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Original Research

Gender Differences–Induced Changes in Serum Hematologic and Biochemical Variables in Mangalarga Marchador Horses After a Marcha Gait Competition

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ABSTRACT

The aim of this study was to investigate whether marcha exercise results in changes in serum hematologic and biochemical variables and whether this condition differs in male and female horses. Thirty-five Mangalarga Marchador horses, 18 males and 17 females were included in the study. Blood samples were obtained before and immediately after a marcha competition. Samples were used for measurement of hematocrit (HCT), hemoglobin concentration (HGB), red blood cell (RBC), total and differential white blood cell (WBC), total protein (TP), albumin (Alb), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), creatine kinase (CK), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine (Crea), and urea (BUN) levels. Data were submitted to analysis of variance using the SAS statistical program, and means were compared by the Tukey test ($P < .05$). In both males and females, RBC, HCT, AST, and GGT activity increased in response to exercise, but there were no significant differences between genders. In females, significant increases in TP, BUN, Crea, CK, and LDH levels were detected after the marcha gait, but no such changes were detected in males. Total WBC concentrations increased in response to the exercise in both, males and females; however, there were no differences between genders. Plasma HGB, Alb, and ALP levels after marcha gait did not differ in both genders. Marcha exercise–induced significant hematologic and biochemical changes in serum of Mangalarga Marchador horses, independently of gender. However, females presented more blood changes after marcha gait. Gender differences should be taken into consideration in experiments with athletic horses.

1. Introduction

With its distinctive and comfortable lateral and diagonal gait, the Mangalarga Marchador horse is one of the most important breeds in Brazil. This breed is used for a specific functional trial called the marcha competition. Characterized by long duration and high metabolic demand, marcha competition is carried out in circles, at constant speed without interruption during the events [1].

It has been shown that physical exercise induces physiological responses to meet increasing metabolic requirements during activities [2]. So, understanding these responses and identifying the behavior of hematological parameters during physical exertion are essential elements in the evaluation of competition horses [3]. In an attempt to evaluate performance and evaluate fitness, a considerable

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number of studies have been carried out to investigate the physiological response of horses to different exercise types [4].

Arabian breed horses that performed the Standard Incremental Exercise Test exhibited high serum levels of the enzymes aspartate aminotransferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH) [5]. Similar results were obtained for the Crioulo breed during adaptation to exercise [6]. Likewise, potassium serum levels increased in Quarter horses after team roping [7], and important physical, biochemical, and hematologic changes were observed in horses after cattle roundups in rodeo competitions [8]. Such findings have been associated with exercise-induced responses in addition to the lack of proper training routines and the unfavorable conditions sometimes observed in rodeo events. Also, Santiago et al. [9] observed increased lactate metabolism in horses after eventing competitions, and Miranda et al. [10] reported high levels of mean hemoglobin, erythrocytes, and leukocytes in horses after team penning.

Studies with Mangalarga Marchador horses after marcha gait have revealed that exercise alters levels of lactate [11] and of insulin and cortisol [12]. However, no research has been carried out under actual competition conditions to investigate how these parameters behave. In this scenario and considering the increasing popularity of marcha gait, the aim of this study was to investigate whether marcha exercise results in changes in serum hematologic and biochemical variables and whether this condition differs in male and female Mangalarga Marchador horses.

2. Materials and Methods

2.1. Horses and Sompetition

This study was approved by the ethics committee on animal experiments of Universidade Estadual do Norte Fluminense Darcy Ribeiro (CEUA/UENF), under protocol 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). Mean temperature was 24°C, and relative humidity was 80%.

Thirty-five Mangalarga Marchador horses, 18 males and 17 females, ranging from 4 to 6 years of age and weighing 399 ± 45 kg were included in the study. Competitions were held in the evenings, when animals performed marcha competition at mean speed between 9 and 12 km/h for 45 minutes on average (20 minutes in the clockwise direction and 20 minutes in the counterclockwise direction), totaling 45 minutes on average (i.e., covering roughly 6–7 km), as defined by the Brazilian Association of Mangalarga Marchador Breeders.

2.2. Blood Samples

Blood samples were obtained by jugular venipuncture before (time T0) and immediately after a marcha competition (time T1). Samples were aspirated into 10-mL syringes and immediately transferred into EDTA tubes for hematological tests. The blood samples for the biochemical assays were transferred into tubes with no anticoagulant. The serum obtained was transferred to Eppendorf tubes and kept at −20°C until biochemical analysis.

Routine hematological parameters: hematocrit (HCT), HGB, red blood cells (RBC), and total white blood cells (WBC) were counted with an automated hematology analyzer (MS4, Auto Hematology Analyzer). Leukocyte differential count was carried out by optical microscopy based on stained blood smears (May-Günwald-Giemsa).

Serum samples were used for the measurements of total protein (TP), albumin (Alb), aminotransferase AST, gamma glutamyltransferase (GGT), CK, alkaline phosphatase (ALP), LDH, creatinine (Crea), and urea (BUN) levels. The serum variables were measured using an automatic clinical chemistry analyzer (E-225-D, Labquest, CELM).

Plasma hemoglobin, TP, Alb, AST, GGT, CK, ALP, LDH, Crea, and BUN levels were analyzed after correction to avoid the influence of hemoconcentration. The values obtained after exercise were recalculated taking into account the changes in total protein concentration according to the following formula:

$$H_{corr} = H \times TP_1/TP_2,$$

H_{corr}—corrected plasma hemoglobin, TP, Alb, AST, GGT, CK, ALP, LDH, Crea or BUN concentration, H—plasma hemoglobin, TP, Alb, AST, GGT, CK, ALP, LDH, Crea, or BUN concentration determined after exercise, TP_1—total protein level before exercise, TP_2—total protein after exercise [13].

2.3. Statistical Analysis

Data were submitted to analysis of variance using the SAS statistical program, and means were compared by the Tukey test ($P < .05$).

3. Results

In both males and females, RBC, HCT, AST, and GGT activity increased in response to exercise, but there were no significant differences between the genders. In females, significant increases in total protein, BUN, creatinine, CK, and LDH levels were detected after the marcha gait (T1), but no such changes were detected in males.

Total WBC concentrations increased in response to the exercise in both males and females, but there were no differences between the genders (Table 1). Plasma hemoglobin, albumin, and ALP levels after marcha gait did not differ in both genders.

4. Discussion

The changes observed in hematological parameters were due to the decrease in plasma volume caused by respiration, redistribution of vascular volume, loss of fluids through sweating, and splenic contraction [4]. In horses, splenic contraction increases erythrocyte levels by up to 40%, stimulating blood oxygenation competence [14]. Contraction of splenic muscles takes place as a response to sympathetic stimulus prompted by exercise