



Serum proteinogram of the Campeiro horse

[Proteinograma sérico de equinos da raça Campeiro]

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ABSTRACT

The aim of this study to measure the fractions of the total serum proteins of the Campeiro horse and identify the influences of biological variants. Blood samples were taken in 138 horses of the breed Campeiro for measuring the concentration of total serum protein by the biuret method. Serum concentrations of protein fractions were measured by electrophoresis using agarose gel. Groups were formed according to age, sex and reproductive condition. The average values of serum fractions: albumin (2.85 ± 0.36 g/dL), alpha 1 (0.28 ± 0.11 g/dL), alpha 2 (0.26 ± 0.08 g/dL) beta 1 (0.57 ± 0.15 g/dL), beta 2 (0.89 ± 0.28 g/dL), gamaglobulinas (1.86 ± 0.34 g/dL), albumin/globulin ratio (0.75 ± 0.18) and 2.5% percentile and 97.5% had slight differences in relation to the reference interval proposed for the species. They observed higher values of alpha 1 and 2 globulins in the group from that had six to eight years old and gamaglobulins in group above 13 years old. Serum protein concentrations were similar in horses and mares and between non-pregnant and pregnant. Sex and pregnancy status did not affect serum proteinogram. Alpha and gamaglobulins have higher values as the age increases. Serum proteinogram of Campeiro horses shows variations that have to be considered in the interpretation of laboratory tests.

Keywords: albumin, clinical pathology, globulins, serum proteins

RESUMO

Este trabalho tem por objetivo mensurar as frações das proteínas totais séricas de equinos Campeiros e identificar as influências de variantes biológicas. Foram colhidas amostras de sangue de 138 equinos, machos e fêmeas da raça Campeiro. A determinação da concentração de proteínas totais séricas foi realizada pelo método de biureto. As concentrações séricas das frações proteicas foram determinadas por eletroforese, utilizando-se gel de agarose. Formaram-se grupos em relação à idade, ao sexo e à condição reprodutiva. Os valores médios das frações séricas albumina ($2,85 \pm 0,36$ g/dL), alfa 1 ($0,28 \pm 0,11$ g/dL), alfa 2 ($0,26 \pm 0,08$ g/dL), beta 1 ($0,57 \pm 0,15$ g/dL), beta 2 ($0,89 \pm 0,28$ g/dL), gamaglobulinas ($1,86 \pm 0,34$ g/dL), relação albumina/globulina ($0,75 \pm 0,18$) e os percentis 2,5% e 97,5% apresentaram diferenças pontuais em relação aos intervalos propostos para a espécie. Observaram-se maiores valores de alfa 1, alfa 2 globulinas, no grupo de seis a oito anos, e de gamaglobulinas, no grupo acima de 13 anos de idade. O proteinograma sérico foi similar entre machos e fêmeas e entre fêmeas vazias e gestantes. Sexo e estado gestacional não afetaram o proteinograma sérico. Alfa e gamaglobulinas têm incrementos em função de idades crescentes. O proteinograma sérico de equinos Campeiros tem variações que devem ser consideradas em exames laboratoriais.

Palavras-chave: albumina, patologia clínica, globulinas, proteínas séricas

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INTRODUCTION

Electrophoresis of serum proteins is a useful diagnostic tool in veterinary medicine, which can provide important information and facilitate the clinical differentiation of diproteinemias (Keren *et al.*, 1999). Various changes in the fractions of serum proteins can occur and these can be indicative of non-specific disease processes or markers in some potential pathological conditions.

The standard physiological electrophoretic determination in different species is useful in differentiation of healthy from unhealthy individuals in which routine tests are not effective for clinical decision making by providing a basis of direction for the choice of laboratory tests more specified (Eckersall, 2008; Mallard *et al.*, 1998).

The fractionation of serum proteins is a useful test in the equine medical clinic routine, but is not yet sufficiently standardized (Riond *et al.*, 2009), in which analogous values of reference that do not fit relevant characteristics to the animal or breed may result in erroneous interpretations.

The Campeiro horse is locally adapted in the *Planalto Serrano Catarinense* region (southern Brazil) and has a characteristic gaited to be credited to exist organic adaptations to the environment, as they were observed in other studies (Fonteque *et al.*, 2016; Souza *et al.*, 2016). Therefore, knowledge of protein fractions that make up the serum protein profile, its proportions and the influence of biological factors associated in sex, age, reproductive period (Miller *et al.*, 1998), are necessary for knowledge of the physiologic standards of the race and its variations. Thus there will be more accuracy in the interpretation of laboratory tests used to aid diagnosis of diseases.

This study aims to measure the serum protein profile through the fractionation of albumin, alpha 1, alpha 2, beta 1, beta 2 and gammaglobulins and the influence of biological variables: age, sex and pregnancy, in adult horses of the Campeiro breed.

MATERIALS AND METHOD

The study was approved of the Santa Catarina State University Animal Ethics Committee (n° 01.05.14). Animal owners gave consent for their inclusion in the study.

Were used 138 horses with a mean age 9.8 ± 5.6 years, being that 14 males (10.15%) and 124 females (88, 85%) registered of the Campeiro breed, from conservation properties in the city of Lages, Curitibaanos, Campos Novos and Concordia in the Santa Catarina State and in the city of Caxias do Sul, in the State of Rio Grande do Sul, located in southern Brazil.

Blood samples were collected by venipuncture of the external jugular in vacuum tubes of 10mL without anticoagulant. After centrifugation, serum was frozen at -20°C and stored in tubes (Eppendorf, Hamburg, Germany) until laboratory analyzes. Measurements of the concentration of total serum protein (TSP) was performed by the biuret method using commercially available reagents. Serum concentrations of protein fractions of albumin, alpha 1, alpha 2, beta 1, beta 2 and gamma globulin were performed by electrophoresis by using the agarose gel method (Celmigel, CELM general agarose gel), Tris buffer pH 9.5 (CELM), and electrophoretic run for 20min in 100V current (System SE-250, CELM). After the procedure, the gel was stained with amido-black 2% and bleached with acetic acid 5%. The proportion of each protein fraction was established by the program software SDS-60 (CELM) of the SE-250 system (CELM) after scanner reading.

To evaluate the influence of factors related to age, sex and pregnancy on the variables studied are, the animals were divided into four groups according to age: three to five years; six to eight years; nine to 12 years and above 13 years, between males and females and also between pregnant and non-pregnant females.

The Shapiro-Wilk test was used to evaluate the normality of the data. Outliers were identified according to the Grubbs test. The reference intervals were calculated using percentiles 2.5%, 50% and 97.5%, supported on the recommendations of guidelines of Clinical Laboratory and Standards Institute (Defining..., 2008) and Geffré *et al.* (2009). The coefficients

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of variation were calculated dividing the standard deviations by their respective means, and the expression as a percentage. It used the Kruskal-Wallis test followed Bonferroni test for multiple comparisons of means to the TSP and its fractions in different age groups. The comparison of males and females and between pregnant and non-pregnant females was performed with the Wilcoxon-Mann-Whitney test. Values of P <0.05 were considered significant.

RESULTS

Table 1 shows the mean values, standard deviation, relative values, and variation coefficient and percentiles of 2.5%, 50 and 97.5% of the concentration of TSP and its different fractions. One outlier was excluded from the

gammaglobulin and two were excluded from the alpha 2 globulins.

In relation to age, there were an increase (P <0.05) in alpha 1 and alpha 2 globulins in the group of six to eight years for the group of three to five years, remains similar in the following groups (Table 2). The gammaglobulin showed markedly increased values in the group of six to eight years, reaching the highest values in the group of animals with 13 years old or older (P <0.05) (Table 2).

The values are of serum protein fractions were similar between males and females and nonpregnant and pregnant mares (P <0.05) (Table 3).

Table 1. Mean values, standard deviations (m±sd), relative values (RV), coefficient of variation (CV) and percentiles (2.5%, 50% and 97.5%) of total serum protein (TSP) and albumin (alb), alpha 1, alpha 2, beta 1, beta 2 and gamma globulins, and the albumin: globulin (A/G) ratio of 138 healthy adults of Campeiro horses

Variable	Mean ± sd	RV	CV	Percentiles		
				2.5%	50%	97.5%
TSP (g/dL)	6.75±0.59	100.00%	09%	5.73	6.75	8.00
Alb (g/dL)	2.85±0.36	42.82%	13%	2.12	2.82	3.54
Alpha1 (g/dL)	0.28±0.11	4.15%	38%	0.13	0.28	0.49
Alpha 2 (g/dL)	0.26±0.08	3.85%	31%	0.16	0.24	0.48
Beta 1 (g/dL)	0.57±0.15	8.44%	27%	0.35	0.54	0.93
Beta 2 (g/dL)	0.89±0.28	13.19%	32%	0.50	0.79	1.52
Gamma (g/dL)	1.86±0.34	27.55%	18%	1.31	1.85	2.54
A:G	0.75±0.18	-	22%	0.42	0.76	1.09

Table 2. Average values and standard deviations of total serum proteins (TSP) and protein fractions: albumin (alb), alpha 1, alpha 2, beta 1, beta 2 and gamma globulins and albumin: globulin (A/G) ratio in different age groups in 138 clinically healthy adult of Campeiro horses

Variables	Age group				P-valor*
	3 to 5 years (n= 34)	6 to 8 years (n= 34)	9 to 12 years (n= 34)	Over 13 years (n= 36)	
TSP (g/dL)	6.76±0.72	6.81±0.68	6.81±0.68	6.97±0.51	ns
Alb (g/dL)	2.83±0.45	2.97±0.36	2.83±0.35	2.75±0.29	ns
Alpha1 (g/dL)	0.23±0.09 ^b	0.31±0.10 ^a	0.29±0.08 ^{ab}	0.29±0.14 ^{ab}	< 0,05
Alpha 2 (g/dL)	0.25±0.07 ^b	0.32±0.09 ^a	0.26±0.09 ^{ab}	0.22±0.05 ^b	< 0,05
Beta 1 (g/dL)	0.54±0.17	0.60±0.18	0.55±0.14	0.59±0.13	ns
Beta 2 (g/dL)	0.95±0.31	0.89±0.24	0.86±0.30	0.85±0.29	ns
Gamma (g/dL)	1.77±0.21 ^{bc}	1.66±0.32 ^c	1.88±0.34 ^b	2.15±0.30 ^a	< 0,05
A:G	0.81±0.17	0.80±0.15	0.77±0.19	0.70±0.14	ns

*Comparison by the Kruskal-Wallis test, where P <0.05 indicates significant effect of age. Different lowercase letters in the row demonstrate significant difference (P <0.05) by the Bonferroni test. ns: not significant.

Table 3. Mean values and standard deviations of total serum proteins (TSP) and protein fractions: albumin (alb), alpha 1, alpha 2, beta 1, beta 2 and gamma globulins and albumin: globulin (A/G) ratio in relation to sex and at the time of gestation in healthy adult of Campeiro horses

Variables	Mares (n= 124)	Horses (n= 14)	Mares	
			Non-Pregnant (n= 74)	Pregnant n= (50)
TSP (g/dL)	6.80±0.59	7.01±0.83	6.65±0.58	7.02±0.56
Alb (g/dL)	2.84±0.33	2.86±0.53	2.92±0.28	2.73±0.38
Alpha1 (g/dL)	0.28±0.11	0.30±0.09	0.25±0.09	0.33±0.12
Alpha 2 (g/dL)	0.26±0.09	0.26±0.04	0.26±0.08	0.27±0.10
Beta 1 (g/dL)	0.57±0.14	0.56±0.23	0.55±0.15	0.59±0.14
Beta 2 (g/dL)	0.92±0.28	0.74±0.23	0.90±0.30	0.89±0.25
Gamma (g/dL)	1.85±0.35	1.94±0.32	1.77±0.35	2.00±0.34
A:G	0.76±0.17	0.82±0.17	0.81±0.16	0.69±0.16

There was no significant difference ($P > 0.05$) between horses and mares and between pregnant and non-pregnant mares by the Wilcoxon-Mann-Whitney test.

DISCUSSION

Mean values and percentiles 2.5% and 97.5% were similar to those proposed reference intervals in Kaneko (1997), except for the lower limits of albumin and alpha 2 globulins that were lower and upper limits of beta 2 and gamma globulins that were higher than those presented above as reference. Comparing the mean values and reference intervals proposed by Riond *et al.* (2009), higher values of beta 2 and gamma globulins and lower values of alpha 2 globulins and albumin/globulin (A/G) were observed, being similar to the others variables for horses of Campeiro breed. The relative values of alpha 1, beta 2 and gamma globulins shared the same differences of their concentrations, compared to results of Riond *et al.* (2009). Numeric distances of the differences of the results of this study in relation to the data of the above mentioned literature were small but sufficient to show different organic responses in the Campeiro breed compared to other racial groups, which may be related both to the environment as intrinsic factors.

The coefficients of variation were relatively high for fractions in alpha and beta globulins, with values close to 30%, demonstrating that modulation of these globulins may be related to the heterogeneity of the environment and/or genetic diversity of animals. Interpretation of the results of this test should be the light of benchmarks possible specific (Faria Filho *et al.*, 2016). However, specific coefficients of

variation information were not found in laboratory tests for better inference.

Age presented effect on protein profile, increases in alpha 1 and alpha 2 globulins were identified in group that had six to eight years in relation to the group of three to five years, and remains similar in the following groups. Showing the improvement of the immune system of the Campeiro horses as immunogenic insults occur over time. Similar results were found by Alberghina *et al.* (2010) when evaluating the proteinogram of healthy goats in which the group of animals of 5-12 years old presented higher concentrations of total protein and α -globulin than the group of 2-4 years of age, also by França *et al.* (2011) in which they identified an increase in total serum protein and gammaglobulin levels in buffaloes at 24 months compared to those at six months of age and by Alberghina *et al.* (2011), who observed an increase of alpha and betaglobulins from six years in dairy cows. The alpha fraction proteins, in most species except ruminants, are the most rapidly migrating globulins in electrophoresis. The alpha 1 antitrypsin protein corresponds to 90% of the normal alpha 1 zone peak and the alpha 2 zone is composed for several proteins such as haptoglobin, alpha-2 macroglobulin and ceruloplasmin, and all the alpha fraction classified as acute phase proteins (Eckersall, 2008). The main function of this group of proteins it is to defend the body against pathological damage, helps restore homeostasis and regulates the different inflammation

(Petersen *et al.*, 2004). In addition to inflammatory conditions, these proteins can be released under normal physiological conditions (Kustritz, 2005).

The approximately 35% increase in the concentration of gammaglobulin in the group of older animals compared to the group of six to eight years, demonstrates the immune competence in older individuals due to exposure of a higher number of antigens. The gammaglobulin fraction is predominantly composed of immunoglobulins of various classes (IgG, IgA, IgM, IgD and IgE), with marked fluctuations concentrations observed during the first weeks of life in dependent mammals of passive transfer process (Schade *et al.*, 2016; Perkins and Wagner, 2015; LeBlanc *et al.*, 1992). As the animal grows, the immune system acquires autonomy to produce its own antibodies, from the adaptive immune system cells and activated B cells in response to exposure to antigen (Jackson and Elsawa, 2015).

All males used in this study were stallions due to the fact that only intact males receive the definitive registration, according to the guidelines of the Brazilian Association of Campeiro Horse Breeders (ABRACCC). The greatest anabolic effects of testosterone to estrogen ratio (Eckersall, 2008), seems not to influence the different protein fractions in Campeiro horses. Similar results were obtained by Cavalcante *et al.* (2012) when studying horses and donkeys. However, a research including castrated animals can be valid for more precise interpretation of fractions of serum proteins.

The pregnant females in this study were grouped regardless of the gestational period in which the data from the first two thirds of pregnancy that is expected to have little physiological impact (Ousey, 2004), seem to have been enough to not reflect in significant differences. Similar results were obtained in Lusitanian mares in the last third of pregnancy compared to postpartum (Agrícola *et al.*, 2008). However, in another study elevations in concentrations of alpha 1, alpha 2 and gamma globulins during the two weeks before labor were identified (Bazzano *et al.*, 2016). In some species, the concentration of total protein decreases in maternal blood during pregnancy due to the reduction in the albumin, although there is a slight increase in globulin. In

bitches, acute phase reaction occurs 21 days after fertilization, an increase of acute phase proteins and C-reactive protein in maternal serum (Vannucchi *et al.*, 2002; Eckersall *et al.*, 1992). Grunberg *et al.* (2011) found lower concentrations of alpha-globulins in pre-partum cows in relation to cows in lactation and in anestrus, it this may be associated with immunoglobulins transfer from the bloodstream to the mammary gland colostrum for its synthesis.

CONCLUSION

The serum proteinogram of Campeiro horses presented specific variations that should be considered in laboratory tests. The alphas and gamma globulins show higher values according to the increase of age. Sex and gestational status did not affect serum proteinogram. The mean values and the proposed ranges can be used in the interpretation of equine serum protein concentrations as a reference for the Campeiro breed and as in comparative studies.

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