Archivos Latinoamericanos de Producción Animal. 2024. 32 (1)
https://doi.org/10.53588/alpa.320101

# Hematological and biochemical reference values for the Brazilian Pony breed

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**Abstract:** The aim of this study was to characterize the hematological profile of the equine Brazilian Pony breed herds in northern of Rio de Janeiro state, Brazil. Blood samples were separated in categories: adult stallions, pregnant mares and non-pregnant mares. The red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hgb), main corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC) and differential count of basophils (Baso), eosinophils (Eos), band cells (Bands), segmented neutrophils (Neut), lymphocytes (Lym), monocytes (Mono), and platelets (Plat) were evaluated. The biochemical parameters of urea (Urea), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), and creatinine were measured in pregnant and non-pregnant mares. Analysis of variance was carried out with difference of hematological parameters between males and females. Also, analysis of variance was used to verify the effect of pregnancy on biochemical parameters. There was effect of sex on RBC, hemoglobin, VCM and MHCH, but PVC and MHC were not affected by sex. Higher means were observed in females. There was an effect of sex on WBC, eosinophils and neutrophils. There was effect of pregnancy on AST, but no effect on urea, GGT and Creatinine. According to the results obtained in this study, it can be concluded that the analysis of the blood count and the biochemical of the Brazilian Pony reveals specificities compared to other equine breeds. Thus, these results could be used as biomarkers for the evaluation of the Brazilian Pony breed in Rio de Janeiro.

**Keywords:** blood, horse, leukogram, pony.

# Valores de referencia hematológicos y bioquímicos para la raza Pony Brasileño Hematología y bioquímica en Pony brasileño

**Resumen.** El objetivo de este estudio fue caracterizar el perfil hematológico de un plantel de equinos de la raza Pony Brasilera en el norte del estado de Rio de Janeiro, Brasil. Muestras de sangre fueron separadas en categorías: sementales adultos, yeguas preñadas y yeguas no preñadas. Fueron evaluados los glóbulos rojos (RBC), el volumen globular (PCV), la hemoglobina (Hgb), el volumen corpuscular principal (MCV), la hemoglobina corpuscular media (MCH), la concentración de hemoglobina corpuscular media (MCHC), los glóbulos blancos (WBC) y el contaje diferencial de basófilos (Baso), eosinófilos (Eos), células en banda (Bands), neutrófilos segmentados (Neut), linfocitos (Lym), monocitos (Mono) y plaquetas (Plat). Los parámetros bioquímicos de Urea (Urea), alanina aminotransferase (ALT), aspartato aminotransferase (AST), gama glutamil transpeptidase (GGT) y creatinina fueron medidos en yeguas gestantes y no gestantes. Fue realizado análisis de varianza para verificar diferencias entre machos y hembras en los parámetros hematológicos y bioquímicos. Fue verificado efecto del sexo sobre RBC, hemoglobina, VCM y MHCH, pero PVC y MHC no fueron afectados por el sexo. Medias mayores fueron observadas en el sexo femenino. Hubo efecto del sexo sobre leucocitos, eosinófilos y neutrófilos. Hubo efecto de la preñez en AST, pero ningún efecto en la Urea, GGT ni Creatinina. De acuerdo con los resultados obtenidos en este estudio, se puede concluir que el análisis del hemograma y la bioquímica del Pony Brasileño revelan especificidades en comparación con otras razas de equinos. Por lo tanto, estos resultados podrían ser utilizados como puntos de referencia para la evaluación de la raza Pony Brasileño.

**Palabras clave:** equinos, leucograma, bioquímica, poni.

Recibido: 2022-02-25. Revisado: 2022-05-09. Aceptado: 2023-12-26

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# Valores de referência hematológicos e bioquímicos para a raça Pônei Brasileiro Hematologia e bioquímica em pôneis brasileiros

**Resumo.** O objetivo neste estudo foi caracterizar o perfil hematológico de um rebanho de equinos da raça Pônei Brasileiro no norte do estado do Rio de Janeiro, Brasil. As amostras de sangue foram separadas em categorias: garanhões adultos, éguas gestantes e éguas não gestantes. Os parámetros avaliados foram glóbulos vermelhos (RBC), volume globular (PCV), hemoglobina (Hgb), volume corpuscular principal (VCM), hemoglobina corpuscular média (HCM), concentração de hemoglobina corpuscular média (CHCM), glóbulos brancos (WBC), e contagem diferencial de basófilos (Baso), eosinófilos (Eos), células em banda (Bands), neutrófilos segmentados (Neut), linfócitos (Lym), monócitos (Mono) e plaquetas (Plat). Os parâmetros bioquímicos de Uréia (Urea), alanina aminotransferase (ALT), aspartato aminotransferase (AST), gama glutamil transpeptidase (GGT) e creatinina foram mensurados em éguas gestantes e não gestantes. A análise de variância foi realizada para comparar parâmetros hematológicos e bioquímicos de equinos machos e fêmeas. . Efeito significativo do sexo foi verificado sobre hemácias, hemoglobina, VCM e MHCH (médias mais elevadas foram observadas em fêmeas), mas PVC e MHC foram semelhantes entre animais machos e fêmeas. O fator sexo também afetou leucócitos, eosinófilos e neutrófilos. A prenhes afetou AST, mas os resultados de Uréia, GGT ou Creatinina foram semelhantes. De acordo com os resultados obtidos neste estudo, as análises do hemograma e da bioquímica do Pônei Brasileiro revelam especificidades entre os fatores em estudo e em comparação com outras raças de cavalos. Assim, esses resultados podem ser utilizados como valores de referência para a raça Pônei Brasileiro.

**Palavras-chave:** cavalos, leucograma, bioquímica, pônei.

## Introduction

The Brazilian Pony breed is a recently created breed, records began in 1970, with the founding of the Brazilian Association of Piquira and Pony Horses (ABCCPONEI, 2022). These horses have been selected since then, and due to their characteristics it is necessary to conservation of the breed, as well as an exhaustive control of mating in order to avoid problems related to inbreeding. The control of animals from the point of view of health is fundamental in any program of genetic improvement and conservation. In association with this, demand is increasing for information about these horses' physiology to support diagnosis of health based on hematological parameters. The literature contains a large amount of information about the hematological and biochemical profile of horses (Zobba *et al*., 2011; Adamu *et al*., 2013; Rukavina *et al*., 2017; Ono *et al*., 2019; Ata *et al*., 2020;), but none is available regarding the Brazilian Pony breed.

Horse breed is the result of natural and artificial selection, and animals of the same breed in different regions can present peculiarities regarding their physiological characteristics (Fonteque *et al*., 2016). The population of the modern horse have particularity comparing to locally adapted breeds of horses subjected to periods of natural selection (Petersen *et al*., 2013). The correct interpretation of hematological and biochemical test results requires specific reference values for studied populations, due to important variations observed in the local environment.

The horse hematological profile reported in the literature (Chiba *et al*., 2016; Kupczynski & Spitalniak, 2015; Wanderley *et al*., 2015) may not be fully applicable under all conditions because it is influenced by multiple factors, including breed, environment and differences in management (Adamu *et al., 2013; Paden et al*., 2014).

The evaluation of blood cells and biochemical hematological profile is part of the clinical assessment routine of horses. Hematological and biochemical blood parameters are important to identifying the breed's physiological variations and are used for clinical diagnosis of infectious and parasitic diseases. They are also used to provide information about the metabolic situation of animals and to monitor recovery in the case of health problems (Adamu *et al*., 2013).

Information about the physiological characteristics of the Brazilian Pony horse breed is limited in the literature. Thus, the aim of this study was to evaluate hematological and biochemical characteristics in clinically healthy stallions and mares of the Brazilian Pony breed.

## Material and Methods

### Animals

The animals, the animals of the Brazilian Pony breed, were raised in the municipality of Campos dos Goytacazes, northern Rio de Janeiro state, Brazil (21o 45 ´15´´ South latitude, 41o 19´28´´ West longitude, and 13 meters above sea level). The feeding of the animals was the same for males and females. All animals were maintained in pastures with Coast Cross grass (*Cynodon dactylon*) throughout the experiment.

According to the scale of Köppen and Geiger, the climate of this region is classified as Aw, with the mean annual minimum temperature of 20° C and mean annual maximum temperature of 36° C). The average annual rainfall is 1,073 mm, with more rainfall in the summer than winter. The precipitation varies 156 mm between the driest month and the wettest month. This experiment was carried out in the autumn period (April to June).

Brazilian Pony animals were evaluated and separated in the following categories: 1- stallions (N= 20, aged 6-13 years); 2- pregnant mares (PM, N= 44, aged 6-19 years) (Figure 1). The animals were clinically examined, performed by veterinarians or specialized techniques, before the experiment and only healthy ones were used. Clinical examination was performed and the body condition score, cardiac frequency and respiratory frequency were measured to determine health. Normal ranges for hematology and plasma chemistry consider the values published by Southwood, L.L. 2013.

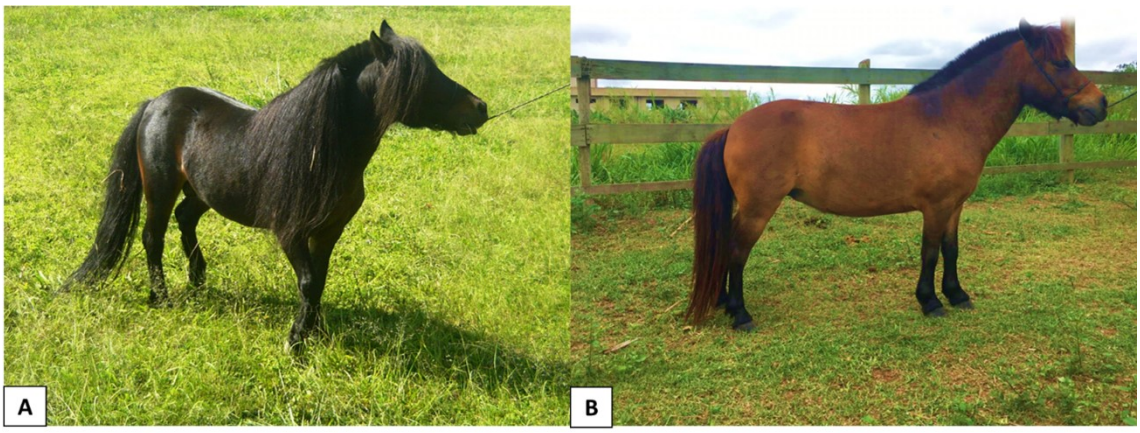


Figure 1. Brazilian Pony breed. Stallion (A). Pregnant mare (B).

### Sample collection and handling

A blood sample was taken from all subjects between 8:00 am and 10:00 am and were then taken once a month between February and November for examination.

Blood samples were obtained from all animals by jugular venipuncture and stored in Vacutainer tubes. For hematological study blood was collected in sterile vial (5mL) using potassium ethylenediaminetetraacetic acid (EDTA-Sigma Chemical Co, St. Louis, MO, USA) as anticoagulant and for collection and preservation of serum 10 ml of blood was collected from jugular vein of each animal in heparinized test tubes. All material was processed in the Clinical Pathology sector (UENF).

The tubes containing blood were kept in slanting position and the blood was allowed to clot. After the blood got clotted, the tubes were transferred to refrigerator at 4 degree centigrade for 12 hours to allow maximum secretion of serum from the clot. Then the serum was pipetted out from the tubes and was centrifuged at 2,500 rpm for 5 minutes to separate unlysed cells and other darts. The clear serum was collected carefully into sterilized vials and stored in the frozen chamber of the refrigerator. No preservatives were added to the serum. Before using the frozen serum for experiment, it was allowed to defrost at room temperature.

The first study compared the males and pregnant mares regarding red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hgb), main corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBC) and differential count basophils (Baso), eosinophils (Eos), band cells (Bands), segmented neutrophils (Neut), lymphocytes (Lym), monocytes (Mono) and platelets (Plat). Hematology assessment was accomplished with an electronic cell counter (MS4®) and the Instant prov (Panótico PA205 Newprov® dye).

The second study compared two stages: the pregnant and non-pregnant mares, with biochemical analysis of urea (Urea), alanine aminotransferase (ALP), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), and creatinine (Creatinine). Were analyzed using standard kits with the aid of a spectrophotometer.

**Statistical analysis**

The data were analyzed using the SAS® software (Statistical Analysis System, University Edition) to verify the mean, median, standard deviation, mean standard error, and maximum and minimum values for each variable. The assumption of normal distribution of all variables was checked (Proc Univariate), and the variables that were not normally distributed (white blood cells, segmented neutrophils, lymphocytes, eosinophils, monocytes) were transformed with ($log10^{+1}$). The means and standard deviations are presented in the original scale.

In the first study involving hematological parame-ters, analysis of variance of repeated measures was carried out with the difference of hematological parameters between males and females using the GLM procedure. Sex effects were considered significant if $P < 0.05$.

In the second study, involving differences of biochemical parameters between pregnant and non-pregnant mares and in the comparison between the stages pregnant mares and non-pregnant mares (nPM), the Tukey test was used at 5 % probability.

## Results and Discussion

Effects were detected of sex on RBC, hemoglobin, VCM and MHCH ($P < 0.05$). Means and standard deviations of hematological parameters in Brazilian Pony horses are presented in Table 1. Only PCV and MCH showed no effect regarding sex ($P > 0.05$).

Table 1. Hematological values (Mean ± standard-desviation) in the Brazilian Pony breed according the sex.

| Parameter | Reference values | Male(N= 20) | Female pregnant(N= 44) |
|---|---|---|---|
| RBC ($x10^6/\mu L$) | 6.2-10.2 | 6.1 ± 1.2[a] | 5.9 ± 1.1[b] |
| PCV (%) | 24-45 | 31.7 ± 4.7[a] | 31.4 ± 4.2[a] |
| Hgb (g/dL) | 11.4-17.3 | 10.8 ± 1.6[a] | 10.4 ± 1.4[b] |
| MCH (pg) | 12.3-19.9 | 17.1 ± 3.5[a] | 17.4 ± 3.4[a] |
| MCV (fL) | 37-58 | 52.1 ± 5.4[b] | 53.6 ± 5.4[a] |
| MCHC (g/dL) | 31-38.6 | 33.4 ± 1.6[a] | 32.8 ± 2.7[b] |

N= number of animals in the study. Values with different letters in the same row are significantly different ($P < 0.05$). Red blood cells (RBC); packed cell volume (PCV); hemoglobin concentration (Hgb); mean corpuscular hemoglobin (MCH); volumetric index (MCV); mean corpuscular hemoglobin concentration (MCHC).

Effects were detected of sex on WBC, eosinophils and neutrophils ($P < 0.05$). However, there were no effects of sex on basophils, band cells, lymphocytes, monocytes and platelets ($P > 0.05$). For the three variables affected by sex (RBC, Hgb and MCHC), the higher means were observed in females (6.1 ± 1.2, 10.8 ± 1.6 and 33.4 ± 1.6 x $10^6$ µ/L respectively) (Table 2).

Table 2. Leukogram values (Mean ± standard-desviation) in the Brazilian Pony breed according the sex.

| Parameter | Reference values | Male(N= 20) | Female pregnant(N= 44) |
|---|---|---|---|
| WBC ($x10^9/L$) | 4.9-10.3 | 6.775 ± 1.778[b] | 7.492± 3.756[a] |
| Baso ($x10^9/L$) | 0-0.3 | 0.005 ± 0.018[a] | 0.005 ± 0.022[a] |
| Eos ($x10^9/L$) | 0-0.8 | 0.213 ± 0.182[b] | 0.283 ± 0.447[a] |
| Bands ($x10^9/L$) | 0-0.2 | 0.025 ± 0.055[a] | 0.441 ± 0.147[a] |
| Neut ($x10^9/L$) | 2.2-8.1 | 3.777 ± 1.224[b] | 4.284 ± 1.794[a] |
| Lym ($x10^9/L$) | 1.7-5.8 | 2.732 ± 1.324[a] | 1.203 ± 0.001[a] |
| Mono ($x10^9/L$) | 0.1-1.0 | 0.247 ± 0.318[a] | 0.245 ± 0.216[a] |
| Plat ($x10^9/L$) | 72-183 | 251.945 ± 417.883[a] | 230.781 ± 125.679[a] |

Values with different letter in the same row are significantly different ($P < 0.05$).
Number of animals (N); white blood cells (WBC); basophils (Baso); eosinophils (Eos); band cells (Bands); segmented neutrophils (Neut); lymphocytes (Lym); monocytes (Mono); platelets (Plat).

The blood biochemistry of mares was evaluated in two categories (non-pregnant and pregnant mares) and we observed effects of the category on ALT and AST ($P < 0.05$). However, there were no effects of mare category on urea, GGT and creatinine ($P > 0.05$). The higher means for AST were observed in non-pregnant mares (Table 3).

Table 3. Biochemistry of non-pregnant and pregnant Brazilian Pony mares.

| | | Mare stage | |
|---|---|---|---|
| Parameter | Reference values | Non-pregnant(N= 18) | Pregnant(N= 44) |
| Urea (mg/dL) | 26-56 | 34.6 ± 8.8[a] | 33.7 ± 9.9[a] |
| ALT (UI/L) | 4-10 | 5.0 ± 1.64[a] | 3.78 ± 1.93[b] |
| AST (UI/L) | 205-555 | 391.3 ± 137.6[a] | 237.9 ± 128.4[b] |
| GGT (UI/L) | 12-45 | 32.5 ± 55.6[a] | 24.9 ± 36.9[a] |
| Creatinine (mg/dL) | 0.6-1.8 | 1.4 ± 0.3[a] | 1.3 ± 1.7[a] |

Values with different letter in the same row are significantly different (P < 0.05).
Number of animals (N); Urea (Urea); alanine aminotransferase (ALT); Aspartate aminotransferase (AST); Gamma glutamyl transpeptidase (GGT).

## Discussion

The means of RBC, hemoglobin and MCHC obser-ved in this study in the Brazilian Pony breed, for males and females were different, where the males presented higher means (Table 1). The male horses were all in reproductive activity. Therefore, during the experiment they presented more anxious behavior and excitement most of time, mainly with the presence of mares, different from the females, who behaved more calmly, with lass movement than stallions. Thus, we can suggest that higher frequency of exercise of males might act to increase the release of RBC into the circulation as result of splenic contraction. And the higher means of Hgb and MCHC can be explained by the fact that the increased activity induced higher oxygen-carrying capacity of the blood to the muscles in activity (Zobba *et al*.,2011). The same authors reported that these hematological variables vary with exercise intensity.

Comparison of the hematological parameters with those reported in other studies showed that the means of RBC, hemoglobin and PCV were lower comparing to the results reported by Andrade *et al*. (2016) studying Brazilian Pony horses from the municipality of Orlando, São Paulo, Brazil. We can suggest these differences were due to different regions where the studies were conducted. The difference of altitude and climate between the regions could cause differences in hematological profiles of animals. The altitude of Orlando is 695 m, higher than Campos dos Goytacazes. According to Vivek *et al*. (2018), hematological profiles of RBC, hemoglobin and PCV tend to increase with the increase of altitude due to greater splenic contraction. In comparison with the study by Zobba *et al*. (2011), with Thoroughbred horses in Italy, the means of RBC, Hgb and PCV of the Brazilian Pony animals in this study were lower, and the mean of MCVC was higher. The higher means of MCHC of the animals we evaluated could be a compensatory reaction to lower values of erythrocytes and hematocrit. We found values of erythrocytes lower than reported by Shawaf *et al*. (2018), who evaluated hematological parameters of Shetland ponies in Saudi Arabia. However, we found higher means of hemoglobin, PCV, MCV, MHC and MCHC. Regarding the leukogram analysis (Table 2), we observed higher means of white blood cell count, eosinophils and neutrophils in females. Some factors can explain this result. Mares' hemogram parameters can be affected by hormonal concentration during their estrous cycle, pregnancy and lactation (Faramarzi *et al*., 2018; Mariella *et al*., 2014) and increase of regulatory T cells after artificial insemination (Awan *et al*., 2016). In contrast, stallions have rarely been found to have eosinophils in the interstitial regions of the testes (Brosnahan 2018).

We observed lower means of leukogram parameters compared with the values presented by Andrade *et al*. (2016), also studying the Brazilian Pony breed (10.7 ± 0.22 x $10^9$/L of WBC, 0.626 ± 0.317 x $10^9$/L of basophils, 4.589 ± 0.697 x $10^9$/L of neutrophils and 4.336 ± 0.701 x $10^9$/L of lymphocytes). These differences could be due to the local climates where the studies were conducted. The present study was performed in a region with a climate characterized by high temperatures, reaching 34° C, and mean annual temperature range from 25 to 28° C (Rua *et al*., 2018). High temperature can cause stress on equines, and this is followed by increased cortisol release (Fenner *et al*., 2016; Rizzo *et al*., 2017).

## Conclusion

The analysis of the blood count and the biochemical of the Brazilian Pony reveals specificities compared to other equine breeds. The data for Brazilian Pony in the present study were obtained in Autumn. Is necessary to consider that the season period can affect the hematological and biochemical parameters. In addition, reference values for young, growing horses should be established separately. It is necessary to further investigate the effects of feed, age, and season. However, the results obtained in this study will be utilized for veterinary practice and appropriate management of Brazilian Pony breed.

**Acknowledgement:** To the owner and employees of pony Farm.

**Conflict of interests:** The authors declare there are no conflicts of interests in the present paper.

**Ethics statement:** This experiment was approved by the Committee on Ethical use of animals (CEUA) of Norte Fluminense State University (UENF) of the state of Rio de Janeiro, according to the standards of the Brazilian Society of Laboratory Animal Science/Brazilian College of Animal Experimentation (SBCAL/COBEA), under protocol number 307.

**Funding Information:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), finance code 001. We are also thankful for the support of fellowship of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and CNE FAPERJ.

**Author contributions: Miguel Alejandro Silva Rua** and **Celia Quirino** Conceived or designed study. **Aylton Bartholazzi Junio** and **Miguel Alejandro Silva Rua**, Performed research. **Celia Quirino** and **Aylton Bartholazzi Junior**, Analyzed data. **Aylton Bartholazzi Junior, Wilder Hernando Ortiz Vega** and **Luis Fonseca Matos**. Wrote the paper. **Luis Fonseca Matos** and **Celia Quirino** Supervision. *Wilder Hernando Ortiz Vega*. Reviewed manuscript.

**Edited by:** Julio Cesar de Souza

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