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Serum protein fractions, hematological and biochemical variables of the Pêga donkey (*Equus asinus*) breed in the first year of age

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ABSTRACT

This study describes changes in serum protein fractions, hematological and biochemical variables of Pêga donkeys, an important Brazilian asinine breed, during their first year of life. Complete blood count, biochemical analysis and serum protein fractionation by electrophoresis were performed on blood samples taken from eight donkeys, three females and five males, collected on the day of birth, at 3rd, 7th, 15th days and monthly until 12 months of life. There were no age-related differences for CK, albumin, magnesium, potassium, chlorides, triglycerides, basophils, band and segmented neutrophils. Sodium concentration remained almost constant. The WBC curve ascended until five months, lymphocytes up to seven months, monocytes until six months, phosphorus rose during the first month, IgA until the third month, and then all previous parameters started to decrease. Urea and ionized calcium concentrations diminished until 15 days, then tended to increase. Eosinophils, total protein, GGT, AST, creatinine, IgG, transferrin, albumin (identified by electrophoresis), haptoglobin, 23 and 138 kDa molecular weight proteins showed an upward trend during this period. RBC, hemoglobin concentration, PCV, platelets, glucose, total calcium, cholesterol, ALT, AP, ceruloplasmin, α_1-acid glycoprotein, 33 kDa molecular weight protein, indirect, direct and total bilirubin, tended to decrease during the first year of life. This is the first report on changes in serum protein fractions, hematological and biochemical variables of Pêga donkeys during the first year of life, and demonstrates that most of these parameters change during this period. The data obtained are useful for clinical routines and as a basis for future scientific investigations into the donkeys' physiology and metabolism.

Key words: biochemistry, clinical pathology, Equidae, hematology, newborn, SDS-PAGE

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Introduction

The Pêga donkey (*Equus asinus*), the most popular Brazilian donkey, is a large breed with Iberian origin, also popular in some other countries in South America, such as Bolivia, Paraguay and Colombia. Its breeding began in Minas Gerais State and, since then, the Pêga breed has been selected for more than two centuries, mainly to produce outstanding saddle-type mules. Nowadays, the Brazilian Association of Pêga Donkey Breeders (ABCJ Pêga) has approximately 2,000 members and about 20,000 mules and donkeys registered (CANISSO and McDONNELL, 2010). However, a limited amount of research into the hematological and serum biochemical variables of this breed have been performed (CAMPOS et al., 1968; GIRARDI et al., 2014; GIRARDI et al., 2015).

Often, veterinarians and owners are faced with difficulty in interpreting biological analysis results from donkeys because of the lack of clearly established reference ranges (PITEL et al., 2006) and much of the necessary information is extrapolated from that existing for horses, which cannot be a valid comparison (DE ALUJA et al., 2001). Despite belonging to the same family (Equidae) and the same genus (*Equus*), horses and donkeys exhibit very different characteristics in their erythrograms (PERDIGÃO DE OLIVEIRA et al., 1974). In general, veterinarians have paid little attention to this species, and in most schools of Veterinary Medicine, donkeys are not included in the study plans (DE ALUJA et al., 2001). Changes in hematological variables for donkey breeds and populations may be influenced by age, sex and the time of sampling, in relation to exercise, geographical and nutritional factors (MORI et al., 2004), and should be considered in the clinical analysis of these animals.

The purpose of this present study was to determine which physiological changes occur in hematological variables, biochemical patterns and serum protein fractions of Pêga donkeys during the first year of life, obtaining results that will guide more accurate diagnoses, help to understand physiological processes, and serve as a basis for future scientific investigations about this species.

Materials and methods

For this study, blood samples were collected from eight donkeys, three females and five males, maintained under field conditions, bred for reproductive purposes, in three herds in São Paulo and Minas Gerais States, Brazil. All farms had the same breeding system, they were fed on pasture, with a supply of feed and mineral mixture when necessary, and appropriate health management.

The animals had blood collected on the day of birth, on the 3rd, 7th, 15th days and monthly until 12 months of life, with the collection period from February 2010 to December 2011. The collections were performed during the morning, in nonfasting animals, only under mechanical restraint, by the closed evacuated system (BD Vacutainer- BD Diagnostics

- Preanalytical Systems, São Paulo, São Paulo, Brazil) using multiple sample needles and plastic tubes of 4 mL volume with ethylenediaminetetraacetic acid dipotassium anticoagulant (K_2EDTA 7.2 mg) for complete blood count; tubes of 4 mL volume with sodium fluoride (NaF 6 mg) and ethylenediaminetetraacetic acid disodium (Na_2EDTA 12 mg) anticoagulants to obtain plasma for glucose measurement; and plastic tubes of 10 mL volume, without anticoagulant, to obtain serum for biochemical analysis and serum protein electrophoresis. The collection was conducted by external jugular venipuncture after proper regional antisepsis, up to the complete capacity of the tube. The samples were homogenized after collection and packed in a cooler with reusable ice packs for temporary storage and transportation. Serum and plasma fractions were separated, as soon as possible, by centrifugation, and biochemical analysis was undertaken on the collection day. For serum protein fractionation, serum samples were stored below freezing at -20ºC, in plastic sterile microtubes of 1.5 mL capacity (MEYER and HARVEY, 1998), because the main biochemical constituents remain stable in this condition (THRALL, 2007). If samples for complete blood count could not be immediately processed, they were stored under refrigeration at 4 °C for a maximum period of 12 hours (JAIN, 1993).

Red blood cells count - RBC ($\times 10^{12}$/L), white blood cells count - WBC ($\times 10^{9}$/L), platelets count ($\times 10^{9}$/L), packed cell volume (PCV - L/L) and hemoglobin concentration - Hb (g/L) were measured using an automated veterinary hematology analyzer (ABXVET-Horiba ABX, Montpellier, Hérault, France). The differential leukocyte count (basophils, eosinophils, bands, segmented neutrophils, lymphocytes and monocytes, $\times 10^{9}$/L) was performed by blood smear analysis, stained by modified Rosenfeld method, using light microscopy. The analyses were performed at the Clinical Pathology Laboratory of the Veterinary Hospital "Governador Laudo Natel", School of Agrarian and Veterinary Sciences, São Paulo State University, Jaboticabal, Brazil.

The following biochemical parameters were measured: alanine aminotransferase - ALT, aspartate aminotransferase - AST, alkaline phosphatase - AP, gamma-glutamyl transferase - GGT, creatine kinase - CK (U/L); total protein, albumin (g/L); urea, glucose, cholesterol, triglycerides, total calcium, phosphorus, magnesium, chlorides (mmol/L); creatinine, total and direct bilirubin (µmol/L), through semi-automatic spectrophotometer analyzer and commercial kits (LabQuest, Labtest Diagnóstica S.A., Lagoa Santa, Minas Gerais, Brazil). Sodium, potassium and inorganic calcium (mmol/L) serum levels were determined by an electrolyte analyzer (Roche 9180 Electrolyte Analyzer, Roche, São Paulo, São Paulo, Brazil). Indirect bilirubin values (µmol/L) were obtained by subtraction of direct bilirubin levels from total bilirubin values. The analyses were performed at the Research Laboratory of the Department of Veterinary Clinic and Surgery, School of Agrarian and Veterinary Sciences, São Paulo State University, Jaboticabal, Brazil.

The serum protein fractionation by sodium dodecyl sulphate - poliacrilamide gel electrophoresis (SDS-PAGE) was performed according to technique described by LAEMMLI (1970). After fractionating, the gel was stained for 10 minutes in coomassie blue solution (50 % methanol, 40 % water, 9.75 % glacial acetic acid, 0.25 % coomassie blue), and then it was placed in a solution of 7 % acetic acid to remove excess dye until the fractions became clear. The fractions concentrations were determined through computerized scanning densitometer (Shimadzu CS 9301, Tokyo, Japan). As reference, a marker solution (SigmaMarker wide range, Sigma-Aldrich, Saint Louis, Missouri, USA) with molecular weight ranging from 6.5 to 200 kilodaltons (kDa), was used. The serum protein fraction concentrations (mg/L) were determined multiplying the percentage of each fraction by the total protein concentration, obtained through semi-automatic spectrophotometer analyzer (LabQuest) by biuret method. These analyses were made at the Research Laboratory of the Department of Veterinary Clinic and Surgery, School of Agrarian and Veterinary Sciences, São Paulo State University, Jaboticabal, Brazil.

Data analysis was performed using a statistical software (SAS 9.1, SAS Institute Inc., Cary, North Carolina, USA), assessing the association between variables and time by linear, quadratic and cubic regression. The level of statistical significance was set at $P<0.05$.

This study was approved by Ethics Commission in Use of Animals of the School of Agrarian and Veterinary Sciences, São Paulo State University, Protocol N° 6369/10.

Results

The results of the hematological variables of Pêga donkey breed during the first year of life are shown in Tables 1 and 2. The serum biochemical profile is described in Tables 3, 4 and 5. Serum protein fractions obtained by electrophoresis are related in Table 6.

There were not age-related differences ($P>0.05$) for values of basophils, bands, segmented neutrophils, CK, creatinine, indirect bilirubin, albumin, triglycerides, sodium, magnesium, potassium, and chlorides.

Hemoglobin concentration ($P<0.05$ $y = -0.0003x^2 + 0.04x - 128.82$), PCV ($P<0.0001$, $y = -0.0002x + 0.15$) and platelets count decreased ($P<0.05$, $y = -0.29x - 109.15$), while the eosinophil count increased ($P<0.001$, $y = 0.001x + 0.18$) during the first year of age. RBC ($P<0.001$, $y = 0.0000003x^3 - 0.0002x^2 + 0.03x + 8.37$) increased in the first 3 months, WBC curve ascended until 134 days old ($P<0.01$, $y = 0.000000x^3 - 0.0006x^2 + 0.12x + 8.23$), lymphocyte means increased up to 4 months ($P<0.0001$, $y = 0.000001x^3 - 0.0006x^2 + 0.10x + 1.48$) and monocyte counts raised until 6 months of age ($P<0.0001$, $y = -0.00000x^2 + 0.002x + 0.29$), and after all started to reduce.

Table 1. Hematological variables (mean ± standard deviation and coefficient of variation - CV) of Pêga donkey (*Equus asinus*) breed, from birth to 12 months of age

Time from birth	RBC ($\times10^{12}$/L)	Hb (g/L)	PCV (L/L)	Platelets ($\times10^{9}$/L)	WBC ($\times10^{9}$/L)
Day 0	9.11 ± 0.84	136.1 ± 13.6	0.475 ± 0.049	404 ± 143	7.9 ± 2.1
Day 3	9.16 ± 1.39	141.3 ± 27.4	0.481 ± 0.102	342 ± 107	8.3 ± 2.5
Day 7	8.27 ± 0.97	126.8 ± 14.9	0.421 ± 0.053	380 ± 148	9.3 ± 2.4
Day 15	7.84 ± 0.63	117.1 ± 12.3	0.390 ± 0.032	521 ± 180	10.0 ± 4.0
1 month	8.74 ± 0.59	120.9 ± 11.5	0.422 ± 0.032	498 ± 223	12.5 ± 5.4
2 months	9.21 ± 0.87	124.8 ± 13.6	0.427 ± 0.042	448 ± 227	12.5 ± 4.7
3 months	9.76 ± 0.95	131.0 ± 15.4	0.444 ± 0.050	338 ± 155	12.9 ± 3.9
4 months	9.45 ± 0.82	128.4 ± 13.3	0.431 ± 0.048	281 ± 90	15.0 ± 4.0
5 months	9.53 ± 1.19	135.0 ± 15.7	0.445 ± 0.060	286 ± 118	16.6 ± 4.5
6 months	9.49 ± 1.02	138.5 ± 19.5	0.456 ± 0.058	286 ± 188	15.0 ± 3.2
7 months	8.52 ± 0.86	127.3 ± 13.5	0.418 ± 0.049	296 ± 135	13.4 ± 2.3
8 months	7.66 ± 1.10	113.6 ± 15.1	0.384 ± 0.055	408 ± 110	12.1 ± 3.4
9 months	7.08 ± 0.80	106.4 ± 14.5	0.357 ± 0.041	416 ± 114	12.9 ± 4.2
10 months	7.22 ± 0.83	109.4 ± 12.0	0.373 ± 0.045	361 ± 44	12.6 ± 3.7
11 months	6.56 ± 0.90	103.7 ± 13.8	0.342 ± 0.049	294 ± 43	12.0 ± 3.3
12 months	7.37 ± 0.71	113.7 ± 14.1	0.381 ± 0.045	303 ± 65	12.6 ± 1.6
CV	9.79	10.95	10.75	38.03	22.63

RBC - red blood cell count, Hb - hemoglobin concentration, PCV- packed cell volume, WBC - white blood cell count.

Table 2. Differential leukocyte count (mean ± standard deviation and coefficient of variation - CV) of Pêga donkey (*Equus asinus*) breed, from birth to 12 months of age

Time from birth	Basophils ($\times10^{9}$/L)	Eosinophils ($\times10^{9}$/L)	Band neutrophils ($\times10^{9}$/L)	Segmented neutrophils ($\times10^{9}$/L)	Lymphocytes ($\times10^{9}$/L)	Monocytes ($\times10^{9}$/L)
Day 0	0.00 ± 0.00	0.09 ± 0.19	0.11 ± 0.06	6.24 ± 2.18	1.25 ± 0.58	0.19 ± 0.08
Day 3	0.03 ± 0.07	0.08 ± 0.07	0.16 ± 0.24	5.29 ± 2.45	2.44 ± 3.06	0.30 ± 0.22
Day 7	0.01 ± 0.02	0.11 ± 0.14	0.14 ± 0.16	6.22 ± 2.67	2.52 ± 0.95	0.31 ± 0.17
Day 15	0.06 ± 0.07	0.22 ± 0.23	0.13 ± 0.13	6.76 ± 3.91	2.51 ± 1.13	0.28 ± 0.10
1 month	0.01 ± 0.02	0.22 ± 0.24	0.11 ± 0.12	7.83 ± 4.43	3.85 ± 1.10	0.50 ± 0.28
2 months	0.00 ± 0.00	0.34 ± 0.41	0.23 ± 0.15	5.78 ± 2.87	5.67 ± 1.47	0.47 ± 0.26
3 months	0.03 ± 0.06	0.35 ± 0.18	0.12 ± 0.14	5.85 ± 2.32	6.17 ± 3.57	0.42 ± 0.18
4 months	0.00 ± 0.00	0.37 ± 0.16	0.08 ± 0.09	6.71 ± 1.71	7.36 ± 2.52	0.50 ± 0.23
5 months	0.02 ± 0.06	0.61 ± 0.30	0.21 ± 0.26	7.67 ± 2.70	7.34 ± 1.74	0.70 ± 0.25
6 months	0.05 ± 0.13	0.56 ± 0.56	0.12 ± 0.14	6.95 ± 1.30	6.89 ± 1.52	0.46 ± 0.19
7 months	0.02 ± 0.06	0.31 ± 0.24	0.12 ± 0.11	6.01 ± 1.81	6.41 ± 1.86	0.54 ± 0.22

Table 2. Differential leukocyte count (mean ± standard deviation and coefficient of variation - CV) of Pêga donkey (*Equus asinus*) breed, from birth to 12 months of age (continued)

Time from birth	Basophils ($\times10^9$/L)	Eosinophils ($\times10^9$/L)	Band neutrophils ($\times10^9$/L)	Segmented neutrophils ($\times10^9$/L)	Lymphocytes ($\times10^9$/L)	Monocytes ($\times10^9$/L)
8 months	0.05 ± 0.13	0.38 ± 0.48	0.14 ± 0.13	5.56 ± 2.47	5.64 ± 2.35	0.32 ± 0.26
9 months	0.02 ± 0.05	0.77 ± 1.33	0.09 ± 0.18	6.91 ± 2.78	4.76 ± 1.93	0.39 ± 0.16
10 months	0.02 ± 0.04	0.93 ± 1.05	0.12 ± 0.10	6.50 ± 2.41	4.58 ± 1.08	0.51 ± 0.13
11 months	0.02 ± 0.05	0.42 ± 0.40	0.09 ± 0.15	5.61 ± 1.74	5.44 ± 1.71	0.45 ± 0.17
12 months	0.00 ± 0.00	0.46 ± 0.35	0.06 ± 0.11	4.78 ± 0.86	6.98 ± 2.00	0.33 ± 0.12
CV	310.20	133.76	114.70	34.86	36.56	46.88

Table 3. Enzyme activity parameters (mean ± standard deviation and coefficient of variation - CV) of Pêga donkey (*Equus asinus*) breed, from birth to 12 months of age

Time from birth	AST (U/L)	AP (U/L)	ALT (U/L)	GGT (U/L)	CK (U/L)
Day 0	175.5 ± 71.3	905.9 ± 336.5	31.43 ± 19.60	63.11 ± 15.70	315.8 ± 191.9
Day 3	225.9 ± 51.6	533.8 ± 129.0	22.92 ± 10.82	65.57 ± 20.65	120.6 ± 36.5
Day 7	208.5 ± 40.1	441.0 ± 101.4	18.99 ± 9.26	63.11 ± 19.50	321.0 ± 247.1
Day 15	186.6 ± 32.1	372.1 ± 65.0	16.37 ± 7.64	57.38 ± 15.30	207.6 ± 121.7
1 month	185.9 ± 33.6	379.4 ± 132.7	11.78 ± 7.80	58.33 ± 20.83	315.0 ± 140.3
2 months	180.7 ± 18.1	344.1 ± 126.6	8.51 ± 3.90	42.08 ± 13.56	266.3 ± 133.3
3 months	214.1 ± 16.7	319.2 ± 89.5	11.79 ± 3.70	44.94 ± 15.54	397.8 ± 383.1
4 months	202.3 ± 29.9	321.3 ± 49.7	11.22 ± 5.60	33.47 ± 14.13	334.2 ± 294.0
5 months	212.8 ± 30.2	272.6 ± 44.0	14.41 ± 10.75	40.16 ± 23.04	188.4 ± 28.2
6 months	228.5 ± 34.5	269.5 ± 43.9	12.44 ± 3.89	34.43 ± 8.18	223.3 ± 95.2
7 months	223.3 ± 36.6	230.1 ± 30.6	13.75 ± 7.37	31.56 ± 8.61	354.6 ± 526.2
8 months	203.0 ± 25.8	244.6 ± 55.9	11.13 ± 3.36	50.68 ± 25.19	245.3 ± 68.6
9 months	203.0 ± 27.7	243.6 ± 93.1	9.82 ± 3.36	47.81 ± 14.02	164.3 ± 56.4
10 months	237.2 ± 37.9	272.5 ± 147.8	9.73 ± 3.62	50.26 ± 42.54	337.8 ± 231.7
11 months	235.4 ± 62.2	231.0 ± 90.7	9.73 ± 3.62	64.49 ± 31.51	314.5 ± 106.7
12 months	213.9 ± 50.4	246.0 ± 56.4	7.85 ± 4.39	40.80 ± 20.34	223.0 ± 78.4
CV	18.28	35.59	51.47	38.49	81.22

ALT - alanine aminotransferase, AST - aspartate aminotransferase, AP - alkaline phosphatase, GGT - gamma-glutamyl transferase, CK - creatine kinase.

Table 4. Serum metabolites levels (mean ± SD and coefficient of variation - CV) of Pêga donkey (*Equus asinus*) breed, from birth to 12 months of age

Time from birth	Creatinine (µmol/L)	Urea (mmol/L)	Cholesterol (mmol/L)	Albumin (g/L)	Indirect bilirubin (µmol/L)	Direct bilirubin (µmol/L)	Total bilirubin (µmol/L)	Triglycerides (mmol/L)	Glucose (mmol/L)	Total Protein (g/L)
Day 0	153.48 ± 35.05	3.71 ± 0.87	4.18 ± 1.83	22.21 ± 2.92	4.33 ± 3.36	4.54 ± 2.78	8.87 ± 5.43	0.45 ± 0.24	8.24 ± 1.48	45.76 ± 9.67
Day 3	129.28 ± 37.43	2.82 ± 1.28	3.96 ± 1.40	23.00 ± 3.69	2.22 ± 1.90	2.88 ± 1.30	5.10 ± 2.66	0.68 ± 0.34	6.59 ± 1.32	54.13 ± 10.74
Day 7	121.88 ± 28.71	2.06 ± 0.94	3.19 ± 1.06	22.65 ± 2.98	1.32 ± 0.76	1.96 ± 1.25	3.27 ± 1.21	0.53 ± 0.20	7.14 ± 1.88	52.04 ± 5.92
Day 15	105.75 ± 11.15	2.19 ± 0.73	2.82 ± 0.71	22.18 ± 2.16	2.25 ± 1.63	1.83 ± 1.49	4.08 ± 2.73	0.61 ± 0.36	6.38 ± 1.16	53.49 ± 4.45
1 month	115.25 ± 22.72	1.87 ± 0.57	2.42 ± 0.44	23.24 ± 2.15	1.45 ± 0.88	1.28 ± 0.60	2.74 ± 1.01	0.62 ± 0.19	5.84 ± 0.60	56.51 ± 4.20
2 months	125.53 ± 30.12	2.47 ± 0.83	2.35 ± 0.49	22.01 ± 2.50	1.52 ± 0.69	1.69 ± 0.89	3.20 ± 0.91	0.45 ± 0.13	4.94 ± 0.30	57.49 ± 6.00
3 months	120.78 ± 15.08	2.73 ± 1.26	2.60 ± 0.53	23.13 ± 2.40	2.25 ± 1.10	1.24 ± 0.45	3.48 ± 1.21	0.52 ± 0.20	4.57 ± 0.56	62.53 ± 5.46
4 months	117.68 ± 9.41	3.38 ± 0.86	2.48 ± 0.58	22.75 ± 3.30	2.40 ± 0.98	1.24 ± 0.53	3.63 ± 0.87	0.61 ± 0.24	4.81 ± 0.71	62.43 ± 7.92
5 months	130.39 ± 16.63	3.73 ± 0.83	2.86 ± 0.66	22.48 ± 4.14	2.66 ± 0.93	1.54 ± 0.33	4.11 ± 1.00	0.52 ± 0.17	4.37 ± 0.49	65.91 ± 7.66
6 months	129.07 ± 16.64	4.49 ± 1.64	3.56 ± 1.76	23.65 ± 4.80	2.84 ± 1.42	1.48 ± 0.75	4.32 ± 1.39	0.62 ± 0.27	4.68 ± 0.63	65.79 ± 5.54
7 months	130.83 ± 13.40	5.15 ± 1.82	3.46 ± 1.14	24.53 ± 3.56	2.29 ± 0.91	1.71 ± 0.59	4.00 ± 0.88	0.52 ± 0.30	4.80 ± 1.13	64.50 ± 5.75
8 months	137.46 ± 19.16	4.96 ± 1.37	2.62 ± 0.78	24.75 ± 2.82	2.31 ± 1.22	1.52 ± 0.54	3.83 ± 1.39	0.47 ± 0.11	4.73 ± 1.10	64.00 ± 3.34
9 months	113.59 ± 8.80	5.25 ± 1.38	2.31 ± 0.66	22.30 ± 3.18	2.18 ± 0.83	1.48 ± 0.80	3.66 ± 1.11	0.71 ± 0.71	4.70 ± 1.00	64.78 ± 4.70
10 months	122.75 ± 15.92	6.85 ± 1.94	2.06 ± 0.56	22.59 ± 2.53	2.32 ± 1.43	1.27 ± 0.49	3.84 ± 1.90	0.45 ± 0.17	4.39 ± 0.83	66.19 ± 6.14
11 months	132.98 ± 23.21	5.53 ± 1.74	2.03 ± 0.56	22.76 ± 2.51	2.52 ± 1.24	1.61 ± 0.70	4.13 ± 1.82	0.50 ± 0.14	4.21 ± 0.74	65.20 ± 6.32
12 months	147.33 ± 30.19	5.66 ± 1.70	2.14 ± 0.57	23.35 ± 2.07	2.65 ± 2.34	1.43 ± 0.54	4.08 ± 2.66	0.73 ± 0.26	4.24 ± 0.49	66.73 ± 6.04
CV	16.48	32.68	30.43	13.38	57.54	55.77	44.56	50.69	15.81	10.05

Table 5. Electrolyte concentrations (mean ± SD and coefficient of variation - CV, mmol/L) of Pêga donkey (*Equus asinus*) breed, from birth to 12 months of age

Time from birth	Total calcium	Phosphorus	Magnesium	Sodium	Potassium	Ionized calcium	Chlorides
Day 0	3.19 ± 0.11	1.72 ± 0.14	0.76 ± 0.14	144 ± 16	4.7 ± 0.7	1.49 ± 0.19	100.49 ± 13.17
Day 3	3.19 ± 0.25	2.59 ± 0.42	0.86 ± 0.20	135 ± 4	4.6 ± 0.4	1.34 ± 0.21	99.50 ± 9.72
Day 7	3.08 ± 0.13	2.99 ± 0.62	0.82 ± 0.12	135 ± 4	4.9 ± 0.3	1.20 ± 0.34	104.85 ± 8.95
Day 15	3.13 ± 0.27	2.78 ± 0.27	0.79 ± 0.17	137 ± 5	5.1 ± 0.5	1.43 ± 0.24	95.90 ± 22.92
1 month	3.11 ± 0.23	2.62 ± 0.11	0.81 ± 0.07	136 ± 3	4.7 ± 0.6	1.36 ± 0.27	95.03 ± 21.69
2 months	3.17 ± 0.28	2.50 ± 0.28	0.78 ± 0.13	136 ± 5	4.9 ± 0.4	1.49 ± 0.20	96.31 ± 7.37
3 months	3.11 ± 0.10	2.38 ± 0.26	0.92 ± 0.18	137 ± 5	4.6 ± 0.3	1.49 ± 0.15	96.09 ± 7.99
4 months	3.14 ± 0.26	2.42 ± 0.40	0.91 ± 0.21	135 ± 3	4.9 ± 0.5	1.56 ± 0.10	99.96 ± 6.84
5 months	3.07 ± 0.13	2.05 ± 0.20	0.85 ± 0.11	138 ± 6	5.3 ± 0.9	1.48 ± 0.21	97.65 ± 7.31
6 months	3.17 ± 0.13	1.97 ± 0.30	0.92 ± 0.18	135 ± 2	4.9 ± 0.5	1.55 ± 0.11	99.26 ± 7.93
7 months	3.10 ± 0.18	1.91 ± 0.23	0.77 ± 0.12	134 ± 2	4.9 ± 0.5	1.56 ± 0.16	100.12 ± 8.56
8 months	3.10 ± 0.15	1.91 ± 0.50	0.83 ± 0.11	134 ± 1	4.7 ± 0.5	1.63 ± 0.10	96.36 ± 2.61
9 months	2.91 ± 0.15	1.81 ± 0.43	0.85 ± 0.15	137 ± 2	4.7 ± 0.5	1.52 ± 0.10	92.52 ± 12.58
10 months	3.06 ± 0.20	1.78 ± 0.25	0.78 ± 0.06	136 ± 5	4.9 ± 0.4	1.61 ± 0.12	99.36 ± 10.10
11 months	3.07 ± 0.23	1.86 ± 0.19	0.84 ± 0.15	137 ± 4	4.9 ± 0.5	1.55 ± 0.21	99.45 ± 7.57
12 months	2.99 ± 0.28	1.71 ± 0.26	0.82 ± 0.09	138 ± 5	4.7 ± 0.5	1.42 ± 0.17	96.47 ± 9.84
CV	6.27	15.37	16.41	3.74	10.80	12.60	11.83

Levels of AP ($P<0.01$, $y = -0.00004x^3 + 0.03x^2 - 5.31x + 593.06$), ALT ($P<0.01$, $y = -0.00000x^3 + 0.001x^2 - 0.25x + 22.88$), plasma glucose ($P<0.0001$, $y = -0.000000x^3 + 0.0002x^2 - 0.05x + 7.27$), serum cholesterol ($P<0.01$, $y = -0.0000003x^3 + 0.0001x^2 - 0.02x + 3.50$), and total calcium ($P<0.05$, $y = -0.0004x + 3.16$) decreased during the observational period. GGT ($P<0.05$, $y = -0.000004x^3 + 0.0003x^2 - 0.53x + 66.55$), direct ($P<0.01$, $y = -0.0000003x^3 + 0.0002x^2 - 0.03x + 2.90$) and total bilirubin ($P<0.05$, $y = -0.0000004x^3 + 0.0003x^2 - 0.04x + 5.33$) showed reduction for 4 months after birth, then increased. The means of AST ($P<0.01$, $y = 0.09x + 195.24$) and total serum protein ($P<0.05$, $y = 0.000001x^3 - 0.0009x^2 + 0.20x + 50.00$) tended to increase with aging. Urea serum concentrations diminished up to 44 days, then tended to increase until the end of observations ($P<0.001$, $y = -0.0000004x^3 + 0.0002x^2 - 0.02x + 2.73$). Serum phosphorus levels raised in the first month, then decreased ($P<0.05$, $y = 0.00000008x^3 - 0.0000x^2 + 0.002x + 2.52$). Ionized calcium levels increased in the first 7 months, tending to decrease in the remainder of the first year ($P<0.01$, $y = -0.00000x^2 + 0.002x + 1.34$).

The electrophoretic separation revealed 10 distinct serum protein fractions, in descending order of molecular weight: immunoglobulin A (IgA), 138 kDa molecular weight protein (MWP_{138}), ceruloplasmin, transferrin, albumin, immunoglobulin G (IgG),

Table 6. Serum protein fractions (mean ± standard deviation and coefficient of variation - CV) of Pêga donkey (*Equus asinus*) breed, from birth to 12 months of age, ordered by decreasing molecular weight

Time from birth	IgA (mg/dL)	MWP_{138} (mg/dL)	Ceruloplasmin (mg/dL)	Transferrin (mg/dL)	Albumin (mg/dL)	IgG (mg/dL)	Haptoglobin (mg/dL)	α_1-acid glycoprotein (mg/dL)	MWP_{33} (mg/dL)	MWP_{23} (mg/dL)
Day 0	46 ± 18	15 ± 16	12 ± 10	181 ± 71	3153 ± 587	854 ± 499	30 ± 12	21 ± 17	19 ± 14	140 ± 40
Day 3	74 ± 26	26 ± 23	19 ± 11	203 ± 51	3575 ± 548	1091 ± 460	50 ± 19	42 ± 18	23 ± 17	279 ± 59
Day 7	120 ± 55	35 ± 28	22 ± 21	238 ± 52	3295 ± 508	868 ± 327	63 ± 51	36 ± 25	15 ± 11	343 ± 58
Day 15	223 ± 59	46 ± 17	23 ± 23	351 ± 62	3129 ± 444	758 ± 190	93 ± 49	35 ± 17	15 ± 7	453 ± 128
1 month	343 ± 90	43 ± 10	16 ± 11	434 ± 104	3216 ± 304	781 ± 118	104 ± 60	31 ± 15	15 ± 5	423 ± 81
2 months	352 ± 63	34 ± 8	13 ± 11	451 ± 92	3369 ± 303	914 ± 421	75 ± 26	19 ± 5	14 ± 2	362 ± 57
3 months	347 ± 110	34 ± 7	15 ± 10	420 ± 62	3622 ± 381	1153 ± 428	85 ± 37	22 ± 9	15 ± 4	404 ± 54
4 months	335 ± 64	30 ± 8	6 ± 4	377 ± 81	3418 ± 294	1460 ± 663	62 ± 22	17 ± 8	14 ± 6	383 ± 68
5 months	327 ± 64	34 ± 13	9 ± 6	383 ± 50	3756 ± 259	1390 ± 572	88 ± 28	22 ± 13	13 ± 6	389 ± 83
6 months	279 ± 59	29 ± 11	12 ± 8	343 ± 87	3458 ± 567	1402 ± 502	59 ± 18	18 ± 11	13 ± 6	379 ± 115
7 months	245 ± 47	35 ± 16	14 ± 9	336 ± 44	3912 ± 471	1268 ± 380	63 ± 17	18 ± 11	13 ± 5	382 ± 84
8 months	232 ± 51	32 ± 14	11 ± 8	359 ± 111	3714 ± 620	1496 ± 394	68 ± 18	15 ± 6	12 ± 5	336 ± 55
9 months	247 ± 64	36 ± 15	12 ± 3	335 ± 82	3848 ± 621	1424 ± 324	91 ± 35	17 ± 6	12 ± 6	331 ± 50
10 months	245 ± 24	43 ± 18	7 ± 3	359 ± 56	3913 ± 478	1505 ± 162	65 ± 16	13 ± 4	15 ± 7	321 ± 69
11 months	183 ± 35	32 ± 19	6 ± 4	331 ± 60	3807 ± 510	1707 ± 234	56 ± 25	13 ± 3	13 ± 9	295 ± 69
12 months	204 ± 67	39 ± 21	9 ± 6	359 ± 84	3667 ± 457	1674 ± 294	48 ± 11	14 ± 5	14 ± 7	311 ± 48
CV	22.50	41.60	75.71	18.99	11.85	31.27	43.41	49.83	50.03	17.46

IgA and IgG - immunoglobulins A and G; MWP_{138}, MWP_{33}, MWP_{23} - 138, 33, 23 kDa molecular weight proteins.

haptoglobin, α_1-acid glycoprotein, 33 kDa (MWP_{33}) and 23 kDa (MWP_{23}) molecular weight proteins. There was not age-related difference ($P>0.05$) for MWP_{138}. IgG concentrations increased ($P<0.0001$, $y = 2.35x + 887.25$) during the first year of age, while IgA showed a marked rising until 112 days, when had a slight decline ($P<0.0001$, $y = 0.00005x^3 - 0.03x^2 + 5.17x + 109.23$). Ceruloplasmin ($P<0.001$, $y = -0.03x + 17.20$), α_1-acid glycoprotein ($P<0.05$, $y = 0.000x^2 - 0.12x + 32.97$), and MWP_{33} ($P<0.05$, $y = -0.01x + 16.36$) decreased during the first year of age. Albumin showed an upward trend during this period ($P<0.0001$, $y = 1.77x + 3295.06$). The haptoglobin curve raised slightly for 145 days, when declined ($P<0.05$, $y = -0.0006x^3 + 0.17x + 65.54$). The values of transferrin ($P<0.0001$, $y = 0.00004x^3 - 0.03x^2 + 3.90x + 243.53$) and MWP_{23} ($P<0.01$, $y = 0.00002x^3 - 0.02x^2 + 2.51x + 294.04$) increased for 3 months after birth, then started to decrease.

Discussion

As described by some authors, RBC (BROWN and CROSS, 1969; ZINKL et al., 1990; ORLANDI et al., 1997; CALDIN et al., 2005; PITEL et al., 2006; VERONESI et al., 2014), hemoglobin concentration (BROWN and CROSS, 1969; ORLANDI et al., 1997; PITEL et al., 2006) and PCV (BROWN and CROSS, 1969; PITEL et al., 2006) decreased with advancing age. As well, SGORBINI et al. (2013) reported decreasing in hemoglobin and PCV values after the first 48 hours of life of Amiata foals. These higher erythrocytic values for younger animals could be related to the greater stress they suffer during sampling, resulting in splenic contraction with release of erythrocytes in bloodstream, as previously observed in horses (PITEL et al., 2006). The platelet count decrease throughout the period agrees with PITEL et al. (2006) and SGORBINI et al. (2013), which observed higher means for the youngest animals of their researches.

The WBC curve profile is similar to that described by BROWN and CROSS (1969), PITEL et al. (2006), SGORBINI et al. (2013) and VERONESI et al. (2014), which reported WBC levels increasing for donkeys in the first months of life. This might be due to the increased bone marrow production related to environment exposure (SGORBINI et al., 2013).

Monocyte counts raised until 6 months of age, when started to reduce, as reported in other studies that described a decreasing trend with aging (CALDIN et al., 2005; PITEL et al., 2006). The eosinophil count increase during the first year is similar to that reported by BROWN and CROSS (1969) and ZINKL et al. (1990), which described an upward trend of this variable with age. This increase is probably due to progressive contact of animals with parasite or allergen antigens since their birth, which induct eosinophil poietic factors formation, mainly interleukin 5 (WEISS and WARDROP, 2010).

As reported in previous studies, there were not age-related differences for values of basophils (FOLCH et al., 1997; VERONESI et al., 2014), segmented neutrophils and

chlorides (VERONESI et al., 2014), CK (ORLANDI et al., 1997; JORDANA et al., 1998; PITEL et al. 2006), creatinine (PITEL et al., 2006), albumin (PITEL et al. 2006; SGORBINI et al., 2013; VERONESI et al., 2014), triglycerides (SGORBINI et al., 2013), sodium (ORLANDI et al., 1997; CALDIN et al., 2005; SGORBINI et al., 2013), magnesium (CALDIN et al. 2005; VERONESI et al., 2014) and potassium (CALDIN et al. 2005; SGORBINI et al., 2013). The band neutrophil count was not associated with time, disagreeing with FOLCH et al. (1997) that observed its decreasing with advancing.

AP levels fell in the first year of age, as reported by CALDIN et al. (2005), PITEL et al. (2006), SGORBINI et al. (2013) and VERONESI et al. (2014). Higher levels of AP for younger animals could be explained by high AP activity of bone origin, due to the intense bone metabolism during body growth, which decreases with advancing age (PITEL et al., 2006). Similar to other authors' observations, levels of AST (PITEL et al. 2006; SGORBINI et al., 2013) tended to increase with aging. The alterations of GGT activity with advancing age disagree with some researchers (ORLANDI et al., 1997; JORDANA et al., 1998; CALDIN et al., 2005; PITEL et al., 2006, VERONESI et al., 2014) which not observed age-related changes in donkeys.

Total serum protein showed an increase with aging, in agreement with reports of PERDIGÃO DE OLIVEIRA et al. (1980), DINEV and KHUBENOV (1986), ZINKL et al. (1990), FOLCH et al. (1997), PITEL et al. (2006), ETANA et al. (2011), and VERONESI et al. (2014). It is likely due to the increasing amount of globulines, since the serum albumin levels did not change during the experimental period, reflecting the gradual immunocompetence improvement in response to environmental challenges.

As found by previous researches, the mean levels of ALT (VERONESI et al., 2014), plasma glucose (ZINKL et al., 1990) and serum cholesterol (CALDIN et al., 2005; PITEL et al., 2006; VERONESI et al., 2014) decreased during the observational period. Increased levels of glucose and cholesterol for younger animals during the first year of age may be due to the kind of feeding, that is done mainly by breastfeeding in the beginning, and naturally change during this period, with increasing forage intake.

The initial reductions of direct and total bilirubin levels were similar to observations of SGORBINI et al. (2013), that reported higher total bilirubin level at birth, remaining constant up to the third week, and VERONESI et al. (2014), that detected lower values of total bilirubin for animals from day two and 21 of life, compared with the period between birth and day one. This could be caused by erytrocyte destruction during perinatal period (SGORBINI et al., 2013).

The early decrease of serum urea concentrations is similar to that reported by SGORBINI et al. (2013), VERONESI et al. (2014) and by PITEL et al. (2006).

The serum phosphorus curve behavior from the first month corroborates some studies (DINEV and KHUBENOV, 1986; ZINKL et al., 1990; ORLANDI et al., 1997; JORDANA et

al., 1998; CALDIN et al., 2005) that described a decreasing trend in its concentration with advancing age. The increase of serum phosphorus levels observed during the first month is in agreement with VERONESI et al. (2014), that observed elevation of these values from second to 21th day after birth. High serum phosphorus levels generally reflect fast bone growth periods like neonatal and youth stages and, after, these levels show a gradual decline with the increase of age (EVANS, 2009). The decrease in serum phosphorus with aging probably corresponds to reduction of bone metabolism (ZINKL et al. 1990).

Reduction in total calcium levels during the first year disagrees with CALDIN et al. (2005), PITEL et al. (2006), SGORBINI et al. (2013), and VERONESI et al. (2014), which have not seen association between age and the concentration of this variable. Ionized calcium levels showed different behavior to total calcium, increasing in the first 7 months. No other study involving age and ionized calcium serum concentration in donkeys was found.

The observations about IgA and IgG agree with PERDIGÃO DE OLIVEIRA et al. (1980), ZINKL et al. (1990) and PITEL et al. (2006), which reported an upward trend in levels of serum globulins in donkeys with advancing age. This is due, firstly, to the colostrum intake (PERDIGÃO DE OLIVEIRA et al., 1980; ECKERSALL, 2008) and, then, to the maturation of the immune system of the neonate, which rapidly gains immunocompetence and begins to synthesize its own immunoglobulins (ECKERSALL, 2008).

As ceruloplasmin is an α_2-globulin (ECKERSALL, 2008), its decreasing trend agrees with CALDIN et al. (2005), which reported higher values of α_2-globulins for younger donkeys. The reduction in ceruloplasmin and α_1-acid glycoprotein, which are α-globulins (ECKERSALL, 2008), contrasts with the finding of PERDIGÃO DE OLIVEIRA et al. (1980) that observed an upward trend of α-globulins in donkeys from birth to eight months of age. Alpha$_1$-acid glycoprotein binds to pharmacological compounds, what can affect the free concentration of drugs (ECKERSALL, 2008). So, it would be interesting to assess if some drugs administered to donkey foals need an increased dose to achieve therapeutic effects in these animals.

The initial increase of haptoglobin concentration, an α_2-globulin (ECKERSALL, 2008), agrees with CALDIN et al. (2005), which described greater mean values of α_2-globulins for animals until one year, compared to other age groups. Also PERDIGÃO DE OLIVEIRA et al. (1980) observed an upward trend of α-globulins in donkey foals from birth to eight months of age. The early increasing trend of transferrin values is similar to that reported by PERDIGÃO DE OLIVEIRA et al. (1980), which observed increasing of β-globulins, fraction containing the transferrin protein, in donkeys from birth to eight months old.

MWP_{138}, MWP_{33} and MWP_{23} proteins are not described in the referred literature, and their functions remain unknown; however, MWP_{23} protein changed significantly throughout the period and this fact warrants further investigations on it.

Discrepancies among this and previous studies can be explained by different electrophoretic techniques used and, logically, by differences of breed, handling, feeding and environment. Additional discussion about the other proteins obtained was not possible because such differentiation, in relation to the changes on the serum protein fractions separated by SDS-PAGE during the first year of life in donkeys, is unprecedented.

To our knowledge, this is the first report on changes of serum protein fractions, hematological and biochemical variables of Pêga donkey throughout the first year of life.

Conclusions

The results obtained indicate that, for Pêga breed donkeys, most of the hematological, serum biochemical variables and serum protein fractions physiologically change during the first year of age and these differences must be considered during the clinical evaluation of the animal. The data obtained are useful for clinical routine and as basis for future scientific investigations about donkeys' physiology and metabolism.

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SAŽETAK

Istraživanje opisuje promjene bjelančevinskih frakcija u serumu, te hematoloških i biokemijskih pokazatelja tijekom prve godine života Pêga magaraca u Brazilu. U uzorcima krvi 8 magaraca, 3 ženke i 5 mužjaka, prikupljenima na dan puljenja, 3., 7. i 15. dan te mjesečno do dobi od 12 mjeseci, provedene su analize kompletne krvne slike, biokemijskih pokazatelja i frakcija bjelančevina u serumu dobivenih elektroforezom. Nije bilo dobno uvjetovanih razlika za CK, albumin, magnezij, kalij, kloride, trigliceride, bazofile, nesegmentirane i segmentirane neutrofile. Koncentracija natrija ostala je gotovo nepromijenjena. Krivulja bijelih krvnih stanica rasla je do 5 mjeseci, limfocita do 7 mjeseci, monocita do 6 mjeseci. Fosfor je rastao tijekom prvog mjeseca, IgA do 3 mjeseca, a nakon toga su svi spomenuti parametri počeli padati. Ureja i koncentracija ioniziranog kalcija smanjivali su se do 15. dana, nakon čega su pokazali tendenciju rasta. Eozinofili, ukupne bjelančevine, GGT, AST, kreatinin, IgG, transferin, albumin (određen elektroforezom), haptoglobin, bjelančevine s molekulskom masom 23 i 138 kDa pokazali su tijekom tog razdoblja trend rasta. Broj eritrocita, koncentracija hemoglobin, PCV, trombociti, glukoza, ukupni kalcij, kolesterol, ALT, AP, ceruloplazmin, α_1-kiseli glikoprotein, bjelančevina s molekulskom masom od 33 kDa, indirektni, direktni i ukupni bilirubin, pokazali su tendenciju opadanja tijekom prve godine života. Ovo je prvo izvješće o promjenama bjelančevinskih frakcija u serumu, te hematoloških i biokemijskih pokazatelja kod Pêga magarca tijekom prve godine života. Pokazano je da se većina pokazatelja u tom razdoblju mijenja. Dobiveni podaci korisni su za kliničku rutinu i predstavljaju osnovu za buduća znanstvena istraživanja fiziologije i metabolizma magaraca.

Ključne riječi: biokemija, klinička patologija, magarci, hematologija, novorođenče, SDS-PAGE