negatively affect performance of horses, causing physiologic and endocrine changes in response to HS (Wagner, 2010). The temperature-humidity index (THI) is a measured used to quantify the degree of HS on farm animals, and for cattle it starts from 72 units (Armstrong, 1994). In horses, thermoneutral zone is between 5-25 °C (Morgan, 1998). Additionally, during the daily work of horses doing recreational activities a great effort by the cardiovascular system is required (Mills et al., 1996). In fact, it has been detected that shortly after the horse starts physical work, blood flow is increased to supply energy and oxygen to the striatum muscle located in the thoracic and pelvic limbs, but this route produces a lot of body heat and can be aggravated if HS conditions are present (Ohmura et al., 2002; Hernández et al., 2011). Animals exposed to intense heat conditions have shown an increase in cortisol concentrations, which is directly related to the level of stress; they also show a decreased in insulin, thyroxine, triiodothyronine and plasma aldosterone (Habeeb et al., 2018; Butterfield et al., 2018). Then, water consumption, rectal temperature, pulse rate, and respiratory rate also increased; finally, the consumption of nutrients and dry matter intake, as well as the rate of passage of food decreased (Kamal et al., 2018a; Kamal et al., 2018b).

Correlations have been observed between climate and physiological responses such as respiratory and heart rates, body temperature, and general metabolism in horses (Arnold et al., 2006; Janczarek et al., 2015). Fortunately, horses have an excellent thermoregulatory system which allows them to release body heat through the skin by sweating; they also use other mechanisms to loose body heat like panting, defecating, and urinating (Mrowka and Reuter, 2016; Polsky and von Keyserlingk, 2017). In a tropical region of the central coast of the Gulf Mexico, Domínguez et al. (2014) evaluated physiological responses of local breed horses to a winter tropical weather, finding that even in winter season, there are periods of heat stress during the morning and afternoon, so comfort is not completely achieved in this tropical scenario. The climate in Colima, a Mexican state located close to the Pacific Ocean is categorized as tropical (Vizcaíno-Vargas et al., 2004), and its effect on European breed horses has not yet been evaluated. Therefore, the objective of this study was to evaluate the effect of winter conditions, divided in three periods, on some physiological responses of Spanish and Friesian breed horses in a tropical ecosystem.

MATERIALS AND METHODS

Location of the study

This study was carried out in two different locations of the municipality Colima, located in the state Colima, Mexico (El Chanal: 19° 17´ 33˝ N, and 103° 42´ 13˝ W; Los Potrillos: 19° 14´ 55˝N, and 103° 41´ 03˝W). The climatic conditions are tropical, with temperatures ranging between 11 and 35 °C, average annual precipitation of 1000 mm, average annual relative humidity of 50%, and 485 m above sea level (Vizcaíno-Vargas et al., 2004; INEGI 2017).

Climatological data

Daily ambient temperature and relative humidity were recorded every 15 minutes by the weather station of the National Water Commission, Colima Delegation, which was located about seven kilometers from the study sites (19° 13´ 06˝ N, and 103° 43´ 15˝ W). Both ambient temperature (AT, ºC) and relative humidity (RH, %) were used to estimate the temperature-humidity index (THI, units) following the formula suggested by Hahn (1999):

$$THI = 0.81 \times AT + RH (AT - 14.4) + 46.4.$$

Experimental animals, diet and livery stables

The duration of the study was 100 d and the winter season was divided into three periods (P1: December 19, 2016, to January 22, 2017; P2: January 23 to February 26, 2017; and P3: February 27 to March 31, 2017). Twelve horses, six Spanish and six Friesian breeds with similar characteristics (age [4 ± 0.9 years old], and body weight [500 ± 50 kg]) were selected to ensure balance between genetic groups. Half of the horses from each breed were in one location (El Chanal) and the other half in other location (Los Potrillos); both places corresponded to the same municipality. Horses were fed a similar diet consisted of a combination of commercial concentrate (350 g/kg as fed), alfalfa hay (600 g/kg as fed), and oat hay (50 g/kg as fed) daily at 0900 and 1800 h, formulated based on NRC (1978). Freshwater was available at all times. All horses were confined to individual stables with dimensions of 4 x 4 m, N-S orientation, and equipped with shades, drinkers and feeders.

Experimental procedure

Physiological responses were obtained twice every week on Monday and Friday at 0800 h. Horses were moved to an adjacent handling sleeve to obtain rectal temperature (RT, °C; Fig. 1a) using a manual thermometer (51-II; Fluke Corporation, Everett, WA, USA) which was assembled to a peak attachment (80 PK-22; Fluke Corporation, Everett, WA, USA). Also, a conventional stethoscope (3M, Littmann Classic III, MA, USA) was used between the third and sixth intercostal space area of the left flank to obtain heart rate (HR, beats/min; Fig. 1c). The respiration rate (RR; Fig. 1c) was determined by observing the number of breaths during 30 s and multiplying it by 2 to obtain breaths per minute (bpm). Finally, capillary refill time (CRT, seconds; Fig. 1b) was obtained by a pressure applied to the gum with the index finger for five seconds until it turned white; if the pressure was removed it can be counted the time until the gum "pink" indicated that the blood has flowed into the capillaries.

Statistical analysis

A total of 360 observations of each physiologic and environmental variables were analyzed; initially, descriptive statistics were estimated using PROC MEANS from SAS (2004). To determine the effect of breed (*B*) and period (*P*) on each response variable, a 2 x 3 factorial arrangement of treatments in a randomized block design was used. The statistical model included the fixed effects of breed and period, as well as their interaction involved (*B x P*). Location was considered as the block factor. Least squares means and standard errors of the means are reported and significance was declared if $P < 0.05$, whereas a trend was accepted to occur if $0.05 < P < 0.10$. All response variables from the experiment were analyzed using the PROC MIXED of SAS (2004).

RESULTS

Environmental conditions

The average values per week of THI, AT, and RH are shown in Fig. 2. Overall, the average THI during all the weeks of the study was above 71 units. However, during weeks 2, 8, and 12, corresponding to each of the 3 periods, respectively, the average THI was above 73 units. At least 5 weeks below or equal to 70 units were also observed within the second and third periods. The average per week of AT remained at approximately 24.2 °C and RH at 53.2%. The maximum and minimum values of THI, AT, and RH by period are shown in Fig. 3. In periods 1 and 3, the maximum average THI reached 79 units, although in period two it decreased by one unit. The minimum average THI during the three study periods was above 63 units. The maximum and minimum averages of RH during the three study periods were above 77 and 27%, respectively. Finally, maximum and minimum averages of AT during all periods were above 33 and 17 °C, respectively. During the complete experiment the mean values for THI, AT, and RH were 71.4 units, 24.3 °C, and 53.4%, respectively (Table 1).

Physiological variables

Daily averages of HR, RR, CRT, and RT were 37.4 beats/min, 17.8 bpm, 2 s, and 38 °C, respectively (Table 1). Also, HR ranged from 35 to 42 beats/min, RR from 11 to 29 bpm, CRT 2 to 3 s, and RT 37.7 to 38.6 °C. The CRT showed no effect ($P > 0.05$, Table 2) by the B x P interaction, nor for any main effect. Although, the interaction effect *B x P* was significant ($P < 0.05$) for the remaining physiological variables. Therefore, averages of the treatment combinations are shown in Table 3. In fact, during the first period both breeds obtained similar responses ($P > 0.05$) in physiological variables (37 beats/min, 17 bpm, and 37.9 °C in HR, RR, and RT, respectively). But during the second and third periods, Spanish and Friesian horses obtained differences ($P < 0.05$) in HR (38.1 and 37.6 beats/min), RR (18.8 and 18.5 bpm, as well as 18.9 and 17.4 bpm), and RT (38.1 and 38.0 °C, as well as 38.0 and 37.8 °C), respectively.

DISCUSSION

Climatic conditions

While the RH observed several peaks during the study, AT and THI showed a steady state. Interestingly, these changes in RH could be explained by equines' preference for using shade during summer mornings in arid conditions (Holcomb et al., 2014; Holcomb and Stull, 2016), showing that environmental conditions can be severe to horses during the morning in extreme environments, which could produce a refractory HS effect, causing in horses accumulated fatigue during the whole period (González-Alonso et al., 1999). This may lead to low performance in their daily activities, although it was not a clearly observed effect on our animals during the morning sampling.

Fig 1. Experimental procedure. Rectal temperature (A); Capillary refill time (B); Heart rate and respiration rate (C).

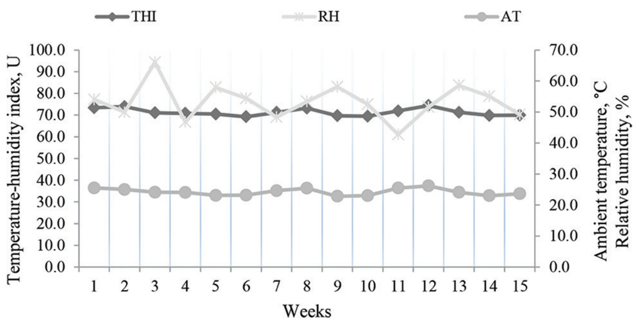

Fig 2. Average values of the temperature-humidity index (THI), ambient temperature (AT), and relative humidity (RH) per week during the experimental period.

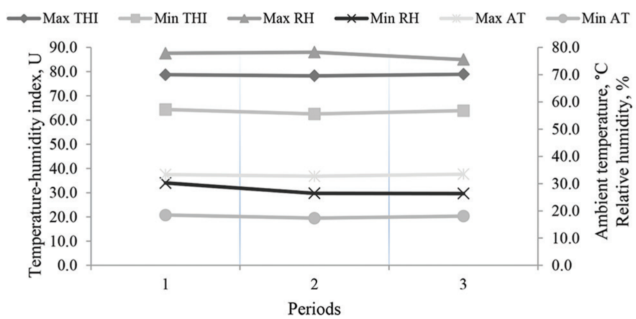

Fig 3. Maximum and minimum values of the temperature-humidity index (THI), relative humidity (RH), and ambient temperature (AT) by periods.

Table 1: Descriptive statistics of the environment and physiological variables recorded in two equine breeds under heat stress conditions during all study.

Items	Mean	SEM	Maximum	Minimum
Environment variables				
Ambient temperature, °C	24.26	1.33	28.10	21.70
Relative humidity, %	53.38	9.08	79.0	32.0
Temperature-humidity index, units	71.43	2.23	78.46	67.27
Physiological variables				
Heart rate, beats/min	37.41	1.14	42.0	35.0
Respiration rate, bpm	17.81	2.39	29.0	11.0
Capillary refill time, seconds	2.01	0.14	3.0	2.0
Rectal temperature, °C	37.97	0.22	38.6	37.7

Horses are physiologically susceptible to warm environments (Holcomb, 2017). In fact, under these conditions, the negative changes observed in physiological responses, such as heart rate, respiratory rate, and rectal temperature are well documented (Janczarek et al., 2015). Truthfully, during all study an average AT of around 24 °C was obtained; per period the maximum AT easily exceeds 30 °C according to other results (Holcomb and Stull, 2016), which means that the animals of both genetic groups exceeded the temperature critical point according to their thermoneutral zone (20-30 °C; Morgan, 1998), thus activating the physiological and behavioral responses to dissipate the heat charge. In fact, the same authors mention that these responses begin before reaching the minimum value of the thermoneutral zone, which generates negative effects on the animals due to the shortened temperature threshold. So horses show signs of fatigue even before obtaining the maximum potential during the working or training time.

Although the average THI was 71 units, up to 7 units of maximum THI above 72 units were detected per period, where mild HS starts. This shows that Spanish and Friesian horses had a mild to moderate HS, according to Wiersma (1990). Certainty, depending on the intensity of climatic factors (i.e., wind speed, solar radiation, relative humidity, ambient temperature), horses can gain or lose body heat (Holcomb, 2017). Then, although the horses may have adapted to the environmental conditions during the study, this can represent a slight disadvantage when starting the daily work, which results in less efficiency. It is important to mention that no variables were collected during the afternoon; however, thermoregulation mechanisms were activated by the horses in the night resulting from the activities performed during the day.

Physiological variables

Capillary refill time, CRT

Results shown in CRT (s 1 and 2) consistently coincides with the nearly two seconds reported by Shawaf et al. (2020) during winter time, which showed an excellent health status in both genetic groups. This is true because they were never reused to the daily work of training.

Heart rate, HR

Under thermoneutral conditions, the average normal heart rate in horses is 33 beats/min (Hodgson, 2014a). In general, the average obtained throughout the study in both groups of animals was four beats/min above this average (Table 1). Coincidentally, the same response was obtained when evaluating the number of beats/min in a transfer trip, since the different horse breeds recorded an average of 40 beats/min at 37 °C ambient temperature (Friend, 2000).

It should be noted that in our study, due to the highly significant $B \times P$ interaction for HR, RR, and RT, and then to the statistically nature of the results, we focused on the discussion of Table 3. Both breeds of horses obtained a HR of 37 beats/min during the first study period. In contrast, during the second and third periods, Friesian horses obtained 1.3 and 2.7% fewer beats/min, respectively, than Spanish horses, suggesting greater efficiency in dissipating body heat. Moreover, both horse breeds were similar in obtaining four beats/min above-normal HR during the first period. Significances were detected between breeds during the second and third periods with approximately 5 beats/minute above normal, except for Friesian horses which during the third period obtained the same HR average as in

Table 2: Main and interaction effects on physiological responses of horses during the study.

Items	Breed				Period					Breed x Period
	Spanish	Friesian	SE	P-Value	I	II	III	SE	P-Value	P-Value
HR[1]	37.7	37.1	0.07	<0.0001	36.9	37.8	37.5	0.09	<0.0001	0.0016
RR[2]	18.2	17.5	0.16	0.0026	16.6	18.7	18.2	0.20	<0.0001	0.0308
CRT[3]	2.0	2.0	0.01	0.2516	2.0	2.0	2.0	0.01	0.0974	1.0000
RT[4]	38.0	37.9	0.01	<0.0001	37.9	38.1	37.9	0.01	<0.0001	0.0095

[924]Heart rate (beats/min); [2]Respiration rate (bpm); [3]Capillary refill time, seconds; [4]Rectal temperature (°C).

Table 3: Interaction effect on physiological responses of horses during the study.

Interaction	n	HR[1]	RR[2]	RT[3]
Period I				
Spanish	60	37.0±0.18[a]	16.7±0.40[a]	37.91±0.02[a]
Friesian	60	36.8±0.18[a]	16.5±0.40[a]	37.90±0.02[a]
Period II				
Spanish	60	38.1±0.18[a]	18.8±0.40[a]	38.14±0.02[a]
Friesian	60	37.6±0.18[b]	18.5±0.40[a]	38.03±0.02[b]
Period III				
Spanish	60	38.0±0.18[a]	18.9±0.40[a]	37.98±0.02[a]
Friesian	60	37.0±0.18[b]	17.4±0.40[b]	37.82±0.02[b]

[1]Heart rate (beats/min); [2]Respiration rate (bpm); [3]Rectal temperature (°C).
[ab]Means by breed per period with different superscript are different ($P < 0.05$).

the first period. Controversially, even 34.2 beats/min have been reported during summer (Janczarek et al., 2015), which slightly disagrees with our results although it was under similar conditions of little or no activity. Considering that HR is higher during the summer than winter by 45% under arid conditions (Brinkmann et al., 2012), everything indicates that under tropical conditions this number can be similar. In fact, horses during winter obtained a similar number of beats/min than the present study (Shawaf et al., 2020). Regular monitoring of HR in horses can help to evaluate physical response respect to HS and other pathologies (Harris et al., 2007). Actually, it has been mentioned that horses under HS conditions reduce the systolic volume and increase HR during moderate exercise, compromising cardiac expenditure (Marlin et al., 1996). Simply providing water baths in horses under HS conditions can help to reduce the negative effects and decreases HR.

Respiration rate, RR

First of all, it is very important to mention that the normal RR in horses is around 14 bpm (Lekeux et al., 2014). On the other hand, Collier et al. (2017) mention that animals under HS can increase their RR above normal value, depending on the intensity and duration of the HS. Thus, both groups of horses obtained an average of almost 18 bpm, that is, at least four breaths higher than normal. Also, maximum and minimum breathing values during the study ranged between 11 to 29 bpm, respectively. This values suggest that some mornings were warmer than others, which obviously is related to the results obtained in environmental variables during the study; so when RR increased, evaporation and cooling was improved, and the more heat-stressed, the more ventilation and energy is used by horses. In fact, in metabolic terms ventilation represents an increment in the use of intracellular adenosine triphosphate (Pösö et al., 2008). In addition, the maximum RR values were twice as high as normal, indicating that horses began working activities with a clear deterioration of their well-being.

Inversely, during the first and second periods, Spanish and Friesian horses had similar RR, obtaining almost 17 and 19 bpm, respectively. This implies an increased between 3 and 5 bpm, which could be a concern given the normal value reported in resting horses. On the other hand, during the third period Friesian horses obtained almost 9% fewer breaths per minute than Spanish horses, which implies a difference above normal value between 24 and 35%, respectively. Even though at the beginning of the study horses had similar body weight and age, differences in RR could be due to the size of Friesian horses, which are known to be larger than Spanish horses. In fact, this may be due to that lungs represent 1% of the body weight and that bronchioles are not well developed in horses since most of the gas exchange takes place in the alveolar unit (Lekeux et al., 2014). The same authors mention that the connective tissue septa between lobules are not complete, allowing collateral ventilation to transfer air between the lobules through accessory pathways to communicate with the bronchioles and alveolar ducts. Unfortunately, the accessory pathways represent a high resistance to airflow, almost 16% of the required volume, which is low compared to the 90% observed in the human lung (Franklin et al., 2012).

Rectal temperature, RT

Maximum and minimum RT were 38.6 to 37.7 °C, respectively. During all study, both breeds obtained an average of almost 38 °C; on this respect, Lekeux et al. (2014) mention that the normal RT in horses is 37 °C, so one additional degree in RT may cause physiological and metabolic unbalances in the body (Janczarek et al., 2015).

On the other hand, similar conditions were obtained during the first period with respect to RT, which was 37.9 °C in both horse breeds. Then, according to an increase of AT and THI during the second period, an increase in RT was also observed in both horse breeds, but Friesian horses were more sensitive in reducing RT by 0.3% than Spanish horses, which implies a difference above the normal value of almost 3%, in both horses' breeds. As a result, this phenomenon was consistently repeated during period three. Some authors mention that an increased in RT is an important sign for detecting HS in horses (Collier et al., 2017). Thus, if RT decreases, thermoregulation process in the animal is not forced (Mrowka and Reuter, 2016). But if body temperature increases, so will oxygen consumption by the mitochondria, mainly during the process of oxidative phosphorylation (Lekeux et al., 2014; Mrowka and Reuter, 2016). A study of Kuntz et al. (2006) showed an increased in DMI of horses to maintain thermoregulation. However, understanding thermoregulation in horses can be difficult, as is evidenced by the wide range in the thermoneutral zone (Morgan, 1998). In fact, although the mechanism appears to be essentially adrenergic-sympathetic, horses have another mechanism to release heat from the body, such as evaporation where through sweating is a redistribution of blood to the surface of the skin, resulting in a highly efficient mechanism (McManus et al., 2011; Hernández et al., 2011; Habeeb et al., 2018). Meanwhile, sweating is under sympathetic nervous control in horses (Hodgson, 2014b). In addition, same author mention that evaporative cooling is an efficient mechanism to reduce the heat gain by the body. Regarding to RT, an increase has been observed throughout the day (Holcomb et al., 2013; Holcomb et al., 2015). Heat by radiation from the floor may contributed to the increase in RT in both breeds of horses, although this is difficult to verify because floor temperatures were not recorded. Assuming the latter, neurons of the preoptic nucleus by way of synaptic connections in the brain through the neurotransmitters glutamate and gamma aminobutyric acid help to regulate body or rectal temperature as well as other physiological responses such as sleep, the sodium-potassium pump or the renin-angiotensin-aldosterone system (Nissen and Renaud, 1994; Henry et al., 2009; McKinley et al., 2015). Thus, under HS conditions, hormones that modulate metabolic rate during thermoregulation such as thyroid hormones and leptin can be affected (Taylor et al., 2014). That is, under HS conditions triiodothyronine and thyroxine hormones may decrease in blood levels (Habeeb et al., 2018). Therefore, according to Holcomb et al. (2015) our horses absorbed environment heat and increased body heat by metabolism. Also, a high correlation ($0.78 < r < 0.96$) has been detected between cortisol levels and RR, HR, and RT (Casella et al., 2016). Hence, although we did not determine cortisol levels, we may expect similar results since an increased in blood cortisol is commonly used as HS indicator (Möstl and Palme, 2002). Finally, work, exercise, or simply daily activities can induce HS, especially if body heat and environmental factors are critical, causing a failure in the thermoregulatory system by anhidrosis, and consequently inadequate welfare.

More research is needed to get a better picture of the negative effects of HS on horses, which can be attained collecting additional physiological variables at different times of the day, as well as other seasons, shade structures, or nutritional strategies to improve equine welfare. In addition, it is necessary to carry out hormonal determinations that reinforce the presence of heat stress in horses.

CONCLUSIONS

Horses of the present study were exposed to mild to moderate heat stress during winter tropical conditions. This level of heat stress produced no changes in physiological responses during the first study period, but during periods two and three Friesian horses were physiologically less affected than Spanish horses. These results can be pertinent in searching for better management practices for the care of recreational horses. Future research could include hormone determinations involved in determining heat stress in horses, as well as different strategies to mitigate the negative effects during and after regular equine work in tropical conditions.

Conflict of interest

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Authors' contributions

Conceived the experiment: Avendaño-Reyes Leonel, and Hernández-Rivera Juan Augusto. Performed field trial: Miranda-Ojeda Marianne Guadalupe, Carrillo-Díaz María Isabel and Ruíz-Ramírez Johnatan Alberto. Performed statistical analysis and interpretation of results: García-Casillas Arturo César and Macedo-Barragán Rafael Julio. Wrote the manuscript: Avendaño-Reyes Leonel, and Hernández-Rivera Juan Augusto. All authors read and approved the manuscript.

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