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Effect of high-intensity ground and pool exercise on cortisol, lactate levels, heart rate, and blood count in Colombian Paso horses

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Abstract

Background: The Colombian Paso Horse develops different gaits and undergoes demanding training, being considered a high-performance athlete. Therefore, it is essential to implement specific training schemes. Aim: This study compared the changes in cortisol concentration, blood lactate levels, heart rate, and blood count induced by exercise performed on a hard surface versus in a pool in Colombian Paso Horses. Methods: A total of 10 Colombian Paso Horses, of both sexes (6 males and 4 females), with an average weight of 350±20 kg, a body condition score of 5-6/9, and an average age of 10±4 years, underwent high-intensity exercise on a hard surface and in a pool, with a 30-day interval between exercise modalities. This was a prospective and descriptive study. Venous blood samples were collected to measure cortisol, blood lactate concentrations, and blood count, and heart rate was recorded before and after exercise for each modality to facilitate comparisons. Results: The variables cortisol, lactate, heart rate, and blood count showed significant changes between pre- and post-exercise periods in both modalities, indicating higher exercise intensity on the hard surface. Conclusion: Pool exercise is a viable alternative for athletic training in the Colombian Paso Horse breed. However, individual responses should be considered, and the advantages and disadvantages of each training modality should be weighed.

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Introduction

The Colombian Paso Horse (CPH) breed is known for its phenotype, elegance, striking appearance, and smooth movement. It demonstrates various gaits (trot, canter, trocha, and paso fino), which are judged in competitions. During any of these gaits, the horse must exert moderate to maximum physical effort and therefore requires rigorous training to perform well. Consequently, it is now regarded as a high-performance athlete (1). Despite advances in understanding the sports conditioning of the CPH, including studies on the minimum and maximum heart rate values, muscle fiber types, and variations in hematological, biochemical, metabolic, and endocrine parameters in horses undergoing field and pool exercise tests (1-5). Different training protocols remain traditional, based on hard surfaces, empirical, and extrapolated from other breeds with various disciplines. However, in recent years, pool training has been implemented as an alternative protocol, offering multiple benefits (5). Cortisol is a steroid hormone produced by the adrenal cortex cells, whose synthesis and release are regulated by activation of the hypothalamic-pituitary-adrenal (HPA) axis through the action of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH). It is widely used as a biomarker for evaluating stress, both in response to disease, pain, and alterations in well-being, and for predicting the prognosis of various equine pathologies such as gastrointestinal and respiratory diseases and clinical cases of sepsis, as well as an indicator of physical fitness and athletic performance in sport horses (6-10). This hormone is characterized by high variability, influenced by individual adaptive capacity and by geographical and environmental factors (11-13). In sports medicine, cortisol is an indicator of the response to exercise and physical conditioning, facilitating the mobilization of energy sources (hyperglycemia), anti-inflammatory effects, and tissue regeneration (14). Additionally, it has been considered a marker of stress induced by physical activity; therefore, understanding the effects of cortisol on exercise is essential to ensure the health and well-being of horses in sports (15,16). Proper management of cortisol levels, along with variables such as lactate concentration and heart rate during intense exercise, is critical to minimize adverse effects and ensure that horses maintain optimal performance without compromising their health (17).

Studies on the acute response of blood cortisol concentration to exercise performed on hard surfaces and in pools in CPH are absent, despite cortisol being an important indicator of physical condition and athletic performance in sport horses. In this regard, this study aimed to compare the changes in cortisol concentration, blood lactate, and heart rate induced by both exercise modalities in CPH.

Materials and methods

Ethical approval

All procedures were approved by the Bioethics Committee for Animal Research of the Faculty of Veterinary Medicine of the Remington University Corporation (No. 11-2022) and carried out in accordance with the relevant laws and guidelines.

Study location

This study was conducted at the facilities of a sports training center located in the tropical region, at 6º05’00.84” North latitude and 75º20’05.23” West longitude, 2,150 meters above sea level, with a relative humidity of 69%, an average environmental temperature of 16ºC, and an annual precipitation of 2,117 mm³.

Study population

A total of 10 CPHs of both sexes (6 females and 4 males), with an average weight of 350±20 kg, body condition score of 5-6/9 (18), and an average age of 10±4 years, were used for this study. All horses were maintained under similar management conditions, housed in stables, and fed a diet based on hay (*Digitaria eriantha*), green forage (Pennisetum purpureum), commercial concentrate (2 kg/day) (Campeón Dorado, Solla, Itagui, Colombia), mineralized salt (60 g/day) (Brío salts, Italcol, Girardota, Colombia), and had access to water ad libitum. All animals had up-to-date sanitary management and periodic dental care, ensuring they were 0clinically healthy.

Experimental protocol

With a 30-day adaptation period before the experiment and under similar management and feeding conditions throughout the study, this study included two types of exercise: hard-surface and pool-based, with a 4-week rest period between modalities. Before and after the execution of both exercise modalities, the horses were evaluated by general clinical examination to determine body temperature (°C), pulse rate, heart rate, and respiratory rate. In the ground training modality, horses underwent a high-intensity exercise regimen. Each session lasted 40 minutes and included 10 minutes of warm-up, 20 minutes of moderate (70-80% of Maximum Heart Rate (MHR) to high-intensity exercise (80-90% of MHR), and 10 minutes of cool-down at a walk. In the swimming modality, horses exercised at moderate intensity (70-80% of MHR) in a circular pool with a 23.3-meter lap. Each session lasted 40 minutes, divided into 10 minutes of warm-up, 20 minutes of intermittent swimming, and 10 minutes of stretching exercises and cool-down at a walk. During the swimming phase, horses performed two consecutive laps in one direction, followed by a one-minute pause before switching direction. They swam two laps to the right, paused, then two laps to the left. This cycle was repeated four times, totaling 16 laps in approximately 20 minutes. Training frequency was three sessions per week for swimming and five for ground training. In this study, ground exercise was performed first.

Blood sample collection

Blood samples were collected before and after each session for each exercise modality from the jugular vein, with prior preparation and an antiseptic protocol at the venipuncture site. The samples were placed in tubes with or without EDTA anticoagulant and transported to the laboratory under refrigerated conditions (4ºC) for analysis. Anticoagulated samples were used for hematological analyses, including complete blood counts, plasma protein quantification (albumin and globulin), and lactate measurement. Non-anticoagulated samples were centrifuged at 3000 rpm for 10 minutes to separate serum and plasma, which were subsequently aliquoted into 1.5 mL Eppendorf tubes and preserved at −20°C until further analysis. The hematological parameters, including red blood cell count, hematocrit, hemoglobin, white blood cells, neutrophils, lymphocytes, eosinophils, and platelets, were measured using a Genrui VH-50 automated hematology analyzer (Genrui-bio, Shenzhen, China). Total and differential plasma proteins were also quantified. Comprehensive quality control procedures were performed during the analysis to ensure the precision and validity.

Heart rate measurement and blood lactate determination

Heart rate was measured using a Suunto Ambit3 vertical® monitor (Vantaa, Finland: Suunto; 2021), and lactate concentration was determined in the field using a NOVA

Lactate Plus portable device (NOVA Biomedical, Waltham, MA, USA), which employs enzymatic-amperometric detection with the lactate oxidase reagent. Both measurements were taken in the pre- and post-exercise periods of each modality.

Serum cortisol analysis

The ELISA assay was performed according to the recommendations of the Cortisol AccuBind ELISA Kit (Monobind Inc). A 25 µL sample of serum was pipetted into each well, and controls/calibrators were added. The negative control and each of the seven calibrators were performed in duplicate. Fifty µL of enzyme-cortisol reagent was added to each well, excluding the blank. The plate was shaken for 30 seconds, and 50 µL of biotin-cortisol reagent was added to each well. The plate was shaken again for 30 seconds, covered with plastic wrap, and incubated at room temperature for 60 minutes. After incubation, the plate was washed three times with 350 µL of wash buffer for each well. The plate was then dried completely, and 100 µL of the working substrate was added to each well. The plate was incubated for 15 min, followed by the addition of 50 µL of stop solution to each well, with agitation for 20 seconds. The plate was read using a spectrophotometer at a wavelength of 450 nm (BioTek ELx800). The sample concentrations (µg/dL) were determined by linear regression from the points generated by each of the seven calibrators.

Statistical analysis

All study data were stored in a Microsoft Excel database (Microsoft, New Mexico, USA) for later analysis using SPSS version 29.0 (IBM, Armonk, New York, USA). Descriptive analysis was performed for all variables, and the Shapiro-Wilk normality test was applied, considering normal distribution if (P>0.05). To identify differences between variables and exercise modalities in the pre- and post-exercise periods, tests for related and independent group comparisons were used. The Student t-test was used when the data were normally distributed, and the Wilcoxon test was used when the data were non-normal. The significant value was set at P<0.05.

Results

In the concentration and behavior of serum cortisol across the periods of each exercise modality, cortisol levels were compared, showing a statistically significant difference between the periods. Higher levels of this analyte were observed in the post-exercise period, especially in the hard-surface exercise modality (Table 1). There was also noticeable variability in cortisol concentration among individuals, even within the periods of each modality.

The comparison of each period between the exercise modalities is shown in table 2, where a statistical difference was observed only between the post-exercise periods. A greater increase was confirmed in the hard-surface exercise modality, coinciding with the highest values of heart rate and lactate concentration, as well as greater changes in the blood count in this same period. However, the difference was also significant between both periods in each modality, as presented in table 3.

Table 1: Mean values (±SD), minimum and maximum values, and confidence intervals of serum cortisol concentration (µg/dl) for each period in both exercise modalities (hard surface and pool) of the 10 Colombian Paso Horses

Period - Modality	Mean	SD	Mín.	Máx.	Confidence Interval 95%		*P value*
					Lower	Upper	
Pre-exercise in the pool	8,51	4,08	1,16	13,51	-3,580	- 0,521	0,014*
Post-exercise in the pool	10,56	3,54	5,13	14,41			
Pre-exercise on the surface	10,05	3,45	4,56	16,06	-5,782	- 2,113	0,001*
Post-exercise on the surface	14	2,09	10,89	17,68			

SD: Standard deviation, Min: Minimum, Max: Maximum, *Statistically significant difference (P<0.05).

Table 2: Comparison of serum cortisol concentration (µg/dl) between the pre- and post-exercise periods of both modalities (hard surface exercise and swimming exercise) for the 10 Colombian Paso Horses

Period - Modality	Mean difference	Confidence Interval 95%		*P value*
		Lower	Upper	
Pre-exercise in the pool Pre-exercise on the surface	-1,546	-5,1034	2,011	0,373
Post-exercise in the pool Post-exercise on the surface	-3,443	-6,2238	-0,663	0,018*

*Statistically significant difference (P<0.05).

Table 3: Means (±SD) of heart rate, blood lactate concentration, and blood count in the pre- and post-exercise periods of the hard surface and pool modalities for the 10 Colombian Paso Horses

Parameters	Exercise on the surface		P value	Swimming exercise		P value
	Pre-exe	Post-exe		Pre-exe	Post-exe	
HR (bpm)	89±18	203±10	0.01*	94±19	215±7.0	0.01*
Lactate (mg/dl)	1.04 (0.88-1.37)+	5.80 (4.35 11.03)+	0.00*	1.29±0.98	2.46±1.11	0.00*†
Erythrocytes (10^6/mm^3)	7.7±0.9	11.4±1.0	0.00*	8.7±1.7	10.4±1.0	0.01*
Hematocrit (%)	35.4±4.0	50.1±6.1	0.00*	35.9±4.1	46.2±4.5	0.00*
Hemoglobin (g/dl)	13.3 (10.9-15.2)+	16.3±2.2	0.12†	12.4±1.4	16.6±1.4	0.00*
Leukocytes (10^3/mm^3)	10.2±2.3	9.8±2.3	0.71	7.1±1.4	7.0±0.8	0.78
Neutrophils (10^3/mm^3)	4.9±2.8	6.4±1.9	0.08	3.1±1.1	3.6±0.8	0.13
Lymphocytes (10^3/mm^3)	3.6±1.7	5.2±1.8	0.03*	3.1±1.0	2.9±1.0	0.49
Eosinophils (10^3/mm^3)	0.1 (0-0.6)+	0 (0-0.1)+	0.12†	0.03 (0-0.3)+	0.3 (0.1-0.1)+	0.13†
Platelets (10^3/mm^3)	240.6±121.5[1]	164.8±48.5	0.13	227.3±38.30	217.6±64.5	0.59
Plasma proteins total (g/dl)	8.2± 2.4	7.9±0.8	0.65	6.8±0.8	7.2±1.3	0.46
Albumin (g/dl)	3.4±0.6	3.8±0.5	0.22	3.4±0.4	3.3±0.5	0.61
Globulins (g/dl)	3.6±0.6	4.1±0.6	0.03*	3.2±0.4	3.2±0.5	0.80

HR: Heart rate, bpm: beats per minute, Pre-exe: Pre-exercise, Post-exe: Post-exercise, †: Wilcoxon test result value, +: Median and interquartile range, *Statistical significance: P value ≤ 0.05.

Discussion

The CPH, in addition to performing various gaits with particular characteristics that demand strenuous physical preparation, is also classified as a high-performance athlete (1) and is globally recognized for its nervous temperament and elevated spirit. These, along with the need for commands from the interaction of the bridle with the mouth to execute its gait, make the rider's weight part of the increased intensity of exercise (19, 20). Therefore, standardized treadmill tests used for physical training in other breeds are limited for this breed (21, 22), with field exercise tests being preferred to assess the effort required for training and competition (19).

The behavior of the lactate and heart rate variables between the pre- and post-exercise periods of both modalities evaluated indicated acute exercise responses. Thus, they are relevant parameters within field exercise tests, as previously described (19). In both the hard-surface and pool exercises, the average heart rate during the post-exercise period exceeded 200 bpm, indicating that the race breeds had reached their anaerobic threshold. However, blood lactate levels did not exceed 4 mmol/L (72 mg/dL), which would typically indicate the same condition (23). Despite this, the results for both variables were consistent with previous studies on the same CPH breed (19). This demonstrates differences in exercise response across breeds and sports disciplines.

Under the conditions of the present study, greater changes in heart rate and lactate concentration were observed in the post-exercise period of the hard-surface modality, indicating greater effort and intensity, which coincided with previously described maximum-intensity exercise in the same breed (19). Meanwhile, during the same post-exercise period in the pool exercise modality, the intensity was comparable to that reported for a swimming protocol in obese CPHs, which resulted in reductions in body fat (5). Thus, sports training programs should be tailored to the specific context, scenario, and characteristics of each breed.

The increase in cortisol in horses after intense exercise is a well-documented phenomenon, essentially linked to the stress response. It plays a key role in mobilizing energy resources, such as glucose, to meet the muscular demands during exercise (24). This increase in cortisol is part of the body's adaptive response to physical effort, facilitating the rapid mobilization of resources to maintain physical performance, especially under high-energy-demand conditions (25). Given the significant increase in cortisol in the post-exercise period, it can be inferred that the exercise in both modalities was intense, with a higher requirement in the hard-surface modality.

Cortisol, in addition to contributing to metabolic function to enhance energy reserves, also exerts cardiovascular effects that help cope with the effort, as well as inducing the inflammatory response associated with changes in the hemo-leukogram and immune system (26). This is related to increases in heart rate and lactate concentration, and alterations in the blood count, observed in the post-exercise period of both modalities, but with a greater difference in the hard-surface exercise. Therefore, cortisol was used as a physiological marker to assess both the horse's physical conditioning and their stress response to exercise under tropical conditions (17,27,28).

The values and the response of serum cortisol to exercise reported in this study should be interpreted with caution due to the wide variability range of the analyte and the elevated concentrations in the CPH breed, compared to other breeds from areas far from the tropics (29), probably due to breed-specific traits, management system, and geographic location.

Additionally, during training and competitions, these horses are subjected to hyperflexion of the neck, both dorsally and ventrally, to enhance the visual appeal and elegance of their gait (30). This posture has been associated with increased cortisol secretion, possibly related to discomfort or stress caused by the training position and restricted head and neck movement (31-33). This behavior likely contributed to the increased cortisol levels observed during hard-surface exercise.

On the other hand, cortisol levels at rest and after exercise varied within the normal range reported for this breed. However, it is essential to consider the circadian rhythm of this hormone as well (29). Similarly, assessing the cortisol index as an indicator of chronic stress (34) should be considered, especially since these horses are kept in stabling conditions, a factor that predisposes them to stress. This assessment would help clarify whether the observed increase in cortisol originates from exercise-induced effort or from chronic stress. Moreover, continuous training has been shown to promote a faster rate of cortisol elimination (15).

The results of the variables analyzed in this study, associated with changes in blood count, showed a greater impact of the exercise response in the hard-surface modality, accompanied by greater stress, as reflected in elevated cortisol concentrations. This greater response to exercise on the hard surface is likely related to the fact that the horses used in this study were not previously trained, which may have increased their physiological stress and, consequently, their cortisol levels. To confirm this interpretation, future studies should include a larger number of conditioned animals and extend the evaluation period, allowing a more precise characterization of the dynamics of hematological and hormonal responses to high-intensity exercise. In contrast, pool exercise, although it also induces measurable physiological changes, appears to generate a milder response and could therefore be a valuable alternative for improving conditioning and maintaining athletic performance with lower physical stress.

Conclusion

The results obtained emphasize the importance of considering individual, environmental, and management factors when evaluating the impact of exercise on CPH. Training in a pool, although it elicits physiological changes in response to effort, does not produce as intense a load as hard-surface exercise, suggesting its usefulness in physical conditioning programs with lower joint impact. Therefore, specific contexts, scenarios, and individual breed factors should be considered in sports training protocols.

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Conflict of interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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تأثير التمارين عالية الكثافة على الأرض وفي حمام السباحة على مستويات الكورتيزول واللاكتات ومعدل ضربات القلب وتعداد الدم في خيول باسو الكولومبية

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الخلاصة

يطور حصان باسو الكولومبي مشيات مختلفة ويخضع لتدريبات شاقة، ويعتبر رياضيًا عالي الأداء. ومن ثم، فمن الضروري تنفيذ خطط تدريبية محددة. قارنت هذه الدراسة التغيرات في تركيز الكورتيزول ومستويات اللاكتات في الدم ومعدل ضربات القلب وتعداد الدم الناجمة عن التمارين الرياضية التي يتم إجراؤها على سطح صلب مقابل تلك التي يتم إجراؤها في حمام السباحة في خيول باسو الكولومبية. خضع ما مجموعه ١٠ خيول باسو كولومبية، من كلا الجنسين (٦ ذكور و ٤ إناث)، بمتوسط وزن ٣٥٠ ± ٢٠ كجم، ودرجة حالة الجسم ٥-٩/٦، ومتوسط العمر ١٠ ± ٤ سنوات، لتمارين عالية الكثافة على سطح صلب وفي حمام سباحة، مع فاصل زمني مدته ٣٠ يومًا بين طرق التمرين. وكانت هذه دراسة استطلاعية ووصفية. تم جمع عينات الدم الوريدي لقياس الكورتيزول وتركيزات اللاكتات في الدم وتعداد الدم، وتم تسجيل معدل ضربات القلب قبل وبعد التمرين لكل طريقة لتسهيل المقارنات. أظهرت متغيرات الكورتيزول واللاكتات ومعدل ضربات القلب وتعداد الدم تغيرات كبيرة بين فترات ما قبل وبعد التمرين في كلتا الطريقتين، مما يشير إلى ارتفاع شدة التمرين على السطح الصلب. تعتبر التمارين الرياضية في حمام السباحة بديلاً قابلاً للتطبيق للتدريب الرياضي في سلالة خيول باسو الكولومبية. ومع ذلك، ينبغي النظر في الاستجابات الفردية، وينبغي الموازنة بين مزايا وعيوب كل طريقة تدريب.