

Haematological and biochemical values of farmed red deer (*Cervus elaphus*)

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ABSTRACT

The objective of this study was to investigate the haematological and biochemical values of clinical significance for red deer (*Cervus elaphus*) and provide data for farmed fawns. Blood samples were collected regularly from 34 fawns and compared with 11 adults. The mean blood haemoglobin (Hb), total erythrocyte count (RBC), packed cell volume (PCV), total leukocyte count (WBC) and percent of segmented neutrophils were significantly lower in fawns. Red distribution width (RDW), total platelet count (PLT), plateletcrit (PCT) and percent of eosinophils and lymphocytes were significantly higher in fawns. A vast majority of biochemical parameters were significantly lower in fawns: blood urea nitrogen (BUN), creatinine (CRE), total protein (tPROT), total bilirubine (tBIL) and glucose (GLU) concentration, aspartate-aminotransferase (AST) and creatin phosphokinase (CPK) activity, while albumine concentration (ALB), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) activity showed a significant increase. These results demonstrate the need to use specific reference intervals for deer of different ages. The values reported here can be used as a starting point for reference range establishment for clinically healthy young farmed red deer in Croatia.

Key words: red deer, fawn, haematology, biochemistry

Introduction

The increasing farming of red deer in Croatia has created a need for haematological and serum biochemical reference data, specially those which concern the young population of animals. Hematologic and serum biochemistry analytes may be used to assess the condition of wild populations, giving indications of disease, nutritional status, habitat quality, and other stressors. The published values are for haematology and biochemistry

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(SLAVICA et al., 2000; ROSEF et al., 2004; POLJIČAK-MILAS et al., 2004; VENGUŠT et al., 2006; GUBTA et al., 2007) and there is less or no data on some haematological parameters, such as red distribution width (RDW), platelet number (PLT), platelet volume (MPV) and plateletcyt (PCT). Differences in normal values may arise because of differences in the population of animals studied (e. g. geographic location, physical activity, age, breed, sex, etc.) and the methodology of the test (e. g. reagents, reaction temperature, equipment, etc.), which all together could cause differences in laboratory data and give highly variable results. Also, published data often do not distinguish reference ranges of laboratory analytes between the different age subpopulation, despite the fact that many values vary with the age of the animal (MEYER and HARVEY, 2004; TOMKINS and JONSSON, 2005). In addition, the restraining methods and sample handling have been found to affect haematological values in many species of deer (CHAPPLE et al., 1991; MONTANE et al., 2003; POLJIČAK-MILAS et al., 2004).

This study was undertaken to compare the haematological and biochemical parameters of red deer in Croatia in relation to their age.

Materials and methods

Haematological and biochemical parameters were determined in venous blood samples from 34 fawns (*Cervus elaphus*) and 11 adult animals of both sexes, both showing no clinical signs of disease. Fawns were aged 4-14 months, and mature animals were aged 2-3 years. The deer habitat was in Baranja, at an altitude of 100 m. All animals were kept on free pasture and sampled once, in August. The blood samples were taken from 07-09 hours. The deer were blood sampled by jugular venepuncture without tranquillization.

Blood was collected with an 18-g needle, using the Vacutainer blood collection system (Becton, Dickinson and Co., Rutherford, New Jersey 07070, USA). The samples were collected into a plain tube for serum with clot activator and EDTA tube for haematology. Blood samples with clot activator were centrifuged ($1,500 \times g$ at $4^\circ C$ for 10 min), within 2 hr of collection, the serum was stored at $-20^\circ C$ prior to biochemistry analysis. Samples for haematology were refrigerated at $+4^\circ C$ and analysed within 24 hr.

The haematological parameters were analysed on a Abbott Cell-Dyn CD 3500 automatised haematology analyser with extended veterinary package application (Abbot Diagnostic division, Mountain View, CA), for the following hematologic variables: haemoglobin (Hb), red blood cell count (RBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), red distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet haematocrit (PCT) and white blood cell count (WBC). Differential leukocyte counts, neutrophils, eosinophils, basophils, lymphocytes and monocytes were determined microscopically on blood smears stained

by Pappenheim, where 100 leukocytes were identified. The smears were examined under light microscopy (Olympus BH2) at 400× magnification. Validation of the method used in measure process shows the following coefficient of variation (CV): Hb- 0,6, RBC 0,8, PCV - 0,8, MCV - 0,2, RDW - 1,5, MCH 0,7, MCHC 0,7. The use of optical count and impedance count assures the correct leukocyte count is always correctly reported, even in the presence of abnormal pathology. CV for WBC was 1.5, and for PLT and PCV 3.6 and 1.2 (YOUNHEE et al., 2007).

Sera were analyzed using an Olympus AU600 biochemistry analyzer and original reagents of the manufacturer for aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine phosphokinase (CPK), gamma glutamyl transferase (GGT), total protein (tPROT), albumin (ALB), urea (BUN), creatinine (CRE), cholesterol (CHOL), triglycerides (TRYG), total bilirubin (tBIL) and glucose (GLU) concentration. Quality control validation of methods shows coefficient of variation (CV) as follows: AST - 1,70, ALP - 1,85, CPK - 1,25, GGT - 1,53, tPROT - 1,63, ALB - 1,5, BUN 2,10, CRE 1,67, CHOL 2,02, TRYG 1,9, tBIL 3,75 and GLU 1,95 (FARR and FREEMAN, 2008).

Means and standard deviations were calculated for animals grouped by age. All measured and calculated parameters were normally distributed. The Student's *t*-test was used to determine statistical differences between young and adult subpopulation, and significance was set at $P < 0.05$. All statistical analyses were performed with the statistical software program Statistica (Statistica 8 for Windows, StatSoft Inc.)

Results

Values reported are the mean ± one standard deviation.

The results of RBC parameters are presented in Table 1. The RDW value was significantly higher in fawns compared to the adult group of animals. Young animals showed significantly lower values of Hb, RBC and PCV, while in MCV, MCH and MCHC values were not significantly different between the two groups.

Table 1. Red blood cell count in fawns and adult red deer

RBC parameter	Fawns (n = 34)	Mean ± 2.5 SD	Adults (n = 11)	Mean ± 2.5 SD
Hb (g/L)	151 ± 15.60*	120-182	193 ± 19.10	155-231
RBC ($\times 10^{12}/L$)	10.2 ± 1.14*	7.4-12.8	12.3 ± 1.14	10-14.5
PCV (L/L)	0.43 ± 0.05*	0.33-0.53	0.55 ± 0.05	0.46-0.65
MCV (fL)	43.0 ± 3.64	35.7-50.3	45.2 ± 2.54	40-50
RDW	21.9 ± 1.14*	19.6-24.1	20.1 ± 0.93	18.3-22.0
MCH (pg)	15.0 ± 1.05	12.9-17.1	15.7 ± 0.63	14.4-16.9
MCHC (g/L)	350.2 ± 10.50	329-371	347.8 ± 14.42	319-377

* = $P < 0.05$

Table 2 show leukocyte parameters: total leukocyte count and differential leukocyte count in 100 cells. Fawns showed significantly increased relative lymphocyte and eosinophil number, while total WBC count and relative percentage of segmented neutrophils were significantly lower. Percentage of monocytes and basophils showed similar values in young and adult animals.

Table 2. White blood cell count in fawns and adult red deer

WBC parameter	Fawns (n = 34)	Mean ± 2.5 SD	Adults (n = 11)	Mean ± 2.5 SD
WBC ($\times 10^9/L$)	4.88 ± 1.00*	2.9-6.9	15.41 ± 4.87	5.7-25.1
Segmented neutrophils (%)	33.3 ± 9.59*	14-52	76.6 ± 10.00	57-97
Lymphocytes (%)	58.2 ± 10.17*	38-79	26.6 ± 8.17	4-37
Monocytes (%)	1.59 ± 2.20	0-6	1.60 ± 2.37	0-6
Eosinophils (%)	5.47 ± 4.67*	0-15	0.80 ± 1.03	0-3
Basophils (%)	1.41 ± 2.05	0-5	0.40 ± 0.84	0-2

* = P<0.05

In Table 3 are the results of platelet count and platelet indices. Fawns showed a significantly increased number of platelets in circulation, which were almost tripled, compared with adults, followed by markedly increased plateletcrits. MPV did not show differences between the two groups.

Table 3. Platelet count and platelet indices in fawns and adult red deer

PLT parameter	Fawns (n = 34)	Mean ± 2.5 SD	Adults (n = 11)	Mean ± 2.5 SD
PLT ($\times 10^9/L$)	746.159 ± *	428-1064	262 ± 118	25-498
MPV (fL)	7.29 ± 1.73	3.8-10.7	8.24 ± 1.86	4.5-11.9
PCT (%)	0.53 ± 0.14*	0.26-0.81	0.27 ± 0.19	0.08-0.34

* = P<0.05

Table 4 summarises the results of measurement of different biochemical analytes. Most of the measured variables were significantly decreased in fawns: BUN, CRE, tPROT, tBIL and GLU concentration. TRYG and CHOL concentrations were similar in the two groups of animals. ALB was slightly increased compared with adults.

Table 4. Biochemical analytes (substrates) in fawns and adult red deer

substrates	Fawns (n = 34)	Mean \pm 2.5 SD	Adults (n = 11)	Mean \pm 2.5 SD
BUN (mmol/L)	5.55 \pm 0.97*	3.6-7.5	11.1 \pm 3.18	4.8-17.5
CRE (μ mol/L)	77.8 \pm 7.60*	63-93	164 \pm 60.94	42.2-286.0
tPROT (g/L)	62.8 \pm 3.41*	56-70	93.9 \pm 6.54	80.8-107.0
ALB (g/L)	28.7 \pm 2.36*	24-33	25.4 \pm 1.97	21.5-29.3
tBIL (μ mol/L)	8.89 \pm 3.27*	2.4-15.4	15.5 \pm 3.99	7.5-23.5
GLUC (mmol/L)	6.34 \pm 0.85*	4.7-8.0	10.1 \pm 3.79	2.5-17.6
TRYG (mmol/L)	0.18 \pm 0.07	0.05-0.32	0.22 \pm 0.11	16-485
CHOL (mmol/L)	1.49 \pm 0.34	0.80-2.18	1.83 \pm 0.91	0.01-3.65

* = P<0.05

The different enzyme activities are presented in Table 5. Fawns had significantly increased activity of GGT and ALP. The activity of AST and CPK were significantly decreased in fawns, while ALT activity did not show any statistically difference between fawns and adults. The most prominent differences were observed in AST, which were 5 times lower in fawns, and CPK, which were almost 30 times lower than those in adult animals. ALP activity was app. 10 times higher in fawns.

Table 5. Biochemical analytes (enzymes) in fawns and adult red deer

enzyme 37° C	Fawns (n = 34)	Mean \pm 2.5 SD	Adults (n = 11)	Mean \pm 2.5 SD
AST (U/L)	60.5 \pm 15.40*	30-91	250.5 \pm 117.44	16-485
ALT (U/L)	30.4 \pm 11.83	7-54	33.5 \pm 8.66	16-51
GGT(U/L)	25.8 \pm 9.38*	7-44	15.5 \pm 9.82	1-24
ALP (U/L)	302.0 \pm 86.40*	129-475	31.6 \pm 10.71	10-53
CPK (U/L)	288.9 \pm 239.30*	1-757	9717 \pm 8428.97	1-26575

* = P<0.05

Discussion

Laboratory data may be interpreted on the level of both the population and the individual animal and can be important for health monitoring of wild species. Blood variables can also be useful predictors of survival in reintroduction and translocation programs (MATHEWS et al., 2006). It is widely accepted that haematological and biochemical values may vary according to sex, age, time of year, physiological factors, geographic location, nutritional status and method of restraint used to obtain the blood sample. Some of these factors no doubt interact to give highly variable results (ASHER et al., 1989; MATTHEWS and COOK, 1991), so documenting reference values for young populations of deer is of particular

importance to enhance our understanding of their rapid physiological development and concomitant changes in mean values of different parameters of diagnostic importance. In the present study haematological and biochemical parameters were measured for different age populations of red deer. To the best of our knowledge some of these measures have not been published previously, such as RDW, PLT, MPV and plateletcrit, at least for the group of young animals. Thus, not all our results could be compared with previously published data, and the new findings in the present study can therefore serve as a starting point for reference range establishment.

In the present study fawns had significantly lower Hb, RBC and PCV values than the adults. Age-related changes in deer have been previously reported: a distinct pattern of increases in RBC, Hb and PCV with age is evident regardless of sex or species. Increases in RBC and haematocrit by 40-60% from birth to age 10 months were noted in deer (THORN, 2000). Investigating RBC and Hb concentrations in free-ranging neonates, CARSTENSEN POWELL and DELGIUDICE (2005) observed about 50-75% values of those reported for captive and free ranging, adult white-tailed deer. Deer are easily excitable as a species, which is often reflected in the hematologic parameters and as a result red cell values are significantly higher in excited deer than in resting deer, likely as a result of splenic contraction (THORN, 2000). Investigating the effect of the method of capture on the haematology and blood chemistry of red deer (*Cervus elaphus*) MARCO and LAVIN (1999) noted significant differences in the haemogram: RBC, PCV, Hb and segmented neutrophil, lymphocyte, monocyte and WBC were higher in animals captured by physical means, which all together create the need for the establishment of different ranges, according to capture methods. On the other hand, ROSEF et al. (2004) did not find any differences in young and adult red deer in fourteen haematological parameters measured. In blood smears we observed the sickling phenomenon. As a laboratory observation it does not appear to have a deleterious effect on the deer. MCV, MCH and MCHC did not appear to vary with age. RDW represents the width of erythrocyte distribution using their MCV. RDW was significantly higher in fawns. Despite wide acceptance of RDW determination in human medicine, in veterinary medicine there is still little information concerning this parameter in clinical practice, especially in wild animals.

We found significant differences in total WBC count and percentages of segmented neutrophils, which were lower in fawns. Investigating the haematology of captive unsedated chital deer (*Axis Axis*), CHAPPLE et al. (1991) also observed higher WBC counts in stags than in hinds. This may be due to the greater activity of the stags, including aggressive interaction, since increased muscular activity increases blood and lymph circulation, sequestering leucocytes in capillary beds into large-vessel blood. Causes of relative leukocytosis in adult deer may be associated with physical exertion, excitement or fear, or certain local or generalized infections (CARSTENSEN POWELL and DELGIUDICE,

2005). The effects of age were also reflected in higher relative lymphocyte and eosinophils count in fawns. The higher eosinophil count seen in fawns may be a reflection of increased exposure to parasites. Opposite to our findings, ENGLISH and LEPHERD (1991) observed lower eosinophil numbers in fawns. Generally, young animals have been shown to have a higher lymphocyte count, which gradually declines to adult values by 3-6 months of age (DUNN, 2000). The percentage of monocytes and basophils in blood smears of fawns were similar to those of adult animals.

There were only minor differences in mean platelet volume between adults and fawns but there were significant differences in the platelet number and plateletcrit, with the former having a marked increase of platelet number and total platelet volume in blood - PCT. The quantification of platelets in peripheral blood is a well-recognized tool in veterinary diagnostics. Recently, new indices related to platelet counts have been provided by haematologic analysers, namely PCT and MPV, where platelet volume represents a marker of platelet function and activation. It is well known that the surfaces of cells are essential for clotting reactions to take place (KHANDEKAR et al., 2006), so we examined plateletcrit, which were significantly increased in fawns indicating greater coagulation capacity. This highlights the importance of establishing reference ranges for individual species if blood variables are to be used to monitor the population condition and/or health of individuals.

We found significant differences in most of the measured biochemical analytes - fawns showed significantly lower concentrations of BUN, CRE, tPROT, tBIL and GLU. The increased concentration of BUN in adults could be the consequence of greater protein catabolism intensity. Investigating laboratory parameters in deer, ENGLISH and LEPHERD (1991) also measured lower glucose and blood urea nitrogen (BUN) in fawns. Lower creatinine concentrations in fawns could be attributed to lower muscle mass, while lower glucose concentration compared with adults could be the consequence of greater stress influences in adults. This can be supported by the extreme increases of muscle enzymes in adults, such as CPK ($34 \times$ higher in adults) and AST ($4 \times$ higher in adults). Elevated glucose levels during the excitement of capture have been demonstrated in many species (CHAPPLE et al., 1991). Comparing the biochemical analytes between young and adult red deer, POLJIČAK-MILAS et al. (2006) found significantly elevated BUN and GLU in young population, while the CRE, ALB, GLU and TRYG concentrations recorded in young population are not in agreement with the results obtained in our study. Opposite to our results, plasma glucose concentration did not appear to vary with age in young male russa deer (TOMKINS and JONSONN, 2005). Fawns had significantly lower total protein concentration compared to the adult group, while albumin concentration was slightly increased. Considering these findings, the young population had a much higher A/G ratio than the adult red deer population. Total protein concentration has been previously

reported to be lower in fawns compared to adults. SLAVICA et al. (2000) reported similar values in different deer species. Investigating effects of age on concentrations of ionized and total magnesium and calcium in healthy horses, BERLIN and AROCH (2008) found significantly decreased concentrations of serum albumin and total protein concentrations in foals, compared with adults. Our results are similar to those obtained by BOUDA et al., (2000), who investigated plasma protein, and packed cell volume (PCV) values in blood samples in young red deer. SZABO et al. (2005) also found lower blood total protein in young animals when compared to adult females. Their results indicate that two main reference ranges for blood values should be considered for marsh deer, for blood obtained from anesthetized or physically restrained individuals.

Between major blood biochemistry values measured in various species in response to stressor, CPK and AST activity have an important place. In fawns they were significantly decreased, which could be the consequence of greater stress influence and greater mechanic damage of tissues in adults during blood sampling. The obtained results indicated that the stressful effect of restraint and handling must therefore be considered when measuring blood parameters in wild animals. CHAPPLE et al. (1991) concluded that greater increases in CK and AST in stags compared with hinds suggested that the stags reacted more vigorously to handling. As animals adapted to regular handling, enzyme concentrations were reduced. Enzymes (ALT, AST, CK, and LDH) appear elevated in many stressed wild ungulates and in those suffering from capture myopathy (KOCK et al., 1987; VASSART et al., 1992), although some authors found that CK and AST levels are the most sensitive indicators of muscular disorders (CHAPPLE et al., 1991). Overall, the most commonly used enzyme indicators of stress in general are CK, AST, ALT and ALP and changes in the activities of these indicators have been successfully used to reveal stress responses in a wide range of animals. MARCO and LAVIN (1999), and VENGUŠT et al. (2006) find greater activities of enzymes in the group of animals captured by physical methods when compared with animals captured by chemical methods. Elevated serum AP levels in fawns are the result of active bone metabolism and growth.

In conclusion, the present study is to report haematology and serum biochemistry values for manually restrained, unsedated adult and juvenile red deer, and is directly applicable to deer managed on farms. The most pronounced changes between young and adults animals were in total white blood cells, lymphocyte and neutrophils percentage, platelet number, plateletcyt and serum levels of BUN, creatinine, GGT and muscle enzymes, where CK activity were extremely increased in adult animals. The distribution of haematological and serum biochemical values was fairly normal, suggesting that the mean values could be representative of normal values for fawns.

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SAŽETAK

Istraženi su klinički značajni hematološki i biokemijski pokazatelji u jelena (*Cervus elaphus*) držanih na farmama. Pretraženi su uzorci od 34 mlada jelena, a rezultati pretrage bili su uspoređeni s rezultatima pretrage uzoraka 11 odraslih jelena. Koncentracija hemoglobina (Hb), broj eritrocita (RBC), hematokrit (PCV), ukupni broj leukocita (WBC) i postotak segmentiranih neutrofila bili su značajno niži u mladim jelena. Distribucija eritrocita po volumenu (RDW), broj trombocita (PLT), trombokrit (PCT) i postotak eozinofila i limfocita bio je značajno viši u mladim jelena. Većina biokemijskih pokazatelja bila je značajno niža u mladim jelena: koncentracija ureje (BUN), kreatinina (CRE), ukupnih proteina (tPROT), ukupnog bilirubina (tBIL) i glukoze (GLU), kao i aktivnost aspartat-aminotransferaze (AST) i kreatin-fosfokinaze (CPK), dok su albumin (ALB), aktivnost alkalne fosfataze (ALP) i gama-glutamil transferaze (GGT) pokazivali značajni porast. Rezultati ukazuju na potrebu uvođenja specifičnih referentnih vrijednosti za jelene različite životne dobi. Dobivene vrijednosti mogu predstavljati ishodišnu točku za uspostavu referentnoga raspona u klinički zdravih mladih jelena uzgojenih na farmama u Hrvatskoj.

Ključne riječi: jelen, hematologija, biokemija
