



Hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise in Colombian Paso horses



Angélica María Zuluaga Cabrera ^{a*}

Maria José Casas Soto ^b

José Ramón Martínez Aranzales ^a

Viviana Elena Castillo Vanegas ^c

Nathalia María del Pilar Correa Valencia ^a

María Patricia Arias Gutierrez ^d

^a Grupo de Investigación CENTAURO, Escuela de Medicina Veterinaria, Universidad de Antioquia, Medellín, Colombia.

^b Normandía Centro Equino, Rionegro, Colombia.

^c Laboratorio Clínico Veterinario Vitalab. Rionegro, Colombia.

^d Universidad CES, Facultad de Medicina Veterinaria y Zootecnia, Medellín, Colombia.

* Corresponding author: angelica.zuluaga@udea.edu.co

Abstract:

The present study aimed to describe the hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise in Colombian Paso horses (CPHs). A standardized field exercise test was carried out on 11 untrained adult CPHs of both sexes. The variables of interest were measured before and after the test (i.e. hematocrit, total plasma proteins, creatine kinase, creatinine, blood urea nitrogen —BUN, aspartate aminotransferase, gamma glutamyl transpeptidase, triglycerides, cholesterol, alkaline phosphatase, cortisol, insulin, blood sugar levels). Evidence of sympathetic-adrenergic

response activation, described for other breeds and equestrian sports disciplines (i.e. hemoconcentration, negative change in plasma volume, slight increase in creatinine and BUN) was found. In addition, evidence of mobilization and use of energy sources such as glucose and triglycerides was found. In conclusion, the increasing-intensity exercise carried out during a standardized field test produced a negative change in plasma volume and the activation of the classic sympathetic-adrenergic response in CPHs.

Key words: Clinical pathology, Equine, Splenic contraction, Training.

Received: 25/11/2020

Accepted: 08/06/2021

Introduction

Standardized treadmill or field exercise tests have allowed to identify the horse's physiological responses and adaptations to exercise. Their characterization and interpretation would later become training indices^(1,2).

Hematological and biochemical parameters are included within the group of variables of interest to be evaluated from such exercise tests. Nevertheless, what has been reported in this regard in horses may differ according to the intensity and duration of the exercise^(3,4). In addition, some findings are not considered as responses or physiological adaptations, but as exercise-induced disorders, including hemolysis and lymphopenia⁽⁵⁾. In Colombian Paso horses (CPHs), there are not enough reports to confirm the expected changes during exercise in animals of this breed.

Due to the increasing demand for professional accompaniment in the training of CPHs, it became important to describe the hematological, biochemical, and endocrine parameters in acute response to increasing-intensity exercise for the breed.

Material and methods

Ethical considerations

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

Study location

The study was carried out in facilities located in a very humid lower montane forest life zone⁽⁶⁾ (2,130 m asl), with an environmental temperature between 12 and 18 °C, and a relative humidity of 69 %.

Animals

Eleven (11) untrained adult CPHs were chosen at convenience. Nine non-pregnant females and two uncastrated males, with a mean of 6.6 ± 4.8 (2.5 to 16) years of age, 371 ± 30 kg of weight and $7/9^{(7)}$ of body condition were included. Animals were clinically healthy on physical examination, with a complete and updated health plan (vaccines and deworming) at the time of the measurements. Regarding the management conditions, the animals were under complete housing and fed on pangola grass hay (*Digitaria eriantha*; 2.5 kg/d on average), green forage (*Pennisetum purpureum*; 30 kg/d on average), commercial balanced feed (2 kg/d on average), mineral salt formulated for horses (100 g/d), and water *ad libitum*.

Field exercise test

A standardized field exercise test was carried out and was composed by four steps with increasing-intensity, also considering moments of rest and recovery. Heart rate (HR) was measured using a monitor reference Ambit 3 vertical (Suunto®, Finland). The protocol used⁽⁸⁾ controlled the intensity of the exercise in 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).

Definition of the hematological, biochemical, and endocrine parameters

A venous blood sample was collected in a tube with EDTA for the measurement of hematocrit (HTC) and total plasma proteins (TPPs) during the moments of rest and at the end of each step of the exercise test. The percentage of change in plasma volume was determined by the concentration of albumin at the moment of rest and at the end of the exercise test⁽⁹⁾.

In addition, samples were taken both in a tube with EDTA and in a dry one, at the moments of rest and of maximum intensity for the behavior of the complete blood count [CBC; i.e. total concentration of erythrocytes, leukocytes, neutrophils, lymphocytes, basophils, monocytes, eosinophils, bands, platelets, hemoglobin, mean corpuscular hemoglobin concentration (MCHC), fibrinogen], and blood chemistry [i.e. creatine kinase (CK), creatinine, blood urea nitrogen (BUN), aspartate amino transferase (AST), gamma glutamyl transpeptidase (GGT), triglycerides, cholesterol, alkaline phosphatase (AP)], hormones (i.e. cortisol, insulin) and blood glucose levels.

Statistical analyses

Descriptive results for non-parametric data were reported as means (ME), interquartile range (IQR), standard deviation (SD), coefficient of variation (CV) for each variable instead. The 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 mean 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.

Results

Hematocrit, total plasma proteins, and plasma volume

The behavior of the HTC during the exercise test was consistent and homogeneous among the study animals, as shown by the low values of the SD for all the variables (Table 1). On the other hand, the TPPs showed slight changes during each step of the exercise test. Albumin was analyzed separately, and given the TPPs values, its values were relatively homogeneous. The mean plasma volume change was -4.65 ± 8.16 L, although three of the animals registered a positive change.

Table 1: Descriptive results of the hematocrit, total plasma proteins, and albumin values in each step of the field exercise test performed on the Colombian Paso horses of study

Moment/Step	Hematocrit (%)			Total plasma proteins (g/dL)			Albumin (g/dL)		
	ME (IQR)	SD	CV	ME (IQR)	SD	CV	ME (IQR)	SD	CV
Rest	37.9 (36.4 - 43.8)	4.52	0.11	6.52 (6.0 - 6.6)	0.42	0.07	3.42 (3.30 - 3.47)	0.18	0.05
Warm-up	42.0 (37.0 - 42.5)	4.62	0.11	6.62 (6.34 - 7.05)	0.41	0.06	3.51 (3.44 - 3.94)	0.28	0.08
Moderate intensity	48.0 (46.0 - 52.6)	5.09	0.11	6.96 (6.25 - 7.06)	0.46	0.07	3.63 (3.57 - 4.15)	0.36	0.10
High intensity	49.7 (47.0 - 53.9)	4.60	0.09	7.14 (6.32 - 7.25)	0.49	0.07	3.69 (3.60 - 3.99)	0.23	0.06
Maximum intensity	51.4 (50.3 - 54.8)	3.67	0.07	7.03 (6.49 - 7.13)	0.48	0.07	3.65 (3.63 - 4.00)	0.18	0.05
Recovery	41.5 (41.0 - 44.9)	2.81	0.07	6.77 (6.07 - 7.13)	0.49	0.07	3.60 (3.49 - 3.94)	0.24	0.07

ME= Means; IQR= Interquartile range; SD= Standard deviation; CV= Coefficient of variation. Reference value for hematocrit⁽¹⁰⁾= 32 - 47%; reference value for total plasma proteins⁽¹¹⁾ = 5.2 - 7.9 g/dL; reference value for albumin⁽¹¹⁾ = 2.6 - 3.7 g/dL.

Hematological parameters

In the hematological parameters before and after the exercise test, there were changes in fibrinogen ($P= 0.004$), the total concentration of leukocytes ($P= 0.0475$) and bands ($P= 0.0002$) (Table 2).

Table 2: Hematological parameters with significant statistical results, measured before and after the field exercise test performed on the Colombian Paso horses of study

Hematological parameter	Moment	ME (IQR)	SD	CV	Ref. value ⁽¹⁰⁾
Fibrinogen, mg/dL	Before	200 (200 – 600)	214.9	0.58	100 - 500
	After	200 (200 – 500)	206.7	0.58	
Total concentration of leukocytes, $10^3/\text{mm}^3$	Before	8.2 (7.1 – 10.5)	1.91	0.21	5.2 - 12.1
	After	9.3 (8 – 12.2)	2.26	0.23	
Total concentration of bands, $10^3/\text{mm}^3$	Before	0.0 (0.0 – 0.08)	0.09	1.95	0 – 14
	After	0.0 (0.0 – 0.08)	0.06	2.5	

ME= Means; IQR= Interquartile range; SD= Standard deviation; CV= Coefficient of variation.

Biochemical parameters

The biochemical parameters were not different ($P>0.05$) for the mentioned steps, with the exception of AP. However, some enzymes showed an increased activity in relation to the physiological concentration (i.e. CK, AST), as presented in Table 3.

Table 3: Biochemical parameters measured before and after the field exercise test performed on the Colombian Paso horses of study

Biochemical parameter	Moment	ME (IQR)	SD	CV	Ref. value
Creatine kinase, U/L	Before	250 (196 - 293)	56.01	0.224	90 - 270 ⁽¹²⁾
	After	279 (247 - 337)	79.11	0.263	
Creatinine, mg/dL	Before	1.54 (1.44 - 1.62)	0.150	0.098	1.2 - 1.9 ⁽¹¹⁾
	After	1.71 (1.62 - 1.99)	0.316	0.175	
Blood urea nitrogen, mg/dL	Before	22.48 (20.37 - 24.45)	2.35	0.105	8 - 27 ⁽¹²⁾
	After	23.7 (22.54 - 26.7)	2.487	0.103	
Aspartate amino transferase, U/L	Before	294 (263 - 341)	56.01	0.186	226 - 366 ⁽¹¹⁾
	After	320 (270 - 356)	61.73	0.189	
Gamma glutamyl transpeptidase, U/L	Before	15 (11.31 - 23.92)	5.49	0.338	4.3 - 13.4 ⁽¹¹⁾
	After	18 (16 - 24.51)	6.55	0.322	
Triglycerides, mg/dL	Before	26.12 (15.5 - 35.8)	23.35	0.707	11 - 52 ⁽¹²⁾
	After	54.3 (35.1 - 63.9)	14.26	0.280	
Cholesterol, mg/dL	Before	114.1 (90.05 - 123.6)	23.10	0.213	51 - 109 ⁽¹²⁾
	After	102.25 (58.3 - 131.15)	40.60	0.393	
Alkaline phosphatase, U/L	Before	343.21 (320.7 - 541) ^a	139.06	0.365	109 - 315 ⁽¹²⁾
	After	335.05 (321.9 - 488.18) ^b	131.90	0.335	

ME= Means; IQR= Interquartile range; SD= Standard deviation; CV= Coefficient of variation.

^{ab} Significant difference when each parameter was compared before and after the exercise test, according to the Wilcoxon signed-rank analysis ($P<0.05$).

Endocrine parameters (hormones and glycemia)

Table 4 shows the hormonal profiles and blood glucose levels obtained during the study, which were similar ($P>0.05$) for the mentioned steps.

Table 4: Behavior of cortisol, insulin, and glucose before and after the field exercise test performed on the Colombian Paso horses of study

Endocrine parameter	Moment	ME (IQR)	SD	CV	Reference value
Cortisol, µg/dL	Before	7.91 (2.21 - 14.39)	6.781	0.780	3.0 - 13 ⁽¹³⁾
	After	7.04 (5.19 - 9.61)	7.318	0.773	
Insulin, IU/mL	Before	40.98 (19.16 - 55.96)	50.84	1.067	4.52 - 33.53 ⁽¹⁴⁾
	After	26.47 (22.92 - 54.56)	18.60	0.535	
Glucose, mg/dL	Before	101 (85 - 153)	35.69	0.313	71 - 130 ⁽¹⁴⁾
	After	134 (101 - 162)	37.89	0.286	

ME= Means; IQR= Interquartile range; SD= Standard deviation; CV= Coefficient of variation.

^{ab} Significant difference when each parameter was compared before and after the exercise test, according to the Wilcoxon signed-rank analysis ($P > 0.05$).

Discussion

Acute responses to exercise, both hematological, biochemical, and endocrine, have been scarcely reported in CPHs. Therefore, the acute physiological changes that occur during exercise in this breed are currently not recognized. This situation represents a disadvantage for the professionals who participate in the sports conditioning processes, since it compels them to work from references that do not correspond to the context of the CPHs. On the other hand, the absence of such information enables the erroneous interpretation of findings in blood tests, although some of the hematological and biochemical variations provide a significant perspective of the pathological conditions that occur from intense exercise⁽¹⁵⁾. Also, it is important to consider that the hematological, biochemical, and endocrine responses by themselves do not describe the athletic capacity of the horse; in fact, parameters such as HTC have not been found to be correlated with metabolic indicators, for example the anaerobic threshold⁽¹⁶⁾.

It is pertinent to clarify that the reference values considered for the present study were contrasted with previous studies carried out in animals of the same breed. The above corresponds to the hemoleukogram values⁽¹⁰⁾, cortisol and insulin concentration⁽¹²⁻¹⁴⁾. The other variables were contrasted with literature^(11,12). The HTC was measured and not calculated, despite using automated equipment.

Exercise is an event of physiological stress, for this reason it is expected the HTC to increase, mainly in moderate, high, and maximum intensity, as occurred in the animals of the present study, exceeding the reference values for rest during the mentioned steps. The behavior observed for HTC is explained by splenic contraction and loss of water during exercise, reflecting hemoconcentration. It takes 30 to 60 sec for splenic erythrocyte release to occur in the presence of increased circulating epinephrine. During the recovery, the sequestration of erythrocytes and leukocytes by the spleen takes approximately 5 min, although their full reserve can take up to 30 min⁽¹⁵⁾. Thus, the return of HTC to its value at rest or during warm-up is explained by this phenomenon, in addition to the blood volume recovery.

In view of the fact that HTC is highly affected by the adrenergic response during exercise (to establish polycythemia due to dehydration), it is recommended to include the total concentration of erythrocytes in the analysis and compare it with the HTC and the concentration of TPPs. In this group of horses, both events were found (hemoconcentration due to splenic release and decrease in plasma volume), as a consequence of dehydration.

The TPPs and albumin showed an increasing behavior during exercise and were found to be augmented (within the reference range) due to the aforementioned loss of water. The concentration of plasma proteins at rest and during exercise, is the result of the interaction of numerous factors, such as the degree of filtration between the intra and extravascular spaces, metabolic demands, neuroendocrine control, nutritional status, and water balance⁽¹⁷⁾.

The plasma volume change found in the present study may be related to the movement of fluids between the different compartments, given the increasing of the hydrostatic pressure generated by the rise in arterial and venous pressure during exercise. In addition, it could be related to the secretion of natriuretic peptide, as the intensity of exercise increases⁽¹⁵⁾.

In humans, it has been reported that plasma volume can decrease due to hemoconcentration or even increase due to hemodilution, depending on the type of exercise performed. The decrease in plasma volume is greater during high intensity exercise^(18,19), and the magnitude of sweating and hydration during exercise can also determine its decrease⁽²⁰⁾. In the present study, a reduction in plasma volume was observed at the end of the exercise test, when horses showed profuse sweating. This corroborates that, as in humans, high intensity exercise and sweating reduce this parameter.

A previous study reported an increase of 5.12 % in plasma volume in endurance horses that competed in an 80 km race, hydrated with 30 L of a solution with sodium chloride and potassium chloride, while, in hydrated horses (with 10 L of the same solution), a decrease in plasma volume of -2.34 % was observed⁽²¹⁾. In this study, the change in plasma volume was -4.65 %, which was expected, since the horses did not hydrate until the final of the exercise test and the sampling were completed. Starling forces explain these findings based on

changes in hydrostatic and oncotic pressures in the vascular and interstitial compartments. During physical activity, a redistribution of cardiac output occurs. Blood flow to the active musculoskeletal system and to the skin tissue increases, and the rise in capillary hydrostatic pressure favors the passage of water and even proteins to the interstitial compartment. These events explain how fluid movement affects plasma volume during exercise⁽¹⁹⁾.

The leukogram obtained from the animals in the present study was significantly different when the values obtained were compared in the rest and recovery steps. Leukocytes show transient alterations in response to increased sympathetic tone. When stored with red blood cells in the spleen, splenic contraction can lead to an increase in the count by approximately 30 %⁽¹⁵⁾. However, its increase is not an indicator of physical condition, rather it is the neutrophil:lymphocyte ratio (10:1) with a shift to the left, is a sign of exhaustion, stress, or overtraining. In the horses of the present study, bands were found in three of the animals, without changes in the neutrophil:lymphocyte ratio. It was not considered a pathological finding, since the values were within the reference range, and it was not accompanied by other changes in the leukogram or at the clinical examination. In addition, values around 50 % of the total neutrophils are sequestered in the capillary spleen beds and are known as marginal pool or splenic reserve. Marginalized neutrophils can be mobilized under certain conditions, including exercise, stress, transport, and exogenous corticosteroids or catecholamine administration, causing variations in the leukogram⁽²²⁾.

Within the biochemical values analyzed herein, the fibrinogen in some animals was found to be remarkable. It should be taken into account that fibrinogen is an acute phase protein, considered as a nonspecific indicator of inflammation⁽²³⁾. It is presumed that some of the animals in the present study experienced some process related to ongoing inflammation that was not reflected in the clinical examination, nor could it be related to leukocyte values. It is understood that the increase in fibrinogen is related to inflammatory processes of infectious or non-infectious origin, so the albumin:globulin ratio may clarify the origin of such increase. In addition, fibrinogen synthesized in the liver in response to an inflammatory process may remain increased—even when the lesions resolved several days ago, registering a peak between 5 and 7 d after the lesion. Therefore, fibrinogen is an indicator of inflammation with absent hemoconcentration.

Blood biochemistry analyzes of the animals in the present study demonstrated that the effort printed on the test is sufficient to increase muscle biochemical activity. It is advisable to add a post-recovery measurement to check if the reestablishment of blood volume alters the concentration of related analytes or if these remain elevated as indicators of muscle injury. The post-test eligible time to detect underlying damage should be anticipated based on the analyte. In racehorses, it is known that 3 d after a competition, some hematic and biochemical values have not yet returned to their reference range⁽²⁴⁾.

Creatinine tends to increase during exercise, due to an augmented use of phosphocreatine and gluconeogenesis, and a decreased glomerular filtration rate⁽¹⁵⁾, being a confident indicator of muscle metabolism and kidney function. The increased use of phosphocreatine evidences the high and maximum intensity work that the study horses experienced.

The BUN did not show a specific trend. This was expected from this parameter since BUN is the result of the urea cycle and the metabolism of nitrogenous products obtained from the diet. Furthermore, it can be reabsorbed in the proximal convoluted tubule in about 30 %, therefore, it is not a good indicator of functionality nor is it a good index of metabolism to be observed after exercise.

The CK is the enzyme in charge of hydrolyzing the reaction that produces ADP and phosphocreatine from ATP. Its increase is mainly associated with a rise in cell permeability due to acidosis or an increase in the use of ATP by the musculoskeletal system. The peak of its production occurs 4 to 12 h after the event that triggers it. The animals in the present study registered an increase in the blood concentration of this enzyme; however, no animal registered an increase related to injury. Nevertheless, no samples were taken during the described release peak, which limits the inference from this enzyme.

Despite being present in several organs, AST is used as a marker of cellular injury in the liver or the musculoskeletal system. Its magnification must be 5 to 100 times greater than the reference value to be useful from the clinical point of view⁽¹⁵⁾. This strengthens the premise that the field exercise test applied to the animals of the present study does not constitute a harmful activity for the musculoskeletal system in healthy horses without previous training.

The biochemical analysis also allowed the recognition of the energy sources that the horses used for this kind of effort. An increased mobilization of glucose and triglycerides was observed. During exercise, glycolysis and lipolysis are activated to obtain energy for muscle contraction^(15,25), especially under aerobic conditions, as occurred in the warm-up and other steps of the exercise test used herein.

It is advisable to include bilirubin quantification to verify the presence of erythrocyte rupture due to the fragility of the membrane that can occur because of changes in blood pH derived from exercise. Hence the importance of accompanying this analysis with the measurement of MCHC. In the present study, bilirubin was not measured, however, MCHC remained within the reference range.

The hormonal activity during exercise is related to the body's energy requirements. The activation of specific endocrine responses is highly dependent on the intensity and duration of exercise, which attempt to preserve the life of the animal through the use of multiple metabolic mechanisms to provide additional energy for muscle contraction⁽²⁶⁾.

Glycogenolysis and gluconeogenesis are activated during exercise, in part, by the release of cortisol, growth hormone, and catecholamines⁽²⁷⁾, while insulin secretion is decreased by the release of catecholamines during exercise⁽¹⁵⁾. In contrast, other authors found that exercise does not influence insulin secretion⁽²⁸⁾. According to the results of the present study, this hormone was found to be elevated before and after the field exercise test. Therefore, metabolic disorders should not be ruled out in the study animals, which were "overweight" at the time of the test, according to the body condition assessment (7/9 on average).

The physical and psychogenic stimuli associated with exercise induce the synthesis and secretion of adrenocorticotropic hormone (ACTH), β -endorphins, and cortisol. In addition, vasopressin —released during physical activity, enhances ACTH secretion. Cortisol measurement should be based on the circadian rhythm and breed, as reported for CPHs⁽¹³⁾. In horses without adequate athleticism, elevated serum cortisol levels affect leukocyte function⁽¹⁵⁾. However, this condition will remain as long as the exercise is strenuous. Contrary to expectations, in the case of the CPHs of study, the serum cortisol concentration remained within the reference intervals, even after the exercise test, although with a plasma concentration sufficient to explain the hyperglycemic and hyperlipemic observed effects.

Future studies on CPHs' sports medicine should propose the identification of physiological changes triggered by physical effort, and thus determine the starting point for the detection of pathologies or as a point of comparison after the application of a prescribed training.

Conclusions and implications

The increasing-intensity exercise performed by the CPHs in the present study produced water loss, as evidenced by the change in plasma volume, with the consequent hemoconcentration, and the slight increase in both creatinine and BUN. The significant differences observed in the leukogram and fibrinogen were apparently produced by individual factors of some animals. Therefore, it cannot be concluded whether the exercise test actually produces physiological responses in this regard. In addition, evidence of anticipated stress response was found from the cortisol value, in the absence of muscle injury. The CPHs considered in the study were suspected of equine metabolic syndrome, although they were not diagnosed for it. These factors must be taken into account when interpreting acute responses and adaptations derived from physical training in horses of this breed.

Acknowledgments

To the Normandía Centro Equino and Mervequus for allowing the testing of their animals and to the sustainability strategy of CENTAURO Research Group (Universidad de Antioquia, Medellín, Colombia) and the Universidad CES (Medellín, Colombia) for the financial support.

Conflict of interests

The authors declare that they have no conflict of interest.

Literature cited:

1. Fraipont A, Van Erck E, Ramery E, Fortier G, Lekeux P, Art T. Assessing fitness in endurance horses. *Can Vet J* 2012;53(3):311-314.
2. De Mare L, Boshuizen B, Plancke L, De Meeus C, De Bruijn M, Delesall C. Standardized exercise tests in horses: current situation and future perspectives. *Vlaams Diergeneeskd Tijdschr* 2017;86(2):63-72.
3. Lekeux P, Art T, Linden A, Desmecht D, Amory H. Heart rate, hematological and serum biochemical responses to show jumping. In: Persson SGB *et al.* ICEEP Publications: Equine exercise physiology 3. 1991:385-390.
4. Zobba R, Ardu M, Niccolini S, Cubeddu F, Dimauro C, Bonelli P, *et al.* Physical, hematological, and biochemical responses to acute intense exercise in polo horses. *J Equine Vet Sci* 2011;31(9):542-548.
5. Masini A, Tedeschi D, Baragli P, Sighieri PC, Lubas G. Exercise-induced intravascular haemolysis in standardbred horses. *Comp Clin Path* 2003;12:45-48.
6. Holdridge LR, Grenke WC, Hatheway WH, Liang T, Tosi JA. Forest environments in tropical life zones: a pilot study, Pergamon Press; 1971.
7. Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. *Equine Vet J* 1983;15(4):371-372.
8. Arias MP, Maya JS, Arango L. Efectos de dos protocolos de entrenamiento sobre el lactato sanguíneo en caballos de paso fino. *Rev Med Vet Zoot* 2019;66(3):219-230.
9. Van Beaumont W, Strand JC, Petrofsky JS, Hipskind SG, Greenleaf JE. Changes in total plasma content of electrolytes and proteins with maximal exercise. *J Appl Physiol* 1973;34(1):102-106.

10. Castillo CA, Tobón M, Cano CA, Mira J, Suárez AP, Vásquez EM. Valores hematológicos en caballos criollos colombianos del valle de Aburrá. In: Corporación Universitaria Lasallista editores. *Perspectivas y avances de investigación de la serie Lasallista investigación y ciencia*. 1st ed. Caldas, Antioquia, CO: Delasalle; 2010:245-262.
11. Kaneko J, Harvey J, Bruss M. *Clinical biochemistry of domestic animals*. 6th ed. David, USA: Academic Press; 2008.
12. Southwood L. *Practical Guide to Equine Colic*. 1st ed. Iowa; USA: Wiley-Blackwell; 2013.
13. Zuluaga AM, Martínez JR. Serum cortisol concentration in the Colombian creole horse. *Rev Colomb Cienc Pecu* 2017;30(3):231-238.
14. Rosas M. Diagnóstico de síndrome metabólico equino y su asociación con signos clínicos de laminitis en el caballo criollo colombiano en la sabana de bogotá [master thesis]. Bogotá, DC: Universidad de la Salle; 2017.
15. Boffi F. *Fisiología del ejercicio en equinos*, 1st ed. Buenos Aires, Argentina: Intermédica; 2007.
16. Valette JP, Barrey E, Wolter R. Multivariate analysis of exercise parameters measured during an incremental treadmill test. In: Persson SGB *et al.* ICEEP Publications: Equine exercise physiology 3. 1991:337-342.
17. Mesa-Rojas MC. Análisis del comportamiento de los parámetros hematológicos en caballos que compiten en carreras de enduro a 2640 M.S.N.M. [undergraduate thesis]. Bogotá, DC: Universidad de La Salle; 2016.
18. Sawka MN, Convertino VA, Eichner ER, Schnieder SM, Young AJ. Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Med Sci Sports Exerc* 2000;32(2):332-348.
19. Sanchis-Gomar F, Lippi G. Physical activity - an important preanalytical variable. *Biochem Med* 2014;24(1):68-79.
20. Mora-Rodríguez R, Fernández VE, Hamouti N, Ortega JF. Skeletal muscle water and electrolytes following prolonged dehydrating exercise: Fluid losses in exercising skeletal muscle. *Scand J Med Sci Sports* 2014;25(3):274-282.
21. Sampieri F, Schott HC, Hinchcliff KW, Gero RJ, Cunilleras JE. Effects of oral electrolyte supplementation on endurance horses competing in 80 km rides. *Equine Vet J* 2006;38(36):19-26.

22. McKenzie EV. Hematology and serum biochemistry of the equine athlete. In: Kenneth H, Andris K, Raymond G editors. Equine sports medicine and surgery. 2nd ed. USA: Elsevier Health Science; 2014:921-929.
23. Polla BS, Cossarizza A. Stress proteins in inflammation. *EXS* 1996;77:375-391.
24. Bos A, Compagnie E, Lindner A. Effect of racing on blood variables in Standardbred horses. *Vet Clin Pathol* 2018;47(34):625-628.
25. Bayly WM, Allen JR, Gollnick PD. Equine exercise physiology I: energy production and substrate utilization. *Equine Vet Sci* 1984;4(3):114-118.
26. Hinchcliff KW, Geor RJ, Kaneps AJ. Equine exercise physiology: the science of exercise in the athletic horse, Elsevier Saunders; 2008.
27. López-Chicharro JL, Fernández-Vaquero A. Fisiología del ejercicio, Ed. Panamericana, 3ª ed. Buenos Aires, Paris. 2006.
28. Noble GK, Silience MN. Diurnal rhythm and effects of feeding, exercise and recombinant equine growth hormone on serum insulin concentrations in the horse. *Equine Vet J* 2013;45(6):745-750.