# Metabolic evaluation of Crioulo horses participating in competitions of 750 km<sup>1</sup>

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**ABSTRACT.**- Amaral L.A., Marchiori M., Martins C.F., Correa M.N. & Nogueira C.E.W. 2013. **Metabolic evaluation of Crioulo horses participating in competitions of 750 km.** *Pesquisa Veterinária Brasileira 33(12):1471-1477.* Departamento de Clinicas, Faculdade de Veterinária, Universidade Federal de Pelotas, Campus Universitário s/n, Pelotas, RS 96010-900, Brazil. E-mail: lo1amaral@gmail.com

The Crioulo breed of horses performs in one of the most physically demanding equestrian competitions, the Marcha de Resistência, which is a contest in which the horses run 750 km in 15 days. The study's aim was to characterize the metabolic responses during this period. We evaluated eleven Crioulo horses in the competition, specifically, two males and nine females. Blood samples were collected 24 hours before the contest and on the 4th, 9th, 11th, 14th and 15th days of competition. We evaluated CK, AST, LDH, glucose, lactate, urea, creatinine, sodium, potassium, chloride, magnesium, total calcium, ionized calcium, total protein, hematocrit and the white blood cell count. At the end of the competition, the mean values of serum AST were 1151±358 IU/ L the mean LDH values were 7418±1695 IU/L and CK was 13,867±3998UI /L. There was a significant increase in urea, creatinine and lactate (p<0.0001). A decrease in the mean values of chloride, sodium, potassium, and total and ionized calcium was observed (p≤0.0002). An evaluation of the total leukocytes and segmented neutrophils (p≤0.0002) revealed their increased values, and decreased values were observed for hematocrit, plasma protein and total lymphocytes ( $p \le 0.0003$ ). The values of glucose, on average, remained constant. Based on these data, we conclude that the Marcha de Resistência competition necessitated a high muscular demand and the depletion of energy and electrolytes, suggesting an inflammatory process in the animals evaluated.

INDEX TERMS: Endurance, horse, exercise, metabolism.

RESUMO.- [Avaliação metabólica de cavalos Crioulos submetidos a uma competição de resistência de 750 km.] O cavalo Crioulo realiza uma das provas de maior exigência física nas modalidades equestres. A *Marcha de Resistência* é uma competição na qual os animais percorrem 750km durante 15 dias. O objetivo desse estudo foi caracterizar as respostas metabólicas ocorridas nesse período. Foram avaliados 11 equinos da raça Crioula inscritos na competição, sendo dois machos e nove fêmeas. Foram efetuadas coletas de sangue 24h antes da prova e no 4º, 9º,

11º, 14º e 15º dia de competição. Foram avaliados a concentração sanguínea de creatininoquinase, aspartato aminotransferase, lactato desidrogenase, glicose, lactato, uréia, creatinina, sódio, potássio, cloreto, magnésio, cálcio total, cálcio iônico, proteínas totais, hematócrito e o leucograma. Ao final da competição os valores médios de AST sérica foram de 1151±358 UI/L, os valores de médios de LDH foram de 7418±1695 UI/L e de CK foi de 13867±3998UI/L. Houve aumento significativo nos valores de uréia, creatinina e lactato (p<0,0001). Foi observada diminuição nos valores médios de cloreto, sódio, potássio, cálcio total e iônico (p≤0,0002). Na avaliação hematológica foi constatada elevação nos valores de leucócitos totais e segmentados (p≤0.0002) e diminuição nos valores de hematócrito, proteína plasmática total e linfócitos (p≤0,0003). Os valores de glicose, em média mantiveram-se constantes. Com base nesses dados conclui-se que a competição Marcha de Resis-

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tência determinou elevada exigência muscular, com depleção energética e eletrolítica, além de sugerir um processo inflamatório nos animais avaliados.

TERMOS DE INDEXAÇÃO: Enduro, equino, exercício, metabolismo.

## INTRODUCTION

The progression in the knowledge of exercise physiology in horses was begun by Procter in 1934. At that time, the study gathered the general knowledge of physiology, biochemical energy and its interaction with other systems with the primary objective of understanding and improving equine sports medicine. After years of research, if has been observed that within the different racial groups, there are numerous features associated with the metabolic response of the horse to exercise; therefore, the racing of a horse or its genetic group influences physiological events that occur during physical activity (Boffi 2006).

The Crioulo breed has the second-highest record of animals in Brazil, and it is known for its versatility and ruggedness, as exhibited in the equestrian's greatest physical test known in the world, the Marcha de Resistência (ABCCC 2011). The *Marcha de Resistência* is a competition with the aim of demonstrating and disseminating the resistance of the Crioulo horse. The test consists of a 750-km course with points of rest and veterinary control. The competition is held on farms inside the Rio Grande do Sul, where the animals are exposed to adverse weather conditions, rough terrain and natural obstacles. The competition is held during a period of 15 days, and all horses are submitted to homogeneous environmental conditions, being kept on native pastures with access to water at will with no medication or replacement minerals. During the competition, the animal's metabolism undergoes a maximum requirement to keep organ systems functioning, but no one knows exactly how the horse's body responds to this challenge. Data on the energy profile, muscles, kidney, liver and electrolytes are essential for understanding the metabolic behavior of these animals. In horses that participate in endurance competitions, these assessments are part of a routine monitoring response to resistance exercise (Evans 2000). Therefore, the aim of this study was to characterize the metabolic responses in Crioulo horses participating in a 750-km contest of strength.

## **MATERIALS AND METHODOS**

This experiment was developed in the Jaguarão municipality of Rio Grande do Sul state (Latitude - 32°33′58″ Longitude - 53°22′33″, Height - 26 meters) during the annual *Marcha de Resistência* organized by the Brazilian Association of Crioulo Horse Breeders. The stages took place on farms located within the municipality.

The competition consisted of twenty-nine Crioulo participants, and the studied population consisted of 11 horses (two males and nine females) with an average body weight of 458±42 kg and 8±4 years of age respectively. The chemical assessment of the pasture in the paddock where the animals were released daily during the competition was determined to consist of 68.7% of dry matter, 5.7% crude protein, 1.1% ether extract, 17.8% crude fiber and 7.3% ash. All animals, 30 days prior to the start of the competition, were treated against endo and ectoparasites and subse-

quently kept in a communal environment with pasture and water ad libitum. During the competition, after each daily step, the animals were kept loose on native grass with water access and prohibited from any therapeutic intervention and supplementation during the competition. This study was approved by the Ethics and Animal Experimentation  $n^{\varrho}$  4602/2010.

All of the animals underwent the collection of blood samples with EDTA, oxalate and without anticoagulant before the start of the competition (day 0) for a hematological and biochemical analysis. The hematological and biochemical evaluations were performed immediately after the end of each stage of the competition on days 4, 9, 11, 14 and 15, as shown in table 1.

The physiological pattern taken as a reference to determine the healthiness of the animals has been described in literature (Kaneco et al. 1997).

Immediately after blood collection, blood glucose was measured by a glucometer (Accutrend ® Performance, Roche Diagnostics, Mannheim, Germany). The samples in tubes without anticoagulant were centrifuged for 5 min at 400 G, and the separated was serum frozen at -20°C for subsequent analysis of urea (UR), creatinine (CR), creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH ), sodium (Na), potassium (K), magnesium (Mg), chlorine (Cl), total calcium (Ca) and calcium ion (Ca). The samples collected in tubes with oxalate were also centrifuged at 400G for 5 min, and the separated plasma was frozen at -20 C for the subsequent measurement of lactate. The determination of plasma lactate was performed by enzymatic method using a commercial kit (Labtest Diagnóstica S.A., Lagoa Santa, Brazil) on semi--automatic biochemical analyzer (Bioplus - BIO 200). The samples were gathered with EDTA and were sent to the clinical pathology laboratory at the Federal University of Pelotas, where they measured the total serum protein (TPP), hematocrit (HT), hemoglobin (Hb) and complete leukocytes. We analyzed Cl, Mg and Ca with the aid of reagents (Labtest Diagnóstica S.A., Lagoa Santa, Brazil) for performing a subsequent spectrophotometry (Labquest - CELM, E-225-D, Salzburg, Austria). The Na, K and Cai were determined with the aid of reagents and a further reading on a diagnostic ion selector (Iselab - Drake, São José do Rio Preto, SP, Brazil). Using the blood samples collected in the vials containing anticoagulant (EDTA), we collected the hematocrit in the microhematocrit tube centrifuged at 14.000 G five minutes later with a particular scale reading. The plasma total protein concentration was determined by refractometry (Atago T2 -NE, Bellevue, WA 98005,USA). The values of the CK activity and the AST LDH methodology kinetics were determined by UV-IFC kits (Labtest Diagnóstica S.A., Lagoa Santa, Brazil) and spectrophotometry (Labquest - CELM, modelo E-225-D, Salzburg, Austria). Urea levels were measured by UV kinetic enzymatic method and creatinine levels were measured by alkaline picrate method, the reading was performed by spectrophotometry (LabQuest - CELM, model E-225-D, Salzburg, Austria) and by using commercial kits (Diagnostic Labtest SA, Lagoa Santa, Brazil).

The statistical analysis were performed using the Statistical Analysis System (SAS Institute Inc. Cary, NC, USA) by analysis of variance with a simple comparison of media through the Tukey test and was considered to be significant if p<0.05.

Table 1. Schedule of speed and distance traveled by the animals on each day of sampling during the Marcha de Resistência

Samples	1	2	3	4	5	6
Day Distance	Day 0* 0 km	4º day 60 km	9º day 40 km	11º day 60 km	14º day 60 km	15º day 40 km
Speed	Rest	2,3 m/s	3,3 m/s	3,3 m/s	Free speed	Free speed

<sup>\*</sup>Day 0 = 24 h before the start of competition-basal.

#### **RESULTS**

Twenty-nine animals participated in the competition, and twenty-three completed the total distance of 750 km. Six animals left the competition, four because of exhaustion and two for claudication.

In the profile determined by the measurement of muscle CK, AST and LDH, it was observed that the levels of CK and LDH before the start of the competition were, on average, 513 IU/L and 922 IU/L, respectively (Fig.1). At the end of the competition (day 15), the mean value of CK was 13,837±3998 IU/L and 2614% higher than the value obtained before the competition. The mean value of AST was 1,150±358 IU/L and 193% higher than the average observed before the competition. LDH averaged 7,418±1,695 IU/L, which was 717% greater than the mean value observed at rest. The biggest turning point in the curve of CK during the competition was observed from the 9th day, and that of the AST was observed only from the 14th day, while that of the LDH was observed from the 11th day.

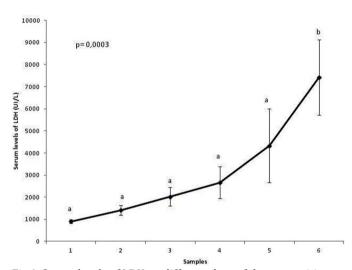


Fig.1. Serum levels of LDH on different days of the competition.

Serum creatinine showed average  $1.37\pm0.06$ mg/dL at rest and exhibited moderate oscillations during the competition (p<0.0001). On the  $14^{th}$  day was measured the highest elevation in serum levels, and this level remained at the end of 15 days, reaching a mean value of  $1.75\pm0.07$ mg/dL.

The serum urea rose at each collection (Fig.2), remaining above the physiological levels from the  $4^{th}$  day of competition, and reaching their highest average levels on the  $15^{th}$  day with a value of  $67.3\pm3.9$ mg/dL, 589% higher than the baseline.

Regarding the electrolytic variables, the chloride average before the competition was  $98 \pm 1.1 \text{mEq/dl}$ , and its value on day 15 was  $86 \pm 3.6 \text{mEq/dl}$ , which was 12.5% lower than at the first collection (p<0.0001). It was observed that 54% (n=6) of the animals had chloride values below the physiological limit prior to the start of the contest. During the competition, the analysis of chloride remained below physiological levels in 51.3% (n=47), and on days 14 and 15, all (n=11) animals showed hypochloremia.

The overall mean serum sodium at rest was 136.8±0.5mEq/dL, and the lowest average values were

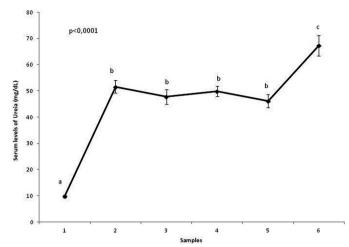


Fig.2. Serum levels of urea on different days of the competition.

observed after the  $11^{th}$  and  $15^{th}$  day of the competition, which was obtained averaging  $130\pm0.9$  and  $131\pm1.1$ mEq/dL, respectively, being below the physiological values and significantly different regarding the collection at rest (p<0.0001). The average potassium observed at rest was  $4.2\pm0.09$ mEq/dL, and this value showed significant decreases on days 4 and 14 of the competition, averaging  $3\pm0.1$  and  $32\pm0.1$ mEq/dL respectively (p=0.0002).

At the end of the competition, the total calcium showed a general mean of  $8.73\pm0.3$ mg/dL. Hypocalcemia was observed in all of the animals before starting the competition, being observed as, on an average,  $9.8\pm0.1$ mg/dL. After the  $4^{th}$  day of competition, there was a significant drop in the serum to an average of  $7.8\pm0.3$ mg/dL, but during collection, these rates increased to  $8.9\pm0.1$ mg/dL and remained constant in subsequent collections. Investigation of the calcium ion revealed that the average was  $1.7\pm0.04$ mEq/dL with a significant drop from the  $4^{th}$  day and was retained in the average until end of the competition. However, it is noteworthy that the observed values were high in relation to the physiological patterns.

The mean serum magnesium at rest was  $1.56\pm0.04$ mg/dL, and on day 4 was  $1.48\pm0.06$ mg/dL, at day 9 was  $1.39\pm0.05$ mg/dL, at 11 days was  $1.33\pm0.06$ mg/dL, at day 14 was  $1.43\pm0.07$ mg/dL, and at 15 days was  $1.42\pm0.1$  mg/dL, with no difference being observed between the collections.

The average of the blood lactate was not changed in the collections of the 4th day  $(1.37\pm0.3 \text{mmol/dL})$ , the 9<sup>th</sup> day  $(1.36\pm0.1 \text{mmol/dL})$  and the  $11^{\text{th}}$  day  $(2.45\pm0.3 \text{mmol/dL})$ , which corresponded to the slower developed steps (2.3 m/s, 3.3 m/s, and 3.3 m/s, respectively). On the  $14^{\text{th}}$  and 15 th day, the values rose (p<0.05) and were above the physiological values of  $5.2\pm0.5$ ,  $42\pm0.9 \text{mmol/dL}$ .

The mean blood glucose levels in the animals at rest was  $67\pm1.3$ mg/dL on day 4,  $76\pm10.2$ mg/dL at 9 days,  $56\pm2.1$ mg/dL at day 11,  $82\pm7.3$ mg/dL on day 14,  $60\pm6.4$ mg/dL on day 15 with a mean value of  $72\pm10.1$ mg/dL and no difference between trials (p=0.1).

In hematology, the mean hematocrit was observed at rest to be  $41.9\pm1.3\%$  with a gradual decrease of the mean values occurring by the  $11^{th}$  day of competition when the observed

average was  $27.4\pm1.1\%$  (p<0.05). Days 14 and 15 showed an increased hematocrit compared with day 11 (p<0.05). In the collections of the 9th and 11th days, the animals were anemic, with a mean value of  $28\pm0.9\%$  being observed. Hemoglobin values showed the same behavior (Table 2).

As with the hematocrit and hemoglobin, the total plasma protein had lower values during the competition (p<0.0001), as shown in Figure 3.

In the evaluation of the average WBC, the total leukocytes at rest were  $11.900\pm503$ , with a significant increase occurring until the  $15^{th}$  day, which had an average of  $16,400\pm645$ . The behavior of the values of the segmented neutrophils was similar, whereas treated neutrophils exhibited leukocytosis (Fig. 4). The average resting segmented neutrophil was  $5900\pm432$ , while on the  $15^{th}$  day, the average rate was  $13,500\pm821$  (p<0.0001). The average number of the animal's lymphocytes before the competition was  $5200\pm366$ . With the passage of the stages of the competi-

Table 2. Mean ± s.e. values for each hematological variable evaluated in the collections made during the Marcha de Resistência

Day of sample	Basal	4º	9º	11º	14º	15⁰
Leukocyte	11945ª	12573ª	14118 <sup>abc</sup>	$13067^{ab}$	$15780^{bc}$	16463°
(/µl)	$(\pm 503,1)$	(±654,6)	(±934,6)	(±727,5)	(±681,9)	$(\pm 645,8)$
Neut. segmented	5955a	$9109^{b}$	$11301^{bc}$	$9102^{b}$	$11893^{bc}$	13435°
(/µl)	$(\pm 432,2)$	(±780,8)	(±822,1)	(±555,3)	(±798,4)	(±821,8)
Lymphocytes	5252a	2918bc	1993 <sup>b</sup>	$3136^{bc}$	3453°	2644bc
(/µl)	(±366,9)	(±243,3)	(±183,9)	(±199,2)	(±438,0)	(±277,8)
Hematocrit	41,9a	35,4 <sup>b</sup>	28,5°	27,4°	$31,6^{ab}$	38,1 <sup>ab</sup>
(%)	$(\pm 1,30)$	$(\pm 1,10)$	$(\pm 0.85)$	$(\pm 0.90)$	$(\pm 1,02)$	$(\pm 1,11)$
Hemoglobin	14,2a	12,4 <sup>b</sup>	9,8°	9,4°	$13,5^{ab}$	$13,0^{ab}$
(g/dl)	$(\pm 0,44)$	$(\pm 0,42)$	$(\pm 0.33)$	$(\pm 0.32)$	$(\pm 0.34)$	$(\pm 0.37)$
PPT (g/dl)	7,1 <sup>a</sup>	$6,4^{b}$	6,2 <sup>b</sup>	6,3 <sup>b</sup>	6,6 <sup>ab</sup>	6,6 <sup>ab</sup>
	$(\pm 0,13)$	$(\pm 0,13)$	$(\pm 0,10)$	$(\pm 0,14)$	$(\pm 0,15)$	$(\pm 0,16)$

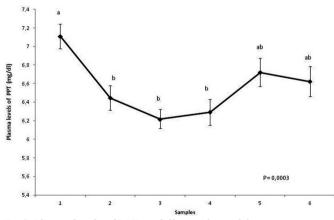


Fig.3. Plasma levels of PPT on different days of the competition.

tion, there was a decrease in the serum levels of these cells, and on the 15th day, the average was  $2600\pm277$  (p<0.0001), as shown in Figure 5.

## **DISCUSSION**

The competition was characterized by a high muscular demand and a depletion of energy and electrolytes as demonstrated by the blood glucose, total plasma protein,

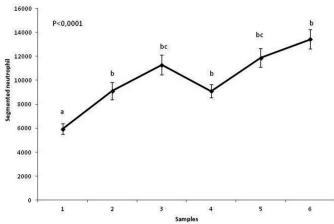


Fig.4. Mean segmented neutrophils on different days of the competition.

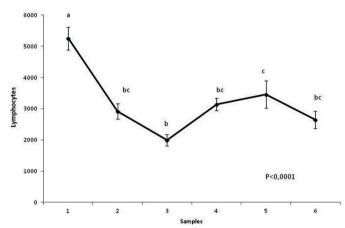


Fig.5. Means of lymphocytes on different days of the competition.

chloride, potassium, magnesium, sodium, total and ionized calcium. The values of CK, AST and LDH were found to be high throughout the competition whereas at the end of the race CK levels have increased 2614%, AST was 193% and LDH increased 717%. Salles (2013) evaluating horses in a 90 km endurance competition, observed CK rise of 83%, 31% in AST levels and 41% in LDH levels. As noted during the *Marcha de Resistência*, the average of increase in these enzymes were much higher, which may characterize an extensive involvement of cell membrane permeability or even rupture of the muscle cells characterizing cases of myositis. For Spinha de Toledo et al. (2001) only high plasma concentrations would have a direct relationship with severe myositis. However, no animal was removed from the competition for suspected muscle injury.

In addition to enzymes related to serious muscle damage, myoglobin is released into circulation; this metabolite is toxic to renal tubules and is responsible for acute renal failure associated with severe myositis(Hamilton et al. 1989). The high levels of CK and LDH shown in Table 2 were not accompanied by significant increases in serum creatinine. These data corroborate with those observed by Noleto (2012) and Santiago et al. (2013), which found no elevation in serum creatinine levels after prolonged exercise. The BUN levels rose from day 4 but remained constant

until the end of the competition. The results of creatinine and urea showed no injury to the kidney in these animals, despite the high levels of muscle enzymes.

The increase in urea levels and the observed decrease in total plasma protein can configure the use of protein catabolism as a source of energy for maintenance of exercise during the *Marcha de Resistência*. In general, exercise of low intensity and long duration has 94% of its energy generated by the consumption of lipids (Boffi 2006). However second Lawrence (1994), is not determined how much the protein is important as fuel for exercise in horses, nevertheless in other animal species is estimated that protein catabolism contributes 5-15% of the oxidative energy production. Increasing concentrations of urea and decreased protein in the blood of endurance horses can quickly reflect protein catabolism in situations of glycogen depletion (FRAPE 1998).

In the evaluated animals, the average blood glucose at rest was observed to be below the reference values, and this trend continued during the year. In other races, such as Arabic and PSI, an inverse trend is described with increased blood glucose levels all during exercise (Balarin et al. 2006, Orozco et al. 2007). The increase of blood glucose is explained by the release of catecholamines and the stimulation to gluconeogenesis and lipolysis.

In this study, we found that animals that had high glucose levels (>100mg/dl) showed signs of fatigue, and a heart rate, body temperature and dehydration elevated> 8%; however, those that left the competition had hypoglycemic (<20mg/dl) energy depletion. Fernandes & Larsson (2000) observed a reduction in blood glycemia levels after an endurance race, however not at such low levels.

The concentration of lactate and the serum concentration of chloride showed the greatest change on the  $14^{\rm th}$  and  $15^{\rm th}$  day, on which the animals showed hypochloremia. These days were the last two days of competition, and because the competition is free of speed steps, the animals reached an average speed of 7 m/s. The concentration of chloride in sweat is twice its concentration in plasma; therefore, the sweat results in an extended loss and a significant reduction in its plasma concentration, as observed by Correa 2010 in horses during resistance exercise.

According to Santos & Gonzáles (2006) the increase in lactate levels above 4 mmol/l is common on high physical intensity competition, however, despite the *Marcha de Resistência* be considered a low intensity competition, at the last days are developed high speeds that characterized a ratio above 4mmol/l in plasma lactate levels, similar result observed by Ferraz et al. (2009) e Caiado et al. (2011)

In general, during endurance competitions, there are symptoms of hypokalemia associated with the fatigue of animals (Rose et al, 1980). In this study, we observed a significant decrease in serum potassium levels (p=0.0002), but the average remained within the physiological limits. In a 40-km endurance competition a decrease in the values of the concentration of potassium ions was observed (Martins et al. 2005), Di Fillippo et al (2009) made a similar observation in 60km enduranceIn the evaluated animals, a low level of total calcium associated with normal or eleva-

ted levels of calcium ion was observed. The total calcium remained below the physiological values in all of the collections that were made; however, the calcium ion, despite remaining undiminished during the competition, remained within the physiological limits. Serum calcium is the sum of three components: the ionized calcium, the one bound to plasma proteins and the complex, with such anions as citrate, phosphate and sulfate. Serum calcium's ionized fraction is the biologically active form and makes up approximately 50% of the total calcium in the blood, whereas the remainder is bound to albumin and other plasma proteins (Silva 2008).

The serum calcium concentration is reduced during exercise, due to its loss in sweat and the intracellular exchanges between muscle cells or because of an increase in calcium binding with other substances in the plasma. Acidosis removes calcium from albumin, increasing the concentration of ionized calcium in the plasma; the opposite phenomenon occurs in alkalosis. Thus, acidosis is accompanied by hypercalcemia and alkalosis by hypocalcemia (Bayly & Kline 2006). However, in these evaluated animals, the findings do not clarify the etiology of the low total calcium levels observed before the beginning of the competition, suggesting that these results are due to the physiological profile of horses trained for endurance. The same way Correa et al. (2011) observed before exercise, decrease in serum calcium levels in animals trained for horseback riding.

Unlike the values of total calcium, magnesium levels remained constant during the competition, but they remained below physiological limits This ion plays an important role in muscle contraction. According to Bayly & Kline (2006), the plasma concentration of magnesium may decrease after prolonged exercise because its concentration on sweat is greater than its plasma concentration. However Sales et al. (2013) demonstrated an increase in the concentration of Mg after 90km endurance, associating the case to an increase in muscle cells permeability, where their concentration is higher. In the present study, despite the high permeability of muscle cells, configured by the increase in CK, AST and LDH, no change was observed in magnesium levels.

During the evaluation, a decrease in serum sodium levels was observed, particularly in free speed steps, where there were higher levels of perspiration in these animals. As with chloride, the sodium concentration in sweat is higher than in plasma, and a greater quantity of this mineral is lost (Bayly & Kline 2006), however with a tendency to remain within the physiological limits, as observed in other studies (Di Filippo et al. 2009, Dumont et al. 2012) In the hematological evaluation, a decrease in hematocrit and hemoglobin on days 4, 9 and 11 was observed with a slight elevation occurring on days 14 and 15. The hematocrit increases because of a hemoconcentration associated with dehydration and also a higher intake of red cells in the bloodstream as a result of the splenic contraction common in this species (Boffi 2006, Di Filippo et al. 2009). However, this finding was not observed in our study. Several animals had anemia reaching values below 25%, which suggests the occurrence of intravascular hemolysis induced by exercise, as observed in human athletes and described in athletic horses (Masini et al. 2003). The occurrence of hemolytic episodes is indicated by the presence of high plasmatic hemoglobin associated with low levels of haptoglobin after exercise. The literature regarding "sports anemia" in horses, particularly exercise-induced hemolysis, is quite limited.

The neutrophil leukocytosis observed in all animals in this study is described in the equine endurance practice and some other modalities (Orozco et al. 2006, Ferraz et al. 2009, Miranda et al. 2011, Santiago et al. 2013). The WBC has been widely used as an indicator of stress in horses. A stressed WBC is characterized by elevated values of total leukocytes, which is accompanied by neutrophilia, lymphopenia and eosinopenia (Bayly & Kline 2006). The endurance is associated with neutrophilic leukocytosis (Snow et al. 1982), which is probably due to the increase of circulating steroids (Bayly & Kline 2006). This finding is confirmed in this study because at the end of the competition, all of the animals presented total leukocyte values above the physiological levels. This result may be related to the fifteen days in which the animals were subjected to exercise stress in the Marcha de Resistência competition.

However lymphocytes showed an inverse behavior. As the competition developed, the lymphocyte values decreased in comparison with the initial collection. These data, as well as neutrophilia, may be linked to immunosuppression caused by the long-term presence of endogenous corticosteroids similar result observed by Miranda et al. (2011). Second Rose & Hodgson (1994), the occurrence of persistent leukocytosis observed in competition suggests physical exhaustion of the animals.

## **CONCLUSION**

Based on these data, it is concluded that competition in the *Marcha de Resistência* led to a high muscular demand with depletion of energy and electrolytes, suggesting an inflammatory process in the evaluated animals.

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