Journal of Animal and Veterinary Advances 17 (3): 66-69, 2018
ISSN: 1680-5593

Biochemical Parameters in Pantaneiro Horse in Extensive Management in the Wetland Area

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Abstract: This research has an objective of evaluate the serum biochemical concentrations of Creatine Kinase (CK), Lactic Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Gamma-Glutamyltransferase (GGT), creatinine, urea, albumin and calcium in Pantaneiro horses created extensively in the Pantanal Region of Brazil. Blood samples from a total of 52 male and female horses were collected and divided into 5 groups according to the age of the animals. The mean values of the serum concentrations were: CK 221.3 U/L± 75.22-287.8 U/L±110.67; LDH 899 U/L±263.8-1264 U/L±83.5; AST 220 U/L-282±54.01 U/L±30.78; GGT15.3 U/L± 3.91-30.6 U/L±10.25; creatinine 0.85 mg/dL±0.13 the 1.20 mg/dL±0.17; urea 32-44.5±5.83 mg/dL±7.04; albumin 2.80 g/dL±0:28-2.43 g/ dL±00.46; calcium 10.75 mg/dL±1.1-11.8 mg/dL±2.62. All groups showed increased values for CK and GGT and decreased values for serum calcium compared from other references from the specie. There was found a wide variation in the LDH values. The age group influenced the serum results of creatinine, albumin and calcium.

Key words: Blood, equine, enzymes, Pantanal, region, urea, albumin

INTRODUCTION

Pantanal has large tracts of land with periods of floods and long periods of drought, forage availability, tropical climate and other peculiar characteristics of the Pantanal require adapted animals within these conditions (Silva *et al*., 2005).

The Pantaneiro horse has become a factor of economic and social importance in the Pantanal ecosystem. It is essential in the handling of cattle and transport being. It is the only horse capable of supporting extended tracks periods in flooded areas (Miserani *et al*., 2002). This animal lives and researches in a region with great natural obstacles due to climatic and topographic characteristics (Ribeiro *et al*., 2004).

The clinical hematology and serum chemistries are commonly used by a variety of reasons in equine medicine such as for clinical routine and in diagnosing diseases (Munoz *et al*., 2010) such as for horses in competition, turning into a decisive tool to monitor the athlete animal (Balarin *et al*., 2005). According to Cardinet (1997), complementary tests are commonly used in equine sports medicine. The main used enzymes to determine the function of muscle are: Creatine Kinase (CK), Aspartate Aminotransferase (AST) and Lactate Dehydrogenase (LDH) (Cardinet, 1997).

The physiological parameters as reference are often based on an animal that lives in temperate climate, good conditions of handling and feeding (Pritchard *et al*., 2009). There are several reasons for the differences in the results of the biochemical values including genetic, nutritional quality, nutritional quantity and availability of water, sweat losses of electrolytes, parasitism and subclinical disease (Pritchard *et al*., 2009). In addition, the physiological effects of labor and the age they begin working can influence the parameters considered "normal" for the species (Pritchard *et al*., 2009).

The literature presents significant differences in the activity of several enzymes in accordance to the location

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where the experiments are performed (Balarin *et al*., 2005). These differences can hind a correct interpretation of the results when these values are used in other regions, demonstrating the importance of standardization of benchmarks for proper interpretation of results (Balarin *et al*., 2005).

This research aimed to evaluate the biochemical serum concentrations of Creatine Kinase (CK), Lactic Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Gamma-Glutamyltransferase (GGT), creatinine, urea, albumin and calcium in Pantaneiro horses created extensively in the Mato Grosso state Pantanal wetlands.

MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use (CEUA-Comissão de Ética no Uso de Animais), the Universidade Federal Fluminense under protocol number 277-2013.

In the study, 57 Pantaneiro horses, males and females were used, divided into 5 experimental groups by age: the First Group (G1) with 10 animals up to 12 months, the Second Group (G2) with 10 animals between 13-24 months, Third Group (G3) 10 animals between 25-36 months, Fourth group (G4) with 10 animals 37-48 months and the Fifth group (G5) with 17 animals over 60 months being males castrated and females. All animals were created extensively in areas containing mimoso grass (*Axonopus purpusii*) and humidicola grass (*Brachiaria humidicola*), supplemented with mineral salt, twice a week. Horses from the group G5 received supplementation of two kilos of commercial mash diet every day due to their research with cattle.

It was collected 10 mL of blood from each animal in vacuum tubes without anticoagulant (Vacuntainer®) by venipuncture of the jugular vein. After collection the samples were centrifuged 700 g for 10 min, obtaining the separation of blood serum. Serum was aliquoted into microtubes and stored -20°C until processing. The serum concentrations were evaluated Creatine Kinase (CK), Lactic Dehydrogenase (LDH), Aspartate Aminotransferase (AST), Gamma-Glutamyltransferase (GGT), creatinine, urea, albumin and calcium.

A semi-automatic biochemical analyzer Bioplus Bio-200® Model was used with the proper commercial kit for each parameter.

It was used for the statistical analysis computer package SPSS Version 18 and the Kruskal-Wallis test was used for quantitative variables. Occurring statistical difference in this test among variables, the Mann-Whitney test to identify whether the groups were different among them was used with significance level of $p = 0.05$.

RESULTS AND DISCUSSION

The age factor interfered with biochemical serum creatinine to all group's results, Into G-G5 of albumin and G1, G4 and G5 of calcium. The other biochemical parameters evaluated did not suffer influence of age.

The mean values and standard deviation of the studied biochemical parameters CK, LDH, AST, creatinine, urea, GGT, albumin and calcium, in Pantaneiro horse clinically healthy are willing in Table 1.

All groups of this study using Pantaneiro horses showed increased values of CK compared to reference values for the species. This result can be explained by the fact that these horses are in constant movement due to the system which they are bred and maintained. According to Pritchard *et al*. (2009), in a study with working equines in Pakistan, an increase in the reference values for CK was found which can be indicative of a chronic low muscle injury. Although, this is reversible, corroborating to Da Cas *et al*. (2000) which states that as the adaptability of animals with the exercise will occur leading to a decrease and stabilization of enzymes. However, Ribeiro *et al*. (2004), found no significant changes in this enzyme when they analyzed serum from horses and mules on a ride in the Pantanal, attributing the increase of these enzymes as a physiological consequence of the exercise performed.

The results found in this study for the LDH values were 899±263.8-1194.6±169.5 U/L, greater values the found by Ribeiro *et al*. (2002) which ranged from 580±217.22- 671.9±166.74 U/L in the Pantaneiro horses in different categories.

Meyer *et al*. (1995) reported that a large variation of LDH concentration in plasma after the exercise may be due to an increased cell membrane permeability, hypoxia and distribution of this enzyme in various tissues. The Pantaneiro horses used in this study were free-living, being in constant movement. Mean values of LDH differed among groups which corroborates with the findings of Correa *et al*. (2010).

The mean values of AST in the groups did not differ significantly, a result similar found by Franciscato *et al*. (2006) in Criollos horses in different categories and ages. AST values compared with Rose *et al*. (1983), Cardinet (1997) and McGowan *et al*. (2002) were within the normal range for the equine species. Toledo *et al*. (2001)

Table 1: Means and standard deviations of blood biochemical values in Pantaneiro horses created extensively in the Mato Grosso state at Pantanal wetlands, Pocone-MT, 2015[1]

Parameters[2]	G1	G2	G3	G4	G5
CK (U/L)	284.6±47.9[a]	235.3±80.79[a]	221.3±75.22[a]	287.8±110.67[a]	229.4±56.9[a]
LDH (UI/L)	1050.5±150.5[a]	1264±83.5[a]	1189.8±259.8[a]	1194.6±169.5[a]	899±263.8[a]
AST (U/L)	237.2±40.71[a]	230±36.72[a]	220±54.01[a]	256±35.4[a]	282±30.78[a]
GGT (U/L)	20.6±22.63[a]	22.9±8.69[a]	26.75±14.42[a]	30.6±10.25[a]	15.3±3.91[a]
Urea(mg/dL)	44.5±7.04[a]	36±8.77[a]	36.5±8.24[a]	33±5.34[a]	32±5.83[a]
Creatinine (mg/dL)	0.85±0.13[a]	1.10±0.29[b]	1±0.15[c]	1.20±0.17[d]	1.20±0.12[e]
Albumin (g/dL)	2.51±0.24	2.43±0.46[a]	2.79±0.39[b]	2.67±0.72[c]	2.80±0.28[d]
Calcium (mg/dL)	11.55±1.63[a]	11.8±2.62	10.95±5.24	10.80±1.16[a]	10.75±1.01[a]

[1]Median values followed by same letters (a or b or c or d or e) on the same line do not differ statistically in test Mann-Whitney the 5%[2]CK: Creatine Kinase; LDH: Lactic Dehydrogenase; AST: Aspartate Aminotransferase; GGT: Gamma-Glutamyl transferase.

studying Thoroughbreds described lower values than the found in Pantaneiros of this study and Oliveira studying Lusitanos horses found higher values than the ones described in this study.

Factors such as climate, feeding, handling, health, intensity and duration of exercise can influence the AST levels described by Da Cas *et al.* (2000). In the groups studied was not observed influence of these factors along with age.

Creatinine in all groups studied, presented values within the reported for equine species (Ducan *et al.* 1994). According to Piccione *et al.* (2009), any factor that influences muscle mass and physical training can affect creatinine production levels.

Serum urea values found in G2-G5 groups are within the normal range of 21.0-43.0 mg/dL described by Ducan *et al.* (1994). However, the mean serum urea of the G1 is above the cited value for equine specie. Mundim *et al.* (2004) found values of 52.53 mg/dL for urea suggesting a possible hemoconcentration in draft horses.

Serum levels of GGT enzyme were increased in all groups when the results were compared to the reference literature. These results were also found by Balarin *et al.* (2005) and McGowan (2008) after a training period in sport horses. Franciscato studying Criollo horses identified an increase of GGT in non-pregnant females.

Haddad *et al.* (2009) demonstrated in a study with sport horses, that serum GGT increases with exercise. It can be an explanation to the high values of GGT of the G4 (30.6 U/L) and G3 (26.75 U/L) groups. Probably, the lower values found of GGT (15.3 U/L) in the G5 group, working animals, indicates an adaptation to the management.

In intensive exercise, there is an increase in the plasma concentration of albumin due to the result of the diversion of the intercellular fluid (Thrall *et al.* 2007) and albumin indicates the degree of dehydration (Carlson and Mansmann, 1974). This study's albumin values remained within the normal range described by Carlson and Mansmann (1994). However, Santos *et al.* (2001) found in horses Pantaneiro values way above the described for equine species. The results found in this study can be explained by intense physical exercise these horses are subjected to.

Santos *et al.* (2001) reported lower serum calcium values in Pantaneiro horses subjected to ride when compared to this study Pantaneiro horses bred in an extensive way in the Mato Grosso state at Pantanal Marshland areas. This is justified by the use of calcium in muscle contraction and its loss by sweating . The warm weather associated with the effort required tends to loss of minerals and electrolytes in sweat.

CONCLUSION

The Pantaneiro horse created extensively in Mato Grosso state at Pantanal ecosystem presents calcium values below value to species. The age interfered with the creatinine levels in all groups and the animals suffered albumin interference age above 12 months and calcium in the animal over 12 and 48 months. The other biochemical parameters not suffered interference age.

ACKNOWLEDGEMENTS

The researchers pleasing to Higher Education Personnel Improvement Coordination (CAPES) and Foundation to support the research of Rio de Janeiro (FAPERJ) for financial support.

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