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Biochemical blood parameters in Balkan donkeys (*Equus asinus*) in Bulgaria

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Abstract

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The Balkan donkey is an autochthonous breed, traditionally raised by livestock breeders on the Balkan Peninsula. Its population in Bulgaria is decreasing, ageing, and even threatened with extinction. Therefore, the awareness of the physiological and pathological conditions of the Balkan donkey is essential for the conservation of the breed.

The present study aimed to investigate a large number of blood biochemical parameters in healthy Balkan donkeys in Bulgaria and to determine their gender- and age-related changes. Blood sera from 73 clinically healthy Balkan donkeys were collected to assay 20 biochemical blood parameters using a Mindray BS-120 biochemical analyzer and an Electrolyte Analyzer/Caretium: XI–921A. Statistical analysis of the gender and age effects on the measured variables was performed with the non-parametric Kruskal-Wallis test.

Regarding gender, significant differences were observed in the following biochemical parameters: Glu, CREA, TG, TP, ASAT, ALAT, ALP, Ca, and Cl. Additionally, significant differences were noted in age for LDH.

The study emphasizes the need to consider animal gender and age when interpreting blood biochemical parameters about the protection of animal genetic resources.

Keywords: Balkan donkeys; Bulgaria; healthy; blood; biochemical parameters
Abbreviations: Total protein (TP); albumin (ALB); creatinine (CREA); urea (UREA); glucose (Glu); cholesterol (CHOL); triglycerides (TG); total bilirubin (TBili); aspartate aminotransferase (ASAT); alanine aminotransferase (ALAT); alkaline phosphatase (ALP); creatine kinase (CK); gamma-glutamyltransferase (GGT); lactate dehydrogenase (LDH); calcium (Ca); phosphorus (P); magnesium (Mg); potassium (K); sodium (Na); chlorides (Cl)

Introduction

According to FAO data, the global donkey population is in decline. In 2000, their number was 43,472 million, including 163 different breeds and breed groups. Six years later, in 2006, the donkey population had declined to 41 million (Vlaeva et al., 2019), with some breeds facing extinction. The trend is similar in Europe, particularly in the Balkan Peninsula (Djoković et al., 2020).

The Balkan donkey is a local breed, traditionally raised by livestock breeders on the extensive highland pastures of the Balkan Peninsula (Stanišic et al., 2015; Djoković et al., 2020). In the past, it was primarily used as a working and draft animal, carrying loads from the mountainous regions, but today its status has a different dimension. In the area, donkeys are reared as companion animals, for tourist attrac-

tions, for light agricultural activities, or as part of a new target for the production of dairy and meat products (Vlaeva et al., 2019). While the Balkan donkey population is generally decreasing, in some countries, such as Croatia and Serbia, they are included in programs for the conservation of animal genetic resources, and their number has increased in recent years (Vlaeva et al., 2019; Djoković et al., 2020). Unfortunately, in other countries of the region, their populations are still diminishing and are even threatened with extinction.

Nevertheless, the interest in donkeys' (*Equus asinus*) welfare and health is steadily growing at a worldwide scale (Coroian et al., 2019). Information on health monitoring and diagnosis of different diseases in donkeys is still scarce. In addition to the primary clinical indicators, haematological parameters and a large number of serum biochemical indices are also necessary for the diagnosis and prognosis of diseases (Kisadere et al., 2019). Although data on haematological and biochemical parameters in donkeys have been recently published (Mori et al., 2003; Girardi et al., 2014; Al Shafei et al., 2015; Laus et al., 2015; Burden et al., 2016; Sedlinská et al., 2018; Ayo et al., 2024), they are from other regions of the world and usually refer to a particular breed traditional to the area. In our country, as well as in most neighbouring countries except Serbia (Stanišic et al., 2015), where local Balkan donkeys are raised, no data on their biochemical blood parameters are currently available.

The present study aimed to assay 20 blood biochemical parameters in healthy Balkan donkeys in Bulgaria and to evaluate changes associated with gender and age.

Materials and Methods

Animals

A total of 73 local Balkan donkeys, originating from eight settlements in three regions of the same geographical area (south Bulgaria), were included in the study. The group comprised 31 male and 42 female donkeys from 8 months to 30 years of age (Group I: n=9, 0 to 5 years; Group II: n=37, six to 15 years; Group III: n=18, 16 to 25 years; Group IV: n=9, 26 to 30 years). All animals were reared on pasture in almost identical living conditions (in the open air during the day and under shelters during the night) and were regularly treated against parasites (helminths). The study was conducted from June to September 2022, following a thorough clinical examination to exclude animals with deviations in clinical status and clinical signs of disease.

Blood for biochemical analysis was collected via jugular venepuncture in Vacutest collection tubes with clot activator (Vacutest Kima srl, Arzergrande, PD, Italy). The blood was left to clot at room temperature, and sera were separated by centrifugation at 3000 rpm for 10 minutes. Blood sera were transferred into cryotubes and frozen at -80 °C.

Blood biochemical parameters

The following biochemical parameters were assayed in blood serum samples: total protein (TP, g/L), albumin (ALB, g/L), creatinine (CREA, μmol/L), urea (UREA, mmol/L), glucose (Glu, mmol/L), cholesterol (CHOL, mmol/L), triglycerides (TG, mmol/L), total bilirubin (TBili, μmol/L), aspartate aminotransferase (ASAT, IU/L), alanine aminotransferase (ALAT, IU/L), alkaline phosphatase (ALP, IU/L), creatine kinase (CK, IU/L), gamma-glutamyltransferase (GGT, IU/L), lactate dehydrogenase (LDH, IU/L), calcium (Ca, mmol/L), phosphorus (P, mmol/L), magnesium (Mg, mmol/L), potassium (K, mmol/L), sodium (Na, mmol/L), chlorides (Cl, mmol/L).

The analyses were conducted in the central clinical laboratory of the University Veterinary Hospital, Faculty of Veterinary Medicine, Trakia University – Stara Zagora, using a biochemical analyzer (Mindray BS-120, China) and an Electrolyte Analyzer/Caretium: XI–921A (China).

Statistical analysis

Data are presented as medians and interquartile ranges (IQRs). The statistical analysis of the effects of gender and age on measured biochemical variables was performed using the non-parametric Kruskal-Wallis test (MedCalc 15.8; Belgium).

Results

Blood biochemical parameters in healthy Bulgarian Balkan donkeys, as well as gender- and age-associated changes, are presented in Tables 1 and 2.

Serum glucose concentration was greater in male donkeys ($P = 0.0128$) compared to females, but there were no statistically significant differences between the different age groups. Age differences in UREA and CREA were neither present, yet serum creatinine differed substantially by gender, with higher values in male donkeys ($P = 0.0046$).

There were no significant differences in lipid profile parameters among the different age groups; however, serum TG levels in jennies exceeded those of males ($P = 0.0150$). Similarly, median TP value in female donkeys was greater ($P = 0.0134$) than that of males, without significant age- and gender-associated deviations in ALB concentrations.

Some of the liver markers (Tbili, GGT) showed no differences based on gender and age. In contrast, the activities of others – ASAT ($P = 0.0463$), ALAT ($P = 0.0205$), and ALP ($P = 0.0008$) – were statistically significantly higher in the

Table 1. Biochemical parameters of female and male Balkan donkeys in Bulgaria

Parameter	Gender						P-value
	Female			Male			
	N	Median	IQR	N	Median	IQR	
Glu	42	5.79	5.28–5.95	31	5.98	5.66–6.31	0.0128
UREA	42	5.100	4.200–5.600	31	5.100	4.625–6.175	
CREA	42	81.00	74.00–95.00	31	96.00	87.00 -102.00	0.0046
CHOL	42	1.95	1.69–2.31	31	1.92	1.68–2.25	
TG	42	0.98	0.76–1.42	31	0.75	0.58–0.99	0.0150
TP	42	73.65	69.00–78.10	31	71.10	66.70–73.85	0.0134
ALB	42	33.20	32.20–34.30	31	33.70	32.33–34.78	
Tbili	42	3.41	2.97–5.04	31	3.33	2.87–5.78	
ASAT	42	344.50	291.00–390.00	31	295.00	258.50–349.75	0.0463
ALAT	42	14.00	12.00–17.00	31	12.00	10.00–14.00	0.0205
GGT	42	55.00	37.00–74.00	31	45.00	37.00–69.25	
ALP	42	825.00	621.00–978.00	31	627.00	417.00–764.50	0.0008
CK	42	259.50	165.00–375.00	31	307.00	224.25–474.00	
LDH	42	731.50	450.00–888.00	31	803.00	567.50–876.50	
Ca	42	3.37	3.26–3.47	31	3.46	3.35–3.53	0.0468
Phos	42	1.13	0.93–1.31	31	1.07	1.00–1.23	
Mg	42	0.85	0.78–0.92	31	0.85	0.78–0.92	
Na	42	135.05	132.30–136.80	31	134.40	130.38–138.03	
K	42	4.09	3.78–4.31	31	4.00	3.67–4.20	
Cl	42	102.50	101.00–105.00	31	105.00	103.00–108.00	0.032

blood of jennies without any age-associated differences. Although serum CK activity in male animals tended to be higher, neither age- nor gender-related significant variations were detected. Serum LDH did not differ significantly between genders; however, its activity was statistically significantly more elevated in the group of young donkeys ($P = 0.0039$), with substantial differences among the age groups ($P_{1-3} = 0.01$; $P_{2-3} = 0.01$; $P_{1-4} = 0.05$).

Serum macro elements (Ca, P, Mg) demonstrated no variations with age ($P>0.05$), but serum calcium in male donkeys exceeded significantly the concentrations in females ($P = 0.0468$). Among blood electrolytes (Na, K, and Cl), only chlorides were substantially greater in males ($P = 0.032$), but none of them exhibited age-associated variations.

Discussion

Gender-associated changes were established for the following parameters: Glu, CREA, TG, TP, ASAT, ALAT, ALP, Ca, and Cl. Age-associated differences were observed for blood LDH. Serum glucose levels were higher in male Bulgarian Balkan donkeys, without age differences. Our values were higher than those reported by other researchers (Mori et al., 2003; Laus et al., 2015; Kisadere et al., 2019; Trimboli et al., 2020), except for Romanian donkeys (Coroian et al., 2019), but the gender pattern was the same. On the contrary, Silva et al. (2024) found higher blood glucose concentrations in female donkeys, probably because pregnant animals were also included. Increased blood glucose levels in young donkeys have been documented in other autochthonous breeds, e.g., Martina Franca (Trimboli et al., 2020) and Miranda (Silva et al., 2024). The blood glucose concentrations may vary due to numerous factors, including age, sex, physiological state, circadian rhythms, diet, stress, blood storage conditions, and duration (Kisadere et al., 2019; Trimboli et al., 2020).

Other important parameters used to evaluate renal function in humans and animals include creatinine and Urea. In our study, no significant age-related differences were found; however, creatinine values were higher in male donkeys. These findings were in line with data published by Girardi et al. (2013), Sedlinská et al. (2017), Kısadere et al. (2018), and Fraczkowska et al. (2021), who also reported higher CREA values in male subjects due to greater muscle mass. On the other hand, Trimboli et al. (2020) found a dramatic decrease in CREA concentrations with ageing. A study in Portugal found higher creatinine values in female donkeys and, conversely, higher urea concentrations in male donkeys (Silva et al., 2024). The inclusion of pregnant female animals in the latter study, as well as gender differences, has probably in-

Table 2. Biochemical parameters of Balkan donkeys in Bulgaria related to age

Parameter P – value	Age groups											
	I			II			III			IV		
	N	Median	IQR	N	Median	IQR	N	Median	IQR	N	Median	IQR
Glu	9	5.97	5.71–6.06	37	5.88	5.60–6.06	18	5.92	5.29–6.39	9	5.75	5.56–6.16
UREA	9	6.00	4.50–7.63	37	5.10	4.20–5.53	18	5.10	4.30–5.40	9	5.10	4.35–6.88
CREA	9	72.00	66.50–98.50	37	86.00	76.75–102.00	18	87.00	81.00–95.00	9	99.00	80.00–109.00
CHOL	9	2.21	1.65–2.95	37	1.97	1.63–2.32	18	1.87	1.72–2.11	9	1.96	1.72–2.49
TG	9	0.99	0.89–1.53	37	0.86	0.66–1.38	18	0.93	0.59–1.34	9	0.78	0.65–1.00
TP	9	70.30	66.65–72.80	37	74.30	69.95–77.73	18	70.45	67.80–74.20	9	71.20	69.53–72.73
ALB	9	33.70	32.08–34.75	37	33.20	32.15–34.63	18	33.25	32.50–34.50	9	33.80	31.90–34.90
Tbili	9	3.33	2.97–5.09	37	3.58	3.19–6.00	18	2.915	2.51–3.54	9	3.64	3.24–7.18
ASAT	9	320.00	255.00–344.00	37	320.00	290.75–370.00	18	344.50	293.00–388.00	9	286.00	241.75–319.75
ALAT	9	11.00	9.500–14.25	37	14.00	12.00–17.25	18	12.50	10.000–16.000	9	11.00	8.50–14.75
GGT	9	56.00	35.500–73.00	37	54.00	40.75–75.25	18	42.00	36.000–77.000	9	42.00	22.75–60.75
ALP	9	553.00	417.250–739.50	37	768.00	549.00–979.75	18	807.00	627.00–918.00	9	693.00	592.00–797.75
CK	9	281.00	197.750–631.75	37	270.00	178.25–384.75	18	352.50	190.00–523.00	9	247.00	176.25–292.75
LDH **0.0039**	9	923.00	787.000–984.25	37	778.00	558.00–891.25	18	553.50	499.00–630.00	9	666.00	349.75–880.00
Ca	9	3.38	3.248–3.49	37	3.37	3.26–3.46	18	3.47	3.44–3.58	9	3.34	3.29–3.48
Phos	9	1.25	1.045–1.50	37	1.17	0.98–1.32	18	1.07	0.92–1.28	9	0.99	0.91–1.10
Mg	9	0.85	0.780–0.908	37	0.85	0.81–0.94	18	0.87	0.77–0.960	9	0.80	0.750–0.883
Na	9	135.30	132.650–138.40	37	133.80	130.60–136.73	18	134.70	133.20–136.60	9	134.90	132.05–138.38
K	9	4.22	3.835–4.33	37	4.09	3.89–4.23	18	3.91	3.69–4.08	9	3.81	3.69–4.18
Cl	9	103.00	98.750–106.50	37	103.00	100.75–105.00	18	105.00	102.00–109.00	9	104.00	103.00–105.25

LDH между групите: $P_{1-3} = 0.01$; $P_{2-3} = 0.01$; $P_{1-4} = 0.05$

fluenced the results (Kısadere et al., 2019; Silva et al., 2024).

In this study, the lipid profile parameters did not demonstrate differences in individual age groups, but serum TG in female animals was significantly higher than that in males. A study in Kyrgyzstan found higher serum CHOL levels in male donkeys, as well as statistically significant differences between younger and older animals (Kısadere et al., 2019). In Martina Franca donkeys, the highest CHOL values were found in animals up to 1 year, while TG was not affected by age (Trimboli et al., 2020). Another large-scale study in donkeys from the Miranda breed in Portugal detected significant between-gender differences for TG and CHOL, with higher values in females (Silva et al., 2024). The differences between this study and ours are likely due to the different age grouping, the relatively small number of animals under 1 year of age, and the absence of pregnant jennies.

Average values of blood TP and Alb of the Bulgarian Balkan donkey population were similar to those reported in donkeys from Brazil (Mori et al., 2003), Italy (Trimboli et al., 2020) and Portugal (Silva et al., 2024), slightly greater than those published in Kyrgyzstan (Kısadere et al., 2019), Romania (Coroian et al., 2019), Italy (Laus et al., 2015) and the UK (Burden et al., 2016), but correspondingly lower than those reported in the Czechia (Sedlinská et al., 2017) and Türkiye (Gul et al., 2007). Our study has identified gender differences in blood TP, with higher values in females, as well as a lack of age and gender differences in ALB concentrations. In Martina-Franca donkeys, TP in blood was higher in older animals (Trimboli et al., 2020), which is contrary to the data reported by Kisadere et al. (2019) and in line with those of Girardi et al. (2014). The differences in studied parameters may be attributed to various agro-climatic regions, hormonal effects, stress, fluid loss due to sweating, and feeding regimens adopted by owners (Mori et al., 2003; Gupta et al., 2016; Kisadere et al., 2019).

In Balkan donkeys in Bulgaria, some liver function markers (Tbili, GGT) did not show differences between genders or age. Others, such as ASAT, ALAT, and ALP, were statistically higher in female donkeys, with no age-related changes observed within individual groups. Kisadere et al. (2019) found higher TBili values in male and younger donkeys. In the same study, serum GGT levels were higher in middle-aged female donkeys. In Portugal, no significant gender and age differences were found for blood Tbili values, whereas GGT activity differed only between genders, being more elevated in male donkeys (Silva et al., 2024).

Average values and gender differences for AST, ALT, and ALP, similar to ours, were found in Kyrgyz donkeys, with higher values in females and young animals (Kisadere et al., 2019). Silva et al. (2024) found no significant differences in serum AST levels with age, despite higher values in young donkeys. However, in contrast to our study, a significant between-gender difference was detected, with males having higher values. Elevated serum AST activity may result from damage to liver, muscle, and myocardial cells, and should be assayed in parallel with ALT and CK values (Silva et al., 2024).

Although serum CK activities in male Balkan donkeys tended to increase, age and sex differences were not statistically significant. Our results were higher than the data cited from Brazil, Kyrgyzstan, and Portugal, and lower than those from the Czech Republic. Gender differences were comparable to those observed for Kyrgyz donkeys. On the other hand, serum LDH values did not differ significantly between males and females; however, statistically higher values were found in the young animals group, which corresponds to reported age differences in Kyrgyz donkeys (Kisadere et al., 2019). Higher median LDH values were observed compared to those of Brazilian and Kyrgyz donkeys, but they were comparable to studies in the Czech Republic (Mori et al., 2003; Sedlinská et al., 2017; Kisadere et al., 2019). Differences in LDH and CK activities may be due to variations in age, sex, lean body mass, and physical activity (Sedlinská et al., 2017; Kisadere et al., 2019; Silva et al., 2024).

In Bulgarian Balkan donkeys, no age differences were observed in the serum levels of macroelements Ca, P, and Mg; however, male animals demonstrated statistically higher serum Ca levels. Our values for macroelements are very close to those published in Italy, Sudan, Nigeria, and the UK (Laus et al., 2015; Al Shafei et al., 2015; Burden et al., 2016; Ayo et al., 2023) and higher than those reported from Kyrgyzstan, Italy, and Portugal (Kisadere et al., 2019; Trimboli et al., 2020; Silva et al., 2024). In Kyrgyz donkeys, the highest Ca concentration was found in female and middle-aged animals, while for phosphorus, it was in middle-aged males. In the Miranda breed, Silva et al. (2024) found no significant differences in serum Ca related to age and sex, while a decrease in P concentration was reported with age. Regarding Mg, the authors described significant sex- and age-related differences, with slightly higher values in male and older animals. Serum Ca levels can be affected by age, physical condition, gender, high haemoglobin levels, season, haemodynamic changes, and measurement errors. Other factors affecting blood P and Mg include pasture sward and animal age (Mori et al., 2003; Kisadere et al., 2019).

The average blood electrolytes Na, K, and Cl concentrations in the Balkan donkeys from Bulgaria are very similar to those observed in other breeds and breed groups, except Sudanese animals, in which Na and K were significantly higher (Al Shafei et al., 2015; Burden et al., 2016; Sedlinská

et al., 2017; Coroian et al., 2019; Trimboli et al., 2020; Silva et al., 2024). In the present study, only chloride levels were elevated in male animals, and no age-related changes were observed in any of the three elements. In contrast, Silva et al. (2024) measured higher Na and Cl levels in female donkeys, also without age differences. Regarding potassium, the same authors reported a sex difference, with slightly higher values in males, and no age-related changes. Bature et al. (2018) found a significant decrease in blood sodium and potassium levels with aging. The opposite trend was affirmed by Girardi et al. (2014), who found that Na and K were not affected by age, which is consistent with our study. The variations in blood electrolytes are probably due to differences in geographical regions (climatic factors, soil composition, botanical composition of the sward), water intake and free access to water sources, working conditions of animals, as well as the samples' storage, assay techniques, and used reagents (Al Shafei et al., 2015).

A limitation of the present study is the uneven distribution of animals in the different groups (female, male, young, middle-aged, and elderly). Unfortunately, the Balkan donkey population in Bulgaria is aging, so middle-aged animals predominate, which limits the rate of age changes. For the same reason, the group of young donkeys included animals up to 5 years of age, as those up to 1 year were too few, which is another drawback. However, considering the endangered status of the breed, we believe the study is representative. Future studies with a larger number of animals and more uniform gender and age groups are needed to summarize the blood biochemical findings, allowing them to serve as reference values.

Conclusions

The Bulgarian donkey is part of a large group of Balkan donkeys, whose population has recently become smaller and is aging, necessitating complex action for its protection. Our study of blood biochemical parameters was performed in healthy animals, and some of these parameters were found to be influenced by the animal's gender and age. These changes would be helpful to veterinarians for the accurate interpretation of clinical results.

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