Ciência Animal Brasileira
Brazilian Animal Science

DOI: 10.1590/1809-6891v26e-80876E

e-ISSN 1809-6891
Veterinary Medicine | Research article

Glycemia and lactacidemia in eventing horses in a high-speed treadmill test

Glicemia e lactacidemia em equinos de Hipismo Completo em teste em esteira de alta velocidade

Caroline Rodrigues[1], Ananda Buzzetti[1], Isabella Nothaft[1] Beatriz Cavalcante[1] Maria Izabel Almeida[1], Cristiane Baldani[1], Fernando Queiroz Almeida*[1]

1 Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Rio de Janeiro, Brazil

*corresponding author: almeidafq@yahoo.com.br

Received: October 17, 2024. Accepted: March 17, 2025. Published: May 15, 2025. Editor: Luiz Augusto B. Brito.

Abstract: This study aimed to evaluate the effects on glycemia and lactacidemia as a function of the time of intake of concentrate ration in horses before a high-speed treadmill test. Fifteen eventing Brazilian Sport horses were used in two experimental trials in a completely randomized design. In the first trial, a glycemic index test was performed with four horses and the glycemic response was evaluated after concentrate ration intake. In the second trial, 12 horses were used, in three experimental treatments (time of supply of concentrate ration before the high-speed treadmill test - 2, 4 and 6h) and four horses / group, and submitted to test on a treadmill. Blood samples were collected for glucose, lactate and heart rate monitoring and, with these results, VL_2, VL_4 and V_{200} were estimated by exponential and linear regression, respectively. The evaluating of horse's performance in the experimental groups it was verified that VL_2, VL_4, and V_{200} were not influenced by the time of supply of the concentrate ration in 2, 4 or 6 hours before the high-speed treadmill test. The time of supply of the concentrate ration before the high-speed treadmill test did not influence the glycemia, lactacidemia and heart rate of the horses before and during the test. Further studies are needed to understand the glucose restoration after exercise in standardized treadmill speed tests.

Key-words: equine; glucose; lactate; performance.

Resumo: Este estudo teve como objetivo avaliar os efeitos do tempo de fornecimento da ração concentrada, antes do teste em esteira de alta velocidade, sobre a glicemia e a lactacidemia em equinos. Foram utilizados quinze equinos Brasileiro de Hipismo da modalidade Hipismo Completo, em dois ensaios experimentais. No primeiro ensaio, foram utilizados quatro equinos em teste de índice glicêmico após consumo de ração concentrada. No segundo ensaio, foram utilizados 12 equinos agrupados em três tratamentos experimentais (tempo de fornecimento da ração concentrada antes do teste em esteira de alta velocidade - 2h, 4h e 6h) e quatro equinos/grupo, submetidos a teste de esforço físico em esteira de alta velocidade. Foram coletadas amostras sanguíneas para dosagem de glicose, lactato e monitorada a frequência cardíaca e, com esses resultados foram estimados a VL_2, VL_4 e a V_{200} por regressão exponencial e linear, respectivamente. Na avaliação do desempenho dos equinos nos grupos experimentais, verificou-que que VL_2, VL_4, e V_{200} não foram influenciadas pelo tempo de fornecimento da dieta concentrada em 2, 4 ou 6 horas antes do teste em esteira de alta velocidade. O horário de fornecimento da ração concentrada antes do teste incremental de velocidade não influenciou a glicemia, a lactacidemia e a frequência cardíaca dos equinos antes e durante o teste. São necessários novos estudos para elucidar os mecanismos fisiológicos envolvidos na reposição da glicose sanguínea após a realização de exercícios físicos em esteira de alta velocidade.

Palavras-chave: equinos; glicose; lactato; desempenho.

1. Introduction

Exercise tests are valuable tools in horse fitness evaluation, as they provide a mechanism for assessing a range of body systems [1]. Measurements of cardiorespiratory and metabolic functions during an exercise test provide information on the athletic performance of horses [2]. The use of a standardized high-speed treadmill allows for greater reproducibility of sports function variables by eliminating the influence of environmental conditions, allowing accurate quantification of respiratory, cardiovascular, hematological, and biochemical variables to assess the physical fitness of horses [3, 4]. The physiological parameters used to assess the physical performance of equine athletes are the speed at which the horse is running when the heart rate (HR) equals 200 bpm (V_{200}); the speed at which the horse is running when the plasma lactate concentration equals 2 mmol/L (VL_2), which indicates the threshold of aerobic metabolism [5]; and the speed at which the horse is running when the plasma lactate concentration equals 4 mmol/L (VL_4), which indicates the threshold of anaerobic metabolism [6, 7]. It is important to consider these parameters when evaluating an animal because HR is not a sufficiently sensitive variable to demonstrate the physical effort required for the exercise performed or to evaluate the conditioning of the horse [8].

Understanding the variations in plasma glucose concentration is fundamental, not only in cases of nutritional disorders, but also in terms of racehorse exercise and performance, since glucose is an important energy source for athletes [9]. As the glycemic response is influenced by several factors such as diet composition, schedule of meal intake before exercise, and the time interval since the previous meal, several studies have been conducted on this subject [10, 11]. Some studies have sought to explain how diet composition modulates hematological and biochemical variables besides HR by manipulating the composition or type of diet provided to race horses [12-15]. The main objective of other studies was to evaluate how the energy reserves in the form of muscle glycogen influence blood glucose concentration and, consequently, the availability of this energy substrate [16]. Other researchers evaluated the influence of feeding schedule manipulations on blood glucose and insulin concentrations during exercise [17].

Considering the importance of glucose regulation mechanisms in exercise physiology, correlating glycemic values with performance results can help understand the mechanisms used by horses to obtain energy during physical activity. Therefore, this study aimed to evaluate the effects of concentrated feed consumption on glycemia and lactacidemia in eventing horses before a high-speed treadmill test.

2. Material and methods

2.1 Ethical approval

This study was approved by the Ethics Committee on Animal Use of the Veterinary Institute of the Universidade Federal Rural do Rio de Janeiro, under (number 6062121118).

2.2 Animals

Twelve Brazilian Sports horses from the Brazilian Army Riding School (four mares and eight geldings) were used in the current study. The mean age of the horses was 10.7 ± 2.6 years (range, 7–15 years),

and their mean weight was 492.8 ± 41.9 kg (range, 440–598 kg). The animals performed daily eventing training activities, namely one hour of intense activity which included walking, trotting, and galloping, as well as jumping on a sand or grass track. When not active, the animals remained confined to 4 ×4 m stalls with feeders and free access to water. All animals used in the experiments were subjected to clinical examination (physical examination and laboratory analysis) to obtain veterinary clearance prior to the study. Subsequently, they were weighed on a digital scale, and the body score was determined using a scale of 0–9 by evaluating the neck, withers, shoulders, ribs, back, and base of the tail [18].

The diet was provided according to the routine feeding protocol of the Brazilian Army Riding School. The horses were fed coastcross hay (Cynodon spp. Coastcross) and commercial concentrated feed distributed five times a day (concentrated feed at 5 am, 1 pm, and 7 pm and hay at 10 am and 3 pm). The amounts of food were those routinely supplied by the Brazilian Army Riding School, i.e., equivalent to 3.0% of body weight (BW) as dry matter at a concentrate: forage ratio of 60:40, considering the daily energy requirement of racehorses within the "heavy work" category, which relates to the intensity of training to which horses were subjected [19] (Table 1).

Table 1. Bromatological composition of equine diet foods, expressed on the dry matter basis.

Food	DM[1]	MM[1]	OM[1]	CP[1]	EE[1]	NFC[1]	NDF[1]	HEM[1]	DE[2]
Concentrate	89.7	8.1	91.6	17.1	8.1	44.5	22.2	12.8	3.24
Coastcross hay	91.2	5.8	94.2	10.4	1.9	9.9	72.1	39.0	1.92

DM: dry matter; MM: mineral matter; OM: organic matter; CP: crude protein; EE: ether extract; NFC: non-fibrous carbohydrates; NDF: neutral detergent fiber; HEM: hemicellulose; DE: digestible energy.
[1] %; [2] Mcal/kg.

The consumption of non-fibrous carbohydrates (NFC), which included starch, through the supply of commercial concentrate was estimated at 2.65 g of NFC/kg of BW using the following formula: %NFC = 100 - (%CP +%NDF +%EE +%MM) described in the NRC (19). Digestible energy (DE) was calculated using the following formula: DE (kcal/kg DM) = 2,118 + 12.18 × (%CP) – 9.37 × (%ADF) – 3.83 × (% HEM) + 47.18 × (%EE) + 20.35 × (% NFC) – 26.3 x (%MM), where R2 = 0.88 [20].

2.3 Glucose curve test

The experimental design used four repetitions (horses) and seven treatments (blood collection times). One sample was collected in the preprandial state (T0) and six samples were collected in the postprandial state (T1 to T7) with the order of sample collection occurring in a completely randomized manner. Blood samples were collected 30 min before the feed was provided (T0) and at 30, 60, 90, 120, 180, 240, and 300 min (T1–T7) after the feed was supplied. The mean BW of the horses was 487.75 kg. The diet was supplied as a concentrated feed in a pelleted form plus coastcross hay, given regularly to horses according to the feeding schedule. On the test day, the concentrate was supplied at 5 am as usual, and the animals remained at rest in stalls until the last sample collection.

2.4 High-speed treadmill test

The experimental design was completely randomized, with 12 horses allocated on three treatments (postprandial period before the test) comprising three groups of four animals. The concentrate was provided at a fixed time (5 am), and the start times of the exercise were 2 h, 4 h, and 6 h after feeding. The mean BW of the horses used in this experiment was 485.5 kg in "Group 2 hours", 476.3 kg in "Group 4 hours", and 516.5 kg in "Group 6 hours".

The horses were previously adapted to exercise on the high-speed treadmill Galloper (Sahinco®, Brazil), three times a week for five weeks an exercise protocol comprising a standardized warm-up phase of 10 min, with 4 min walking at a speed of 1.8 m/s and 6 min trotting at 4.0 m/s. The incremental speed phase consisted of 1 min for each speed increased to a gallop at 6, 8, and 10 m/s. After the tests, gallop deceleration was standardized by returning to trotting at a speed of 4.0 m/s for 1 min and 1.8 m/s for 5 min, followed by 25 min of walking on a lead as an active cool-down [3]. During the treadmill test, the horses wore a HR monitor (Polar Equine, Polar®, Finland) that was positioned on the chest, in the apex beat area, after the hair in the area was moistened with a solution containing water and ethanol before the horse entered the treadmill. At the end of the test, the results were transferred to a computer using infrared transmission with the interface provided by the Polar® software and HR values were recorded.

Before exercise, the animals were aseptically prepared for venous catheterization using the left jugular vein as the collection site. An extension line was attached to the intravenous catheter to collect blood from the moving animals. The extension line was filled with anticoagulant solution (heparin sodium in 0.9% sodium chloride solution) and fixed with a 0.1 × 1.8 m cotton bandage and 10.1 × 4.5 m white waterproof tape. Blood samples for glucose and lactate analyses were collected in Vacutainer tubes containing sodium fluoride. The samples were centrifuged at 300 rpm for 10 min for serum separation and aliquot removal. Analyses were subsequently performed using a spectrophotometer (A5) and commercial glucose (Labtest®) and lactate (Lactate K084-2, Bioclin) reagents. Blood samples were collected before the beginning of the exercise test, during the test at each speed change, at the end of the test, and 5, 15, and 30 min after the test.

2.5 Statistical analysis

Analyses were performed using R software (Vienna, Austria, 2018). The means, standard deviations, and 95% confidence intervals were calculated for each variable and group at each assessed time and speed. The glycemic index test was performed after calculating the mean, standard deviation, and 95% confidence interval for the mean plasma glucose concentration in the four horses at each time point, and the area under the curve (AUC) vales were calculated individually using Microsoft Office Excel software using the integration function.

Data on the plasma lactate concentration during the exercise test were subjected to exponential regression analysis to estimate VL_2 and VL_4. Data on HR in the galloping phase of the exercise test were subjected to linear regression analysis to estimate V_{200}. Data were analysed using analysis of variance (ANOVA) and means were compared using the Fischer test ($\alpha<0.05$). To compare the groups with respect to the different parameters (glucose, lactate, and HR as a function of time), the Shapiro–Wilk test of

normality was used to ascertain the normality of data distribution, which was found to be non-normal, except for the results of glucose after the high-speed treadmill test. The Kruskal-Wallis test ($p<0.05$) was used when the data distribution was non-normal. The Wilcoxon test ($p<0.05$) was used for pairwise comparisons of the groups, and the p-values of these tests were corrected using the Bonferroni method. The Friedman test ($p<0.05$) was used to compare schedules (times) and speeds and the Wilcoxon test ($p<0.05$) was used for pairwise comparisons of the groups.

3. Results

3.1 Glucose curve test

The average intake of commercial concentrated feed for each meal was 1.95 kg/dry matter (DM), equivalent to 1.78 g CNF/kg BW. The glucose concentration baseline (prior to feeding) was 75.3 (±10.8) mg/Dl. The values obtained at 30, 60, 90, 120, 180, 240, and 300 minutes postprandial were 75.8 (±8.2), 81.3 (±5.3), 80.3 (±8.1), 70.5 (±3.8), 65.8 (±8.1), 71.3 (±8.3), and 68 (±5.0) mg/dL respectively, corresponding to 4.2, 4.2, 4,5, 4.5, 3.9, 3.7, 3.9, and 3.8 mmol/L (Figure 1).

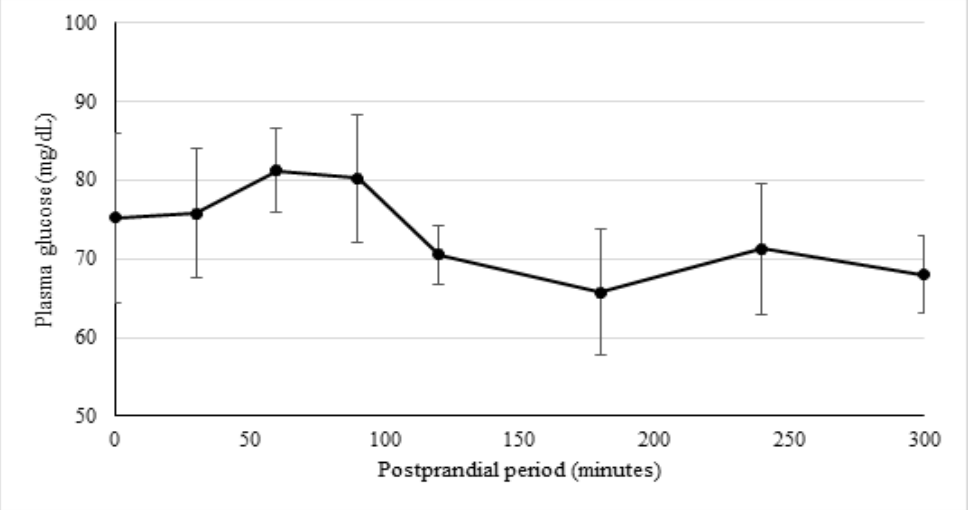

Figure 1. Mean values of plasma glucose (mg/dL) in the postprandial period in horses fed with commercial concentrated feed.

No significant differences ($p=0.06$) were observed between the blood glucose values. Peak blood glucose level occurred between 60 and 90 minutes after concentrate diet intake, with values of 81.3 and 80.3 mg/dL, respectively. The mean value of AUC in the postprandial period was 627 (± 28) mmol × min/L and the individual values of the AUC for each horse were 617, 663, 596, and 633 mmol × min/L.

3.2 High-speed treadmill test

The values obtained from the measurement of plasma glucose during the exercise test were not influenced by the feeding time of the concentrate feed ($p>0.05$). However, significant differences were observed between the different speeds ($p<0.05$) (Figure 2). Mean plasma glucose concentrations were obtained before and during the high-speed treadmill test at speeds 0, 1.8, 4, 6, 8, and 10 m/s. The respective values were 89 (±16), 84 (±14), 76 (±15), 75 (±16), 74 (±16), and 73 (±19) mg/dL in "Group 2 hours"; 80 (±15), 70 (±19), 64 (±14), 58 (±17), 59 (±17), and 60 (±14) mg/dL in "Group 4 hours"; and 79 (±13), 78 (±10), 80 (±10), 81 (±11), 83 (±13), and 86 (±15) mg/dL in "Group 6 hours".

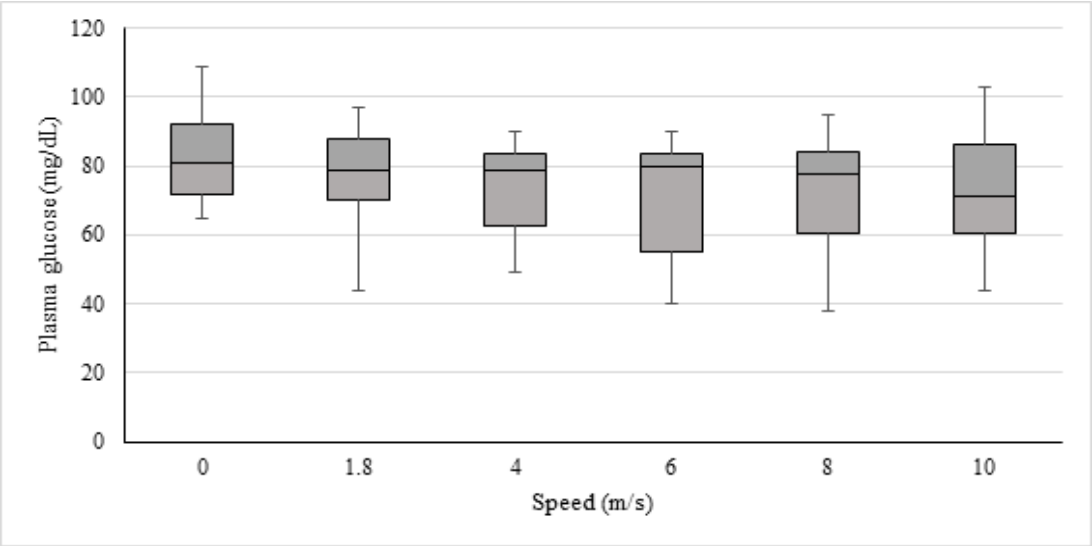

Figure 2. Plasma glucose during the exercise test.

The mean plasma lactate levels at rest (baseline) were 1.36 mmol/L. Mean lactate plasma concentrations were obtained during the high-speed treadmill test at speeds of 0, 1.8, 4, 6, 8, and 10 m/s. The respective values were 1.17; 1.32; 1.49; 2.34; 3.36, and 5.65 mmol/L in "Group 2 hours"; 1.76; 1.75; 2.63; 3.52; 4.39, and 6.14 mmol/L in "Group 4 hours"; and 1.15; 1.33; 1.82; 2.69; 5.0, and 6.80 mmol/L in "Group 6 hours". During the high-speed treadmill test, the correlation between plasma lactate concentration at different speeds (0, 1.8, 4, 6, 8, and 10 m/s) and the experimental groups showed that there was no significant differences among the three groups ($p>0.05$); however, differences were found between the speeds ($p<0.05$) (Figure 3).

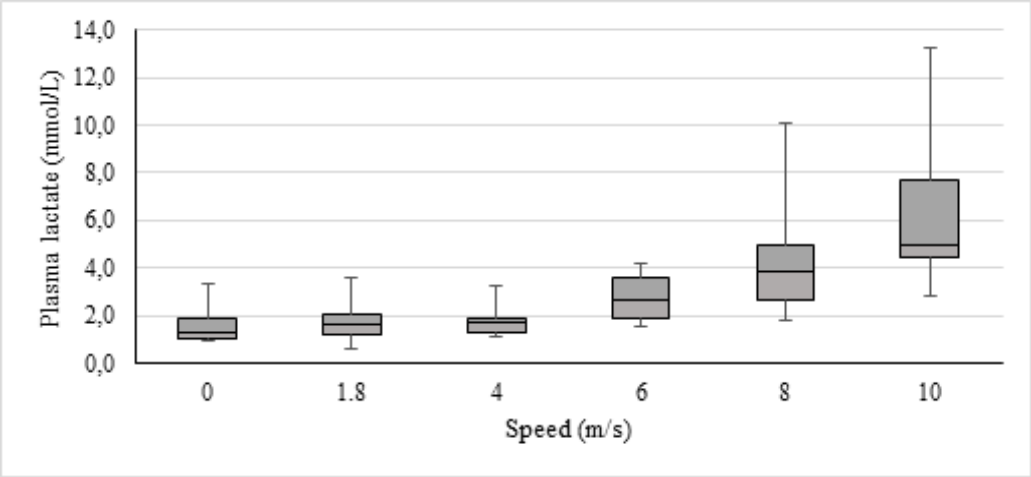

Figure 3. Plasma lactate (mmol/L) during the exercise test.

The plasma lactate concentrations obtained in the high-speed treadmill test were adjusted using the following exponential equation: In "Group 2 hours," the equation 0.9774exp (1.1633x) ($R2 = 71\%$) ($p<0.05$) was obtained. In "Group 4 hours," the equation 1.519exp (1,1366x) ($R2 = 61\%$) ($p<0.05$) was obtained. In "Group 6 hours" the equation 0.979exp (1.194x) ($R2 = 73\%$) ($p<0.05$) was obtained. There

were no significant differences between the groups in terms of VL2 or VL4 (p>0.05; Table 2). The estimates of VL_2 were as follows: 4.7 m/s in "Group 2 hours"; 2.2 m/s in "Group 4 hours"; and 4.0 m/s in "Group 6 hours". The estimates of VL_4 were as follows: 9.3 m/s in "Group 2 hours"; 7.6 m/s in "Group 4 hours"; and 7.9 m/s in "Group 6 hours".

Table 2. Mean values and standard deviation (SD) of VL2, VL4, and V200 in eventing horses as a function of the time of supply of the concentrated diet (2, 4, and 6 hours) before the high-speed treadmill test.

Pre-test concentrated feed	VL_2 (m/s)	VL_4 (m/s)	V_{200} (m/s)
Group 2 hours	4.9 ± 1.8a	9.4 ± 2.6a	10.2 a
Group 4 hours	3.5 ± 1.3a	7.6 ± 2.4a	9.9 a
Group 6 hours	4.0 ± 1.3a	8.1 ± 2.2a	13.3 a

Means followed by the same letters did not differ from each other in relation to groups, according to Fisher's test (p>0.05)

The HR did not differ (p>0.05) between the groups; however, it differed depending on speed (p<0.05). The mean heart rates obtained during the high-speed treadmill test at speeds of 0, 1.8, 4, 6, 8, and 10 m/s were 49, 100, 120, 162, 181, and 198 bpm in "Group 2 hours"; 46, 116, 135, 170, 182, and 203 bpm in "Group 4 hours"; and 49, 105, 131, 166, 176, and 184 bpm in "Group 6 hours", respectively.

Only speeds corresponding to galloping (6, 8, and 10 m/s) were selected to estimate V_{200} because, in this phase, there was a linear relationship between the HR and speed, resulting in a linear regression equation. There were no differences in the mean values of V_{200} between the experimental groups (p>0.05 ; Table 4). Estimated V_{200} values were 10.2 m/s ($\hat{Y}$ = 108.1974 + 9.0329X [r2 = 53.0, p<0.05]) in "Group 2 hours"; 9.9 m/s ($\hat{Y}$ = 121.9211 + 8.4868 X [r2 = 21.0, p<0.05]) in "Group 4 hours", and 13.3 m/s ($\hat{Y}$ = 137.5833 + 4.6875X [r2 = 30.0, p<0.05]) in "Group 6 hours".

Feeding time of the concentrate feed affected the plasma glucose concentration after the high-speed treadmill test, mainly in "Group 4 hours" (p<0.05). In addition to presenting significant differences at different speeds (p<0.05), the blood glucose values obtained showed differences in the distribution of results five minutes after the end of the physical exercise test (p<0.05) between groups, more specifically between "Group 6 hours" and "Group 4 hours" (p<0.10).

The mean values of plasma lactate after the high-speed treadmill test (0, 5, 15, and 30 min post-test) did not differ (p>0.05) between the experimental groups; however, a significant reduction was observed over the post-test time (p<0.05; Table 3).

Table 3. Mean values and standard deviation of the plasma concentration of glucose (mg/dL) and lactate (mmol/L) in eventing horses after the high-speed treadmill test.

Post-test time (minutes)	Plasma glucose (mg/dL)	Plasma lactate (mmol/L)
	Group 2 hours	
0	72,5 ± 18,7^{Aa}	5,65 ± 2,32^{Aa}
5	73,0 ± 15,2^{ABa}	5,98 ± 4,29^{Aa}
15	77,6 ± 9,3^{Aa}	4,20 ± 3,30^{Ab}
30	84,0 ± 8,5^{Aa}	3,32 ± 2,27^{Ac}
	Group 4 hours	
0	60,0 ± 14,1^{Aab}	6,48 ± 2,64^{Aa}
5	57,0 ± 8,0^{Bb}	5,62 ± 3,42^{Aa}
15	70,6 ± 6,7^{Aab}	2,68 ± 1,33^{Ab}
30	79,0 ± 7,6^{Aa}	2,16 ± 0,95^{Ab}
	Group 6 hours	
0	85,8 ± 15,2^{Aa}	6,80 ± 4,30^{Aa}
5	87,8 ± 13,4^{Aa}	5,89 ± 4,63^{Aa}
15	83,0 ± 9,2^{Aa}	3,63 ± 2,69^{Ab}
30	88,3 ± 11,9^{Aa}	2,45 ± 1,08^{Ac}

Means followed by different capital letters differ for the experimental groups according to the Kruskal–Wallis test ($p<0.05$) and the Bonferroni test ($p<0.10$). Means followed by different lowercase letters differ from each other with respect to time after the Friedman ($p<0.05$) and Wilcoxon tests ($p<0.10$).

After the high-speed treadmill test, no significant differences were observed in HR ($p>0.05$) of the horses in all three test groups, with heart rate significantly decreasing as speeds decreased from 10 m/s to 4 m/s and further to 1.8 m/s ($p<0.05$). Average heart rates decreased rapidly after the high-speed treadmill test, from 184, 150 and 98 bpm at speeds of 10, 4, and 1.8 m/s, respectively (Figure 4).

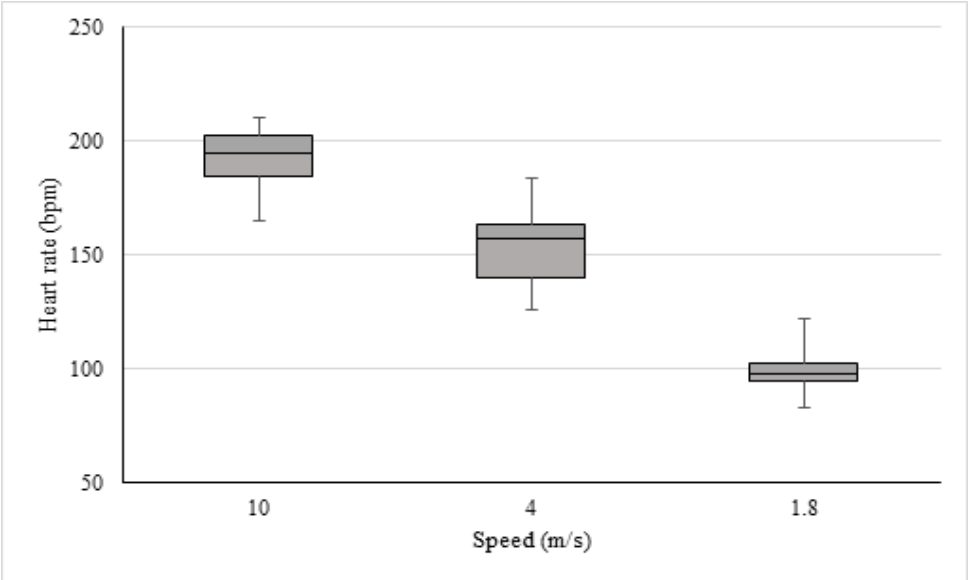

Figure 4. Heart rate (bpm) after the exercise test.

4. Discussion

During exercise, several physiological alterations occur that efficiently produce energy to maintain physical effort, such as transient hyperglycemia [21, 22]. Glycemic values also vary in the postprandial period [23]. Typically, the glucose curve has a continuously increasing slope, and after a peak, the plasma glucose concentration decreases markedly when the diet contains high levels of soluble carbohydrates (24). Because of the essential role of nutrition in the postprandial glucose curve, it is important to identify the associations between nutrition, biochemical parameters, and physical fitness.

The blood glucose levels at rest obtained herein were within the physiological range for horses (75–115 mg/dL) [25] and within the acceptable range for racehorses (70–140 mg/dL) [26]. The intake of different amounts of starch may influence blood glucose peaks [13, 23]. When diets had a lower amount of starch (0.3 g of starch/kg BW), the peak was observed 75 ± 17 minutes after feed intake, and when diets had a higher amount of starch (more specifically 2.0 g of starch/kg BW), the peak occurred only 120 ± 42 minutes after intake. In the present study, with horses consuming approximately 1.78 g CNF/kg BW, the peak plasma glucose was observed between 60 and 90 min, with mean values of 81.3 and 80.3 mg/dL, respectively. The results obtained in the postprandial blood glucose test align with those reported in other studies in which the diet consisted mostly of soluble carbohydrates [24]. A continuous increase in the concentration of plasma glucose and its reduction after a peak was expected owing to the action of insulin after the glucose level peaked [27]. In addition, horses consuming commercial diets containing 0.8 and 1.1 g of starch/kg BW present mean AUC values of 559 ± 49 and 732 ± 162 mmol x min/L [13], respectively, which were similar to the mean value obtained in the present study, 627 ± 28 mmol x min/L.

The glucose curve for horses in "Group 6 hours" had an increasing slope in comparison to those in "Group 2 hours" and "Group 4 hours", whereas a decreasing curve was expected [26]. It is assumed that the group whose feed was provided 6 hours before exercise had increased glycogenolysis and gluconeogenesis compared to the other groups, which led to a higher availability of glucose in the blood of horses in this group.

As shown in the present study, plasma glucose concentration tends to decrease during the first moments of exercise owing to the mobilization of this energy substrate by skeletal muscles [28]. Subsequently, an increase in the activity of hormones that regulate energy metabolism, mainly catecholamines and glucagon, leads to an increase in plasma glucose concentration through glycogenolysis and gluconeogenesis, which is of paramount importance in maintaining glycemia during physical exertion [29, 30]. Five minutes post-exercise tests, plasma glucose values were higher in horses fed 6 h before the test (87.8 mg/dL) than in those fed 4 h before the test (57 mg/dL) (Table 3). This suggests that the timing of feed supply may influence glucose restoration in the bloodstream after effort. Knowing that normal plasma glucose in equine athletes at rest ranges from 70 to 140 mg/dL [26], further studies should be performed with insulin and glucagon dosages to better understand glycemia during and after exercise tests.

Baseline plasma lactate levels in horses typically range from 0.5 to 1.5 mmol/L [26], and the value obtained in this study was within this range. The correlation between lactate levels and speed is graphically expressed in the form of an exponential curve according to exercise intensity [26]. The results indicated that there was no change in VL_2 and VL_4 associated with the feeding schedule; thus, there was

no influence on the performance of the horses during the test. However, anaerobic metabolism was predominant during most of the exercise test, especially in "Group 4 hours" and "Group 6 hours", which had VL_4 values lower than those in "Group 2-hours" as lactate concentration in the muscle increases when there is not enough oxygen available [31]. In eventing horses, the mean VL_2 after the initial exercise test has been estimated at 5.9 m/s and mean VL4 at 7.6 m/s [5]. The values obtained in the present study were similar; however, the protocol used in the high-speed treadmill test differed with regard to time and speed, which may explain the differences in the results.

The horses in the three experimental groups showed a decreasing pattern of lactacidemia five minutes after the end of the high-speed treadmill test. Some studies have reported a reduction in lactate levels in the bloodstream during the recovery phase, because this compound can be used for the synthesis of glucose and glycogen [26]. Additionally, the fact that these horses are well trained may also contribute to this reduction, as training promotes metabolic adaptations that enhance the efficiency of lactate removal and utilization [40]. This improved metabolic efficiency allows for faster conversion of lactate to glucose, facilitating post-exercise glucose restoration.

The reference range for horse HR is between 28 and 45 bpm [32]; however, in the present study, the mean baseline was 47.9 bpm, which was measured before the physical exertion test was performed. There is a positive linear correlation between HR and speed, depending on exercise intensity, resulting in an increasing slope [1, 5]. The high baseline HR value obtained can be explained by psychogenic factors, such as anxiety triggered in the horse when it undergoes the same repetitive processes that precede exercise. Another reason could be that the HR monitor was placed on the horses after they were removed from the stalls, whereas other team members applied the protective material for physical activity. This may have induced a sympathetic nervous system response that led to an increase in the pretest HR [33].

The V_{200} value obtained in the present study was similar to that of a study that used eventing horses under similar training conditions ($V_{200} = 9.02 \pm 1.45$ m/s) [34], but higher than those obtained in two other studies that also used in eventing horses, wherein the V200 equalled 8.5 m/s [37] and the V200 varied between 8 and 9 m/s [38]. The speed reached by a horse when its HR reaches 200 bpm is known as the V_{200}. It is a performance indicator that serves as a parameter for comparison between individuals. Animals with better physical fitness tend to have a higher V_{200}, that is, a higher speed is required to reach a heart rate of 200 bpm [39]. After the physical exertion test, the heart rate decreases rapidly in the first minute and then gradually [1]. The heart rate after a physical exercise test or competition can be used to assess the horse's recovery, and if the heart rate is maintained above 130 bpm for 10 min in the recovery phase, it indicates that the horse is insufficiently trained or has some clinical disorder such as atrial fibrillation, respiratory infection, or lameness [1]. In the present study, it was observed that at 6 minutes post-test, the average heart rate of horses fed 2, 4, and 6 hours before the test were 99, 100, and 98 bpm, respectively, corroborating that described for horses with adequate and healthy conditioning [1].

5. Conclusion

The feeding schedule did not influence blood glucose levels in the horses before and during the high-speed treadmill test; however, it was able to alter the restoration of post-test glycemia. Further studies are needed to assess the influence of glucose replacement after exercise in standardized speed

tests. The time of supply of the concentrated feed before the high-speed treadmill test also did not have a significant effect on HR or lactate concentration. The evaluation of the performance of each experimental group showed that VL_2, VL_4, and V_{200} were not affected by the feeding schedule at 2, 4, or 6 h before the high-speed treadmill test.

Supplementary material

Graphical Abstract (only available in the electronic version).

Conflicts of interest statement

The authors declare that there is no conflict of interest

Data availability statements

The data will be provided upon request.

Author contributions

Conceptualization: C. M. C. Rodrigues and F. Q. Almeida. Investigation: C. M. C. Rodrigues, A. P. Buzzetti, I. T. Nothaft. Methodology: C. M. C. Rodrigues, I. T. Nothaft, and B. C. Moreira. Formal Analysis: C. M. C. Rodrigues, C. D. Baldani, M. I. V. Almeida. Project management: F. Q. Almeida. Writing (original draft): C. M. C. Rodrigues, B.C. Moreira. Writing (review and editing): B.C. Moreira and F. Q. Almeida.

Acknowledgments

The authors are grateful to the Foundation for Research Support of the State of Rio de Janeiro (FAPERJ), the members of the research team of the Equine Performance Evaluation Laboratory (LADEq), and the Brazilian Army Riding School for their unconditional support in the accomplishment of this research.

References

1. Evans DL. Exercise testing in the field. In: Hinchcliff KW, Kaneps AJ, Geor RJ, editors. Equine Sports Medicine and Surgery. 1st ed. London: Saunders; 2004. p. 19-31. ISBN: 978-0-7020-2857-1

2. Hines MT. Clinical approach to commonly encountered problems. In: Reed SM, Bayly WM, Sellon DC, editors. Equine Internal Medicine. 4th ed. Amsterdam: Elsevier Health Sciences; 2017. p. 232-310. PMCID: PMC7158300

3. Castejón F, Riber C, Santisteban R, Tovar P, Trigo P, Agüera Y. Valoración ergométrica y muscular en cinta rolante. In: Valoración morfofuncional en el plan de mejora del pura raza español-caballo andaluz. Junta de Andalucía, Ministerio de Educación y Cultura; 1995. p. 100-12. ISBN: 978-84-92521-89-0

4. Nankervis EJ, Launder RCM, Murray RC. The Use of Treadmills Within the Rehabilitation of Horses. J Equine Vet Sci. 2017; 53:108-15. DOI: https://doi.org/10.1016/j.jevs.2017.01.010

5. Souza BG, Veiga CCP, Oliveira GF, Ferreira AMR, Almeida FQ. Avaliação de um programa de treinamento para cavalos de concurso completo de equitação: efeitos sobre a frequência cardíaca e a curva de lactato. Rev Bras Med Vet. 2013; 35(4):385-91. Disponível em: https://bjvm.org.br/BJVM/article/view/623

6. Muñoz A, Santisteban R, Rubio MD, Vivo R, Agüera EI, Escribano BM, *et al*. The use of functional indexes to evaluate fitness in the Andalusian horse. J Vet Med Sci. 1997; 59(9):747-52. DOI: https://doi.org/10.1292/jvms.59.747

7. Hodgson DR, McGowan CM. An Overview of Performance and Sports Medicine. In: Hodgson DR, McGowan CM, McKeever KH, editors. The Athletic Horse: Principles and Practice of Equine Sports Medicine. 2nd ed. Elsevier Saunders; 2014. p. 1-8. DOI: https://doi.org/10.1016/B978-0-7216-0075-8.00010-1

8. De Mare L. Standardized exercise tests in horses: current situation and future perspectives. Vlaams Diergen Tijdschr. 2017; 86(2):63-72. DOI: https://doi.org/10.21825/vdt.v86i2.16290

9. Gobesso AAO, Etchichury M, Tosi H. Resposta plasmática de glicose e insulina em equinos alimentados com diferentes fontes de amido. Braz J Vet Res Anim Sci. 2009; 46(4):324-31. URI: http://hdl.handle.net/11449/71450

10. Gleeson M. Biochemistry of Exercise. In: Micheli LJ, editor. The Encyclopaedia of Sports Medicine. 1st ed. Sage Publications, Inc; 2013. p. 36-58. DOI: https://doi.org/10.1002/9781118692318.ch3

11. Brøkner C, Austbø D, Næsset JA, Blache D, Knudsen KEB, Hansen HH, Tauson AH. Glycaemic and insulinemic response to dietary carbohydrates in horses. Acta Vet Scand. 2016; 58(Suppl 1):69. DOI: https://doi.org/10.1186/s13028-016-0244-1

12. Pagan JD, Harris PA. The effects of timing and amount of forage and grain on exercise response in thoroughbred horses. Equine Vet J. 1999; 30:451-7. DOI: https://doi.org/10.1111/j.2042-3306.1999.tb05264.x

13. Vervuert I, Voigt K, Hollands T, Cuddeford D, Coenen M. Effect of feeding increasing quantities of starch on glycaemic and insulinaemic responses in healthy horses. Vet J. 2009; 182:67-72. DOI: https://doi.org/10.1016/j.tvjl.2008.04.011

14. Jansson A, Lindberg E. A forage-only diet alters the metabolic response of horses in training. Anim. 2012; 6(12):1939-46. DOI: https://doi.org/10.1017/S1751731112000948

15. Oliveira CA, Azevedo JF, Miranda ACT, Souza BG, Ramos MT, Costa APD, *et al*. Hematological and blood gas parameters response to treadmill exercise test in eventing horses fed different protein levels. J Equine Vet Sci. 2014; 34(11-12):1279-85. DOI: https://doi.org/10.1016/j.jevs.2014.09.007

16. Jose-Cunilleras E, Hinchcliff KW, Lacombe VA, Sams RA, Kohn CW, Taylor LE, Devor ST, *et al*. Ingestion of starch-rich meals after exercise increases glucose kinetics but fails to enhance muscle glycogen replenishment in horses. Vet J. 2006; 171(3):468-77. DOI: https://doi.org/10.1016/j.tvjl.2005.02.002

17. Pagan JD, Burger I, Jackson SG. The long-term effects of feeding fat to 2-year-old Thoroughbreds in training. Equine Vet J. 1995; 18:281-8. DOI: https://doi.org/10.1111/j.2042-3306.1995.tb04949.x

18. Henneke DR, Potter GD, Krieder JL. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J. 1983; 15:371-2. DOI: https://doi.org/10.1111/j.2042-3306.1983.tb01826.x

19. National Research Council. Nutrients requirements of horses. 6th ed. Washington, DC: National Academy of Science; 2007. 341 p. DOI: https://doi.org/10.17226/11653

20. Pagan JD. Advances in Equine Nutrition. Nottingham: Nottingham University Press; 1998. p. 71-6. ISBN-13: 978-1897676837

21. Mircean M, Giurgiu G, Mircean V, Zinveliu E. Serum cortisol variation of sport horses in relation with the level of training and effort intensity. Bull USAMV-CN. 2007; 64:488-92. https://journals.usamvcluj.ro/index.php/veterinary/article/view/2481

22. Coelho CS, Gama JA, Lopes PF, Souza VR. Glicemia e concentrações séricas de insulina, triglicérides e cortisol em equinos da raça Mangalarga Marchador após exercício físico. Pesq Vet Bras. 2011; 31(9):756-60. DOI: https://doi.org/10.1590/s0100-736x2011000900006

23. Hansen TL, Rankins EM, Bobel JM, McKinney M, Hackmann TJ, Warren LK. Postprandial blood glucose and insulin responses of horses to feeds differing in soluble fiber concentration. J Equine Vet Sci. 2020; 88:102963. DOI: https://doi.org/10.1016/j.jevs.2020.102963

24. Healy HP, Siciliano PD, Lawrence LM. Effect of concentrate form on blood and gastric fluid variables in ponies. Equine Nutr Physiol Soc. 1995; 15(10):423-8. DOI: https://doi.org/10.1016/S0737-0806(06)81833-2

25. Kaneko JJ, Harvey JW, Bruss ML. Clinical biochemistry of domestic animals. 6th ed. New York: Academic Press; 2008. 928 p. DOI: https://doi.org/10.1016/B978-0-12-370491-7.00001-5

26. McGowan CM., Hodgson DR. Hematology and biochemistry. In: Hodgson DR, McKeever KH, McGowan CM. The Athletic Horse: Principles and Practice of Equine Sports Medicine. 2nd Edition. Elsevier Saunders. Missouri, USA. 2014. p. 56-68. ISBN: 978-0-7216-0075-8

27. Bertin FR, Taylor SD, Bianco AW, Sojka-Kritchevsky JE. The effect of fasting duration on baseline blood glucose concentration, blood insulin concentration, glucose/insulin ratio, oral sugar test, and insulin response test results in horses. J Vet Intern Med. 2016; 30:1726-31. DOI: https://doi.org/10.1111/jvim.14529

28. Trilk JL, Lindner AJ, Greene HM, Alberghina D, Wickler SJ. A lactate-guided conditioning programme to improve endurance performance. Equine Vet J Suppl. 2002; 34:122-5. DOI: https://doi.org/10.1111/j.2042-3306.2002.tb05403.x

29. McKeever KH. The endocrine system and the challenge of exercise. Vet Clin North Am Equine Pract. 2002; 18:321-53. DOI: https://doi.org/10.1016/S0749-0739(02)00005-6

30. Ferraz GDC, Teixeira-Neto AR, Pereira MDC, Linardi RL, Lacerda-Neto JCD, Queiroz-Neto AD. Influência do treinamento aeróbio sobre o cortisol e glicose plasmáticos em equinos. Arq Bras Med Vet Zootec. 2010; 62(1):23-9. DOI: https://doi.org/10.1590/S0102-09352010000100003

31. Rose RJ, Ilkiw JE, Sampson D. Changes in blood gas: acid–base and metabolic parameters in horses during three-day event competition. Res Vet Sci. 1980; 28(3):393-8. DOI: https://doi.org/10.1016/S0034-5288(18)32733-4

32. Boffi F. Fisiología del Ejercicio en Equinos. 1st ed. Buenos Aires: Inter-Médica; 2007. 320p. ISBN: 978-9505553143

33. Prates RC, Rezende HHC, Lana AMQ, Borges I, Moss PCB, Moura RS, Rezende ASC. Heart rate of Mangalarga Marchador mares under marcha test and supplemented with chrome. Rev Bras Zootec. 2009; 38:916-22. DOI: https://doi.org/10.1590/S1516-35982009000500019

37. Garcia TR, Rezende ASCD, Santiago JM, Almeida FQD, Fonseca MG, Munõz A. Venous hemogasometry and blood electrolytes in Mangalarga Marchador mares submitted to aerobic training. Ciênc Agrotec. 2013; 37(6):559-65. DOI: https://doi.org/10.1590/S1413-70542013000600009

38. Kowal RJ, Almosny NRP, Cascardo B, Summa RP, Cury LJ. Avaliação dos valores de lactate e da atividade sérica da enzima creatina quinase em cavalos (Equus caballus) da raça Puro-Sangue-Inglês (PSI) submetidos a teste de esforço em esteira ergométrica. Rev Bras Ciênc Vet. 2006; 13(1):13-9. DOI: http://dx.doi.org/10.4322/rbcv.2014.259

39. Bayly W, *et al*. Equine Sport Medicine and Surgery: basic and clinical sciences of the equine athlete. 1st ed. Philadelphia: Saunders Company; 2004. p. 27-56. DOI: https://doi.org/10.1016/B978-0-7020-2671-3.50009-X

40. Seeherman HJ, Morris EA. Comparison of yearling, two-year-old and adult Thoroughbreds using a standardised exercise test. Equine Veterinary Journal, 1991; 23:175-184. DOI: https://doi.org/10.1111/j.2042-3306.1991.tb02750.x