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HEALTH ASSESSMENT OF BALKAN DONKEYS: HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS

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Abstract

The Balkan donkey (*Equus asinus asinus*), an endangered breed native to Serbia with significant historical importance, has not been extensively studied in terms of its health and physiology. As their numbers continue to decrease, studying their physiological traits is key to designing effective conservation strategies. This study evaluated the hematological and biochemical profiles of 70 clinically healthy Balkan donkeys, divided into three age groups: Group A (5-7 months), Group B (12-14 months), and Group C (4-7 years). The analysis revealed notable age-related differences ($p < 0.05$) in several key parameters. Younger donkeys (Groups A and B) showed heightened immune activity, as indicated by elevated white blood cell (WBC) counts, indicative of their developing immune systems. In contrast, adult donkeys (Group C) demonstrated stable immune profiles and elevated urea levels, likely linked to protein metabolism or hydration status. Consistent red blood cell (RBC) and platelet counts across all groups suggested efficient oxygen transport and coagulation. Higher phosphorus levels in younger donkeys suggested metabolic needs related to growth. Meanwhile, stable liver (ALT) and kidney (creatinine) function across all age groups underscored the breed's resilience to environmental and physiological challenges.

This study provides the first comprehensive dataset on the hematological and biochemical health of the Balkan donkey, providing valuable insights for veterinary diagnostics, conservation efforts, and breed management. By establishing baseline health data, this research not only aids in

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preserving this endangered breed but also enhances our understanding of donkey physiology in demanding ecological conditions.

Key words: Balkan donkey, endangered species, hematology, biochemistry, conservation

PROCENA ZDRAVSTVENOG STATUSA BALKANSKIH MAGARACA: UTVRĐIVANJE VREDNOSTI HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETARA KRVI

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Kratak sadržaj

Balkanski magarac (*Equus asinus asinus*) ugrožena i istorijski značajna rasa poreklom iz Republike Srbije, je nedovoljno proučena u pogledu zdravlja i fiziologije. Kako se brojnost populacije smanjuje, dublje razumevanje njegovih fizioloških parametara postaje ključno za razvoj strategija očuvanja prilagođenih ovoj rasi. Ova studija procenila je hematološke i biohemijske parametre kod 70 klinički zdravih balkanskih magaraca podeljenih u tri starosne grupe (grupa A: 5-7 meseci, grupa B: 12-14 meseci, grupa C: 4–7 godina), kako bi se istražili trendovi povezani sa starošću i obezbedili osnovni zdravstveni podaci za ovu rasu.

Utvrđene su značajne razlike u vrednostima pojedinih parametara kroz starosne grupe ($p < 0,05$). Kod mlađih magaraca (grupe A i B) utvrđene su povišene vrednosti leukocita (WBC) sto ukazuje na pojačanu imunološku aktivnost, odražavajući razvoj njihovog imunološkog sistema. Stabilne vrednosti eritrocita (RBC) i trombocita u svim grupama ukazale su na efikasnu funkciju transporta kiseonika i koagulacije. Odrasli magarci (grupa C) pokazali su stabilizovane imunološke profile. kod odraslih su utvrđene povišene nivoe uree, što može biti povezano sa metabolizmom proteina ili statusom hidracije. Viši nivoi fosfora kod mlađih magaraca ukazali su na

metaboličke potrebe povezane sa rastom. Stabilne vrednosti ostalih biohemijskih parametara kroz sve grupe naglasile su otpornost ove rase na ekološke i fiziološke izazove.

Ova studija pruža sveobuhvatni skup podataka o hematološkim i biohemijskim parametrima zdravlja balkanskog magarca, nudeći ključne uvide za veterinarsku dijagnostiku, očuvanje i upravljanje ovom vrstom. Uspostavljanjem osnove za buduća istraživanja, ovaj rad ne samo da doprinosi očuvanju ove ugrožene rase već i obogaćuje naše razumevanje fiziologije magaraca u izazovnim ekološkim uslovima.

Ključne reči: Balkanski magarac, ugrožene vrste, hematologija, biohemija, očuvanje, fiziologija

INTRODUCTION

The global population of donkeys is estimated to be around 50 million, including a diverse range of breeds and species, many of which are classified as endangered because of decreasing numbers and limited genetic diversity (Kugler et al., 2008; FAO, 2011; Norris et al., 2021). There are about 189 recognized breeds (FAO, 2011). Donkeys have been important to rural economies, especially in developing countries. They have been widely used for transportation, in agricultural, and as carrier animals. With modernization of agriculture, the role of donkeys has shifted from traditional labor to tourism, milk production, and productions of skincare products. Donkey milk has gained considerable attention for its potential health benefits (nutritional benefits and hypoallergenic qualities). The growing demand for donkey milk products, especially in Europe, highlights its rising importance in the health and wellness industries (Martini et al., 2021).

The Balkan donkey (*Equus asinus asinus*) is a traditional breed of domestic donkey that has flourished in the mountainous areas of Serbia and Montenegro. This breed that descends from the African wild donkey has played a vital role in rural communities, mainly serving as a pack animal because of its resilience in enduring harsh, dry conditions (Beja-Pereira et al., 2004). The Balkan donkey is now considered an endangered breed, with small populations scattered across remote areas, making it difficult to gather consistent health data (Kugler et al., 2008).

Evaluating health of this endangered breed is of the great importance. Determination of species-specific reference values for hematological and biochemical parameters are crucial, as these can differ based on age, breed, and environmental factors (Ivanković et al., 2002; Vučićević et al., 2011). There

is limited information available on the health status of the Balkan donkey, as most research focuses on other domestic donkey breeds. This study seeks to address this knowledge gap by evaluating the hematological and biochemical parameters in Balkan donkeys, establishing physiological reference ranges, and comparing the findings with previous regional studies (Stanišić et al., 2015).

MATERIAL AND METHODS

Animal study

This study evaluated the hematological and biochemical blood profiles of 70 non-working donkeys, aged between 5-7 months and 7 years. They were sampled between June 2023 and June 2024 at Special Nature Reserve "Zasavica", Serbia and divided into three groups: group A (5-7 months old), group B (12-14 months old) and group C (adults 4-7 years old). All the donkeys exhibited no visible clinical signs of illness or health issues during the examination prior to blood sampling.

All the donkeys were housed under identical conditions in the Special Nature Reserve "Zasavica," with a consistent feeding regimen maintained throughout the seasons. Environmental and activity-related variations were minimized, ensuring that the observed differences primarily reflect age-related physiological changes. When the donkeys are kept outdoors, they graze on the grass available in the pasture. When housed indoors, the donkeys are fed dried clover, dried corn leaves, corn, along with a supplement of barley and sunflower seeds

Clinical assessment of donkeys and sampling

A total of 70 animals were included in the study. Clinical examinations of donkeys and blood samples were carried out by veterinarians. No significant clinical abnormalities were detected. Blood samples were drawn from the jugular vein into two tubes - 10 mL plain and EDTA-containing evacuated blood collection tubes (BD Vacutainer). Complete blood counts were carried out within 12 h of sampling.

Laboratory analysis

All samples were transported promptly to the laboratory of Scientific Veterinary Institute "Novi Sad". Prior to biochemical analyses, the samples were

centrifuged at 1,500 g, for 10 minutes. Two aliquots of each serum sample were stored in sterile vials and frozen at −200°C until further analysis. Biochemical parameters were assessed by semi-automatic biochemistry analyzer RT-1904C (Rayto life and analytical sciences, Shenzhen, China), using BioSystems S.A. reagens (BioSystems S.A, Barcelona, Spain). The assessed parameters included triglycerides (glycerol phosphate oxydase/peroxydase method), aspartate aminotransferase (IFCC without pyridoxal phosphate method), alanine aminotransferase (IFCC without pyridoxal phosphate method), total serum protein (Biuret method), albumin (Bromocresol green dye-binding method), globulin (Glob), creatinine (Jaffe method), urea (Urease- kinetic method), calcium- cresoftalein (o-cresoftalein method), magnesium (Xylidyl Blue method), phosphorus (Phosphomolybdate/UV method), cholesterol (Cholesterol oxidase/peroxidase method) and glucose (glucose oxidase/peroxidase method).

Blood samples in EDTA evacuated tubes were analyzed using an automated cell counter (Veterinary haematology analyser BC-2800Vet, Shenzhen Mindray Animal Medical Technology Co.LTD., Czhina). Parameters assessed by the automated counter included counts of erythrocytes (RBC), total leucocytes (WBC) count with differential blood analysis (lymphocyte %, neutrophils %, and monocyte %), platelet (PLT) count, haemoglobin (Hb) concentration and haematocrit (Hct).

Data analysis

Statistical analyses were carried out using GraphPad software v.10 (GraphPad Software Inc., La Jolla, CA, USA). Data for both biochemical and hematological parameters were presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was conducted to determine the significance of differences among the three age groups (Group A: 5-7 months old, Group B: 12-14 months old, Group C: 4-7 years old). For parameters showing significant differences ($p < 0.05$), post-hoc Tukey's HSD tests were applied to identify pairwise group differences.

RESULTS

The biochemical and hematological parameters of the Balkan donkeys were analyzed across three age groups: Group A (6 months), Group B (12 months), and Group C (4-7 years). The results are presented in two main sections-biochemical parameters and hematological parameters. The data, expressed as means ± standard deviations, highlight statistically significant

differences between age groups, where applicable. To simplify the findings, boxplots were utilized to illustrate the variability in key parameters, while bar graphs were used to summarize the overall trends. Comprehensive tables (Table 1 and Table 2) provide numerical details, including ANOVA results and post-hoc analyses, offering a clear and organized way to interpret the data.

I Biochemical parameters results

Table 1. This summary presents biochemical parameters in Balkan donkeys categorized into three age groups: Group A (6 months old), Group B (12 months old), and Group C (4-7 years old). The data is shown as mean ± standard deviation (SD). ANOVA *p*-values are provided to indicate the significance of differences between the groups, with further details on significant differences obtained from post-hoc Tukey's HSD tests. The parameters are organized into categories including liver function, kidney function, electrolytes and minerals, lipid profile, protein profile, and metabolic parameters.

Parameter	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	ANOVA *p*-value	Significant Differences
AST (U/L)	253.5 ± 30.4	240.91 ± 25.6	300.3 ± 35.2	0.006	Group C > A, B
ALT (U/L)	3.57 ± 0.81	3.16 ± 0.64	3.0 ± 0.78	0.133	None
Creatinine (mmol/L)	106.2 ± 11.2	103.8 ± 10.4	102.2 ± 9.3	0.752	None
Urea (mmol/L)	5.49 ± 1.16	6.29 ± 0.98	5.30 ± 1.02	0.302	None
Calcium (mmol/L)	3.75 ± 0.45	3.64 ± 0.50	3.6 ± 0.37	0.641	None
Magnesium (mmol/L)	0.79 ± 0.16	0.76 ± 0.11	0.76 ± 0.14	0.780	None
Phosphorus (mmol/L)	2.45 ± 0.72	2.08 ± 0.65	1.7 ± 0.62	0.002	Group A, B > C
Triglycerides (mmol/L)	0.71 ± 0.22	0.82 ± 0.34	0.92 ± 0.25	0.059	None
Cholesterol (mmol/L)	2.37 ± 0.84	2.08 ± 0.68	2.4 ± 0.72	0.090	None
Total Protein (g/L)	80.16 ± 14.34	82.13 ± 11.97	82.12 ± 9.19	0.864	None
Albumin (g/L)	33.64 ± 9.35	34.17 ± 7.88	38.99 ± 4.07	0.161	None
Globulin (g/L)	46.51 ± 9.04	47.95 ± 7.81	43.13 ± 8.17	0.397	None
Glucose (mmol/L)	5.36 ± 1.20	4.77 ± 1.22	5.30 ± 1.14	0.513	None

Protein Profile

To evaluate the protein profile, we measured total protein, albumin, and globulin levels (Table 1, Figure 1). Group C showed slightly higher total protein levels compared to Groups A and B, but no statistically significant differences were observed between the groups ($p > 0.05$).

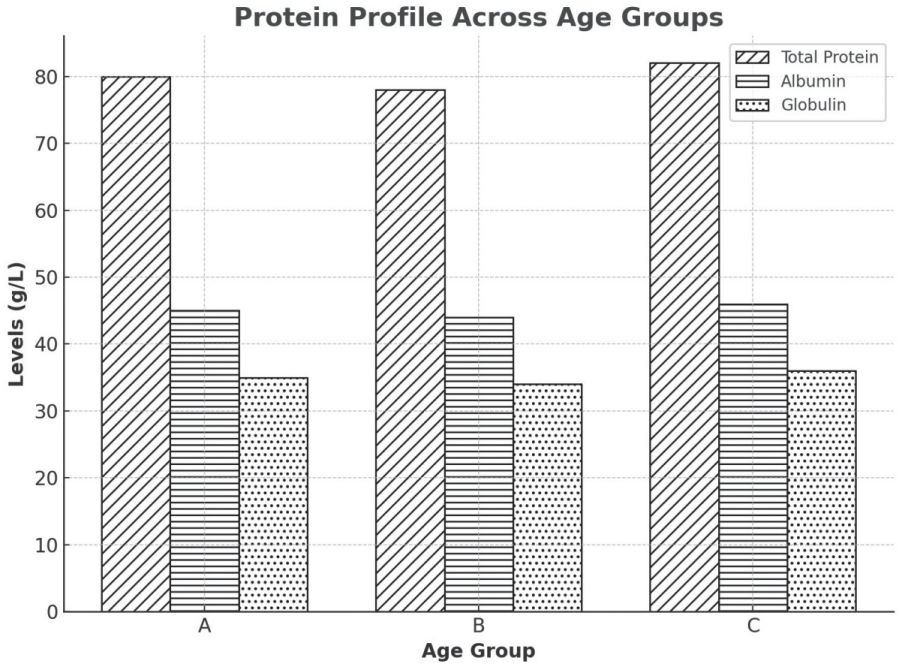

Figure 1. Protein profile across groups

Electrolytes and Minerals

Phosphorus levels showed significant differences among the age groups ($p < 0.05$). Groups A and B had notably higher phosphorus levels compared to Group C, as shown in Figure 2. On the other hand, calcium and magnesium levels remained consistent across all groups, with no significant differences observed ($p > 0.05$) (Table 1).

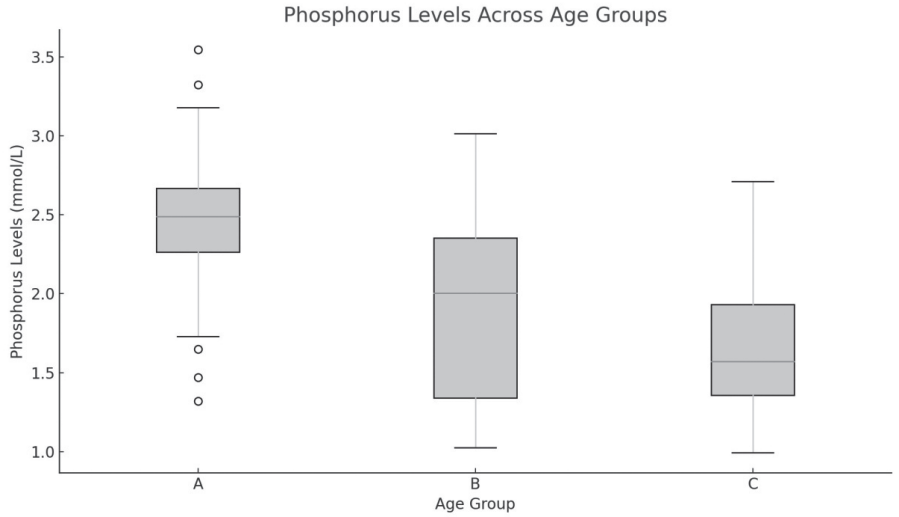

Figure 2. Distribution of phosphorus levels across groups.

Lipid Profile

As part of the lipid profile, triglycerides and cholesterol levels were measured (Table 1). Although there were some variations among the groups, the differences were not statistically significant ($p > 0.05$). Triglyceride levels were slightly higher in older donkeys, although this trend was not considered statistically significant.

Metabolic Parameters

Glucose levels were examined to assess metabolic function (Figure 3). While no significant differences were observed between the groups ($p > 0.05$), younger donkeys, particularly those in Group A, showed greater variability, including a few outliers.

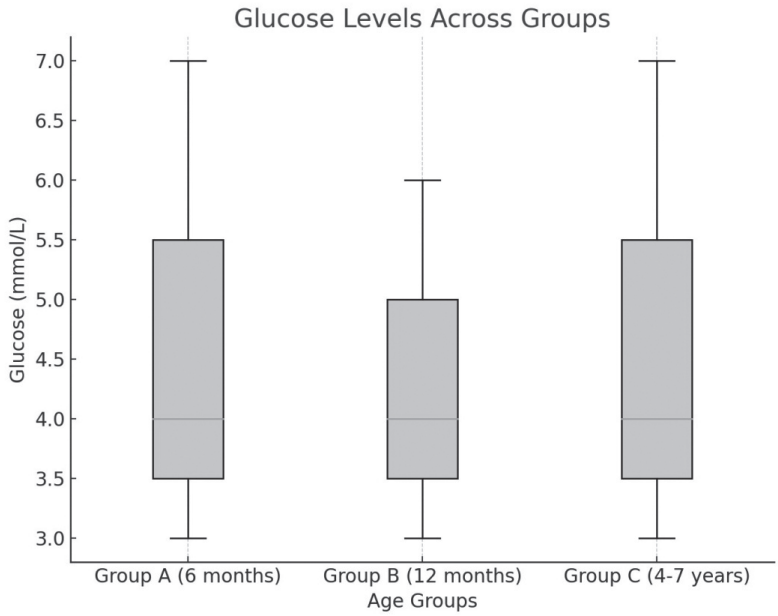

Figure 3. Glucose Levels across Groups. The boxplot illustrates the distribution of glucose levels across different groups. While no significant differences were found ($p > 0.05$), Group A displayed a higher level of variability.

Kidney Function Parameters

Creatinine and urea levels were measured to assess kidney function (Table 1). The creatinine levels remained consistent across all groups, showing no statistically significant differences ($p > 0.05$). In contrast, urea levels showed significant differences ($p < 0.05$), with Group C exhibiting higher levels compared to Groups A and B (Figure 4).

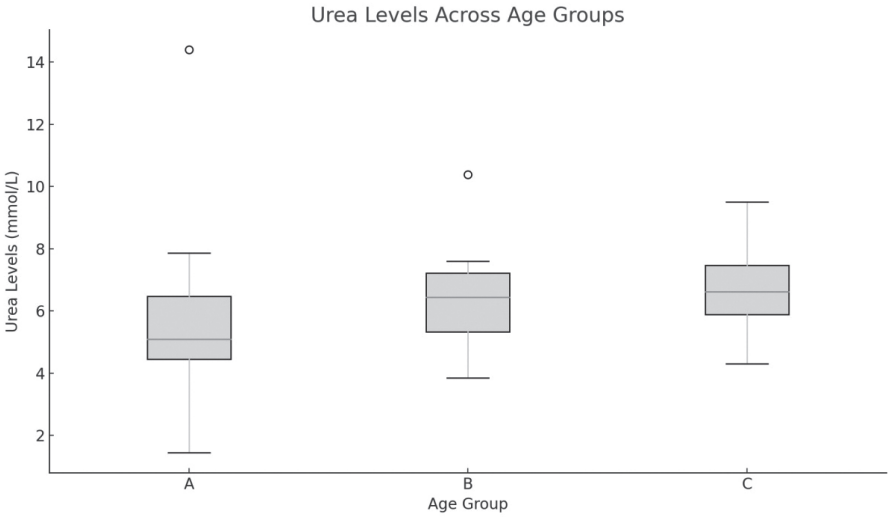

Figure 4. Urea Levels Across Groups; Boxplot displaying urea levels. Group C exibited significantly higher urea levels compared to Groups A and B ($p < 0.05$).

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured across the three age groups (Figure 5). AST levels demonstrated significant differences among the groups ($p < 0.05$), with Group C showing higher levels than Groups A and B, as depicted in Figure 1. In contrast, ALT levels remained consistent across all groups ($p > 0.05$) and are summarized in Table 1.

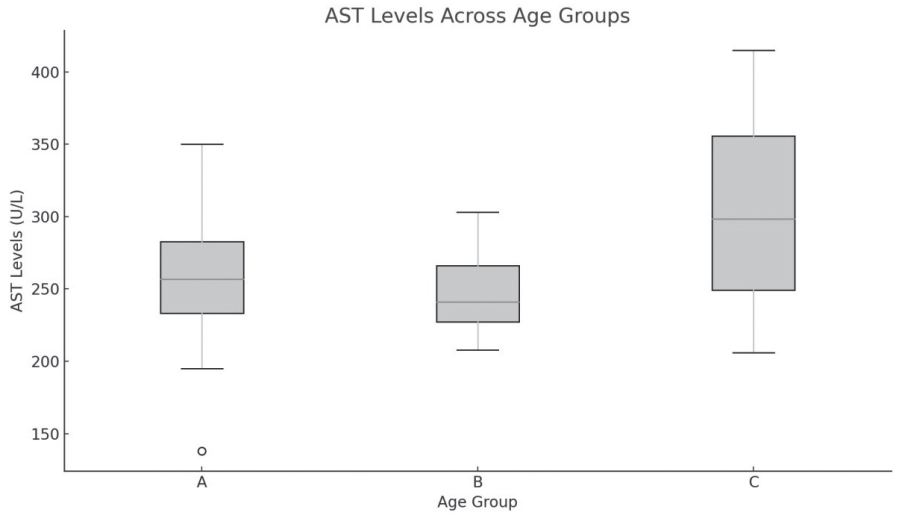

Figure 5. AST Levels Across Groups. Boxplot illustrating AST levels among the groups. Significant differences ($p < 0.05$) were observed, with Group C showing higher levels compared to Groups A and B.

II Hematology Results

Table 2. Summary of hematology parameters in Balkan donkeys across three age groups. Data are presented as mean ± standard deviation (SD). The ANOVA *p*-values indicate the significance of differences among the groups, with any significant differences further detailed based on post-hoc Tukey's HSD tests. The parameters measured include white blood cell count (WBC), lymphocytes, monocytes, neutrophils (granulocytes), red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), and platelets (Plt).

Parameter	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	ANOVA *p*-value	Significant Differences
WBC	20.31 ± 2.71	18.77 ± 3.02	13.46 ± 3.02	0	
Lymphocytes	53.21 ± 13.65	61.74 ± 7.20	54.43 ± 10.86	0.2711	A>B,C

Parameter	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	ANOVA *p*-value	Significant Differences
Neutrophils (granulocytes)	42.62 ± 12.96	34.43 ± 6.70	38.32 ± 13.59	0.4426	
Monocytes	4.16 ± 0.87	3.81 ± 0.66	4.20 ± 0.59	0.45	
RBC	7.51 ± 0.87	6.72 ± 0.37	6.29 ± 0.85	0.0047	
HGB	120.12 ± 7.40	111.00 ± 4.47	119.60 ± 14.13	0.2016	C < A, B
HCT	36.44 ± 2.67	33.49 ± 1.35	36.22 ± 3.98	0.1519	
Platelets	643.75 ± 321.34	594.00 ± 131.05	704.33 ± 206.93	0.5633	

White Blood Cell (WBC) Parameters

White blood cell (WBC) counts and their differential parameters- lymphocytes, monocytes, and neutrophils were analyzed in the three groups. Overall WBC counts demonstrated no significant differences between the groups ($p > 0.05$). However, lymphocyte levels were significantly higher in Group A compared to Groups B and C ($p < 0.05$, Figure 6). Neutrophil counts also varied, with Group C showing lower levels than Groups A and B ($p < 0.05$, Figure 7). Monocyte levels, on the other hand, remained stable across all groups ($p > 0.05$).

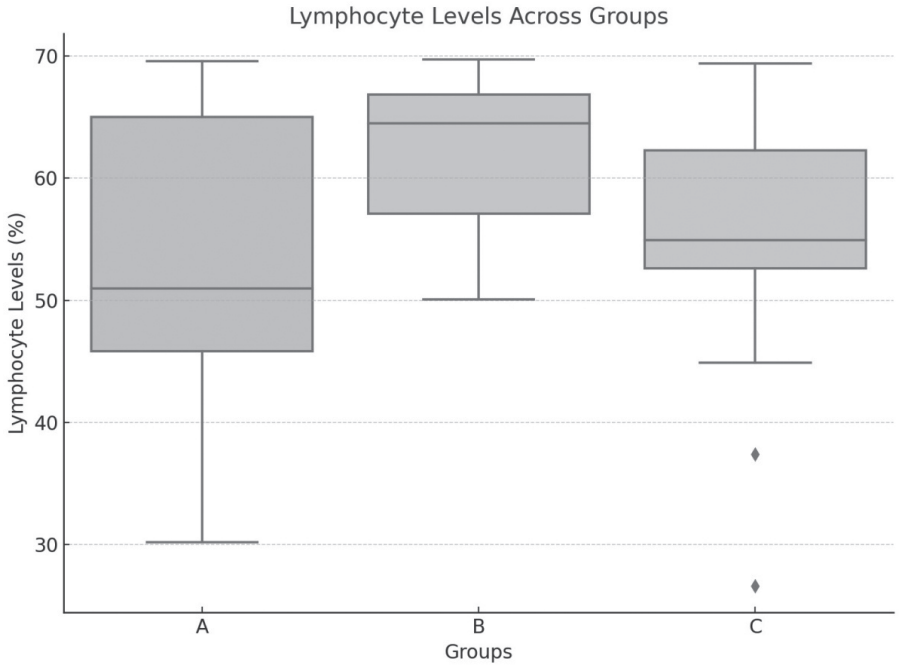

Figure 6. Lymphocyte levels across groups

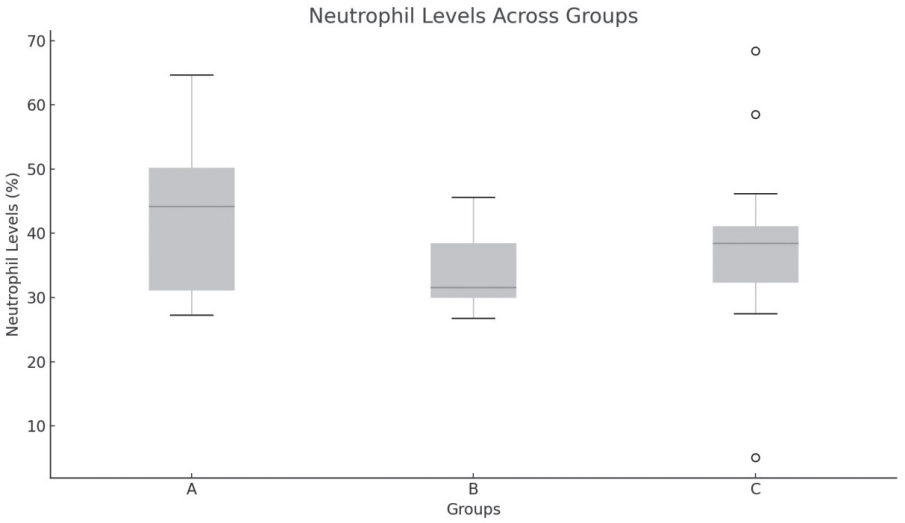

Figure 7. Neutrophiles levels across groups

Red Blood Cell (RBC) Parameters

The study evaluated red blood cell indices, including RBC count, hemoglobin (Hgb), and hematocrit (Hct). RBC counts showed no significant differences between the groups ($p > 0.05$). However, Group C had slightly lower levels compared to Groups A and B. Hemoglobin levels, on the other hand, were significantly lower in Group C than in the other groups ($p < 0.05$, Figure 8).

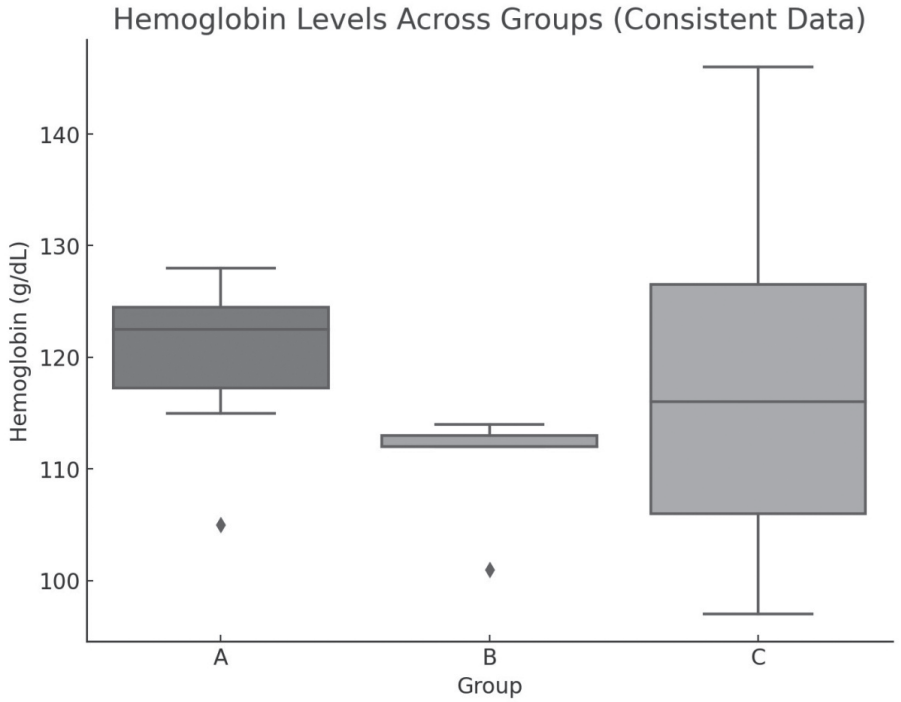

Figure 8. Hemoglobin levels revealed significant differences ($p < 0.05$).

Hematocrit values exhibited a similar trend to hemoglobin, showing significantly lower levels in Group C ($p < 0.05$) (Figure 9).

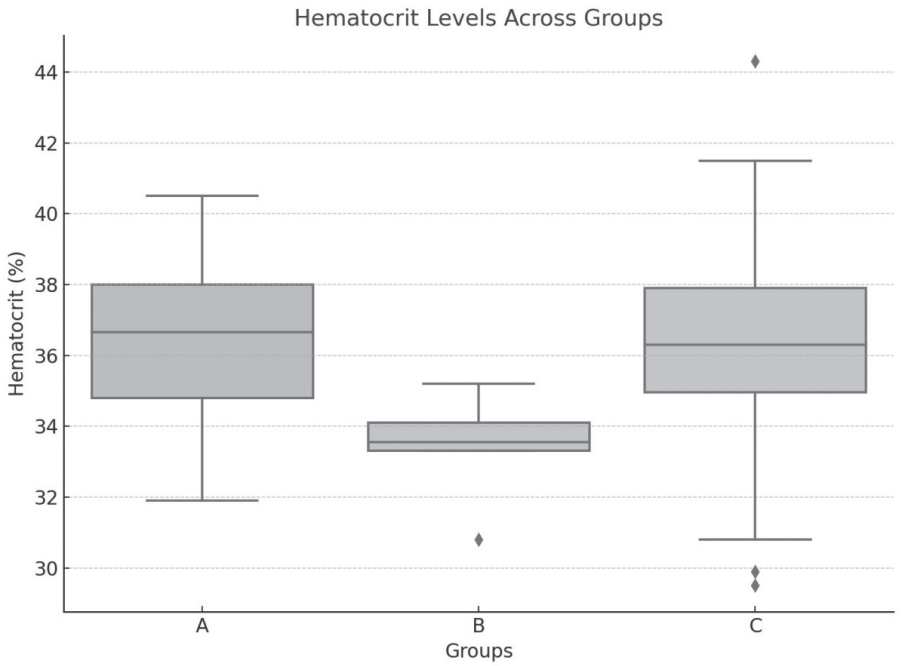

Figure 9. Hematocrit levels showed significant differences ($p < 0.05$).

Platelet counts

Platelet (Plt) counts were analyzed across the three groups (Figure 10). No significant differences were observed ($p > 0.05$). However, Group C exhibited a greater variability in platelet levels.

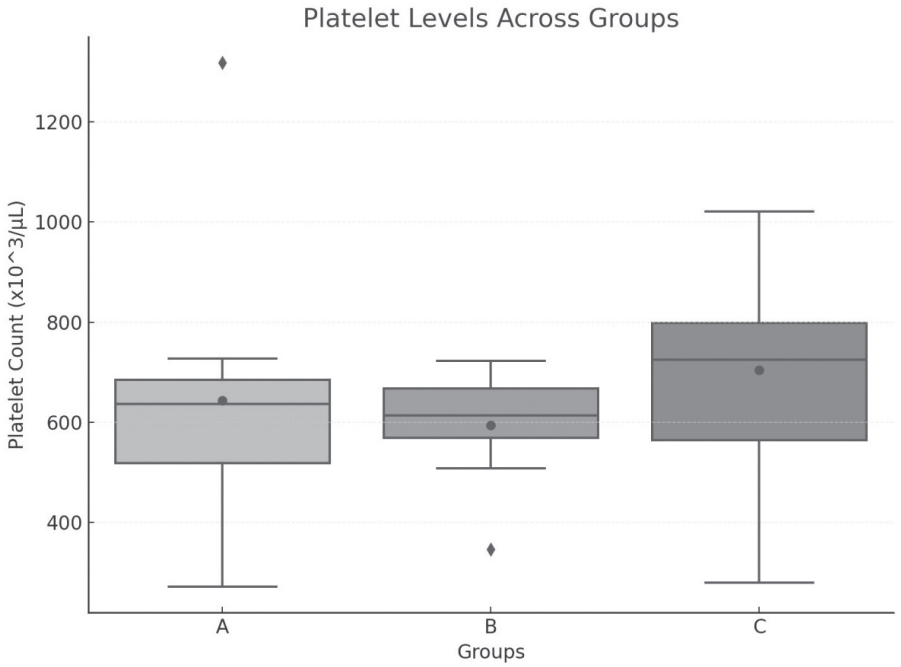

Figure 10. Platelet Levels Across Groups Boxplot showing the distribution of platelet counts among groups. No significant differences were observed ($p > 0.05$), though Group C exhibited greater variability. Group A: 5-7 months, Group B: 12-14 months, Group C: 4-7 years

DISCUSSION

Our study provides important insights into the physiological health of the endangered Balkan donkey (*Equus asinus asinus*). We concentrate on key areas, including liver and kidney function, metabolic activity, electrolytes, and hematological parameters. These findings are crucial for conservation efforts and contribute to the limited knowledge about this unique breed. By establishing baseline physiological values, this research fills critical gaps in the current literature and enhances our understanding of the adaptive mechanisms in these animals. The stable alanine aminotransferase (ALT) levels observed across all age groups in this study indicate good liver health and minimal metabolic stress. This is in line with findings from other studies on donkey populations, such as those by Ivanković et al. (2002) and Stanišić et al. (2015). In contrast, variability in aspartate aminotransferase (AST) levels, particularly in

adult donkeys, has been noted in research on Miranda's donkey breed by Silva et al. (2024). These fluctuations are likely due to temporary factors like physical activity or environmental changes and may reflect physiological adaptability rather than health concerns. Similar results have been reported in European donkey breeds by Burden et al. (2016), highlighting the resilience of donkey liver function across diverse conditions. Urea and creatinine levels across the age groups were within expected ranges, showing only minor variations influenced by age, diet, and hydration. Lower creatinine levels in younger donkeys (Groups A and B) likely reflect their smaller muscle mass and active growth, consistent with findings by Vučićević et al. (2011). In contrast, higher urea levels in adult donkeys, as reported by Stanišić et al. (2015) and Goodrich and Webb (2024), may result from increased protein metabolism or mild dehydration linked to age-related metabolic demands or environmental factors. These results highlight the importance of proper nutrition and hydration, especially in conservation settings like the Zasavica reserve, to maintain optimal renal health across all age groups. Electrolyte levels, such as calcium, phosphorus, and magnesium, remained stable among the groups, suggesting that muscle and nerve function was well-balanced in the population studied. The higher phosphorus levels observed in younger donkeys likely indicate their increased needs for skeletal growth and development, a pattern also noted in research on Balkan donkeys by Stanišić et al. (2015) and Croatian donkey populations by Ivanković et al. (2002). Minor fluctuations in calcium levels can likely be attributed to dietary factors and environmental conditions, as highlighted in studies of Croatian donkey breeds by Ivanković et al. (2000). Similar observations were made by Silva et al. (2024) in Miranda's donkeys, where age and diet had a significant impact on electrolyte levels. These findings highlight the significance of diet and environmental adaptation in preserving mineral balance across various life stages.

There were clear age-related metabolic changes in glucose, cholesterol, and triglyceride levels. Glucose levels varied greatly among the three groups, likely due to dietary factors or stress. In adult donkeys, cholesterol levels rose, indicating metabolic adjustments, a trend that was also observed in different breeds of donkeys as reported by Caldin et al. (2005), Trimboli et al. (2020) and Silva et al. (2024). Lower triglyceride levels in younger donkeys may indicate less fat intake during their growth periods. These observations are in line with broader research, including the work of Burden et al. (2016), which highlights metabolic adaptations across different age groups. Our primary focus was to examine age-related differences. We noticed that sex can also influence certain biochemical parameters. However, due to highly imbalanced sex ratio among the sampled donkeys, an accurate statistical analysis specifically addressing

sex-related effects was not feasible. Despite this limitation, our findings suggest notable metabolic differences between males and females. Female donkeys had significantly higher total protein, albumin, calcium, and urea levels, indicating potential differences in protein metabolism and mineral regulation. Male donkeys exhibited higher triglycerides, cholesterol, ALT, and glucose, pointing to variations in lipid metabolism, carbohydrate processing, and liver function. These findings highlight the importance of considering both age and sex in assessing donkey health. Regarding the impact of gender to the analyzed parameters, Silva et al., 2024 observed significant differences between groups (females versus males). This study supports our findings, demonstrating that gender significantly influences certain biochemical parameters in donkeys. Recognizing these differences is essential for accurately assessing their health and ensuring proper management and care.

Hematological Parameters

Hematological analysis showed notable age-related trends, especially in white blood cell (WBC) counts. Neutrophil levels were highest in Group A, indicating the increased innate immune activity commonly seen in younger animals as they adjust to environmental challenges. Differential WBC counts showed a peak in lymphocyte levels in Group B, suggesting enhanced adaptive immune activity during early adulthood. Monocyte levels remained consistent across all groups, aligning with their function in tissue repair and immune monitoring. Younger donkeys in Groups A and B displayed higher overall WBC levels, likely due to active immune development and responses to environmental pathogens. These results are in line with the findings of Stanišić et al. (2015), who noted age-related variations in WBC counts in Balkan donkeys, and Sedlinská et al. (2016), who observed similar patterns in younger donkeys from Czech and Slovak populations. Conversely, the lower WBC counts seen in Group C (adults) indicate a stable and efficient immune system, typical of immunological maturity in equids. The red blood cell (RBC) parameters, including hemoglobin (HGB) and hematocrit (HCT), showed little variation among age groups, indicating a stable capacity for oxygen transport in the donkeys studied. The slightly elevated RBC and HGB levels in younger donkeys (Group A) likely reflect their increased oxygen demands during growth, which aligns with the observations of Laus et al. (2015), Burden et al. (2016) and Sedlinská et al. (2016) who noted similar trends in healthy donkeys and age-related physiological adaptations in equids. The consistent HCT levels across all groups further highlight the adaptability and resilience of the red blood cell system in meeting metabolic needs throughout different life stages.

Platelet counts showed no significant differences among the age groups, suggesting consistent thrombopoietic activity and coagulation function throughout the population. The minor variability noted in Group A could be due to physiological changes occurring during early growth stages. This finding is consistent with the research by Stanišić et al. (2015), which indicated stable platelet parameters and established reference ranges for Balkan donkeys, reinforcing the idea of hematological stability across various age groups.

CONCLUSION

This study provides valuable insights into the physiology of the Balkan donkey breed, contributing to the existing body of literature on donkey health and conservation. Our results emphasize liver and kidney function, stable metabolic parameters, and age-related trends in blood health that showcase the remarkable adaptability of these animals. The increased white blood cell counts in younger donkeys indicate active immune responses during their growth, while the consistent red blood cell and platelet parameters across different ages highlight stable physiological characteristics that are clearly not influenced by age. This research marks a significant advancement in understanding the physiological health of the endangered Balkan donkey breed, with a focus on liver and kidney function, metabolic health, and blood parameters across various age groups. Although the findings offer valuable baseline data and insights into the adaptability of these animals, they do not provide enough information to establish definitive reference values for the breed. Future studies should further explore how environmental factors, seasonal shifts, and genetics influence metabolic adaptations in donkeys, contributing to better conservation strategies and improved veterinary care for this endangered breed. These preliminary findings establish a strong foundation for future studies focused on developing breed-specific reference values. They also highlight the need for continued research and customized conservation strategies that address the unique nutritional, environmental, and physiological needs of the Balkan donkey. By building on this foundation, future research can enhance veterinary diagnostics and contribute to the long-term preservation of this endangered breed.

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Author's Contribution:

SS, MŽ – the idea of the publication, organisation of work, writing the manuscript.MŽ, VG – laboratory analysis for all the samples.

SS, MŽ, VG, MŽB, DL, SL, DB reviewed the manuscript and participated in the final draft of the manuscript.

Competing interest

There is no competing interest existing. The manuscript is not intluenced by any personal or financial relationship with people or organizations.

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