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Effect of Marcha Exercise on Serum Electrolytes and Acid–Base Balance in Mangalarga Marchador Horses

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A B S T R A C T

The marcha competition is a functional evaluation, without similar in the world. In this competition, the animals perform marcha at average speed between 9 and 12 km/hr for 50 minutes, covering roughly 6 to 7 km with no rest. The aim of this study was to evaluate and compare the effect of batida gait competition on acid–base balance in Mangalarga Marchador horses during an official marcha competition. The study was conducted on 24 Mangalarga Marchador horses, 12 stallions, and 12 mares (aged 4–6 years and 399 $\pm$ 45 kg of body weight). Venous blood samples were collected before and after competition. Blood samples were analyzed for acid–base balance, as well as biochemical and electrolyte parameters. In stallions, a significant ($P < .05$) postexercise increase in pH, partial pressure of oxygen, bicarbonate concentration, base excess, and glucose was noted. Decrease ($P < .05$) in pCO_2 (partial pressure of carbon dioxide), chloride and ionized calcium were observed after exercise only in stallions. No changes ($P > .05$) in hematocrit, hemoglobin, sodium, and potassium were observed after exercise in all animals. The changes in the acid–base balance and electrolyte profile of the Mangalarga Marchador stallions after marcha competition resulted in hypochloremic metabolic alkalosis. Marcha competition did not cause acid–base disturbance in mares. This study enables an evaluation and comparison of physical effort caused by the marcha exercise on acid–base balance in Mangalarga Marchador horses and provides further evidence of the existence of gender differences in sports horses.

1. Introduction

The Mangalarga Marchador horse is the most important and numerous Brazilian equine breed, and its natural gait is marcha rather than trot [1,2]. There are approximately 350,000 registered Mangalarga Marchador horses in Brazil. Other Brazilians breeds have gaits with similar characteristics, such as Campolina, Mangalarga, and Piquira [1].

The marcha is a comfortable four-beat lateral and diagonal gait with moments of triple support and no suspension [3]. The ideal range of speed of the marcha gaits is 3.0 to 4.0 m/s because this speed allows maximum stability, with significant moments of triple support and a four-beat sequence and the absence of aerial phase [4]. The Mangalarga Marchador horses have at least two natural types of four-beat gaits. The picada gait is a broken pace with little vertical movement of the rider. The batida gait is a broken trot with significant movements of the legs in a diagonal pattern and with a vertical rider's movement [1].

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Mangalarga Marchador horses have been submitted to different sporting disciplines, including endurance races, cavalcades, and marcha competitions [2]. The marcha competition is a functional evaluation of Mangalarga Marchador horses, without similar in the world [5]. The effort is predominantly aerobic and moderate-intensity submaximal, where horses must perform four-gaited exercise at 13 km/hr for approximately 50 minutes with no rest [2,3,5].

The effects of marcha exercise on a number of physiological, hematological, and biochemical parameters in the Mangalarga Marchador horse have been reported [1,6,7]. During the four-beat gait challenges, the red blood cells, hematocrit (HCT), hemoglobin (HGB), white blood cells, and total plasma protein rose and remained high after the 4-hour rest [6]. After a simulated marcha field test, significant increases were observed in heart rate, respiratory rate, HCT, total protein, and glucose (GLU) concentration [1]. It was demonstrated that picada horses expend more energy when compared with batida horses at the same speed [1]. Finally, marcha exercise induced significant hematologic and biochemical changes in serum of Mangalarga Marchador horses, independently of gender [7]. However, in this study, females presented more blood changes after marcha gait [7].

Currently, there is a lack of information about the effect of the marcha competition on acid–base balance in Mangalarga Marchador horses. Therefore, it has been hypothesized that horses in marcha exercise have significant changes in venous blood gas analysis. The aim of this study was to evaluate the effect of marcha exercise on acid–base balance and biochemical parameters of Mangalarga Marchador horses in an official competition and whether this differs in male and female horses.

2. Materials and Methods

2.1. Horses and Competition

All procedures applied in this study were approved by the Ethics Committee for Tests with Animals of Universidade Estadual do Norte Fluminense Darcy Ribeiro (number 2014/250). Evaluations were carried out during a marcha gait in a rodeo park in the city of Campos dos Goytacazes, Rio de Janeiro, Brazil (south latitude 20°48′21″, west longitude 40°38′52″ and altitude 13 m). The mean temperature during the competitions was 26°C, and relative humidity was 72%.

Twenty-four Mangalarga Marchador horses (12 stallions and 12 mares), ranging from 4 to 6 years of age and weighing 399 ± 45 kg, were included in the study. Competitions were held in the afternoons, when animals performed batida gait at average speed between 9 and 12 km/hr for 50 minutes (25 minutes in the clockwise direction and 25 minutes in the counterclockwise direction), covering roughly 6 to 7 km, as defined by the Brazilian Association of Mangalarga Marchador Breeders (ABCCMM).

2.2. Blood Samples

Jugular blood samples were collected 30 minutes before the competition and 05 minutes after the horses finished the competition. Blood was collected in sterile test tubes for serum (5 mL) and anaerobically in 2-mL heparinized syringes for gasometric examinations. Analyses of acid–base balance parameters were tested up to 5 minutes after blood collection, whereas hematological parameters were tested up to 2 hours later. The blood samples for serum were centrifuged at 3,000g for 15 minutes at room temperature (2 hours after collection), and the serum samples were frozen (−20°C) until analysis.

Analysis of parameters determining acid–base balance was performed according to methodology of blood gasometric examinations. In order to determine the acid–base balance, the following parameters were analyzed using portable analyzer (I-Stat Analyzer, Abbot Point of Care Inc, East Windsor, New Jersey, with CG8+ cartridges): pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), concentration of bicarbonates (HCO_3^-), extracellular base excess (BE) and concentrations of sodium, potassium, ionized calcium (iCa^{++}), HCT, HGB, and GLU. Blood pH measurements (pH, pO_2, and pCO_2) were corrected according to the rectal body temperature. The chloride (Cl^-) was done using an ion-selective (Iselab, Brazil).

2.3. Statistical Analysis

All the results were expressed as means and standard error. The data obtained were subjected to analysis of variance using the SAS statistical program. The statistical model was as follows:

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

where Y_{ij} is the dependent variable; μ is the overall mean; α_i is the effect of blood sampling before competition and after competition ($I = 1$ and 2); and ε_{ij} is the random residual error.

The differences among animal (stallions and mares) means were tested for significance using Fisher's test. Effects were considered significant at a probability of $P < .05$.

3. Results

The values of horses' blood gasometric parameters before the competition demonstrated the acid–base balance state (Table 1). Postexercise, the values of pH, pO_2, HCO_3^-, and BE showed a significant increase in stallions ($P <$ .05), whereas the values of pCO_2 decrease significantly ($P <$ .05) in these same animals.

Mean values of blood hematological indices in horses are presented in Table 2. No significant differences ($P > .05$) were observed between before and after exercise levels of HCT and HGB in stallions and mares.

In the case of the examined electrolytes, only Cl and Ca concentrations showed a significant difference after exercise. The values of Cl and Ca decrease in stallions ($P < .05$). The changes in Na values were not confirmed statistically, but a decrease tendency after competition was noted in both stallions and mares. Postexercise, the K concentration increases in stallions and decreases in mares; however, the differences were not statistically significant (Table 2).

Table 1
Parameters of acid–base balance before and after a marcha competition (mean ± standard error).

Parameters	Animals	
	Stallions	Mares
pH		
Before	7.391 ± 0.011[B]	7.418 ± 0.008
After	7.456 ± 0.014[A]	7.482 ± 0.012
pCO_2 (mm Hg)		
Before	43.176 ± 1.843[A]	42.075 ± 1.202
After	40.133 ± 2.046[B]	40.767 ± 1.758
pO_2 (mm Hg)		
Before	32.833 ± 3.103[B]	33.182 ± 1.874
After	35.800 ± 3.469[A]	36.917 ± 2.767
HCO_3^- (mmol L^{-1})		
Before	26.200 ± 0.968[B]	27.400 ± 0.813
After	32.317 ± 1.703[A]	27.567 ± 0.991
BE (mmol L^{-1})		
Before	2.333 ± 0.558[B]	3.917 ± 0.434
After	5.333 ± 1.333[A]	4.250 ± 0.880

Abbreviations: BE, base excess; HCO_3^-, bicarbonate; *p*CO2, partial pressure of CO2; *p*O2, partial pressure of O2.
Means followed by different capital letters in columns differ significantly between times (Fisher test at $P < .05$); means followed by different lowercase letters in rows differ significantly between groups (Fisher test at $P < .05$).

Blood GLU concentration increased after the marcha competition ($P < .05$) in stallions.

4. Discussion

The significant increase of pH in stallions after the marcha exercise was probably caused by increase pCO_2 elimination due to hyperventilation. HCO_3^- and BE could be connected with the excessive Cl^- loss through perspiration during the marcha competition. Chloride is the most abundant anion in extracellular fluid, and Cl^- and HCO_3^- are the only important resorbable anions in renal tubular fluid. Loss of Cl^- ions from serum results in an increased secretion of HCO_3^- ions, in order to maintain serum neutrality [8–11]. However, the extent of the changes in acid–base balance and electrolyte concentration will depend to a great degree on the environmental temperature and humidity during the exercise [8,10,11]. Similar results have been reported after endurance rides [9,12].

In the present study, the hyperventilation observed immediately after the exercise was similar to the reported in horses after high-speed treadmill [8,13], cross-country races [14], and in combined driving disciplines, including dressage, marathon, and obstacle driving [15]. Immediately after the end of exercise, physical and chemical stimuli, such as excitation and caloric stress, increase the respiratory frequency and thus diminish the level of pCO_2 and increase the pO_2 [16]. The hyperventilation is expected due to the higher oxygen demand by the active muscle tissue [8,17]. However, when equines are exercised at maximal speed, the locomotor-respiratory coupling in quadrupeds represents a mechanical limitation to the increase in alveolar ventilation, thus resulting in an increase in pCO_2 [18].

Marelli et al [19] reported that supplementation with soy oil reduced the occurrence of compensatory hyperventilation, by benefiting the thermoregulation of Mangalarga Marchador mares after marcha competitions. According to the authors, adequate training of the animals also reduces the occurrence of hyperventilation. Training promotes structural adaptations that improve the oxygenation of blood in the lungs, the efficiency of its transmission to the tissues, and the ability to transport O_2 [19].

In this study, regardless of the sex, the marcha competition did not alter significantly the HCT and HGB concentrations. These two variables respond to the same sympathetic stimulus, through the adrenergic mechanism, which produces contraction of the spleen musculature, releasing a larger number of red blood cells, and hence HGB, in the bloodstream [20,21]. The degree of splenocontraction depends on the oxygen required by the active muscles [22]. Hematocrit and HGB are influenced both by the intensity of exercise and the individual excitation caused by the competitive environment [23] and are considered a good index of cardiovascular and metabolic adjustments to physical effort [24].

The mild decrease observed in Ca concentration in the blood samples from the stallions could have been caused by their loss with the sweat. The reduction in the blood concentration of Ca is consistent with the hypertonic nature of horse sweat [12,25]. Besides the intensity of the exercise, the concentration of these ions in the sweat depends on the environmental temperature and relative air humidity [8]. Another explanation for the reduction of the Ca values is their intracellular movement, which is necessary for their use in muscle function [25]. Calcium bonds with troponin molecules for actin-myosin activation, permitting efficient muscle contraction during exercise [26]. The reduction of Ca after exercise can also be related to complexation of

Table 2
Concentration of hematological, biochemical, and electrolytes parameters before and after a marcha competition (mean ± standard error).

Parameters	Animals	
	Stallions	Mares
HCT (%)		
Before	40.000 ± 2.503	42.583 ± 1.328
After	40.667 ± 3.997	40.000 ± 0.853
HGB (g/dL)		
Before	13.617 ± 0.848	14.392 ± 0.437
After	13.850 ± 1.353	13.640 ± 0.297
GLU (mmol L^{-1})		
Before	87.333 ± 11.563[B]	105.000 ± 6.695
After	119.167 ± 18.865[A]	144.000 ± 10.681
Na (mmol L^{-1})		
Before	138.167 ± 0.477	137.333 ± 0.414
After	136.667 ± 1.820	136.583 ± 0.645
K (mmol L^{-1})		
Before	3.217 ± 0.224	4.042 ± 0.111
After	4.000 ± 1.008	3.533 ± 0.129
Ca (mmol L^{-1})		
Before	1.120 ± 0.200[A]	1.449 ± 0.064
After	0.986 ± 0.160[B]	1.228 ± 0.067
Cl (mmol L^{-1})		
Before	95.07 ± 1.18[A]	92.22 ± 1.64
After	87.06 ± 1.50[B]	89.38 ± 0.67

Abbreviations: GLU, glucose; HCT, hematocrit; HGB, hemoglobin.
Means followed by different capital letters in columns differ significantly between times (Fisher test at $P < .05$); means followed by different lowercase letters in rows differ significantly between groups (Fisher test at $P < .05$).

these ions with organic and inorganic anions and linkage with albumin [14]. Decrease in Ca has been reported during polo competition by Zobba [25].

No differences were noted in Na and K concentration in blood of Mangalarga Marchador horses after marcha competition (Table 2). Similar results were reported in horses submitted to maximum intensity exercises [14] as well as submaximal tests [12,15,26]. Suitable preparation of animals in relation to the physical effort to be exerted results in ideal ionic balance, as often found in finalist animals in competitions [26]. High electrolyte losses are usually noted in poorly trained horses, which get tired easily [15].

The increase in blood GLU concentration observed in this study was similar to that detected in other investigations during light, moderate, and intense exercise, including after a standard field gaited test in Mangalarga Marchador horses [27]. However, other researchers observed that GLU did not change in the same Brazilian-gaited horses after marcha simulation tests [28]. However, in that study, the average test time was only 18.88 minutes [28], not 50 minutes, as defined by the ABCCMM and performed in this study.

Glucose concentration in horse blood during exercise is subject to changes depending on intensity and duration of the effort [27]. Blood GLU concentration increased progressively during exercise, peaking at the end [29]. At the start of exercise, GLU and glycogen in the muscles are the main sources of energy for muscular contraction, so their levels are depleted. Afterward, the blood GLU concentration tends to increase due to the higher rate of gluconeogenesis [30]. The athletic horses that exhibited good performance were able to utilize GLU more effectively than those that showed poor performance [31].

The observation of different hematological and biochemical results in function of sex after the same physical effort, like the findings in this study, has been described by other authors [7,20,32]. These findings reveal the need to take gender into consideration in experiments with sports horses [20]. Data obtained from populations of mixed sexes should be evaluated with caution, since they may not represent the individual reality of males and females.

5. Conclusions

In conclusion, this study demonstrated that marcha competition results in acid–base and electrolytic imbalance only in Mangalarga Marchador stallions. Therefore, the gender differences in studies of sports horses should be taken into consideration.

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P.A.D.F., L.P.M., and M.A.D.M. participated in the design of the study, supervised the experiments, revised and submitted the final manuscript. L.P.M., L.M.M., L.M.F.R., I.S.V., and R.M.S.P. participated in the study from the early phase to the end of data collection. M.A.D.M., L.M.F.R., and L.P.M. carried out the acid–base and biochemistry studies. P.A.D.F. and M.A.D.M. performed statistical analysis and prepared the final draft of the manuscript. All authors were involved in discussing and interpreting the data described in this study, critically read the manuscript, and approved its final version.

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