

HEMATO-BIOCHEMICAL RESPONSE TO EXERCISE WITH ERGOMETRIC TREADMILL, MOUNT TRAINING AND COMPETITION IN JUMPING HORSES

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RESUMO: Este estudo objetivou avaliar as variações hematológicas e bioquímicas que ocorrem em diferentes protocolos de exercícios em equinos de salto. Foram utilizados 17 equinos da raça de salto Brasileiro de Hipismo, entre 5 e 12 anos. Todos os animais foram submetidos aos seguintes protocolos de exercício: repouso (controle); esteira ergométrica (40 min a 5 m/s); treinamento montado (40 min, sendo 10 min ao passo, 20 min ao trote, 10 min ao galope); e competição (prova de salto a 350 m/min, obstáculos com 1,20 m de altura e percurso de 430 m). Foram realizadas coletas de sangue e verificação da frequência cardíaca com os animais em repouso (controle) e imediatamente após o término dos protocolos de exercício. As avaliações sanguíneas abrangeram contagem de eritrócitos e leucócitos totais, hematócrito, concentração de hemoglobina, proteínas plasmáticas totais, creatina quinase (CK), lactato desidrogenase (LDH), aspartato transaminase (AST), fosfatase alcalina (FA), lactato, glicose, bicarbonato, ureia, creatinina, sódio e potássio. Em relação ao grupo controle, o exercício em esteira ergométrica apresentou variações significativas apenas em hematócrito, frequência cardíaca, potássio e creatinina. O treinamento montado e a competição apresentaram aumentos nas concentrações de proteína, CK, LDH, FA e potássio com relação aos demais protocolos de exercício. Hemoglobina e lactato aumentaram apenas no grupo de competição. A concentração de glicose reduziu em todos os grupos experimentais quando comparada ao grupo em repouso. A frequência cardíaca, a creatinina, número de eritrócitos, hematócrito e FA apresentaram aumento progressivo conforme a intensidade de exercício. Não houve alterações nos valores de AST, ureia e sódio. Conclui-se que os diferentes protocolos de exercício em equinos de salto promoveram diferentes respostas nos parâmetros hemato-bioquímicos.

Palavras-chave: enzimas clínicas; fisiologia do exercício

ABSTRACT: The aim of this study was to evaluate the variations promoted by different kinds of exercise on hematological and biochemical parameters of jumping horses. Seventeen Brazilian Jumping horses (Brasileiro de Hipismo breed) from 5 to 12 years old were used. All animals were submitted to the following exercise protocols: rest (control group), ergometric treadmill (40 minutes at a speed of 5 m/s), mount training (40 minutes consisting of 10 minutes walking, 20 minutes trotting and 10 minutes running on field conditions) and jumping competition (speed of 350 m/min, height of obstacles 1.20 m and 430 m length). Venous blood samples were drawn from the jugular vein and heart rate were verified at rest (control) and immediately after the exercise. The erythrocyte and leucocytes count, packed cell volume, hemoglobin concentration, creatine kinase (CK), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), sodium, potassium, bicarbonate, total plasma protein (TPP), urea, creatinine were determined. The treadmill group showed higher values of PCV, heart rate and creatinine compared to control group. Mount training group and jump group promoted an increase of total protein, CK, LDH, ALP, lactate, creatinine and potassium. Hemoglobin and lactate increase only in jump competition. Glucose concentration decreased in all groups of exercise practice. Heart rate, erythrocyte counting, PCV, ALP and creatinine increased in a positively relation with the exercise intensity. No alterations were observed in AST, urea and sodium. It is concluded that different exercise protocols in jumping horses promoted different responses on hemato-biochemical parameters.

Key Words: clinical enzymes; exercise physiology

INTRODUÇÃO

In the last years the interest for exercise physiology in horses has been growing in the search for a better knowledge on the responses to several types of exercises (Sabev, 2011). However, many gaps in this subject still remain to know in the perspective to obtain evaluation tools for training techniques (Kowal et al., 2006).

Early works concerning exercise and training evaluation in horses only considered total blood count, but thereafter biochemistry parameters were included in the performance study of the athlete horse (McKenzie et al., 2014). Analysis of hematological and biochemical parameters are useful during training for driving the intensity and the type of the effort appropriate to the athletic capacity of each animal.

The use of ergometric treadmill in horses took along several advantages not only in diagnosis aspects, but also as an important support during training activities (McGowan et al., 2002; Prince et al., 2002). Ergometric treadmill allows a better study of the impact promoted by a determined exercise pattern, as it can be measured speed, constancy and duration of the exercise (Seeherman, 1991).

The present study evaluates the use of common techniques of training in ergometric treadmill, in traditional exercise and in competition through the variations observed in hematological and biochemical parameters in jumping horses.

MATERIAL E MÉTODOS

In this work seventeen jumping horses of both sexes and with ages between 5 to 12 years were submitted to three different situations of exercises. All the animals were trained to the practice of traditional and treadmill exercise, mounting exercise and competitions.

The horses were maintained in stables belonging to an equestrian society in Porto Alegre (southern Brazil), feeding a commercial concentrate twice a day, supplemented with hay and having water ad libitum. All the horses were clinically evaluated and only healthy animals participated in the experiment. All the animals were submitted to each one of the following treatments: (1) Rest: maintained resting in the stable for at least 24 hours (control group). (2) Ergometric treadmill (Equiboard model ET01, São Paulo, Brazil): training exercise with slope of 0° for 40 minutes and constant speed of 5 m/s; (3) Traditional mount training during 40 minutes (10 minutes walking, 20 minutes trotting and 10 minutes running on field conditions) e (4) Competition: jumping competition at a mean speed of 350 m/min, obstacles with 1.20 m height and extension of 430 m. The interval among each treatment was at least 12 days. The experiment was performed during the spring season, when the historical environmental temperature of Porto Alegre presents intervals from 15°C to 30°C and relative humidity between 65 to 80%.

During rest and immediately after finishing the treatments, heart rate was measured in all animals using a cardiac monitor (Datascope model Passport 2, The Physician's Resource for Medical Equipment, USA). Blood samples were taken from the jugular vein using in vacuum tubes with sodium fluoride for determining glucose and lactate, vacuum tubes with EDTA for total blood count and bicarbonate, vacuum plain tubes for determining enzymes (AST, ALP, LDH and CK), electrolytes (Na, K), urea and creatinine.

Blood counting includes packed cell volume (PCV) obtained by microhematocrit technique (Jain, 1986), hemoglobin by the cyanometahemoglobin method, and erythrocyte and leukocyte counting by

microscopy Neubauer chamber, using as diluents Hayem solution for erythrocyte counting and Türk solution for leukocyte counting with magnification of 1:200 and 1:20, respectively. Total protein was determined by refractometry and determination of CK, AST, LDH, ALP, lactate, glucose, urea and creatinine were done by colorimetric techniques using Cobas Integra (Roche). Sodium and potassium were determined by potentiometry using selective electrodes (Integra 400, Roche). Bicarbonate concentration was obtained by gasometry (Rapidlab 348, Bayer).

The statistical analysis included one-way Anova for repeated measures among groups and variables differences compared using the Tukey test. The considered level of probability was 5%.

RESULTS

The values of the hematological and biochemical parameters of the jumping horses in the exercise protocols are showed in Table 1. Erythrocyte counts increased significantly with the increment of the exercise effort. The same tendency was observed with PCV values. The difference between PCV at rest and in competition was tenfold increase. Hemoglobin concentration was higher only in the competition group. Leukocyte counting did not show any difference among the groups. Heart rate increased proportionally with the exercise intensity. The heart rate value of the horses in competition was fivefold increase compared with the values at rest.

Table 1. Hematological and biochemical parameters in jumping horses immediately after being submitted to different types of exercise: ergometric treadmill (40 min), mount training (40 min) and jump competition. The values are expressed as mean \pm standard deviation. In all cases N=17.

Parameter	Rest (control)	Ergometric treadmill	Mount training	Jump competition
Heart rate (bpm)	36.6 ^a \pm 3.5	60.0 ^b \pm 16	124.0 ^c \pm 8	184.3 ^d \pm 8.3
Erythrocytes (10^9 /mL)	8.18 ^a \pm 0.73	8.55 ^b \pm 0.54	8.84 ^b \pm 0.69	9.54 ^c \pm 1.26
PCV (%)	32.64 ^a \pm 3.02	36.88 ^b \pm 3.48	39.29 ^b \pm 3.80	43.23 ^c \pm 6.56
Hemoglobin (g/dL)	12.3 ^a \pm 1.43	11.24 ^a \pm 1.32	12.91 ^a \pm 1.16	14.07 ^b \pm 1.86
Leukocytes (μ L)	8,217 \pm 1,216	8,300 \pm 1,107	7,988 \pm 1,462	8,241 \pm 1,450
Total protein (g/L)	60.1 ^a \pm 3.5	58.7 ^a \pm 3.4	66.6 ^b \pm 4.2	66.7 ^b \pm 4.7
CK (U/L)	64.3 ^a \pm 16.2	77.8 ^a \pm 30.6	103.0 ^a \pm 34.1	91.7 ^a \pm 42.2
LDH (U/L)	224.2 ^a \pm 75.4	249.1 ^a \pm 142.8	262.0 ^a \pm 79.0	201.3 ^a \pm 47.3
AST (U/L)	144.5 \pm 53.3	143.7 \pm 40.8	125.2 \pm 17.8	128.7 \pm 34.0
ALP (U/L)	109.5 ^a \pm 34.0	100.0 ^a \pm 20.5	143.9 ^b \pm 28.9	150.6 ^b \pm 37.1
Lactate (mmol/L)	0.60 ^a \pm 0.15	0.63 ^a \pm 0.12	0.56 ^a \pm 0.17	3.14 ^b \pm 1.93
Glucose (mmol/L)	5.46 ^a \pm 0.652	4.81 ^b \pm 0.576	4.53 ^b \pm 0.741	4.98 ^b \pm 1.12
Bicarbonate (mmol/L)	22.8 \pm 1.8	23.9 \pm 2.8	22.0 \pm 5.2	22.5 \pm 4.2
Urea (mmol/L)	6.50 \pm 1.93	6.58 \pm 1.92	6.29 \pm 1.32	6.32 \pm 1.15
Creatinine (μ mol/L)	107.8 ^a \pm 6.2	112.3 ^a \pm 4.4	137.0 ^a \pm 15.9	140.5 ^a \pm 13.2
Sodium (mmol/L)	136.29 ^a \pm 3.56	136.4 ^a \pm 4.22	136.12 \pm 2.64	136.29 \pm 3.56
Potassium (mmol/L)	3.55 ^a \pm 0.29	3.81 ^a \pm 0.52	4.10 ^b \pm 0.22	4.09 ^b \pm 0.43

Different letters indicate significant difference ($P < 0.05$) among exercise protocols. PCV: packed cell volume, CK: creatine kinase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase.

Total protein values were higher in the groups of training and competition, compared with the groups at rest and treadmill. The CK activity was greater in the mount training group compared with the competition group, and the treadmill exercise did not differ from the horses at rest. The animals in mount training had the highest values of LDH. The AST activity did not vary among all types of exercises. The ALP activity was higher in horses submitted to mount training and competition, compared with the animals at rest and in treadmill exercise.

The concentration of lactate was greater only in the competition group which had fivefold increase in relation to the other groups. The glycemia values were higher in the horses at rest compared with the animals submitted to exercise. Bicarbonate, sodium and urea values did not differ in any group. Creatinine values had an increase which was gradual with the intensity of exercise. Potassium values were higher in the horses submitted to mount training and competition compare with the animals at rest or in the ergometric treadmill.

DISCUSSION

The present work compares different exercise protocols with focus on hemato-biochemical changes in jumping horses. The exercises had a progressive intensity (ergometric treadmill → mount training → jump competition) having the rest condition as control group. Heart rate evaluation confirms the progressive intensity of the exercise protocols. The progressive increments of the exercises were similar to those detected by Voss et al. (2002) and Sabev (2011).

Erythrocyte counting and PCV significantly augmented with the exercise intensity, with higher values in jump competition. Even though Ferraz et al. (2009) mentioned that PCV increases with the intensity of the exercise, as a consequence of hemoconcentration caused by dehydration, and erythrocyte counting augments in response to spleen contraction, the results of the present work clearly show that jump competition demands higher circulation of blood red cells, compared to training (mounted or treadmill). Hemoglobin values were significantly higher only in the jump competition group. Aguilera-Tejero et al. (2000) found a hemoglobin increment in horses after intensive exercise varying from 11.8 ± 0.5 g/dL at rest to 18.0 ± 0.6 g/dL. Hemoglobin increase is a physiological response to exercise in order to increase the tissue oxygenation capacity of the blood (Rose et al., 1980). The present results suggest that erythrocyte counting and PCV seem to be more sensible indicators that respond to moderate exercise, while hemoglobin is an indicator that responds to more intense exercise.

Leukocyte counting did not show any difference among groups, in contrast with the findings of Ferraz et al. (2009), who observed increase of total leukocytes in horses at rest ($8,730 \pm 360/\mu\text{L}$) compared with horses after

exercise ($11,280 \pm 520/\mu\text{L}$). This increment was attributed to catecholamine's action released after vigorous exercise. However, this is a transitory effect with a maximum duration of 30 min (Paludo et al., 2002) and was not observed in the present experiment.

Total protein concentration was higher in horses submitted to mounting exercise and to jump competition, compared to horses at rest or in treadmill exercise. Rose et al. (1980) also found an increase of total protein in horse (from 68.0 ± 4.7 g/L before exercise to 73.1 ± 6.2 g/L after jump competition), attributed to a moderated degree of dehydration.

Activities of muscular enzymes, namely creatine kinase (CK), aspartate transaminase (AST) and lactate dehydrogenase (LDH) have been used as indicators of muscle lesions (Balarin et al., 2005). In the case of CK, some transient increments have been observed after exercise due to higher permeability membrane without any muscle lesion (Gondin et al., 2013). CK activity was higher in the groups of mounting exercise and jump competition compared to rest and treadmill groups. Those findings agreed with data observed by Andrews et al. (1995) and Robinson (2003). CK response in athletic horses depends on training condition, being higher in animals in initial phase of training and in harder and longer exercises (Harris et al., 1998). In polo ponies, Ferraz et al. (2010) found plasma CK increments of 35% after 6 hours of a training match returning to baseline after 12 h. Those authors suggest a period of 24 h after a physical effort to clinically evaluate CK values in athletic horses, considering that this enzyme has a plasma half-life of 6 h.

LDH in horses at rest diminished in a progressive way as training condition of the animals is more advanced (McKenzie et al., 2014). In

those animals, low increase due to exercise might be observed. In the present work LDH was higher only in the mounting group, which is unexpected considering that the jump competition is a harder exercise. Anyway, the relatively low LDH increase in more severe exercises revealed a good physical conditioning of the animals. McGowan *et al.* (2002) also did not find differences in LDH values in horses submitted to treadmill exercises.

Values of AST were not different among groups in the present study, which is a characteristic of a good athletic condition in animals submitted to exercise (Harris *et al.*, 1998). Andrews *et al.* (1995) found that AST activity increases after a three day long competition compared to values at rest and Snow *et al.* (1982) observed moderate increment in AST after an endurance riding of 80 km, attributed to dehydration and intravascular hemolysis. Those findings revealed that AST activity may be augmented in cases of extreme exercise demand in well conditioned horses. Lack of increment responses in plasma enzymes may be due to moderated exercise and increased values due to exercise generally return to their basal levels after 30 min at rest (Thomassian *et al.*, 2007, McGowan, 2008).

Alkaline phosphatase (ALP) activity was higher in mounting and competition groups. Rose *et al.* (1980) and Williamson *et al.* (1996) reported increases in ALP in horses participating of endurance riding, suggesting that the intensity of the exercise lead to fluid losses.

Although many works show increments in plasma enzymes after exercises of several degrees of intensity, in general, those increments may reach 50% of baseline and remain within the reference limits. Marked enzyme increments have been observed only in

horses with suspected pathologic muscle damage (McGowan, 2008).

Lactate is an important indicator of exercise performance (Pösö, 2002), because adapted animals have more stability of serum concentrations than non-adapted animals. Alterations in the values of serum lactate may indicate fatigue as a consequence of an increased anaerobic muscle catabolism during exercise (McGowan *et al.*, 2002). In the present experiment, animals training on treadmill or mounting exercise did not have increments in lactate, but after jump competition, the serum concentration was 5 fold the mean of the other groups ($P < 0.05$), indicating the intensity of the exercise. Guhl *et al.* (1996) found a positive correlation between lactate concentration and intensity of training and Thomassian *et al.* (2005) detected lactate alterations in horses trained at speed higher than 8 m/s (in the present experiment speed in treadmill was 5 m/s). Ferraz *et al.* (2010) found an increment of 29 fold in plasma lactate of ponies submitted to a training match compared to the resting situation, however returning to the baseline after 6 h post-exercise. This variation in plasma lactate demonstrates that the aerobic condition might recover few hours after severe exercise.

Glucose concentration in serum reduced after exercise in all the groups of this experiment, as a consequence of energy demand and depletion of hepatic glucose. More intense or longer exercises as endurance riding or complete races may cause hyperglycemia (Fernandes and Larsson, 2000), which is considered a physiological response.

Bicarbonate concentrations in all groups of exercised horses were within the physiological range (Robinson, 2003) without any difference in relation to the rest group. This finding was surprising in the jump competition group,

as an increase in lactate may result in acidosis (Aguilera-Tejero et al., 2000). It is probable that lactate increase may be less drastic than trotting or gallop because the lower duration of jumping competition. In addition, the stability of bicarbonate may result from an adequate compensation or physical adaptation of the animals (Foreman et al., 1996).

Serum urea also did not show alterations with exercise in the horses of this experiment, similarly to the finding of Fernandes et al. (2010) with Thoroughbred horses submitted to exercise. In contrast, creatinine values increased in a progressive manner according to the intensity of exercise, in agree to the observed by Fernandes et al (2010). This phenomenon may be explained by a reduction in the plasma volume and an increase in the muscle protein catabolism (Rose et al., 1980). The rise in creatinine occurs before any rise in urea because urea excretion by kidney is significantly reduced during exercise.

Sodium concentrations did not vary among groups of horses, as in the work of Fernandes et al. (2010) studying horses submitted to different intensity exercises. Matsui et al. (2002) found sodium losses of 23% of mineral requirements through sweat in horses submitted to intense exercise, but they suggest that an additional sodium supplement is not necessary to compensate such losses. Crocomo et al. (2009) found serum sodium decreases in Thoroughbred horses after intense exercise attributed the sodium sweat losses. Aguilera-Tejero et al. (2000) observed rise in sodium after a jump competition, as a consequence of water loss, confirmed by hyperproteinemia. It seems that hypernatremia will occur only in more intense and longer exercises, as it was observed in ponies submitted to high intensity effort (Ferraz et al., 2010).

Potassium behavior was different than sodium. Higher values of potassium were found in horses submitted to mounting exercise and jump competition, compared to rest and treadmill groups. Aguilera-Tejero et al. (2000) and Piccione et al. (2007) mentioned that potassium increase may occur in intense exercise as a consequence of dehydration and may accompany the increment in total protein, as observed in the present work. Schott et al. (2002) cited that increase in potassium may be due, not only to plasma volume reduction, but also to cation exchange from inner cell fluid in lactate metabolic acidosis compensation.

CONCLUSION

It is concluded that physical exercise intensity cause different hemato-biochemical alterations in jump horses. Erythrocytes, PCV, ALP, creatinine and heart rate increase progressively with the exercise intensity. Hemoglobin and lactate increase only in jump competition. Total protein, CK and potassium increase in mounting exercise and jump competition. Glucose decreases in all the exercise protocols, while no alterations are observed in AST, urea and sodium.

NOTE

The research was approved by the Ethical Committee of the Federal University of Rio Grande do Sul (Project n° 9934).

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