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Fitness of Eventing Horses Submitted to Interval Training

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Abstract

The fitness of eventing horses was assessed in field tests through the use of heart and respiratory rates and blood biochemical parameters. The first stage (S1) lasted three days and consisted of the following three tests: the incremental speed test (IST), the incremental jumping test (IJT), and the jumping course (JC). Following the first stage, horses were submitted to a six-week interval training program and were then submitted to another evaluation, the second stage (S2), of the same tests as in the first stage. Lower heart rate (HR) values were observed during the incremental speed test in S1 than in S2. The enzymes creatine kinase (CK), aspartate transferase (AST), and lactate dehydrogenase (LDH) showed significant speed-related reductions in the second stage in comparison with the first stage. HR increased significantly during the incremental jumping test in the upper obstacle heights (55 and 70 cm). Respiratory rate showed a significant decrease in the last two laps and at 10 min after exercise in the second stage, compared to the first stage. Values of LDH were lower in the second stage than in the first stage, and AST values were significantly lower at 55 cm and 10 min after exercise in S2. During the jumping course, RR had better recovery in the second stage than in the first stage, and glucose decreased during the course in both stages. The enzymes CK and AST presented higher levels at 10 min after exercise in the second stage than in the first stage. The field tests can be utilized for evaluating the fitness of eventing horses.

Keywords:

Equine; field test; exercise physiology; incremental test

1. Introduction

It is well established that physical exercise induces transient alterations in the horses' homeostasis that need to be restored by cardiovascular, respiratory, metabolic, and musculoskeletal adaptations [1]. In order to introduce horses to the specific procedures needed for clinical evaluation, conditioning must be gradual [2]. Fitness and its evaluation are important parts of all equestrian disciplines, and the standardization of exercise tests is recognized as being valuable for monitoring the training progress [3]. Accordingly, as per Arfuso *et al.*, understanding the physiological adaptations of both athletes that are necessary for the execution of such difficult exercise is crucial for a correct evaluation of athletic performance, as well as for adapting and improving an adequate training program. Field tests simulate speeds, gaits, surfaces, and environments similar to the diverse components of eventing competitions, specifically preserving their individual biomechanics [4,5]. Information obtained by stress tests must provide values which are repeatable, objective, and valid, allowing for a standardization of the results [6].

Physical training promotes physiological changes in the anaerobic energy system, resulting in increased levels of resting anaerobic substrates and the amount and activity of key enzymes that control the anaerobic phase of glycogen fractionation [7]. Among the parameters considered for the evaluation of athletic performance in horses, cardiovascular parameters are the most studied as they represent good indicators of fitness level and workload effort. Heart rate evaluation is relatively simple, and in the equine athlete, it is essential to be evaluated before, during, and after exercise in order to quantify the intensity of the workload and the horse's fitness, thereby determining the effects of such exercise on its cardiovascular system [4,8–10].

Plasma lactate concentration is the variable which best correlates with the animal's performance, providing additional information on the athlete's actual condition [11]. Lactate production and its association with heart rate and speed represent the main parameters utilized for estimating the efficiency of training protocols in equine athletes, expressed as V4 [12]. The velocity where the blood lactate concentration reaches a certain level is defined as VLa. The quantitative variable determining the speed with which the lactate concentration reaches 4 mmol/l is described as V4 or Vla4. Using this variable offers the investigator a reliable way to determine physical fitness and performance in the equine athlete [11].

Enzymes used as clinical markers of muscle lesions include creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), which leak into the blood circulation due to microtraumas and increases of sarcolemma permeability [13,14].

The current study aims to evaluate the athletic metabolism and fitness of eventing horses, recruited from military use, via an interval training protocol through the use of incremental speed, incremental jumping, and jumping course tests.

2. Materials and Methods

2.1. Location

The experiment was carried out in the 3rd Guard Cavalry Regiment of the Brazilian Army, in Porto Alegre, Rio Grande do Sul, Brazil.

2.2. Animals

Animal Use Ethics Committee approved the work in question. The protocol number is 004/2017 at the Federal University of Pampa, Dom Pedrito, Brazil/RS. Fifteen mixed gender horses of the Brazilian Sport Horse (Brasileiro de Hipismo) and crossbreeds from the Coudelaria do Rincão Stud were used, which were owned by the Brazilian Army. The horses selected for the experiment were destined for Eventing–Level 1, specifications according to the Federation Equestrian International (FEI). All equine training options were at the same performance level. The animals underwent testing and training at the beginning of the competition season.

The average age of the horses was 6.5±1.0 years, and the average weight and height were 508.9±44.9 kg and 1.62±0.48 meters, respectively. Daily nutritional management consisted of supplying 4 kg of alfalfa hay, 4 kg of commercial concentrate (18% protein) offered twice a day, and 2 kg of oat grain once a day. The water was provided ad libitum. The animals were stabled in the 3rd Cavalry Regiment of Guard, and, prior to the experiment, they were submitted to clinical and hematological examination to ensure their health. During the entire period of the experiment, the animals remained healthy without lesions and with daily veterinary monitoring.

2.3. Experimental Design

Parameters were measured at two different times with a temporal interval of six weeks between the first three tests: incremental speed test, incremental jumping test, and jumping course, and the second stage with a repetition of the same three tests. The first test for initial fitness evaluation (stage 1) occurred in the week preceding training. The second evaluation (stage 2) occurred at the end of the sixth week of training. Stage 1 was carried out at the end of May with temperatures varying between 18^{o}C and 28^{o}C, and stage 2 was carried out 6 weeks later, at the end of July, with a temperature variation between 12^{o}C and 22^{o}C.

Animal preparation was conducted prior to all tests, involving the placement and fixation (suture and instant glue) of a flexible intravenous catheter1 14G gauge and a catheter extension with heparinized saline solution (10.000 UI/L). Blood collections were performed before heating the animals (basal) in the intervals between each stage of the tests and 10 minutes after the end of the test. Two tubes were utilized, one containing gel and clot activator and the other sodium fluoride. Immediately after the collections, the blood was centrifuged and separated, plasma and serum, in 2ml tubes for freezing and subsequent analysis. Each rider monitored the speed determined in the tests by the cell phone attached to the forearm, through the KerClockItSport application connected to the Polar Equine H7 heart rate monitor2 fitted to the animals via Bluetooth. The same app stored heart rate, speed, and distance travelled.

2.3.1. Test 1 Protocol: Incremental Speed Test (IST)

Before testing, animals performed a warm-up of 10 min of walking and 10 min of trotting and galloping. The incremental speed test was performed at five different speeds according to **Table 1**. Each lap covered a length of 850 meters in an open circular grass track, with 2 min intervals between laps. Each rider monitored the speed in real time through the use of the KER ClockIt Sport3 app on a smartphone fixed to the rider's forearm, which was also connected to the heart rate frequency meter.

The incremental speed test was used as a test for jumping horses according to the methodology performed by Munk [15]. Blood samples were taken before the animals were warmed (basal) in the intervals between each step of the test and 10 min after exercise.

2.3.2. Test 2 Protocol: Incremental Jumping Test (IJT)

The incremental jumping test was conducted on a 20 x 60 m outdoor sand track, with two rows of five simple vertical obstacles along the lengths spaced at 3 m distances. Three sets of 90 seconds each were performed. The obstacle heights were 40, 55, and 70 cm with elevation between steps. Before commencing the test, horses were warmed up in the same manner as in Test 1, followed by six jumps using the same heights.

1 Disposable intravenous catheter, model 14G, Descarpack, Brazil.
2 Polar Equine H7 Heart Rate Sensor Electrode Base Set, Polar Electro, EUA.
3 KER ClockIt Sport App, Kentucky Equine Research, EUA. Available at ker.com.

Table 1: Incremental Speed Test applied to horses in an open circular track with grass floor.

	850m Circular Track				
Lap	1st	2nd	3rd	4th	5th
Speed	240 m/min	320 m/min	400 m/min	480 m/min	560 m/min

2.3.3. Test 3 Protocol: Jumping Course (JC)

The jumping course was performed as per **[15]** and was modified to suit the initial sport level of the animals. The height of the course was 1 meter, and the total distance of the course was 380 meters, with 11 jumps, with two of them double and performed at a speed of 325 m/min. All animals were warmed up in the same manner as in Test 2.

2.4. Training Protocol

The horses were trained during a period of 6 weeks, with an average duration of 50 min per day. The interval training was performed three days a week based on the protocol used for jumping horses by Munk **[15]** and modified according to Cavalcanti **[16]** to fit the exercises to the eventing category (**Table 2**).

Training 1 consisted of flat track of 850 m, 90 seconds in V4**, 90 seconds of walking, and 4 repetitions. Training 2 included exercises of jumps with varied combinations, with lines (straight or in curve) of 4 to 8 strides of gallop, and "doubles" combination with 1, 2, or 3 strides of gallop. Combinations used "vertical" (max 1.05 m), "oxer," or parallel type obstacles (maximum 1.05 m x 1.20 m). Training 3 comprised a ring 20 x 60 m, 6 obstacles on the longer sides, 90 seconds of jumping, 90 seconds of walking, speed of 325 m/min, and 4 repetitions. Free training normally consisted of flexing exercises, initially with 10 min of step with free reins, performing figures, changing hands, making circles of 15 and 20 meters, serpentine, and taking advantage of the entire length of the arena to change the direction. These same figures are performed at a trot for 25 minutes and at a gallop for 15 minutes.

2.5. Statistical Analysis

The parameters were measured at two different times with a six-week time interval, between the first 3 tests (TIV, TIS, and PS) and a second stage with the repetition of the same 3 tests (TIV, TIS, and PS). The desired comparison was to confront each of the seven parameters in these two situations. The T-test for paired samples was used because the experimental setup allowed for a "before" and "after" of each animal. Besides the T-test for paired samples, a one-way analysis of variance test (ANOVA) was utilized for evaluating the parameters within each training.

3. Results

3.1. Test 1: Incremental Speed Test

In the incremental speed test, an average increase on HR was found in all stages according to speed increase. The HR values in stage 1 were lower than in stage 2. We obtained significant difference in HR at velocities of 240 m/min, 320 m/min, 480 m/min, and 10 min after exercise ($p < 0.05$; $p < 0.01$; $p < 0.05$; $p < 0.05$, respectively). **Figure 1** shows average results of variables between HR, RR, and speed found in both stages of the test. The RR of the animals tested was higher in stage 1 compared to stage 2. The reduction of this variable proved to be statistically significant in all speeds ($p < 0.01$). The level of plasma lactate had no difference between the two stages. The values of glucose during the test were different only on the 240 m/min speed of stage 2 compared to stage 1 ($p < 0.002$). **Table 3** shows the results obtained in the measurement of lactate and glucose. The measurement of muscle enzymes CK, AST, and LDH showed, in almost totality, a significant decrease at the speeds of stage 2 compared to stage 1 ($p < 0.01$). At the speed of 560 m/min and 10 min after exercise, in stage 2, CK blood level showed no significant decrease. At this same speed, there was no significant decrease in the AST value. These data are presented in **Figure 2.**

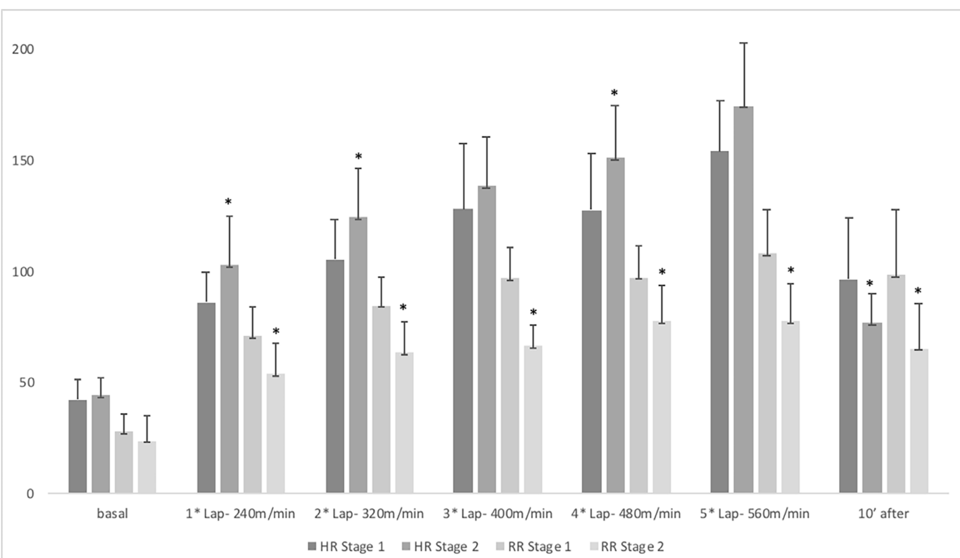

Figure 1: Average Heart Rate (HR) and Respiratory (RR) during IST. * above each bar denotes significant difference ($p < 0.05$) between stages.

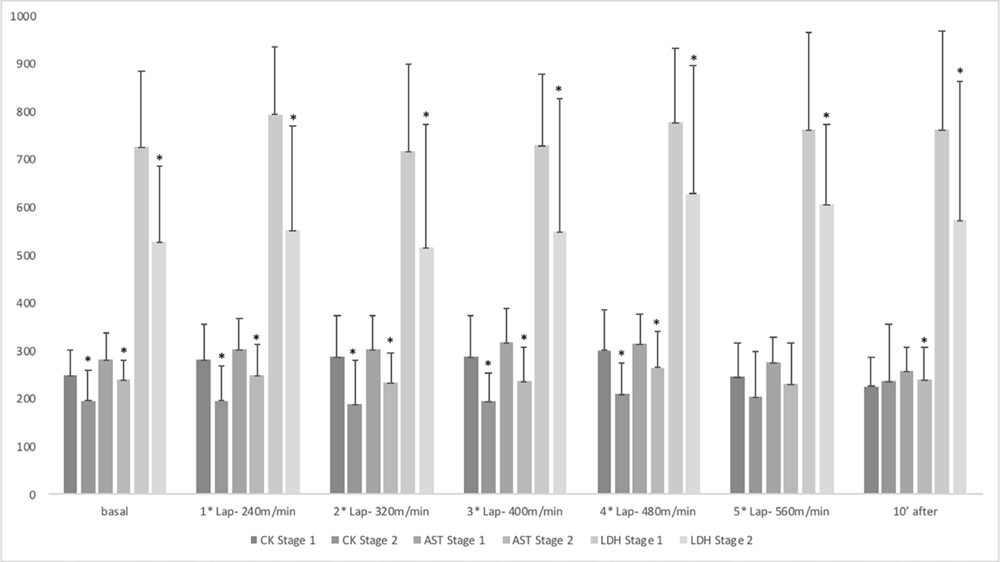

Figure 2: Average values of CK, AST and LDH enzymes during IST. * above each bar denotes significant difference ($p < 0.02$) between stages.

Table 2: Weekly Interval Training Program applied to horses on sand floor, six days a week in the mornings shift.

Weekly Training Program						
Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Training 1	Free	Training 2	Free	Training 3	Jumping Course	Rest

Training 1: Flat track 850m, 90 seconds in V4**, 90 seconds at walk, 4 repetitions.
Training 2: Exercises of jumps with varied combinations, with lines (straight or in curve) of 4 to 8 strides of gallop, and "doubles" combination with 1, 2 or 3 strides of gallop. Combinations using "vertical" (max 1.05m), "oxer" or parallel type obstacles (max 1.05m X 1.20m).
Training 3: Ring 20 x 60m, 6 obstacles on the bigger sides, 90 seconds jumping, 90 seconds walking, speed of 325m/min, 4 repetitions.
Free: Flexion.
* Warm up: 10 min walking, 20 min trotting and galloping. For training 3 plus 8 warm up jumps.
**V4 – corresponds to the speed at which blood lactate reached 4mmol/1 concentration, value determined in the Test 1.

Table 3: Lactate and Glucose values during Test 1 – IST.

	Basal		1st Lap 240m/min		2nd Lap 320m/min		3rd Lap 400m/min		4th Lap 480m/min		5th Lap 560m/min		10 min after exercise	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Lactate	2.1	1.9*	2.9	2.5	2.9	2.8	3.8	3.2	4.4	4.4	7.2	5.7	4.7	3.2
	±0.3	±0.2	±1	±0.7	±0.8	±1.4	±1.2	±1.0	±1.4	±1.7	±2.4	±1.7	±1.6	±1.2
Glucose	63.6	89.5*	74.3	80.6*	79.3	81.8	84.8	83.6	88.4	86.3	95.7	94.2	85.1	96.2
	±26.6	±9.5	±12	±7.5	±12.6	±6.6	±8.1	±6.7	±10.3	±7.7	±10.9	±7.5	±24.5	±4.8

* demonstrates significant difference ($p < 0.04$) between stages.

3.2. Test 2: Incremental Jumping Test (IJT)

The heart rate in test 2 demonstrated a significant increase in heights of 55 and 70 cm ($p < 0.04$ and $p < 0.02$), respectively. Respiratory rate showed a significant decrease in the last two laps, 55 and 70 cm, and 10 min ($p < 0.001$; $p < 0.001$; $p < 0.002$, respectively) after exercise of stage 2 in comparison with stage 1. **Figure 3** presents the average values obtained for HR and RR.

Lactate and glucose values did not statistically differ between the two stages. However, glucose curve showed similar characteristics between the stages, with a decrease in the first lap, and according to the increment of exercise, there was an increase of blood level.

The CK enzyme showed no significant difference between stages. However, LDH indicated a significant decrease ($p < 0.004$) in all values of the measurements of stage 2 in comparison with stage 1. The AST values decreased significantly at 55 cm and 10 min after exercise from stage 2 ($p = 0.009$; $p = 0.04$) as given in **Table 4**.

3.3. Test 3: Jumping Course

The heart rate showed no difference between the two stages. The respiratory rate had a faster recovery 10 min after exercise, with a significant decrease ($p = 0.02$) in stage 2. The average values of HR and RR are given in **Table 5**.

The lactate values did not show the same increase comparing the two stages during the course. According to **Table 6**, a significant difference ($p = 0.001$) between the two stages was observed. The glucose values decreased during the course but did not show significant variations in any of the stages according to **Table 6**.

Muscle enzymes CK and AST had a significant increase ($p = 0.01$ and $p < 0.001$, respectively) in basal values and at 10 min/Ae during stage 2 compared to stage 1. The LDH values only showed significant difference in baseline samples. These data are presented in **Table 7**.

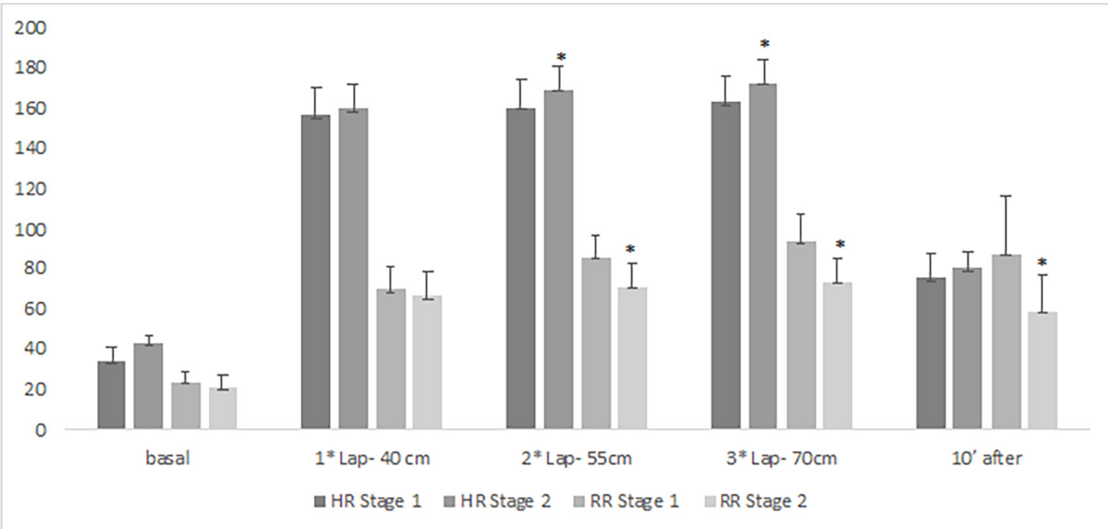

Figure 3: Average values of HR and RR during IJT. * above each bar denotes significant difference ($p < 0.04$) between stages.

Table 4: CK, AST and LDH enzymes values from Test 2 – IJT.

	Basal		1ª Lap 40 cm		2ª Lap 55 cm		3ª Lap 70 cm		10 min after exercise	
	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
CK	166.8 +37.6	148.7* +42.5	164.6 +34.1	234.1 +189	171.6 +43.7	179.5 +225	171.4 +40.6	166.8 +195.4	177.8 +52	250.3 +222
AST	270.7 +39.9	201.3* +64.4	286.8 +51.2	284.9 +78.8	298.6 +47.5	287.2* +73.5	296.3 +50.4	277.7 +78.6	287.8 +46.2	273.4* +50.9
LDH	645.2 +175.23	468.3* +166.4	674.3 +172	541.0* +195.3	688.2 +205.3	546.2* +116.8	730.2 +239.1	508.8* +163.6	683.4 +231.8	524.4* +136.3

Average values of CK, AST and LDH during IJT, * denotes significant difference ($p < 0.04$) between stages.

Table 5: Average HR and RR values during Test 3 - JC.

	Basal		Jumping Course 1m		10 min after exercise	
	S1	S2	S1	S2	S1	S2
HR	39.0 ±10.4	43.0 ±4.6	178.6 ±17.3	171.4 ±10.8	54.0 ±13.6	59.5 ±13.3
RR	27.0 ±4.6	24.0 ±7.6	58.3 ±9.0	56.1 ±13.4	49.6 ±19.6	36.3* ±13.3

Average HR and RR values during JC, * denotes significant difference ($p < 0.02$) between stages.

Table 6: Average Lactate and Glucose Values evaluated in Test 3.

	Basal		Jumping Course 1m		10 min after exercise	
	S1	S2	S1	S2	S1	S2
Lactate	1.9 ±0.4	1.6 ±0.7	3.4 ±1.2	2.3* ±1.3	2.2 ±0.6	1.9 ±0.8
Glucose	86.1 ±4.7	91.9* ±12.3	83.1 ±10.6	81.2 ±16.1	89.1 ±9.8	91.3 ±13.2

Average lactate and glucose values during JC, * denotes significant difference ($p > 0.02$) between stages.

Table 7: CK, AST, and LDH enzymes Values during Test 3 – Jumping Course

	Basal		Jumping Course 1m		10 min after exercise	
	S1	S2	S1	S2	S1	S2
CK	130.4	242.1*	158.4	210	145.5	195.4*
	±36	±213.9	±58.1	±112.1	±41.5	± 57.4
AST	226.9	268.1*	256.3	289.9	244.6	308.0*
	±37.5	±58.2	±40.6	±75.9	±37.8	±49.9
LDH	485.1	596.4*	567.5	630.1	542.2	561.1
	±148.7	±130.5	±123.2	±244.7	±118.9	±137.1

Average CK, AST and LDH enzymes values during JC, asterisk denote significant difference ($p < 0.01$) between stages.

4. Discussion

4.1. Test 1: Incremental Speed Test

The heart rate values contrasted with those found in horses stabled in horse riding schools that showed heart rate of 140 bpm/min at speeds of 270 to 390 m/min **[17]**. The same authors reported that horses in high levels of conditioning when reached the speeds of 420 to 480 m/min had HR values similar to those found during stage 2 of IST, 138 and 151 bpm/min.

The increase of HR in S2 is justified by training because the cardiovascular system was adjusted to the increased physical activity of skeleton muscle and metabolic demand during the second test, readjusting blood flow with the purpose of increasing the availability of oxygen and energetic substrates for ATP synthesis, transporting the products of elimination, carbon dioxide, hydrogen ions, and lactate, and regulating the homeostasis of the heat generated by muscular work **[18]**. In the present study, HR difference was observed in stage 2 at three distinct speeds of 240 m/min, 320 m/min, and 480 m/min, as well as at 10 min after exercise. However, in the study with eventing equine training, CCI* category, during progressive treadmill test, no difference was observed in the HR values between the group of trained and untrained equines, only finding difference in the recovering period, two minutes after exercise **[19]**.

Physical activity can be compared to a ventilatory pump, in which the increase of speed would lead to the almost linear increase in ventilation per minute, corresponding to the tidal volume multiplied by the respiratory rate, where both would increase according to the animal's gait **[20]**. This increase, compatible with gait, was observed in both stages of IST. Studies performed with an incremental speed test reported higher respiratory frequencies at speed of 480m/min in comparison with the two stages of the present study, which were found to be 97 and 98 mov/min, respectively **[21]**. The significant RR decrease at all speeds in S2 compared to S1 is justified by respiratory dynamics during exercise, which must increase to maintain the pressure of oxygen and blood pH at physiological levels, all while minimizing the metabolic cost of the respiratory muscles **[22]**. The V4 of the present study was determined individually during S1 for each horse to perform training 1 at this speed. The average speed corresponding to V4 in S1 was 480 m/min, and similar values were found by Azevedo comparing V4 values between field and treadmill tests in progressive stress test **[23]**, Munk evaluating the effect of 3 different interval trainings on jumping horses determined V4 at 490 m/min **[15]**. Lower values were found by Bitschnau *et al.* **[3]** and Soares **[24]**, where V4 = 426 ± 54 m/min and V4 = 425.83 ± 59.00 m/min, respectively. One of the expected effects of an equine training protocol is the increase in the aerobic potential of the skeleton muscles, resulting in a greater workload capacity supported until the lactate starts to accumulate (anaerobic threshold); that is, the data curve of lactate by speed moves to the right in well-conditioned animals **[12,25,26]**.

As a consequence of the physiological adaptations, there is a reduction of lactate plasma concentration in horses submitted to interval trainings **[27]**, a parameter that was not observed in the present study possibly due to the short time of the protocol to which they were submitted. The glucose concentration during all laps on S1 showed a significant increase. It is believed that this increase is due to the increase in the glycogenolysis rate, due to the need for glucose. Andrews *et al.* demonstrated that the increase of glucose concentrations in equines submitted to eventing competition was higher in comparison with the group of horses submitted to endurance competition **[28]**. The response of equines submitted to high intensity and long duration exercises was the increase of glycemia **[14]**.

Glucose values in IST, lower at the onset of progressive gallop and higher during recovery period, may be due to increased glycogenolysis induced by the tissues' need for glucose and release of catecholamines [29].

It is possible to observe in the present study that despite of CK values between stages 1 and 2, the physiological values described by Pritchard *et al.* [30], which vary between 123 and 358 UI/L for healthy and resting horses, were not exceeded.

In the current study, a significant decrease in CK activity until the fourth lap in S2 was observed in comparison with S1. Training reduces the activity of enzymes resulting from exercise, and resistance training in horses leads to a decrease in CK production [31]. Thoroughbreds horses from 2 to 6 years old presented in the end of a treadmill exercise stress test higher values of CK than basal [31]. This difference was not found in this study.

The activity of the muscle enzyme is usually low in plasma as they are inside the myocyte; however, after exercise or muscle injury, activity increases significantly due to increased cell permeability, cellular necrosis, deficient elimination, or increased synthesis [32].

When evaluating horses of eventing discipline during the progressive stress test, Santiago observed that serum concentrations of AST increased in the progressive gallop, followed by a reduction during the recovery phase, and reported a reduction in serum concentrations of AST in the final phase of the training [33]. Similar data were found in the present study.

LDH concentration in S2 was lower in comparison with S1 with only 6 weeks of training. Divergent data were found in eventing horses submitted to prolonged training where no changes were observed, before and after exercise, in plasma concentrations of LDH [34,35].

4.2. Test 2: Incremental Jumping Test

The principle of sports training is the specificity, where the effect of physical activity exerted is specific for the muscle fibers involved in the exercise [36]. Previous research has indicated that the height of obstacles [37] as well as the height and speed [38] will influence heart rate and blood lactate concentration in horses.

In the current study, an increase of HR was observed when the exercise was started in the two stages of the IJT in the first lap (40 cm). This dynamic was also observed by evaluating jumping horses in a similar incremental test, where an increase in heart rate was observed right after the start of the exercise, attributed to adrenergic discharge [24,29,39].

The HR values found in this study were higher than those reported by Soares [24] where jumping horses with superior and inferior performance and average age of 11 years were compared. A possible justification for these results would be that young or untrained horses presented higher heart rate during exercise compared to older or trained horses [39] and that the maximum heart rate (HR Max) in horses is reduced with age [18]. It should be noted that the average age of the animals in the present study was 6.5 years. Repeating the same evolution of the IST, the IJT also showed a significant difference with a decrease in HR at the second lap, third lap, and 10 min after exercise in S2.

High-intensity exercise is associated with producing energy mainly by anaerobic metabolic pathways (both lactic and alactic) [40]. The higher the intensity of exercise, the higher the amount of lactate and hydrogen ions (H^+) produced. In the study in question, the increase of lactate occurred from the first lap (40 cm), and in the following laps, no a significant increase was observed in its concentration. The average maximum lactate concentration was 3.8 mmol/L before the training period and 2.8 mmol/L after training, as shown in a study conducted by Munk [15]. In the present study, there were no differences in lactatemia in the comparison before and after the 6-week training period, with an average value observed of 3.05 mmol/L between the stages.

Plasma glucose levels also did not show any alteration after the training program conducted by other researches [41]. During S2, the initial reduction of glucose concentration was identified. Ferraz *et al.* [39] reported a decrease in glycemia at the beginning of the incremental test, followed by an increase stimulated by elevated stress intensity, catecholamines, and glucagon action, which act on the liver promoting glycogenolysis.

The CK values found in S2 are in agreement with the physiological values reported by [30] and [41] between 100 and 300 U/L. Training tends to attenuate serum CK and AST alterations over time, although this depends on the intensity and frequency of training. The plasma activity of CK and AST enzymes was also measured in BH crossbred equines submitted to IJT. Baseline values and values after exercise were higher than those found in the present study [24], with the values after exercise being above the physiological reference values.

There is a great variation of the physiological values of LDH. Studies of different equestrian sports have reinforced the importance of establishing reference value for horses of different disciplines and breeds and mainly under tropical climatic conditions [42,43].

Several factors regulate glycolytic pathway activity, including oxygen availability, LDH activity, and the magnitude of the ATP/ADP ratio [44]. LDH is a cytoplasmic enzyme that catalyzes the conversion of pyruvate to lactate at the end of anaerobic glycolysis [45].

A significant decrease in LDH concentration in comparison between the stages was verified in the IJT results. In the same way, in another study evaluating LDH concentration after training, the values presented a significant decrease. According to these authors, the serum LDH values in horses at rest decrease progressively as the animal adapts to training [46]. For example, plasma malate (MDH) and lactate dehydrogenase (LDH) activities change with formation and reflect alterations in oxidative capacity and lipid mobilization [47].

4.3. Test 3: Jumping Course

In jumping competitions, horses are normally in speeds between 300 and 450 m/min with heart rate of 150 bpm/min at the beginning and 190 bpm/min at the end of the course [29]. The cardiac parameters are compatible with those found in the present study where the speed of 325 m/min was determined during the realization of the course. The metabolic demands of these animals are very different from race and resistance breeds, and undoubtedly anaerobic metabolism plays a major role in a jumping course, although the speed and the duration are lower compared to racehorses [48–51].

However, immediately after the exercise and for a few minutes, although tidal volume progressively approaches resting values, respiratory rate remains high [52,53]. Evaluating the recovery rate in the present study, a significant reduction in S2 is observed, suggesting that the acidemia may continue to influence respiratory activity during these periods. Moreover, it is likely to be an evaporative respiratory contribution to dissipating thermal load induced by exercise [54,55], especially due to the difference in temperature and humidity between the stages.

A significant decrease in the plasma lactate concentration was observed immediately after jumping course at S2. In a study conducted during a jumping course with obstacles with equal height and superior to 100 cm, and also a six-week training, the results obtained were 3.4 mmol/L in stages 1 and 3.1 mmol/L in stage 2 [15].

The average lactate values found 10 min after the course in both stages were approximately 2 mmol/L. Although the speed and duration of this type of event are low, the increase in HR associated with increased plasma lactate and the speed in the approach of jumps [29,55] represents an increase in the exercise intensity, with greater explosion and the use of anaerobic metabolism [48,56,57].

A reduction of blood glucose concentration was observed immediately after the two stages of T3. Depending on the intensity, duration, and type of physical exercise, the principal regulatory systems including nervous, cardiovascular, endocrine, and respiratory systems are called upon to work in concert in order to re-establish homeostatic equilibrium [4,40,58].

The comparison between the two stages allowed the standardization of the tests with repeatability, objectivity, and validity, presenting significant differences in the values of the main markers of the physiological dynamics of the exercise. The differences in the values between the stages were sufficiently significant to show the importance of a specific training for equines destined to eventing discipline.

5. Conclusion

The interval training protocol positively affected the physical conditioning of athlete horses destined to eventing discipline, and the field tests were feasible and compatible with the specificity of the modality. The incremental tests presented important results for the sports evaluation of the animals tested through physiological and biochemical parameters.

Authors' Contributions

Juliana – MSC student and performed most of the field tests and also lab tests (all)

Nelson – laboratory tests advisor, co-advisor

Rafael – Army officer in charge of providing the horses

William – help during field tests

Bruna- laboratory tests (lactate)

Ana Carolina – lab tests biochemistry (CK, AST and LDH)

Adriana – MSC advisor, supervisor at field tests

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Data Availability Statement

The data supporting the findings of this study are available within the article.

Conflicts of Interest

All the authors declare no conflicts of interest.

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Ethical Approval

Animal Use Ethics Committee approved the study. The protocol number is 004/2017 at the Federal University of Pampa - Dom Pedrito - Brazil/RS.

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