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Original Research

Hematological and Biochemical Changes in Mangalarga Marchador Horses After a Four-Beat Gait Challenge in Three Different Distances

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ABSTRACT

The "marcha" is a comfortable equestrian four-beat gait characterized as a submaximal exercise. Four healthy Mangalarga Marchador horses of 6–7 years of age were assessed under field conditions covering distances of 12.7, 19.3, and 38.8 km. Blood samples were collected before, during, and at the end of the exercise, as well as after 4 hours of rest, to measure the hematological, biochemical, and enzymatic biomarkers' variations. The following measurements were recorded: red blood cells (RBCs); hematocrit (HT); hemoglobin (HB); red cell dispersion width (RDW); white blood cells (WBCs); absolute lymphocytes count (LYM); relative lymphocytes count (LYMr); total plasmatic protein (PPT); creatine kinase (CK); uric acid (UA); dehydrogenase lactic (LDH); and aspartate aminotransferase (AST). Significant alterations related to the different distances covered were observed for lymphocytes (LYM and LYMr), which were lower ($P < .05$) when horses were exercised for 38.8 km. The physical exercise caused significant alterations ($P < .05$) in all biomarkers investigated, except for AST. During the four-beat gait challenges, the RBCs, HT, HB, RDW, WBCs, and PPT rose and remained high after the 4-hour rest. At the same time, the UA, LDH, and CK were greater during the exercise but returned to basal values after the 4-hour rest. The four-beat gait challenges promoted significant variations on the hematological and biochemical markers in Mangalarga Marchador horses, as well as a decrease in the horses' lymphocyte number after 38.8 km of exercise.

1. Introduction

The comfortable lateral and diagonal gait, with moments of triple support, is known in Brazil as "marcha" and is present in important horse breeds of this country [1]. The marcha activity is characterized as an equestrian submaximal exercise [2,3]. Several studies have demonstrated that this particular exercise can alter biomarkers and physiological parameters in horses [1–4].

It is widely accepted that moderate physical activity promotes good health in both humans and animals. On the other hand, severe exercise can result in health problems such as immune suppression, for example the study by Folson et al [5]. Previous research studying the effects of physical exercise on homocysteinemia in horses, as well as the effects of the nonprotein amino acid on lymphocyte proliferation [6], concluded that physical exercise reduces immune responses and causes an imbalance in redox

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status. Additionally, intense physical exercise can induce the degranulation of neutrophils and, consequently, an increase in the plasma concentration of enzymes with pro-oxidative and proinflammatory properties related to muscle damage [7].

Exercise tests represent a tool to assess physical condition of horses through the analysis of the physiological changes which occur in response to the exercise performed [8,9]. Depending on its intensity and duration, the exercise has effects on physiological parameters [10], and even different types of gaits performed by the same breed can affect the animal's physiological responses to exercise in a different manner [1].

Currently, there is a lack of information related to physiological responses of horses to the four-beat gait challenge in different workloads. Therefore, the aim of the present study was to measure variations in hematological, biochemical, and enzymatic biomarkers of physical exercise among horses in response to four-beat gait challenges covering three different distances.

2. Materials and Methods

2.1. Animals

Four healthy Mangalarga Marchador gelding horses, with ages from 6 to 7 years, under the same management routine, were used in the present study. The animals were fed *Pennisetum purpureum* grass (approximately 15 kg/d), and a pelleted concentrated diet (approximately 5.0 kg/d; digestible energy, 2.8 Mcal; crude protein, 12%; ethereal extract, 2.5%). The pellets provided 60% of the energy requirements for horses performing exercises of low and medium intensity [11]. All procedures used in the present study were approved by the Institutional Animal Care and Use Committee of the Universidade Federal Rural de Pernambuco under protocol number 026/2013.

2.2. Exercise Tests

The exercise was performed under field conditions, in a typical dirt farm road (flat unpaved road), with horses exercising at a four-beat (marcha) gait during more than 90% of the test, with a small period of walking pace at the beginning and/or the end of the test. The tests took place in the morning, between 7 AM and 10 AM, with the horses covering three different distances: 12.7, 19.3, and 38.8 km. The horses performed at all distance tests, with 3 days of rest between each test. The physical activity ceased for 1 hour at the midpoint of each distance, and water was provided to all horses during this interval. In addition, to determine both the velocity and the distance covered accurately, "Global Positioning System" monitored the horses.

2.3. Region and Weather Conditions

The four-beat gait tests were performed at the Northeastern of Brazil (latitude:7.9742° south; longitude: 34.9976° west; 109 m above sea level), and all tests occurred under temperatures ranging from 28°C to 32°C, and humidity between 60% and 80%, during the summer.

2.4. Blood Sampling

Blood samples were collected from the jugular vein, and the material was placed into two tubes, one without anticoagulant and the other containing heparin. Samples were collected at four different time points: at rest, with horses fasted in the stalls (phase 1); at the middle of the exercise length (phase 2); at the end of the exercise (phase 3), and after a 4-hour rest (more than 4 hours of recovery—phase 4).

Red blood cells (RBCs), hematocrit (HT), hemoglobin (HB), red cell dispersion width (RDW), white blood cells (WBCs), total count of lymphocytes (LYM), and percentage of lymphocytes (LYM%) were assessed using an automatic cellular counter (Model pocH-100iv; Sysmex Corp). Tubes with heparin were centrifuged (3,000 rpm, 5 minutes) for plasma collection. The total plasmatic protein (TPP) was analyzed using manual refractometry. The tubes without anticoagulant were centrifuged (3,000 rpm, 5 minutes) for serum collection, frozen at −20°C, and then analyzed using semiautomatic equipment (Doles D 250, DOLES) to determine the concentrations of creatine kinase (CK), uric acid (UA), dehydrogenase lactic (LDH), and aspartate aminotransferase (AST).

2.5. Statistical Model and Analyses

All results were analyzed by two-way analysis of variance (ANOVA), with one factor being the distance covered and the other factor being the phase of exercise. Tukey test was performed for means' comparison with the SigmaStat Software 3.0. When significant statistical differences among treatments were not observed, the one-way ANOVA was performed. Results are expressed as means ± last square of standard error and were considered significant at $P < .05$.

3. Results

The horses performed at an average speed of 11.4 km/hr (approximately 3.2 m/s) during the three different distance tests. At this speed, four-beat gaited horses perform the typical marcha gait [1]. During the experiment, no injuries or diseases were observed in the horses studied.

Table 1 displays the mean values observed in the present study for lymphocytes during the different exercise phases, the different distances, and the interactions among phases and distances. Table 2 presents the mean values observed for the hematological and serum biomarkers during the different phases of the exercise tests.

Lymphocyte (LYM and LYM%) was the only marker that changed ($P < .05$) as a result of the different distances performed by the horses in the present study. The exercise phases promoted significant variations ($P < .05$) in all biomarkers measured, with the exception of AST.

White blood cells rose during the exercise (phases 2 and 3), but the observed increase was not significant ($P > .05$). At phase 4 (4 hours of recovery phase 4 h