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CLINIC AND SURGERY

Metabolic responses of jumping horses training on an inclined treadmill

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Respostas metabólicas dos equinos de salto em treinamento na esteira inclinada

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ABSTRACT: This study assessed the metabolic responses of seven show-jumping horses subjected to an inclined treadmill training. The horses were trained daily in 50-minute morning sessions, five days a week, which included dressage and flexion exercises, and jumping gymnastics. The treadmill training occurred in the afternoon for 16 weeks, with two phases. In both phases, horses were trained twice weekly, with three-day intervals between sessions. The first phase lasted six weeks with walking (1.7 m/s) and treadmill inclines of 0%, 7%, and 13%. The second phase lasted ten weeks with similar training frequency and higher intensity, with treadmill at a 13% incline. Blood samples were collected before and after the treadmill training and, during training sessions to determine AST, CK, LDH, uric acid, glucose and lactate. Data was analyzed by ANOVA using a split-plot design, with evaluation time points (before and after training) as plots and blood collection times during the training session as subplots. Means were compared using Tukey's test. No difference in CK and uric acid were observed before and after training ($P > 0.05$). Both variables were affected by collection time ($P = 0.009$), reaching peak values 12 h post-training. Differences of LDH were observed before and after treadmill training. No differences were observed in the AST concentration. Glucose and lactate did not differ before and after the treadmill training. The treadmill training of show-jumping horses was a safe protocol without evidence of muscle injury.
Key-words: equine, exercise, muscle enzymes, treadmill.

Respostas metabólicas dos equinos de salto em treinamento na esteira inclinada

RESUMO: Este estudo teve como objetivo avaliar as respostas metabólicas de sete cavalos de salto da raça Brasileiro de Hipismo, submetidos a treinamento complementar em esteira ergométrica em plano inclinado. Para avaliar as respostas metabólicas, os cavalos eram treinados diariamente em sessões matinais de 50 minutos, cinco vezes na semana, incluindo exercícios de adestramento, flexionamento e salto. Os cavalos foram submetidos a treinamento em esteira ergométrica no período da tarde durante 16 semanas, em duas fases. Além disso, em ambas as fases, os cavalos treinaram duas vezes por semana, com intervalos de três dias entre as sessões. A primeira fase durou seis semanas com locomoção ao passo (1,7 m/s), com sessões de treinamento na esteira com inclinação de 0%, 7% e 13%. A segunda fase durou dez semanas, com treinamento similar, intensidade maior e esteira na inclinação de 13%. Amostras de sangue foram coletadas antes e depois do treinamento complementar e, durante as sessões de treinamento foram avaliados AST, CK, LDH, ácido úrico, glicose e lactato. Os resultados foram analisados por ANOVA, em delineamento de parcelas subdivididas, com os momentos de avaliação antes e depois do treinamento analisados como parcelas e os tempos de coleta de sangue analisados como subparcelas. As médias foram comparadas pelo teste de Tukey. No entanto, não houve diferença na concentração de CK e ácido úrico antes e depois do treinamento complementar ($P > 0,05$). Contudo, houve efeito do momento da coleta de sangue durante as sessões de treinamento para ambas as variáveis ($P = 0,009$), atingindo valores máximos 12 horas após a sessão de treinamento. Ademais, diferenças nas concentrações de LDH foram observadas entre antes e após o treinamento complementar ($P < 0,05$). Entretanto, não foi observada diferença na concentração sanguínea de AST e a glicose e o lactato não diferiram antes e depois do treinamento ($P > 0,05$), respectivamente. Portanto, o protocolo de treinamento complementar em esteira inclinada com cavalos de salto demonstrou ser seguro com adaptação metabólica e sem evidências de lesão muscular.
Palavras-chave: equino, enzimas musculares, exercício.

INTRODUCTION

Jumping horses undergo specific training programs and their assessment usually requires a combined approach, including a standardized exercise test and a more subjective evaluation of behavior and locomotion. One of the primary goals of athletic training in horses is to improve musculoskeletal strength, thereby increasing resistance to injury. Physical exercise markedly influences the physiology

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of athletic animals, as high-intensity effort and conditioning stimuli trigger broad cellular and molecular responses (CHANDA & PETCHDEE, 2022; PLISAK et al., 2023). Competitions impose a substantial physical demand frequently associated with transient inflammatory and metabolic disorders that can trigger injuries (KLOBUČAR et al., 2019). However, many injuries are preceded by inadequate adaptation of muscle or tendon tissues to exercise, an imbalance between subclinical damage and cellular repair activity, or both (PUGLIESE et al., 2020). Consequently, injuries reduce athletic performance, muscle capacity, and lead to financial losses (RIBEIRO FILHO et al., 2018).

High speed treadmills' had been widely used for training, assessment, rehabilitation, and biomechanics studies in horses, as it allows exercise protocols to be standardized under controlled conditions. It enables the stimulation of walk, trot, and gallop, in any desired combination, while providing safe and uniform locomotor conditions. In addition, treadmill exercise allows precise control of speed, acceleration, deceleration, incline, duration, and distance according to the intended workload (THOMASSIAN et al., 2007; PUGLIESE et al., 2020; LEWCZUK & MAŚKO, 2024). Inclined treadmill exercise shifts the horse's weight backward, increasing pelvic limb loading and the force generated by these limbs (ROBERT et al., 2000). According to DENOIX (2014), uphill locomotion demands requires concentric contraction of the propulsive muscles to generate a resultant force vector directed both forward and upward, displacing the horse's center of mass against gravity. A similar biomechanical principle applies to jumping exercise. Furthermore, MCDONOUGH et al. (2002) showed that when Thoroughbred horses run on a treadmill with a 10% incline, maximal oxygen consumption increases by approximately 20% compared with running on a level treadmill, with metabolic thresholds occurring at proportionally higher absolute intensities, allowing the horse to sustain greater effort before reaching metabolic fatigue. Together, these biomechanical and metabolic effects support the use of inclined treadmill exercise as a complementary conditioning strategy for show jumping horses, which require well-developed musculature to generate sufficient power and impulsion to overcome obstacles effortlessly.

Horses have morphological characteristics suited to strength and speed and these attributes can be developed through training. Conversely, inadequate or excessive training demands routine may compromise animal welfare, increase the risk of musculoskeletal injury, and negatively affect athletic performance, potentially resulting in the lost competitive season, wasted investment, and depreciation within the equine industry (HODGSON et al., 2014a; 2014b).

Serum levels of aspartate aminotransferase (AST), creatine kinase (CK), lactate dehydrogenase (LDH), glucose, lactate, and uric acid (UA) are well-established biomarkers in equine exercise physiology. These biomarkers provide relevant information on muscle response (AST, CK, LDH), energy metabolism (glucose and lactate), and oxidative status (UA) during physical exercise (CASTEJÓN et al., 2006; TEIXEIRA-NETO et al., 2008; FERRAZ et al., 2008; KRUMRYCH, 2010). These parameters offer a practical and clinically relevant approach for monitoring performance, physical adaptation, and metabolic responses in sport horses. This study evaluated the metabolic response of jumping horses undergoing complementary training on an inclined treadmill.

MATERIALS AND METHODS

Animals and experimental design

Seven Brazilian Sport Horses, two females and five geldings, with an average age of 12 ± 4 years and an average body weight of 498 ± 58 kg, were used in this study. The horses were part of the Instructor Riding Course at the Brazilian Army Riding School in the show-jumping discipline. All horses followed a daily training routine lasting approximately 50 minutes. Weekly training included outdoor exercises at walk, trot, canter and gallop, jumping courses and pole gymnastics to improve physical conditioning, flexibility, strength and balance. Jumping exercises were performed on sand or grass tracks during outdoor sessions. Sundays were reserved for rest. The standard training routine followed two main criteria: intensive jumping was not performed on consecutive days and jumping sessions were limited to two per week.

The horses underwent clinical examinations to assess their health status before and throughout the study. Body weight was measured, and body condition scores were evaluated according to HENNEKE et al. (1983). The animals were housed in 4 × 4 m stalls equipped with feeders and automatic drinkers, with free access to water. They were fed a commercial concentrate ration and Coastcross hay at amounts equivalent to 2% of body weight on a dry matter basis. The diet was divided into four daily meals: concentrate was provided at 05:00 and 18:00, and hay was provided at 14:00 and 21:00.

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Training protocol

Animals were selected at the beginning of the training season and acclimated to the treadmill (Galloper 5500, Sahinco®, Palmital, São Paulo, Brazil) before the beginning of the complementary training protocol (Figure 1). Treadmill acclimation lasted three weeks, during which the horses become able to walk safely at 1.6 m/s under 0%, 7%, and 13% incline (Figure 2). After this adaptation period, the complementary training protocol was initiated.

The inclined treadmill training protocol was specifically developed for this study. The selection of incline levels was informed by values reported in the literature and by equipment constraints. Gradients of treadmill inclination ranging from 6% to 10% have been employed in previous equine studies to evaluate neuromuscular and metabolic responses during treadmill locomotion (ROBERT et al., 2000; CROOK et al., 2010; TAKAHASHI et al., 2014). Treadmill a 10% incline shown to significantly increase hindlimb muscle recruitment and metabolic cost of locomotion in horses (McDONOUGH et al., 2002; CROOK et al., 2010). Based on this evidence, a gradient of treadmill inclination ranging from 0% to 13% was adopted as the minimum and maximum gradient available on the treadmill used in this study.

Training sessions were conducted on Tuesday and Friday afternoons, between 13:00 and 18:00, in a temperature-controlled treadmill room (18-22 ºC). The training program lasted 16 weeks and was divided into two phases:

Phase 1 of the complementary training - The first phase lasted six weeks. Horses performed exercise protocol on the treadmill twice weekly with three-day intervals between sessions. On the first weekday of training, the session began with a 4 min warm-up walk at 1.7 m/s on a flat treadmill, followed by 6 min at a trot at 3.8 m/s, with the final 3 min at a 3% incline. Horses then walked for 2 min on a flat treadmill, followed by 2 min on a treadmill at a 7% incline, and completed the session with 5 min of cool-down at a walk on a flat treadmill. On this day, horses covered 2,694 m in 19 min (Table 1).

On the second training day of the week, the session began with the same warm-up protocol. Thereafter, horses performed three sequential walking bouts at 1.7 m/s, each lasting 4 minutes and consisting of 2 minutes on a flat treadmill followed by 2 minutes on a treadmill at 7% incline. The session ended with a 5 minutes cool-down at walk on a flat treadmill. On this day, horses covered 3,510 m in 27 min.

Phase 2 of the complementary training - The second phase lasted 10 weeks and was characterized by increased exercise intensity. Horses performed exercise protocol on the treadmill twice weekly, with three-day intervals between sessions.

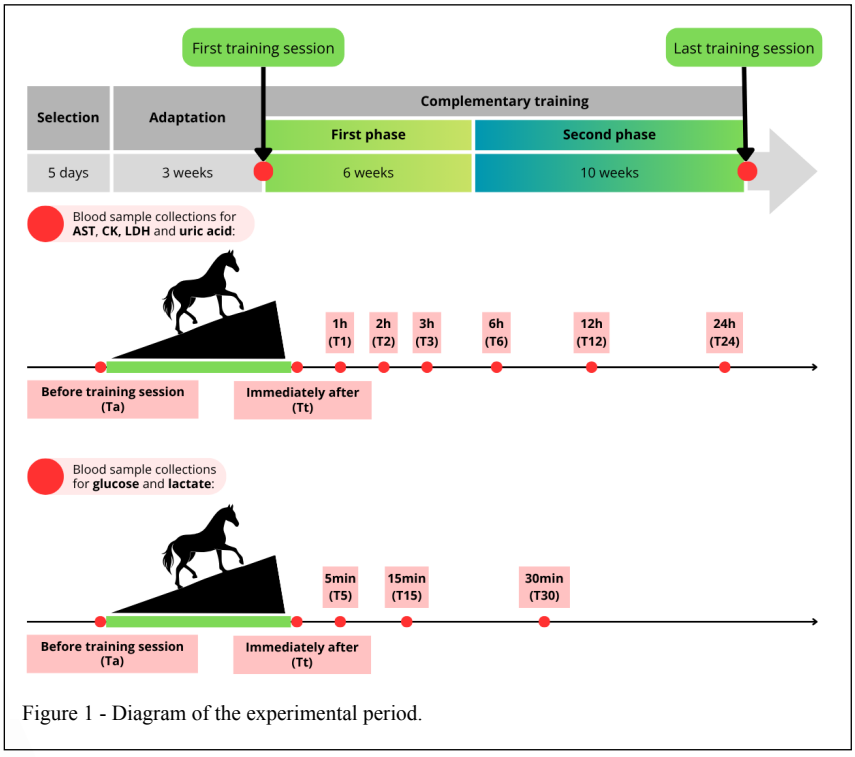


Figure 1 - Diagram of the experimental period.

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Non-Inclined Treadmill



7% inclined treadmill



13% inclined treadmill

Figure 2 - Horse on the inclined treadmill.

Each session training began with the same warm-up protocol used in Phase 1 and ended with five minutes cool-down at walk on a flat treadmill. After warm-up, horses performed three sequential walking bouts at 1.7 m/s. The first bout lasted 4 min and consisted of 2 min on a flat treadmill followed by 2 min on a treadmill at 7% incline, whereas the following two bouts lasted 5 min each and consisted of 2 min on a

Table 1 - Complementary treadmill training protocol during Phases 1 and 2.

Gaits	--------Speed (m/s)--------	Treadmill Inclination (%)	-----Distance (meters)-----	----Duration (minutes)----
----------Phase 1----------				
----------First day of the week----------				
Walk	1.7	0	408	4
Trot	3.8	0	684	3
Trot	3.8	3	684	3
Walk	1.7	0	204	2
Walk	1.7	7	204	2
Walk	1.7	0	510	5
Total	--	--	2694 m	19 min
----------Second day of the week----------				
Walk	1.7	0	408	4
Trot	3.8	0	684	3
Trot	3.8	3	684	3
Walk	1.7	0	204	2
Walk	1.7	7	204	2
Walk	1.7	0	204	2
Walk	1.7	7	204	2
Walk	1.7	0	204	2
Walk	1.7	7	204	2
Walk	1.7	0	510	5
Total	--	--	3510 m	27 min
----------Phase 2----------				
----------Twice-weekly----------				
Walk	1.7	0	408	4
Trot	3.8	0	684	3
Trot	3.8	3	684	3
Walk	1.7	0	204	2
Walk	1.7	7	204	2
Walk	1.7	0	204	2
Walk	1.7	13	306	3
Walk	1.7	0	204	2
Walk	1.7	13	306	3
Walk	1.7	0	510	5
Total	--	--	3714 m	29 min

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flat treadmill followed by 3 min on a treadmill at 13% incline. Each session lasted 29 min, during which horses covered 3,714 m.

Blood sample collection

For biochemical analyses, blood samples were collected by jugular venipuncture using vacutainer tubes containing K2-EDTA, clot activator, or sodium fluoride, according to the analyte to be measured. Collections were performed on the first training day of Phase 1 and on the last training day of Phase 2, while horses were maintained on their regular diets and feeding schedules. Samples for AST, CK, LDH, and uric acid analyses were collected before, immediately after the training session, and at 1, 2, 3, 6, 12, and 24 hours post-exercise. Samples for glucose and lactate analyses were collected before exercise, immediately after the training session, and at 5, 15, and 30 min post-exercise.

After collection, blood samples were immediately centrifuged at 150 x g for 10 minutes to obtain plasma and serum. Aliquots were transferred to properly labeled Eppendorf tubes and stored at the appropriate temperature until analysis. Analyses were conducted using a BTS 310 analyzer (Biosystems®, Curitiba, PR, Brazil) with specific reagent kits (Labtest®, Lagoa Santa, MG, Brazil).

Statistical analyses

Blood biochemical results (AST, CK, LDH, uric acid, glucose, lactate) from treadmill-trained horses were assessed for normality and homogeneity of variances using the Shapiro-Wilk and Bartlett tests, respectively. When the results did not meet the assumptions for ANOVA, logarithmic transformation was performed. The biochemical parameters were analyzed by ANOVA ($\alpha = 0.05$) using a split-plot design according to the following statistical model:

$$Y_{ijk} = \mu + A_i + T_j + e_{ij} + C_k + (TC)_{jk} + e_{ijk}$$

where Y_{ijk} is the observed value of the biochemical variable; μ is the overall mean; A_i is the random effect of animal; T_j is the fixed effect of evaluation moment, before and after complementary training; e_{ij} is the main plot error; C_k is the fixed effect of blood collection time during the training session; $(TC)_{jk}$ is the interaction between evaluation moment and collection time; and e_{ijk} is the residual error. When significant effects were observed, means were compared using Tukey's test. Statistical analyses were performed using R CORE TEAM (R software, version 3.6.3).

RESULTS AND DISCUSSION

No differences were observed in blood AST concentration before and after the treadmill training program ($P > 0.05$) or among the evaluated blood collection times ($P > 0.05$) Similarly, CK and uric acid concentration did not differ before and after training protocol ($P > 0.05$). However, blood collection time during the training sessions significantly affected both CK and uric acid concentrations, with peak values observed 12 hours post-exercise ($P = 0.015$ and $P = 0.009$, respectively) (Table 2).

Performance evaluation based on hematological and biochemical indicators requires consideration of the metabolism demands of exercise in horses from different equestrian disciplines (MASKÓ et al., 2021). Blood concentrations of

Table 2 - Mean values of blood concentration of aspartato aminotransferase (AST), creatin kinase (CK), and uric acid in horses during treadmill training sessions.

Blood collection time	----------AST (IU/L)----------	----------CK (IU/L)----------	-------Uric acid (mg/dL)-------
Before training	315.2 ± 169.0	140.4 ± 53.7^{b}	0.57 ± 0.1^{ab}
Immediately after training	321.7 ± 130.7	170.9 ± 67.6^{ab}	0.55 ± 0.1^{ab}
After training - 1 hour	315.6 ± 169.1	157.8 ± 83.8^{ab}	0.51 ± 0.1^{b}
After training - 2 hours	320.9 ± 165.5	161.7 ± 63.9^{ab}	0.55 ± 0.1^{ab}
After training - 3 hours	320.2 ± 165.7	148.9 ± 68.8^{ab}	0.49 ± 0.2^{b}
After training - 6 hours	310.0 ± 129.3	172.1 ± 75.6^{ab}	0.54 ± 0.1^{ab}
After training - 12 hours	314.0 ± 165.8	185.3 ± 91.8^{a}	0.60 ± 0.1^{a}
After training - 24 hours	306.7 ± 184.1	141.2 ± 55.8^{b}	0.51 ± 0.1^{ab}
P value	0.89	0.015	0.009

Means followed by different letters in the column differ from each other according to the Tukey test ($\alpha = 0.05$).

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CK and AST may vary according to breed, age, exercise type and duration, environmental conditions and management practices (GOMIDE et al., 2006; THOMASSIAN et al., 2007; CAIADO et al., 2011). Training can attenuate the increase in serum AST activity after exercise. Similarly, increases in CK are influenced by the training protocol and the type of exercise. Recreational horses may exhibit greater post-exercise increases in CK than endurance horses, suggesting that lower physical conditioning can enhance enzyme release (WITKOWSKA-PIŁASZEWICZ et al., 2019; MIGLIO et al., 2020). Conversely, well-planned and controlled training programs can result in moderate increases in CK after physical activities, suggesting adaptation to exercise (CARDINET, 1997; THOMASSIAN et al., 2007; SALES et al., 2013). PADILHA et al. (2017) reported that CK blood levels in Brazilian Sport horses returned to reference ranges within 24 hours after exercise, whereas LDH and AST concentrations returned to baseline within up to 72 hours.

Changes in CK and AST activity are sensitive markers of muscle fatigue and adaptation to exercise, and may vary considerably according to sport modality and level of physical conditioning (MASKÓ et al., 2021). Furthermore, increases in AST after exercise may be associated with increased cell membrane permeability in different tissues, and do not necessarily indicate tissue injury (SALES et al., 2013). THOMASSIAN et al. (2007) observed that AST peaked 24 h after physical activity, while CAIADO et al. (2011) reported significantly lower values before and immediately after the exercise, with the highest value recorded before exercise (210.2 IU/L). Similarly, MASSÁNYI et al. (2022), evaluating horses subjected to a 15-min inclined treadmill protocol comprising two uphill steps at 6.7 km/h (10 minutes at 3º followed by 5 minutes at 6º) reported AST values of 274.3 IU/L before exercise, 254.8 IU/L during exercise, and 302.1 IU/L at the end of the session. These values are comparable to those observed in the present study, in which AST peak at 321.7 IU/L at the end of the exercise.

Normally, creatine kinase (CK) is measured alongside AST, as these enzymes provide complementary information for the diagnosis of muscle injury. CK is usually the first enzyme to increase after muscle injury, rising within 4-8 h, peaking between 12 and 24 h, and returning to baseline within 96 h (CARDINET, 1997; BENESI et al., 2009; DI FILIPPO et al., 2016). The late elevation of AST and CK was also highlighted by THOMASSIAN et al. (2007), who confirmed that the late elevation may indicate muscle injury, with CK peaking between 3 and 6 h and AST between 12 and 24 h after exercise. Regarding AST, peak values in the blood occur between 24 and 36 h after muscle injury because of its half-life of approximately eight days (MATTOSINHO et al., 2017).

In the present study, CK activity peaked 12 h after the training session (185.3 ± 91.8 IU/L) and decreased significantly at 24 h post-training (141.2 ± 55.8 IU/L). These findings suggested that in healthy Brazilian sport horses undergoing treadmill training, CK activity peaks approximately 12 h after exercise and returns to pre-exercise levels within 24 h.

The significant increase in CK concentration 12 hours post-exercise may reflect acute muscle adaptation or altered membrane permeability resulting from the varying physical demands of each training session, without evidence of long-term enzyme accumulation. Muscle damage is hypothesized to occur when post-exercise CK activity exceeds 100% of the resting (baseline) value, i.e., when CK activity more than doubles following exercise, reflecting sarcolemma disruption and myofibrillar necrosis rather than a transient physiological adaptation (VALBERG et al., 1993; CARDINET, 1997). In the present study, the mean CK increase from baseline (140.4 ± 53.7 IU/L) to the 12-hour post-exercise peak (185.3 ± 91.8 IU/L) corresponded to an increase of approximately 32%, well below this threshold. The post-exercise increase observed in the present study is therefore consistent with this physiological profile, supporting the interpretation that the variation observed may represent an expected physiological adaptation to exercise rather than evidence of clinical muscle damage. This is consistent with MASSÁNYI et al. (2022), who reported average CK values of 266.16 IU/L before exercise, 299.7 IU/L during exercise, and 276.06 IU/L at the end of exercise, without visible clinical changes in the horses throughout the experiment. While CK provides an early, transient signal that returns to baseline within a short period, AST follows a different release pattern that is determined less by exercise duration and more by the severity of cellular injury. In horses, the ratio of cytoplasmic AST (cAST) to mitochondrial AST (mAST) is greater than in other mammals, and it has been postulated that a greater magnitude of serum AST increase (via release of mAST in addition to cAST) occurs specifically with more severe or irreversible cell injury, rather than as a function of exercise duration (JONES & BLACKMORE, 1982; BILLINGS et al., 2021). Because of its slower release kinetics and longer half-life compared to CK, AST

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is considered a marker of past or prolonged muscle damage/hypoperfusion, as discussed further below (MASKÓ et al., 2021). In the present study, AST concentrations did not differ significantly before and after training (P > 0.05), which, together with the CK increase remaining well below the 100% damage threshold, reinforces the interpretation that the treadmill training protocol did not induce clinically relevant muscle injury.

According to MELO et al. (2013), uric acid concentrations in athletic horses range from 0.030 to 0.035 mmol/L. During endurance exercise, uric acid levels have been positively correlated with lactate, CK and heart rate, and higher concentrations have been observed in metabolically imbalanced horses, supporting its role as a marker of sustained post−exercise metabolic stress and consistent with the results of the present study (CASTEJON, 2006; ADAMU et al., 2012). CASTEJON et al. (2006) also reported increased uric acid concentrations in horses that did not complete a race, associating this response with prolonged energy imbalance, metabolic acidosis, and, in severe cases, cellular fatigue. Uric acid is a byproduct of ATP degradation and may increase after strong effort because of adenosine 5'-triphosphate breakdown. Therefore, it can be used as a marker of relative exercise intensity. In addition, uric acid concentrations may help faster as fitness level increases (ESCRIBANO et al., 2013; EVANS, 2000).

In the present study, the uric acid blood peak observed at 12 hours post-exercise may be attributed to sustained purine degradation via xanthine oxidase activity in peripheral tissues, as described by RÄSÄNEN et al. (1996). Additionally, oxidative stress may persist during recovery, maintaining elevated uric acid levels even after other metabolic parameters return to baseline (SOARES et al., 2011). SCHUBACK & ESSÉN-GUSTAVSSON (1998) reported increased uric acid concentration between 20 and 30 minutes after exercise, whereas RÄSÄNEN et al. (1996) observed peaks up to 6 hours post-exercise. The later peak observed may also reflect individual variation in enzymatic clearance or antioxidant replenishment dynamics (SAUTIN & JOHNSON, 2008). Since uric acid concentrations did not differ before and after the training program, this may suggest a limited impact on purine-related oxidative metabolism. However, as uric acid is an indirect and non-specific marker, and specific oxidative stress markers were not assessed in this study, this interpretation should be considered with caution.

Regarding LDH, a significant difference was observed between the mean before and after the training protocol (P = 0.007), but not between the blood collection times (P > 0.05) (Table 3). DIAS et al. (2009) and SANTOS et al. (2015) evaluated LDH concentrations in Brazilian Sport Horses after a show jumping competitions and reported significant increases, with mean values of 201.29 IU/L and 265.53 UI/L, respectively. THOMASSIAN et al. (2007) observed higher LDH values and suggested that the variation may be related to the characteristics of the horses evaluated, which could influence the activity of muscle enzymes, justifying the higher variation also observed in the present study. Considering these associations, along with differences among breeds and exercise types, LDH does not appear to be specific to skeletal muscle tissue. ASSENZA et al. (2016) observed a significant increase in LDH activity 10 minutes after the end of each showjumping competition compared to the values before the first competition day, probably due to the increase in physical effort and enzymatic activity, concluding that LDH activities has a longer half-life compared to CK, in fact, peaking 24 hours after exertion and potentially remaining elevated for 48 hours post-exercise.

BENESI et al. (2009) reported that the values of AST, CK, and LDH do not show significant variations after 24 months of age in the Brazilian Sport Horse breed. DI FILIPPO et al. (2016) observed that LDH was similar to that in the present study at the basal level, with an average value of 347.8 IU/L, while the values after training were significantly higher compared to other authors, reaching 877.3 IU/L. Some studies have shown that CK and LDH, being located in the cytoplasm, can leak out when cell membrane permeability changes. Small fluctuations in CK activity may occur during exercise due to increased muscle membrane permeability, especially in prolonged or strenuous exercises (ALLEN et al., 2016). This

Table 3 - Average blood concentration values of desidrogenase lactica (LDH) in horses before and after the complementary treadmill training protocol.

Training protocol	LDH (IU/L)
Before treadmill training protocol	312.8 ± 30.6[b]
After treadmill training protocol	393.9 ± 45.2[a]
P value	0.007

Means followed by different letters in the column differ significantly by Tukey's test (α = 0.05).

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behavior is also observed when CK is related to different degrees of muscle injury. Conversely, AST, which is located within mitochondria, increases only with more severe mitochondrial injury. Beyond the magnitude of injury itself, AST elevation may also reflect prolonged exercise duration or tissue hypoperfusion, as demonstrated in horses subjected to hypotensive stress independent of exercise (DUKE et al., 2006), as well as exposure to hot and humid environmental conditions that exacerbate cellular stress (BROWNLOW & MIZZI, 2023).

No significant differences were observed in the plasma glucose concentration before and after the treadmill training protocol ($P > 0.05$). However, plasma glucose concentrations varied significantly among blood collection times ($P = 0.029$) (Table 4). In contrast, lactate concentration did not differ before and after treadmill training ($P = 0.24$) or among blood collection times ($P = 0.51$). The glycemic response may be related to gluconeogenic activity, mainly through pathways associated with lipid mobilization, as well as hepatic glycogenolysis. These mechanisms can influence glucose fluctuations according to exercise types, intensity, duration, and levels of physical stress. During physical activity, the energy demand of skeletal muscles increases, and during high-intensity or long-duration exercises, oxygen availability may become insufficient to fully support aerobic energy production, increasing reliance on alternative metabolic pathways. MASSÁNYI et al. (2022) suggested that variations in glucose concentrations may also be influenced by different diets or more intensive training programs, which require greater intake of soluble carbohydrates.

In response to exercise, skeletal muscles increase anaerobic energy production, thereby raising lactate levels; consequently, lactate levels are expected to increase (CAIADO et al., 2011). The increase in blood lactate concentrations is an indirect indicator of the onset of metabolic acidosis, a condition whose variation during physical activity is related to the horse's physical conditioning and depends on the intensity and duration of effort, and training level (GOMIDE et al., 2006). The present study observed no predominance of energy production via the lactic anaerobic pathway, in agreement with the findings of GOMIDE et al. (2006), who state that this condition occurs at values above 4 mmol/L. LÉGUILLETTE et al. (2020) observed similar lactate values during a 5 m/s canter (0.6 ± 0.2 mmol/L), which were significantly lower than those after an 11 m/s gallop (7.4 ± 2.1 mmol/L). VINCZE et al. (2010) state that in show jumping competitions, with jumps up to 100 cm in height and a minimum average speed of 300 m/min, there is no substantial anaerobic energy supply. Over time, the increased workload from jumping higher obstacles may alter the ratio of aerobic to anaerobic metabolic pathways, thereby increasing the workload for the equine athlete, even over smaller obstacles and during basic flat work (LÉGUILLETTE et al., 2020).

CONCLUSION

The results of this study indicated that the complementary treadmill training protocol applied for show-jumping horses induced transient and reversible metabolic responses, consistent with those of moderate-intensity exercise and physiological adaptation. The CK and uric acid blood concentration peaked 12 hours post-exercise and returned to baseline within 24 hours, suggesting muscle adaptation rather than damage. The LDH increased over the training period, reflecting greater cumulative metabolic demand, while AST remained stable throughout. Glucose and lactate concentrations were consistent

Table 4 - Mean values of blood glucose and lactate concentration in horses during treadmill training session.

Blood collection time	----------Glucose (mg/dL)----------	----------Lactate (mmol/L)----------
Before training	91.4 ± 10.8^{ab}	1.18 ± 0.4
Immediately after training	78.2 ± 11.2^{ab}	1.22 ± 0.4
After training - 5 minutes	84.3 ± 7.9^{b}	1.16 ± 0.3
After training - 15 minutes	92.8 ± 16.4^{a}	1.09 ± 0.3
After training - 30 minutes	86.6 ± 16.3^{ab}	1.08 ± 0.4
P value	0.029	0.51

Means followed by different letters in the rows differ significantly according to Tukey's test ($\alpha = 0.05$).

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with aerobic energy predominance, indicating that exercise intensity did not exceed the animals' anaerobic threshold. Collectively, these metabolic responses demonstrated that complementary inclined treadmill training is a safe and effective strategy for improving the metabolic fitness of show-jumping horses without inducing muscle damage.

DATA AVAILABILITY STATEMENT

The raw data are available from the author.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

Conceptualization design, APB and FQA; methodology and sample collection, APB, ITN and JAG; statistical analysis: APB and BCF; writing – original draft preparation, APB and BCM; writing – review and editing, BCF, FQA and JAG; funding, FQA. All authors have read and agreed to the published version of the manuscript.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The animal study protocol was approved by the Ethics Committee of the Universidade Federal Rural do Rio de Janeiro (CEUA/IV/UFRRJ), number: 6062121118.

DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

The authors declare that they did not use artificial intelligence to realize this study and prepare the manuscript.

REFERENCES

ADAMU, L. et al. Metabolic responses in endurance horses during racing in relation to uric acid profile, leucocytes, heart rate and plasma biochemical parameters. **Veterinární Medicína**, v.57, n.11, 2012. Available from: <http://vetmed.agriculturejournals.cz/artkey/vet-201211-0004_metabolic-responses-in-endurance-horses-during-racing-in-relation-to-uric-acid-profile-leucocytes-heart-rate.php>. Accessed: Sept. 24, 2026. doi: 10.17221/6466-VETMED.

ALLEN, K. J. et al. Exercise testing in the equine athlete. **Equine Veterinary Education**, v.28, n.2, p.89-98, 2016. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.1111/eve.12410>. Accessed: Sept. 24, 2026. doi: 10.1111/eve.12410.

ASSENZA, A. et al. Serum muscle-derived enzymes response during show jumping competition in horse. **Veterinary World**, v.9, n.3, p.251-255, 2016. Available from: <https://www.veterinaryworld.org/Vol.9/March-2016/5.html>. Accessed: Sept. 24, 2026. doi: 10.14202/vetworld.2016.251-255.

BENESI, F. J. et al. Biochemical profile of some plasmatic enzymes in BH (Brasileiro de Hipismo) filies bred Colina in São Paulo State. **Brazilian Journal of Veterinary Research and Animal Science**, v.46, n.4, p.288-295, 2009. Available from: <https://revistas.usp.br/bjvras/article/view/26777>. Accessed: Sept. 24, 2026. doi: 10.11606/issn.1678-4456.bjvras.2009.26777.

BILLINGS, A. et al. Laboratory Markers of Muscle Injury. In: WALTON, R. M. et al. (Eds.). **Equine Hematology, Cytology, and Clinical Chemistry**. 2ed. Wiley-Blackwell, cap.10, p.119-141, 2021. Available from: <https://onlinelibrary.wiley.com/doi/10.1002/9781119500186.ch10>. Accessed: Sept. 24, 2026. doi:_10.1002/9781119500186.ch10.

BROWNLOW, M.; MIZZI, J. X. An overview of exertional heat illness in Thoroughbred racehorses: pathophysiology, diagnosis, and treatment rationale. **Animals**, v.13, n.4, p.610, 2023. Available from: <https://www.mdpi.com/2076-2615/13/4/610>. Accessed: Sept. 24, 2026. doi: 10.3390/ani13040610.

CAIADO, J. C. C. et al. Influence of physical exercise on equine activities of AST and CK and plasma concentration of lactate in Quarter horses submitted to team roping. **Pesquisa Veterinária Brasileira**, v.31, n.5, p.452-458, 2011. Available from: <https://www.scielo.br/j/pvb/a/FgvmbKdR8qLzNDsVPg83vsv/?lang=pt>. Accessed: Sept. 24, 2026. doi: 10.1590/S0100-736X2011000500014.

CARDINET, G. H. Skeletal muscle function. In: KANEKO, J. J. et al. **Clinical Biochemistry of Domestic Animals**. London: Academic Press, 5ed., p.407-440, 1997.

CASTEJÓN, F. et al. Uric acid responses to endurance racing and relationships with performance, plasma biochemistry and metabolic alterations. **Equine Veterinary Journal**, v.38, Supl.36, p.70-73, 2006. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2006.tb05516.x>. Accessed: Sept. 24, 2026. doi: 10.1111/j.2042-3306.2006.tb05516.x.

CHANDA, M.; PETCHDEE, S. Cardiac morphology changes in horses as a response to various types of sports. **Journal of Applied Animal Research**, v.50, n.1. p.453-459, 2022. Available from: <https://www.tandfonline.com/doi/full/10.1080/09712119.2022.2097245>. Accessed: Sept. 24, 2026. doi: 10.1080/09712119.2022.2097245.

CROOK, T. C. et al. The effect of treadmill speed and gradient on equine hindlimb muscle activity. **Equine Veterinary Journal**, v.42, p.412-416, 2010. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.2010.00222.x>. Accessed: Sept. 24, 2026. doi: 10.1111/j.2042-3306.2010.00222.x.

SciELO Preprints - This document is a preprint and its current status is available at: https://doi.org/10.1590/0103-8478cr20250050

DENOIX, J. M. **Biomechanics and Physical Training of the Horse**. CRC Press, Boca Raton, FL. 2014. 186p.

DI FILIPPO, P. A. et al. Gender differences – induced changes in serum hematologic and biochemical variables in Mangalarga Marchador horses after a marcha gait competition. **Journal of Equine Veterinary Science**, v.43, p.18-22, 2016. Available from: <https://www.sciencedirect.com/science/article/pii/S0737080616300363?via%3Dihub>. Accessed: Sept. 24, 2026. doi: 10.1016/j.jevs.2016.04.093.

DIAS, D. C. R. et al. Effect of a vitamin e and selenium oral supplementation on hematological parameters, muscular injuries markers enzymes and peroxidation indices of biomolecules in show jumping horses. **Ciência Animal Brasileira**, v.10, n.3, p.790-801, 2009. Available from: <https://www.cabidigitallibrary.org/doi/full/10.5555/20103014249>. Accessed: Sept. 24, 2026.

DUKE, T. et al. Clinical observations surrounding an increased incidence of postanesthetic myopathy in halothane-anesthetized horses. **Veterinary Anaesthesia and Analgesia**, v.33, n.2, p.122-127, 2006. Available from: <https://pubmed.ncbi.nlm.nih.gov/16476002/>. Accessed: Sept. 24, 2026. doi: 10.1111/j.1467-2995.2005.00189.x.

ESCRIBANO, B. M. et al. Genetic analysis of haematological and plasmatic biochemical parameters in the thoroughbred Spanish horse exercised on a treadmill. **Animals**, v.7, n.9, p.1414-1422, 2013. Available from: <https://www.sciencedirect.com/science/article/pii/S1751731113000955?via%3Dihub>. Accessed: Sept. 24, 2026. doi: 10.1017/S1751731113000955.

EVANS, D. L. **Training and Fitness in Athletic Horses**, 1st ed.; Rural Industries Research and Development Corporation: Kingston, Australia, 2000. 88p.

FERRAZ, G. C. et al. Blood lactate threshold reflects glucose responses in horses submitted to incremental exercise test. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.60, p.256-259, 2008. Available from: <https://www.scielo.br/j/abmvz/a/zD6Hw3JMZb9JdBgHwznT7Bx/?lang=en>. Accessed: Sept. 24, 2026. doi: 10.1590/S0102-09352008000100035.

GOMIDE, L. M. W. et al. Blood lactate concentrations of horses competing in the resistance phase of 3-day combined training event. **Ciência Rural**, v.36, n.2, p.509-513, 2006. Available from: <https://www.scielo.br/j/abmvz/a/zD6Hw3JMZb9JdBgHwznT7Bx/?lang=en>. Accessed: Sept. 24, 2026. doi: 10.1590/S0103-84782006000200022.

HENNEKE, D. R. et al. Relationship between condition score, physical measurements and body fat percentage in mares. **Equine Veterinary Journal**, v.15, p.371-372, 1983. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.1983.tb01826.x>. Accessed: Sept. 24, 2026. doi: 10.1111/j.2042-3306.1983.tb01826.x.

HODGSON, D. R. et al. Tendon, ligament, bone, and cartilage: anatomy, physiology, and adaptations to exercise and training. In: HODGSON, D. R. et al. **The Athletic Horse Principles and Practice of Equine Sports Medicine**. Elsevier, 2.ed. p.202-242, 2014a.

HODGSON, D. R. et al. Hematology and Biochemistry. In: HODGSON, D. R. et al. **The Athletic Horse Principles and Practice of Equine Sports Medicine**. Elsevier, 2nd ed. p.56-68. 2014b.

JONES, S.; BLACKMORE, D. J. Observations on the isoenzymes of aspartate aminotransferase in equine tissues and serum. **Equine Veterinary Journal**, v.14, n.4, p.311-316, 1982. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.1982.tb02439.x>. Accessed: Sept. 24, 2026. doi: 10.1111/j.2042-3306.1982.tb02439.x.

KLOBUČAR, K. et al. Changes in biochemical parameters in horses during 40 km and 80 km endurance races. **Acta Veterinaria**, v.69, n.1, p.73-87, 2019. Available from: <https://www.researchgate.net/publication/333034901_Changes_in_biochemical_parameters_in_horses_during_40_km_and_80_km_endurance_races>. Accessed: Sept. 24, 2026. doi: 10.2478/acve-2019-0005.

KRUMRYCH, W. Blood antioxidant defence in horses during physical exercises. **Bulletin Veterinary Institute in Pulawy**, v.54, p.617-624, 2010. ISSN (Print): 0042-4870. Available from: <https://scholar.google.com/citations?view_op=view_citation&hl=pl&user=ZbfQFTQAAAAJ&citation_for_view=ZbfQFTQAAAAJ:qjMakFHDy7sC>. Accessed: Sept. 19, 2025.

LÉGUILLETTE, R. et al. Comparison of physiological demands in Warmblood show jumping horses over a standardized 1.10 m jumping course versus a standardized exercise test on a track. **BMC Veterinary Research**, v.16, p.182, 2020. Available from: <https://doi.org/10.1186/s12917-020-02400-9> Accessed: Sept. 24, 2026. doi: 10.1186/s12917-020-02400-9.

LEWCZUK, D.; MAŚKO, M. Inclined treadmill short-duration exercise for walk regularity of recreation horses, **Journal of Veterinary Behavior**, v.76, p.8-12, 2024. Available from: <https://www.sciencedirect.com/science/article/pii/S1558787824000844?via%3Dihub>. Accessed: Sept. 24, 2026. doi: 10.1016/j.jveb.2024.10.002.

MASSÁNYI, M. et al. Changes in haematological and biochemical parameters in blood serum of horses during exposition to workload stress. **Heliyon**, v.8, e12241, 2022. Available from: <https://www.sciencedirect.com/science/article/pii/S2405844022035290?via%3Dihub>. Accessed: Sept. 24, 2026. doi: 10.1016/j.heliyon.2022.e12241.

MASKÓ, M. et al. The physical activity-dependent hematological and biochemical changes in school horses in comparison to blood profiles in endurance and race horses. **Animals**, v.11, 1128, 2021. Available from: <https://pubmed.ncbi.nlm.nih.gov/33920044/>. Accessed: Sept. 24, 2026. doi: 10.3390/ani11041128.

MATTOSINHO, R. O. et al. Hematological alterations and serum biochemistry of equine athletes. **Revista de Ciência Veterinária e Saúde Pública**, v.4, n.1, p.82-91, 2017. Available from: <https://periodicos.uem.br/ojs/index.php/RevCiVet/article/view/35706>. Accessed: Sept. 24, 2026. doi: 10.4025/revcivet.v4i1.35706.

McDONOUGH, P. et al. The effect of treadmill incline on maximal oxygen uptake, gas exchange and the metabolic response to exercise in the horse. **Experimental Physiology**, v.87, n.4, p.499-506, 2002. Available from: <https://physoc.onlinelibrary.wiley.com/doi/10.1111/j.1469-445X.2002.tb00063.x>. Accessed: Sept. 24, 2026. doi: 10.1111/j.1469-445X.2002.tb00063.x.

MELO, S. K. M. et al. Hematological parameters and blood biochemistry in pleasure horses in tropical region. **Ciência Animal Brasileira**, v.14, n.2, p.208-215, 2013. Available from: <https://revistas.ufg.br/index.php/vet/article/view/16484>. Accessed: Sept. 24, 2026. doi: 10.5216/cab.v14i2.16484.

SciELO Preprints - This document is a preprint and its current status is available at: https://doi.org/10.1590/0103-8478cr20250050

MIGLIO, A. et al. Metabolic and biomolecular changes induced by incremental long-term training in young thoroughbred racehorses during first workout season. **Animals**, v.10, n.2, p.317, 2020. Available from: <https://www.mdpi.com/2076-2615/10/2/317>. Accessed: Sept. 24, 2026. doi: 10.3390/ani10020317.

PADILHA, F.G.F. et al. Blood biochemical parameters of Brazilian sport horses under training in tropical climate. **Brazilian Journal of Animal Science**, v.46, n.8, p.678-682, 2017. Available from: <https://www.scielo.br/j/rbz/a/V9byg5fKYVwy3wpGk3LLvxH/?lang=en>. Accessed: Sept. 24, 2026. doi: 10.1590/S1806-92902017000800008.

PLISAK, U. et al. Changes in novel anti-inflammatory cytokine concentration in the blood of endurance and race horses at different levels of training. **Computational and Structural Biotechnololgy Journal**, v.21, p.418-424, 2023. Available from: <https://spj.science.org/doi/10.1016/j.csbj.2022.12.016>. Accessed: Sept. 24, 2026. doi: 10.1016/j.csbj.2022.12.016.

PUGLIESE, B. R. et al. Effect of fatigue on equine metacarpophalangeal joint kinematics — a single horse pilot study. **Journal of Equine Veterinary Science**, v.86, p.102849, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0737080619305982?via%3Dihub>. Accessed: Sept. 24, 2026. doi: 10.1016/j.jevs.2019.102849.

R CORE TEAM. **R: A language and environment for statistical computing**. Vienna: R Foundation for Statistical Computing, 2021. Available from: <https://www.R-project.org/>. Accessed: May, 10, 2021.

RÄSÄNEN, L. A. et al. Accumulation of uric acid in plasma after repeated bouts of exercise in the horse. **Comparative Biochemistry and Physiology Part B**: Biochemistry and Molecular Biology, v.114, n.2, p.139-144, 1996. Available from: <https://www.sciencedirect.com/science/article/pii/0305049196000223?via%3Dihub>. Accessed: Sept. 24, 2026. doi: 10.1016/0305-0491(96)00022-3.

RIBEIRO FILHO, J. D. et al. Clinical and laboratory assessment of Mangalarga Marchador horses submitted to marcha exercise. **Brazilian Journal of Veterinary Medicine**, v.40, n.1, e47418, 2018. Available from: <https://bjvm.org.br/BJVM/article/view/474>. Accessed: Sept. 24, 2026. doi: 10.29374/2527-2179.bjvm47418.

ROBERT, C. et al. The effects of treadmill inclination and speed on the activity of two hindlimb muscles in the trotting horse. **Equine Veterinary Journal**, v.32, n.4, p.312-317, 2000. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.2746/042516400777032246>. Accessed: Sept. 24, 2026. doi: 10.2746/042516400777032246.

SALES, J. V. F. et al. Endurance horses' finalists: Expression of Mg+2, CK, AST and LDH in horse finalists of endurance race. **Pesquisa Veterinária Brasileira**, v.33, n.1, p.105-110, 2013. Available from: <https://www.scielo.br/j/pvb/a/q9NB7zGdBY6BXwRmQBn89zv/?lang=pt>. Accessed: Sept. 24, 2026. doi: 10.1590/S0100-736X2013000100019.

SANTOS, V. P. et al. Hemato-biochemical response to exercise with ergometric treadmill, mount training and competition in jumping horses. **Archives of Veterinary Science**, v.20, Supl.1, p.1-8, 2015. Available from: <https://revistas.ufpr.br/veterinary/article/view/36617>. Accessed: Sept. 24, 2026. doi: 10.5380/avs.v20i2.36617.

SAUTIN, Y. Y.; JOHNSON, R. J. Uric acid: the oxidant-antioxidant paradox. **Nucleosides, Nucleotides, and Nucleic Acids**, v.27, n.6-7, p.608-619, 2008. Available from: <https://www.tandfonline.com/doi/abs/10.1080/15257770802138558>. Accessed: Sept. 24, 2026. doi: 10.1080/15257770802138558.

SCHUBACK, K.; ESSÉN-GUSTAVSSON, B. Muscle anaerobic response to a maximal treadmill exercise test in Standardbred trotters. **Equine Veterinary Journal**., v.30, n.6, p.504-510, 1998. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.1998.tb04526.x>. Accessed: Sept. 24, 2026. doi: 10.1111/j.2042-3306.1998.tb04526.x.

SOARES, J. C. M. et al. Biochemical and antioxidant changes in plasma, serum, and erythrocytes of horses before and after a jumping competition. **Journal of Equine Veterinary Science**, v.31, n.7, p.357-360, 2011. Available from: <https://www.sciencedirect.com/science/article/pii/S0737080611001092?via%3Dihub>. Accessed: Sept. 24, 2026. doi: 10.1016/j.jevs.2011.03.017.

TAKAHASHI, T. et al. The Effects of inclination (Up and Down) of the treadmill on the electromyogram activities of the forelimb and hind limb muscles at a walk and a trot in Thoroughbred horses. **Journal of Equine Science**, v.25, n.4, p.73-77, 2014. Available from: <https://www.jstage.jst.go.jp/article/jes/25/4/25_1410/_article>. Accessed: Sept. 24, 2026. doi: 10.1294/jes.25.73.

TEIXEIRA-NETO, A. R. et al. Alterations in muscular enzymes of horses competing long-distance endurance rides under tropical climate. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.60, p.543-549, 2008. Available from: <https://www.scielo.br/j/abmvz/a/Y764yD6vRRnm4Y4hR8GZqHp/?lang=en>. Accessed: Sept. 24, 2026. doi: 10.1590/S0102-09352008000300004.

THOMASSIAN, A. et al. Serum activities of aspartate aminotransferase, creatine kinase and lactate dehydrogenase in arabian horses submitted to standard incremental exercise test. **Brazilian Journal of Veterinary Research and Animal Science**, v.44, n.3, p.183-190, 2007. Available from: <https://revistas.usp.br/bjvras/article/view/26637>. Accessed: Sept. 24, 2026. doi: 10.11606/issn.1678-4456.bjvras.2007.26637.

VALBERG, S. et al. Muscle histopathology and plasma aspartate aminotransferase, creatine kinase and myoglobin changes with exercise in horses with recurrent exertional rhabdomyolysis. **Equine Veterinary Journal**, v.25, n.1, p.11-16, 1993. Available from: <https://beva.onlinelibrary.wiley.com/doi/10.1111/j.2042-3306.1993.tb02893.x>. Accessed: Sept. 24, 2026. doi: 10.1111/j.2042-3306.1993.tb02893.x.

VINCZE, A. et al. Effect of age and event on post exercise values of blood biochemical parameters in show jumping horses. **Acta Agraria Kaposváriensis**, v.14, n.2, p.185-191, 2010. Available from: <https://journal.uni-mate.hu/index.php/aak/en/article/view/1998> Accessed: Sept. 24, 2026. doi: 10.31914/aak.1998.

WITKOWSKA-PIŁASZEWICZ, O. et al. Changes in serum amyloid A (SAA) concentration in Arabian endurance horses during first training season. **Animals**, v.9, n.6, p.330, 2019. Available from: <https://www.mdpi.com/2076-2615/9/6/330>. Accessed: Sept. 24, 2026. doi: 10.3390/ani9060330.

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