

Comparative hematological and biochemical values in pregnant and non-pregnant red, *Cervus elaphus*, and fallow deer, *Dama dama*, females

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Abstract. The hematological and biochemical parameters were determined in blood of chemically immobilized pregnant and non-pregnant free ranging red, *Cervus elaphus*, and fallow, *Dama dama*, deer. In both species a marked reduction of red blood cell count, hemoglobin concentration and hematocrit, as well as higher concentration of triacylglyceride, cholesterol, creatinine and alanine aminotransferase activity were detected in pregnant animals. Significant differences in blood parameters were determined between the two cervid species. The red deer hinds had higher hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin value, higher count of neutrophils and lymphocytes and a total white blood cells count, glucose, albumin, triacylglyceride and urea concentration, whereas fallow deer does had higher alanine aminotransferase activity and cholesterol concentration. The marked differences in blood glucose values were noted between pregnant animals regarding the species. Pregnant hinds had lower lymphocyte count than non-pregnant ones, while pregnant does had more than twice lymphocyte count in their blood than non-pregnant animals. Moreover, pregnant fallow deer does had rather high eosinophile count in the blood. The results provided by this research may facilitate better assessment of health status in free ranging red and fallow deer.

Key words: fallow deer does, red deer hinds, pregnancy, hematology, serum biochemistry

Introduction

The hunting grounds in the Republic of Croatia have been populated by the autochthonous red deer species and imported fallow deer species. The Islands of Brijuni were inhabited by fallow deer since the nineteenth century and from there they spread to continental parts of the Republic. During the years of Croatian War of Independence (1990–1995), the part of deer game population which had suffered by war events, escaped to the more peaceful parts of the Croatian country or moved to the nearby states. Therefore, in order to increase the number of animals and maintain the ecologic equilibrium at the hunting grounds, some deer were imported from Hungary. In the Republic of Croatia the deer game farming has recently attracted huge interest, especially towards the development of tourist hunting. The knowledge in blood constituents is important to assess the health status at the deer population (F o w l e r & M i l l e r 2003). Some data on biochemical parameters in fallow deer on Brijuni Islands

and at the continental area are available from studies by Slavica et al. (2000) and Poljičak-Milas et al. (2004, 2006). The hematological parameters were observed at the restricted group of animals from the same area (Janicki et al. 2000). The data of hematology and blood biochemistry in pregnant and non-pregnant deer females are scarce. It is well known that during the pregnancy some metabolic changes occur that may alter physiological range of blood constituents (Jainudeen & Hafez 1994, El-Sherif & Assad 2001, Khan & Ludri 2002). Therefore, we have hypothesized the influence of the gravidity and nutrition on hematological and biochemical parameters in blood of the deer species as it was confirmed in the domesticated ruminants. The aim of our study was to evaluate the selected biochemical and hematological parameters in pregnant red and fallow deer in respect to physiology of the gravidity considering different nutrition and disease disposal.

Material and Methods

The study was conducted on 30 free-ranging fallow deer does, *Dama dama* and 40 red deer hinds, *Cervus elaphus*, aged between two and five years, from different hunting grounds in continental part of Croatia. These hunting grounds are part of public land, and have optimum water supply, soil and climate conditions. The food disposal is mostly sufficient to maintain deer population throughout the year. However, in winter hay is provided as a supplemental feeding. Blood samples of fallow deer does were collected during the first half of March, and for red deer hinds during the first half of May at year 2002. Blood was taken from the jugular vein, immediately after recumbence under the effect of the “Vienna” sedative solution. The solution consists of zolazepam and tiletamine (2.2 mg/kg “Zoletil®”, Virbac, France) in combination with xylazine hydrochloride (1.5 mg/kg “Rompun”, Bayer, Leverkusen, Germany), and it was administered from a distance using the dist-inject CO₂ powered gun (Dist-Inject, Basel, Switzerland).

The blood samples for biochemical analyses were taken in the test tubes with a suppressor containing gel and congeal activator (“Becton-Dickinson”, Meylan cedex, France) whereas commercial tubes with ethylenediaminetetraacetic acid (K₃E 15%) were being used for the hematological analyses. The blood samples were refrigerated and transported on ice (+ 4 °C) to the laboratory, when they were processed within six hours from the time of collection. Animals were weighed on the field, and the age of the animals was estimated from degree of eruption and wear of molar teeth (Wagenknecht 1984). The pregnancy was determined by ultra sonic examination using a “Sonovet 2000” (Medison Co. LTD, Korea). Red deer hinds were between the 21st and 24th week of gestation, while fallow deer does were in the last stages, between the 29th and 32nd week of gestation. The difference in the stadium of gestation is due to the difference in the rutting season and estimated conception period between the red and fallow deer.

After coagulation at room temperature the serum was extracted by centrifugation at 1200 g for 10 minutes. Biochemical parameters were determined by standard methods on an automatic analyzer “Technicon RA-1000” (Technicon Instruments Corp., Tarrytown, New York), according to International Union of Pure and Applied Chemistry (IUPAC 1997). Reagents for all analyses, including bovine low and high reference value serum, were obtained from Randox Laboratories (Ardmore, Antrim, UK). Glucose concentration was measured with Hexokinase, albumin with Bromocresol green, and total serum protein with Biuret endpoint reaction. Cholesterol concentration was determined by Cholesterol Oxidase (CHOD PAP) and triacylglyceride by Lipase/GPO-PAP colorimetric method.

Creatinine concentration was determined with Alkaline picrate without deproteinization and urea with Urease kinetic method. Alanine aminotransferase (ALT) activities were determined at 25°C by an optical test, without a Pyridoxal-5-Phosphate. Hematological parameters were determined using hematological counter SERONO – 9120 Baker System. Differential leukocyte count were determined microscopically on blood smears stained by May Grünwald-Giemsa stains.

Statistical analysis was carried out using ANOVA/MANOVA (STATISTICA 6.1, StatSoft Inc. 2003 USA) with the species and pregnancy status as the main effect, and hematological and biochemical parameters as dependent variable. Distribution of each variable was tested by Shapiro-Wilks' W test. The significant differences between means were evaluated by Fisher LSD test.

Results

The comparative biochemical and hematological parameters from pregnant and non-pregnant red and fallow deer, are presented in Tables 1 and 2. Red deer hinds had higher blood glucose concentration than fallow deer does, furthermore pregnant red deer hinds had higher glucose concentration than non-pregnant ones. However, pregnant fallow deer does had exceptionally low glucose concentration in comparison with non-pregnant does. Both, albumin and urea concentration in the blood of non-pregnant red deer hinds were higher than in the pregnant ones, whereas diametrically opposite finding was determined in fallow deer does. Other measured biochemical parameters, triacylglyceride, cholesterol and creatinine concentration as well as ALT activity were higher in pregnant females of both species than in non pregnant ones. Results also showed that red deer hinds, pregnant and non-pregnant, had higher triacylglyceride, but lower cholesterol level in their blood than fallow deer does (Table 1).

Results of hematological analyses showed that pregnant females of both species had lower red blood cells count (RBC), hemoglobin concentration (Hb) and hematocrit level (Ht) than non-pregnant females. However, red deer hinds, pregnant and non-pregnant, had higher

Table 1. Comparison of serum biochemical parameters in adult female animals.

Biochemical parameters	ADULT FEMALE ANIMALS			
	Non-pregnant		Pregnant	
	Red deer n = 29	Fallow deer n = 19	Red deer n = 4	Fallow deer n = 4
Body weight (kg)	75.34 ± 10.03	40.75 ± 4.11 ^{****}	119.75 ± 4.50 ^{****}	42.08 ± 6.10 ^{b****}
Glucose (mmol/l)	11.74 ± 3.03	9.05 ± 2.30 ^{***}	22.25 ± 1.24 ^{c***}	4.72 ± 0.26 ^{b, d***}
Total protein (g/l)	74.41 ± 4.38	70.58 ± 9.32	73.05 ± 4.87	75.00 ± 3.56
Albumin (g/l)	39.82 ± 4.31	36.26 ± 3.68 ^{****}	31.02 ± 1.96 ^{c***}	43.25 ± 3.40 ^{b, d***}
ALT (μkat/l)	0.39 ± 0.11	0.46 ± 0.14 ^{a*}	0.51 ± 0.07 ^{c**}	0.61 ± 0.08 ^{d**}
Triacylglyceride (mmol/l)	0.58 ± 0.29	0.33 ± 0.08 ^{****}	0.89 ± 0.13 ^{c**}	0.43 ± 0.06 ^{b*** d*}
Cholesterol (mmol/l)	1.66 ± 0.36	1.91 ± 0.19 ^{****}	2.06 ± 0.26 ^{c*}	3.14 ± 0.53 ^{b, d**}
Creatinine (μmol/l)	135.45 ± 13.76	131.26 ± 3.43	183.50 ± 21.98 ^{c**}	156.00 ± 14.70 ^{b* d**}
Urea (mmol/l)	9.76 ± 1.72	6.39 ± 3.37 ^{****}	6.67 ± 2.24 ^{c*}	11.10 ± 0.60 ^{b*** d***}

Data are expressed as mean ± standard deviation; *P < 0.05, **P < 0.01, ***P < 0.001: a = significant differences between non pregnant red and fallow deer females; b = significant differences between pregnant red and fallow deer females; c = significant differences between pregnant and non-pregnant red deer females; d = significant differences between pregnant and non-pregnant fallow deer females.

Table 2. Comparison of hematological parameters in adult female animals.

Hematological parameters	ADULT FEMALE ANIMALS			
	Non-pregnant		Pregnant	
	Red deer n = 36	Fallow deer n = 26	Red deer n = 4	Fallow deer n = 4
RBC ($10^{12}/l$)	11.92 ± 1.52	11.56 ± 0.94	9.38 ± 0.88 ^{a**}	8.80 ± 0.65 ^{d***}
Hb (g/l)	171.15 ± 18.13	152.00 ± 12.11 ^{a***}	150.25 ± 18.29 ^{c*}	122.75 ± 8.87 ^{b*,d***}
Ht (%)	61.08 ± 7.05	53.92 ± 5.03 ^{a***}	54.27 ± 5.33 ^{c*}	36.77 ± 4.17 ^{b*,d***}
MCV (fl)	51.29 ± 2.68	47.03 ± 4.77 ^{a***}	57.39 ± 4.11	41.17 ± 1.74 ^{b,d***}
MCH (pg)	14.39 ± 0.81	13.23 ± 0.97 ^{a***}	16.30 ± 0.92 ^{c*}	13.77 ± 1.06 ^{b**}
MCHC (%)	279.73 ± 15.24	282.62 ± 16.50	282.81 ± 20.14	312.25 ± 6.98 ^{b*,d***}
WBC ($10^9/l$)	10.90 ± 5.22	3.44 ± 0.8 ^{a***}	8.59 ± 1.98	4.95 ± 0.91 ^{b*,d*}
Neutro. seg. ($10^9/l$)	5.82 ± 1.17 (43 – 67%)	2.7 ± 0.09 ^{a**} (76 – 81%)	5.22 ± 0.67 (53 – 73%)	2.41 ± 0.08 ^{b**} (37 – 78%)
Lympho. ($10^9/l$)	4.77 ± 1.01 (32 – 57%)	0.83 ± 0.18 ^{a**} (19 – 42%)	3.33 ± 0.63 ^{c*} (27 – 45%)	1.83 ± 0.76 ^{b*,d*} (16 – 58%)
Eosinophils ($10^9/l$)	0.08 ± 0.06 (0 – 2%)	0.02 ± 0.02 (0 – 2%)	0.06 ± 0.05 (0 – 2%)	0.64 ± 0.53 (2 – 30%)

RBC = Red blood cells count, Hb = hemoglobin concentration, Ht = hematocrit, PCV = packed cell volume, MCH= mean cell hemoglobin, MCV = mean cell volume, MCHC = mean cell hemoglobin concentration, WBC = total leukocyte count, neutro. seg. = segmented neutrophils, lympho. = lymphocytes. Data are expressed as mean ± standard deviation; *P < 0.05, **P < 0.01, ***P < 0.001: a = significant differences between non pregnant red and fallow deer females; b = significant differences between pregnant red and fallow deer females; c = significant differences between pregnant and non-pregnant red deer females; d = significant differences between pregnant and non-pregnant fallow deer females. Data for segmented neutrophils, lymphocytes, and eosinophils are also expressed as minimum and maximum relative differential count.

Hb and Ht level than fallow deer does. Calculated red blood cells parameters showed higher mean cell volume (MCV) and mean cell hemoglobin (MCH) in red deer hinds, pregnant and non-pregnant, than fallow deer does, but pregnant fallow deer does had lower MCV value than non-pregnant ones, whereas pregnant red deer hinds had slightly higher MCV than non-pregnant hinds. Among pregnant females, pregnant fallow deer does had higher mean cell hemoglobin concentration (MCHC) than red deer hinds, and further pregnant fallow deer does had higher MCHC than non-pregnant does. Total leukocyte count (WBC) was the highest in non-pregnant red deer hinds, while in pregnant hinds reduced WBC count was determined. Fallow deer does had lower WBC than red deer hinds, with the lowest WBC value in non-pregnant fallow deer females. The higher neutrophile and lymphocyte count was detected by the differential white blood cells count in red deer hinds than fallow deer does. There were no differences between pregnant and non-pregnant females regarding neutrophile count, but pregnant hinds had lower lymphocyte count than non-pregnant ones and conversely, pregnant does had more than twice as high as lymphocyte count in their blood than non-pregnant animals. Moreover, pregnant fallow deer does had exceptionally high eosinophile count in the blood (Table 2).

Discussion

Several biochemical parameters were chosen to complete the results of previous research conducted on red and fallow deer females. The total serum proteins (TP) concentration of

both species groups in the study ranged over similar values and mainly was in the range of the previously published data. However, in farmed red deer hinds a higher upper protein range was observed (Knox et al. 1988, Zomborszky et al. 1996). On the other hand, Kent et al. (1980) noticed lower serum protein values in red deer hinds, and Chapman & Chapman (1980) in fallow deer does, but the blood samples in both studies were taken during the winter months. During our research the blood was collected over the spring and possibly higher TP values in blood were detected as a result of the abundant fresh spring forage. Kolb et al. (1995) also found higher TP and urea values in May as a result of an increased protein intake. Comparison of the albumin concentrations revealed significantly higher values in the red deer hinds than in the fallow deer group, but in the pregnant animals, significantly higher albumin concentration was observed in fallow deer does.

Furthermore, the differences in urea concentration between the particular female groups in our study correspond to albumin concentration differences. The urea concentration significantly increased in the pregnant fallow deer whereas in pregnant red deer hinds the urea concentration decreased in relation to non-pregnant hinds. The level of rumen degradable proteins (RDP) and the ratio of RDP/RUP (rumen undegradable protein) in the diet respectively may affect the blood urea values (Maréjka et al. 2004). Moreover, the water supply might have been insufficient. There is an evidence of an increase in blood TP, albumin and urea concentration as the pregnancy progress (El-Sherif & Assad 2001). Rodríguez et al. (1996) found that glomerular filtration and urea clearance were significantly reduced during late pregnancy. It may also cause the rise of urea concentration in pregnant fallow deer does used in our research. Furthermore, the creatinine concentration was significantly higher in pregnant than in non-pregnant females. El-Sherif & Assad (2001) also revealed an increase in blood creatinine concentration during the gestation of Barki sheep. Creatinine is formed from creatine or creatine phosphate, and creatine appears in the urine after break down of muscle during pregnancy and in the case of hyperthyroidism (Beitz 2004). Thyroid activity is known to be higher in the pregnant females causing the catabolism of body proteins (Jainudeen & Hafez 1994). Accordingly, in our research an increase of urea and creatinine concentration in pregnant animals could have been a consequence of the higher protein demands in late pregnancy which could have led to reduced ability of kidneys to eliminate excess urea and creatinine from plasma.

Moreover, the measured transaminase activity was higher in pregnant red and fallow deer females than non-pregnant ones. The higher activity of transaminases might indicate impairment in muscle (Cory cycle) and liver cells due to rapid gluconeogenesis associated with pregnancy. Beitz (2004) stated the effect of adrenal corticoids on mobilization of amino acids from body proteins during pregnancy, which is associated with an increased rate of hepatic deamination.

Opposite to the rise in albumin and urea concentration, significantly lower glucose concentration in pregnant fallow deer does was observed. The glucose concentration in fallow deer hinds was similar to those from other studies on the same species (English & Lephord 1981, Kolb et al. 1995). However, the glucose concentration in red deer hinds was higher than reported by Knox et al. (1988) and Marco & Lavín (1999). The glucose concentration might depend on degree of sedation. Some authors described an increase in glucose concentrations in reindeer immobilized by the use of adrenergic alpha-2 anesthetics, which inhibit the release of insulin and increase glucose output from the liver (Soveri et al. 1999). Perhaps the higher amount of xylazine injected to the red deer females because of their larger body mass might result in hyperglycemia. Interestingly,

the glucose concentration in pregnant red deer hinds was significantly higher than in non-pregnant hinds, while in pregnant fallow deer does concentration of glucose was more than 50% lower than in non-pregnant specimens. Khan & Ludri (2002) also found significantly higher concentrations of glucose in non-pregnant goats than in pregnant ones with a tendency to decline in the group of the pregnant animals towards the end of gestation. Schluhmbohmer et al. (1997) proved that insulin responsiveness was significantly reduced in sheep during late pregnancy, which led to decreased glucose turnover and uptake by muscle and fat tissues. During the pregnancy the output of adrenocorticotropic hormones, glucocorticoids and adrenaline for breakdown of liver glycogen is amplified. The released hormones act in mobilizing amino acids from body proteins and the concomitant conversion of α -ketoacids to glucose (Beitz 2004).

Comparing our results with results of Marco & Lavín (1999) on the same deer species, a higher triacylglyceride concentration were detected in the present study. On the other hands, the triacylglyceride concentration of fallow deer females in this study were lower than in the fallow deer females from the Mediterranean part of Croatia (Poljičak-Milas et al. 2004). We could presume that differences in triacylglyceride values in the blood of investigated females, compared to other relevant studies, were partly nutritionally related. Contrary to the triacylglyceride concentration, which was higher in red deer females, higher cholesterol concentrations were established in the fallow deer females. The measured cholesterol concentration in red deer hinds was very similar to the results of Knox et al. (1988) and Marco & Lavín (1999), as well as cholesterol level in the sera of female fallow deer does corresponded well with the literature data for adult fallow deer (Dhindsa et al. 1975). Additionally, the cholesterol concentrations were significantly higher in pregnant females than in non-pregnant group of animals in both deer species. Significantly higher cholesterol concentrations were also reported in pregnant cows supposedly to sustain pregnancy (Kanecko 1980). That might also have been important to maintain the pregnancy of deer females in our study.

Regarding hematological parameters, the red blood cell number (RBC) in red deer hinds corresponded to other reported data for sedated free ranging hinds (Haigh & Hudson 1993, Marco & Lavín 1999). However, Zomborszky et al. (1996) and Peinado et al. (1999) reported lower RBC count in sedated, farmed red deer females. The measured hematological parameters in fallow deer does agree with the values of the free ranging sedated fallow deer, presented by Janicki et al. (2000). Likewise in red deer females, the results from other studies on the sedated farmed fallow does have shown lower mean RBC values (Peinado et al. 1999). Cross et al. (1988) stated that spleen is contracted due to adrenaline release when manual restraint or a crush is used. The fear and excitement, and consequently higher adrenalin release and spleen contraction at time of sedation, could have an impact in both species groups on total RBC. In comparison with non-pregnant females, pregnant red deer hinds and fallow deer does had decreased RBC, hemoglobin and hematocrit values. Similar results have been observed in pregnant mares, sows, sheep and bitches (Jain 1993). The results during the last stage of gestation could have been attributed to the hemodilution effect resulting from an increase in plasma volume. This phenomenon was also observed in pregnant Danish landrace goats (Mbasaa & Poulsen 1991) and Baladi goats during late pregnancy (Azab & Abdel-Maksoud 1999). The observed hemodilution in late pregnancy of domestic animals may have a physiological importance as it reduces the blood viscosity, thereby greatly increasing the blood flow in the small blood vessels (Guyton & Hall 1996). Thus, hemodilution

may improve the blood flow through placental blood vessels, especially in late pregnancy to increase the transfer of nutrients and oxygen to the fetus.

In the blood of pregnant females the RBC, hematocrit and total hemoglobin decreased, but the calculated blood parameters, mean cell hemoglobin (MCH) in red deer hinds, and mean cell hemoglobin concentration (MCHC) in fallow deer does increased. Similarly, M b a s s a & P o u l s e n (1991) observed that MCH and MCHC increased during late pregnancy of Danish landrace goats, and A z a b & A b d e l - M a k s o u d (1999) detected elevated MCH in Baladi goats. This could prevent a marked decrease in total oxygen carrying capacity of circulating blood.

Red deer hinds had higher total leukocyte count (WBC) than fallow deer does. Moreover, detected WBC count in red deer hinds was higher in comparison with data reported in previous studies (H a i g h & H u d s o n 1993, M a r c o & L a v í n 1999). Present results of WBC count for red deer females were more similar to reference values in domesticated ruminants (F e l d m a n et al. 2000). Z o m b o r s z k y (1996) had also detected high WBC count in red and fallow deer females, especially during lactation period. Present results of WBC count in fallow deer does showed lower values than in the mentioned investigation, closely related to the results obtained by K o v á č et al. (1997) and J a n i c k i et al. (2000). Clinical examinations of females did not show any signs of illness, and moreover red deer hinds were in a good body condition. Total white blood cell count and especially differential neutrophile and lymphocyte count in deer species shows a marked variability dependent on numerous physiological factors and animal handling. C r o s s et al. (1988) observed that sedation with xylazine hydrochloride significantly lowered the values of RBC count, Hb concentration and plasma viscosity, but elevated the lymphocyte count. On the other hand, catecholamines can cause a transient leukocytosis with neutrophilia, by shifting cells from marginal pool into circulating pool (J a i n 1993). The fear and excitement, and consequently higher adrenalin release at the time of sedation, as well as used sedative solution could have had an impact in both species groups on total and differential WBC count. There were no differences between pregnant and non-pregnant females regarding neutrophile count, but pregnant fallow deer does had more than twice lymphocyte count than non-pregnant ones, and exceptionally high eosinophile count in their blood that might have been related to endoparasite infestation.

When summarizing the presented results, recorded differences of biochemical values between pregnant and non-pregnant females: glucose, cholesterol and creatinine concentration, as well as ALT activity, indicate intensive metabolic activity related to increased demands of growing fetal tissue. At the same time, the investigated pregnant fallow deer does were in weaker body condition. Therefore they should activated physiological mechanisms in terms of lipid and protein mobilization to ensure higher energy demands associated with the progression of pregnancy and therefore more susceptible to endoparasite infestation, which was documented with higher lymphocyte and especially eosinophile count.

A c k n o w l e d g e m e n t s

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