Metabolic and physiological changes during and after vaquejada exercise in horses

[*Adaptações metabólicas e fisiológicas durante e após o teste de vaquejada em cavalos*]

"Artigo Científico/Scientific Article"

Monica Miranda **Hunka**, Luzilene Araújo **Souza**, Thaís Helena Sougey de **Almeida**, Priscilla Cristina Rolim **Nery**, Helena Emília Cavalcanti da Costa Cordeiro **Manso**, Hélio Cordeiro **Manso Filho***

[1]Núcleo de Pesquisa Equina, Departamento de Zootecnia, Universidade Federal Rural de Pernambuco, Recife-PE, Brasil
*Autor para correspondência/Corresponding author: E-mail: helio.mansofo@ufrpe.br

Abstract
Physiological and metabolic evaluations of equine sports have been conducted to define parameters or biomarkers for performance evaluation, clinical diagnosis and predict fatigue and overtraining. Actually, the *vaquejada* is the most important equine sport in Brazil, and these horses are repeatedly submitted to short-duration physical exertion, during 2 or 3 alternate or consecutive days, requiring adequate conditioning for this equestrian modality. The objective of this study was to test the hypothesis that *pulls* horses and *helper* horses undergo physiological and metabolic changes during *vaquejada* races. Eighteen Quarter Horses were used, including 12 *pull* horses and 6 *helper* horses. Hemograms and the following analyses were performed: glucose, lactate, cholesterol, triglycerides, NEFA, total proteins, albumin, creatine kinase, gama glutamyl transferase and cortisol. A heart rate monitor was used to measure the heart rate, velocity and travel distance of each animal. Statistical analyses were performed using ANOVA and Tukey's tests, with P established at 5%. *Pull* horses had higher heart rate values, but the velocities attained by the two groups of horses were similar. There was an increase in heart rate, glucose, lactate, non-esterified fatty acids and cortisol in both *pull* and *helper* horses after racing, but these levels returned to resting values 30 minutes after the end of exercise. It was concluded that the *vaquejada* field test promoted significant changes in haematological, biochemical biomarkers and in the heart rate of both groups of horses.

Palavras-chave: anaerobic exercise; biomarkers; cortisol, equestrian sports lactate.

Resumo
Avaliações metabólicas e fisiológicas para modalidades equestres tem sido realizadas para definir parâmetros ou biomarcadores para análise de desempenho, diagnósticos clínicos e para predizer fadiga e treinamento exaustivo. Atualmente, a vaquejada é o esporte equestre mais importante no Brasil, estes cavalos são submetidos a esforços físicos repetidos e de curta duração, durante 2 ou 3 dias consecutivos, necessitando de condicionamento adequado para esta modalidade. Objetivou-se testar a hipótese de que cavalos de *puxar* e de *esteira* sofrem desafios metabólicos e fisiológicos diferentes, porque as atividades propostas para os dois são diferentes, durante as corridas de vaquejada. Foram utilizados 18 cavalos, sendo 12 de *puxar* e 6 de *esteira*. Foram realizadas as seguintes análises; hemograma, glicose, lactato, colesterol, triglicerídeos, ácidos graxos não esterificados (AGNE), proteínas totais, albumina, creatina quinase, gama glutamil transferase e cortisol. Foi utilizado um monitor de frequência cardíaca (FC) para medir: FC, velocidade e distância percorrida por cada animal. Foram utilizados os testes de ANOVA e Tukey com significância estabelecido em 5%. Os cavalos de *puxar* apresentaram FC mais elevada porém as velocidades atingidas pelos dois grupos de cavalos foram similares. Houve aumento da FC, glicose, lactato, AGNE e cortisol nos dois grupos de cavalos após as corridas, porém esses níveis retornaram aos valores de repouso 30 minutos após o fim do exercício. Conclui-se que o teste de simulação de vaquejada promove mudanças significativas em biomarcadores hematológicos e bioquímicos, como também na FC dos dois grupos de cavalos.

Keywords: biomarcadores; cortisol; exercício anaeróbio; hemograma; lactato.

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Introduction

Physiological and metabolic evaluations of different equine sports have been conducted to define parameters or biomarkers for performance evaluation, clinical diagnosis and predict the fatigue and overtraining (Tateo et al.,2008). Therefore, it is important to understand the involved physiological and metabolic processes and possible adaptations necessary for different types of exercise around the World, especially in some regional equine sports, which are not well-evaluated and this may causes some misconception about the animal welfare.

In different countries in South America, like Brazil, Colombia and Mexico, the *Vaquejada* or *Coleada* are typical equine sport and they are the major modality or Quarter-Horses (QH), producing a large equine industry in these region (BRASIL, 2016). Actually, the *vaquejada* is the most important equine sport in Brazil, and during this sport two horses and a bovine run on a sand track and simultaneously (Hunka et al., 2017). These horses are repeatedly submitted to short-duration physical exercise, during 2 or 3 alternate or consecutive days (Santiago et al., 2013; Hunka et al., 2017), requiring adequate conditioning for this equestrian modality. Thus, an experiment was developed to test the hypothesis that physiological, haematological and biochemical changes in *pull* and *helper* horses used in this equestrian modality would occur during and after their *vaquejada*, because their activity during the modality are different. The findings may be used to improve nutrition and training practices of this group of athletes, contributing to their welfare.

Material and Methods

These animals were from same horse training center (8°19'39''S, 35°42'20''W), had similar general management program and are submitted to the positive stimulations described in the five domains model (Mellor, 2017**)** were applied to improve horses' welfare. It was used eighteen adult Quarter horses, including 12 *pull* horses (6.4 ± 1.1 years old; ~500 kg) and 6 *helper* horses (7.2 ± 1.5 years old; ~500 kg). Also, the horses received the same training and feeding program with Tifton hay (*Cynodon dactylon*), water and mineralized salt *ad libitum* and 4 kg/day of commercial concentrate (15% crude protein, 12% fat, 3.85 Mcal DE/kg), following the NRH (2007) for this level of exercise. Also, they have individual stall and dry-lots (~2 hectares), which is used regularly for all horses after their training at list for 2 hours per day. All the animals were followed by the veterinarian in order to evaluate claudication or other clinical problem.

The *vaquejada* field test (VFT) was performed as described in the literature and followed the manual for good practice in equine sports and events (Hunka et al., 2017; BRASIL, 2017). Briefly, the VFT consisted of a *pull* and a *helper* horse running on a soft sand track (150 length) with a bovine, one cowboy and his horse gallop and work to pull the bull down (pull horse) and another cowboy and his horse (*helper* horse) help the first cowboy to keep the bull running in line (Santiago et al.,2013). The *pull* horses (n=12) participated in one cycle (3 races). The *helper* horses (n=6) participated in two cycles with a five-minute interval between each cycle (Figure 1). The cycle began when the two horses and the 1st bull left the corral's gate, immediately after the bull ran through the corral gate, and the cycle ended when the 3rd bull was pulled down in the score lane and the *pull* horses had stopped. Each bull runs only on time.

Blood samples were collected by jugular venipuncture, with vacuum tubes with EDTA and without anticoagulant, at five times for *pulling* horses (pre-test (overnight fastening), after the first cycle, and in recovery after 15, 30 and 240min), and at six times for *helper* horses (pre-test (overnight fastening), after the first and the second cycles, and in recovery after 15, 30 and 240 min) (Figure 1). In all cases, all horses recovered inside their stalls, where they had free access to water, mineralized salt and Tifton hay. These blood samples were analysed using an haematology analyser (Sysmex pocH-100iV, Roche Diagnóstica do Brasil, Brazil), and glucose, lactate, triglycerides, non-esterified fatty acids, total proteins, albumin, creatine kinase and gamma-glutamyl transferase were analysed using a biochemical analyser (D250, Doles, Brazil). Cortisol analysis was performed using an ELISA (Bioclin Mindray MR-96A, China) with a commercial Accubind kit (Monibind Inc., Lake Forest, CA, USA).

A heart rate monitor (Polar V800 Equine Science, Polar®, Finland), equipped with a global positioning system, was used to record the speed, the distance, and the heart rate (HR) during the cycles of *pull* and *helper* horses. The recorded data was analysed by Polar Flow Sync applicative (Polar®, Kempele, Finland) determining: maximum (HRmax), medium (HRmed) and minimum heart rate (HRmin), maximum (Smax) and average (Savr) speed in the cycle and total time of the cycle duration. Also this applicative was used to evaluate HR variation by zones: zone 1 (Z-1<150 bpm), zone 2 (Z-2: between 150 and 170 bpm), and zone 3 (Z-3>170 bpm).

All results were analysed by ANOVA and by Tukey's test, with the P-value established at 5%. The results were evaluated with SigmaPlot 13.0 (Systat Software, Inc, USA) and are expressed as the mean ± average standard error.

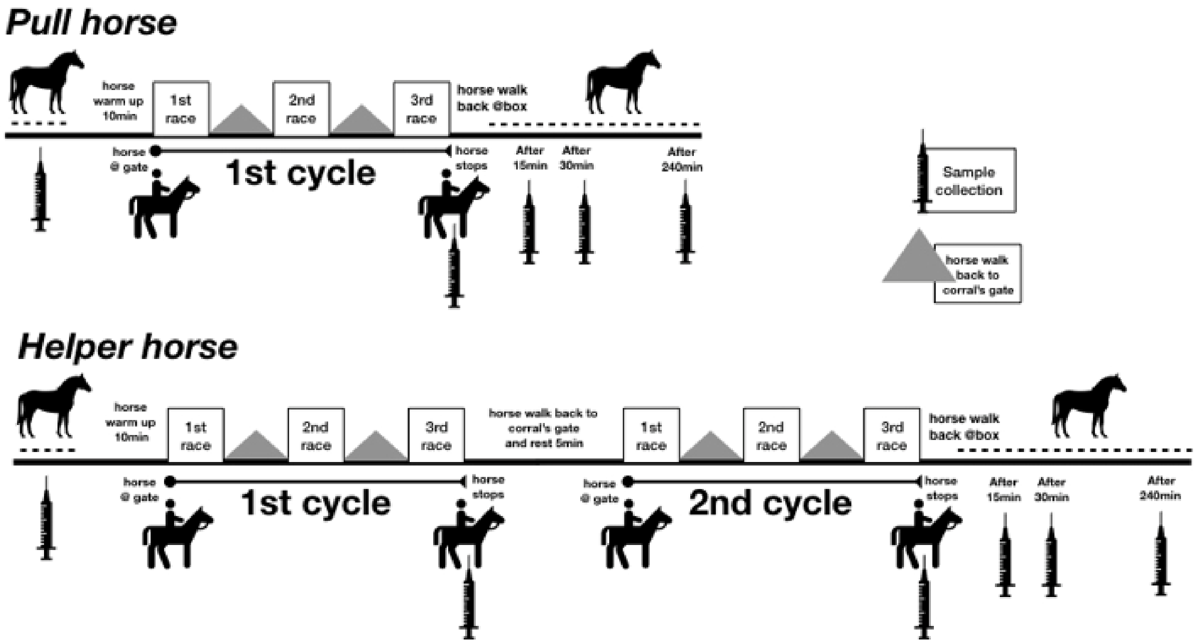

Figure 1. The *Vaquejada* Simulation Test.

Results

The analysis of aspects of the HR showed that *pull* and *helper* horses had different responses during the vaquejada exercise. The *pull* horses had higher HRmed, HRmin and percentage of HR>170 bpm when compared with *helper* horses (P<0.05). In contrast, *helper* horse had large frequency of HR<150bpm in both cycles. The HRmax was higher in *pull* and *helper* (1st cycle) and there is no differences between cycle 1 and 2 in *helper* horses (P>0.05) (Table1.). The frequency of HR between 150 and 170bpm and speed (maximum and medium) between both horses during their races were not different (P>0.05).

Also *pull* horses had significative variation during their exercise, where higher HR ~201bpm but after 15min it dropped to ~60bpm (Table 2). The *helper* horse indeed had variations of their HR but maximum HR was observed at the cycle 1, around 193bpm, and dropped to near 177bpm, but without differences between cycles, and at the 15min time point of the recovery period the HR was around 63bpm. (Table 3). There was no difference between pre-test and 240min HR in both groups of horses (P>0.05).

The biochemical and haematological results observed in *pull* horses (Table 2) and *helper* horses (Table 3) indicate that cortisol, red blood cells, haematocrit, haemoglobin, glucose, lactate and NEFA increased after the cycles in both groups of athletes (P<0.05) and returned to the pre-test values until 30 minutes of recovery. Conversely, the triglyceride decreased after the cycles in *pull* horses (P<0.05) but there were no changes in *helper* horses (P>0.05). Additionally, the triglyceride of the *pull* horses returned to the pre-test values at the end of the evaluation period (P>0.05). Finally, all horses finished their races without any signal of claudication or other clinical problem, and during the next 48 hours they were followed by the veterinarian, which did not report any changes in their health status.

Table 1. Results of heart rate, heart rate frequency, speeds and duration of cycles in horses submitted to the vaquejada field test.

Parameter	*Vaquejada* Horses		
	Pull horse	Helper horse	
		Cycle 1	Cycle 2
HR minimum, bpm	114.82±3.83[a]	80.67±7.62[b]	92.17±13.36[a,b]
HR medium, bpm	158.82±1.49[a]	143.50±4.46[b]	138.33±5.75[b]
HR maximum, bpm	201.50±4.31[a]	193.50±5.98[a,b]	177.83±4.05[b]
%HR <150 bpm	39.09±4.79[b]	61.33±5.28[a]	71.17±4.70[a]
%HR 150-170 bpm	26.27±6.14	19.33±1.89	11.33±2.60
%HR >170 bpm	34.64±4.20[a]	9.75±3.90[b]	17.50±5.13b
Maximum speed, m/s	8.80±0.36	8.41±0.54	9.21±0.28
Average speed, m/s	1.96±0.11	1.78±0.12	1.98±0.10
Duration, minutes	6.01±0.61[a]	7.82±0.95[a]	4.79±0.24[b]

Note: different letters in the same row indicate significance (P<0.05) by Tukey's test; HR: heart rate; n = 12 (pull horses) and n = 6 (helper horses).

Table 2. Results of heart rate, heamatological and biochemical biomarkers in pull horses submitted to the v*aquejada* field test.

Parameter	Periods of samples collections				
	Pre-test	After the cycle	+15 min	+30 min	+240 min
Heart rate, bpm	39.00±1.93[c]	201.50±4.31[a]	59.67±2.44[b]	48.17±2.15[c]	39.67±1.43[c]
Cortisol, nmol/L	12.69±1.38[b]	27.59±7.45[a,b]	34.49±7.73[a]	22.35±3.59[a,b]	13.24±3.59[b]
RBC x10^6/µL	9.21±0.26[b]	12.49±0.31[a]	10.11±0.38[b]	9.24±0.22[b]	8.97±0.26[b]
Hemoglobin g/dL	14.33±0.38[b,c]	19.52±0.41[a]	15.77±0.62[b]	14.34±0.30[b,c]	13.93±0.42[c]
Haematocrit %	42.61±1.12[b,c]	58.88±1.18[a]	47.47±1.82[b]	43.02±0.89[b,c]	41.67±1.23[c]
Glucose, mmol/L	5.15±0.16[d]	7.30±0.39[a,b]	7.79±0.43[a]	7.07±0.36[a,b,c]	5.79±0.29[c,d]
Lactate, mmol/L	0.75±0.10[c]	6.39±0.60[a]	3.43±0.50[b]	1.57±0.33[c]	0.83±0.13[c]
Triglycerides mmol/L	0.33±0.02[a,b]	0.21±0.02[c]	0.27±0.05[a,b,c]	0.29±0.03[a,b,c]	0.35±0.02[a]
NEFA, mmol/L	0.18±0.02[b,c,d]	0.26±0.02[a,b,c]	0.34±0.04[a]	0.28±0.04[a,b]	0.11±0.02[d]
Total Proteins, g/L	80.00±5.80	85.20±4.20	78.90±3.60	71.40±4.60	75.70±2.80
Albumin, g/L	59.40±3.30	65.50±2.30	58.20±1.90	57.20±2.00	57.00±2.40
GGT, U/L	33.05±1.49	36.98±1.58	31.82±1.28	32.44±1.53	31.64±2.16
CK, U/L	229.67±38.97	270.10±37.74	312.04±77.76	283.09±55.83	236.65±23.03

Note: different letters in same row indicate P<0.05 by Tukey's test; NEFA: Non-esterified fatty acids, CK: creatine kinase, GGT: Gamma-glutamyl Transferase.

Table 3. Results of heart rate, heamatological and biochemical biomarkers in helper horses submitted to the v*aquejada*field test.

Parameter	Periods of samples collections					
	Pre-test	**After Cycle 1**	**After Cycle 2**	**+15 min**	**+30 min**	**+240 min**
Heart rate, bpm	47.00±5.65^{c}	193.50±5.98^{a}	177.83±4.05^{a,b}	63.33±4.67^{c}	55.33±5.51^{c}	41.67±4.39^{c}
Cortisol, nmol/L	23.45±6.07^{a,b}	41.94±.48^{a,b}	47.45±6.34^{a,b}	51.04±11.31^{a}	33.66±4.69^{a,b}	18.21±4.41^{b}
RBC, x10^{6}/µL	8.77±0.37^{c}	11.43±0.49^{a}	11.43±0.33^{a,b}	9.24±0.44^{c}	8.81±0.31^{c}	8.84±0.42^{c}
Hemoglobin, g/dL	14.35±0.70^{c}	18.63±0.54^{a}	18.60±0.35^{a,b}	15.15±0.81^{c}	14.40±0.55^{c}	14.10±0.67^{c}
Haematocrit %	41.77±1.91^{c}	54.78±1.86^{a}	54.70±0.17^{a,b}	44.08±2.15^{c}	41.88±1.44^{c}	41.88±1.81^{c}
Glucose, mmol/L	5.32±0.35^{c}	8.38±0.66^{a}	7.24±0.54^{a,b,c}	7.77±0.79^{a,b}	6.52±0.42^{a,b,c}	5.36±0.42^{c}
Lactate, mmol/L	0.93±0.04^{b}	2.72±0.91^{a,b}	3.42±0.74^{a}	1.63±0.59^{a,b}	0.94±0.26^{b}	1.01±0.15^{b}
Triglycerides, mmol/L	0.27±0.03	0.23±0.05	0.22±0.07	0.20±0.04	0.27±0.04	0.31±0.02
NEFA, mmol/L	0.18±0.01b	0.42±0.08^{a}	0.28±0.03^{a,b}	0.28±0.02^{a,b}	0.27±0.05^{a,b}	0.17±0.03^{b}
Total Proteins, g/L	73.80±9.00	80.90±10.20	78.10±9.30	78.40±9.60	75.30±10.60	64.80±6.40
Albumin, g/L	57.40±2.00	64.10±2.50	58.80±4.20	61.00±2.80	63.70±3.70	59.40±2.30
GGT, U/L	26.81±1.45	28.93±1.74	27.46±0.95	25.63±0.97	25.92±1.00	26.02±0.73
CK, U/L	156.02±16.1^{b}	266.17±23.1^{a,b}	270.48±42.7^{a}	216.35±34.1^{a,b}	202.00±15.0^{a,b}	180.82±18.4^{a,b}

Note: different letters in same row indicate P<0.05 by Tukey's test; NEFA: Non-esterified fatty acids, CK: creatine kinase, GGT: Gamma-glutamyl Transferase, n = 6.

Discussion

Little information has been available on the mechanism that *vaquejada* horses, *pull* and *helper*, spent energy and respond to the physiological and metabolic challenges during and after a vaquejada exercise. Here we showed that during this type of exercise, the *pull* horse accumulated more lactate and had lower NEFA concentration when evaluate data from *helper* horses. This last group also showed large increase in cortisol and NEFA concentration. However, during the recovery period the HR and some blood and biochemical biomarkers showed similar variation patterns, with these parameters returning to pre-test concentration values between 15 and 30 minutes during recovery period, showing that both groups of horses were well conditioned to this kind of exercise. However, the degree of variation was specific to each athletic group, suggesting that these animals exert distinct efforts during *vaquejada*, and need specific training and nutrition.

In the present study, significant differences were observed in HRmed and HRmin, with the highest values observed in *pull* horses when compared with *helper* horses during their cycle 1. The *pull* horses make a final sprint (~13 m) to pull down the bovine and, in contrast, the *helper* horse works as a cutting horse type exercise, when the bull leave the gate and during whole race, until stop. This aspect was reflected by a higher %HR>170 bpm in *pull* horses, which was ~3.5x higher than that of the *helper* horses in cycle 1. The *helper* horses had a higher %HR<150 bpm (*Pull* horses ˜39% and *Helper* horses ˜61%), which indicated that they do exercise are more aerobic than *pull* horse exercises. The analysis of HRmax, which was different between the groups of *vaque-*

jada horses, clearly confirms this differential caloric expenditure. This results confirm previous papers that indicates similar differences but with large number of horses (Hunka et al., 2017). Apparently this increase in HR was due to a proportional increase in the load, regardless of speed, like was described in the literature (Piccione et al., 2013).

In the present study, the HR were close to those previously found with the use of a HR monitor with GPS, showing that *pull* horses reached a HRmax of ~210 bpm and *helper* horses ~196 bpm, with a Smax of ~9 m/s (Hunka et al., 2017), which was similar to other QH sports like reining and 3 barrels (Rammerstorfer et al., 1998; Kästner et al., 1999; Souza et al., 2018). In other studies with *vaquejada* horses, the HRmax reached approximately 100 bpm for *pull* and *helper* horses, with Smax similar to those of the present study, but this value was obtained using a stethoscope (Santiago et al., 2013; Azevedo et al., 2014; Souza et al., 2018), which it was not precise to determine HR during intense exercise. The utilization of the heart rate monitor makes this evaluation precise and the real-time data collected may be used to characterize the type of exercise by zone.

Evaluating the glucose and lactate adjust predictably during high-intensity and short-duration exercises, as observed in racehorses (Judson et al., 1983; Desmecht et al., 1996; Godoi et al., 2010), with the accumulation of lactate favoring gluconeogenesis during the recovery period, as observed in *vaquejada* horses. The actual results were similar to other studies (Santiago et al., 2013; Azevedo et al., 2014), but one study with *pull* horses only did not showed large elevation in lactate and glucose after the exercise that was not well described (Souza et al., 2018). In the present experiment, both groups of *vaquejada* horses showed a similar pattern in their glucose and lactate curves, with a high increase in their post-cycle concentrations (lactate: *pull* ~8.5x; *helper* ~6.7x; glucose: *pull* and *helper* - ~1.5x), which were expected because the intensity of the vaquejada exercise and was similar to other QH sport (Ramalho et al., 2012; Sousa et al., 2018). Also, it is important observe that both groups of *vaquejada* horses had glucose concentration elevated after their races, indicating these horses still have energy stores after 3 or 6 races and apparently they were well conditioned for this kind of exercise (Santiago et al.,2013).

The NEFA and triglycerides are a less effective energy source for short duration and high intensity exercise because of the slow rate at which they can be oxidized for energy production by the muscular system (Brandi et al., 2008; Li et al., 2012), however during *Vaquejada* races there are some degree of repetition, especially when the horses went to the finals. Also, it is known that NEFA and triglycerides concentrations are directly proportional to exercise duration (Hambitzer and Bent, 1988). In this current model of evaluation, the two groups of horses showed similar patterns of NEFA changes after VFT, with a significant increase up to 15 minutes after the end of the effort and a return to fasting/resting values after 4 hours. However, these elevation in the NEFA concentration was larger in *helper* horse, which was expected because these group of horse run more times and training with more repetition, favouring the aerobic exercise. The increase in NEFA after exercises were associated with both different degree of lipolysis and with the haemoconcentration in the initial recovery phase, which was stimulated by the repetition of races and elevation of cortisol in both horses. These current results demonstrated that *vaquejada* horses might require fat supplementation to increase their body fat reserves to fully meet caloric demands during race repetitions (Hambitzer and Bent, 1988).

Exercise invariably induces physiological stress and results in increased circulating cortisol and other hormones concentrations, and this process is important to increase the availability of energy substrates through the mobilization of glucose and free fatty acids (Rizza et al., 1982; Djurhuus et al., 2002). It has also been shown that cortisol may be influenced by the presence of bovines in the competition (Tadich et al., 2013), which is an important factor in *vaquejada* races but expected that this influence was not major component of cortisol elevation because these horses do training regularly with bovines. The current results showed that cortisol increased in both *pull* (~2.7x) and *helper* horses (~2.2x) when the resting/fasting concentration were compared to the peak, 15 minutes after the end of the exercise, however during recovery period cortisol concentration retuned to values similar to pre-test.

Red blood cells changes rapidly due to splenic contraction, which promotes a rapid increase in the number of circulating red cells (Piccione et al., 2007), producing a change in plasma volume and an increase the oxygen transport for different tissues. This process was observed in the *pull* and *helper* horses used in the current study, and the haematocrit increased ~1.4x and ~1.3x in *pull* and *helper* horses, respectively, after the races, and returned to values similar to those during fast/rest after 15 minutes of recovery. Also, results obtained here were similar to other western modality exercises that used Quarter Horses and simulate exercise with bovines in farms (Binda et al., 2016; Sousa et al., 2018).

Assessment of enzymes may aid in the detection of subclinical muscle injury and may be used to predict the fatigue in athletic horses (Valberg et al., 1993; Kim et al., 2005). It should be emphasized that the muscle rapidly releases CK after the challenge, but it is expected that there will be a recovery 2 hours after the effort (Muñoz et al., 2002) and its concentration was not great than 400UI (Valberg et al., 1993), which was not observed in current *pull* or *helper* horses. These results were similar to other ones that showed light increase in CK concentration after exercise in health horses (Santiago et al., 2013; Binda et al., 2016; Souza et al., 2018). Horses that do intense exercise and after 4 hours shows CK concentration above 400U/L or more may have significant degree of muscle lesions (Valberg et al., 1993), which compromises their future performance. Also, one important factor during horse training is early detection of the fatigue and very few studies produced association with GGT and fatigue (Kim et al., 2005). We have few studies about association between GGT and fatigue and here we did not observed significant variation in GGT concentration in both group of horses, but horses used were well trained. These biomarkers should be important to access their training status and their well-being but more studies are needed to improve our knowledge about the fatigue and blood enzymes.

Conclusion

The *vaquejada* field test promotes significant changes in haematological and biochemical biomarkers and in the heart rate of horses, thus demonstrating the physiological adaptations that occur in short-duration and high-intensity exercises in these groups of athletic horses. A different pattern identified for the two types of horses' performed efforts during *vaquejada* exercise. It is also observed a rapid recovery of the studied parameters to the pre-test levels, which suggest that the animals were adapted to the type of the exercise.

Conflicts of Interests

The authors declare they have no conflicts of interests with regard to the work presented in this report.

Ethics Committee

The protocol for the experimental procedures was approved by the Ethics Committee on the Use of Animals (UFRPE, CEUA#105/2015).

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References

Azevedo, N.M.S.; Azevedo, M.V.; Lima, P.F.; Manso Filho, H.C. Analysis of tracheal secretion in healthy horses undergoing a vaquejada simulation test. **Open Journal of Veterinary Medicine**, 4: 232-238, 2014.

Binda, M.B.; Teixeira, F.A.; Carvalho, R.S.; Macedo, L.P.; Conti, L.M.C.; Manso Filho, H.C.; Coelho, C.S. Effects of 3-barrel racing exercise on electrocardiographic and on blood parameters of quarter horses. **Journal of Equine Veterinary Science**, 47: 71–76, 2016.

Brandi, R.A.; Furtado, C.E.; Martins, E.N.; Freitas, E.V.V.; Lacerda-Neto, J.C.; Queiroz-Neto, A. Efeito de dietas com adição de óleo e do treinamento sobre a atividades muscular de equinos submetidos à prova de resistência. **Acta Scientiae Animal Science**, 30(3):307-315, 2008.

Desmecht D.; Linden A.; Amory H.; Art P.; Lekeux P. Relationship of plasma lactate production to cortisol release following completion of different types of sporting events in horses. **Veterinary Research Communication**, 20(4):371-379, 1996.

Djurhuus, C.B.; Gravholt, C.H.; Nielsen, S.; Menghel, A.; Christiansen, J.S.; Schmitz, O.E.; Moller, N. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. **American Journal of Physiology Endocrinology Metabolism**, 283: E172-E177, 2002.

Godoi, F.N.; Almeida, F.Q.; Migon, E.X.F.; Almeida, H.F.M.; Monteiro, A.B.F.; Santos, T.M. Performance of eventing horses fed high fat diet. **Revista Brasileira Zootecnia**, 39(2):335-343, 2010.

Hambitzer, R.; Bent, E. Effect of long distance exercise on free fatty acids in blood plasma of Arab horses. **Journal of Veterinary Medicine**, 35:622-625, 1988.

Hunka, M.M.; Lima, L.C.F.L.; Souza, L.A.; Silva, C.J.F.L.; Silva, E.R.R.; Manso, H.E.C.C.C.; Manso Filho, H.C. Heart rate and velocity in *vaquejada* horses during field tests. **Comparative Exercise Physiology**, 13(1):25-30, 2017.

Judson, G.J.; Frauenfelder, H.C.; Mooney, G.J. Training and blood biochemistry. In: Hinchcliff, K.; Geor, R.; Kaneps, A. **Equine exercise physiology**. 1st ed. Philadelphia: Saunders, 1983. p.408-415.

Kästner, S.B.R., Feige, K., Weishaupt, M.A.; Auer, J.A. Heart rate and hematological responses of Quarter Horses to a reining competition. **Journal of Equine Veterinary Science**, 19(2):127-131, 1999.

Kim, J.; Hinchcliff, K.W.; Yamaguchi, M.; Beard, L.A.; Markert, C.D.; Devor, S.T. Exercise training increases oxidative capacity and attenuates exercise-induced ultrastructural damage in skeletal muscle of aged horses. **Journal of Applied Physiology**, 98:334-342, 2005.

Li, G.; Lee, P.; Mori, N.; Yamamoto, I.; Arai, T. Long term intensive exercise training induces to a higher plasma malate/lactate dehydrogenase (M/L) ratio and increased level of lipid mobolizations in horses. **Veterinary Research Communication**, 36:149-155,2012.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. MAPA. **Revisão do estudo do complexo do agronegócio do cavalo.** Disponível em: http://www.agricultura.gov.br/assuntos/camarassetoriaitematicas/documentos/camarassetoriais/equideocultura/anosanteriores/revisaodoestudodocomplexodoagronegociodocavalo> acessed 25 nov. 2018.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. MAPA. **Manual de boas práticas para o bem-estar animal em competições equestres**. Disponível em: <http://www.agricultura.com.br/animal/bem-estar-animal>accessed 07feb2017.

Mellor D.J. Operational Details of the Five Domains Model and Its Key Applications to the Assessment and Management of Animal Welfare. **Animals**, 7(60):1-20, 2017.

Muñoz, A.; Riber, C.; Santisteban, R.; Lucas, R.G.; Castejón, F.M. Effect of training duration and exercise on blood-borne substrates, plasma lactate and enzyme concentrations in Andalusian, Anglo-Arabian and Arabian breeds. **Equine Exercise Journal**, 34:245-251, 2002.

Nutrient Requirements of Horse, **National Research Council**, 6th ed. Washington: The National Academy Press,2007,360p.

Piccione, G.; Giannetto, C.; Fazio, F.; Di Mauro, S.; Caola, G. Haematological response to different workload in jumper horses. **Bulgarian Journal Veterinary Medicine**, 10(4):21-28, 2007.

Piccione, G.; Messina, V.; Bazzano, M.; Gianneto, C.; Fazio, F. Heart rate, net cost of transport, and metabolic power in horse subjected to different physical exercises. **Journal of Equine Veterinary Science**, 33:586-589, 2013.

Ramalho, L.O., Caiado, J.C.C., Souza, V.R.C.; Coelho, C.S. Glicemia e concentrações séricas de insulina, triglicérides e cortisol em equinos da raça quarto de milha e mestiços usados em provas de laço em dupla. **Brazilian Journal of Veterinary Research and Animal Science**, 49: 318-324, 2012.

Rammerstorfer, C., Potter, G.D., Cudd, T.A., Gibbs, P.G., Vamer, D.D.; Householder, D.D. Physiological responses of mature Quarter Horses to reining training when fed conventional and fat supplemented diets. **Journal of Equine Veterinary Science**, 18(3):175-183, 1998.

Rizza, R.A.; Manadrino, L.J.; Gerich, J.E. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action. **Journal Clinical Endocrinology Metabolism**, 54:131-138, 1982.

Santiago, T.A.; Manso, H.E.C.C.C.; Abreu, J.M.G.; Melo, S.K.M.; Manso Filho, H.C. Blood biomarkers of the horse after field *Vaquejada* test. **Comparative Clinical Pathology**, 23 (3):769-774, 2013.

Sousa, R.A.; Silva, G. A.; Rêgo, G.M.S.; Gonçalves Neto, J.R.; Gottardi, F.P.; Machado, L.P. Effect of vaquejada exercise on the physiological and biochemical profiles of sporadic competitors and atletic horses. **Acta Veterinaria Brasilica**, 12: 17-23,2018.

Souza, L.A.; Hunka, M.M.; Nery, P.C.R.; Coelho, C.S.; Manso, H.E.C.C.C.; Manso Filho, H.C. The effect of repeated barrel racing on blood biomarkers and physiological parameters in Quarter Horses. **Comparative Exercise Physiology**, 14 (1): 47-54, 2018.

Tateo, A., Valle, E., Padalino, B., Centoducati, P., Bergero, D. Change in some physiologic variables induced by Italian traditional conditioning in Standarbred yearling. **Journal of Equine Veterinary Science**, 28: 743-750,2008.

Tadich, T.A.; Araya, O.; Solar, F.; Ansoleaga, N.; Nicol, C.J. Description of the responses of some blood Constituents to Rodeo exercise in Chilean Creole horses. **Journal of Equine Veterinary Science**, 174-181,2013.

Valberg, S.; Johnson, L.; Lindholm, A.; Holmgren, N. Muscle histopathology and aspartate aminotransferase, creatine kinase and myoglobin changes with exercise in horses with recurrent exertional rhabdomyolysis. **Equine Veterinary Journal**, 25:11-16, 1993.