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Hydration status and biochemical profile of Quarter Horses in vaquejada training

Status de hidratação e perfil bioquímico de Quarto de Milha em treinamento de vaquejada

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ABSTRACT

The hydration status and biochemical profile were assessed and compared in two Quarter Horses groups (pull and helper), at three times (T) of a vaquejada training: at rest, before exercise (T0); after three sprints or races (T1); after an hour of rest from sprints (T2). There was no significant difference between the evaluation times in each group nor between the groups in the times evaluated for the parameters: capillary refill time, skin turgor, color and humidity of the mucosa, total plasma proteins, creatinine, urea and osmolarity, creatine kinase, aspartate aminotransferase, total calcium, phosphorus, and total magnesium. There was increase in the hematocrit and plasma glucose in both groups at T1, but without differences among the groups. The plasm lactate increased in T1. Only for plasma lactate there were changes between the groups, being higher in the pull at T1. It was concluded that, in response to the imposed exercise, the pull and helper groups showed similar hydration status with only slight dehydration, and maintained their biochemical rates preserved, except lactatemia which increased in both, but higher in the pull, suggesting that this group performs more intense anerobic metabolism in the vaquejada.

Keywords: Hydration; Biochemistry, Equine, Exercise; Vaquejada.

RESUMO

O status de hidratação e o perfil bioquímico foram avaliados e comparados em dois grupos de equinos Quarto de Milha (puxador e auxiliar), em um treino de vaquejada. Os parâmetros foram avaliados em três tempos (T): em repouso, antes do exercício (T0); após três sprints (T1); e após uma hora de descanso dos sprints (T2). Não houve diferença significativa entre os tempos de avaliação para um mesmo grupo e nem entre os grupos para os parâmetros: tempo de enchimento capilar, turgor da pele, cor e umidade da mucosa, proteínas plasmáticas totais, creatinina, ureia, osmolaridade, creatina quinase, aspartato aminotransferase, cálcio total, fósforo e magnésio total. O hematócrito e a glicose aumentaram em ambos os grupos no T1. O lactato plasmático aumentou em T1 nos grupos e somente neste parâmetro houve diferença significativa entre os grupos, sendo maior no grupo puxador no T1. Concluiu-se que, em resposta ao protocolo de exercício, ambos os grupos apresentaram status de hidratação similar com leve desidratação, e mantiveram suas taxas bioquímicas preservadas, exceto a lactatemia, que aumentou em ambos, porém, maior no puxador, sugerindo que nesse grupo o metabolismo anaeróbico é de maior intensidade nesta modalidade.

Palavras-chave: Hydratação; Bioquímica, Equino, Exercício; Vaquejada.

INTRODUCTION

The vaquejada is an equestrian cultural and sporting test that is very common in Brazil, with its official circuits authorized by the Associação Brasileira de Criadores de Cavalos Quarto de Milha (ABQM). In this modality, two cowboys, the “helper” and the “pull”, riding their horses, race side by side with a steer between them on a sand track with the objective of making it fall down on the track by pulling the tail. During the sprint, the helper rider assists in the work of guiding and maintaining the steer aligned, and in passing the steer’s tail - protected by a tail shield – to the pull cowboy who should pull the steer down within the limits of the track (ABVAQ, 2016; MELO *et al*., 2022).

In the vaquejada there is a large demand on the horses to exert physical effort, with fast starts, sudden changes in direction and stops, and strength while pulling the steer down (LOPES *et al*., 2009; MENESES *et al*., 2014; SOUZA *et al*., 2017). Thus, as in other Western-type horse contests, the exercise the horse performs in the vaquejada is classified as high intensity short duration, demanding fast energy production and intake to support the big muscular effort, that makes the exercises predominantly anaerobic (BARBOSA *et al*., 2016).

The anaerobic mechanisms include principally anaerobic glycolysis, with adenosine triphosphate (ATP) synthesis and no need for oxygen. In this process, lactic acid is produced and accumulated, and the muscle glycogen is quickly exhausted, both with consequences for the glycogen. A horse breed that is outstanding for these requirements is the quarter horse, mainly because it has IIX type muscle fibers, that

contract quickly, are good for glycogen storage (VERMEULEN *et al*., 2017), have low oxidative capacity and high glycolytic capacity that favor the execution of this exercise (LOPES et al., 2009; RODRIGUES *et al.*, 2016).

Exercise stimulates mechanisms in the horses of cardiovascular and respiratory responses, essential for oxygen and substrate supply to the cells (ARRUDA *et al*., 2015), and large heat production that needs to be dissipated by transpiration, because it causes immediate body heating varying according to the climatic conditions and can create heat stress in the athlete (LOPES *et al*., 2009; FONSECA, 2014). Furthermore, functional alterations can be triggered in the hydration, electrolytic and enzyme status related to the muscle effort, so it is important to assess physical and laboratory parameters such as biomarkers of the adaptation and athletic condition of the horse to the type of exercise, in training or in horse tests (PADILHA *et al*., 2017; HUNKA *et al*., 2018).

Understanding of the dynamics of the sport and the various aspects that can be determining in the success or failure of the chase and pulling down the steer on the competition track is fundamental for athlete training (MARIZ *et al*., 2023). However, although fairly common in Brazil, only a few reports present physiological and laboratory results for horses in vaquejada practiced in the country. Considering the importance of obtaining more results that express the organic responses of horses in training or vaquejada competitions for their application in the physical conditioning of these animals, the present study assessed the hydration status and biochemical parameters associated to the muscle work in healthy horses adapted to vaquejada competition, verifying the recovery or not of the parameters after three vaquejada sprints, and comparing the results between the pull and helper horses.

MATERIAL AND METHODS

The experiment was carried out on two consecutive mornings in a vaquejada park: Haras Parque Marinho, located in the municipality of São Luís, Maranhão state, Brazil, Latitude: 2° 31' 51" South, Longitude: 44° 18' 24" West and 3.66 m altitude. The sprints started on the first day at 8 hours (h):45 minutes (min.), at 28 °C temperature and 93% relative air humidity, and ended at 12h:15min., with 31 °C temperature and 64% relative air humidity. On the next day, the sprints started at 8h:25 min, at 27 °C temperature and 94% relative air humidity and ended at 12h:35 min., at 30 °C temperature and 66% relative air humidity.

A total of twelve Quarter Horse was used, eight male gelding horses and four females, with body weight between 389 and 462 kilograms (kg) body weight, aged from sevem years three months to eleven years eight months. Six horses made up a set with the cowboy in the pull function (6 males) and six in the helper function (two males and four females), and three pull and three helper horses were used on each one of the two consecutive days. All the horses were already adapted to vaquejada competition, in good health conditions according to the normal limits of the parameters: mucosa color and moisture, capillary refill time, skin turgor time, heart and respiratory hate, rectal temperature, normal miction and defecation, normal hemogram, leucogram, total plasma proteins and fibrinogens.

No horse had presented colic in the previous 60 days and no horse presented lameness at the time of the selection examination for the study. The animals were kept in individual masonry stalls ranging from 16.0 and 18.0 square meters (m^2) in size. The horses´ diet was based on conditions of moderate degree exercise, directed for approximate intake of 3% dry matter/day, as they were in training and were not in a competition period. *Tifton* (*Cynodon spp*.) hay as bulk was placed in the stall in hay baskets twice a day (6h00min and 17h30min), with free access in 24 h, in a total of approximately 0.5 kg bulk/100 kg body weight/day. As supplementation, pelleted commercial concentrate was supplied containing a minimum 120 g kg^{-1} crude protein and 3.400 kcal kg^{-1} digestible energy (Equivita vaquejada – Integral Mix), in a total of approximately 1% body weight/day, divided in three daily meals at 6:00h, 13h30 p.m. and 18h30min. Another supplement supplied was mineral salt (Coequi plus© – Tortuga): 100g/day, in an individual salt cellar in the stall. Water was supplied *ad libitum*, in individual water dispensers in the stalls.

The assessment times (T) of the physical parameters and sample collection for laboratory exams on the horses were: T0 (fifteen minutes before starting to warm up the horse for 10 minutes before the first sprint, with the horse not yet saddled); T1 (immediately after the three sprints); and T2 (one hour after T1, with the animals resting in the stall, but without water or food). Between the T0 collection and the first sprint, the horse was conditioned with a gentle warmup by trotting for 10 minutes. The rest after the third sprint took place with the horses in their stalls without feeding. No horse received water or any fluid 30 minutes before T0 and until T2. Immediately after T2, water *ad libitum* was made available in the water dispenser in the stall.

The following clinical parameters were evaluated: color and humidity of the mucosa, capillary refill time, and skin turgor time, according to SPEIRS (1999). Blood samples were collected after cleaning the skin, by puncturing the jugular vein, and placed in flasks containing sodium fluoride to obtain plasma (vacuum siliconized flask – 4.0 ml – sodium fluoride – Vacuette), and in a siliconized flask with anticoagulant to obtain serum (vacuum siliconized flask – 4.0 ml - without anticoagulant – Vacuette), and also, in a flask containing EDTA, to carry out the hemogram and obtain hematocrit (packed cell volume) (vacuum siliconized flask – 4.0 ml – EDTA – Vacuette). Hematocrit (packed cell volume) was determined using the micro-hematocrit method (JAIN, 1986), and the total plasmatic protein (TPP) was determined using refractometry (COLES, 1984).

The serum and plasma aliquots were kept frozen at -20 °C. In these, the serum concentrations were measured of creatinine, blood urea nitrogen (BUN), total calcium (tCa), total magnesium (tMg), phosphorous, creattine kinase (CK) and aspartate aminotransferase (AST), and in the plasma, plasma glucose and plasma lactate concentrations, in a automatic biochemical multianalyzer (Chermray 120 Full Automatic Biochemical Analyzer – Rayto, Chine). Plasma osmolarity was determined using freezing-point depression (Advanced Micro-Osmometer Model 3320 - Advanced Instruments Inc., Norwood/MA, USA). All laboratory parameters were measured at the Clinical Pathology Laboratory of Maranhão State University (UEMA), São Luís, Maranhão State, Brazil.

The means and standard deviations of the variables were calculated. For analyzes between times in the same group, the data were submitted to ANOVA and the Tukey test, and at the same time between groups, to the Student's t-test, both with 5% probability. The analyzes were carried out using the statistical analysis program 9.4/2015 (Statistical Analysis System Institute - SAS Institute, SAS/STAT, USA).

RESULTS AND DISCUSSION

All the horses tolerated well the activity performed and there was no abnormal performance in any of the animals before, during or after carrying out the exercise sessions. The physical parameters of the horses before the exercise were within the limits of normality (SPEIRS, 1999).

Regarding the TPC and TTP there were no differences in the same group ($P>0.05$) or between the groups ($P>0.05$) in the assessment times (Table 1), and the color of the conjunctival mucosa and moisture of the oral mucosa remained pink and moist,

respectively, in all the times, indicating normality. Pink and moist mucosa, TPC < 2 seconds and TTP up to 3 seconds, are within the limits that indicate absence of dehydration (SPEIRS, 1999). Gomes *et al*. (2020) also did not find variation in the TPC of quarter mile horses after two barrels races, where like the vaquejada, the exercise is high intensity and short duration. Considering that the variation in the groups was slight and did not alter the TPC to limits higher than normal, it can be considered that the exercise did not cause dehydration in the horses in either of the groups. This was probably due to the animals already being conditioned to the vaquejada tests, that require more intense effort than the degree of effort proposed in the training in the present study.

Regarding the hematocrit, there were differences in each group ($P<0.05$), in the assessment times, where the hematocrit increased after the sprints at T1 ($P<0.05$) in relation to T0 and T2 (Table 1). But there was no variation between the groups in the times assessed. Opposite results were obtained to Lopes *et al*. (2009) who did not find an significant increase in hematocrit of the horses after vaquejada. However, this increase has been described in some studies on horses after vaquejada exercise (BINDA *et al*. 2016, SOUSA *et al*. 2018, HUNKA *et al.* 2018), and may be justified by the fact that the horses have a good reserve of erythrocytes in the spleen (6 to 12 liters), and this, responding to the stimuli that alter the sympathetic activity and release of catecholamines under the influence of the exercise, releases the erythrocytes to the blood, transporting oxygen in the erythrocytic hemoglobin to the tissues. This is reflected in the total red cell count, hematocrit, and hemoglobin concentration (WICKLER e ANDERSON, 2000). Another factor that justifies the rise in hematocrit, is the loss of liquid due to sweating and respiration and exchange of fluids in the plasma to the tissue with an increase in temperature during the exercise, that may be further influenced according to a higher environmental temperature, that reduced the blood plasma volume, generating hemoconcentration (BÖNING *et al*., 2012). Thus, taken together, the increase in erythrocytes and reduction in liquid in the blood are here considered as accounting for the mild hemoconcentration of horses in the present study.

The loss of moisture in the mucosa caused by effort during training or intense exercise tests was reported as a factor responsible for the increase in the TPP, creatinine and BUN concentrations in horses. Thus, they serve as biomarkers that help in the assessment of the hydration status of these animals, and their concentrations can increase when the plasmatic liquid decreases (FERNANDES e LARSSON, 1994). In the present study, the concentration of TPP (Table 1), creatinine and BUN (Table 2) did not increase

(P<0.05). But Santiago *et al.* (2013) observed significant differences in horses doing vaquejada shortly after the exercise, with increased TPP and creatine in the pull horses but only increased PPT in the helper horses, while no alteration for urea was observed in either group. In comparison with the results obtained here, although TPP had a slight increase in T1, and slightly greater in T2 (Table 1), this was not enough to cause a significant change in its concentrations, due to the small loss of fluid from the body and, consequently, low hemoconcentration signaled by the hematocrit obtained. This also influenced the fact that creatinine and BUN concentrations did not vary (Table 2).

Another factor is the production of substances resulting from the muscle metabolism that can cause increase in creatinine, such as degradation of muscle creatin to creatinine and decrease in this urinary excretion during muscle activity (KREIDER *et al.*, 2017). However, the values of these parameters did not vary above the normal limits in the time studies, so it can be considered that the degree of effort in the exercise was not so intense as to cause fluid losses considered, as also, they did not substantially alter CK production resulting from the exercise, influencing, subsequentially, to not increasing the serum creatinine.

Table 1 – Mean values and standard deviations of capillary refill time, skin turgor time, color and humidity of the mucosa, hematocrit and total plasma proteins of Quarter Horses in vaquejada training

Parameter (unit)	Equine	Evaluation time		
		T0	T1	T2
Capillary refill time (second)	Pull	$1.10^{Aa} \pm 0.22$	$1.30^{Aa} \pm 0.45$	$1.10^{Aa} \pm 0.22$
	Helper	$1.30^{Aa} \pm 0.27$	$1.40^{Aa} \pm 0.22$	$1.30^{Aa} \pm 0.27$
Skin turgor time (second)	Pull	$2.20^{Aa} \pm 0.45$	$2.30^{Aa} \pm 0.45$	$2.20^{Aa} \pm 0.45$
	Helper	$2.10^{Aa} \pm 0.22$	$2.25^{Aa} \pm 0.42$	$2.30^{Aa} \pm 0.45$
Color and humidity of the mucosa	Pull	Rose and moist	Rose and moist	Rose and moist
	Helper	Rose and moist	Rose and moist	Rose and moist
Hematocrit (%)	Pull	$37.40^{Ba} \pm 6.35$	$45.20^{Aa} \pm 7.66$	$34.80^{Ba} \pm 3.49$
	Helper	$34.20^{Ba} \pm 2.86$	$43.80^{Aa} \pm 4.92$	$35.60^{Ba} \pm 3.29$
Total plasma proteins ($g\ dL^{-1}$)	Pull	$6.99^{Aa} \pm 0.43$	$7.12^{Aa} \pm 0.39$	$7.44^{Aa} \pm 0.98$
	Helper	$6.72^{Aa} \pm 0.33$	$7.04^{Aa} \pm 0.26$	$6.96^{Aa} \pm 0.36$

T0: before the start of training: T1: immediately after three runs; T2: 1h after T1. Different uppercase letters in the same line indicate different values from each other (P<0.05) by Tukey's test. Different lowercase letters in the same column indicate different values from each other (P<0.05) by Student's t-test.

Source: the authors (2024)

The plasmatic glucose concentration increased in the two groups of horses in T1 ($P<0.05$) – although they remain within the normal reference limits – but there were no differences between them in any of the times ($P>0.05$) (Table 2). Unlike the results in the present study, Souza *et al*. (2018) did not observe differences for blood glucose between before and after vaquejada exercise in pull horses. It is possible that good glycemic stabilization of the animals in the pre-exercise, associated to a previous adaptation of the animals to the type of effort imposed on them, that was less than the higher stress levels the animals were submitted to in previous competitions that caused them bigger adrenalin, collaborated so that only a discrete and non-significant increase in plasmatic glucose occurred, because adrenalin promotes glycogenolysis during exercise and is directly related to the stress intensity (NAKATA *et al*., 1999).

The results for glucose obtained in the present study corroborated with those of other authors. Hunka *et al*. (2018) also observed that in pull and helper horses the glucose concentration increased after one and two sprints, respectively, and returned to the pre-test values after 30 minutes recovery for the helpers, and later for the pull horses. urthermore, they highlighted that both groups also had high glucose concentrations after the races, indicating that the animals had energy reserves after the races, a fact also verified in this study (Table 2), however, with glycemic values with lower averages. Lopes *et al*. (2009) and Soares *et al*. (2021) reported increased glucose in horses after vaquejada sprints during competition. These authors considered that there is a rapid metabolic mobilization to generate an energy supply (glucose) for the horses making the effort in the vaquejada. This increase would be in function of the release of the main action of cortisol (glucocorticoid) in response to exercise, that has an effect antagonistic effect to insulin. Reduction in insulin release becomes limited to capturing glucose by the muscle cell, and with the increase in circulating glucagon, favors increase in the plasmatic glucose levels (HYYPPÃ, 2005 apud RAMALHO *et al*., 2012).

The osmolaridade was not statistically different among the times or between the groups ($P<0.05$) (Table 2). This suggests that the fluid losses through sweating were also not significant to influence this parameter and subsequentially, the osmolarity, corroborating with Kreider *et al*. (2017), who also considered that the increase in osmolarity and reduction in blood plasma volume are, respectively, condition for the increase in ADH (antidiuretic hormone, vasopressin) secretion and release in the circulation, that stimulates reabsorption in the kidneys, helping partially in liquid

conservation in the body. Therefore, the renal adjustment of water and walt excretion corrects the plasmatic volume and adjusts the plasmatic osmolarity.

Santiago *et al*. (2013) considered that there may be different responses in the energetic metabolism between the pull and helper horses when the energy is made available and used during the exercise, as it is derived from fat stores in the body to supply the constant energy demand imposed by the physical effort. These authors also observed a bigger variation in the glucose in the pull horses shortly after the first sprint, while for the helper horses this occurred only after the second sprint. In the face of this finding, the researchers considered that the energy in the pull horses probably came from liver and muscle stores, and that the helper horses needed to convert a little fat and lactate to glucose during their type of exercise. However, the glucose values found in the horses studied by Santiago *et al*. (2013) were below the normal limits before the exercise, but increased when the exercise stopped, leading them to attribute gluconeogenesis as an endogenous collaborator in generating fuel for the muscles during exercise. Simões *et al.* (1999) also reinforced that the rises in blood glucose in phases after exercise would be related to the effects of the catecholamines and glucagon in the liver. Thus, it is considered that these physiological mechanisms are also those that justify the increase in plasmatic glucose in pull and helper horses in T1.

Table 2 – Mean values and standard deviations of creatinine, blood urea nitrogen (BUN), osmolarity, plasma glucose and lactate of Quarter Horses in vaquejada training

Parameter	Equine	Evaluation time		
		T0	T1	T2
Creatinine (mg dL^{-1})	Pull	$1.30^{Aa} \pm 0.14$	$1.56^{Aa} \pm 0.18$	$1.46^{Aa} \pm 0.21$
	Helper	$1.44^{Aa} \pm 0.17$	$1.56^{Aa} \pm 0.32$	$1.48^{Aa} \pm 0.16$
BUN (mg dL^{-1})	Pull	$32.80^{Aa} \pm 5.81$	$35.80^{Aa} \pm 5.07$	$33.60^{Aa} \pm 5.68$
	Helper	$30.20^{Aa} \pm 4.49$	$31.60^{Aa} \pm 8.50$	$31.40^{Aa} \pm 4.28$
Plasma glucose (mg dL^{-1})	Pull	$74.80^{Ba} \pm 7.92$	$91.00^{Aa} \pm 6.60$	$80.40^{ABa} \pm 8.68$
	Helper	$81.60^{Ba} \pm 1.95$	$98.80^{Aa} \pm 12.39$	$88.00^{Ba} \pm 11.11$
Osmolarity (mMol L^{-1})	Pull	$269.31^{Aa} \pm 12.45$	$277.32^{Aa} \pm 5.70$	$271.30^{Aa} \pm 7.28$
	Helper	$266.24^{Aa} \pm 6.09$	$280.66^{Aa} \pm 26.75$	$279.87^{Aa} \pm 12.70$
Plasma lactate (mMol L^{-1})	Pull	$0.61^{Ba} \pm 0.23$	$8.65^{Aa} \pm 2.078$	$1.46^{Ba} \pm 0.66$
	Helper	$0.53^{Ba} \pm 0.05$	$6,24^{Ab} \pm 1.73$	$1.35^{Ba} \pm 0.63$

T0: before the start of training: T1: immediately after three runs; T2: 1 h after T1. Different uppercase letters in the same line indicate different values from each other ($P<0.05$) by Tukey's test. Different lowercase letters in the same column indicate different values from each other ($P<0.05$) by Student's t-test.
Source: the authors (2024)

Lactic acid is dissociated in protons H^+ and lactate (FERGUSON *et al*., 2018), and the plasmatic lactate concentration is commonly used to infer on the level of lactic acid in the blood (SANTOS, 2019). In both the pull and helper horse groups the lactate plasmatic concentration increased in T1, differentiating from T0 and T2 ($P<0.05$) (table 2). Increase in blood lactate after high intensity short duration exercise was also observed in horses shortly after vaquejada (BINDA *et al*., 2016; SOUZA *et al*., 2019), barrel racing (RODRIGUES *et al*., 2016; SOUZA *et al*., 2018; GOMES *et al*., 2019; GOMES *et al*. 2020a; Gomes et al., 2020b) and double roping (PEREIRA et al., 2018). Increase in lactic acid can lead to fatigue or early muscle damage and is more common in athletes little conditioned to the type of exercise (VERMEULEN *et al*., 2017). However, some studies have shown that this increase happening in equines adapted to high intensity short duration exercise, but who showed a considerable reduction in lactate after rest, but who showed a considerable reduction in plasma lactate after resting for 30 min. (BINDA *et al.*, 2016) and 60 minutes from exercise (GOMES *et al*., 2020a).

The reason for this increase in the present study may be justified based on the physiological responses inherent to the type of exercise. The main metabolic pathways for energy production are anaerobic, starting with the use of the phosphocreatine stocks, followed by anaerobic glycolysis, with consequent lactate formation (PEREIRA *et al*., 2018). In high intensity short duration exercises, such as those mentioned above, horses do not make an adequate correlation between the respiration and locomotion mechanisms, and there long pauses in respiration during the sprint. Thus, the blood flow is unable to supply sufficient oxygen and energy for ATP production, so it doesn't respond quickly to the high demand that the type of energy requires. Thus, the organism processes the anaerobic mechanism of energy supply, in which ATP is the main product from muscle glycogen degradation. In the final stage of this degradation, pyruvate is converted to lactate in a reaction catalyzed by the lactate dehydrogenase (SECANI e LÉGA, 2009).

There lactate concentration decreased after rest (T2) compared to T1, but there was still a significant difference between these times ($P<0.05$), and the means in T2 were also higher than in T0 ($P<0.05$) (table 2). Similar results were reported in other studies after vaquejada exercise (LOPES *et al*., 2009; SANTIAGO *et al*., 2013; BINDA *et al*., 2016; SOUZA *et al*., 2018). This characteristic can be justified based on doing continuous and more frequent exercises in training and competitions, so that the organism adapts and responds better regarding capacity to metabolize the increased lactate in the blood after

physical effort. Furthermore, it also collaborates for the fibromuscular development of the athlete horse, especially the IIA type fiber, that has good oxidative capacity (SANTIAGO *et al*., 2013). Pereira *et al.* (2018) observed in double roping horses that exercised in regular training, that lactate decreased after 30 min. They considered this to be a positive effect of training on the physical conditioning of the animals. The horses in the present study were well-conditioned athletes and did vaquejada training and competitions, with adequate physical and muscle size. Therefore, it is probable that they were pre-adapted with good capacity to recompose the blood lactate more quickly, reducing its increase just 60 min. from the end of the exercise.

There were differences between groups in relation to plasma lactate, being higher in the pull group at T1 ($P<0.05$) (Table 2). Similar results were obtained by Hunka *et al.* (2018) in Quarter Horses who carried out a field vaquejada test. These researchers outlined a cycle of three sprints for the pulling group and two cycles of three sprints for the auxiliary group. The pulling group had a much higher lactate concentration after one cycle than that of the assist group after completing two cycles, and lactate recovery to normal levels in both groups was recorded 30 min. after the end of the sprints. It is likely that the reason for the greater lactatemia in the pull horse is the greater effort to which it is subjected by its cowboy in the function of taking down the reins on the track, which means that anaerobic metabolism is more intense in horses that perform this function.

In the present study, the CK and AST concentrations did not differ among the times nor between the groups of pull and helper horses ($P>0.05$), and in both there was only a discrete increase shortly after exercise (T1) that increased a little more after one hour resting (T2), but with values within the limits referred to as normal in all the three assessment times (Table 3).

The CK concentration is more specific for muscle necrosis than AST, the simultaneous determination of AST and CK in horses has very valuable diagnostic potential and helps in the prognosis, because of the different rates of disappearance of its activities in serum or plasma (KINGSTON, 2008 apud PATELLI, 2016). The effect of exercise and training on the serum enzymatic activity of the muscle is difficult to determine. The results vary in the literature due to the differences for type, intensity and duration of the exercise carried out and physical conditioning of the animals and the different modalities (SOUZA *et al.*, 2016). For example, similar results to the present study for CK and AST were observed by SOUSA *et al.* (2018) in horses during vaquejada competition, both in a group of horses that competed sporadically and in a group of horses

that did vaquejada regularly. The authors reported that in both groups there was a discrete non-significant raise for these enzymes immediately after sprinting, with later decrease of their concentration at 30 and 120 min., and at no time were there concentrations above normal limits. However, Souza *et al.* (2017) reported significant increase for CK, but not for AST, in horses that did only one sprint of simulated vaquejada.

Lopes *et al*. (2009), Santiago *et al.* (2013) and Souza *et al*. (2017) observed increased CK concentration in vaquejada horses after exercise, that did not occur for AST. To the contrary, Souza *et al*. (2018) reported that the enzymatic AST levels increased significantly in vaquejada horses immediately after three vaquejada sprints, and not more after 50 min. (end of assessment) when this decreased. On the other hand, the CK concentrations also did not present statistical difference during the times assessed.

Table 3 – Mean values and standard deviations of creatine kinase (CK), aspartate aminotransferase (AST), total calcium, phosphorus and total magnesium concentrations of Quarter Horses in vaquejada training

Parameter	Equine	Evaluation time		
		T0	T1	T2
CK ($U\ L^{-1}$)	Pull	$162.60^{Aa} \pm 22.06$	$176.80^{Aa} \pm 28.38$	$181.60^{Aa} \pm 34.44$
	Helper	$156.40^{Aa} \pm 34.87$	$172.25^{Aa} \pm 21.27$	$173.20^{Aa} \pm 65,24$
AST ($U\ L^{-1}$)	Pull	$243.80^{Aa} \pm 12.76$	$269.80^{Aa} \pm 21.00$	$256.80^{Aa} \pm 19.59$
	Helper	$257.00^{Aa} \pm 49.68$	$270.25^{Aa} \pm 63.53$	$252.40^{Aa} \pm 25.50$
Total Calcium ($mMol\ L^{-1}$)	Pull	$2.81^{Aa} \pm 0.28$	$2.78^{Aa} \pm 0.21$	$2.96^{Aa} \pm 0.22$
	Helper	$2.92^{Aa} \pm 0.42$	$3.09^{Aa} \pm 0.86$	$3.01^{Aa} \pm 0.10$
Phosphorum ($mg\ dL^{-1}$)	Pull	$2.29^{Aa} \pm 0.21$	$2.45^{Aa} \pm 0.30$	$2.12^{Aa} \pm 0.34$
	Helper	$2.28^{Aa} \pm 0.34$	$2.35^{Aa} \pm 0.28$	$2.34^{Aa} \pm 0.22$
Total magnesium ($mg\ dL^{-1}$)	Pull	$0.91^{Aa} \pm 0.10$	$0.91^{Aa} \pm 0.15$	$0.94^{Aa} \pm 0.18$
	Helper	$1.00^{Aa} \pm 0.11$	$0.97^{Aa} \pm 0.22$	$0.94^{Aa} \pm 0.07$

T0: before the start of training: T1: immediately after three sprints; T2: 1 h after T1. Different uppercase letters in the same line indicate different values from each other ($P<0.05$) by Tukey's test. Different lowercase letters in the same column indicate different values from each other ($P<0.05$) by Student's t-test.
Source: the authors (2024)

Studies on horses in other equestrian modalities have also reported results for CK and AST in relation to exercise. Binda *et al*. (2016), Gomes *et al*. (2019) and Gomes *et al.* (2020b) also did not observe variation in the CK and AST enzymes in horses before and after barrel racing exercise. Patelli et al. (2016) observed increase in CK before and after separation and barrel racing tests, but there was no difference in relation to the type of test and, regarding AST, they verified increase of this enzyme only after exercise in

the separation modality. Assessing the serum CK, AST and amyloid A protein (PAA) in ten horses that jumped one-meter-high obstacles, Carvalho Filho *et al*. (2019) did not detect significant difference for AST in show jumpers in the pre-exercise and at 30, 60 and 120 min. after exercise, but increase in CK 30 min. after the end of the exercise, that stabilized after 24 h.

Hunka et al. (2018) found only a slight increase in CK in pulling and helping horses after one cycle and two cycles of vaquejada, respectively, but without reaching muscle injury thresholds, and attributed this to the fact that the animals were in good physical condition for this activity. Similarly, the horses in the present study also were adapted and in continuous vaquejada activity, with good adaptation and good muscular condition. According to Barralet e Ricketts (2002), repetitive and adequate exercises induce physiological adaptation of the horses to stressful exercise, reducing extracellular alterations that are damaging to the muscle cells. Therefore, as CK and AST levels are related to exercise type and intensity, it is possible that at the end of three sprints, the intensity had not reached a degree of effort beyond the physical conditioning to which the animals were already adapted and did not significantly influence the variation in concentration of these enzymes.

The times when there was increase in serum CK and AST in horses by laboratory analyses differ among studies. In the present study, the last CK and AST concentrations were assessed one hour after the exercise. According to Thomassian *et al*. (2007), there is a quick transitory increase in these enzymes shortly after exercise, proportional to the exercise intensity, and indication of myopathy due to effort signaled by these enzymes only occurs after the exercise, with enzyme peak between 3 and 6 hours for CK and between 12 and 24 hours for AST. Corroborating this author, Rodrigues *et al*. (2016) verified increase in the mean serum CK in quarter horses after four hours resting after the end of the simulated barrel racing exercise, with even bigger concentrations than the means observed shortly after the end of the exercise. However, Souza *et al.* (2018) did not observe an increase indicative of CK and AST in pull horses after the vaquejada exercise. According to Michima *et al*. (2010), the CK and AST activity has a mean positive linear correlation with the distance run, that is, the greater the distance and workload, the greater is the demand for skeleton muscle work, and may derive only from the increase in extravasation of the integral muscle fibers in function of the increase in muscle activity, without alteration in the muscle cell integrity. Furthermore, increase in AST is not specific to the muscle cell, although this and the hepatocytes

are its main sources, and only when there is a tissue lesion will it subsequently increase (SILVA *et al*., 2007). As the horses in both the groups finished the training in good physical condition and without clinical signs that would indicate myopathies, a physiological extravasation of the enzyme justifies better its slight increase in the muscle enzymatic activity after exercise, that was not significant, perhaps because the exercise was not over long distance although the horses had made three sprints.

The tCa values did not vary in the groups nor between the assessment times (P>0.05) (Table 3). The results also corroborated with Inoue *et al*. (2002) who observed that the serum calcium concentration remained stable in pure bred mares shortly after exercise. However, it differed for after exercise because a quick reduction was observed in the calcium values after the horses´ rest. They considered that the increase in serum calcium (hypercalcemia) was due to hemoconcentration in the animals, but it may have been supplied later by the increase in circulating calcitonin. Similarly to the results of the present study, increases in tCa were not observed at the end of the exercise (T2). Thus, it is possible that the tCa concentration increased temporarily during and at the end of the exercise in the horses in the present study, but also increased the calcitonin secretion, that prevented hypercalcemia.

In high intensity short duration exercise such as the barrel racing test, Gomes *et al*. (2019) detected that the tCa did not vary in the horses. But in English thoroughbred horses that galloped over distance of two meters (m), an increase in calcium was reported by Crocomo *et al.* (2009). According to Titto *et al*. (2009), little calcium is lost through sweating in the short duration intense exercises, because only a small quantity of liquid is lost through sweating. Thus, as the dehydration in the horses in the present study was low, the water loss cannot have been intense and so did not cause significant calcium loss in the sweat, that reflected in normality limits for calcium the times studied.

The phosphorus concentration also did not vary (P<0.05) among the times or between the groups (Table 3). The results did not corroborate with Gomes *et al*. (2020b) found an increase in serum phosphorus in horses after two three-barrel races, and a decrease in it after a one-hour rest, and Santos *et al*. (2019) observed increased in this eletrolyte in horses after vaquejada, that decreased and was only recovered after 50 minutes rest from the activity. The authors attributed the increase to the release of phosphate ion by the dephosphorylation of the ATP from the metabolic mechanisms in the exercise performed, to provide high energy for muscle contraction. Moreira *et al*. (2015) verified increase in this variable in horses after mounted police duties and reported

that this fact may have been due to reduced phosphorus glomerular filtration rate (TFG) due to the exercise, since this reduced the renal, peripheral and muscle blood flow. Furthermore, there is big phosphorus reabsorption in the kidney tubules as the TFG decreases, that collaborates so that the phosphorus blood levels may not vary or vary very little. Thus, it is possible that this fact also influenced the non-variation in the serum phosphorus in the horses in the present study.

The tMG mean values did not differ in the groups or among the times assessed (P>0.05) (Table 3). These results corroborated with with the results by Crocomo *et al*. (2009) in English Thoroughbred Horses doing long duration high intensity exercise (racing) because they also did not observe alterations in the serum magnesium in the horses after the activity. The tMg is involved in several metabolic processes, especially in the muscle tissue where, similarly to tCa and phosphorus, it is released from the sarcoplasmic reticule in the muscle contraction process and recomposed when the stimulation from the exercise stops (CANDIDO, 2016). Thus, the exercise intensity, the physical demands and muscle contraction in the horses in the present study may not have been as intense as in modalities with higher intensity and duration such as racing and marching, so that the phosphorus and magnesium concentration remained leveled after the exercise compared to those measured before exercise and after rest. Thus, can be considered that alteration in the serum magnesium seemed to occur according to the physical conditioning of the athlete horse and in accordance with type, intensity, and duration of the exercise.

CONCLUSIONS

Both the pulling and helping Quarter Horses showed similar responses in most of the physiological and biochemical parameters evaluated, keeping them within normal limits, characterizing that they presented good physical conditioning to the degree of effort imposed on them in the proposed training design.

In both groups, exercise caused a slight change in hydration status, characterized by a slight increase in hemoconcentration, signaled by hematocrit, and hyperlactatemia signaled by an increase in plasma lactate, but which were recovered after an hour of rest from exercise. characterizing that these changes were transient and due to sweating and muscular effort, respectively.

The higher hyperlactatemia in the group of pulling horses, immediately after the sprints, suggests that the use of these horses in this role causes more intense anaerobic

metabolism in them than the horses used as assistants in the vaquegada equestrian activity, characterizing that they are subjected to greater muscular effort due to the physical force imposed by the cowboy-puller to bring down the steer on the track.

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