Blood plasma proteins and protein fractions in roe deer *Capreolus capreolus* L.

Białka oraz frakcje proteinowe osocza krwi sarny europejskiej *Capreolus capreolus* L.

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Abstract

The aim of the research was to investigate some selected biochemical blood parameters in roe deer (*Capreolus capreolus* L.). The experiment covered 15 from 2 to 3-year-old bucks from Kuyavian-Pomeranian Voivodeship. The animals were shot by individual hunters on the shooting grounds during the hunting season of 2008/2009 (in the accordance with the Journal of Laws No 48).
The material for the research was blood plasma obtained after centrifuging full, nonhemolyzed blood. The blood was collected from the zygomatic vein directly to the test tubes with EDTA and transported in cooling conditions to the laboratory. After transporting the samples of blood to a certified analytical laboratory, the following elements of the obtained blood plasma were examined: ceruloplasmin – using turbidimetric method; transferrin – using immunoturbimetric method; troponin- using a third generation assay on an Elecsys; total protein, albumin, globulin – using spectrophotometric method and total iron – using colorimetric method. The results were statistically analyzed, i.e. the correlation between the parameters was measured by means of Pearson's correlation coefficient. The analysis of the results revealed a number of statistically significant relations between the parameters under the investigation, especially among the compounds directly responsible for metabolism of iron and copper. A statistically important positive correlation was observed between ceruloplasmin and ferritin ($r = 0.563$; $P \leq 0.05$) and a negative one between transferrin and troponin ($r = -0.609$; $P \leq 0.05$). Moreover, the content of transferrin – an iron-binding protein – was 0.17 g/l, while the concentration of iron was 58 µmol/l. The content of ceruloplasmin – a protein responsible for metabolism of copper – was very low (0.036 g/l). The level of proteins in the blood plasma of the animals under the research was approximately 72 g/l, with the share of albumins about 46%. The albumin-globulin ratio was 0.86.

Keywords: biochemical blood parameters, game, roe deer

Streszczenie

Celem pracy było zbadanie wybranych parametrów biochemicznych krwi sarny europejskiej (*Capreolus capreolus* L.). Badaniami objęto 15 (2÷3 letnich) kozłów z regionu kujawsko-pomorskiego. Zwierzęta zostały odstrzelone przez indywidualnych myśliwych polujących w obwodach łowieckich w sezonie łowieckim 2008/2009 (zgodnie z zapisem w Dz. U. Nr 48). Materiał do badań stanowiło osocze krwi uzyskane po odwirowaniu pełnej, niezhemolizowanej krwi. Krew została pobrana z żyły jarzmowej bezpośrednio do probówek z EDTA i w warunkach chłodniczych przewieziona do laboratorium. Po przewiezieniu próbek krwi do certyfikowanego laboratorium analitycznego, w pozyskanym osoczu oznaczono: ceruloplazminę – metodą turbidymetryczną, transferynę – metodą immunoturbidymetryczną, troponinę – przy użyciu testu trzeciej generacji (Elecsys); białko całkowite, albuminy, globuliny – metodą spektrofotometryczną oraz żelazo całkowite – metodą kolorymetryczną. Wyniki opracowano statystycznie tzn. do zbadania współzależności między cechami zastosowano korelację Pearsona. Analiza wyników wykazała wiele istotnych statystycznie zależności pomiędzy badanymi parametrami głównie w obrębie związków bezpośrednio odpowiedzialnych za metabolizm żelaza i miedzi w organizmie. Odnotowano dodatnią istotną statystycznie korelację pomiędzy ceruloplazminą a ferrytyną ($r = 0,563$; $P \leq 0,05$) i ujemną pomiędzy transferyną i troponiną ($r = -0,609$; $P \leq 0,05$). Ponadto zawartość transferryny-białka wiążącego żelazo-kształtowała się na poziomie 0,17 g/l, natomiast poziom żelaza wynosił 58umol/l. Z kolei zawartość ceruloplazminy- białka odpowiedzialnego za metabolizm miedzi-była na bardzo niskim poziomie (0,036 g/l). Poziom protein w osoczu badanych zwierząt wynosił w przybliżeniu 72g/l, z czego albuminy stanowiły około 46%. Natomiast stosunek zawartości albumin do globulin wynosił 0.86.

Słowa kluczowe: biochemiczne parametry krwi, dziczyzna, sarna

Streszczenie szczegółowe

Celem pracy było zbadanie wybranych parametrów biochemicznych krwi sarny europejskiej (*Capreolus capreolus* L.). Badaniami objęto 15 kozłów (2÷3 letnich) z regionu kujawsko-pomorskiego. Zwierzęta zostały odstrzelone przez indywidualnych myśliwych polujących w obwodach łowieckich w sezonie łowieckim 2008/2009 (zgodnie z zapisem w Dz. U. Nr 48). Zwierzęta objęte doświadczeniem nie wykazywały objawów świadczących o toczącym się procesie chorobowym. Materiał do badań stanowiło osocze krwi uzyskane po odwirowaniu pełnej, niezhemolizowanej krwi. Krew została pobrana z żyły jarzmowej bezpośrednio do probówek z EDTA i w warunkach chłodniczych przewieziona do laboratorium. Analizę osocza krwi przeprowadzono w czasie nie dłuższym niż 24 godziny od momentu pobrania. Próby krwi oznaczane były w certyfikowanym laboratorium analitycznym ALAB należącym do 10 Wojskowego Szpitala Klinicznego w Bydgoszczy. Po przewiezieniu próbek krwi do certyfikowanego laboratorium analitycznego, w pozyskanym osoczu oznaczono: ceruloplazminę – metodą turbidymetryczną, transferynę – metodą immunoturbidymetryczną, troponinę – przy użyciu testu trzeciej generacji (Elecsys); białko całkowite, albuminy, globuliny –

metodą spektrofotometryczną oraz żelazo całkowite – metodą kolorymetryczną. Wyniki opracowano statystycznie tzn. do zbadania współzależności między cechami zastosowano korelację Pearsona. Analiza wyników wykazała wiele istotnych statystycznie zależności pomiędzy badanymi parametrami głównie w obrębie związków bezpośrednio odpowiedzialnych za metabolizm żelaza i miedzi w organizmie. Odnotowano dodatnią istotną statystycznie korelację pomiędzy ceruloplazminą a ferrytyną (r = 0,563; P≤0,05) i ujemną pomiędzy transferyną i troponiną (r = -0,609; P≤0,05). Ponadto zawartość transferryny-białka wiążącego żelazo-kształtowała się na poziomie 0,17 g/l, natomiast poziom żelaza wynosił 58umol/l. Z kolei zawartość ceruloplazminy- białka odpowiedzialnego za metabolizm miedzi-była na bardzo niskim poziomie (0,036 g/l). Poziom protein w osoczu badanych zwierząt wynosił w przybliżeniu 72g/l, z czego albuminy stanowiły około 46%. Natomiast stosunek zawartości albumin do globulin wynosił 0.86. Stężenie ferrytyny we krwi sarn kształtowała się na poziomie 0,37 ng/l, zaś zawartość troponiny wynosiła 0,043 ng/l.
Nie ulega wątpliwości, że wszystkie poddane analizie parametry mają znaczący wpływ na ogólny stan zdrowia osobników z gatunku *Capreolus capreolus* L., a co za tym idzie mogą mieć wpływ na preferencje siedliskowe tych zwierząt.

Introduction

Biochemical and hematological analyses, especially in the case of farm animals, allow early diagnosing of diseases, selecting a proper supplementation and diet and help to assess the general condition of the organism's homeostasis. Regular control of such parameters as acute-phase proteins, performed by experienced breeders, enable to notice early symptoms of diseases, parasite infections or other processes in the organism before any external manifestations occur (Noordhuizen 2002; Szymańska-Czerwińska and Bednarek 2007). A correct interpretation of the results of blood test, with consideration of all additional factors such as sex, age, diet, lactation, pregnancy etc., can serve as a useful tool for improving the efficiency of production as well as for minimizing the potential losses (Grove-White 2004). The same research can be implemented in hunting economy and breeding of wild animals. Those analyses, however, cannot serve diagnostic aim when alarming symptoms have already occurred or the pathological changes appear. Constant control enables to notice all disturbances of the organism's homeostasis prior to the disease onset and in consequence, allows to take proper and timely measures, such isolation of the animal from the rest of the herd or applying an adequate supplementation or drugs (Mordak 2008).Thorough biochemical blood tests of farm and wild animals give opportunity of precise assessment of their health. In the case of wild animals, rapid changes to the blood composition can be associated with the contamination of their habitat. Roe deer (*Capreolus capreolus* L.) is a species widespread in Europe and a part of Asia. It is strictly bound to the forest and meadow areas, and as a result – often exposed to the substances commonly used in such places: pesticides, chemical fertilizers and other compounds applied in agriculture (Pielowski 1999).

The aim of the study was to mark the selected biochemical blood parameters in roe deer and investigate the correlations between the parameters under the research.

Materials and methods

The experimental material comprised roe deer (*Capreolus capreolus* L.) from Kuyavian-Pomeranian Voivodeship, shot in the hunting season 2008/2009. The investigations involved 15 from 2 to 3-year-old males roe deer coming from Polish region dominated by food, chemical, electromechanical, timber and paper industries. Agricultural lands constitute 65% of the area. The research animals were hunted in their natural environment. The animals were shot by individual hunters on the shooting grounds within the voivodeship. In the accordance with the Decree of the Ministry of Environmental Protection, Natural Resources, and Forestry from 16 March 2005 (Journal of Laws No 48, item 459), the bucks were shot between 11 May and 30 September. The animals under the investigation did not show any symptoms of diseases. The age of the animals was determined on the basis of their dentition, i.e. on the basis of the extent to which some parts of teeth were developed or worn. Roe deer are herbivores, feeding mostly on green parts of diverse plant species, especially grasses, herbs as well as tree leaves and buds. Due to the fact of obtaining the animals from their natural environment, no control over the quantity and quality of the food intake was possible, thus no chemical analysis of it was performed.

The material for the research was blood plasma obtained after centrifuging full, nonhemolyzed blood. The blood was collected from the zygomatic vein directly to the test tubes with EDTA and transported in cooling conditions to the laboratory.

The analysis of blood plasma was performed no later than 24 hours from collecting the samples. The marking took place in a certified analytical laboratory ALAB at the 10th Military Research Hospital in Bydgoszcz. All tests were performed accordingly to the norms of laboratory proceedings of this institution. In all blood plasma tests the following parameters were marked: ceruloplasmin – using turbidimetric method; transferrin – using immunoturbimetric method; troponin- using a third generation assay on an Elecsys; total protein, albumin, globulin – using spectrophotometric method and total iron – using colorimetric method.

In the case of the parameters meeting the assumptions of normal distribution and of homoscedasticity, the correlation was investigated by means of Pearson's correlation coefficient. The results obtained were processed statistically using Statistica 8.0 (StatSoft USA) software.

Results

Table 1 presents the mean values and the standard deviation of the parameters under the investigation. The concentration of ferritin in blood of roe deer was 0.37 ng/l, while the content of troponin was 0.043 ng/l. Moreover, the content of transferrin – an iron-binding protein – was 0.17 g/l, while the concentration of iron was 58 µmol/l. The content of ceruloplasmin – a protein responsible for metabolism of copper – was very low (0.036 g/l). The level of proteins in the blood plasma of the animals under the research was approximately 72 g/l, with the share of albumins about 46%. The albumin-globulin ratio was 0.86 (Fig.1).

Table 1. The content of selected biochemical blood parameters in roe deer ($\bar{x}$, s)

Tabela 1. Zawartość wybranych parametrów biochemicznych krwi sarny europejskiej ($\bar{x}$, s)

Parameters	roe deer (n=15)	
	$\bar{x}$	s
Ceruloplasmin [g/l]	0.04	0.01
Ferritin [ng/l]	0.37	0.06
Transferrin[g/l]	0.17	0.07
Troponin [ng/l]	0.04	0.01
Serum Iron[umol/l]	58.27	7.78
Total protein [g/l]	72.33	10.68

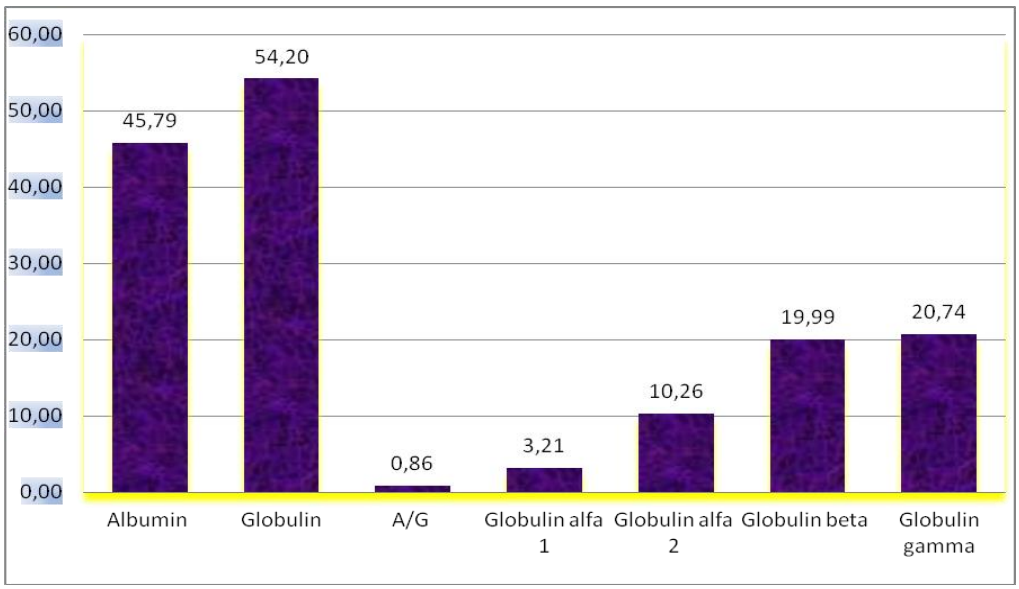

Fig. 1 The content of selected of some roe deer blood plasma proteins [%]

Rys. 1 Zawartość wybranych białek osocza krwi sarny europejskiej [%]

Table 2. Values of Pearson's correlation coefficient (γ_{xy}) for selected biochemical blood parameters in roe deer

Tabela 2. Współczynniki korelacji Pearsona(γ_{xy}) pomiędzy wybranymi parametrami biochemicznymi krwi sarny europejskiej

	Ferritin [ng/l]	Transferrin[g/l]	Troponin [ng/l]	Serum iron[umol/l]	Total protein [g/l]	Albumin [%]	Globulin [%]	A/G [%]	Globulin alfa 1 [%]	Globulin alfa 2 [%]	Globulin beta [%]	Globulin gamma [%]
Ceruloplasmin [g/l]	0.563*	-0.108	0.301	0.342	0.031	-0.244	0.244	-0.224	-0.063	-0.150	0.108	-0.355
Ferritin [ng/l]		-0.049	0.403	0.387	-0.202	-0.218	0.218	-0.301	0.029	0.207	0.228	0.032
Transferrin[g/l]			-0.609*	-0.253	0.291	-0.071	0.071	-0.037	0.120	-0.679*	0.422	-0.008
Troponin [ng/l]				0.152	-0.445	-0.121	0.121	-0.177	-0.083	0.380	0.003	-0.203
Serum iron[umol/l]					-0.004	-0.407	0.407	-0.429	-0.066	0.184	0.270	0.271
Total protein [g/l]						-0.411	0.411	-0.296	0.211	-0.523*	0.131	0.561*
Albumin [%]							-1.000	0.979*	0.227	0.279	-0.742*	-0.543*
Globulin [%]								-0.979*	-0.227	-0.279	0.742*	0.543*
A/G [%]									0.259	0.247	-0.485*	-0.521*
Globulin alfa 1 [%]										-0.122	-0.177	-0.077
Globulin alfa 2 [%]											-0.542*	0.049
Globulin beta [%]												0.286

Correlations marked with (*) are significant at P≤0.05

The study also covered the analysis of correlation between the parameters under the research and the results are presented in Table 2. As it can be noticed on the basis of the obtained values, among the compounds directly responsible for metabolism of iron and copper, a statistically significant positive correlation between ceruloplasmin and ferritin ($r = 0.563$; $P \leq 0.05$) and a negative correlation between transferrin and troponin ($r = -0.609$; $P \leq 0.05$) occurred. Other statistically important correlations are presented in Table 2.

Discussion

Due to the lack of norms concerning the content of the parameters under the investigation in blood of wild living roe deer, the only way to analyze the results obtained in terms of potential pathological changes or other dysfunctions of the individuals was to compare them with the standards used for farm animals (Mordak 2008).

On the basis of the norms developed for cows, in blood of roe deer a substantial deficit of transferrin, an iron-binding protein, was found, as the standard values reach 1.37 g/l – 3.72 g/l. In blood of the animals under the investigation a considerably elevated level of iron was noted, which is fully justified by the large deficit of transferrin, a protein responsible for the element's metabolism. This situation, however, does not necessarily suggest a morbid condition. Taking into account the time of collecting the samples as well as the fact that all the bucks were shot at the beginning of the hunting season, it can be assumed that the disorders in the metabolism of iron can be an effect of impoverishment of the organism after winter. Moreover, the elevated content of iron as noted in the own research, according to Roy (1983), has a negative effect on absorption of copper and lowers its reserves in liver. In the research by Lachowski et al. (Lachowski et al. 1994) on the adaptation of cattle to the conditions of insufficient levels of copper, the concentration of ceruloplasmin in dry cows ranged from 0.357 g/l for control group to 0.964 g/l in the case of experimental group. Furthermore, in the results by Iwańska and Strusińska (Iwańska and Strusińska 1994), who analyzed blood from heifers, this protein's content was 0.216 ÷ 0.393 g/l. In both cases the obtained values were almost 10-fold higher than the own results on roe deer. However, it should be taken into account that the levels of this parameter can also be affected by numerous other factors, such as age (Kim et al. 2002; Kim 2008), pregnancy (Louro et al. 2001), diet or living conditions.

As for the rest of the parameters marked within this study, i.e. total protein or other plasma protein fractions, the levels met the norms. For total protein the standard values are 60-80 g/l (Kuleta 2005), while for albumins >30 g/l and for globulins <50 g/l (Whitaker et al. 2005). The obtained values of total protein in the own research were similar to the ones presented by Yokus and Cakir (Yokus & Cakir 2006). The level of proteins in blood plasma of cows in various stages of pregnancy reached an average value of 78.3 g/l, while in heifers it was slightly lower, i.e. 75.4 g/l. Poljicak-Milas et al. (Poljicak-Milas et al. 2004) conducted similar research on fallow deer (*Dama dama* L.), in order to investigate how the biochemical composition of blood changes dependently on the habitat (the Briuni islands and the mainland – Croatia) and diet. The results of laboratory tests revealed significant differences in the content of total protein and albumins, which in the animals from the islands were 62.5 g/l and 35.22 g/l, respectively, while in the animals from the mainland they were 71.42 g/l

and 40.71 g/l. The results in the latter group are comparable to the ones obtained in the own research on roe deer. Other researchers obtained similar results for fallow deer (Chapman and Chapman 1980; English and Lepherd 1981; Slavica et al. 2000) and red deer (Haigh and Hudson 1993). Other, in vivo research on blood plasma of wild animals in captivity, were performed by Peinado et al. (Peinado et al. 1999). The experimental material covered numerous species such as: *Cervus axis*, *Cervus dama*, *Cervus elaphus hippelaphus*, *Cervus unicolor*, *Elaphurus davidianus*, *Bison bonasus*, *Boselaphus tragocamelus*, *Ovis musimon*, *Connochaetes taurinus* and *Ammotragus lervia*. In all species under the investigation the level of total protein ranged from 63.8 g/l to 74.5 g/l, which is similar to the values obtained in the own research on roe deer *Capreolus capreolus* L., where they ranged from 68 to 72 g/l. However, the level of albumins in the animals under the research was significantly higher and it oscillated between 76.1% and 55.4%, which affected the albumin to globulin ratio, ranging from 2.8 to 1.3, with superiority of results in the higher part of the range. An important aspect of assessing the condition of the animals is the measurement of the concentration of selected metal ion binding proteins, i.e. ceruloplasmin, transferrin, ferritin or troponin. The results obtained allow to estimate the reserve of the selected elements in the animal's body and in the case of their deficiency – to determine the cause.

The obtained correlations between the proteins reveal the interrelations between the elements. The deficiency of Mg stimulates the secretion of parathormone and in consequence, the release of Ca from bones and the rise of its concentration in blood, which may manifest in an increased level of troponin.

Among others, in the research a negative correlation between A/G ratio and globulin fraction was found (Table 2). The results of numerous studies confirm such relation, i.e. along with the rise in the albumin/globulin ratio the production of globulin fraction drops and the level of albumins rise (Zimmerman et al. 2010). This tendency was also found in the own research, which is confirmed by the positive correlation ($r=0.979$; $P \leq 0.05$) between A/G ratio and the level of albumins (Table 2). Investigating the A/G ratio can be also useful in assessment of the animals' health, as its increased or lowered value may indicate the onset of a disease.

The correlations obtained in the study are a supplement to the basic research.

The presented research on the biochemical composition of blood of roe deer *Capreolus capreolus* L. are one of the few analyses in this field so far. The results of such studies on wild animals, though not easy to perform, can provide many valuable information both on the species itself and its habitat.

Conclusion

In conclusion, animal blood tests are important procedures in terms of controlling their health and the data necessary for their proper interpretation are still scarce. It is however beyond questioning that all parameters analyzed in this study have a great impact on general health conditions of the individuals of *Capreolus capreolus* L. species, and as a result, they may also affect their preferences regarding their habitat.

References

Chapman, D.I., Chapman, N.G., (1980) Some serum constituents of fallow deer (*Dama dama*). Research in Veterinary Science, 29(1), 105–107

English, A.W., Lepherd, E.E. (1981) The haematology and serum biochemistry of wild fallow deer (*Dama dama*) in New South Wales. Journal of Wildlife Diseases,17(2), 289–295

Grove-White, D. (2004) Healthcare in the modern dairy herd. Practice, 26(7), 368-376. DOI:10.1136/inpract.26.7.368

Haigh, J.C., Hudson, R.J. (1993) Farming wapiti and red deer. St. Louis: Mosby-Year Book.

Iwańska, S., Strusińska, D. (1994) Współzależność między zawartością β-karotenu i składników mineralnych w dawce pokarmowej a koncentracją Ca, P, Fe, Zn, Cu w surowicy cieląt. In: Związki mineralne w żywieniu zwierząt, Konferencja naukowa 8-9. IX Poznań, 1994, pp. 99–106.

Kim, C., Park, J., Kim, J., Choi, C., Kim, Y., Chung, Y., Lee, M., Hong, S., Lee, K. (2002) Elevated serum Ceruloplasmin levels in subjects with metabolic syndrome. A population based study. Metabolism, 51(7), 838–842.

Kim, L. (2008) Age-related change in ceruloplasmin content in W/SSM rats. Bulletin of Experimental Biology and Medicine, 146(6), 680–681.

Kuleta, Z. (2005) Choroby cieląt. Olsztyn: Wydawnictwo Uniwersytetu Warmińsko-Mazurskiego.

Lachowski, A., Gehrke, M., Węsierski, K., Kuczyńska, I. (1994) Proces adaptacji bydła do środowiska niedoborowego pod względem miedzi. Związki mineralne w żywieniu zwierząt. Konferencja naukowa 8-9 IX 1994, Poznań, pp. 121–125.

Louro, M., Cocho, J., Tutor, J. (2001) Assessment of copper status in pregnancy by means of determining the specific oxidase activity of ceruloplasmin. Clinica Chimica Acta, 312(1–2), 123–127

Mordak, R. (2008) Podstawowe parametry biochemiczne i hematologiczne w monitorowaniu zdrowia bydła. Życie Weterynaryjne, 83, 572–576

Noordhuizen, J.P.T.M. (2002) Veterinary monitoring for herd health, quality control and regulatory purposes. XXII World Buiatrics Congress, Hannover, pp. 52–197

Peinado, V.I., Celdran, J.F., Palomeque, J. (1999) Blood Biochemistry Values in Some Wild Ruminants in Captivity. Comparative Haematology International, 9(4), 175–181

Pielowski, Z. (1999) Sarna. Warszawa: WŚ.

Poljicak-Milas, N., Slavica, A., Janicki, Z., Robić, M., Belić, M., Milinković-Tur, S. (2004) Serum biochemical values in fallow deer (*Dama dama* L.) from different habitats in Croatia. European Journal of Wildlife Research, 50(1), 7–12. DOI: 10.1007/s10344-003-0003-2

Roy, J.H.B. (1983) Problems of calf rearing in connection with their mortality and optimal growth. Livestock Production Science, 10(4), 339–349. DOI:10.1016/0301-6226(83)90018-0

Slavica, A., Janicki, Z., Barić-Rafaj, R., Kolić, E., Manojlović, L., Dezdek, D. (2000) Biochemical blood analysis of the fallow deer (*Dama dama* L.) from the Brijuni islands. Croatia. Veterinarski Arhiv,70, 193–199

Szymańska-Czerwińska, M., Bednarek, D. (2007) Białka ostrej fazy i ich znaczenie w ocenie dobrostanu zwierząt. Życie Weterynaryjne. 82, 1002–1005

Whitaker, D.A., Macrae, A.I., Burrough, E. (2005) Nutrition, fertility and dairy herd productivity. Cattle Practice, 13, 27–32

Yokus, B., Cakir, U. (2006) Seasonal and physiological variations in serum chemistry and mineral concentrations in cattle. Biological Trace Element Research.109(3), 255–266. DOI: 10.1385/BTER:109:3:255

Zimmerman, K.L., Moore, D.M., Smith, S.A. (2010) Species specific hematology. Schalm's veterinary hematology, 6th ed., Ames, Iowa: Wiley-Blackwell, 852-917