

Biochemical and haematological profile of donkeys in the Czech and Slovak Republics: influence of age and sex

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Abstract

The aim of this study was to evaluate the importance of taking into account the age and sex of the donkeys while assessing individual haematological and biochemical variables. Material used for the study were blood samples collected from 112 clinically healthy donkeys (37 males and 75 females) aged from 0.5 to 30 years old. Selected haematological and biochemical variables were examined and the influence of sex and age was analyzed by multivariate analysis. Significant differences were found in some of biochemical and haematological variables between groups. The results of this study can be useful for appropriate clinical interpretation of laboratory results in donkeys.

Blood analysis, serum analysis, jenny, jack, foals

Biochemical and haematological examination of the blood is routinely used in preventive medicine, diagnosis of disease and verification of treatment success in all kinds of animals. Individual species have specific physiological ranges of blood variables, donkeys included. Zinkl et al. (1990) and Jordana et al. (1998) proved that donkey laboratory findings are not completely comparable with reference to other species nor even to, their closest relatives, horses.

Scientific reports show that a large number of factors can affect blood biochemical and haematological indices even within one species. Changes due to geographic location, nutritional status or the effect of management practice have already been proved (Jordana et al. 1998; Mushi et al. 1999; Sow et al. 2012; Sedlinska et al. 2016).

The influence of the donkey's age and sex has also already been reported (Folch et al. 1997; Jordana et al. 1998; Mori et al. 2003, 2004; Girardi et al. 2013, 2014). On the other hand, there is a study which investigated about 4000 donkeys without proof that biochemical variables are affected by the age and sex (French and Patrick 1995).

A previous study by Sedlinska et al. (2016) established normal reference values of biochemical and hematological indices of donkeys in the Czech and Slovak Republics (Central Europe); those values can be useful for clinical routines, allowing veterinarians to establish appropriate interpretations of laboratory data. This study aims to evaluate how important it is to take into account the donkey's age and sex while assessing individual haematological and biochemical variables.

Materials and Methods

Blood serum collected from 112 clinically healthy donkeys (37 males and 75 females) aged from 0.5 to 30 years and housed in different locations and conditions (26 different owners) in the Czech and Slovak Republics were examined for selected haematological and biochemical indices.

Blood was collected by jugular venipuncture into 10 ml tubes for biochemistry and 2.5 ml ethylenediamine tetraacetic acid (EDTA) tubes for haematology. The haematological indices examined were: red blood cells

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Table 1. Biochemical and haematological profile of donkeys: influence of age.

	0-3 years n=54					3-10 years n=33					Above 10 year n=18					F	P
	Mean	Med	Perc 2.5%	Perc 97.5%	SD	Mean	Med	Perc 2.5%	Perc 97.5%	SD	Mean	Med	Perc 2.5%	Perc 97.5%	SD		
WBC (G/l)	11.31	11.30	5.90	18.80	2.92	9.78	9.85	4.90	15.50	2.57	10.28	10.20	6.30	15.80	2.71	0.494	0.484
RBC (T/l)	6.93	6.87	5.43	8.52	0.89	6.04	5.82	4.48	8.52	1.02	5.72	5.66	4.52	7.16	0.77	15.996	<0.001
Hb (g/l)	124.81	125.00	91.00	159.00	25.67	126.93	125.50	98.00	177.00	17.69	122.76	123.00	98.00	154.00	16.35	0.034	0.853
HCT (l/l)	0.35	0.35	0.27	0.44	0.05	0.36	0.35	0.28	0.50	0.05	0.34	0.34	0.28	0.43	0.04	0.019	0.890
MCV (fl)	51.30	51.30	42.60	60.20	4.79	59.38	59.65	47.80	67.50	4.73	60.05	60.30	52.90	65.70	3.04	44.186	<0.001
MCH (pg)	18.55	18.50	14.90	22.80	1.91	21.16	21.80	16.80	23.60	1.67	21.49	21.70	18.20	22.50	1.04	35.278	<0.001
MCHC (g/l)	358.40	358.50	313.00	384.00	15.72	356.70	355.50	333.00	395.00	11.43	358.00	357.00	337.00	382.00	12.92	0.00	0.981
PLT (G/l)	221.75	211.50	53.00	423.00	84.63	229.57	219.50	63.00	448.00	81.44	194.59	173.00	70.00	299.00	64.59	0.149	0.700
Neutrophil bands (%)	0.82	1.00	0.00	4.00	1.18	0.62	0.00	0.00	4.00	1.08	1.00	0.00	0.00	6.00	1.80	2.147	0.146
Neutrophil bands (g/l)	0.11	0.06	0.00	0.53	0.19	0.07	0.00	0.00	0.46	0.13	0.12	0.00	0.00	0.95	0.25	0.856	0.357
NEU (%)	35.66	35.00	14.00	67.00	14.50	37.00	37.50	9.00	66.00	15.75	47.41	50.00	17.00	79.00	14.13	8.487	0.004
NEU (g/l)	4.02	3.39	1.58	10.92	2.08	3.71	3.74	0.68	8.48	1.86	4.75	5.00	1.73	7.98	1.52	3.288	0.073
LYM (%)	56.43	59.00	1.00	82.00	18.47	53.13	51.50	1.00	88.00	22.09	39.12	39.00	11.00	71.00	13.69	12.841	<0.001
LYM (g/l)	6.32	6.89	0.09	10.15	2.54	5.23	5.45	0.07	11.16	2.48	3.94	3.74	1.11	7.24	1.50	12.677	<0.001
MON (%)	1.23	1.00	0.00	5.00	1.59	0.73	0.50	0.00	3.00	0.91	1.24	1.00	0.00	5.00	1.52	0.128	0.721
MON (g/l)	0.15	0.07	0.00	0.70	0.22	0.07	0.03	0.00	0.41	0.10	0.13	0.09	0.00	0.63	0.19	0.380	0.539
EOS (%)	4.30	3.00	0.00	15.00	4.29	5.13	4.50	0.00	13.00	4.21	6.50	4.50	2.00	16.00	4.70	4.803	0.031
EOS (g/l)	0.49	0.41	0.00	1.70	0.50	0.51	0.44	0.00	1.42	0.40	0.67	0.45	0.15	1.89	0.58	3.441	0.067
BAS (%)	0.09	0.00	0.00	2.00	0.40	0.10	0.00	0.00	1.00	0.31	0.12	0.00	0.00	1.00	0.33	0.907	0.343
BAS (g/l)	0.01	0.00	0.00	0.17	0.04	0.01	0.00	0.00	0.14	0.03	0.01	0.00	0.00	0.11	0.04	0.914	0.341
TP (g/l)	75.17	75.35	44.50	89.90	10.35	78.35	78.30	49.60	91.40	7.55	78.26	79.00	59.60	95.90	7.79	4.009	0.048
ALB (g/l)	31.85	32.15	20.30	38.70	4.83	33.94	32.90	20.90	43.30	4.31	31.85	31.90	25.70	40.30	3.90	0.129	0.7199
CREA (µmol/l)	112.79	110.55	68.50	161.80	28.63	114.18	105.60	65.20	159.50	28.76	109.79	105.90	76.40	189.00	30.07	0.500	0.481
ALP (µkat/l)	3.85	3.85	2.01	6.57	1.13	3.36	3.33	1.78	6.29	1.09	4.08	3.72	1.86	7.52	1.65	1.163	0.283
AST (µkat/l)	6.03	5.92	3.74	9.02	1.56	6.14	5.61	3.30	13.90	2.03	6.21	6.02	3.73	9.79	1.43	0.000	0.997
CK (µkat/l)	5.21	5.04	2.27	10.38	2.09	6.64	6.62	3.12	11.79	2.21	6.88	6.76	3.35	10.82	2.63	6.692	0.011
GGT (µkat/l)	1.52	1.47	0.30	2.80	0.62	1.67	1.63	0.27	2.70	0.54	1.81	1.94	0.95	2.94	0.61	2.633	0.108
LDH (µkat/l)	9.73	8.95	4.35	18.31	3.79	11.08	11.30	3.63	16.52	3.25	12.38	11.95	5.07	21.71	4.42	1.244	0.267

Table 1. Biochemical and haematological profile of donkeys: influence of age.

	0-3 years n=54					3-10 years n=33					Above 10 year n=18				
	Mean	Med	Perc 2.5%	Perc 97.5%	SD	Mean	Med	Perc 2.5%	Perc 97.5%	SD	Mean	Med	Perc 2.5%	Perc 97.5%	SD
TAG (mmol/l)	3.47	0.91	0.18	52.35	15.99	5.82	1.04	0.21	103.00	22.27	0.97	1.01	0.36	1.78	0.52
CHOL (mmol/l)	2.36	2.10	1.40	3.10	1.62	2.14	2.10	0.80	3.70	0.66	2.16	2.20	1.20	3.00	0.59
Ca (mmol/l)	2.93	3.09	1.71	3.44	0.45	3.11	3.12	2.02	3.55	0.25	3.03	3.01	2.58	3.43	0.22
P (mmol/l)	1.90	1.96	0.98	2.68	0.42	1.71	1.63	0.91	2.53	0.45	1.57	1.57	0.87	2.26	0.39
Na (mmol/l)	134.36	135.70	105.00	143.70	8.54	131.95	134.75	96.30	143.90	10.95	135.54	138.00	120.00	142.80	7.00
K (mmol/l)	5.15	5.20	4.10	6.50	0.75	5.40	5.40	3.50	9.40	1.36	5.16	5.20	3.80	6.30	0.66
LAC (mmol/l)	7.99	7.72	2.42	14.13	2.93	7.77	7.87	1.42	13.70	2.70	8.62	8.46	5.70	15.36	2.58

WBC (white blood cells), RBC (red blood cells), Hb (hemoglobin concentration), HCT (hematocrit), MCV (mean cell volume), MCH (mean cell hemoglobin), MCHC (mean cell hemoglobin concentration), PLT (platelets), NEU (segmented neutrophils), LYM (lymphocytes), EOS (eosinophils), BAS (basophils), TP (total protein), ALB (albumin), CREA (creatinine), ALP (alkaline phosphatase), AST (aspartate aminotransferase), CK (creatinine kinase), GGT (gamma-glutamyltransferase), LDH (lactate dehydrogenase), TAG (triacylglycerols), CHOL (cholesterol), Ca (calcium), P (phosphorus), K (potassium), Na (sodium), LAC (lactate), Mean (arithmetic mean), Med (median), Perc (percentile), SD (standard deviation), F and P- results of GLM multivariate analysis

(RBC), white blood cells (WBC), haemoglobin concentration (Hb), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelets (PLT), segmented neutrophils (NEU), neutrophil bands, lymphocytes (LYM), monocytes (MON), eosinophils (EOS), and basophils (BAS). Indices were calculated using the haematological analyzer Celltac alpha MEK 6318 (Nihon Kohden, Tokyo, Japan). For each sample, a peripheral blood smear from fresh blood was prepared, stained by the Diff-Quick method and a differential leukocyte count was examined microscopically.

Samples for biochemistry were allowed to clot at room temperature and serum was separated by centrifugation at 3000 g for 15 min. Serum samples were analyzed for metabolites: total protein (TP), albumin (ALB), creatinine (CREA), alkaline phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), triacylglycerols (TAG), cholesterol (CHOL), calcium (Ca), phosphorus (P), potassium (K), sodium (Na) and lactate (LAC). All analyses were performed at the Central Clinical Laboratory of the University of Veterinary and Pharmaceutical Sciences Brno by the biochemical analyzer DPC Konelab 20i® (Thermo Fisher Scientific Oy, Vantaa, Finland).

The effects of age (continuous variable) and sex (categorical variable) on blood indices were analyzed by multivariate analysis (GLM) in the software Statistica for Windows.

Results

The results of biochemical and haematological profiling are listed in Table 1. The results of biochemical and haematological profiling divided according to the sex (male or female donkey) are listed in Table 2. In the study, some significant differences ($P < 0.05$) were found: RBC, MCV, MCH, NEU, LYM, EOS, CK, serum phosphorus in relation to age, and MCV, MCH, TP, CREA in relation to sex.

Discussion

In relation to age, significant differences were found in the following variables: RBC, MCV, MCH, NEU, LYM, EOS, CK, and serum phosphorus.

The significant decrease of RBC with age, which was found in this study, had also been described previously

Table 2. Biochemical and haematological profile of donkeys: influence of sex.

	Male n=36				Female n=69							
	Mean	Med	Perc 2.5%	Perc 97.5%	SD	Mean	Med	Perc 2.5%	Perc 97.5%	SD	F	P
WBC (G/l)	10.78	11.10	5.90	18.90	2.75	10.61	10.75	5.50	17.60	2.90	0.048	0.826
RBC (T/l)	6.77	6.96	4.88	8.52	0.98	6.30	6.26	4.52	8.13	1.04	3.878	0.052
Hb (g/l)	127.42	126.00	99.00	177.00	18.11	123.93	123.00	91.00	159.00	23.63	0.572	0.451
HCT (l/l)	0.35	0.35	0.28	0.50	0.05	0.35	0.35	0.27	0.45	0.05	0.000	1.000
MCV (fl)	52.11	51.50	40.80	62.50	5.58	56.82	57.25	45.00	65.70	5.81	16.227	<0.001
MCH(pg)	18.89	18.40	14.90	22.30	1.98	20.35	20.70	15.40	23.60	2.14	10.515	0.0016
MCHC(g/l)	360.70	357.00	337.00	395.00	12.29	356.38	357.50	313.00	383.00	14.61	2.09	0.151
PLT (G/l)	216.06	217.00	47.00	461.00	85.43	221.15	207.00	77.00	423.00	78.85	0.106	0.745
Neutrophil bands (%)	0.77	1.00	0.00	5.00	1.02	0.80	0.00	0.00	6.00	1.38	0.00004	0.995
Neutrophil bands (g/l)	0.09	0.07	0.00	0.51	0.13	0.10	0.00	0.00	0.95	0.21	0.023	0.879
NEU (%)	38.09	38.00	17.00	79.00	14.17	38.04	38.00	9.00	66.00	15.92	0.067	0.797
NEU (g/l)	4.07	3.39	1.97	11.34	1.93	4.05	3.95	0.68	8.48	1.96	0.042	0.838
LYM (%)	51.91	59.00	0.00	75.00	21.58	52.79	51.00	14.00	88.00	19.01	0.270	0.604
LYM (g/l)	5.62	6.03	0.00	11.16	2.81	5.56	5.39	1.76	10.15	2.38	0.0398	0.842
MON (%)	1.03	1.00	0.00	5.00	1.29	1.10	1.00	0.00	5.00	1.48	0.075	0.785
MON (g/l)	0.10	0.07	0.00	0.47	0.13	0.13	0.07	0.00	0.70	0.21	0.494	0.484
EOS (%)	3.97	3.00	0.00	15.00	4.35	5.34	4.00	0.00	16.00	4.31	2.032	0.157
EOS (g/l)	0.43	0.26	0.00	1.89	0.47	0.57	0.45	0.00	1.76	0.48	1.722	0.193
BAS (%)	0.13	0.00	0.00	2.00	0.42	0.09	0.00	0.00	1.00	0.34	0.277	0.5997
BAS (g/l)	0.01	0.00	0.00	0.21	0.04	0.01	0.00	0.00	0.14	0.03	0.220	0.6399
TP (g/l)	78.95	77.90	67.60	95.90	6.76	75.49	77.30	44.50	91.40	10.11	4.195	0.048
ALB (g/l)	33.16	32.70	25.00	40.10	3.72	32.17	32.45	20.30	40.30	4.99	1.130	0.290
CREA(μmol/l)	122.56	122.00	11.30	189.00	33.32	107.54	103.00	68.50	150.00	24.56	7.0799	0.009
ALP (μkat/l)	3.89	3.76	1.86	7.31	1.16	3.65	3.51	1.78	6.57	1.27	1.104	0.296
AST (μkat/l)	5.92	5.39	3.30	11.39	1.93	6.18	6.08	3.89	9.79	1.56	0.520	0.472
CK (μkat/l)	5.42	5.11	2.27	10.46	2.20	6.18	5.56	3.09	11.79	2.35	2.005	0.1599
GGT (μkat/l)	1.59	1.61	0.27	2.70	0.61	1.63	1.53	0.30	2.94	0.60	0.046	0.831
LDH (μkat/l)	9.60	9.71	3.63	15.68	3.04	11.08	10.91	4.35	21.71	4.10	2.754	0.100
TAG (mmol/l)	1.15	1.03	0.23	2.70	0.55	5.44	0.86	0.21	102.00	21.43	1.210	0.275

Table 2. Biochemical and haematological profile of donkeys: influence of sex.

	Male n=36					Female n=69									
	Mean	Med	Perc 2.5%	Perc 97.5%	SD	Mean	Med	Perc 2.5%	Perc 97.5%	SD	F	P			
CHOL (mmol/l)	2.34	2.35	1.40	3.00	0.42	2.21	2.00	1.20	3.70	1.51	0.217	0.642			
Ca (mmol/l)	2.98	3.09	1.60	3.44	0.41	3.02	3.11	1.96	3.52	0.35	0.149	0.700			
P (mmol/l)	1.85	1.91	1.01	2.68	0.45	1.75	1.73	0.91	2.53	0.43	0.776	0.380			
Na (mmol/l)	135.06	137.00	102.00	143.90	7.84	133.04	135.30	100.00	143.70	9.83	0.962	0.3299			
K (mmol/l)	5.29	5.05	3.70	9.40	1.12	5.19	5.40	3.80	6.70	0.86	0.187	0.666			
LAC (mmol/l)	8.15	8.29	1.42	15.36	3.15	7.97	7.64	2.42	13.70	2.62	0.222	0.639			

WBC (white blood cells), RBC (red blood cells), Hb (hemoglobin concentration), HCT (hematocrit), MCV (mean cell volume), MCH (mean cell hemoglobin), MCHC (mean cell hemoglobin concentration), PLT (platelets), NEU (segmented neutrophils), LYM (lymphocytes), MON (monocytes), EOS (eosinophils), BAS (basophils), TP (total protein), ALB (albumin), CREA (creatinine), ALP (alkaline phosphatase), AST (aspartate aminotransferase), CK (creatinine kinase), GGT (gamma-glutamyltransferase), LDH (lactate dehydrogenase), TAG (triacylglycerols), CHOL (cholesterol), Ca (calcium), P (phosphorus), K (potassium), Na (sodium), LAC (lactate), Mean (arithmetic mean), Med (median), Perc (percentile), SD (standard deviation), F and P - results of GLM multivariate analysis

by several other authors (Zinkl et al. 1990; Folch et al. 1997; Caldin et al. 2005; Laus et al. 2015). Some previous studies (Zinkl et al. 1990; Caldin et al. 2005; Laus et al. 2015) reported this alteration as associated with the smaller size of red cells in young donkeys that they explained by a slight deficiency of iron. In this study, significantly lower values of MCV and MCH were found in donkeys under 3 years old, but the iron level was not measured.

A significantly lower content of LYM was also found in donkeys older than 10 years, which is similar to that reported by Folch et al. (1997).

The NEU values in this study were higher in donkeys older than 10 years, which is not in agreement with previous reports by Folch et al. (1997) and Caldin et al. (2005) who reported a reduction of this variable with aging.

The EOS in our study tended to increase with age, which is in agreement with other studies (Zinkl et al. 1990; Girardi et al. 2014) but at odds with Folch et al. (1997) who reported a decrease of this variable with aging. We can presume that this increase in EOS may be attributed to a higher parasitic burden with age or progressive contact with allergen antigens.

The influence of age on the biochemical properties was revealed. The level of TP was greater for older animals, which is similar to other studies, where an increase level of total protein with age was described (Zinkl et al. 1990; Folch et al. 1997; Girardi et al. 2013), possibly due to the increasing amount of globulins.

This study found an increasing content of CK associated with aging. This can be due to the difference in muscle mass (due to different exercise) between the studied groups. However, no effect of age on CK was found in other studies (French and Patrick 1995; Girardi et al. 2013).

This study also found a significant difference in the values of plasmatic phosphorus concentrations in relation to age. Significantly higher values were found in donkeys up to 3 years of age, which confirmed previous studies (Zinkl et al. 1990; Jordana et al. 1998; Caldin et al. 2005; Girardi et al. 2013). This is probably due to an increased bone metabolism in younger animals.

In relation to sex, significant differences were found in the following indicators: MCV, MCH, TP, and CREA. In this study significantly higher ($P < 0.05$) values of MCH and MCV were found in female animals. This is in complete contrast to the results of the study by Babeker and Bdalbagi (2014) who found significantly higher values of MCH and MCHC in male donkeys. Their study also reports significantly lower values of Hb in female donkeys and slightly higher levels of RBC in male animals, which was also confirmed in our study. The lower RBC in females was probably because female oestrogens decrease erythropoietin production.

Girardi et al. (2013) report that males show greater values of CREA. This is also in agreement with our study where males have higher CREA. This can be due to bigger muscle tissue in males compared to females.

Data in our study showed significant differences in several biochemical and haematological analytes between groups of 112 healthy donkeys divided by age and sex. This analysis can help with future investigations on the influence of age and sex on haematological and biochemical indices under different conditions. An appropriate interpretation of laboratory results can help to improve the veterinary care of donkeys. However, there are some discrepancies in this field. On the one hand, many previous studies (mentioned above) describe varying indices for different sexes and ages in a population of donkeys (Folch et al. 1997; Jordana et al. 1998; Mori et al. 2003, 2004; Girardi et al. 2013, 2014). On other hand, in the study of French and Patrick (1995) based on a population of approximately 4,000 donkeys, no differences in biochemical indices in relation to age and sex were found. Additionally, Laus et al. (2015) found no differences between sexes in biochemical and haematological properties, and Yakubu and Chafe (2008) found no correlation between the haematological indices and sex. More investigation in this field is needed.

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