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HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF CAPTIVE FALLOW DEER (*Dama dama*) IN A ZOO ENVIRONMENT

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Supporting Information

ABSTRACT: Accurate health assessment of wild, semi-captive, or domesticated animals is essential for their well-being. Despite this necessity, limited studies have been conducted on deer species, and there is a paucity of information on the hemato-biochemical parameters of different deer species globally. Present study aimed to fill this gap by determining the hematological and serum biochemical parameters of fallow deer (*Dama dama*) maintained in semi-captivity within zoo environments for the first time in Bosnia and Herzegovina. Present research involved six healthy male fallow deer, aged 2 to 5 years. The deer were immobilized using xylazine hydrochloride and ketamine hydrochloride, and blood samples were collected from the external jugular vein. The hematological parameters measured included RBC, PCV, HGB, MCV, MCH, MCHC, RDW, RETIC, WBC, WBC differential, PLT, MPV, PDW, and PCT. Biochemical parameters included glucose, urea, creatinine, albumin, triglycerides, cholesterol, and enzymes (AST, ALT, ALKP, and GGT) activities. The results showed the higher glucose and urea concentrations and the same values for creatinine, triglycerides, and enzyme activities when compared to some previous reports. These findings highlighted the importance of considering handling methods and environmental conditions when interpreting biochemical parameters, contributing to improved health assessments and management practices for deer in captivity.

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Abbreviations: ALB: Albumin; ALKP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BASO: Basophils; CHOL: Cholesterol; CREA: Creatinine; EDTA K: Ethylenediaminetetraacetic Acid Potassium Salt; EOS: Eosinophils; GGT: Gamma-Glutamyl Transferase; GLU: Glucose; HGB: Hemoglobin; LYM: Lymphocytes; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Corpuscular Volume; MONO: Monocytes; MPV: Mean Platelet Volume; NEU: Neutrophils; PCT: Plateletcrit; PCV: Packed Cell Volume; PDW: Platelet Distribution Width; PLT: Platelets; RBC: Red Blood Cells; RDW: Red Cell Distribution Width; RETIC: Reticulocytes; TG: Triglycerides; WBC differential: White Blood Cells Differential; WBC: White Blood Cells.

INTRODUCTION

Accurately assessing the health of wild, semi-captive, or domesticated animals is crucial for their wellbeing. However, there have been very few studies conducted on deer species, and limited reports exist on the haemato-biochemical parameters of different deer species worldwide (Gupta et al., 2007; Sinanović et al., 2013; Vukšić et al., 2016).

While normal hematological and serum biochemical values for several deer species are limited in the literature, some studies have established reference ranges for certain wild species. For instance, Rosef et al. (2004) provided hematological and serum biochemical reference values for free-ranging red deer (Cervus elaphus atlanticus) in Norway. Similarly, Miller et al. (2013) reported biochemical and hematologic reference values for free-ranging, chemically immobilized wild Norwegian reindeer (Rangifer tarandus tarandus) during early winter. Additionally, Karpiński et al. (2023) presented hematology, and serum chemistry values for free-ranging roe deer (Capreolus capreolus) in Poland. These studies provide valuable baseline data for health assessments and disease diagnosis in these species. However, for other deer species lacking specific reference values, comparisons are often made using baseline data from domestic small ruminants such as sheep and goats (Gupta et al., 2007).

The fallow deer (*Dama dama*) is a native Eurasian wild species of cervid (Pastrana et al., 2022) and among the most common cervid species in Europe and the most widely distributed cervid globally. Although the fallow deer has been introduced to most parts of Europe, it is native only to southern Anatolia, Sicily, southern Italy, and the southern Balkan peninsula. However, distribution data for the fallow deer in Bosnia and Herzegovina is not available, as they are only found in reserves (Bijl and Csányi, 2022). Fallow deer are the most common deer species found in both wild and captive environments in Bosnia and Herzegovina.

Several studies have identified differences in blood values among deer, which can be attributed to various factors including farming conditions, management practices, and sampling techniques. Methods such as collection from pasture, yarding, drafting, indoor confinement, isolation, and catheterisation have been shown to induce stress in deer (Vengušt et al., 2006). These variations in blood values can also result from genetic, environmental, nutritional, and physiological factors, as well as the stress of capture and the influence of different blood sampling techniques (Vengušt et al., 2006).

Assessing the health of wild, semi-captive, or domesticated animals is vital, yet studies on deer species and their haemato-biochemical parameters are limited (Gupta et al., 2007). Differences in blood values among deer have been linked to farming conditions, management, and sampling techniques (Vengušt et al., 2006). In the absence of specific data, comparisons are often made with baseline values from domestic small ruminants like sheep and goats (Gupta et al., 2007).

Fallow deer are the most common deer species found in both wild and semi-captive environments in Bosnia and Herzegovina. They are residents of forested areas and are also kept in semi-captivity in zoos. The data reported here detail the typical hematological and serum biochemical parameters of clinical importance for a deer species raised in captivity. Specifically, these parameters pertain to fallow deer maintained in captivity within zoo environments.

MATERIALS AND METHODS

The research was conducted on six male fallow deer (*Dama dama*), aged between 2 and 5 years, housed in the zoo in Sarajevo – Pionirska dolina, Sarajevo, Bosnia and Herzegovina; 43°52'41.8"N 18°24'44.1"E; elevation 518 m). The animals were healthy, well-fed, and clinically healthy. The deer were immobilized using a combination of 100 mg xylazine hydrochloride and 300 mg ketamine hydrochloride. Following immobilization, blood was collected from the external jugular vein. For hematological analysis, blood samples were collected into tubes containing the EDTA K as an anticoagulant. Hematological analysis was conducted within 2 to 3 hours after sampling using an automated veterinary hematology analyzer – The ProCyte IDEXX, PRC 1025236.

The following parameters were determined: red blood cells (RBC), packed cell volume (PCV), hemoglobin (HGB), the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), the mean corpuscular hemoglobin concentration (MCHC), the red blood cell distribution width (RDW), reticulocyte count (RETIC), white blood cells (WBC), white blood cells differential(WBC-D), the platelets (PLT), the mean platelet volume (MPV), the platelet distribution width (PDW), and the plateletcrit (PCT). Plain tubes were used to collect serum for the analysis of biochemical parameters, including glucose, urea, creatinine, albumin, triglycerides, cholesterol, and the activity of enzymes AST, ALT, ALKP, and GGT. Serum biochemical parameters were determined by the IDEXX Catalyst One veterinary chemistry analyzer, REF 89-92525-00.

Ethical Regulations and animal welfare

In present study, all procedures involving animals were conducted in accordance with recognized standards for animal welfare and ethical research. The fallow deer (*Dama dama*) were housed in managed care within a zoo environment, specifically in the Pionirska Dolina Zoo in Sarajevo, Bosnia and Herzegovina. Their health and well-being were closely monitored by experienced veterinarians, ensuring that all animals were kept in optimal conditions and handled according to established welfare protocols. Blood sampling was performed as part of routine veterinary care, and all immobilization and handling techniques were aimed at minimizing stress and discomfort to the animals. As the blood sampling occurred during routine health monitoring, it did not require specific approval from an ethical committee. However, all efforts were made to follow best practices for animal care and welfare, and the animals were never subjected to unnecessary pain or distress.

Statistical analysis

The results were analysed statistically. The values are presented throughout as a mean value and standard deviation (SD). Statistical analysis was performed by means of the SPSS package (SPSS Inc., Chicago, Illinois, USA).

RESULTS AND DISCUSSION

The hematological and biochemical analysis of six male fallow deer (*Dama dama*) maintained in a zoo environment revealed several notable findings. The glucose and urea concentrations were higher compared to some previous studies, while creatinine, triglyceride, and enzyme activity levels remained consistent with prior reports. The hematological parameters, including RBC, PCV, HGB, MCV, MCH, and MCHC, aligned closely with previously published values.

Table 1 - Mean values of haematology parameters in male fallow deer

Parameters	Mean ± SD
RBC (×1012/L)	10.81 ± 2.06
PCV (L/L)	44.3 ± 37.1
HGB (g/L)	143.3 ± 23
MCV (fL)	41.2 ± 1.48
MCH	14.27 ± 0.6
MCHC	34.67 ± 0.59
RDW	31.97 ± 2.8
%RETIC	0
RETIC	1.13 ± 1.27
WBC (×109/L)	1.95 ± 0.94
%NEU	56.27 ± 6.26
%LYM	23.03 ± 2.47
%MONO	5.6 ± 2.1
%EOS	13.23 ± 2.43
%BASO	1.87 ± 0.25
NEU	1.07 ± 0.44
LYM	0.46 ± 0.26
MONO	0.11 ± 0.06
EOS	0.27 ± 0.17
BASO	0.04 ± 0.02
PLT	265.33 ± 92.45
MPV	7.77 ± 0.51
PDW	6.55 ± 0.35
PCT	0.21 ± 0.07

Table 2 - Mean values of biochemistry parameters in male fallow deer	
Parameters	Mean ± SD
GLU (mmol/L)	9.71 ± 2.35
UREA (mmol/L)	6.31 ± 2.04
CREA (µmol/L)	127 ± 20.58
ALB (g/L)	48 ± 2.67
TG (mmol/L)	0.52 ± 0.46
CHOL (mmol/L)	1.99 ± 0.12
AST (U/L)	99 ± 36.78
ALT (U/L)	36 ± 5.87
ALKP (U/L)	212 ± 19.87
GGT (U/L)	38 ± 6

Existing literature provides data on the blood parameters of deer which vary depending on the sampling technique used, including chemical immobilization (Peinado et al., 1999; Poljičak-Milas et al., 2004), physical restraint (Rehbein et al., 1999), or post-culling (Vengušt et al., 2002). The RBC values determined by present research are slightly lower than those determined by Tajchman et al. (2023) and the values determined by Vukšić et al. (2016), but correspond to the values determined by Venguš et al. (2006) for deer after sedation and culling.

Present study measured PCV, HGB, MCV, MCH, and MCH levels in deer, aligning closely with the findings with the values reported earlier (Vengušt et al., 2006) following sedation and culling. Comparable values were also reported by Kováč et al. (1997), as well as by Barić Rafaj et al. (2011) in red deer. Vukšić et al. (2016) noted that the hemoglobin concentration ranged from 157.00 to 164.00 g/L, averaging 160.50 g/L, which was similar to the concentration observed in present study but higher than that reported by Vengušt (2002). Present research identified significantly lower WBC values compared to other studies (Vengušt et al., 2006; Vukšić et al., 2016). Vukšić et al. (2016) determined the average leukocyte count in adults (7.07 x 10^9/L), which was higher compared to young deer, indicating that age influences the value of this parameter.

The PLT value was determined in fallow deer to be 265.33 ± 92.45 , while Vukšić et al. (2016) reported a mean PLT value of 161.78. Additionally, Barić-Rafaj et al. (2011) found a PLT value of 262 ± 118 in adult red deer. The significant difference between present findings and those of Vukšić et al. (2016), might be attributed to variations in sampling methods, environmental conditions, the health and physiological status of the animals, or differences in the populations studied. Interestingly, present PLT values are closer to those reported by Barić-Rafaj et al. (2011) for adult red deer, suggesting that species differences or age-related factors might play a role. Further investigation is needed to understand these discrepancies and to establish more comprehensive reference ranges for fallow deer and other cervids in different environments.

Red cell distribution width (RDW) measures the variation in erythrocyte size using their MCV (Mean Corpuscular Volume). Baric-Rafaj et al. (2011) in their study on farmed red deer, determined that the RDW was significantly higher in fawns. Although RDW determination is widely accepted in human medicine, there is limited information about this parameter in veterinary medicine, particularly concerning wild animals. The values for WBC and the differential blood count are presented in Table 1. Vukšić et al. (2016) determined a WBC value of 7.07 in adult fallow deer. Barić-Rafaj et al. (2011) found a WBC value of 15.41 \pm 4.87 in 11 adult red deer. Vengušt et al. (2006) reported WBC values in fallow deer as follows: 9.1 \pm 1.2 for restrained deer, 3.6 \pm 0.9 for tranquilized deer, and 2.9 \pm 1.3 for shot deer. In contrast, present research determined a WBC value of 1.95 \pm 0.94.

These differences highlight the significant variability in WBC counts depending on factors such as species, handling methods, and the physiological state of the animals. Present findings, which show lower WBC values compared to other studies (Vengušt et al., 2006) may be influenced by the specific conditions under which our samples were taken. This underscores the importance of considering these variables when interpreting hematological data and establishing reference ranges for wildlife.

Present research also determined values of some biochemical parameters (Table 2). Vengušt et al. (2006) determined the glucose values in deer as follows: $2.9 \pm 0.4 \text{ mmol/L}$ for restrained deer, $8.5 \pm 2.1 \text{ mmol/L}$ for tranquilized deer, and $7.5 \pm 3.2 \text{ mmol/L}$ for deer that were shot. These values indicate that glucose levels vary significantly depending on the method of restraint or sedation. The glucose values determined in present research correspond to the values found for tranquilized deer. However, in reserch conducted by Vengušt and Bidovec (2002) authors described lower glucose values compared to those determined in present research. This discrepancy highlights potential differences in environmental conditions, handling, or physiological states of the deer between the studies.

The serum glucose concentration in fallow deer may exhibited significant individual variation (Rehbein et al., 1999; Slavica et al., 2000). Wilson and Pauli (1983) reported similar results in red deer. Compared to domestic ruminants, deer have higher serum glucose levels, which may be due to their more nervous temperament or higher metabolic rate (Wilson and Pauli, 1983).

Present research determined urea concentration of $6.31 \pm 2.04 \text{ mmol/L}$ in deer, which presents an interesting comparison to the values obtained by other studies (Vengušt et al., 2006). While present methodology also involved the use of sedation, obtained values are somewhat different from those reported by Vengušt et al. (2006). In previous studies, the urea values were reported as follows: $9.8 \pm 3.2 \text{ mmol/L}$ for restrained deer, $8.1 \pm 0.7 \text{ mmol/L}$ for tranquilized deer, and $6.5 \pm 1.6 \text{ mmol/L}$ for deer that were shot. These values indicate that the method of handling and sedation significantly impacts the biochemical parameters measured in deer. For instance, the highest urea concentration was observed in restrained deer, likely due to the stress response elicited by physical restraint. Tranquilized deer showed slightly lower urea levels, which can be attributed to the calming effects of sedation that reduce stress-induced metabolic changes. Deer that were shot had the lowest urea concentrations, potentially due to the rapid physiological changes occurring at the time of death, affecting metabolic waste levels. Although present research employed sedation similar to the aforementioned studies, the urea concentration we observed ($6.31 \pm 2.04 \text{ mmol/L}$) is somewhat different from the $8.1 \pm 0.7 \text{ mmol/L}$ reported for tranquilized deer in research conducted by Vengušt et al. (2006). This discrepancy could be due to differences in sedation protocols, environmental conditions, or the physiological status of the animals at the time of sampling. These variations underscore the importance of considering the method of animal handling and specific research conditions when interpreting biochemical parameters.

This research determined that the concentrations of creatinine and triglycerides were similar to those reported by Vengušt et al. (2006). This consistency suggests that, despite some variations in urea levels, the biochemical responses related to creatinine and triglycerides in present study align with existing literature (Vengušt et al., 2006). This finding reinforces the reliability of present methods and the comparability of present results with previous research. This research determined that the enzyme activities were similar to those reported in other studies (Vengušt et al., 2006; Sinanović et al., 2013). This similarity indicates that the enzymatic responses observed in present study align well with existing literature (Vengušt et al., 2006; Sinanović et al., 2013), further validating our methods and ensuring the comparability of present results with prior studies in this field.

Although Sinanović published a preliminary report in 2013 (Sinanović et al., 2013) on certain biochemical parameters in deer, present research is the first study in Bosnia and Herzegovina to determine both hematological and biochemical parameters in deer blood. The obtained values of hematological and biochemical parameters relate to deer

that were sedated for sampling purposes. Numerous authors mention significant differences in the frequency of the tested parameters depending on whether the animals were sedated, restrained, or shot and then sampled (Vengušt et al., 2006). Some researchers have proposed that two separate ranges of reference blood values should be established for wild animals, based on the capture method used (Vengušt et al., 2006).

CONCLUSION

This study provides essential baseline data on the hematological and biochemical parameters of captive fallow deer in Bosnia and Herzegovina. The findings highlight significant variations in these parameters depending on the method of restraint and sedation used during sampling. The glucose and urea concentrations determined in present research were higher than those reported in some previous studies, indicating potential influences from environmental conditions, handling techniques, and physiological states of the deer. Despite these variations, the consistency observed in creatinine, triglyceride concentrations, and enzyme activities with existing literature suggests that present methods are reliable and comparable to another research in the field. The hematological parameters such as RBC, PCV, HGB, MCV, MCH, and MCHC levels aligned closely with earlier findings, further validating our approach. Also, this study is the first comprehensive report in Bosnia and Herzegovina to document both hematological and biochemical parameters in deer blood, specifically under sedation. Present results emphasize the importance of standardized protocols in wildlife health assessments to ensure accurate and comparable data across different studies and regions. Establishing separate reference ranges for wild animals based on capture methods, as proposed by some researchers, could enhance the precision of health evaluations and contribute to better wildlife management and conservation practices. The results emphasize the importance of standardized protocols in wildlife health assessments to ensure accurate and comparable data across different studies and regions. Establishing separate reference ranges for wild animals based on capture methods, as proposed by some researchers, could enhance the precision of health evaluations and contribute to better wildlife management and conservation practices. In summary, present study underscores the need for continuous monitoring and evaluation of deer health in both wild and captive environments. The data generated from this study will serve as a valuable reference for veterinarians and wildlife biologists in assessing the health and well-being of fallow deer and other cervid species.

DECLARATIONS

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Authors' contributions

N.Hadžimusić contributed to the design of the study, data analysis, and the writing of the manuscript. N.Hadžimusić, A.Livnjak, DŽ.Hadžijunuzović-Alagić and V.Škapur were responsible for sample collection, laboratory analysis, and manuscript review. All authors have contributed to the interpretation of the data and approved the final manuscript for submission.

Ethics committee approval

As the blood sampling was performed during routine veterinary health checks and involved no invasive procedures beyond standard care. The study strictly adhered to internationally recognized animal care guidelines, ensuring minimal stress and maximum welfare for the animals throughout the study.

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Consent to publish

All authors have read and approved the final version of the manuscript and give their consent for publication.

Competing Interests

The authors declare that there are no competing interests regarding the publication of this paper.

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