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Blood lactate concentrations and heart rates of Colombian Paso horses during a field exercise test

Angélica M. Zuluaga Cabrera [a,*], Maria J. Casas Soto [b], José R. Martínez Aranzales [a], Nathalia M. Correa Valencia [a], María P. Arias Gutiérrez [d]

[a] *Centauro Research Group, School of Veterinary Medicine, Faculty of Agrarian Sciences, Universidad de Antioquia, Calle 70 No. 52-21, AA 1226, Medellín Colombia*
[b] *Normandía Equine Center, Rionegro Colombia*
[d] *Universidad CES, Medellín Colombia*

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ABSTRACT

Information on performance indices in Paso horses is scarce. Field exercise tests are necessary to recreate the exertion that occurs during training and competition.

To describe blood lactate concentrations and heart rates of untrained Colombian Paso horses (CPHs) in response to a field exercise test.

A 30-minutes-long standardized field exercise test was carried out on 11 untrained adult CPHs of both sexes. Blood lactate concentration (BLConc) and heart rate (HR) were measured before, during each step of the test, and at recovery. The BLConc and HR were used to calculate the HR at which a BLConc of 4 mmol/L or anaerobic threshold (HRL_4) was reached.

The HR during the field exercise test increased according to the protocol used. The BLConc during the test was variable and, despite having been increasing like the HR, the distribution of the values in each step of the test was remarkably dispersed. The mean blood lactate clearance (BLClear) percentage was 56.3 ± 16, similar in most animals. The HRL_4 was reached at a notably different HR among individuals (132 to 251 bpm).

The field exercise test protocol used herein is useful to assess BLConc and HR changes in acute response to exercise in CPHs. It would be useful to evaluate training kinetics with other parameters including cell blood count and muscle enzymes.

1. Introduction

Understanding the specific physiological effects of the effort the horse experiences in each sport discipline allows improving the conditioning and training processes. In addition, its biological characterization is a key determinant of performance indices, used to identify the appropriate physical status (Allen, Van & Franklin, 2016). The physical training in horses induces physiological adaptations necessary to compete and withstand the intensity of the different tests, with a minimum risk of injury and ensuring accurate behavior and psychogenic indicators (Kupczyński et al., 2018).

Standardized treadmill tests have been widely used for this purpose, allowing the animal to become fatigued by increasing the speed and slope of the treadmill, thus determining the maximum effort and metabolic expenditure required (Allen et al., 2016).

In Paso horses, the use of the treadmill is limited, as in other equestrian disciplines (Allen et al., 2016; Fraipont et al., 2012), since it is not possible to replicate the gait pattern of these animals without the rider (Novoa-Bravo, Bernal-Pinilla & García, 2021). In the specific case of Colombian Paso horses (CPHs), the use of the treadmill is not possible because the gait performance occurs when the bit interacts with the horse's mouth, and because the rider is usually overweighted, increasing the intensity of the exercise. Therefore, it is necessary to evaluate CPHs through field-based tests, allowing to recreate the exertion that occurs during training and competition, accomplishing performance indices for the sporting discipline.

A performance test is essential in the development of a training program for the sport horse, because it allows to determine the athletic status of the animal —based on the physiological variables related to cardiac and metabolic activity, and to evaluate the effects of exercise on the acid-base status. This test simplifies the athlete characterization and allows training strategies to be focused on reducing weaknesses and

* Corresponding author.
E-mail address: angelica.zuluaga@udea.edu.co (A.M.Z. Cabrera).

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strengthening the athletic abilities.

Previous reports have described methods that allow evaluating the horse's progress throughout training, such as cardiac stress tests and hematological and biochemical profiling (Piccione, Giannetto, Fazio, Di Mauro & Caola, 2007). The evaluation of the exercise impact through blood parameters can provide practical information on the effectiveness of a training program (Kupczyński, Śpitalniak, Zwyrzykowska--Wodzińska & Soroko, 2018) and allows to determine if there are cardiovascular, hematological, and metabolic alterations induced by it. However, the changes derived from exercise in each discipline and breed are different and must be characterized specifically.

It is not clear whether the CPH reaches the anaerobic threshold (HRL_4) when the heart rate (HR) is close to 200 bpm as has been described in racehorses (Hodgson, McGowan & McKeever, 2014). Nor is it known if the blood lactate concentration (BLConc) increases proportionally with the HR.

Traditionally, in CPHs the anaerobic threshold has been calculated using the speed at which the horse reaches a BLConc of 4 mmol/L; however, this is not possible for the breed due to the differences in its gait performance. It is not useful to measure V_4 (speed at which a BLConc of 4 mmol/L is achieved) as well, as it is done in other equestrian disciplines, because speed (distance covered per unit of time) does not increase as a result of improved performance. When a CPH improves its performance, it increases the number of strokes or steps per minute, independent of distance covered. Therefore, to establish the moment in which the animal exercises under anaerobic metabolism, the HR should be used instead of the speed.

Hematological, acid-base, and metabolic changes that occur as a result of a maximum-intensity exercise have been characterized in some equestrian sports, through standardized field tests (Fortier, Goachet, Julliand & Deley, 2015; Fraipont et al., 2012; Munsters, Van Iwaarden, Van Weeren & Sloet Van, 2014). Nevertheless, this information is not available for the CPH so far. In this sense, this study aimed to describe blood lactate concentrations and heart rates of untrained CPHs in response to a field exercise test.

2. Materials and methods

2.1. Ethical declarations

The procedures carried out on the animals of study were approved by the Comité de Ética para la Experimentación con Animales (CEEA) of Universidad de Antioquia (Act #122, February 5, 2018).

2.2. Study location

The facilities where the study was carried out correspond to a "Very Humid Lower Montane Forest" life zone (Holdridge et al., 1971), located at 2130 m above sea level, with an environmental temperature between 12 and 18 °C, and a relative humidity of 96%.

2.3. Animals

Eleven untrained adult (>6 months-old) CPHs, with a mean age of 6.6 ± 4.8 (2.5 - 16 y), a mean weight of 371 ± 30 kg, and a mean body condition score of 7/9(Henneke et al., 1983) were chosen at convenience. The study population included nine non-pregnant females and two uncastrated males. The gaits corresponded to paso fino ($n = 5$), trot ($n = 1$), and trocha ($n = 5$). Animals were clinically healthy on physical examination, with a complete and updated health plan (vaccines and deworming) at the time of the study (data not shown). The animals were under complete housing and fed on pangola grass hay (95% dry matter *Digitaria eriantha*; 2.5 kg/d on average), green forage (20% dry matter *Pennisetum purpureum*; 30 kg/d on average), commercial balanced feed (2 kg/d on average), mineral salt (100 g/d), and water *ad libitum*.

2.4. Field exercise test

A 30-minutes-long standardized field exercise test, composed of four steps with increasing-intensity —including rest periods and a final recovery was carried out. The HR was measured using an Ambit 3 sensor (Suunto®, Finland). The HR monitor was validated with another device and an electrocardiograph (data non-published). The protocol used consider controlling the intensity of the exercise at each step (warm-up, 58 to 65% of the maximum HR + moderate intensity, 65 to 75% of the maximum HR + high intensity, 75 to 85% of the maximum HR + maximum intensity, ≥ 85% of maximum HR) and, at every step a rest-period of 1 min was experimented (Arias, Maya & Arango, 2019) (Table 1). The theoretical maximum HR reported for the breed is 221 ± 17 beats per minute (bpm) (Arias, Sánchez, Duque, Maya & Becerra, 2006).

The track used was 35 m in length × 20 m in width, and the surface was covered by dry silt as those used in competitions. Under these conditions, 5 min of continuous exercise were guaranteed in each stage of the test.

2.5. Blood lactate concentration and clearance curve

A venous blood sample was obtained from the jugular vein and collected in a tube with EDTA, using a vacuum system. Samples were collected at rest, immediately after each step, and 10 min after the end of the test (recovery). A total of 10 μl were collected to measure the BLConc, using the Nova Plus® portable device (Nova biomedical, USA). The BLConc and HR were used to calculate the HR at which a BLConc of 4 mmol/L or HRL_4 was reached. The blood lactate clearance (BLClear) was calculated using the BLConc at the end of the maximum intensity step (BL_{max}) and at the recovery (BL_{recov}), using the equation $[(BL_{max} - BL_{recov})/BL_{max}]$.

2.6. Statistical analyses

Descriptive results were reported for all variables, as median, interquartile range (IQR), standard deviation (SD), and coefficient of variation (CV). Shapiro-Wilk test was used to normalize the research data. A Wilcoxon signed-rank test or *u*-test for paired samples (non-parametric alternative to *t*-test), with a confidence level of 95% was used to compare the median range of two related or paired samples for each horse in the study and for each variable of interest. The statistical software Stata 16.0 (StataCorp, 2020, College Station, Texas, USA) was used for all analysis. The HRL_4 was calculated using the equation produced by the scatter plot and the exponential trend generated in Microsoft Excel® as previously reported by the literature (Fraipont et al., 2012). The R^2 of the equations obtained ranged between 0.82 and 0.97.

3. Results

3.1. Heart rate

The HR increased according to the protocol used for the field exercise test (Table 1). During recovery, this index was similar to that during warm-up (Fig. 1).

3.2. Blood lactate concentration

The BLConc during the field exercise test was variable. Despite having been increasing like the HR, the distribution of the values in each step of the test was remarkably dispersed. Elevated blood lactate values were found in some of the individuals during the moderate, high, and maximum intensity steps (Fig. 1), which affected the median value of each step, as can be seen in the IQR, SD, and CV reported herein Table 2. Neither the BLConc nor the HR were significantly different when the medians of the test steps were compared. In addition, it was determined

Table 1
Scheme of the field exercise test used in the Colombian Paso horses of study.

	Initial heart rate	Warm-up	Moderate intensity	High intensity	Maximum intensity	Recovery
Intensity (% of maximum heart rate)	<50	58 to 65	65 to 75	75 to 85	≥ 85	**
Length (in minutes)	ǂ	15	5	5	5	10

** = Heart rate at 10 min after the end of the test, walking the animal; ǂ = Length of the preparation of the specimen for testing.

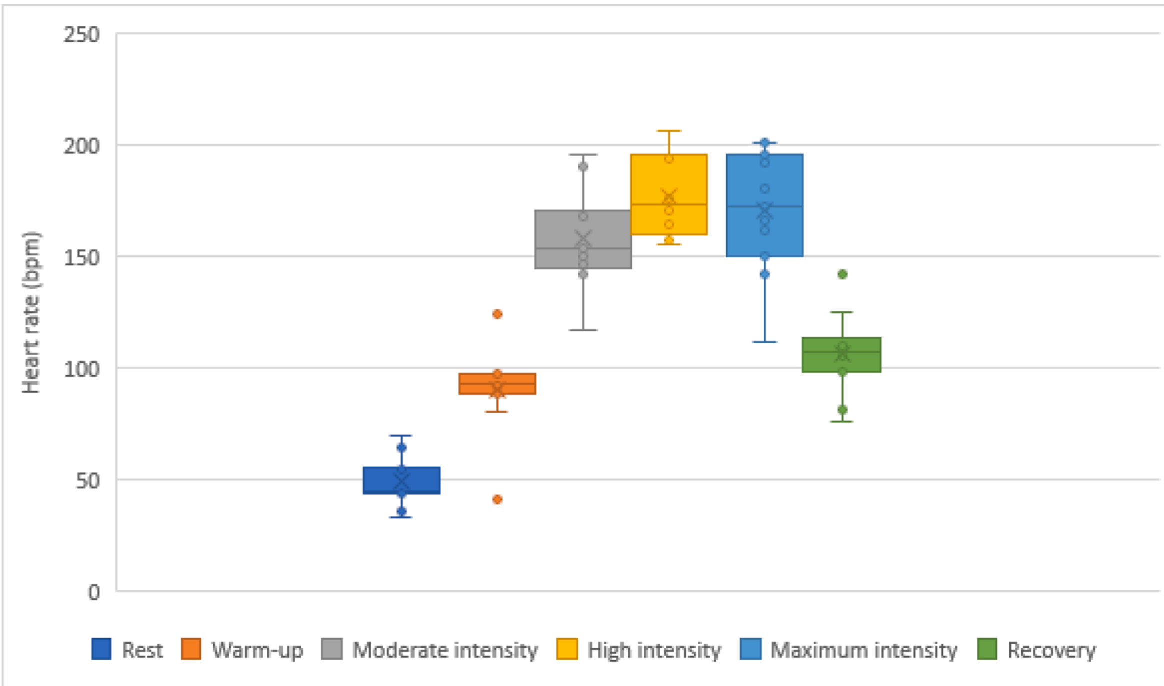

Fig. 1. Distribution of heart rate values obtained at each step of the field exercise test used in the Colombian Paso horses of study.

Table 2
Heart rate and blood lactate concentration results obtained at each step of the field exercise test used in the Colombian Paso horses of study.

Step	Heart rate (bpm)			Blood lactate concentration (mmol/L)		
	Median (IQR)	SD	CV	Median (IQR)	SD	CV
Initial (<50% of the maximum HR)	45 (44 – 54)	11.3	0.23	0.6 (0.5 – 1.1)	0.53	0.67
Warm-up (58 to 65% of the maximum HR)	93 (88 – 97)	19.6	0.22	0.8 (0.6 – 2.2)	1.06	0.78
Moderate intensity (65 to 75% of the maximum HR)	154 (145 – 171)	22.5	0.14	2.7 (1.2 – 3.8)	1.54	0.57
High intensity (75 to 85% of the maximum HR)	173 (160 – 196)	18.0	0.10	4.2 (2.2 – 5.6)	2.33	0.52
Maximum intensity (≥ 85% of the maximum HR)	172 (150 – 192)	28.0	0.16	5.1 (3.1 – 9.3)	3.13	0.55
Recovery	107 (98 – 110)	18.3	0.17	1.9 (1.1 – 3.6)	1.32	0.57

IQR = Interquartile range, SD = Standard deviation, CV = Coefficient of variation, HR = Heart rate.

that the mean BLClear percentage was 56.3 ± 16. Fig. 2

3.3. *Anaerobic threshold and blood lactate clearance*

The $HRL_4$was reached at a notably different HR among individuals (132 to 251 bpm). In addition, the BLClear produced during the field exercise test was similar in most of the animals (Table 3).

4. Discussion

CPH is the most popular breed in Colombia and has been scarcely studied as an athlete. However, it has recently been included within the group of athlete horses, since when it performs in its gait, it experiences a physical effort of moderate to maximum intensity (Arias et al., 2006) and is under arduous training to execute it.

Some physiological features related to the physical working capacity of this type of horse have been described, such as its muscular composition (Enríquez, Granados, Arias & Calderón, 2015) and the average and maximum HR (Arias et al., 2006). Nevertheless, there are no other findings related to physical working capacity for the breed, other than theoretically and in accordance with what has been described for other equestrian disciplines. Such capacity has been also determined by the size of the heart (Young et al., 2002) and the horsés willingness to work (McBride & Mills, 2012).

Research on CPHs have reported HRs according to gait and have defined a theoretical maximum HR of 221 ± 17 bpm (Arias et al., 2006). However, the information remains scarce and does not allow the training protocols to be designed on a technical and specific basis.

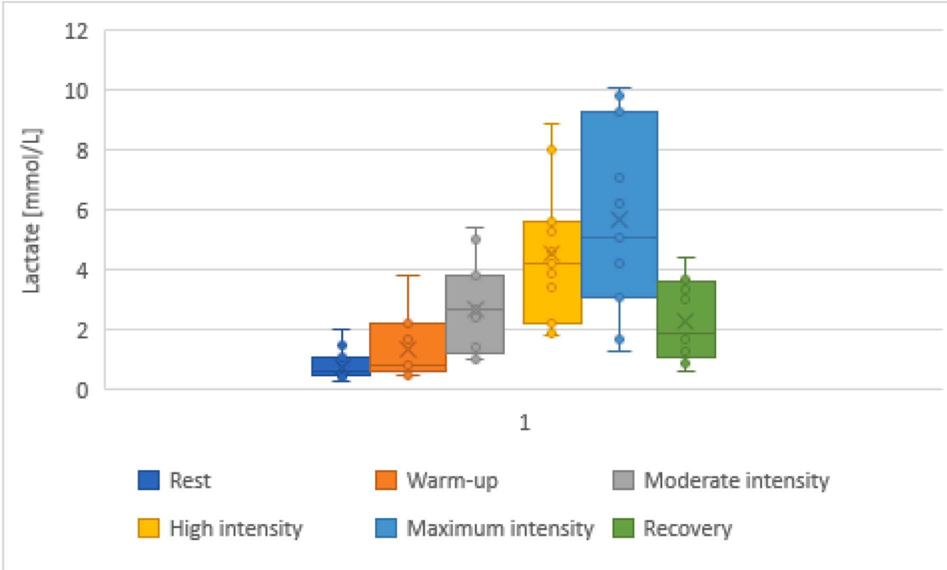

Fig. 2. Distribution of blood lactate values obtained at each step of the field exercise test used in the Colombian Paso horses of study.

Table 3
Heart rate at which the anaerobic threshold was reached, and the percentage of blood lactate clearance in the Colombian Paso horses of study (presented individually).

Animal	HRL_4 (bpm)	Blood lactate clearance (%)
Horse 1	223	58
Horse 2	178	58
Horse 3	132	56
Horse 4	160	60
Horse 5	140	63
Horse 6	215	63
Horse 7	168	22
Horse 8	184	69
Horse 9	251	65
Horse 10	165	74
Horse 11	205	31

HRL_4 = Anaerobic threshold, bpm = Beats per minute.

Furthermore, little is known about the physiological limits that are reached with the performance of these sports activities. Consequently, it is relevant to develop research around the characterization of these animals' athletic activity to improve their performance and well-being conditions.

Although HRL_4 is not as widely used as V_4, this parameter is valuable when the displacement speed does not reflect an improvement in sports performance, especially in horses that perform activities as particular as CPHs, since during competitions, the judges do not evaluate how long a participation lasts. It has been argued that HRL_4 is an interesting value, to be applied at different ages of the horse, regardless of the training stage (Courouce, Chrétien & Valette, 2002).

The present study has confirmed that the field exercise test is a useful approach to evaluate the cardiac and metabolic response in CPHs, using few and inexpensive equipment, as has been reported for Enduro horses under field and treadmill-based protocols (Fraipont et al., 2012). The study reported that the aerobic (V_2 and HRL_2) and anaerobic thresholds (V_4 and HRL_4) in Enduro horses were different under treadmill and field-based protocols, despite having been studied in the same group of animals. Such results indicate that the presence of the rider is decisive in the intensity of the exercise. In the CPHs of the present study, the effect of the rider on exercise intensity was not explored, which should be an aspect to examine in depth (Allen et al., 2016).

As previously reported, the maximum HR of each horse in the present study was different. This situation results from individual peculiarities in cardiac capacity and size, and mental willingness to work (central fatigue) (McBride & Mills, 2012). It should be noted that not all individuals reached the theoretical maximum HR for the Paso horse (221 ± 17 bpm); therefore, it is important to determine the individual maximum HR of each animal, and thus design the training in consideration of working volumes that are equivalent among individuals, using methods such as relative HR, percentage of HR reserve (%HRR), or percentage of oxygen consumption reserve (%VO_2R) (Mann, Lamberts & Lambert, 2013). In addition, to maintain the increasing intensity of exercise to adequately visualize the physiological changes is suggested, in accordance with the sequential activation of the response mechanisms to physical effort. The above highlights the importance of validating field exercise tests that are reproducible and specific for the type of physical effort performed by a certain group of competing horses.

In this sense, untrained horses were used herein because the physiological responses to exercise at different intensities was not known for the breed; therefore, there was no baseline information to evaluate the evolution in the application of any training protocol. On the other hand, CPHs are usually trained under different conditions and intensities, according to the riders' expertise. Therefore, trained animals have previously non-controlled physiological adaptations which would include study bias. Changes occurring under controlled conditions are the subject of other research approaches.

After 10 min of recovery, the HR did not seem to agree with the HR obtained at rest, although it was similar to the one obtained during warm-up. This finding is explained by the phenomenon of cardiac recovery, which occurs in two phases: rapid (adrenal sympathetic response) and slow recovery, that in conjunction with the normalization of blood volume, splenic contraction, and thermoregulation, can take a few hours to return to the frequency and rhythm of rest of the animal (Lindner, Esser, López & Boffi, 2020). This is how the HR recovery is usually rapid in the first minute after exercise (Evans, 2007), and then slowly decreases over several hours (depending on the intensity of the exercise), until reaching the resting values (Lindner et al., 2020). In addition, the HR recovery time at rest values has been reported to be greater in untrained horses compared to trained ones (Hodgson et al., 2014), as could be registered in the CPHs of the present study.

Handling of horses after exercise also determines HR recovery time. Horses that walk with the rider at recovery have been reported to have a faster recovery at rest than those that remain inactive (Lindner et al., 2020). In the present study, the horses walked with the rider for the entire recovery time.

In the case of CPHs, adaptation to different environments is essential, since, HRs were not altered by external factors such as noise or the presence of other animals. According to the authors' experience, the temperament of these horses and their response to external stimuli influence HR variations that are not characteristic of the physical effort carried out *per se*.

As the field exercise test increases, the HR increases linearly with respect to the displacement speed in horses other than Paso ones, due to the increase in the release of catecholamines associated with exercise (Hodgson et al., 2014; Nagy, Murray & Dyson, 2014). In contrast, the HR increases in relation to exercise time and to the speed of gait-related effort in CPHs, according to the number of strokes per minute.

According to the authors' experience, HR can also increase depending on the surface on which the horse exercises, since the sound of its horseshoes produces a state of excitement for the breed. The present study was carried out in a closed space, without an audience and without the use of the resonance track to avoid such effect.

The *stable* state was not identified or interpreted during the present study, since this parameter must be reached by the animal spontaneously. Nevertheless, the exercise intensity was controlled through HR monitoring during the test.

Lactate production was markedly different among individuals and test steps. The study group consisted of untrained animals, of which differences in aerobic capacity for athletic performance are expected and, therefore, differences in the use of anaerobic metabolism during exercise are expected as well. The lactate values recorded in the high and maximum intensity steps agree with those reported in the literature for horses under field-based tests (Capacho & Arias, 2019) and exceeded the HRL_4, as recommended in a maximal intensity test, which means that metabolic behavior is related to the intensity of exercise in this type of animal.

The blood lactate measurement must be preceded by an adequate pre-analytical phase. The use of venous or arterial blood is indistinct, but the type of container, additive, and storage time and temperature of the sample is decisive. Red blood cells obtain energy (as ATP) only through anaerobic glycolysis due to the absence of mitochondria (Boffi, 2007), and when using plasma glucose, lactic acid is produced, among other residues. For this reason, it is recommended to perform the measurement immediately after taking the sample in a vacuum tube with EDTA, using a portable meter (Allen et al., 2016; Henderson, 2013), as carried out in the present study. Delays in testing can result in lowered glucose values and falsely elevated blood lactate-related values.

The BLConc in a horse at rest must be below 1 mmol/L (Allen et al., 2016; Henderson, 2013). In fact, the resting BLConc has been reported before and after applying two training protocols, with 0.92 mmol/L as a maximum value in CPHs (Arias et al., 2019). Small increases in this concentration occur as the intensity of the exercise increases, and at the moment in which the production exceeds the removal, the BLConc increases exponentially (Allen et al., 2016;(Neto and Espinosa, 2009).

The rate at which lactate accumulates depends on animal inherent factors, including the cardiac delivery rate of oxygen to the working muscle (DO_2), the ability of the muscle cell to extract oxygen (VO_2), and the rate of lactate metabolism in the muscle cell during exercise. These factors are limited by the physiological characteristics of the horse as an individual —and also by age, but can be improved with training (Neto and Espinosa, 2009). These physiological differences among individuals explain why both BLConc and BLClear were different in the study animals. The study group included young and adult animals, of which it is known that some muscle fibers can change the type of metabolism depending on the training received (Enríquez et al., 2015), which could explain the results obtained herein. Hence, the importance of individually performing the field exercise test and avoiding transferring results from one animal to another, despite the fact that they are under similar handling conditions. In addition, it is advisable to directly measure —and not to calculate, variables such as HR, blood lactate, and VO_2 (Allen et al., 2016).

Through incremental exercise tests, the HR and the rate at which lactate accumulation begins above 4 mmol/L, is determined. At this point, it is considered that anaerobic metabolism predominates in energy generation (Neto and Espinosa, 2009). In CPHs, the incremental test allows knowing the anaerobic threshold, but not as a function of the speed of movement but rather of the intensity of the exercise. Consequently, the performance indicator used in the present study was HRL_4. This occurs because the speed of movement is not related to the athletic performance in CPHs, and therefore, it is not directly related to the intensity of the exercise.

The reaching of the HRL_4 is indicative of an imbalance between anaerobic cellular metabolism and the reuse of lactate. Most of the study animals reached such a threshold below 200 bpm, and some of them even reached it when they were exercised at low intensity. This means that their ability to exercise without fatigue was low for about 30 min (test duration). This information is relevant for the breed, since they are usually used for horseback riding, long distances are covered and periods of more than 30 min are performed in consideration of the gait (Palacio, De la Osa, Arias, Zuluaga Araque & Gómez, Restrepo, 2013).

The speed to reach the HRL_4 in some of the CPHs in the study can be explained by the high proportion of type II muscle fibers, as has been previously reported for the breed (Enríquez et al., 2015). Despite rapidly reaching the HRL_4, most of the study animals showed BLClear close to 60%. This can be explained by variations between the proportion of muscle fibers IIA and IIX of each horse and by the oxidative capacity of type I and IIA fibers, capable of reusing lactate as an energy source during recovery through gluconeogenesis, and its consequent removal of blood circulation (Enríquez et al., 2015). The interpretation of BLConc must be made according to its metabolism, given its synthesis and removal. Since lactate is a carbohydrate, its blood concentration is dynamic and it can be reused through hepatic and muscular gluconeogenesis (Henderson, 2013; López & Fernández, 2006).

The HRL_4 and BLClear did not show any tendency, confirming that the aerobic capacity during exercise and the activation of the BLClear mechanisms are independent, and therefore, they are characteristics that need to be objectively promoted through training.

Regarding BLClear, it is important to note that decreased renal perfusion also affects lactate elimination (Henderson, 2013). Profuse sweating was noted in the study animals at the end of the test, indicating that they experienced dehydration and possibly decreased renal perfusion.

The recommended way to calculate HRL_4 is by promoting an increase in intensity and a BLConc greater than 4 mmol/L (Fraipont et al., 2012). However, some animals will not achieve this due to their high aerobic capacity or efficient clearance mechanisms, making the calculation of HRL_4 difficult to define.

The pauses that are added to the scheme of a field exercise test both in the field and on the treadmill, are discussed, since they can promote the activation of lactate removal mechanisms and at the end do not show the more faithful measurement of this analyte. For this reason, some authors recommend measuring BLConc at rest and at the end of the test, without pauses between steps (Allen et al., 2016).

The standardization of a field exercise test for a particular discipline is useful for the evaluation of disorders induced by exercise (Allen et al., 2016), since it allows knowing the behavior of performance indices in a healthy horse and to compare it with a non-healthy one.

Given the limited number of animals included in the present study, it is not possible to infer the results in the CPH population. However, it is a baseline for the construction of breed-specific sporting parameters.

In conclusion, the field exercise test is useful to assess BLConc and HR changes in acute response to exercise in CPHs. It would be useful to evaluate training kinetics with other parameters, including cell blood count and muscle enzymes. To the best of our knowledge, this is the first report on the athletic performance using metabolic parameters such as HRL_4 in Paso horses, suggesting it as a potential indicator of conditioning and evolution of physiological adaptations to training, comparing the animal with itself.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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