

# Hematological and biochemical values in Breton breed horses in Brasília-DF

Valores hematológicos e bioquímicos em equinos da raça Bretão em Brasília-DF

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## Abstract

Due to the scarcity of information on Breton horses, the objective was to study hematobiochemical values of this breed. Blood samples were collected from 29 Bretons, males and females, of different ages, in Brasília-DF, distributed into groups, according to age, without distinction of sex (G1): animals from 4 to 9 years old (n=16) and (G2): from 10 to 26 years old (n=13). The horses were also distributed into males and females for comparisons between the sexes. Values for red blood cells, hemoglobin, creatinine, and urea were statistically higher in females. Fibrinogen was higher in males. Lymphocyte values were higher in G1, but mean corpuscular volume, monocytes, neutrophils, and GGT in G2 were higher than G1. The hematocrit value differed between the ages of the females and was higher than that of the males, while the older male animals showed higher values than the young animals. Females presented lower platelet values than males, with older females having higher platelet values than younger females, in the same way as males. G1 females had the highest leukocyte values. The leukocyte values in males of G2 were higher than those of G1. This same behavior occurred for lymphocytes, eosinophils, and creatine kinase. Considering the albumin and aspartate aminotransferase variables, females had the highest values in the group of animals aged 4 to 9 years. Bretons are considered cold-blooded animals, which is consistent with the observed blood count values. However, it is concluded that these horses have biochemical values similar to warm-blooded breeds.

**Keywords:** hematology, Breton, biochemical analysis, hematobiochemical profile.

## Resumo

Devido à escassez de informações sobre equinos da raça Bretão, objetivou-se estudar valores hematobioquímicos da raça. Foram coletadas amostras de sangue de 29 Bretões, machos e fêmeas de diferentes idades, em Brasília-DF, distribuídos entre grupos, segundo idade, sem distinção de sexo (G1): animais de 4 a 9 anos (n=16) e (G2): de 10 a 26 anos (n=13). Os mesmos também foram distribuídos em machos e fêmeas para comparação entre os sexos. Valores para hemácia, hemoglobina, creatinina e ureia foram estatisticamente maiores nas fêmeas. Fibrinogênio foi maior nos machos. Valores de linfócitos do G1 foram maiores, mas o volume corpuscular médio, monócitos, neutrófilos e GGT do G1 foram menores que do G2. Valor do hematócrito difere entre idades das fêmeas e foi superior ao dos machos, os animais machos mais velhos apresentaram valores superiores aos jovens. As fêmeas apresentaram valores de plaquetas menores que os machos, sendo que as mais velhas apresentaram valores de plaqueta maiores que as mais jovens, da mesma forma que os machos. No G1, as fêmeas apresentaram os maiores valores de leucócitos. Os valores de leucócitos nos machos do G2 foram maiores que os do G1. Esse mesmo comportamento ocorreu para linfócitos, eosinófilos e creatinaquinase. Já para as variáveis albumina e aspartato aminotransferase, no grupo de animais de 4 a 9 anos, as fêmeas tiveram os maiores valores. Bretões são animais de sangue frio, o que condiz com os valores do hemograma observados. Porém, conclui-se que estes equinos apresentam valores bioquímicos similares aos de sangue quente.

**Palavras-chave:** hematologia, Bretã, análise bioquímica, perfil hematobioquímico.



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## Introduction

The use of horses is widespread throughout the world, and is based on specific characteristics of each breed, considering the purpose for which it is used. In Brazil, equideoculture is represented by several imported and national breeds (Associação Brasileira de Criadores do Cavalo Bretão, 2021). In order to obtain animals to pull artillery equipment, the Brazilian Army imported Breton stallions, and from subsequent extension programs, the breed was recognized and there was growing dissemination of these animals in the Brazilian equestrian environment (Cavalry Regiment of Guard [RCG], 2021).

The complete blood count (CBC) is a test that allows assessment of the animal's health (Dias et al., 2011; Miknienė et al., 2014), through evaluation of erythrocytes, leukocytes, and platelets, with the quantitative analysis performed by automated analyzers and qualitative analysis from the observation of blood smears (Cowell & Tyler, 2002). The biochemical profile is used in the indication of adaptive processes of the organism, in energy, protein, and mineral metabolism, in addition to providing subsidies in the interpretation of liver, kidney, pancreatic, bone, and muscular functioning (González & Silva, 2006).

Some factors influence hematological and biochemical parameters. These factors may be responsible for variations, such as the particularities of each animal: species, breed, age, and sex (Oliveira et al., 2016). Age is an important factor, resulting in different reference values for parameters in adult, juvenile, or neonate animals. Extrinsic factors refer to the management and environment in which horses are reared: climate, photoperiod, stress, and training (Oliveira et al., 2016; Paludo et al., 2002; Rezende et al., 2006). Several studies have been carried out to show the importance of the hematological and biochemical pattern for each region and breed, and it is extremely important to be aware of these alterations, which contribute to the clinical diagnosis.

Considering that there is a lack in the national literature of laboratory values for Breton horses, the current work seeks to establish the hematological and biochemical values of the breed, comparing them with the standards of the species, according to different age groups and sexes.

## Material and methods

Twenty-nine Breton horses were used, males and females of different ages, belonging to the 32nd Field Artillery Group (GAC) of the Brazilian Army, in Brasília (DF). The animals were kept in individual pens, measuring 2.80 x 4.00 m, with side walls 1.90 m high, allowing communication between the pens. The animals received alfalfa hay (*Medicago sativa*) as voluminous twice a day (10 am and 4 pm) and commercial concentrate divided into three times a day (4 am, 1 pm, and 8 pm), with free access to water and mineral salt. The animals were adapted to the environment, climate, and handling. Previous examinations were performed, and no animals showed alterations in clinical parameters (heart rate, respiratory rate, and temperature), in addition, no animals were under or overweight, and the diet was adequate for the animals. The daily activities of the animals included cannon traction, trotting (15 to 20 km), and cart traction. The animals were divided into two experimental groups, according to age, without distinction of sex (G1): animals from 4 to 9 years old (n=16) and (G2): animals from 10 to 26 years old (n=13). The same animals were also divided into two experimental groups, males (n=22) and females (n=7), for comparisons between the sexes.

The research in question was approved by the UNICEPLAC Ethics Committee on the Use of Animals (License 021/2016). All blood samples were collected in the morning, after the supply of food, before the animals started work, by venipuncture of the jugular vein, with 40x12 needles and 5mL syringes. The needles were uncoupled and the volume transferred to two tubes, one containing ethylenediaminetetraacetic anticoagulant (EDTA) and the other without anticoagulant. Every procedure was preceded by local antisepsis with iodized alcohol. Subsequently, the samples were conditioned in styrofoam containing ice, and immediately sent to the Clinical Analysis Laboratory of UNICEPLAC for hematological and biochemical analysis.

The blood with EDTA was analyzed using SDH-5 equipment (Labtest®- Brazil) to check the following hematological standards: hematocrit (Ht), mean corpuscular volume (MCV), red blood cell count (RBC), hemoglobin (Hb), of mean corpuscular hemoglobin (MCHC), and total leukocyte count (WBC), quantitatively and semi-automatically. Blood smears were also performed on glass slides and stained with panoptic to perform the differential leukocyte count and platelet count

(PLT). To measure total protein and fibrinogen, a portable refractometer was used. To analyze the biochemical profile, the samples without EDTA were centrifuged to obtain the serum, and the following variables were analyzed: aspartate aminotransferase (AST), creatine kinase (CK), urea, creatinine, gamma glutamyl transferase (GGT), total plasma proteins (TP), and albumin, which were measured with automatic equipment Labmax 100 (Labtest® - Brazil) using the respective Labtest® reagents.

To test the effect of the factors Age (group 1 = 4 to 9 years and group 2 = 10 to 26 years) and Sex (Female and Male) on various biochemical and hematological measurements collected from Breton horses, analysis of variance was used. Since in this experiment different measures are collected from the same individual (measurements in triplicate) and there is an imbalance between the number of individuals in the group of females and males, the use of classical analysis of variance (ANOVA) could undermine the assumptions about independence between observations, normal distribution of errors, and homoscedasticity of variance, and consequently generate unreliable results. Thus, to ensure the quality of the final statistical results, it was decided to include the repeated measure on the same individuals as a random effect in the analysis of variance, which is characterized as Mixed ANOVA. When the normal distribution did not accommodate the variability of the data well, better probability distributions were sought through generalized linear models (ANODEV). Whether using ANOVA or ANODEV, classical or mixed, the objective was to separate the biochemical and hematological variables as follows: 1) variables influenced only by sex 2) variables influenced only by age 3) variables influenced by sex and age, without interaction 4) variables influenced by sex and age, with interaction. The analyses were developed in the R statistical language program, and the significance level adopted in all analyses was 5%.

## Results

The results obtained for the Breton breed, irrespective of sex and age, are shown in Tables 1-2, where the hematological and biochemical range can be observed.

Regarding sex, the variables RBC, Hb, fibrinogen, creatinine, and urea presented statistical differences between females and males of the animals under study, as shown in Table 3. In most cases, on average, the values were higher in females, with the exception of fibrinogen, which was higher in males.

Regarding age, the lymphocyte values of G1 were statistically higher ( $p < 0.0001$ ) than the values of G2. The values of MCV ( $p < 0.0001$ ), monocytes ( $p = 0.003855$ ), neutrophils ( $p < 0.0001$ ), and GGT ( $p = 0.00335$ ) in G1 were lower when compared to G2 (Table 4).

The variables Ht, PLT, and MON were influenced by age and sex, with no interaction (Table 5). For Ht, in both age groups, females showed statistically higher values than males. The males in G2 showed higher values than the males in G1, unlike the female age groups, which statistically showed the same value. For platelets, females had lower values than males within each age group. In both sexes, G2 demonstrated higher values than G1. In relation to MON, males showed higher values than females, within each age group. However, when comparing animals of the same sex between age groups, G2 presented higher MON values.

Some variables were influenced by sex and age, with interactions between them (Table 6). Within G1, females had the highest LEU values, with the opposite occurring for G2, where males had the highest values. This same behavior occurred for the absolute values of lymphocytes and eosinophils, for the relative values of eosinophils, and for CK. Differently, for the albumin and AST variables, while for G1, females obtained the highest values, in G2 they had the lowest values.

## Discussion

For each breed there is a specific hematobiochemical profile according to origin and functionality. In this study, Breton animals were used, which, according to Pađen et al. (2014) are cold-blooded animals. The blood count values observed in the Breton horses used in this work match this classification, considering that these horses have a docile temperament and a low metabolic rate (Orozco et al., 2007). The samples were analyzed in triplicate to increase reliability and eliminate undesirable effects (Anjos, 2008), making it possible to establish the variability (standard deviation) in the analysis technique and safe limits for significant data. To avoid the disadvantage of blood

**Table 1.** Mean  $\pm$  standard deviation values of hematological data in Breton horses, regardless of age and sex.

| Variables                                     | (Mean $\pm$ Standard deviation) | Interval         |          |                 |
|---|---------------------------------|------------------|----------|-----------------|
| RBC ( $\times 10^6$ /mm <sup>3</sup> )        | 7.24 $\pm$ 0.94                 | 6.44 - 9.41      |          |                 |
| Hb (g/dL)                                     | 11.11 $\pm$ 1.34                | 10.30 - 14.56    |          |                 |
| Ht (%)  | 35.19 $\pm$ 3.82                | 32.96 - 46.85    |          |                 |
| MCV (fL)                                      | 47.78 $\pm$ 8.58                | 46 - 55          |          |                 |
| MCHC (g/dl)                                   | 31.40 $\pm$ 0.7                 | 30.8 - 32.73     |          |                 |
| TP (g/dL)                                     | 6.84 $\pm$ 0.48                 | 6.4 - 7.8        |          |                 |
| Fibrinogen (mg/dL) (mg/dL) dL <sup>-1</sup> ) | 0.43 $\pm$ 0.28                 | 0.2 - 1          |          |                 |
| PLT ( $\times 10^3$ )                         | 124,200 $\pm$ 30,030            | 100,000 - 87,750 |          |                 |
| LEUC ( $10^3$ / $\mu$ L)                      | 7,571 $\pm$ 1,560               | 6,670 - 11,248   |          |                 |
|   | <b>%</b>                        | <b>Absolute</b>  | <b>%</b> | <b>Absolute</b> |
| NEU ( $10^3$ / $\mu$ L)                       | 60.54 $\pm$ 12.65               | 4.613 $\pm$ 1481 | 51 - 86  | 3.515 - 7.987   |
| LYMP ( $10^3$ / $\mu$ L)                      | 35 $\pm$ 12                     | 2.629 $\pm$ 1034 | 27 - 65  | 1.853 - 5.231   |
| MON ( $10^3$ / $\mu$ L)                       | 1.34 $\pm$ 1.24                 | 98 $\pm$ 90      | 0 - 4.77 | 0 - 343         |
| EON ( $10^3$ / $\mu$ L)                       | 2.9 $\pm$ 2.53                  | 228 $\pm$ 219    | 1 - 9.77 | 74 - 858        |
| BAS ( $10^3$ / $\mu$ L)                       | 0.17 $\pm$ 0.43                 | 11 $\pm$ 29      | 0 - 1.77 | 0 - 99          |

RBC = red blood cell; Hb = hemoglobin; Ht = hematocrit; MCV = Mean Corpuscular Volume; MCHC = mean corpuscular hemoglobin concentration; TP = total plasma proteins; PLT = platelets; LEUC = leukocytes; NEU = neutrophils; LYMP = lymphocytes; MON = monocytes; EON = eosinophils; BAS = basophil.

**Table 2.** Mean  $\pm$  standard deviation values of biochemical data in Breton horses, regardless of age and sex.

| Variables          | (Mean $\pm$ Standard deviation) | Interval        |
|--------------------|---------------------------------|-----------------|
| Proteins (g/dL)    | 8.32 $\pm$ 0.70                 | 7.86 - 9.55     |
| Albumin (g/dL)     | 2.95 $\pm$ 0.28                 | 2.77 - 3.56     |
| Urea (mg/dL)       | 35.01 $\pm$ 12.92               | 26.00 - 73.15   |
| Creatinine (mg/dL) | 1.48 $\pm$ 0.31                 | 1.18 - 2.17     |
| GGT (U/L)          | 17.95 $\pm$ 8.66                | 13.00 - 43.05   |
| AST (U/L)          | 341 $\pm$ 101                   | 278.25 - 700.63 |
| CK (U/L)           | 195.88 $\pm$ 79.61              | 142.00 - 468.40 |

GGT = gamma glutamyl transferase; AST = aspartate aminotransferase; CK = creatine kinase.

**Table 3.** Means  $\pm$  standard deviation of measurements that showed an effect of sex, according to the analysis of variance.

| Variables                              | Females                        | Males                          |
|--|--------------------------------|--------------------------------|
| RBC ( $\times 10^6$ /mm <sup>3</sup> ) | 7.53 $\pm$ 0.91 <sup>a</sup>   | 7.14 $\pm$ 0.94 <sup>b</sup>   |
| Hb (g/dL)                              | 11.54 $\pm$ 1.06 <sup>a</sup>  | 10.97 $\pm$ 1.41 <sup>b</sup>  |
| Fibrinogen (mg/dL)                     | 0.34 $\pm$ 0.23 <sup>b</sup>   | 0.45 $\pm$ 0.29 <sup>a</sup>   |
| Creatinine (mg/dL)                     | 1.66 $\pm$ 0.32 <sup>a</sup>   | 1.39 $\pm$ 0.26 <sup>b</sup>   |
| Urea (mg/dL)                           | 40.33 $\pm$ 17.14 <sup>a</sup> | 34.18 $\pm$ 12.24 <sup>b</sup> |

RBC = red blood cell; Hb = hemoglobin. Different letters indicate a significant difference ( $P < 0.05$ ).

collection using the vacuum system, a 40x12 needle and a 5 mL syringe were used for collection, with blood transfer to the tubes in a short time interval, in order to prevent hemolysis, as there was no pressure exerted by the vacuum, preventing cells from colliding due to the high speed,

**Table 4.** Means  $\pm$  standard deviation of measurements that showed an effect of age, according to analysis of variance.

| Variables      | G1 (4 to 9 years)                                 | G2 (10 to 26 years)                                |
|----------------|---|--|
| MCV (fL)       | 45.15 $\pm$ 10.54 <sup>b</sup>                    | 51.03 $\pm$ 3.42 <sup>a</sup>                      |
| MON (absolute) | 78.52 $\pm$ 72.97<br>(median = 72.5) <sup>b</sup> | 122.97 $\pm$ 104.70<br>(median = 106) <sup>a</sup> |
| LYMP           | 40.46 $\pm$ 11.74 <sup>a</sup>                    | 28.62 $\pm$ 9.58 <sup>b</sup>                      |
| NEU            | 55.25 $\pm$ 11.80 <sup>b</sup>                    | 67.05 $\pm$ 10.74 <sup>a</sup>                     |
| NEU (absolute) | 4267.09 $\pm$ 1197.03 <sup>b</sup>                | 5037.68 $\pm$ 1706.09 <sup>a</sup>                 |
| GGT            | 15.13 $\pm$ 6.04 <sup>b</sup>                     | 20.41 $\pm$ 10.94 <sup>a</sup>                     |

MVC = mean corpuscular volume; MON = monocytes; LYMP = lymphocytes; NEU = neutrophils; GGT = gamma glutamyl transferase. Different letters indicate significant statistical difference ( $P < 0.05$ ).

**Table 5.** Means  $\pm$  standard deviation of measurements that showed an effect of age and sex (no interaction), according to analysis of variance.

| Variables | G1 (4 to 9 years)               |                                 | G2 (10 to 26 years)              |                                  | P-Value<br>Age; Sex   |
|-----------|---------------------------------|---------------------------------|----------------------------------|----------------------------------|-----------------------|
|           | Females                         | Males                           | Females                          | Males                            |                       |
| HT (%)    | 36.92 $\pm$ 3.46 <sup>a</sup>   | 33.79 $\pm$ 1.99 <sup>b</sup>   | 37.01 $\pm$ 4.47 <sup>a*</sup>   | 35.65 $\pm$ 4.91 <sup>b*</sup>   | 0.000224;<br>0.001105 |
| PLT       | 102.71 $\pm$ 16.29 <sup>b</sup> | 122.36 $\pm$ 33.11 <sup>a</sup> | 116.39 $\pm$ 19.73 <sup>b*</sup> | 137.33 $\pm$ 27.94 <sup>a*</sup> | 0.00400;<br>0.00715   |
| MON       | 0.75 $\pm$ 0.62 <sup>b</sup>    | 1.11 $\pm$ 1.06 <sup>a</sup>    | 0.89 $\pm$ 0.78 <sup>b*</sup>    | 2.00 $\pm$ 1.49 <sup>a*</sup>    | 0.003855;<br>0.023030 |

HT = hematocrit; PLT = platelets; MON = monocytes. Different letters compare sex within each age group. \*Same-sex animals that differ between age groups ( $P < 0.05$ ).

**Table 6.** Means  $\pm$  standard deviation that showed an effect of age and sex (with interaction), according to analysis of variance.

| Variables   | G1 (4 to 9 years)                  |                                   | G2 (10 to 26 years)               |                                    |
|-------------|------------------------------------|-----------------------------------|-----------------------------------|------------------------------------|
|             | Females                            | Males                             | Females                           | Males                              |
| LEU         | 8940.83 $\pm$ 1761.20 <sup>a</sup> | 7305.00 $\pm$ 873.40 <sup>b</sup> | 6958.89 $\pm$ 562.30 <sup>b</sup> | 7527.00 $\pm$ 2032.75 <sup>a</sup> |
| LYMP (abs.) | 4026.54 $\pm$ 913.35 <sup>a</sup>  | 2808.27 $\pm$ 914.87 <sup>b</sup> | 2153.69 $\pm$ 770.28 <sup>a</sup> | 1997.50 $\pm$ 624.05 <sup>a</sup>  |
| EOS         | 4.08 $\pm$ 2.39 <sup>a</sup>       | 2.94 $\pm$ 2.67 <sup>b</sup>      | 2.22 $\pm$ 1.72 <sup>b</sup>      | 2.57 $\pm$ 2.60 <sup>a</sup>       |
| EOS (abs.)  | 365.54 $\pm$ 212.59 <sup>a</sup>   | 216.11 $\pm$ 202.57 <sup>b</sup>  | 150.52 $\pm$ 116.35 <sup>b</sup>  | 209.29 $\pm$ 250.95 <sup>a</sup>   |
| Albumin     | 2.91 $\pm$ 0.34 <sup>b</sup>       | 2.97 $\pm$ 0.26 <sup>a</sup>      | 2.99 $\pm$ 0.19 <sup>a</sup>      | 2.88 $\pm$ 0.29 <sup>b</sup>       |
| AST         | 300.83 $\pm$ 102.86 <sup>b</sup>   | 361.78 $\pm$ 114.59 <sup>a</sup>  | 363.25 $\pm$ 164.22 <sup>a</sup>  | 319.63 $\pm$ 61.76 <sup>b</sup>    |
| CK          | 288.50 $\pm$ 152.06 <sup>a</sup>   | 192.11 $\pm$ 67.43 <sup>b</sup>   | 173.56 $\pm$ 75.01 <sup>b</sup>   | 177.67 $\pm$ 38.33 <sup>a</sup>    |

LEU = leukocytes; EOS = eosinophils; AST = aspartate aminotransferase; CK = creatine kinase. Different letters indicate that the means between the sexes within each age group differ statistically at the 5% level of significance.

and the needle gauge minimized damage to cellular elements and the blood vessel, as reported by Brockus (2011). According to research carried out by Paludo et al. (2002) and Rezende et al. (2006), Bretons are adapted to the confinement and the exercises allied to the dry and hot climate of the Federal District. The collection of samples before exercise and in the morning, in an environment to which the animals were accustomed, reduced the chances of liquid losses through sweating and respiratory tract, or of splenic contraction due to stress, consequently avoiding the reduction of blood plasma volume, hemoconcentration, and increased hematocrit (Oliveira et al., 2016).

The studies closest to the current research for Bretons, without distinction of sex and age, are by Paludo et al. (2002), who studied Thoroughbred Race, Brazilian Equestrian, and Breton horses between 4 and 13 years old. As in the current methodology, the Breton group that was evaluated at 6:00 am, before exercise, presented similar values; Leuc of 6.41 ( $\pm 0.96 \text{ mm}^3$ ), RBC 6.67 ( $\pm 1.28 \times 10^6 \text{ mm}^3$ ), Hb 10.4 ( $\pm 0.63 \text{ g/dL}$ ), Ht 32.17 ( $\pm 5.78\%$ ), and TP 6.33 ( $\pm 0.42 \text{ g/dL}$ ).

Although no studies have compared leukograms of warm-blooded and cold-blooded horses, the ranges found for monocytes (0-343  $\text{mm}^3$ ), basophils (0-99  $\text{mm}^3$ ), and neutrophils (3515-7987  $\text{mm}^3$ ) for animals of the same breed as those used in the current study, regardless of age and sex, were lower than described by Grondin and Dewitt (2010) for warm-blooded animals (0-1000  $\text{mm}^3$ ; 0-290  $\text{mm}^3$ ; 2260-8580  $\text{mm}^3$ , respectively). In stressed animals, physiological leukocytosis occurs due to the release of corticosteroids and epinephrine, which generates the release of neutrophils from the marginal compartment to the peripheral circulation, so adaptation to the environment and manipulation were important in this study. When these variables are compared with those of the Hucul pony, animals also considered cold-blooded, the Bretons have higher values, as observed in the study carried out by Cywińska et al. (2015), who determined reference intervals for these animals: MON 0.02 ( $\pm 0.05 \text{ mm}^3$ ), NEU 6.8 ( $\pm 1.8 \text{ mm}^3$ ), and BAS 0.07 ( $\pm 0.09 \text{ mm}^3$ ); while for Bretons, the values found were: MON 1.34 ( $\pm 1.24 \text{ mm}^3$ ), NEU 60.54 ( $\pm 12.65 \text{ mm}^3$ ), and BAS 0.17 ( $\pm 0.43 \text{ mm}^3$ ).

Differently from what was expected, Hb values were higher in females, as normally the highest values in horses are found in males, due to their greater need for oxygen for the musculature, which is more robust in males. The values of RBC, Hb, creatinine, and urea were higher in females. According to Fonteque et al. (2016), there was a statistical difference when comparing the fibrinogen indices between males 363.22 ( $\pm 96.03 \text{ mg/dL}$ ) and females 348 ( $\pm 91.83 \text{ mg/dL}$ ) of the Campeiro breed, also observed for Bretons, where males showed values of 0.45 ( $\pm 0.29 \text{ mg/dL}$ ) and females 0.34 ( $\pm 0.23 \text{ mg/dL}$ ).

In the current study, a statistically ( $p=0.0619$ ) higher difference was observed in serum concentrations of circulating urea among females 40.33 ( $\pm 17.14 \text{ mg/dL}$ ) in relation to males 34.18 ( $\pm 12.24 \text{ mg/dL}$ ), regardless of age. Kaneko et al. (2008), who did not specify breed, sex, or age of horses, reporting values (10-24  $\text{mg/dL}$ ), considerably lower than measured in the current work.

Andreazzi et al. (2015) mention a value of 1.51 ( $\pm 0.22 \text{ mg/dL}$ ) for creatinine in mares without distinction of breed, a value close to that measured in the mares of this work, which presented 1.66 ( $\pm 0.32 \text{ mg/dL}$ ). In the study by Matrone et al. (2007) with English Purebred (EPB) females and males, cite the value of 1.68 ( $\pm 0.18 \text{ mg/dL}$ ). In both studies, the values differ from those of the males found in this study, which obtained a value of 1.39 ( $\pm 0.26 \text{ mg/dL}$ ). The values mentioned by Kaneko et al. (2008) (1.2 to 1.9  $\text{mg/dL}$ ) were the closest to the study. There were no significant differences between the horses in this study and the other results obtained in other studies; although there are changes between the sexes, the variations are within the standards described in the literature.

The values of MCV ( $p<0.0001$ ), monocytes ( $p=0.003855$ ), neutrophils ( $p<0.0001$ ), and GGT ( $p=0.00335$ ) of G1 were lower when compared to G2. Concerning G1, the result was close to the GGT enzyme found by Rico et al. (1977), 13 ( $\pm 6 \text{ U/L}$ ) in horses without distinction of sex, breed, or age. Balarin et al. (2005), in a study using 30 male and female EPB horses at rest and submitted to different types of intensity exercise, females aged 36 to 48 months obtained a value of 14.71 ( $\pm 3.69 \text{ UI/L}$ ). The same was observed with 180-day-old foals of the Campeiro and Pantaneiro breeds 14.7 ( $\pm 1.32 \text{ U/L}$ ) cited by Vieira et al. (2018) in his work, which we can consider as a result close to G1. It is noted that the measurements obtained for the GGT enzyme in this study in G1, compared with the authors mentioned, are within the standard, with no significant discrepancies between the values.

For the GGT value found in G2, 20.41 ( $\pm 10.94 \text{ UI/L}$ ), the closest results were those found by Vieira et al. (2018) in 60-day-old Campeiro and Pantaneiro foals 20.58 ( $\pm 5.43 \text{ U/L}$ ) and by Balarin et al. (2005), in females (36 to 48 months), with a value of 24.48 ( $\pm 7.13 \text{ UI/L}$ ), but under different conditions from the animals studied herein. Other values found for males aged between 24 and 36 months were 11.85 ( $\pm 2.55 \text{ UI/L}$ ) and for females 11.78 ( $\pm 2.32 \text{ UI/L}$ ), for both groups of animals, from 36 to 48 months the males showed values of 27.94 ( $\pm 8.41 \text{ UI/L}$ ) and 26.65 ( $\pm 8.40 \text{ UI/L}$ ). It is observed that these presented lower and higher means when compared

with the results of G1 and G2 in the current study. It is worth mentioning that the values found by Balarin et al. (2005) and used to compare with those found in the current study, were for horses at rest before performing exercise at intensity.

In the current study, there was no statistical difference in age for the variable RBC, where generally the response of older individuals may present a reduction in the action of sympathetic nervous activity and/or circulating catecholamines, resulting in levels of distinct splenic contraction, determining variation in the number of red blood cells. In the studies by Cebulj-Kadunc et al. (2002) with Lipizzaner, the red blood cell count in males,  $8.16 (\pm 0.06 \times 10^6 / \text{mm}^3)$  was higher than in females,  $7.53 (\pm 0.07 \times 10^6 / \text{mm}^3)$ , differently from Bretons, where RBC values were higher in females,  $7.53 (\pm 0.91 \times 10^6 / \text{mm}^3)$ , than in males,  $7.14 (\pm 0.94 \times 10^6 / \text{mm}^3)$ . Also according to this study, the Ht (H<sub>1-1</sub>) of Lipizzaners was higher than in the horses with warm blood (0.37-0.49) and lower (0.32-0.52) than horses with cold blood, which is explained due to the fact that the warm-blooded horses have a smaller RBC size and, consequently, a greater number, when compared to the cold-blooded horses. For the Bretons (32.96 - 46.85) this coincides with the findings for the animals with cold blood, consistent with the citation that these animals have lower hematimetric values, in addition to differing between groups, with values for females being higher than for males, and the animals of G2 (10-26 years) presenting higher values than those of G1 (4-9 years).

Satué et al. (2017) point out that the normal value of platelets in horses varies between 100-350,000/ $\mu\text{L}$ . Comparing between the Bretons, the females had lower platelet values than the males, but when comparing the results of the females of different ages, the older animals (10-26 years) had higher platelet values than the younger animals (4-9 years), in the same way as the males, where the older males presented higher values than the younger males.

G1 lymphocyte values were statistically ( $p < 0.0001$ ) higher than G2 values, which is expected, as the leukocyte response to corticosteroids may vary according to age, being higher in young, more stressed animals (Paludo et al., 2002). For monocytes and lymphocytes (absolute), males of both age groups showed higher values than females, however, in G2 males the values were highest. This same behavior occurred for the variables eosinophils (% and absolute) and CK, and eosinophilia was expected, since in older animals it may be associated with prolonged exposure to the parasites.

The CK enzyme has the function of phosphorylation of creatine and actively participates in energy metabolism of various tissues, including muscle tissue. After exercise or muscle injury, it is rapidly released into the bloodstream and its activity peaks 4-12 hours later (Kingston, 2008). According to Balarin et al. (2005), the measurement obtained for CK was  $185.37 (\pm 39.70 \text{ UI/L})$  for males aged 24 to 36 months, a lower mean value than the G1,  $192.11 (\pm 67.43 \text{ UI/L})$  males described here. Females of the same age group had a mean of  $170.26 (\pm 47.55 \text{ UI/L})$ , thus the other females of 36 and 48 months of age showed a value of  $172.83 (\pm 47.04 \text{ UI/L})$ , similar results for both sexes of the G2 in the current study, mainly for the females that presented values of  $173.56 (\pm 75.01 \text{ UI/L})$ . The other females of 36 and 48 months of age had a mean of  $187.10 (\pm 86.80 \text{ UI/L})$ , a mean close to that of G1 males,  $192.11 (\pm 67.43 \text{ UI/L})$ . Faced with a muscle injury, the joint analysis of the serum activity of AST and CK makes it possible to identify the time elapsed since the injury. In addition to muscle enzymes, other parameters can be used to assess exercise intensity and the animal's general health, such as urea, creatinine, and albumin (Paludo et al., 2002).

Regarding the albumin and AST variables, in the G1 group, females had the highest values, and in the G2 group the values were lower (Table 6). Matrone et al. (2007) obtained the measurement of  $318.1 (\pm 129.5 \text{ UI/L})$  for AST, a result close to G2 for males  $319.63 (\pm 61.76 \text{ UI/L})$ . In the study by Balarin et al. (2005), the mean of  $300.99 (\pm 84.94 \text{ UI/L})$  for females aged 36 to 48 months was similar to the value of females from G1  $300.83 (\pm 102.86 \text{ UI/L})$ . Considering the results referring to the other ages used by Balarin et al. (2005), AST measurements were lower for both females and males in this study. Kaneko et al. (2008), obtained a mean of  $296 (\pm 70 \text{ U/L})$ , demonstrating the minimal differences between the Bretons and other authors.

Regarding albumin, Breton females had higher values in the G1 group than the G2 group, this being close to that measured for mares considered as cold-blooded  $28.53 (\pm 2.18 \text{ g/dL})$ , in the study of Pađen et al. (2014).

## Conclusions

It is concluded that Breton horses present hematological values similar to animals considered cold-blooded and biochemical values similar to those considered warm-blooded, as well as variations in relation to animal age and sex. Thus, these characteristics must be taken into account when interpreting the blood count and biochemistry in this breed, in horses that are in conditions similar to those of the current study.

## Ethics statement

The research has been approved at the UNICEPLAC Ethics Committee on the Use of Animals (License 021/2016).

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## Conflict of interests

No conflicts of interest have been declared.

## Authors' contributions

FFCBC, BHVM, PAS, TGM, GAS and RCC - Development of methodology; preparation and writing the initial draft. HCAT - Application of statistical study data, Review and Editing manuscript. FFCBC, BHVM, TGM and RCC - Writing, Review and Editing manuscript.

## Availability of complementary results

Place where the study was conducted: the work was carried out 32nd Field Artillery Group (GAC) of the Brazilian Army in Brasília, DF, Brazil and Laboratório de Patologia Clínica Veterinária/ Departamento de Medicina Veterinária, UNICEPLAC, Brasília, DF, Brazil.

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