R. Bras. Zootec., 51:e20210111, 2022
https://doi.org/10.37496/rbz5120210111

Biometeorology and animal welfare
Full-length research article

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Assessment of a *vaquejada* horse training protocol based on laboratory clinical parameters

Daniel Ânderson de Souza Melo[1], Juliano Martins Santiago[2]*, Jorge Eduardo Cavalcante Lucena[1], Luís Artur Cordeiro Costa[1], Ana Clara Sarzedas Ribeiro[3], Andreza Correia da Silva[2], Iaçanã Valente Ferreira Gonzaga[1]

[1] Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Ciência Animal e Pastagens, Garanhuns, PE, Brasil.
[2] Universidade Federal Rural de Pernambuco, Unidade Acadêmica de Serra Talhada, Serra Talhada, PE, Brasil.
[3] Universidade Federal Rural de Pernambuco, Programa de Pós-Graduação em Sanidade e Reprodução de Animais de Produção, Garanhuns, PE, Brasil.

***Corresponding author:**
jmartinssantiago@yahoo.com.br

Received: June 4, 2021
Accepted: February 14, 2022

How to cite: Melo, D. A. S.; Santiago, J. M.; Lucena, J. E. C.; Costa, L. A. C.; Ribeiro, A. C. S.; Silva, A. C. and Gonzaga, I. V. F. 2022. Assessment of a *vaquejada* horse training protocol based on laboratory clinical parameters. Revista Brasileira de Zootecnia 51:e20210111.
https://doi.org/10.37496/rbz5120210111

ABSTRACT - The objective of this study was to assess a training protocol employed in the Brazilian Northeast region for fitness conditioning of *vaquejada* horses. For 12 months, 24 Quarter Horses were evaluated under a completely randomized split-plot experimental design in which the plots comprised three age groups: horses at two, three, and four years of age. The split plots were made up of six fitness tests carried out every other month. The fitness test protocol consisted of five levels of protocol exercises on a standard *vaquejada* track. Prior to the tests with fasted animals, we collected blood samples to determine muscle enzymes (aspartate aminotransferase, creatine kinase, and lactate dehydrogenase). During the tests, heart rate, speed, and distance run were recorded using a heart rate monitor. Next, the results were used to calculate speed at which each horse reached 150 bpm (V_{150}), speed at which each horse reached 200 bpm (V_{200}), maximum heart rate (HR_{max}), maximum speed (V_{max}), recovery time needed for the heart rate of horses to return to half the maximum value reached during the fitness tests ($HR_{50\%}$), and recovery time needed for the heart rate of horses to return to baseline values (HR_{basal}). No difference was found among the age groups for V_{150}, HR_{max}, V_{max}, $HR_{50\%}$, HR_{basal}, or muscle enzymes. By the final stage of training, the V_{200} of the three-year-old horses was higher than that of the four-year-old foals. During training, all groups exhibited increases in serum concentrations of muscle enzymes and reductions in efficiency to recover heart rate after exercise. The training protocol assessed is unable to maintain proper fitness for competitions throughout the year.

Keywords: enzyme, equine, muscle

1. Introduction

The versatility of horses allows them to be used both in farming activities and in various sports modalities. In the Brazilian Northeast region, *vaquejada* is the most popular and widespread equestrian modality (Torres et al., 2020). *Vaquejada* trials are an intense test of the athletic qualities of horses, which are exposed to physical and metabolic risks (Lopes et al., 2009).

The races are practiced by two athletes who, mounted on their horses, gallop for a distance of 100 m aligned with a bovine and, upon reaching the scoring zone, attempt to drop it to the ground by pulling on its tail, which is properly lined with tail protection to prevent lesions (ABVAQ, 2016; ABVAQ, 2017).

Each rider plays a specific role. The *esteira* or trailing rider is in charge of positioning the animal on the track and of grabbing its tail and quickly handing it to the partner. After the bovine falls to the ground within the track limits, the *esteira* is also responsible for not allowing the animal to go over the boundaries when standing up. The *puxador* or leading rider is in charge of pulling the bovine by its tail and dropping it to the ground within the track limits (ABVAQ, 2016).

The continual increase in the number of equestrian sports enthusiasts is accompanied by higher skill and fitness requirements for athlete horses. In this context, horse breeders, competitors, and, in particular, trainers must always be aware of enhancements in animal fitness techniques, since adopting proper technical and physical training is essential for the animals to reach their maximum sports potential (Rose and Hodgson, 1994).

An effective training protocol leads to a horse's improved athletic performance based on continuous physical effort with a gradual increase in intensity, always followed by regular periods of rest. Training may result in increased capacity to carry out physical activity and adaptation to effort overload during sports events, thus reducing the risk of injuries, especially of the musculoskeletal system (Graaf-Roelfsema et al., 2007; Ferraz et al., 2010).

This study aimed to assess a training protocol employed in the Brazilian Northeast region for fitness conditioning of *vaquejada* horses.

2. Material and Methods

Research on animals was conducted according to the institutional committee on animal use (005/2019).

The trial was carried out in the municipality of Garanhuns, PE, Brazil (8°54'34.5" S, 36°30'58" W, and 842 m altitude) and lasted for 12 months (January to December), during which the protocol adopted for fitness training of *vaquejada* horses was assessed. A sample of 24 healthy Quarter Horses was used, including eight males and 16 females with a mean weight of 450±21.32 kg and ages between two and four years.

The animals were assigned to three experimental groups based on age group and training level. Group I comprised five two-year-old fillies in their first year of training and not yet competing; Group II was made up of 12 three-year-old horses (four male horses and eight females) in their second year of training and not yet competing; and Group III comprised seven four-year-old animals (four full male horses and three females) in their third year of training that started to compete from the seventh month of monitoring (July to December).

2.1. Feed management

The animals were kept in 16 m^2 individual masonry pens and were fed Tifton hay (*Cynodon* spp.) at 6:00 and 18:00 h at a sufficient amount so that it remained available to the horses 24 h a day (*ad libitum*). They were also fed 6 kg of pelletized commercial concentrate containing 120.00 g/kg crude protein and 3,750.00 kcal/kg digestible energy split into three daily meals at 7:00, 12:00, and 19 h. Water and a mineral lick were provided *ad libitum*.

2.2. Fitness training

All horses were monitored at the same time intervals (January to December of the same year). Horses were trained from Monday through Saturday for all 12 months of the year, with sporadic periods of rest (e.g., for injuries, holidays, and celebrations). At the property, all animals underwent specific training for each age group (Table 1). The two-year-old animals began their first year of training, the three-year-old horses were in their second year of training, and the four-year-old horses were in their third year of training. Especially in the initial training phase (with the two-year-old horses), the

Table 1 - Fitness training protocol employed for the monitored *vaquejada* horses

Age (years)	Type of exercise	Site	Weekly frequency	Period of the day	Daily duration (min)
2.0	Flexing	Pens	Three days	Morning	Variable[1]
	Long-reining	Round arena	Three days	Morning	20 to 30
	Beginning of mounted exercises[2]	Round arena/ Outdoor areas[3]	Six days	Morning	20 to 30
2.5	Walks	Outdoor areas	Six days	Afternoon	30
	Exercises trotting and galloping	*Vaquejada* track	Six days	Morning	20
	Commands with mouthpieces[4]	*Vaquejada* track	Six days	Morning	20
3.0	Walks	Outdoor areas	Six days	Afternoon	30
	Exercises trotting and galloping	*Vaquejada* track	Six days	Morning	20
	Commands with mouthpieces	*Vaquejada* track	Six days	Morning	20
	Exercise with bovines[5]	*Vaquejada* track	Two days	Afternoon	40
4.0	Walks	Outdoor areas	Three days	Afternoon	30
	Exercises trotting and galloping	*Vaquejada* track	Six days	Morning	30
	Commands with mouthpieces	*Vaquejada* track	Six days	Morning	30
	Exercise with bovines	*Vaquejada* track	Three days	Afternoon	60

[1] Dependent on individual response to stimuli, assessed by the trainer.
[2] Exercises for spin, side movements, backward circles, and picking up the correct lead.
[3] Streets, roads, and trails inside and outside the property.
[4] Exercises for spin, circles, and side movements using *vaquejada* mouthpieces.
[5] Aligning the horse at the gate, running the track with the bovine, and cornering, spinning, and dropping the bovine within the scoring zone.

training sessions were gradual and rational, respecting the horses' individual time and responses to stimuli and exercises.

2.3. Field test

Every other month, horses were subjected to a progressive effort field test for a total of six tests (Rezende et al., 2012; Garcia et al., 2013). To achieve greater standardization, the same rider was prioritized to ride the horses, always in the same order and at the same time of day. Immediately prior to each horse being tested, the uncontrollable external factors of ambient temperature and relative air humidity were recorded using an Incoterm® digital thermohygrometer.

Fitness tests were carried out on a standard *vaquejada* track covered with a 40 cm layer of sand. The track was 160 m in length, with an initial width of 30 m and a final width of 50 m.

The test protocol consisted of five successive stages of progressive exercises. In each stage, the horses covered 340 m within the *vaquejada* track: the first lap around the track was completed walking (mean speed of 6.2 km/h), the second and third laps trotting (mean speed of 9.4 km/h), the fourth lap at a canter (mean speed of 16.9 km/h), and the fifth lap at a gallop at maximum effort (mean speed over the entire track of 33.2 km/h and mean maximum speed at isolated moments of 70.1 km/h). During the first 20 min of the recovery period after the tests, horses were cooled down by walking while led by the halter. They were monitored for the first 60 min of the recovery period after the tests.

2.4. Sample collection and processing

Horses' heart rates were monitored during and after the fitness tests using a heart rate monitor. Prior to the tests, a Soft Equine Polar® elastic strap containing sensors was fitted to the thorax of the animals near the brisket region. Next, a Bluetooth Polar H10 device was attached to the elastic strap. A mobile phone with GPS connected to the monitor via Bluetooth was fitted to the arm of the rider to record and store data on the heart rate, speed, and travel distance of the animals during the tests. Heart rate was also monitored during the first 60 min of the recovery period.

The time the horses took for each stage of the fitness tests (walking, first trotting, second trotting, canter, and gallop) and the distance traveled in each lap (340 m for each stage of the test) were used to calculate the mean speed of each horse during the different stages of the test. Mean speed and heart rate of the horses in each stage of the test were used to yield a polynomial regression equation for each animal (Rezende et al., 2012), which enabled us to estimate the speed at which each horse reached 150 and 200 beats per minute (bpm), i.e., V_{150} and V_{200}, respectively.

After the fitness tests, time and heart rate of the horses during the recovery period were used to yield a new polynomial regression equation, through which we estimated the time it took for the heart rate of each individual horse to return to half the maximum value reached during the tests ($HR_{50\%}$) and the recovery time needed to return to baseline values (HR_{basal}).

In the morning of each day of testing, baseline blood samples were collected via venipuncture of the jugular vein of the fasted animals using needles for vacuum collection and vacuum tubes with no anticoagulant. Those samples, stored in isothermic boxes with recyclable ice, were immediately taken to the laboratory and centrifuged at 1,540 *g* for 20 min using a FANEM Excelsa II 206-BL centrifuge to separate the serum. Next, 0.5 mL aliquots of serum were stored in polypropylene Eppendorf tubes, properly identified, and frozen at −18 °C for analyses of muscle enzymes.

Samples were used to determine serum concentrations of aspartate aminotransferase, creatine kinase, and lactate dehydrogenase enzymes using Labteste commercial reagent kits (LDH Liquiform, CK-NAC Liquiform, and AST Liquiform) and a BIOPLUS BIO-2000 semi-automated spectrophotometer.

Ambient temperature and relative humidity of the air (RH, %) data collected were used to estimate the thermal comfort index (CI) according to the following equation (Jones, 2009): CI = (T °F + RH%), in which T °F = temperature in degrees Fahrenheit. The temperature in °C was converted into °F using the formula: (T °C × 9/5) + 32 = °F.

2.5. Statistical analyses

A completely randomized experimental design was used in a split-plot scheme composed of the plots comprising the three experimental groups and the split plots comprising the six fitness tests performed over the 12 months of training:

$$y_{ijk} = o + \alpha_i + \delta_{ik} + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk},$$

in which y_{ijk} = observed value for the variable under study referring to the *k*-th repetition of the combination of the *i*-th level of experimental group with the *j*-th level of the fitness test; o = overall average; α_i = effect the *i*-th level of experimental group on the observed y_{ijk} value; δ_{ik} = residual effect of the experimental groups; β_j = effect of the *j*-th level of the fitness test on the observed y_{ijk} value; $(\alpha\beta)_{ij}$ = effect of the interaction of the *i*-th level of experimental group with the *j*-th level of the fitness test; and ε_{ijk} = residual effect of the fitness tests.

As the two-year-old horses in the early training stage lacked the skills to be tested while being ridden on the *vaquejada* track, Group I was not considered when analyzing the parameters obtained during the fitness tests.

Heart rate, speed, and recovery time were assessed using a completely randomized split-plot experimental design, with the plots comprising two horse groups: three-year-olds (12 animals) and four-year-olds (seven animals). The split plots were made up of the six fitness tests performed over the 12 months of training.

The V_{150}, V_{200}, maximum heart rate (HR_{max}), maximum speed (V_{max}), $HR_{50\%}$, and HR_{basal} were subjected to analysis of variance and test of mean (Tukey's test) or regression analyses using the statistical software SISVAR version 5.6.

Since the blood samples to determine muscle enzymes were collected before the fitness tests, i.e., they were not influenced by the tests, the group of two-year-old horses could be considered. The muscle enzyme evaluation employed a completely randomized split-plot design, with the plots comprising the three experimental groups and the split plots comprising the six fitness tests.

Among the three muscle enzymes studied, only the results of aspartate aminotransferase exhibited normal distribution. The results for creatine kinase and lactate dehydrogenase displayed logarithmic transformation. The results of the three muscle enzymes were subjected to analysis of variance, and means were compared by test of mean (Tukey's test) at 5% probability using the statistical software SISVAR version 5.6.

In addition, during the 12 months of monitoring, information was gathered on the overall health status of the animals and, specifically for the horses already competing in *vaquejada* trials, the frequency of participation and results obtained were recorded.

3. Results

All horses completed the 12 months of training. The exercise routine was briefly interrupted for eight horses in Groups II (three years old) and III (four years old). The interruptions were caused by orthopedic lesions (two animals), muscle lesions (four animals), skin wounds (one animal), and orchiectomy (one animal). In addition, the four-year-old horses, which started competitions after the seventh month of monitoring, competed on average in 1.5 competitions per month.

During the six fitness test sessions, ambient temperatures ranged from 22.65 to 30.15 °C, relative air humidity from 27.46 to 65.9%, and the thermal comfort index from 111.1 to 145.06. In the third, fourth, and sixth fitness test sessions, horses exercised within recommended thermal comfort conditions (≤130) (Jones, 2009) (Table 2).

No difference (P>0.05) was found between the three-year-old and four-year-old horses regarding the speed at which they reached V_{150}, HR_{max}, V_{max}, $HR_{50\%}$, or HR_{basal}.

The values of V_{150} were the same in the second, fifth, and sixth tests. In the fourth test, carried out after eight months of training, V_{150} values were lower (14.27 km/h) than in the third test (19.73 km/h), which was carried out after six months of training (Table 3). In contrast, HR_{max} and V_{max} were similar in all six fitness tests at 204.3 bpm and 70.3 km/h, respectively, on average.

Over a year of fitness training, a horse's recovery capacity, i.e., the time it takes for clinical parameters to return to baseline values, is expected to improve. However, an assessment of such capacity after the fitness tests showed that over 12 months of training, the horses progressively lost recovery efficiency, as both $HR_{50\%}$ and HR_{basal} values increased between the first and sixth tests (Figure 1).

A difference was found between Groups II (three years old) and III (four years old) regarding V_{200} (Table 4). Up until the fourth fitness test, V_{200} values in Group III were equal to or higher than those of Group II. However, from the fifth fitness test onwards, i.e., after ten months of training, that order was inverted. In addition, while the V_{200} of Group II was the same in all fitness tests, that value decreased for Group III over the 12 months of training.

Table 2 - Mean ambient temperature (°C), relative air humidity (%), and thermal comfort index (CI) recorded during the six fitness tests

Variable	Fitness test					
	1st	2nd	3rd	4th	5th	6th
Temperature (°C)	26.2	30.15	27.82	22.65	28.15	28.68
Humidity (%)	65.9	58.56	38.53	41.64	48.84	27.46
CI	145.06	144.83	120.60	114.36	131.51	111.1

No difference was found among the groups in serum concentrations of aspartate aminotransferase (P = 0.157) or creatine kinase (P = 0.256). However, the concentrations of both muscle enzymes increased in all three groups over the training period. At two months of training, the mean concentration of aspartate aminotransferase was 199.26 U/L, but it increased by 58% to 314.32 U/L at 12 months. Likewise, the mean concentration of creatine kinase at two months of training was 170.00 U/L and

Table 3 - Mean values of performance indices V_{150}, HR_{max}, and V_{max} of *vaquejada* horses subjected to six field fitness tests over 12 months of training (n = 19)

Variable	Fitness test						P-value	CV (%)
	1st	2nd	3rd	4th	5th	6th		
V_{150} (km/h)	-	15.95ab	19.73a	14.27b	15.66ab	19.20ab	0.009	27.9
HR_{max} (bpm)	205.65	204.38	200.00	208.18	207.69	199.91	0.458	6.9
V_{max} (km/h)	-	70.50	71.12	66.79	70.11	73.15	0.330	11.5

V_{150} (km/h) - speed at which the horses reached a heart rate of 150 beats per minute in kilometers per hour; HR_{max} (bpm) - maximum heart rate in beats per minute; V_{max} (km/h) - maximum speed in kilometers per hour; CV - coefficient of variation; (-) unregistered value.
Means followed by different letters in the rows differ among the fitness tests according to Tukey's test (P<0.05).

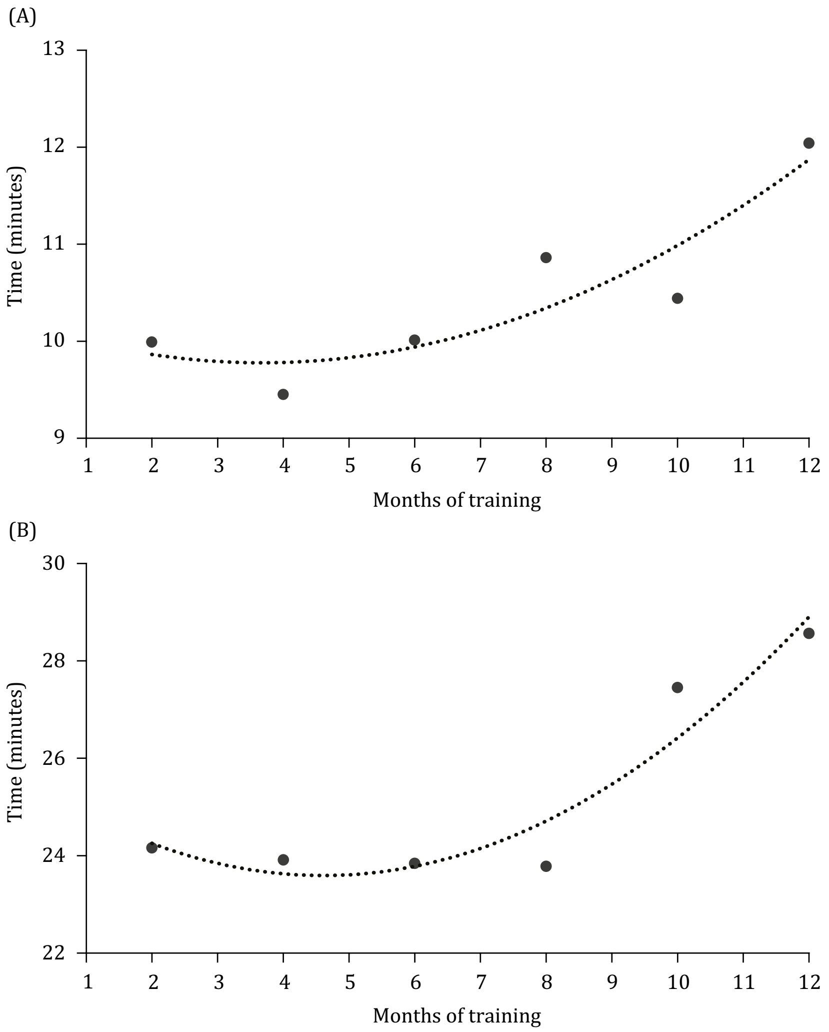

Figure 1 - (A) Recovery time needed for the heart rate of *vaquejada* horses to return to half the maximum value reached during the fitness tests ($HR_{50\%}$) ($y = 0.0303x^2 - 0.2227x + 10.188$; $R^2 = 0.8232$) and (B) recovery time needed for the heart rate of *vaquejada* horses to return to baseline values (HR_{basal}) (n = 19) ($y = 0.0971x^2 - 0.8949x + 25.654$; $R^2 = 0.9066$).

the highest mean value of 259.05 U/L was reached at ten months of training. It then decreased to 214.55 U/L at 12 months of training (Figure 2).

Serum concentrations of lactate dehydrogenase also did not differ among the groups (P = 0.1430). However, unlike the other two muscle enzymes studied, serum concentrations of lactate dehydrogenase varied over the training period, with the lowest values at two months of training (493.63 U/L), the highest at four months (809.27 U/L), and a reduction at 12 months (585.96 U/L) (Table 5).

Table 4 - Mean values of performance index V_{200} of *vaquejada* horses of different age groups subjected to six fitness field tests over 12 months of training (n = 19)

Group	Fitness test					P-value	CV (%)
	2nd	3rd	4th	5th	6th		
3 years old	26.33Aa	26.91Ba	26.33Aa	26.37Aa	31.30Aa	0.001	19.1
4 years old	25.58Aab	36.15Aa	25.02Aab	18.73Bb	22.34Bb		

V_{200} (km/h) - speed at which the horses reached 200 bpm.
Means followed by different lowercase letters in the rows differ among the fitness tests according to Tukey's test (P<0.05).
Means followed by different capital letters in the columns differ among the age groups according to Tukey's test (P<0.05).

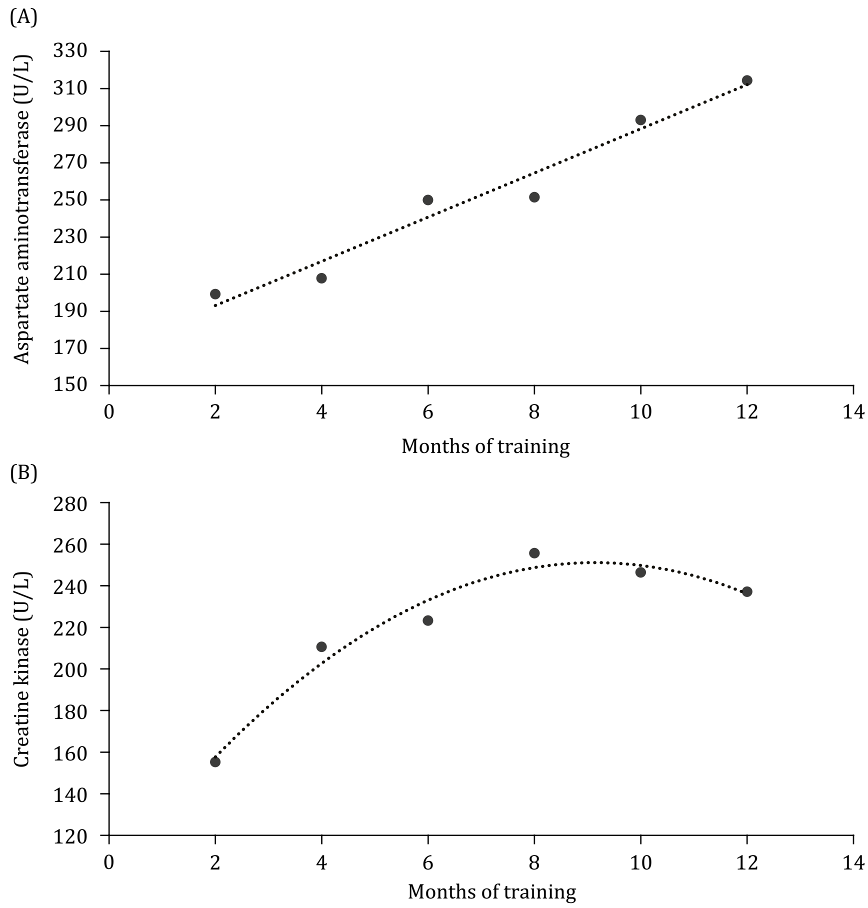

Figure 2 - (A) Mean serum concentrations of aspartate aminotransferase (U/L) of horses during the 12 months of *vaquejada* training (y = 11.896x + 169.35; R^2 = 0.9607) and (B) mean serum concentrations of creatine kinase (U/L) of horses during the 12 months of *vaquejada* training (n = 24) (y = −1.8332x^2 + 33.509x + 97.983; R^2 = 0.9656).

Table 5 - Mean serum concentrations of lactate dehydrogenase (U/L) of horses of different age groups monitored every other month during *vaquejada* training (n = 24)

Age group	Fitness test						P-value	CV (%)
	1st	2nd	3rd	4th	5th	6th		
2 years old	550.09	839.55	665.95	767.20	904.73	622.50		
3 years old	457.03	734.36	642.25	679.07	736.98	576.43	<0.001	2.79
4 years old	523.08	926.42	805.82	680.40	714.17	579.07		
Averages	493.6c	809.3a	693.5a	696.2ab	762.4a	585.9b		

Means followed by different letters in the rows differ among the fitness tests according to Tukey's test (P<0.05).

4. Discussion

The CI indicates the level of comfort an individual feels under certain thermal conditions. In horses, CI values up to 130 indicate thermoregulation will not be impacted by ambient temperature and humidity, values between 131 and 150 indicate the animals will be able to exercise as long as they are provided liquids, and values above 150 indicate the thermoregulation mechanisms will be compromised or will not work, requiring exercise to be interrupted (Jones, 2009). Based on those figures, the CI values recorded in the present study indicate the thermoregulation of the horses was not compromised during the fitness tests.

Similar V_{150} values of three- and four-year-old horses may have resulted from the same training protocol being adopted for both groups. On the farm, the animals in those groups were subjected to exercise sessions from Monday through Saturday in the morning and afternoon both in outdoor areas and on the *vaquejada* track. What differentiated those groups was the greater technical experience of the older horses in the track activities with bovines and the fact that they were already competing professionally.

Since fitness training aims at, among other things, inducing adaptative responses in the cardiovascular system of horses (Souza et al., 2013) and having horses with better fitness reach the frequency of 150 bpm at higher speeds than untrained individuals (Bitschnau et al., 2010), it was expected that V_{150} values would increase across the 12 months of training.

The similar V_{150} values in the second, fifth, and sixth fitness tests may be associated with external, uncontrolled-for factors. Heart rate may have been influenced by variables such as the level of excitation of the animal, pain, or fear. Humid environments and high temperatures also increase cardiac debt (Janzekovic et al., 2010). However, the ambient temperature on the day of the third test was approximately 5 °C higher than that on the day of the fourth test, when horse performance was worse (the mean RH values were very close on both days). That invalidates the hypothesis that environmental factors uncontrolled for in the present study impacted animal performance. Furthermore, the CI on the days of those tests remained within the reference values, which suggests that the lower performance of Group III horses in the fourth fitness test must be related to their fitness and not to environmental conditions.

The training protocol adopted could be considered satisfactory as it allowed all horses to complete the 12 months of training and provided sufficient fitness for them to take part in competitions. However, the V_{150} results suggest the fitness gains were not impressive.

Every horse is born with a maximum heart rate, beyond which an increase in exercise speed will not be accompanied by an increase in heart rate, i.e., it plateaus (Evans, 2004). Since training does not change the maximum heart rate of a horse but the speed at which heart rate is reached, the similarity of estimated HR_{max} values in all six fitness tests was expected. Moreover, that similarity indicates the horses actually reached HR_{max}, thus validating that the field test protocol employed as a progressive fitness test was able to push horses to maximal effort.

The coefficient of variation further confirms that horses reached HR_{max} during exercise. The observed coefficient of variation (6.9) indicates low variability in results in relation to the calculated average, which indicates sufficient data reliability. Low coefficients of variation were also found for V_{150}, V_{200}, and V_{max}.

Although it is understandable that HR_{max} did not vary among the fitness tests as it is related to the biological limit of the species, V_{max} was expected to increase at each test since the physiological goals of training include muscle preparation, with muscular fiber development matching muscle tissue to the target exercise speed and duration (Cavalcanti, 1993). Alternatively, the training adopted may not have increased V_{max} but instead extended the time during which the horse could exercise at V_{max}. In any case, confirming this hypothesis would require the horses to have been subjected to a different fitness test protocol.

Increasing the recovery capacity of horses after physical effort, with a quicker return of clinical parameters to baseline values, is among the goals of training. To demonstrate the influence of fitness on horse heart rate recovery, researchers have assessed sports horses and reported that, 2 min after exercise, professional animals should reach $HR_{50\%}$. In contrast, heart rate in amateur animals remains above $HR_{50\%}$ 2 min after effort (Bitschnau et al., 2010). The present research observed that the horses took increasingly longer to return to $HR_{50\%}$ and HR_{basal} during the fitness tests.

Horse training must include a phase defined as anaerobic for the animal to develop the strength required to run at V_{max}, with high-intensity, short-duration effort and intervals between activities for heart rate recovery (Rose and Hodgson, 1994). In that sense, the training adopted on the farm may have failed to impose the required intensity or the rest time between exercise sessions may have been insufficient to increase the time of heart rate recovery.

Environmental conditions of temperature and humidity also impact the return to HR_{basal} in horses (Evans, 2000; Santos et al., 2002; Younes et al., 2014). In hot environments, the heart rate of athlete horses often remains close to HR_{max} since the circulatory system also plays a thermoregulatory role (Powers and Howley, 2017). The increase in sweating and peripheral vasodilation leads to hypotension, which, in turn, is compensated by tachycardia. However, in the second fitness test, when heart rate recovery was the quickest, environmental conditions were more severe (ambient temperature of 30.15 °C and relative humidity of 58.56%) than in the sixth test, when recovery was the slowest (ambient temperature of 28.68 °C and RH of 27.46%). Therefore, similar to the findings regarding V_{150}, it appears unlikely that environmental conditions were responsible for the gradual loss of efficiency in horse recovery after the fitness tests.

The four-year-old horses in Group III, besides being subjected to the training protocol on the farm, were also competing in professional *vaquejada* trials, especially in March and November, when the most important competitions of the sport take place. A study assessing the stress of horses competing in *vaquejada* found that those animals had physical, biochemical, and hematological alterations (Lopes et al., 2009). The authors associated the alterations with the stress of competitions, which often take place during the hottest times of day, as well as with transportation, the lack of an appropriate training routine, and unsatisfactory environmental conditions in *vaquejada* parks.

Therefore, the fact that horses from Group III reached V_{200} at lower speeds than those in Group II from the fifth fitness test onwards (in October, after ten months of training) (Table 3) may be related to the greater physical strain due to participation in *vaquejada* trials. This suggests that horses already competing should be subjected to a different training routine than three-year-old horses, particularly when they need better fitness, i.e., just before the main competitions. However, the V_{200} results of Groups II and III were similar during the period around March, when important vaquejada competitions are held. Since the animals were still in early training in March, the exercise routine on the farm and participation in competitions may not yet have harmed the fitness of older horses.

The "Potro do Futuro" (Foal of the Future), one of the *vaquejada* competitions regulated by the Associação Brasileira de Criadores de Cavalo Quarto de Milha (ABQM; Brazilian Association of

Quarter Horse Breeders), does not set a minimum age cut-off for participant horses. The competition was created in 2003 for horses up to five years old as of July 1, with no minimum age for animal attendance. The "Derby Brasileiro de Vaquejada" (Brazilian *Vaquejada* Derby) has established minimum and maximum ages for competing horses of three and seven years as of July 1 (ABQM, 2019a; ABQM, 2019b). However, the minimum age to take part in trials should be revised since the performance of four-year-old animals subjected to a routine of training and competitions decreases over time, which will likely impact the duration of their competitive career.

Serum levels of muscle enzymes tend to progressively decrease as the animal adapts to the exercise it is subjected to (Rudolph et al., 1993). Therefore, the enzyme activity of the horses was expected to decrease over the fitness tests.

The progressive increase in serum concentrations of aspartate aminotransferase and creatine kinase in the horses during training may have been related to the gradual increase in workload during sessions. Corroborating that idea, a study that monitored ten Thoroughbred racehorses during the first four months of the competition season reported serum concentrations of 353.5, 414.5, 759, and 1,405.5 U/L for aspartate aminotransferase and of 261, 255, 269, and 470.5 U/L for creatine kinase in December, January, February, and March, respectively (Mack et al., 2014). The authors attributed the increases to the elevation of training exercise intensity.

Nonetheless, the present study found that all aspartate aminotransferase values recorded over the 12 months of monitoring remained within the normality range of 150-500 U/L (Rudolph et al., 1993; Rose and Hodgson, 1994; Mack et al., 2014). Likewise, all creatine kinase values remained within the reference range of 60-330 U/L (Rose and Hodgson, 1994; Latimer et al., 2011).

Another possible cause for the increase in serum concentrations of aspartate aminotransferase and creatine kinase is the intensification of training on the eve of competitions both for beginners in *vaquejada* trials and those already competing professionally. Although the state of Pernambuco holds *vaquejada* trials the whole year, one of the main official competitions of the ABQM takes place in November, precisely when some of the highest concentrations were found for both enzymes.

Short intervals between competitions may also contribute to the increase in serum concentrations of muscle enzymes in horses. A study found higher concentrations of muscle enzymes in horses that competed on two consecutive weekends than in those that competed on only one weekend (Assenza et al., 2016). The authors suggested that the proximity between competitions did not allow enough time for the animals to recover. That hypothesis is supported by the fact that horses commonly attend *vaquejada* trials on consecutive weeks. A study of 1,271 *vaquejada* horses found that 3.3% of the animals took part in trials less than once a month, 16.9% competed once a month, 34.1% competed twice a month, 20.6% competed three times a month, and 25.0% competed every weekend (Torres et al., 2020).

Unlike aspartate aminotransferase and creatine kinase, the variation observed in serum concentrations of lactate dehydrogenase prevented deeper interpretations, indicating that this enzyme may not be very effective in monitoring physiological alterations derived from exercise and training. This idea is supported by a study on Mangalarga Marchador horses subjected to marcha tests for different distances. The researchers reported little variation in lactate dehydrogenase concentration at different times of collection, with mean concentrations of 337.5 U/L at fast, 519.12 U/L during the tests, 435.57 U/L by the end of the tests, and 382.81 U/L 4 h after exercise (Wanderley et al., 2015).

Training horses with the goal of maintaining fitness to attend *vaquejada* trials throughout the year does not seem to be advantageous. A more appropriate strategy would prioritize the participation of horses in the main *vaquejada* competitions and adopt a training protocol that provides a gradual increase in exercise intensity so that the animals reach maximum fitness exactly during the period of the main events.

5. Conclusions

The training protocol assessed is unable to maintain proper fitness of horses for competitions throughout the year.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.M. Santiago. Data curation: J.M. Santiago. Formal analysis: J.M. Santiago, J.E.C. Lucena and L.A.C. Costa. Funding acquisition: J.M. Santiago and J.E.C. Lucena. Investigation: D.A.S. Melo, J.M. Santiago, J.E.C. Lucena, L.A.C. Costa, A.C. Silva and I.V.F. Gonzaga. Methodology: D.A.S. Melo, J.M. Santiago, J.E.C. Lucena, L.A.C. Costa, A.C.S. Ribeiro, A.C. Silva and I.V.F. Gonzaga. Project administration: J.M. Santiago and J.E.C. Lucena. Resources: J.M. Santiago. Supervision: J.M. Santiago and J.E.C. Lucena. Visualization: J.M. Santiago. Writing-original draft: D.A.S. Melo, J.M. Santiago and J.E.C. Lucena. Writing-review & editing: D.A.S. Melo, J.M. Santiago and J.E.C. Lucena.

Acknowledgments

The authors thank the Universidade Federal Rural de Pernambuco (UFRPE) for the financial support (protocol 015/2018), the Programa de Pós-Graduação em Ciência Animal e Pastagens of the UFRPE, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship.

References

Assenza, A.; Marafioti, S.; Congiu, F.; Giannetto, C.; Fazio, F.; Bruschetta, D. and Piccione, G. 2016. Serum muscle-derived enzymes response during show jumping competition in horse. Veterinary World 9:251-255. https://doi.org/10.14202/vetworld.2016.251-255

ABQM - Associação Brasileira de Criadores de Cavalo Quarto de Milha. 2019a. Regulamento geral de concursos e competições da raça Quarto de Milha. Available at: <https://abqm.com.br/app/webroot/documentos/15.021.7-regulamentodecompeticoesdaabqm-janeiro2019.pdf>. Accessed on: Sept. 27, 2020.

ABQM - Associação Brasileira de Criadores de Cavalo Quarto de Milha. 2019b. Circular: 10° Congresso e Derby Brasileiro de Vaquejada. Bezerros. Available at: <https://abqm.com.br/app/webroot/documentos/circularatualizada15.02.pdf>. Accessed on: Sept. 27, 2020.

ABVAQ - Associação Brasileira de Vaquejada. 2016. Regulamento geral da vaquejada. Available at: <https://drive.google.com/file/d/1J38Zil0uYMCqa1IC9umCXf3cwBdUppbF/view>. Accessed on: July 06, 2022.

ABVAQ - Associação Brasileira de Vaquejada. 2017. Regulamentação à utilização do equipamento protetor de cauda para bovinos em vaquejadas. ABVAQ, João Pessoa.

Bitschnau, C.; Wiestner, T.; Trachsel, D. S.; Auer, J. A. and Weishaupt, M. A. 2010. Performance parameters and post exercise heart rate recovery in Warmblood sports horses of different performance levels. Equine Veterinary Journal 42:17-22. https://doi.org/10.1111/j.2042-3306.2010.00260.x

Cavalcanti, P. C. 1993. Equitação global: concurso completo de equitação. Editora Nobel, São Paulo.

Evans, D. L. 2004. Exercise testing in the field. p.19-31. In: Equine sports medicine and surgery. Hinchcliff, K. W.; Kaneps, A. J. and Geor, R. J., eds. Saunders, London.

Evans, D. L. 2000. Training and fitness in athletic horses. Rural Industries Research and Development Corporation, Sydney.

Ferraz, G. C.; Teixeira-Neto, A. R.; Pereira, M. C.; Linardi, R. L.; Lacerda-Neto, J. C. and Queiroz-Neto, A. 2010. Influência do treinamento aeróbio sobre o cortisol e glicose plasmáticos em equinos. Arquivo Brasileiro de Medicina Veterinária e Zootecnia 62:23-29. https://doi.org/10.1590/S0102-09352010000100003

Garcia, T. R.; Rezende, A. S. C.; Santiago, J. M.; Almeida, F. Q.; Fonseca, M. G. and Munõz, A. 2013. Venous hemogasometry and blood electrolytes in Mangalarga Marchador mares submitted to aerobic training. Ciência e Agrotecnologia 37:559-565. https://doi.org/10.1590/S1413-70542013000600009

Graaf-Roelfsema, E.; Keizer, H. A.; van Breda, E.; Wijnberg, I. D. and van der Kolk, J. H. 2007. Hormonal responses to acute exercise, training and overtraining: A review with emphasis on the horse. Veterinary Quarterly 29:82-101. https://doi.org/10.1080/01652176.2007.9695232

Janzekovic, M.; Prisenk, J.; Mursec, B.; Vindis, P.; Stajnko, D. and Cus, F. 2010. The art equipment for measuring the horse's heart rate. Journal of Achievements in Materials and Manufacturing Engineering 41:180-186.

Jones, S. 2009. Horseback riding in the dog days. Animal Science e-News 2:1-7.

Latimer, K. S. (ed.). 2011. Duncan and Prasse's veterinary laboratory medicine: clinical pathology. 5th ed. Wiley-Blackwell, Ames, IA.

Lopes, K. R. F.; Batista, J. S.; Dias, R. V. C. and Soto-Blanco, B. 2009. Influência das competições de vaquejada sobre os parâmetros indicadores de estresse em equinos. Ciência Animal Brasileira 10:538-543.

Mack, S. J.; Kirkby, K.; Malalana, F. and McGowan, C. M. 2014. Elevations in serum muscle enzyme activities in racehorses due to unaccustomed exercise and training. Veterinary Record 174:145. https://doi.org/10.1136/vr.101669

Powers, S. K. and Howley, E. T. 2017. Fisiologia do exercício: teoria e aplicação ao condicionamento e ao desempenho. 9.ed. Editora Manole, São Paulo.

Rezende, A. S. C.; Trigo, P.; Lana, A. M. Q.; Santiago, J. M.; Silva, V. P. and Montijano, F. C. 2012. Yeast as a feed additive for training horses. Ciência e Agrotecnologia 36:354-362. https://doi.org/10.1590/S1413-70542012000300012

Rose, R. J. and Hodgson, D. R. 1994. An overview of performance and sports medicine. p.49-62. In: The athletic horses: principles and practice of equine sports medicine. Hodgson, D. R. and Rose, R. J., eds. Saunders, Philadelphia.

Rudolph, W.; Goic, M.; Matta, S. and Segovia, P. 1993. Variación de las isoenzimas de hidrogenasa láctica posterior a um ejercicio en equinos fina sangre de carrera con diferentes períodos entrenamiento. Archivos de Medicina Veterinaria 25:57-65.

Santos, S. A.; Crispim, S. M. A.; Soares, A. C.; Mauro, R. A.; Pereira, M. and Sereno, J. R. B. 2002. Grazing patterns of Pantaneiro horse. An element of adaptability to the Pantanal region, Brazil. Archivos de Zootecnia 51:129-138.

Souza, B. G.; Veiga, C. C. P.; Oliveira, G. F.; Ferreira, A. M. R. and Almeida, F. Q. 2013. Avaliação de um programa de treinamento para cavalos de concurso completo de equitação: efeitos sobre a frequência cardíaca e a curva de lactato. Revista Brasileira de Medicina Veterinária 35:385-391.

Torres, P. B.; Santiago, J. M.; Lucena, J. E. C.; Lima, G. S.; Melo, D. A. S.; Moraes, A. V. M.; Silva, A. C. and Bezerra, D. G. N. 2020. Characterizing the vaquejada horse herd in the Brazilian state of Pernambuco. Bioscience Journal 36:2133-2141. https://doi.org/10.14393/BJ-v36n6a2020-48845

Wanderley, E. K.; Bem, B. S. C.; Melo, S. K. M.; Gonzales, J. C.; Manso, H. E. C. C. C. and Manso Filho, H. C. 2015. Hematological and biochemical changes in Mangalarga Marchador horse after a four-beat gait challenge in three different distances. Journal of Equine Veterinary Science 35:259-263. https://doi.org/10.1016/j.jevs.2015.01.009

Younes, M.; Robert, C.; Cottin, F. and Barrey, E. 2014. Genetic component of endurance ability. Equine Veterinary Journal 46:15-15. https://doi.org/10.1111/evj.12267_46