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Hematological and blood chemistry values of donkeys (*Equus africanus asinus*) in different management systems

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Summary: In order to test the hypothesis that donkeys, housed in different management systems, present minimal variations in the concentration of various blood values, an experiment was carried out to evaluate blood values in Northeastern donkeys at maintenance, of both sexes, in different management systems. Sixty-two Northeastern donkeys were used, of both sexes, adults, healthy and housed in three management systems: Limoeiro (LIM), Natal (NAT) and Mirandiba/Salgueiro (MS), three different regions in the Northeast of Brazil. Body condition score, red blood cell count, haemoglobin, hematocrit, MCV, MCHC, leukocytes, lymphocyte, other white cells, RDW-CV, RDW-SD, platelets, MPV, total plasma protein, albumin, urea, creatinine, uric acid, glutamine, glutamate, triglycerides, total cholesterol, non-esterified fatty acids, AST, ALT, AP, CK, GGT, Ca, P, Cl, Fe, Na, K and Se were evaluated. Results were submitted to ANOVA with two factors (sex and rearing site) and to Tukey test, considering $p<0.05$. Results showed no difference in body score ($p>0.05$) and, in blood biomarkers, it was observed that RDW-CV and MPV were higher in females ($p<0.05$). In G-MS, red blood cells, Hb, HT and lymphocytes were lower than in G-LIM and G-NAT ($p<0.05$). In G-LIM, MCV and RDW-SD were lower ($p<0.05$). In relation to metabolic biomarkers, high concentrations of UA, TRIG, TC, NEFA, Ca and P ($p<0.05$) were observed in females. Higher concentrations of UA, TRIG, TC, P and Cl were detected in G-LIM ($p<0.05$), and G-NAT had the highest values for URE, GLU and CK ($p<0.05$). Results indicated that, even when kept under different management systems, donkeys may present some haematological and blood chemistry values with similar concentrations, and the few differences found may not present clinical significance for the evaluation of these animals. It was concluded that donkeys may present significant variations in different blood and metabolic parameters, when they are of different sexes and/or housed in different management systems. The red series, electrolytes and trace elements were not very sensitive to identify variations related to gender and/or rearing site groups. However, the count of lymphocytes, the combination of MVC with RDWs, CK, as well as concentrations of NEFA and triglycerides, can be used to better understand the adaptations of different management conditions or breeding systems, since they can be modified by the evaluated conditions, contributing to a better understanding of the state of health or disease of the animals of that species.

Keywords: creatine kinase, glutamine, heamogram, glutamine, mineral, NEFA

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Introduction

The maintenance of donkeys in farms or breeding centers is not common around the world, as the majority of the population regularly rears these equids without much interest (*Rocha* et al. 2018). This situation has made it difficult to characterize various blood biomarkers, which are important for the understanding of health and disease states of these animals. Exceptions can be found in donkeys' rearing for the production of mules or in breeds protected due to the risk of extinction and in 'sanctuaries' (*Gravena* et al. 2010, *Fantuz* et al. 2013).

Donkeys are rustic animals well adapted to the diverse conditions of arid and semi-arid regions, being used in many activities as working animals (*Nobre* 1999). Brazilian breeds evolved from animals brought by the Portuguese and other migrants to the different regions and thus were submitted to years of selection in different environments, forming three breeds with different zootechnical characteristics. The Northeastern are small ones (130 kg) and are a typical north African type, Pêgas are middle size (250 kg) and is used to produce saddle mules because they are a gaited breed and, finally, the Brazilians are large ones (330 kg) that are used to produce draft mules for agriculture working (*Nobre* 1999, *Mariante* and *Cavalcante* 2008). However, in recent decades, these animals have lost their importance due to the strong agriculture mechanization and the increasing urbanization of Brazil, especially of the Northeast region, where donkeys represented an important animal labor force. Hence, many of these animals were abandoned in Caatinga and in small urban areas. Then, to avoid road accidents and unbalanced reproduction, governments began to seize donkeys in gathering centers or sanctuaries, bringing new challenges for their rearing and breeding.

In this sense, the analyzes of blood, hematological and biochemical biomarkers may provide important information about donkeys and may be used as indicators of health and nutritional conditions, and also to assess the effects of physical exercises (*Manso Filho* et al. 2009, *Ferreira* et al. 2017). Thus, the knowledge of blood biomarkers has been used for the development of exercise programs for working animals and nutritional programs for the preservation of animals in "sanctuaries". Due to the current characteristics of donkeys' breeding and maintenance, which makes it difficult to obtain

and analyze blood biomarkers, and to test the hypothesis that donkeys housed in different management systems in the Northeast region present minimal variations in the concentration of various blood values. A study was developed aiming at evaluating body score and blood parameters in Northeastern donkeys of different sexes and kept in different management systems. The characterization of these parameters may contribute to better understand the effects of gender and management systems on these animals, as well as the adaptations to their ecosystems.

Material and methods

Animals and management system

Sixty-two Northeastern donkeys, at maintenance, of both sexes, adult, healthy and within racial standards were used (*Nobre* 1999). They were housed in three management systems: Group Limoeiro (G-LIM, 7°52'52" South; 35°29'40" West), Group Natal (G-NAT, 5°48'07" South, 35°14'27" West) and Group Mirandiba/Salgueiro (G-MS; 8°6'57" South, 38°43'46" West).

G-LIM and G-NAT animals were managed on a semi-intensive basis and fed good quality chopped elephant grass (Pennisetum purpureum), supplemented with common salt. However, G-LIM had free access to mixed pasture with rest areas (~10 hectares), whereas G-NAT only had access to rest areas without extra forage (~2 hectares). In contrast, G-MS were kept in Caatinga forest (190 hectares), which is a semi-arid region with very short rain season (< 800 mm/year of rain), and fed on native vegetation, mostly dry leaves of leguminous trees and grass, without salt supplementation. All animals received water ad libitum. It should also be noted that pregnant or lactating females, young animals (< 3 years), old animals (>15 years) and animals with any sign of disease (chronic, acute or with locomotion problems) were excluded from this evaluation.

Body condition score

It was used the method described in literature that classifies animals into five categories: 1 or cachectic, 2 or thin, 3 or ideal, 4 or overweight, 5 or obese (*Comac* 1997).

Sampling and laboratory analysis

Blood samples were collected one time from each donkey, after overnight fastening, by a vacuum collection system for haematological and blood chemistry analyzes during same week of the year, in November, before the rain season in all three places. The haematological analyzes were performed in a period of less than 24 hours after collection and in a semi-automated haematological equipment (Roche® Poch 100iv), measuring: red blood cells (RBC), haemoglobin (Hb), haematocrit (HT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), leukocytes (LEU), lymphocytes (LINF), other white cells, red cell distribution width – coefficient of variation (RDW-CV), red cell distribution width – standard deviation (RDW-SD), platelets, mean platelet volume (MPV). Biochemical chemistry were performed on semi-automated equipment (Doles® D-250) using commercial kits, to measure: total plasma proteins (TPP), albumin (ALB), urea (URE), creatinine (CREAT), uric acid (UA), alkaline phosphatase (ALP), creatine kinase (CK), gamma-glutamyltransferase (GGT), triglycerides (TRIG), total cholesterol (CHOL), non-esterified fatty acids (NEFA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), calcium (Ca), phosphorus (P), chlorine (Cl), iron (Fe). Sodium (Na) and potassium (K) determinations were performed in an automatic biochemical analyzer (Labmax® 240). For the analysis of glutamine (Gln) and glutamate (Glu), an aliquot of the samples was immediately acidified and neutralized, and the analysis was carried out using the enzymatic method (*Manso Filho* et al. 2009). For the quantification of Selenium (Se), serum samples were processed by microwave-assisted digestion in a microwave oven (MARS-Microwave System) and, for the quantification of Se, an hydrided generation atomic absorption spectrometry (HGAAS, Varian/Spectr AA-220) was used.

Statistical analysis

The program SigmaStat 13.0 for Windows (Systat Software Inc., USA) analyzed the results obtained, using ANOVA, with two factors (gender and rearing site), and the Tukey test. In both cases, the level of significance was set at 5%. All data are expressed as mean +/- average standard error.

Results

Evaluations have shown that adult donkeys at maintenance, even when kept under different management systems, may present many blood and metabolic biomarkers with similar concentrations ($p > 0.05$), and the few differences found may not have clinical significance for the evaluation of these animals. ANOVA analysis with two factors did not indicate interaction among gender groups and rearing site groups for all parameters analyzed ($p > 0.05$).

It can also be observed that in the management systems where the samples were obtained, adaptation to fat accumulation was similar since no differences were observed in the body score ($p > 0.05$), both among gender-related groups and among rearing site groups. However, analyzing blood anisocytosis biomarkers, it was observed that RDW-CV and MPV were higher in females than in males ($p < 0.05$) (Table 1). In G-MS, RBC, Hb, HT and lymphocytes were lower than in G-LIM and G-NAT ($p < 0.05$), and in G-LIM MCV and RDW-SD presented the lowest values ($p < 0.05$).

Among the metabolic biomarkers, there were significant variations between the gender groups for UA, TRIG, CHOL, NEFA, Ca and P, with the highest concentrations being observed in females ($p<0.05$) (Tables 2 and 3). Among these biomarkers, when animals were compared considering the rearing site groups, significant differences were observed for UA, URE, GLU, TRIG, CHOL, NEFA, CK, P and Cl (Tables 2 and 3). The highest values for UA, TRIG, CHOL, P and Cl were detected in G-LIM ($p<0.05$). G-NAT had the highest values for URE, GLU and CK ($p<0.05$) (Tables 2 and 3).

Table 1 Results of Body Condition Score and haematological values in Northeastern donkeys, analyzed by two-way ANOVA (gender group and rearing site group).

Haematological biomarkers	Gender Group		Rearing Site Group		
	Male (n=32)	Female (n=30)	G-LIM (n=18)	G-NAT (n=18)	G-MS (n=26)
Body Condition Score	2.60 ± 0.08	2.36 ± 0.09	2.38 ± 0.11	2.55 ± 0.12	2.51 ± 0.09
Red Blood Cells, $x10^{12}$/L	6.51 ± 0.20	6.79 ± 0.27	7.19 ± 0.27^{a}	7.18 ± 0.36^{a}	5.89 ± 0.15^{b}
Haemoglobin, mmol/L	7.61 ± 0.24	7.81 ± 0.29	7.94 ± 0.29^{a}	8.56 ± 0.42^{a}	7.58 ± 0.20^{b}
Haematocrit, L/L	0.361 ± 0.011	0.367 ± 0.013	0.371 ± 0.013^{a}	0.407 ± 0.018^{a}	0.328 ± 0.090^{b}
MCV, fL	55.54 ± 0.63	54.42 ± 0.76	51.93 ± 1.02^{b}	57.12 ± 0.76^{a}	55.65 ± 0.51^{a}
MCHC, mmol/L	21.11+ 0.09	21.27+0.09	21.36+0.11	20.96+0.16	21.23+0.07
RDW-SD, fL	41.78 ± 0.46	42.28 ± 0.65	40.27 ± 0.65^{b}	43.45 ± 0.70^{a}	42.25 ± 0.58^{ab}
RDW-CV, %	18.22 ± 0.20^{B}	18.95 ± 0.26^{A}	18.93 ± 0.28	18.22 ± 0.34	18.57 ± 0.26
Platelets, $x10^{9}$/L	217.43 ± 16.2	183.23 ± 13.70	215.33 ± 25.42	219.33 ± 16.77	178.11 ± 14.54
MPV, fL	9.05 ± 0.26^{B}	10.22 ± 0.42^{A}	9.05 ± 0.42	9.28 ± 0.38	10.05 ± 0.42
Leukocytes, $x10^{9}$/L	10.50 ± 0.35	10.07 ± 0.48	10.26 ± 0.33	10.44 ± 0.44	10.24 ± 0.60
Lymphocytes, $x10^{9}$/L	3.43 ± 0.24	3.27 ± 0.23	3.20± 0.30^{ab}	4.05 ± 0.34^{a}	2.98 ± 0.23^{b}
Other White Cells, $x10^{9}$/L	7.06 ± 0.37	6.80 ± 0.41	7.06 ± 0.43	6.35 ± 0.39	7.26 ± 0.52

Different letters in the same row indicate P<0.05 (Tukey's test), uppercase for gender group and lowercase for rearing site group. G-LIM: Limoeiro Group; G-NAT: Natal Group; G-MS; Mirandiba /Salgueiro Group; MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; RDW-SD: red cell distribution width – standard deviation; RDW-CV: red cell distribution width – coeficiente of variation; MPV: mean platelet volume.

Table 2 Results of blood chemistry values in Northeastern donkeys, analyzed by two-way ANOVA (gender group and rearing site group).

Metabolic Biomarkers	Gender Group		Rearing Site Group		
	Male (n=32)	Female (n=30)	G-LIM (n=18)	G-NAT (n=18)	G-MS (n=26)
Total Plasmatic Protein, g/L	7.32 ± 0.12	7.57 ± 0.13	7.56 ± 0.18	7.67 ± 0.13	7.20 ± 0.14
Albumin, g/dL	3.30 ± 0.12	3.52 ± 0.14	3.59 ± 0.12	3.51 ± 0.13	3.22 ± 0.18
Ureia, mmol/L	10.00 ± 0.42	8.78 ± 0.53	8.10 ± 0.35^{b}	11.30 ± 0.69^{a}	9.01 ± 0.50^{a}
Creatinine, μmol/L	97.24 ± 2.65	86.63 ± 6.19	91.94 ± 9.72	93.71 ± 4.42	91.06 ± 4.42
Uric Acid, mmol/L	0.64 ± 0.01^{B}	0.93 ± 0.11^{A}	1.07 ± 0.21^{a}	0.64 ± 0.01^{b}	0.64 ± 0.01^{b}
Glutamine, mmol/L	0.26 ± 0.01	0.25 ± 0.02	0.29 ± 0.01	0.25 ± 0.02	0.23 ± 0.02
Glutamate, mmol/L	0.13 ± 0.001	0.12 ± 0.001	0.13 ± 0.01^{ab}	0.14 ± 0.01^{a}	0.12 ± 0.01^{b}
Total Cholesterol, mmol/L	1.79 ± 0.07^{B}	2.48 ± 0.22^{A}	2.91 ± 0.32^{a}	1.77 ± 0.07^{b}	1.82 ± 0.11^{b}
Triglycerides, mmol/L	0.48 ± 0.10^{B}	2.28 ± 0.54^{A}	2.43 ± 0.76^{a}	0.42 ± 0.13^{b}	1.26 ± 0.37^{ab}
NEFA, mmol/L	0,87 ± 0,12^{B}	1,36 ± 0,23^{A}	1,77 ± 0,35^{a}	0,72 ± 0,11^{b}	0,91 ± 0,14^{b}
AST, UI/L	334.20 ± 19.63	351.88 ± 21.48	325.35 ± 17.58	412.03 ± 26.95	306.84 ± 23.08
ALT, UI/L	20.72 ± 2.67	39.18 ± 9.11	43.83 ± 15.16	30.53 ± 3.58	19.24 ± 2.45
ALP, UI/L	479.76 ± 23.65	493,88 ± 30.11	469.69 ± 33.62	535.66 ± 28.12	464.33 ± 32.77
GGT, UI/L	101.47 ± 2.91	99.97 ± 5.79	98.41 ± 8.15	111.12 ± 3.72	95.17 ± 3.97
CK, UI/L	157.47 ± 18.28	143.71 ± 17.69	129.21 ± 20.33^{b}	215.58 ± 30.99^{a}	120.93 ± 11.05^{b}

Different letters in the same row indicate P<0.05 (Tukey's test), uppercase for gender group and lowercase for rearing site group. G-LIM: Limoeiro Group; G-NAT: Natal Group; G-MS; Mirandiba /Salgueiro Group; NEFA: non-esterified fatty acids, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline aminotransferase, GGT: gamma-glutamyltransferase, CK; creatine kinase.

Discussion

The body condition score (BCS) is used as a reference for the evaluation of nutritional and exercise programs, both for donkeys and horses. The results found for BCS in the present experiment were below the ideal values indicated for the species, which is around 3, on a scale ranging from 1 to 5 (*Caldin* et al. 2005). However, it should be noted that the values described as reference are related to animals reared in regions with large access to food supply to keep their body score around 3, like England. Then, values below 3, close to 2.5, might probably be indicated for animals in an extensive or semi-intensive management system, where they do more physical efforts when looking for food or competing for their food. It is important remember that management system and the amount of exercises interfere on BCS values (*Gravena* et al. 2010). However, the combination between BCS with blood and metabolic biomarkers in the present experiment may indicate that current donkeys were well adapted to this management systems in Brazil. In addition, it is important to observe that all animals, males and females, are in same pasture and there is no separation during the food delivery, and in this system may interfere in the characteristic of the body score.

Evaluations of blood values may be used to determine the general state of health of animals and are related to the management system. In the present experiment, when comparing the animals grouped by the rearing site, significant variations were observed in RBC, Hb and HT, with G-LIM and G-NAT presenting higher mean values when compared to G-MS, which was expected because in Caatinga conditions the availability and quality of food is more variable than in systems supplemented with chopped elephant grass (Pennisetum purpureum). The availability of food, particularly fiber, contribute to the equidae's microbiota and the production of B-complex vitamins and volatile fatty acids, which stimulate the erythropoiesis and energy production for animal maintenance (*Julliand* and *Grimm* 2016). However, there were no differences when the animals were grouped by sex ($p > 0.05$). It should be noted that the haematological profile of different breeds of donkeys in Brazil requires further studies, but in the current experiment, RBC, Hb and HT found were within the range of variation described for different breeds (RBC: $5.50-6.82\times10^{12}$/L; Hb: 7.52–8.07g/dL; HT: 0.33–0.37L/L) (*de Oliveira* et al. 1974, *Camac* 1997, *Jordana* et al. 1998, *Lording* 2008).

Different parameters are used to determine the red blood cell anisocytosis index, MCV being the most used. However, due to the way RDW-SD is measured, it has become a more reliable parameter to determine erythrocyte response (*Gameleira* et al. 2011, *Garba* et al. 2015). It has also recently been shown that the combination of MCV and MCHC with RDWs may produce a better understanding and classification of anemia in horses (*Lording* 2008) and it can be applied to donkeys. In the current animals, MCV resembles those described for Italian donkeys (adults: 54fL; youngsters (1–3 years): 49fL) (*Caldin* et al. 2005), and it is also within the values described for other donkey breeds (*Mori* et al 2004, *Tesfaye* et al. 2014). There are few reports of RDW in donkeys, but there is one description of RDW-CV in donkeys (adults: 18%, youngsters 19%) (*Caldin* et al. 2005), which was similar to the current study. It should be reminded that RDW measurement has only recently become a practice due to the greater diffusion of automatic equipments, facilitating the combined analysis of RDWs with MCV and MCHC.

Leukocytes (LEU) are also indicative of the health status of the animals and are regularly used in equine monitoring. In Northeastern donkeys, LEU did not present differences (10×10^9/L) ($p > 0.05$) among the groups, and also remained within the values described in the literature for this species in different breeds around the world (*Jordana* et al. 1998, *Lording* 2008, *Giraldo* et al. 2013, *Garba* et al. 2015). In these latter publications, LEU ranges from 7×10^9/L to 11.72×10^9/L in healthy animals (*Boudreaux* and *Ebbe* 1998, *Garba* et al. 2015). In the present experiment, Lymphocytes (LINF), which is important to understand the immunological responses of the animals, varied according to rearing site ($p < 0.05$), being higher in G-NAT (4.0×10^9/L) and lower in G-MS (3.0×10^9/L), but no variations were observed on gender-related groups ($p > 0.05$) (3.3×10^9/L), being similar to those in literature (3.4×10^9/L) (*Lording* 2008). These variations in the different studies for LEU and LINF may be associated with different analysis methodologies and ani-

Table 3 Results of electrolytes and trace elements in Northeastern donkeys, analyzed by two-way ANOVA (gender group and rearing site group).

Electrolytes and trace elements	Gender Group		Rearing Site Group		
	Male (n=32)	Female (n=30)	G-LIM (n=18)	G-NAT (n=18)	G-MS (n=26)
Calcium, mmol/L	2.25 ± 0.01^{B}	2.13 ± 0,01^{A}	2.27 ± 0,02	2.27 ± 0.01	2.30 ± 0.01
Phosphorus, mmol/L	1.49 ± 0,02^{B}	1.64 ± 0.06^{A}	1.75 ± 0.09^{a}	1.56 ± 0.02^{b}	1.45 ± 0.01^{b}
Iron, µmol/L	13.82 ± 0.97	10.92 ± 1.29	12.41 ± 2.12	13.87 ± 1.19	15.72 ± 1.14
Chloride, mmol/L	87.28 ± 0.78	94.67 ± 4.60	101.44 ± 7.19^{a}	84.70 ± 1.60^{b}	87.80 ± 0.75^{b}
Sodium, mmol/L	139.08 ± 1.00	132.74 ± 4.74	134.02 ± 7.65	139.84 ± 1.20	134.74 ± 1.95
Potassium, mmol/L	4.74 ± 0.11	4.39 ± 0.28	4.57 ± 0.42	4.77 ± 0.11	4.39 ± 0.19
Selenium, µmol/L	0.40 ± 0.02	0.46 ± 0.04	0.48 ± 0.04	0.40 ± 0.03	0.41 ± 0.05

Different letters in the same row indicate $P<0.05$ (Tukey's test), uppercase for gender group and lowercase for rearing site group. G-LIM: Limoeiro Group; G-NAT: Natal Group; G-MS; Mirandiba/Salgueiro Group.

mal management systems. Finally, the LINF/LEU relationship should always be monitored due to its importance, and LINF should represent 20% to 40% of leukocytes (*Caldin* et al. 2005, *Carrick* and *Begg* 2008,), results similar to those found in the current experiment.

Platelets are responsible for the initial temporary interruption of blood flow after vascular injury, and when there is thrombocytopenia, they are usually associated with a pre-existing disease or serious nutritional disorders. The PLAT was similar in all the groups ($p > 0.05$), however in horses different authors showed significant differences for PLAT between males (160×10^9/L) and females (190×10^9/L) (*Boudreaux* and *Ebbe* 1998, *Giraldo* et al. 2013). Other authors demonstrated PLAT variation in donkeys, from 150–220 to 330×10^9/L (*de Oliveira* et al. 1974). However, it was indicated that PLAT may vary around $100–350 \times 10^9$/L in cold-blooded horses, ponies and donkeys without comment differences between males and females (*Lording* 2008, *Laus* et al. 2015). In the present study, there was difference between males (9fL) and females (10fL) for mean platelet volume ($p < 0.05$), different from those described for horses (*Boudreaux* and *Ebbe* 1998). MPV indicates the heterogeneity of the platelet volumes dependent on the release of young platelets for circulation through cytoplasmic fragmentation of megakaryocytes (*Girardi* et al. 2014). In Italian donkeys, it was demonstrated that MPV was around 7.5 fL (*de Oliveira* et al. 1974) and, in horses, MPV value was between 3.0 and 6.0 fL (*Boudreaux* and *Ebbe* 1998, *Jodana* et al. 1998). In the current study, results are more elevated than these publications, so further studies are needed to identify the possible causes of these variations, facilitating the interpretation of the health status of donkeys.

Biomarkers of energy metabolism are important indicators of the state of nutrition and health of animals. The total CHOL (1.55–2.60 mmol/L) and TRIG (0.68–1.02 mmol/L) in donkeys are well described in the literature (*Lee* et al. 1995, *Mori* et al. 2003, *Laus* et al. 2015, *Barbosa* et al. 2016), different from NEFA. Both TRIG and NEFA are related to management system and food availability, different from total CHOL, so they should be used to evaluate the nutritional status of animals under different conditions. Thus, it should be remembered that lipolysis, which broken TRIG in NEFA and glycerol, rises when animals are not receiving enough food to maintain blood glucose and during high stress conditions, like when animals compete for food. These processes may contribute to changes TRIG, NEFA and glycerol concentrations (*Muller* et al. 1995, *Fantuz* et al. 2013, *Ferreira* et al. 2017). Also, it important remember that concentration of fat in animal food and the level of exercise may interfere in TRIG, NEFA and glycerol concentrations, but to understand this process more studies are need in this specie, principally in donkeys' under stress.

Non-esterified fatty acids concentration in donkeys may range from 0.15 to 0.60 mmol/L, depending on nutritional status, age and sex (*Simenew* et al. 2011, *Girardi* et al. 2014). The mean values of NEFA found in the present experiment were high (0.70–1.77 mmol/L) in relation to the values described by *Chiofalo* et al. (2012) and *Simenew* et al. (2011) (0.11–0.26 mmol/L), but the current results reflected the management system that Northeastern donkeys were being reared, even if they presented other biomarkers (RBS, HT, TPP and ALB) within normality. Also it was observed that NEFA was significantly different between males (0.26 mmol/L) and females (0.11 mmol/L) (*Chiofalo* et al. 2012, *Simenew* et al. 2011), which it was also observed in present experiment, but inversely. Furthermore, *Barbosa* et al. (2016), demonstrated that, when adult male and female donkeys are better fed, NEFA concentration decreases but TRIG did not change. Therefore, NEFA determination, associated with TRIG, may be a good indicator of donkeys' nutritional status and should always be used to evaluate the general state of these animals, even though it is not a method regularly used in practice.

Determination of different biomarkers of protein metabolism, such as total plasma proteins, albumin and urea, may serve as an indication of the nutritional status of animals. *Brinkmann* et al. (2013), observed that, when horses are submitted to adverse nutritional conditions, lipolysis and proteolysis increase to maintain energy demand, reducing total plasma protein (TPP) and albumin (ALB) (*Muller* et al. 1995). The results of the evaluations indicated no significant differences for TPP and ALB, and the concentrations described for donkeys of different breeds and in different management systems were similar (TPP: 6–8 g/L; ALB: 2–4 g/L) (*de Oliveira* et al. 1974, *Kirschvink* et al. 2008, *Lording* 2008, *Chiofalo* et al. 2012). Still evaluating protein biomarkers, no significant changes were observed in URE and CREAT, but UA showed differences between males and females ($p < 0.05$), around 30% higher in females. In the rearing site groups, there were significant differences in URE and UA, where the highest URE were found in G-NAT (1.07 mmol/L) and G-LIM (0.64 mmol/L). It should also be noted that the values found in all the groups were within those described for the species (URE: 3.33–8.16 mmol/L); CREAT: 79.6–141.4 µmol/L) (*de Oliveira* et al., 1974, *Mori* et al., 2003, *Chiofalo* et al. 2012), but these authors did not describe UA values, which could be important to increase the understanding of these biomarkers in the process of protein catabolism. Recently, *Girardi* et al (2014), described UA for Brazilian donkeys (0.04–0.10 mmol/L), without differences between males and females, results which are lower than the ones presented in this experiment. The UA are important natural antioxidant and animals with higher UA may have more protection from the negative effects of the stress (*Ferreira* et al. 2017).

The concentration of Glutamine and Glutamate decreases in blood and tissues during catabolic states and lactation, compromising the nutrition of enterocytes and cells of the immune system, so it is considered a conditionally essential amino acid (*Routledge* et al. 1999, *Manso* et al. 2015). The Gln in donkeys was inferior to that described for horses (Gln: 0.45–0.80 mmol/L; Glu: 0.06–0.20 mmol/L) (*Routledge* et al. 1999, *Manso Filho* et al. 2009), representing less than 50% when compared to horse concentrations. In the present experiment, Gln did not show any difference between groups ($p > 0.05$), unlike Glu, which although there was no difference between the gender-related group, there was a significant difference in the rearing site groups, being higher in G-NAT. Finally, it should be remembered that Gln is the most abundant free amino acid in the body and its excessive intake is related to protein degradation, mainly of muscle tissue, which can lead to reduced locomotion to search for food in animals confined or under extensive rearing arrangements. In this sen-

se, the concentration of Gln and Glu should be evaluated for a better understanding of this process in donkeys, because the variation depends on muscle tissue production and enterocytes consumption and are important for the immune system.

Different enzymes present in the blood of the animals serve as biomarkers of the metabolism of different organs, being widely described for donkeys in literature, and the possible modifications in their concentrations reflect pathologies (*Mori* et al. 2003, *Chiofalo* et al. 2012, *Brinkmann* et al. 2013, *Fantuz* et al. 2013). In Northeastern donkeys, there were no significant differences in AST, ALT, GGT and FA among the groups, unlike CK, which was higher in G-NAT (215 UI/L) when compared to G-LIM (129 UI/L) and G-MS (120 UI/L) ($p < 0.05$), but no differences were observed between males and females ($p > 0.05$). Finally, it should be noted that CK is widely used for the identification of muscle injuries in athletic and working horses, with concentrations above 400 UI/L after 4 hours of physical exertion, indicating muscle injury (*Julliand* and *Grimm* 2016).

Minerals and electrolytes serve as biomarkers for the assessment of metabolic balance and are important intermediary compounds in metabolism. In the current experiment, significant differences were observed in the rearing site groups for P and Cl, which was higher in G-LIM ($p < 0.05$), and in the gender-related groups for Ca and P, which was higher in females ($p < 0.05$). However, the values for all analyzed biomarkers were close to the variations reported for donkeys from several localities in literature (*de Oliveira* et al. 1974, *Kirschvink* et al. 2008, *Chiofalo* et al. 2012, *Fantuz* et al. 2013, *Mori* et al. 2013), with the exception of Se, which apparently is lower than the described for donkeys that were being supplemented with a mixture of different minerals that included Se (*Kirschvink* et al. 2008) but being at the lowest values described in mares and foals (*Lee* et al. 1995). It should be remembered, however, that these biomarkers can only be modified in blood under extreme conditions. Therefore, no changes were expected in their concentrations in the blood of the donkeys evaluated, since all of them were healthy animals.

Conclusion

It was concluded that donkeys may present significant variations in different blood and metabolic biomarkers, when they are of different sexes and/or housed in different management systems. The red series and minerals were not very sensitive to identify variations related to gender and/or rearing site groups. However, the body score, the count of lymphocytes, MVC plus RDWs, CK, as well as concentrations of NEFA and triglycerides, may be used to better understand the adaptations of different management conditions.

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Conflict of interest

None of the authors have any conflict of interests.

Animal Welfare Statement

The UFRPE-CEUA Ethics and Animal Welfare commission authorized this research by protocol 23082.007851/2007.

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