



## Short Communication

# Blood biochemical parameters of Brazilian sport horses under training in tropical climate

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**ABSTRACT** - This study aimed to provide baseline data for the serum biochemical parameters of Brazilian sport horses undergoing physical training for eventing to be used as a practical guide. A total of 139 blood samples were collected by vacuum venipuncture of the jugular vein from healthy horses in training. Tubes without anticoagulant were used for biochemical analysis. Means and standard deviations were determined for aspartate aminotransferase (AST) (306.27±84.66 U/L), creatine kinase (CK) (181.06±80.38 U/L), urea (29.51±5.82 mg/dL), creatinine (1.44±0.20 mg/dL), gamma glutamyl transferase (GGT) (14.58±6.55 U/L), calcium (13.22±0.59 mg/dL), and albumin (2.86±0.16 g/dL). The specific reference ranges were: AST, 197-454 U/L; CK, 116-290 U/L; urea, 22-42 mg/dL; creatinine, 1.2-1.8 mg/dL; GGT, 9-26 U/L; calcium, 12.2-14.2 mg/dL; and albumin, 2.6-3.1 g/dL. Beneficial effects of the training program on the horses were observed and these effects did not lead to any changes in the biochemical parameters studied. The environment may influence the serum biochemical parameters, particularly AST and CK, of Brazilian sport horses.

Key Words: enzymes, horses, serum biochemistry

## Introduction

The Brazilian sport horse came into existence in 1977 by crossing the Thoroughbred, Hanoverian, and Westfalen breeds (Dias et al., 2000). Reference standards for athletic horses bred and kept in tropical climates, however, have not been determined.

Blood biochemical parameters are indicative of health status of athletic horses affecting performance and presence of any disorder. Hence, physiological response to training during exercise program has to be evaluated. Such assessment generally includes the analysis of fluids, electrolytes, and renal, muscle, and liver functions (Hodgson et al., 2014).

Muscle injury is indicated by elevated levels of aspartate aminotransferase (AST) and creatine kinase (CK)

(Hodgson et al., 2014). Assessment of the activity of these two enzymes is routinely used to monitor the effects of exercise on athletic horses (Dias et al., 2011). Urea and creatinine levels are used to assess kidney function. Urea is a product of protein metabolism. Urea is produced by the liver and transported by the blood to the kidneys for excretion in the urine (Champe and Harvey, 1996). Creatinine is derived from creatine and phosphocreatine (Champe and Harvey, 1996). The values of urea and creatinine may increase in response to dehydration and exercise (Hodgson et al., 2014).

Liver function is indicated by the levels of gamma-glutamyl transferase (GGT) (Hodgson et al., 2014). Balarin et al. (2005) found no differences in GGT activity in Thoroughbreds undergoing different intensities of training. The range of calcium levels is very narrow and calcium levels tend to remain in the normal range, even in animals on a diet with imbalance of calcium and phosphorus. Calcium may also be lost in sweat, but changes in calcium concentration most often occur as a result of kidney or intestinal diseases (Hodgson et al., 2014). Albumin is particularly important in determining the hydration of the animal, especially after intense exercise or under heat conditions (Hodgson et al., 2014).

The objective of this study was to provide baseline data for the serum biochemical parameters of Brazilian sport

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horses training for eventing to be used as a practical guide for the evaluation of the animals in tropical climates and adapted to such conditions.

## Material and Methods

The study was conducted in Rio de Janeiro, RJ, and in Niterói, RJ, Brazil.

The mean values for maximum and minimum temperatures during the year of the study were 29 and 21.3 °C, respectively. The mean relative humidity was 72.25% (INMET, 2016). For this period, the highest and lowest values of the temperature-humidity index (THI) were 80 and 68, respectively. The procedures in this study were evaluated by the local Ethics Committee for the Use of Animals (CEUA) and approved under the case no. 276/2013.

The animals were kept in 16-m<sup>2</sup> (4 × 4 m) masonry stalls with free access to water. Their diet consisted of coastcross hay (*Cynodon dactylon* (L.) Pers), commercial concentrate provided three times a day, and 50 g of mineral salt provided once a day with the concentrate. Their training program consisted of flatwork, aerobic conditioning, and jumping exercises on both track and natural obstacles.

Blood samples were collected by vacuum venipuncture of the jugular vein in 10-mL tubes without anticoagulant (Vacuplast®). Study subjects included 139 healthy Brazilian sport horses, castrated males and females, aged 3 to 22 years. All horses were in training. Biochemical analysis of CK, AST, urea, creatinine, GGT, calcium, and albumin was performed. A 48-h rest was given to the horses before collection to avoid changes in variables caused by training.

For the biochemical analysis, the anticoagulant tubes were centrifuged for 10 min at 3000 rpm to obtain the serum. The analyses of CK and AST were performed by a BioSystems BTS 310 spectrophotometer (Biosystems®)

and urea, creatinine, GGT, calcium, and albumin analyses were performed by a LabMax 240 Premium (Labtest®) spectrophotometer.

Software Statistical Package for the Social Sciences (SPSS), version 17, was used for statistical analysis and the determination of minimum and maximum reference values was performed using the 5 and 95% percentiles of the analyzed parameters.

## Results and Discussion

The means of the AST (306.27±84.66 U/L), CK (181.06±80.38 U/L), urea (29.51±5.82 mg/dL), creatinine (1.44±0.20 mg/dL), GGT (14.58±6.55 U/L), calcium (13.22±0.59 mg/dL), and albumin (2.86 ± 0.16 g/dL) were within the normal limits reported in earlier reports (Hodgson et al., 2014) (Table 1). Results revealed that training, in general, causes no harm to the Brazilian sport horses that live and train in a tropical climate. These results are very similar to those found by Gupta et al. (2002) in adult Kathiawari horses, an Indian breed very well adapted to their agro-climatic zones. This observation is particularly pertinent to this study, as the climates of Brazil and India are similar.

The specific reference ranges were determined as follows: AST, 197-454 U/L; CK, 116-290 U/L; urea, 22-42 mg/dL; creatinine, 1.2-1.8 mg/dL; GGT, 9-26 U/L; calcium, 12.2-14.2 mg/dL; and albumin, 2.6-3.1 g/dL. Comparison of these specific reference ranges for Brazilian sport horses with the literature values (Hodgson et al., 2014) revealed that the AST and calcium level ranges for the national horses have a higher minimum and maximum limit. Urea and GGT have lower limits than those described by Hodgson et al. (2014).

Souza et al. (2016) found wider reference ranges for AST, CK, urea, and creatinine in Campeiro horses than those observed in the Brazilian sport horses of this work.

Table 1 - Means, standard deviations, medians, and reference ranges<sup>1</sup> for AST, CK, urea, creatinine, GGT, calcium, and albumin in healthy Brazilian sport horses under training

Biochemical parameter	Unit	Mean±SD	Median	Reference range for the biochemical parameters in Brazilian sport horses		Published range for the biochemical parameters studied	
				Minimum limit	Maximum limit	Minimum limit	Maximum limit
AST	U/L	306.27±84.66	293.82	197	454	150	400
CK	U/L	181.06±80.38	170.00	116	290	100	300
Urea	mg/dL	29.51±5.82	29.00	22	42	24	48
Creatinine	mg/dL	1.44±0.20	1.43	1.2	1.8	1.1	1.8
GGT	U/L	14.58±6.55	13.00	9	26	10	40
Calcium	mg/dL	13.22±0.59	13.23	12.2	14.2	10.8	13.2
Albumin	g/dL	2.86±0.16	2.85	2.6	3.1	2.6	3.8

AST - aspartate aminotransferase; CK - creatine kinase; GGT - gamma glutamyl transferase; SD - standard deviation.

<sup>1</sup> Published ranges according to Hodgson et al., 2014.

This difference can be explained by the homogeneity in management of the horses used in this study, which have the same diet, housing, and training protocol.

Blood values are affected by different factors such as individual variations, breed, and level of training (Barrelet and Ricketts, 2002). It is important to note the differences in normal values found in this study as compared with other reports (Hodgson et al., 2014; Souza et al. 2016). Comparison of the data reveals differences in these ranges, suggesting that differences among the animals studied should be taken into account. Although descending from European-bred warmbloods, Brazilian sport horses exhibit some unique characteristics because they are raised and trained in Brazil.

The horses evaluated in this study have different characteristics than horses of the same breed living in other countries because they live under different weather conditions and have different training regimens. Backhouse (2000) emphasizes that horses of European origin such as warmbloods, living in hot, humid environments can experience heat stress, thus outlining the importance of studying these animals. Rosenberg et al. (1983), regarding THI, classify the score of 80 as danger with a high influence over the performance of an athletic horse. Safety measures must be done to avoid substantial losses as the comfort of the animals decreases and the horses are more prone to show signs of heat stress.

Comparison of the mean values of the muscle enzymes AST and CK between the Brazilian sport horses in this study and Canadian Thoroughbreds (Lumsden et al., 1980) reveals differences that might be attributable to the heat in Brazil [AST,  $306.27 \pm 84.66$  U/L (197-454 U/L) vs.  $165 \pm 33.6$  U/L (99-231 U/L), respectively; CK,  $181.06 \pm 80.38$  U/L (116-290) vs.  $44.5 \pm 22.3$  U/L (1-88 U/L), respectively]. When Brazilian sport horses were subjected to incremental tests in the field and on a high-speed treadmill, their CK and AST levels peaked 6 h after exercise (Azevedo et al., 2014). The exercise they underwent for the tests was similar to their daily training in the field. This finding suggests that training causes microlesions in skeletal muscle. Therefore, results indicate that Brazilian sport horses, training and living in a tropical climate, should be given a rest period of 48 h before serum testing to avoid the influence of exercise on study results. These results were supported by the observation that AST, CK, and creatinine levels in equine police service animals did not return to baseline until 24 h after the service was stopped (Krujic et al., 2014).

A relationship between muscular alterations and the duration and intensity of the exercise has been reported (Teixeira-Neto et al., 2008). Monitoring during the recovery

period showed that CK levels returned to pre-ride values 24 h after exercise and lactate dehydrogenase and AST level returned to baseline values 72 h after the horses competed in a long-distance endurance ride under tropical conditions. Under similar conditions, the Brazilian sport horses in this study exhibited baseline mean values of CK and AST after a 48-h rest period. This difference in recovery time can be explained by the difference in exercise type, with the Brazilian sport horses undergoing high-intensity exercise for a shorter period of time. Similar results were found by Fazio et al. (2014) in show jumpers.

Assessment of muscle-damage enzymes is fundamental to the monitoring of possible injuries during the training of animals (Dias et al., 2011). In the present study, no horse showed clinical signs of rhabdomyolysis, although there was an increase in enzyme values above the normal range in a few cases (AST: 12.2%,  $n = 17$  and CK: 4.3%,  $n = 6$ ). One hypothesis is that these particular horses have insipid health, being more prone to muscle injuries even without the appearance of clinical signs of rhabdomyolysis; even after a 48-h rest, CK and AST did not return to values within the normal range due to the characteristics of this breed (Hodgson et al., 2014).

Comparison of the blood parameters of Brazilian sport horses with those of Thoroughbreds that regularly participate in official competitions (Noletto et al., 2016a), may indicate that training for eventing is less demanding, explained by the lower mean values of AST and CK. The higher enzyme levels are caused by an increase in the permeability of muscle cells caused by the intensity and duration of the training. Another explanation is that the training protocol of the Brazilian sport horses of this study was more efficient than the Thoroughbreds studied by Noletto et al. (2016a), which led to more adapted horses.

Exercise and training programs can lead to physiological changes that result in modifications of several blood clinical chemistry parameters (Adamu et al., 2013; Fazio et al., 2014; Binda et al., 2016). The knowledge of reference ranges for these parameters for a group of athletic horses living under similar conditions can allow for more accurate monitoring of their status and appropriate adaptation of their training. Another important factor to consider in the monitoring of serum biochemistry in athletic horses is overtraining syndrome. This condition leads to an increase in the muscle enzymes AST and CK and also GGT (McGowan and Whitworth, 2008). Therefore, the monitoring of these parameters must be regular, as overtraining syndrome is common in horses undergoing a strenuous training program, as with the Brazilian sport horses studied here. Comparison of the data from these horses to the reference

values reported in the literature revealed that none of the animals had enzyme levels or clinical signs consisted with this condition.

Mangalarga Marchador horses have resting values of GGT lower than Brazilian sport horses (Noieto et al., 2016b). Differences in values are likely to occur because of breed differences and the type of training the animals undergo. Pettersson et al. (2007) and Fazio et al. (2014) reported that GGT levels correlate with the transient increase in work by the liver in response to anaerobic exercise such as eventing. In Mangalarga Paulista horses, another Brazilian breed, the urea ranged between 24.81 to 41.23 mg/dL and creatinine from 1.12 to 1.86 mg/dL (Neves et al., 2005). These values are close to those observed in the Brazilian sport horses of present study. In both studies, the animals were healthy and kept under tropical conditions. The mean creatinine level found by Botelho et al. (2012) was much higher than that of Brazilian sport horses in the present study ( $4.1 \pm 2.3$  mg/dL vs.  $1.44 \pm 0.20$  mg/dL). The authors explain that the increased levels in their findings resulted from the technique they used in horses that show no signs of renal failure. Equines of the Mangalarga Marchador breed had mean serum urea concentrations of 4.06 mmol/L (72 mg/dL) in males and 3.49 mmol/L (54 mg/dL) in females (Melo et al., 2013). These concentrations are higher than the reference values reported by Hodgson et al. (2014) and those found in this study of Brazilian sport horses.

Thoroughbred mares were found to have an average value of serum calcium of 2.89 mmol/L (36 mg/dL) (Lumsden et al., 1980), which is higher than that of Brazilian sport horses, and this can be explained by the fact that those mares were pregnant at the time of collection and calcium is essential for homeostasis of the fetus. Zobba et al. (2011) observed a decrease in the calcium levels of polo ponies after intense exercise, returning to near the baseline by 30 min after the effort. Any serum calcium changes that may have occurred in these Brazilian sport horses would not have been observed, since the blood collection was taken after 48 h of rest.

Higher values of albumin in the present study than earlier reported value (Noieto, 2012) can be explained by the fact that these horses are efficient in sweating for thermoregulation and the value of albumin level increases through dehydration.

The tropical climate of Brazil has been an important influence in the adaptation of athletic horses born and raised under such conditions. There are satisfactory effects of the training program on the horses studied and these effects did not lead to any changes in the biochemical

parameters evaluated. Values of AST and CK observed are an adaptation of the Brazilian sport horses to the conditions of an equatorial country as the biochemical parameters were within the normal range. The present study establishes the minimum and maximum limits of the normal ranges of several biochemical parameters in Brazilian sport horses undergoing training.

## Conclusions

This study revealed that the environment may influence the serum biochemical parameters, particularly AST and CK, of Brazilian sport horses.

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