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HEMATOLOGICAL PARAMETERS AND BLOOD BIOCHEMISTRY IN PLEASURE HORSES IN TROPICAL REGION

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ABSTRACT

The objective of this study was to determine haematological variables (red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and coefficient of variation of the distribution curve of red blood cells) and blood biochemistry (total plasma proteins, fibrinogen, urea, creatinine, uric acid, total cholesterol and triglycerides) of pleasure horses. Therefore, we evaluated 46 *Mangalarga Marchador* horses, 32 males and 14 females at different ages (6.83±0.65 years). The animals were kept stabled at different pleasure horses training centers, which presented similar nutritional management and conditioning regimen. We evaluated the sex group (male and female) and age groups (younger than five years, between five and ten years, over ten years). The results indicated differences in the mean corpuscular hemoglobin concentration related to sexual group (P<0,05), and in the variation of total serum protein concentration (P<0,05) related to the age groups. The results suggested that there are important differences in biochemical and hematological parameters in riding horses toward sex and age group, except for mean corpuscular hemoglobin concentration and total plasma proteins concentration, which do not compromise physical performance of these animals.

KEYWORDS: biochemistry; equine**;** erythrogram; sport.

ÍNDICES HEMATIMÉTRICOS E BIOQUÍMICA SANGUÍNEA NO CAVALO DE CAVALGADA EM CONDIÇÕES TROPICAIS

RESUMO

Objetivou-se por meio deste ensaio determinar as variáveis hematológicas (contagem de glóbulos vermelho, hemoglobina, hematócrito, volume corpúscular médio, hemoglobina corpuscular média, concentração hemoglobínica corpuscular média e coeficiente de variação da curva de distribuição das hemácias) e a bioquímica (proteínas plasmáticas totais, fibrinogênio, ureia, creatinina, ácido úrico, colesterol total e triglicérides) no sangue de cavalos de cavalgada. Para tanto, 46 equinos da raça Mangalarga Marchador, sendo 32 machos e 14 fêmeas, de idades diferentes (6,83±0,65 anos) foram avaliados. Os animais eram mantidos estabulados em diferentes centros de preparo de cavalos de cavalgada que apresentavam programa alimentar e regime de condicionamento similares. Avaliaram-se o grupo sexual (machos e fêmeas) e os grupos etários

(menores de cinco anos, entre cinco e dez anos, maiores de dez anos). Os resultados indicaram diferenças na concentração hemoglobínica corpuscular média referente ao grupo sexual (P<0,05) e diferença na variação da concentração das proteínas plasmáticas totais referente aos grupos etários (P<0,05). Os resultados permitem concluir que não há diferenças importantes nos parâmetros bioquímicos e hematológicos em cavalo de cavalgada em relação ao sexo e à faixa etária, com exceção da concentração hemoglobínica corpuscular média e da concentração das proteínas plasmáticas totais, as quais, entretanto, não comprometem a performance física dos animais.

PALAVRAS-CHAVE: bioquímica; equino; eritrograma; esporte.

INTRODUCTION

Mangalarga Marchador is one of the most important and most numerous breed in Brazil. This breed originated from the southern Minas Gerais (COSTA et al., 2005), and came from the crossing of the stallion Alter Real, present from D. João VI to Baron of Alfenas with the herd of Junqueira family, of Iberian origin. The breed is readily identified by its characteristic gait, the ambling gait, instead of the trot. This walking is present in a few breeds around the world, but it is very appreciated in Brazil by riders who use their animals for pleasure ridings.

The blood count and biochemical tests are complementary and represent important tools to aid in the diagnosis of diseases in the equine clinic (KANEKO, 1997), besides being useful for evaluating aspects of horses' athletic ability. Knowing the blood constituents forms the basis for assessing the pathological changes facilitating the diagnosis of different types of problems in animals (HARVEY et al. 1984). It is known that laboratory tests results may change due to age, sex, physical activity of the animal, location and other factors (MEYER & HARVEY, 1998; ZHANG et al., 1998).

Therefore, the objective of this study was to determine the hematological and biochemical variables, in association with sex and age, of fit riding horse in tropical conditions.

MATERIAL E MÉTODOS

This study was approved by the Ethics Committee of the Federal Rural University of Pernambuco, under number 23082.007851/2007.

We used 46 *Mangalarga Marchador* horses, 32 males and 14 females at different ages (6.83 ± 0.65 years). The animals were stabled in three different centers of pleasure ride horses preparation in the metropolitan area of Recife - PE (8º3'0"S; 34º53'44"O). The animals received similar food program, constituted of commercial concentrate (approximately 5.0 kg / day / animal; 14.0% of crude protein, 3.5% ether extract and 2.9 Mcal of digestible energy), fresh elephant grass *(Pennisetum purpureum Schumach),* chopped in a forage chopper (approximately 20.0 kg / day / animal), mineral salt (Coequi Plus - Tortuga ®) and water *ad libitum*. The animals had similar physical conditioning regime, which consisted of at least three weekly rides, using walking or ambling gait, through distances of 10 to 15km on different types of floors and with altitude ranging from 50 to 150 meters above sea level. The transported load (weight of the horseback rider and the harness) did not exceed 100kg.

For analysis, we divided the animals into groups by sex (castrated and intact males and females) and by age (group I: animals younger than five years; group II: animals between five and ten years; and group III: animals older than ten years).

To determine the body condition score we used the method described by COSTA et al. (2001), which has a scale of 1 to 7, 1 being the cachectic animal and 7 the obese animal.

Blood samples were collected by venipuncture of the jugular in vacuum tubes (BD Vacuntainer ®) with anticoagulant, with the animals at rest. Samples were packed and sent under refrigeration to the laboratory for analyzes. Total blood was used for hematological analyzes: red blood cells count (RBC), hemoglobin (Hb), hematocrit (HT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width coefficient of variation (RDW-CV), being determined in automated hematology analyzer (Veterinary Hematology Analyzer pocH-100iV Diff, Sysmex®).

Subsequently, the samples were centrifuged (3000 rpm for five minutes) to obtain the plasma and to perform biochemical analyzes: urea, creatinine (CREAT), uric acid (URIAc), total cholesterol (T-CHOL) and triglycerides (TG), being determined through use of commercially available kits (DOLES® Reactant) in biochemistry semi-automatic equipment (Doles D250, DOLES®). The determination of total plasma protein (TPP) was performed by manual refractometry and the determination of fibrinogen (FIBRI) was performed by the technique of precipitation by heat in water bath at 57°C for three minutes (KANEKO et al. 1998).

For statistical analysis, all the results were analyzed by t test or ANOVA with P at 5% and, when necessary, the Holm-Sidak test was used as a post doc with P also at 5% (SigmaStat® 3.0).

RESULTS AND DISCUSSION

We observed difference in the values of MCHC, when the results were evaluated according to the sex group, being lower in males than in females (Table 1), but there were no differences in the remaining parameters (Table 2). When the results were evaluated according to the age group, we verified differences (P <0.05) in the TPP concentration, with lower values in animals of group I (under five years) than in animals of the other groups (Table 3), but no differences were observed in the other variables (Table 4) for the same group. Tables 1 and 2 present the results obtained in the variables analyzed according to sex and Tables 3 and 4, the results obtained in the variables analyzed according to age.

Table 1. Mean and standard deviation of the body condition score, hematologic and hematimetric indices according to the sex group of *Mangalarga Marchador* horses in the metropolitan area of Recife-PE

Analyzed Variables:	Sex Group	
	MALE (N = 32)	FEMALE (N = 14)
Body score	4.00 ± 0.10	4.18 ± 0.13
RBC (X 10^3/L)	8.20 ± 0.23	7.95 ± 0.43
Hemoglobin (g / dL)	12.62 ± 0.29	12.86 ± 0.58
Hematocrit (%)	36.0 ± 0.01	34.0 ± 0.14
MCV (Fl)	43.88 ± 0.40	43.8 ± 0.94
MCHC (g / dl)	35.43 ± 0.13^{A}	36.13 ± 0.31^{B}
RDW-CV (%)	19.89 ± 0.18	19.67 ± 0.32

RBC: red blood cells count; MCV: mean corpuscular volume; MCHC: mean corpuscular hemoglobin concentration; RDW: coefficient of variation of the average size of erythrocytes. Different letters on the same line indicate P <0.05 by t test.

Table 2. Mean and standard deviation of the concentration of total plasma protein, fibrinogen, urea, creatinine, uric acid, total cholesterol and triglycerides according to the sex group of *Mangalarga Marchador* horses in the metropolitan area of Recife-PE

Analyzed Variables:	Sex Group	
	MALE (N = 32)	FEMALE (N = 14)
Total plasma protein (g/L)	69.87 ± 8.30	67.54 ± 14.50
Fibrinogen (g/L)	3.74 ± 0.42	4.00 ± 0.88
Urea (mmol/L)	4.06 ± 0.48	3.49 ± 0.21
Creatinine (mmol/L)	92.76 ± 2.27	96.48 ± 5.87
Uric Acid (mmol/L)	0.031 ± 0.002	0.033 ± 0.002
Total cholesterol (mmol / L)	2.28 ± 0.15	2.67 ± 0.38
Triglycerides (mmol / L)	0.31 ± 0.02	0.32 ± 0.05

Different letters in the same line indicate P <0.05 by t test.

The values found in this study are similar to values reported in the literature for healthy animals in different conditions (KANEKO et al., 1998; PERRY, 2009), even though the animals were studied in a tropical region and were managed in a particular way. It should be emphasized that these results represent a specific group of working horses that is widespread in the country. *Mangalarga Marchador* horses are not used in regular ambling gait competitions, but in horseback riding, which require a physical conditioning for low to medium intensity and enduring exercises.

Table 3. Mean and standard deviation of the concentration of total plasma protein, fibrinogen, urea, creatinine, uric acid, total cholesterol and triglycerides according to the age group of *Mangalarga Marchador* horses in the metropolitan area of Recife-PE

Analyzed Variables:	Age Group		
	GROUP I (N = 18)	GROUP II (N = 18)	GROUP III (N = 10)
Total plasma protein (g / L)	66.82 ± 1.06 ^{B}	70.25 ± 1.34 ^{A}	71.40 ± 0.94 ^{A}
Fibrinogen (g / L)	4.00 ± 0.55	3.82 ± 0.68	3.33 ± 0.99
Urea (mmol / L)	3.80 ± 0.62	4.03 ± 0.61	3.81 ± 0.25
Creatinine (mmol / L)	88.55 ± 3.55	94.41 ± 3.07	102.54 ± 6.24
Uric Acid (mmol / L)	0.034 ± 0.0031	0.030 ± 0.0016	0.033 ± 0.0031
Total cholesterol (mmol / L)	2.34 ± 0.18	2.48 ± 0.31	2.38 ± 0.33
Triglycerides (mmol / L)	0.36 ± 0.03	0.27 ± 0.03	0.31 ± 0.05

Age groups: *group I* - younger than five years, *group II* - between five and ten years, *group III* - older than ten years. Different letters in the same row indicate P <0.05 by Holm-Sidak test.

Table 4. Mean and standard deviation of the body condition score, hematologic and hematimetric indices according to the age group of *Mangalarga Marchador* horses in the metropolitan area of Recife-PE

Analyzed Variables:	Age Range		
	GROUP I (N = 18)	GROUP II (N = 18)	GROUP III (N = 10)
Body score	4.03 ± 0.15	4.03 ± 0.06	4.15 ± 0.20
RBC (X 10 ^{3} / L)	8.58 ± 0.38	7.95 ± 0.30	7.58 ± 0.25
Hemoglobin (g / dL)	12.94 ± 0.47	12.33 ± 0.37	12.78 ± 0.51
Hematocrit (%)	36.0 ± 0.01	35.0 ± 0.01	34.0 ± 0.01
MCV (Fl)	42.82 ± 0.58	44.27 ± 0.62	44.27 ± 0.62
MCHC (g / dL)	35.48 ± 0.22	35.51 ± 0.22	36.10 ± 0.28
RDW-CV (%)	20.08 ± 0.27	19.92 ± 0.23	19.16 ± 0.30

Age groups: *group I* - younger than five years, *group II* - between five and ten years, *group III* - older than ten years. RBC: red blood cells count, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, RDW-CV: coefficient of variation of the average size of erythrocytes. Different letters in the same row indicate P <0.05 by Holm-Sidak test.

The variations observed in this experiment in the parameters analyzed, both in comparisons between the sex groups and among the age groups, were not expected, confirming the study by NEVES et al. (2005), because all evaluated animals were adults and were submitted to similar feeding program and conditioning regimen. It is noteworthy that the mean values observed for the analyzed variables were within the normal range for horses (KANEKO et al., 1998; PERRY, 2009). These findings confirm the good health of the animals evaluated and may serve as reference for saddled horses under similar conditions.

The assessments carried out in this experiment revealed differences in MCHC (P <0.05), with the highest value observed in females, disagreeing with VEIGA et al. (2006), who analyzed the hematological values of 142 Criollo

horses and observed higher MCHC in males. However, the results agree with VEIGA et al. (2006), who found higher values of MCHC for racing animals, which is explained by the need for greater oxygen supply to tissues under higher metabolism, and differ from the findings of CONCEIÇÃO et al. (2001), who studied Quarter horses.

MCHC values are associated with higher or lower percentage of hematocrit, whose variations, according to different authors, are related to the physical activity as a result of the mobilization of the blood reserves from the spleen and also dehydration that develops in enduring exercises, such as horseback riding (TEIXEIRA NETO, 2004; PICCIONE et al., 2007; PUOLI FILHO et al., 2007; BELLI, 2008).

Horses in Group I (under five years old) presented higher values for hemoglobin and hematocrit compared to other age groups, but there were no statistical differences, although the animals in this experiment were often managed and stayed in a quiet environment. According to DUNCAN et al. (1994), as very young animals are more excitable, they may present higher hematological values. On the other hand, several studies (HARVEY et al., 1984; DINEV & KHUBENOV, 1986; ALMEIDA & SILVA, 1995; MEYER & HARVEY, 1998; VEIGA et al., 2006) detected reduction in hematocrit percentage and hemoglobin concentration in young animals compared to adult animals. This variation is due to hemodilution in young animals. In the current study, plasma volume was not assessed, which could be important in determining the actual value of RBC indices in riding horses.

There were no significant differences in hematocrit values regarding sex, agreeing with the results reported by DINEV & KHUBENOV (1986), but disagreeing with the findings of VAN HEERDEN et al. (1990), who verified a higher hematocrit value in females. CEBULJ-KADUNC et al. (2002) also found higher hemoglobin values for males, which were not observed in this study, corroborating VEIGA et al. (2006).

We observed that the RDW values, which assesses the heterogenicity level of red cells was similar to that described by EASLEY et al. (1985), but lower than those described for Thoroughbred training horses and foals (BALARIN et al., 2006), both compared with animals grouped by sex and by age. In this study, we did not obverse differences between sex groups, corroborating the study by BALARIN et al. (2001).

We verified differences in total plasma protein concentration when animals were compared according to age group, with the highest values found in horses over 10 years (approximately 71.5 g/L). These results are related to senility, because it is widely known that younger animals have a higher amount of body water and that, with senility, this buildup decreases and hence the total plasma proteins concentration can be altered (MANSO FILHO et al., 2008). It is also important to remember that studies about long distance exercises and of different degrees of intensity revealed increases in plasma proteins after the activity (SANTOS et al., 2001; SANTOS, 2006; PUOLI FILHO et al., 2007). By analyzing the concentration of total plasma proteins in the blood, we did not verify differences in fibrinogen concentration, indicating a good state of health of the animals, since variations in the plasma fibrinogen concentration can contribute positively to an early diagnosis of infectious and inflammatory states (DI FILLIPO et al., 2009).

Evaluations of creatinine and urea concentrations can be used to assess protein metabolism as well as renal function of animals. Despite its importance, few studies evaluate the influence of sex and age on these parameters. We observed that urea, creatinine and uric acid concentrations were within the normal range for the species, and were similar to results reported in the literature (KANEKO et al. 1998).

A study carried out by WANDERLEY et al. (2010) with animals of the same breed showed similar mean urea (4.0 mmol/L) and creatinine (94 mmol/L) concentrations in fasted animals to those described in this study. ROSE et al. (1979), BAUER et al. (1984) and EDWARDS et al. (1989), in spite of using different designs to assess the influence of the age factor on creatinine levels, observed the influence of the evolution of age on the variability of this component. Finally it should be

emphasized that the type of management and feeding can influence the values of these markers of protein metabolism in animals. Furthermore, the results of this study are similar to those of NEVES et al. (2005), who studied clinically healthy *Mangalarga Paulista* horses that were separated according to age and sex. The values found for urea and creatinine according the sex and age groups were similar.

The lipids reserves in the blood of horses can be evaluated by determining the triglycerides and total cholesterol concentrations; however, the triglycerides is the most important of them, because it is a source of energy for athlete animals as well as animals in other physical conditions. The values we found for total cholesterol and triglycerides concentration are similar to those described both as reference values, regardless of breed (KANEKO et al., 1998; PERRY, 2009), and as reference values for *Mangalarga Marchador* horses (WANDERLEY et al., 2010). The riding horses did not receive any kind of supplement or concentrate with a high percentage of fat, but they present body condition score around 4, indicating good body fat reserve.

Lipids present in the blood are important for animals that perform exercises with low intensity and low to medium duration, which leads to a significant use of fat as an energy source for muscle work.

The results obtained for the concentration of triglycerides are similar to those of horses at rest after exercise, as reported by WANDERLEY et al. (2010); however, the values of total cholesterol concentration were higher, disagreeing with the study described by SLOET VAN OLDRUITENBORGH-OOSERBAN et al. (2002), who observed that the total cholesterol concentration decreased significantly after exercise in horses fed diets containing 1.5% ether extract.

In a study by HYYPPÄ (2005), the post-exercise recovery of serum triglyceride was slow. According to the author, one hour after the end of the exercise there is an increase in insulin release, which, in turn, inhibits lipolysis at a time when the serum lipid concentrations were already low due to its use as an energy source. This increased concentration of triglycerides is expected as a result of blocking the insulin action and the hyperglycemic effect generated by circulating catecholamines and cortisol due to physical exertion. Thus, there is a negative energy balance, similar to what occurs when a horse is subjected to fasting, with lipolysis and mobilization of other energy sources (DURHAM, 2006; DUGAT et al., 2010).

CONCLUSIONS

Mangalarga Marchador horses used for riding did not show haematological and/or biochemical alterations, when evaluated according to sex and age. The differences observed for the variable MCHC, regarding the sex group, and for TPP, regarding the age group, do not impair the physical performance of animals.

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