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Archives of Veterinary Science, v. 11, n. 2, p. 40-44, 2006
Printed in Brazil
ISSN: 1517-784X

HEMATOLOGIC AND BIOCHEMICAL PARAMETERS IN THREE HIGH PERFORMANCE HORSE BREEDS FROM SOUTHERN BRAZIL

(Parâmetros hematológicos e bioquímicos em três raças de cavalos de alta performance do Sul do Brasil)

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ABSTRACT – The hematological and biochemical parameters are useful tools for clinics and feeding management of athlete equines. The population of high performance horses consists of different breed groups, displaying specific phenotypic and metabolic characteristics related to the type of sport activity they perform. In the state of Rio Grande do Sul, in the South of Brazil, racing, jumping, polo, endurance, reigning, and dressage are the main activities. This study investigated the hemato-biochemical parameters in three high performance horse breeds from Southern Brazil. A total number of 154 horses belonging to the breeds Thoroughbred, Brasileiro de Hipismo, and Criollo, were selected for this study. Within each breed, samples were collected from males (n=12) and non-pregnant females (n=12) of two ages: 1 to 3 years of age (n=12) and over five years of age (n=12). Hematological (total count of erythrocytes and leukocytes, blood cell volume, hemoglobin, and differential count of leukocytes) and biochemical (lactate, fructosamine, glucose, cholesterol, total protein, albumin, globulins, fibrinogen, urea, calcium, magnesium, phosphorus, and enzymes LDH, AST, GGT, and CK) parameters were analyzed. Significant differences were observed in hematological and biochemical parameters, except for calcium and albumin, among breeds. There was no significant effect of age or sex within breed. This study shows that the local population, the breed and the type of sport activity are important variables to be considered in the analysis of blood parameters of horses.

Key-words: horses; hemato-biochemical profile; Brazilian breeds.

RESUMO – Os parâmetros hematológicos e bioquímicos são ferramentas que auxiliam na clínica e no manejo alimentar do eqüino atleta. Na população eqüina de alta performance existem diferentes grupos raciais que apresentam características fenotípicas e metabólicas específicas, relacionadas com o tipo de atividade esportiva que exercem. Atualmente, no Rio Grande do Sul (Sul do Brasil), são reconhecidas as modalidades esportivas de corrida, salto, polo, enduro, provas de rédeas e provas funcionais. O presente trabalho estudou os parâmetros hemato-bioquímicos em três raças de eqüinos de alta performance no Sul do Brasil. Um total de 154 animais das raças Puro Sangue Inglês, Brasileiro de Hipismo e Crioula foram selecionados para este estudo. Em cada grupo racial foram obtidas amostras de machos (n=12) e fêmeas não gestantes (n=12) e de duas faixas etárias: de 1 a 3 anos (n=12) e de mais de cinco anos (n=12). Foram analisados parâmetros hematológicos (contagem total de eritrócitos e leucócitos, hematócrito, hemoglobina e contagem diferencial de leucócitos) e bioquímicos (lactato, fructosamina, glicose, colesterol, proteína total, albumina, globulinas, fibrinogênio, uréia, cálcio, magnésio, fósforo e enzimas LDH, AST, GGT e CK). Foram observadas diferenças significativas nos parâmetros hematológicos e bioquímicos, exceto para cálcio e albumina, entre os grupos raciais. Não houve efeito significativo da idade e do sexo dentro da mesma raça. O presente trabalho mostra que o fator racial e o tipo de atividade esportiva são variáveis importantes que devem ser consideradas na análise de parâmetros sangüíneos em eqüinos.

Palavras-chave: cavalos; perfil hemato-bioquímico; raças brasileiras.

Introduction

The hemato-biochemical profile of high performance horses has been proposed as a marker of their physiology and training status (MARTINS *et al.*, 2005). The correct interpretation of these data requires reference values of the studied populations, due to important environmental, genetic, or management variables (LESAFFRE, 1979). Many stud farms in Southern Brazil are specialized in high performance

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horses of several breeds. Blood markers of energy, protein, and mineral metabolism, as well as tests to assess liver and muscle functions, give support to the practice of preventive medicine in animal breeding (KANEKO *et al*., 1997). The blood profile of horses can be influenced by their temperament, which classifies animals as "hotbloods", "warmbloods" or "coldbloods" (DRAPER, 1999). Thoroughbreds, classified as hotbloods, are frequently investigated as to their blood composition, whereas this information is scarce in literature for the Brasileiro de Hipismo (BH) and Criollo breeds, which are classified as warmbloods.

The Criollo horse is a breed derived from Spanish horses, and it is widely bred in South America. These horses have adapted to this environment for centuries, and are known for their hardiness, high fertility, and longevity (CASTRO *et al*., 2004). The jumping horse breed with highest dissemination is the Brasileiro de Hipismo (Brazilian jumping horse – BH), characterized by good aerobic resistance (DIAS *et al*., 2000), with stout and homogenous muscles.

This study aimed at evaluating the hematological and biochemical parameters in three high-performance horse breeds from Southern Brazil.

Materials and Methods

A total number of 144 clinically healthy horses belonging to three high-performance breeds, Thoroughbred racing horses, Brasileiro de Hipismo (BH) jumping horses, and Criollo, were selected. Each breed group (n=48) was divides in subgroups of 12 animals according to sex (males and non-pregnant females), and age (1-3 years old, and over 5 years old). All horses were kept in stalls with fresh water and hay all day and meals of concentrates. Only the Criollos had access to pasture daily.

Blood samples were obtained by jugular puncture in vacuum tubes without anti-clotting agent for biochemical analyses, and with EDTA 10% for the CBC. Serum was obtained by centrifugation (2500 rpm, 15 minutes) and stored at -20ºC until analyses were performed.

In the CBC, erythrocyte and total leukocyte counts were carried out in semi-automatic cell counter adapted to this species (Celm 530, Celm, Brazil) and differential leukocyte count by optical microscope using specific staining (Panótico Rápido LB stain, Laborclin, Brazil). Fibrinogen determination was carried out by technique of heating at 56ºC, according to JAIN (1993).

Biochemical analyses were performed by spectrophotometric methods using diagnosis kits (Labtest, Brazil; Randox, UK) in semi-automatic apparatus (Metrolab 1600, Argentina). The analytes and methods (in parenthesis) were as follows: glucose (glucose-oxidase), lactate (lactate-oxidase/ aminoantipyrine), fructosamine (nitrotetrazolium reduction), cholesterol (cholesterol oxidase), total protein (biuret), albumin (bromocresol green), urea (urease), creatinine (alkaline picrate), calcium (phtalein purple), inorganic phosphorus (ammonium molibdate), magnesium (sulfonide magon), aspartate aminotransferase (UV kinetic), gama-glutamil transferase (p-nitroaniline), creatine kinase (UV kinetic with N-acetylcysteine) and lactate dehydrogenase (pyruvate-lactate UV kinetic).

Statistical analyses included analysis of variance, carried out using the software SAS version 2001, to verify the effect of breed on each blood parameter. The t-test was used to compare the means of the effect of age and sex. Breed means were compared by the test of Tukey.

Results

TABLE 1 shows the results of hematological parameters of the three studied breeds. TABLE 2 presents blood biochemical parameters. No effects of age or sex were observed on hematological or biochemical data within each breed group ($p>0.05$).

TABLE 1 – HEMATOLOGICAL PARAMETERS IN THREE HIGH PERFORMANCE HORSE BREEDS IN THE STATE OF RIO GRANDE DO SUL (SOUTHERN BRAZIL, 2005).

Parameters	Breed		
	Thoroughbreds	BH	Criollo
N	48	48	48
Erythrocytes (10^6/µL)	$9.00^{c} \pm 0.84$	$7.84^{a} \pm 0.86$	$8.59^{b} \pm 1.08$
Packed cell volume (%)	$39.5^{c} \pm 3.8$	$33.35^{a} \pm 4.0$	$37.4^{b} \pm 4.9$
Hemoglobin (g/dL)	$12.98^{b} \pm 1.17$	$11.33^{a} \pm 1.39$	$12.45^{b} \pm 1.61$
Total leukocytes (/µL)	$9,731^{a} \pm 1,714$	$7,612^{c} \pm 1,740$	$11,132^{b} \pm 2,068$
Band neutrophils (/µL)	$21^{b} \pm 47$	$7^{a} \pm 23$	$6^{a} \pm 41$
Segmented neutrophils (/µL)	$5,411^{a} \pm 1,317$	$4,134^{b} \pm 1,162$	$5,371^{a} \pm 1,677$
Eosinophil (/µL)	$136^{a} \pm 136$	$191^{a.b} \pm 209$	$269^{b} \pm 291$
Basophile (/µL)	0^{a}	$17^{b} \pm 40$	0^{a}
Monocyte (/µL)	$277^{b} \pm 232$	$161^{a} \pm 161$	$138^{a} \pm 143$
Lymphocyte (/µL)	$3,887^{a} \pm 1,277$	$3,102^{b} \pm 1,054$	$5,347^{c} \pm 2,125$
Fibrinogen (g/dL)	$2.0^{a} \pm 0.3$	$2.4^{b} \pm 0.6$	$2.4^{b} \pm 0.6$

Values with different letters are significantly different among breeds ($p<0.05$) by the test of Tukey. BH= Brasileiro de Hipismo (Jumping horses).

TABLE 2 – BLOOD BIOCHEMICAL PARAMETERS IN THREE HIGH PERFORMANCE HORSE BREEDS IN THE STATE OF RIO GRANDE DO SUL (SOUTHERN BRAZIL, 2005).

Metabolite	Breed		
	Thoroughbreds	BH	Criollo
N	48	48	48
Glucose (mmol/L)	6.33 [c] ± 0.54	5.82 [b] ± 12.5	4.35 [a] ± 0.64
Fructosamine (mmol/L)	3.39 [c] ± 0.42	2.77 [b] ± 0.58	2.24 [a] ± 0.30
Lactate (mmol/L)	1.82 [b] ± 0.53	1.35 [a] ± 0.42	1.18 [a] ± 0.43
Cholesterol (mmol/L)	2.67 [a] ± 0.54	3.01 [a] ± 0.80	4.47 [b] ± 1.88
Total protein (g/L)	62.52 [a] ± 3.72	63.27 [a] ± 9.3	76.09 [b] ± 9.0
Albumin (g/L)	36.08 [a] ± 1.90	30.96 [a] ± 3.14	31.76 [a] ± 4.93
Globulin (g/L)	32.43 [a] ± 3.51	32.31 [a] ± 8.59	44.33 [b] ± 8.75
Urea (mmol/L)	5.42 [a] ±1.46	6.46 [b] ± 2.54	6.80 [b] ± 2.05
Calcium (mmol/L)	2.89 [a] ± 0.20	2.90 [a] ± 0.22	3.01 [a] ± 0.48
Magnesium (mmol/L)	0.95 [b] ± 0.14	0.84 [a] ± 0.08	0.97 [b] ± 0.11
Phosphorus (mmol/L)	1.23 [a] ± 0.20	1.17 [a] ± 0.23	1.47 [b] ± 0.44
LDH (U/L)	462 [a] ± 127	583 [b] ± 200	548 [a.b] ± 193
AST (U/L)	286 [a.b] ± 83	277 [a] ± 67	357 [b] ± 255
GGT (U/L)	31.8 [b] ± 12.4	16.7 [a] ± 6.7	17.0 [a] ± 10.3
CK (U/L)	129 [a] ± 62	209 [a.b] ± 112	276 [b] ± 263

LDH: lactate dehydrogenase. AST: aspartate transaminase. GGT: gama-glutamil transferase. CK: creatine kinase. Values with different letters are significantly different among breeds ($p<0.05$) by the test of Tukey. BH= Brasileiro de Hipismo (Jumping horses).

Discussion

The use of laboratorial parameters is required to assess health, nutritional and training status of horses (GOMIDE *et al.*, 2006). In the present study, no influence of sex was observed in the hematological or biochemical parameters. VEIGA (2003), studying Criollo horses, observed differences between males and females in hematological indexes, total leukocytes, neutrophils, lymphocytes, and basophils. We found that Thoroughbreds displayed higher erythrocyte counts, but not higher leukocyte counts than other breeds. Criollo horses presented the highest leukocyte counts among the studied breeds. TYLER *et al.*(1987) recorded higher erythrocyte count for hot-blooded breeds as compared to coldbloods. In the present study, the hematological behavior of the Criollo horse was intermediate between the two other breeds (hotbloods and coldbloods).

The leukocyte values found for the three studied breeds are within the interval found in literature (MUÑOZ *et al.*, 1996), despite the significant difference among the breeds observed in the present study, in which the interval in leukocyte count was 8,300 to 20,000/mL. Although other authors (SCHALM, 1979) observed that older horses (>8 years) had higher leukocyte values than younger horses, in the present study no difference was observed between the analyzed age ranges. SNOW *et al.* (1983) reported no effect of age on hematological parameters of Thoroughbreds.

The differences in hematological values among breeds are reported in literature, emphasizing erythrocyte value differences between Thoroughbreds and coldbloods (MORRIS, 2000). Studies with Andalusian horses, an ancestor of the Brazilian Criollo horse, show higher hematological values as compared to those found for the Criollo horse in the present studies (MUÑOZ *et al.*, 1996), suggesting that the adaptation process generated physiological changes.

The concentration of fibrinogen found is consistent with the values in literature (ANDREWS *et al.*, 1994), and it was lower in Thoroughbreds as compared with the other breeds. This disagrees with other authors (CAMPBELL *et al.*, 1981), who did not find significant differences in fibrinogen values between Thoroughbreds and other breeds.

All values found in the differential leukocyte count are consistent with those described in literature (KRAMER, 2000). Most leukocyte types presented differences among breeds. It is noted a higher lymphocyte number in Criollo horses, which may be associated to the animal's immunity, along with the higher value of globulins.

Respiratory rate and blood lactate are the most sensitive physiological parameters indicating training status (MARC *et al.*, 2000). Lactate metabolism is linked

to glucose metabolism (KANEKO *et al*., 1997). In this study, a direct relationship was found between these two metabolites, as well as significant differences among the three studied breeds ($p<0.01$). Thoroughbreds presented the highest glucose and lactate blood levels, whereas Criollo horses had the lowest. The lactate and glucose levels determined in this study are similar to those reported for Thoroughbreds (DAVIS e EVANS, 2000).

Fructosamine is a glycosylated blood protein, which reflects blood glucose levels from a period up to three weeks before blood collection (KANEKO *et al*, 1997). In the present study, fructosamine levels were directly related to glucose and lactate levels, with a significant correlation between fructosamine and glucose ($r= 0.45$; $p<0.001$), and between fructosamine and lactate ($r= 0.30$; $p<0.001$). However, negative correlations were found between cholesterol and fructosamine ($r= -0.38$; $p<0,001$), glucose ($r= -0,42$; $p<0,001$), and lactate ($r= -0,18$; $p<0,05$).

No difference in albumin was detected among the three studied breeds, but Criollo horses presented higher globulin concentration ($p<0.01$) as compared to the others, which may be related to the immune condition of this breed that is very well adapted to Brazilian conditions. Thoroughbreds presented the lowest urea values, whereas Criollo horses had the highest values. This may be related to the protein metabolism and diet type, although some studies do not show differences in biochemical parameters as a function of dietary protein content (GREPPI *et al*., 1996).

Out of the evaluated mineral levels, only calcium levels did not present differences among breeds. Magnesium content was lower in BH, whereas phosphorus was higher in Criollo horses. Phosphorus and magnesium blood levels are related to diet, physical activity, and environmental conditions (WEISS *et al*., 2002).

Enzymes are used in equine medicine to assess muscle and liver functions (KANEKO *et al*., 1997). CK, a muscle specific enzyme, was significantly related to LDH ($r= 0.59$; $p<0.001$), but not to AST ($r= 0.08$), both present mainly in the liver and in the muscle. The effect of exercise type on CK, AST, and LDH values has been reported in horses, indicating that higher intensity exercises are related to higher enzyme serum levels (CÁS, 1998). In the present study, there were differences in enzyme value between Thoroughbreds and the other two breeds. Criollo horses presented higher CK and AST levels, which may be related to the higher exercise intensity to which they are submitted.

Conclusion

There is significant difference among breeds in most biochemical parameters, suggesting differential clinical management of the different breeds of high performance athlete horses. The differences in the parameters presented by Criollo horse are possibly due to their adaptation to the environment and to the type of physical activity. The evaluated age ranges, and sex do not determine significant differences in hematological and biochemical parameters.

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Recebido para publicação: 24/05/2006
Aprovado: 28/09/2006