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Biochemical blood profile of red deer (*Cervus elaphus*), reared in the conditions of Polissya Volyn

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HR: Conceptualization; Project management; Methodology; Formal analysis; Research; Data curation; Writing — original draft, review & editing; Visualization.

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It was found that the biochemical parameters of blood of red deer (*Cervus elaphus*) reared in the conditions of Polissya Volyn, in general, correspond to the main patterns characteristic of this population, although they have some fluctuations. Thus, we have shown that the content of total protein, its fractional composition, concentration of glucose, urea and creatinine in the blood serum of English deer blood line and Latvian crossbreed deer were within the reference values characteristic of this species of wildlife. It was found that the activity of ALT and GGT was higher in animals of English origin, and AST and ALP — in Latvian crossbred deer. The content of Ca, P, Mg and Na ions in the blood was also higher, respectively, by 9.4, 28, 22.7 and 4%. It was found that against the background of stress caused by physical method of capturing animals for blood sampling, the level of total protein and its albumin fraction. The concentration of urea was by 22.5%, glucose — by 18.4% and creatinine by 6.5% higher than in the group of animals with sedation. Under technological stress in deer, the activity of ALT and AST in the blood increases (by 11 and 5.5%) and ALP and GGT (by 9.1 and 20.0%), respectively. The study of blood biochemical parameters in young animals (3–4 months old) revealed that the content of total protein in females is 6.4% higher than in males. At the same time, the activity of AST activity in the blood serum of males was 17.4% higher than in young females. The concentration of Ca ions in males was 32.4% higher than in females, and concentration of P, on the contrary, was lower in males. The ratio of Ca to P in the blood of males was 1.57, and in females — 1.30. It was found that the content of total protein in animals increased with age and was the highest in adult red deer. While the concentration of the studied macronutrients (Ca, P, Na, K) had a higher level in the blood of young animals. While the concentration of the studied macronutrients (Ca, P, Na, K) had a higher level in the blood of young animals.

Key words: deer, stress, blood, biochemical parameters

Introduction

The intensification of hunting management in Ukraine was conditional nature even before the full-scale invasion of the Russian Federation. Unfortunately, in terms of the level of development of the hunting industry, we were inferior even to the countries of Eastern Europe [12]. Among the main problems that hinder the sustainable development of the industry are: low and ineffective level of immunization of wild animals; death of animals due to the spread of dangerous Infectious diseases; deterioration of the habitat of game animals as a result of climate change and negative anthropogenic activities; use of pesticides and use of modern mechanisms in agriculture in violation of environmental requirements; insufficient environmental requirements; insufficient scientific and methodological support. Today,

as a result of military aggression, there are also significant losses of biodiversity and destruction of natural habitats of wildlife species, pollution of territories by explosive ordnance, violation of natural ecosystems, soils, water bodies and water bodies and water management systems [10]. Therefore, for the sustainable and efficient use of hunting fauna requires the development and application of a number of urgent and promising measures for the conservation, reproduction and maintenance of favorable condition of biodiversity of natural ecosystems, adaptation to climate change and pollution, which leads to the degradation of natural biocenoses. An important aspect of this action plan is to ensure the restoration and of such an action plan is to ensure the restoration and maintenance of wildlife populations in accordance with the indicators of their optimal number and sex and age structure of wildlife populations. And, in particular, the breeding of wild game ungulates, including red deer, in semi-free conditions, taking into account the results of in-depth study of biology, ethology and ecology of animals to improve their comfort zone [5].

The physiological processes and functions of an animal are constantly influenced by environmental factors. The animal's body and blood, in particular, react in a special way react to each environmental factor in a special way [4, 13, 14]. Knowledge of the range of reference values of blood parameters and their determination for individual species and their determination for individual game species is key to monitoring individual and herd health as part of the overall environmental monitoring. The indicators characterizing the state of wild populations of animals and red deer, in particular, may be influenced by the habitat, housing conditions, breed, sex, age, physical activity, diseases, stress, etc [14, 17].

The method of animal immobilization is also important [2, 11, 15]. It is believed that the very stress to which deer are exposed during blood sampling can significantly affect the can significantly affect the results obtained [3]. At the same time, there are few reports in the scientific literature, there are few reports on the reference biochemical parameters of blood of red deer, as well as the effects of anesthesia or muscle relaxation. The published data differ significantly [1]. Most of the described indicators are obtained from animals, when tranquilizers were used.

The aim of this stage of work was to find out the impact of blood sampling methods on red deer for the study of biochemical parameters against the background of different conditions of animal husbandry, their age, sex and population type.

Materials and Methods

The experiment was conducted in the conditions of the farm "Amila", which is geographically located in Western Polissya (Radovychi village, Kovel district, Volyn region) and specializing in the production of region) and specializes in red deer breeding since 2017. The farm breeds deer of three populations (English, Hungarian and Eastern European) and two crossbreeds (Latvian and Lithuanian). The system of red deer rearing in the farm conditions involves a whole range of biotechnical, zootechnical and veterinary sanitary measures [9, 16, 18]. It usually includes, in addition to assessing the general condition of the of the animal, determination of its body weight (at a certain stage of development), coprological examination of feces for the presence of helminth eggs or larvae and selective blood tests for morphological and biochemical parameters.

To perform the experimental part of the work 7 groups of deer (n=10) were selected. In particular, groups D1–D3 were formed from females of the English population, aged 2–3 years (enclosed — the area of the fenced watering from watering troughs; existing feeding systems and provision of agrotechnical and veterinary measures). Deer of experimental groups D1 and D2 were subjected to prophylactic deworming with *Ivomec*®, at the rate of 1 ml per animal, subcutaneously. Animals of group D3 were not dewormed. Animals in groups D4 (English population) and D5 (Latvian crossbreed) were also 2–3 years old. Groups D6 (males) and D7(females) — fawns of the English population of 3–4 months of age, which were kept in pens with females (mothers) in fenced areas.

We used two methods of contact with deer to collect blood samples. Thus, from deer D1 and D2 groups and young animals of D6 and D7 groups, blood for research was collected after driving them through the appropriate system of mazes from the transition corridors and fixing them in a special box. It is worth noting that with this form of capture wild animals are subjected to technological stress, which is likely to have an impact on the results obtained.

Animals of D3, D4 and D5 of the experimental groups, which were kept in a semi-free were subjected to a medical method of immobilization. To create the effect of sedation and muscle relaxation and 30-minute immobilization of deer, we used the xylazine-containing drug *Xyla* (Interchemie Verken "De Adeyaar" Esti AS, Estonia). It was administered to animals using a special DAN inject gun in a dose of 1 ml per 100 kilo. The time from drug administration to the start of blood sampling was 30–45 min.

Blood for the study was taken from deer by puncture of the jugular vein. Blood was collected in separate vacuum tubes "Vacutest" (red lid, with silicon dioxide as an activator, using a double-sided needle "Voles"). Were taken 10 ml of blood to obtain serum. Biochemical analysis of serum was performed using a *Humalyzer 3000* analyzer and standard certified test kits "Human Diagnostics Worldwide" (Germany).

Results and Discussion

During the study of biochemical parameters of red deer blood, we confirmed the data of many scientists that they are relatively stable constants, characteristic of individual population subfamilies, may vary depending on geographical habitat, age, sex, physiological condition, type and nature of feeding (feeding), time of year [4, 13].

It has been established that they are largely subject to fluctuations depending on the specifics of blood sampling from animals. Stress factors usually disrupt the established homeostasis in this species of wildlife and can provoke numerous disorders, including slower growth rates, metabolic disorders, immunodeficiency's, and often cause various diseases [8].

The main stress factors in livestock production, including among deer when rearing them both in free (aviary) and semi-free conditions (farm), are considered to be changes in feeding regime, regrouping, introduction, climatic and temperature factors etc.

In the course of our research, we found that against the background of a stressful factor (running animals through long mazes to a stationary fixation box) in the blood serum of deer of the first experimental group (D1) increased the content of total protein (P<0.05) and its albumin fraction (P<0.05), compared to similar indicators of animals of the third experimental group, which were immobilized with a xylazine-containing preparation (table 1).

According to some researchers [1], this trend is apparently the result of the following the use of sedatives can change the permeability of capillaries and cause, to a certain extent, hemodynamic to a certain extent, hemodilution, loss of proteins and especially albumin.

As a result of experimental studies, we noted that the concentration of urea in the blood serum of deer of group D1 was higher than that of group D3 by 1.4 mmol/L or 22.5%. It is important that the data characterizing the content of the studied substance in deer of group D1 slightly exceeded the reference values for this species of wildlife. It is known that the concentration of urea in the blood of animals can be subject to significant fluctuations and depends on the time of year and the structure of the probable diet. The observed increase in the level of urea in the blood serum of deer (group D1 and D2) is likely to be the result of catabolic processes that occurred during animal feeding, concentrated feed mixtures on the farm. In our studies, the level of urea in the blood of deer subjected to chemical immobilization was slightly lower than that of the physical method of capturing animals.

Similar changes were observed in the concentration of glucose in the blood serum of deer. Its content is entirely related to animal feeding, but other factors, such as stress. We have found that the glucose concentration in the blood of D3 deer which were reared in farm conditions, under *Xyla* sedation was 18.4% (P<0.05) lower than that of animals of group D1. Some scientists who have studied such patterns tend to believe that the depression of the central and efferent nervous system, against the background of the use of adrenergic drugs, may be accompanied by the release of insulin and the glucose output from the liver [1, 14].

We have found that the level of creatinine in the blood serum of deer, against the background of technological stress associated with intense movement and muscle exertion, tended to increase (by 6.5%) in animals of group D1 compared to the analogues of group D3. In this series of experiments, we also analyzed the biochemical parameters of deer blood against the background of preventive deworming. According to the results of the spring (2021) parasitological examination of red deer feces of different ecotypes, mainly eggs of strongyles of digestive tract infections (*Trichostrongylus axei*, *Ostertagia ostertagia*, *Cooperia ancophora*, *Chabertia ovina*) and larvae of strongyles of respiratory tract infections (*Muellerius capillaris*), which are also characteristic of other ungulate species, were found [6, 7]. According to the experimental scheme, deer of group D1 were treated with an anthelmintic, while group D2 was not. The method of catching animals for blood sampling was the same. The deer were driven to a stationary box and biomaterial was obtained during fixation.

Table 1. Dynamics of some biochemical parameters of blood serum of red deer under different methods of their immobilization (M±m, n=10)

Parameter	Reference values	Animal group		
		D1	D2	D3
Protein total, g/L	63.6–66.3	68.2±2.7	62.8±4.1[11]	65.7±7.8*
Albumin, g/L	35.7–37.5	37.9±1.4	33.9±3.0[11]	35.4±4.0*
Globulins, g/L	27.2–29.5	30.3±3.3	28.9±2.6	30.3±3.4
α-globulin, g/L	10.1–10.9	10.5±0.9	11.9±0.7	11.2±0.3
β-globulin, g/L	4.8–5.7	5.6±0.6	4.6±0.4	5.9±0.8
γ-globulin, g/L	11.8–13.5	14.2±1.0	12.4±0.9	13.2±0.5
A/G ratio	—	1.25±0.08	1.17±0.09	1.17±0.07
Glucose, mmol/L	8.5–9.7	11.3±0.6	10.3±0.6	9.2±0.5*
Urea, mmol/L	5.1–6.0	7.6±0.44	7.7±0.9	6.2±0.5
Creatinine, μmol/L	147.6–159.4	174.3±6.5	184.4±11.4[11]	163.6±14.4**

Note. Reference values are given according to O. Rosef et al. for animals immobilized with a neuroplegic agent. In this and other tables: * — P<0.05; ** — P<0.01 (D3 vs. D1); [1] — P<0.05; [11] — P<0.01 (D2 vs. D1)

As a result of the studies (table 1), it was found that in non-dewormed deer of group D2 against the background of an apparently chronic development of the pathological process caused by helminths, the protein synthesizing function was suppressed. The content of total protein in the blood serum of animals of this group was 8% lower (P<0.05) than in animals treated with *Ivomek*. At the same time, the observed decrease in total protein was mainly due to a decrease in its albumin fraction (P<0,01). The concentration of glucose in the blood of non-dewormed animals tended to decreased, and the creatinine level increased slightly.

It is well known that the activity of certain enzymes is an indicator of stress in animals. We found that the activity of ALT, AST, ALP, and GGT had significant deviations in blood obtained from animals subjected to different immobilization methods (table 2). Thus, in deer under chemical sedation with *Xyla*, the activity of transaminases (ALT and AST) was lower by 11.0 and 5.5% (P<0.01; P<0.05) than in animals subjected to exhaustive running to the fixation box through the corridor system.

A similar trend was characteristic of alkaline phosphatase (ALP) and gamma glutamyltransferase (GGT). Against the background of technological stress in the blood of deer of group D1 the concentration of these enzymes exceeded that of animals of group D3 by 9.1 and 20.0% (P<0.05). It was noted that the studied indicators of enzyme activity in animals of group D3 (under chemical sedation) were within the limit values determined for this group of adult wild animals. As for the indicators of animals of group D1 animals that went beyond average values, we agree with the opinion of some authors [3, 8], that the reference constants of biochemical digital expressions have significant differences in deer with different methods of capturing animals (physical fixation or anesthesia) and require additional research.

The electrolyte balance of blood and tissues in the body of deer is extremely important, as it ensures appropriate osmosis, metabolic processes, bone growth and is the basis of the body's homeostasis. For this species of animal, it is also peculiar in that it promotes the growth of horns (antlers). We studied the concentration of Calcium, Phosphorus, Magnesium, Sodium and Potassium ions in the blood serum (table 3). It was found that their levels were within the within the reference values determined for red deer. At the same time, there were no significant deviations in animals subjected to different methods of immobilization were not noted.

According to many scientists, the course of metabolic processes in the body of deer depends on many factors. We have previously described the results of research on deer body weight depending on the population. It was shown that the difference in body weight between animals of the same sex and age was up to 36 kg. In particular, the animals of the Latvian crossbreed were not only heavier in weight compared to the English population, but also taller, and more massive. The males had more horny processes etc.

In our opinion, it was interesting to find out the biochemical parameters of blood in deer of different lines that were raised on a farm in the Western Polissya of Ukraine. For this purpose, blood of animals from two populations: English and Latvian crossbreeds were studied (table 4).

Table 2. The activity of individual enzymes of red deer blood serum under physical and chemical method of their immobilization (M±m, n=10)

Parameter	Reference values	Animal group		
		D1	D2	D3
ALT U/L	51.7–56.7	60.2±3.7	63.4±6.2	53.6±4.0**
AST U/L	55.0–63.3	66.9±4.0	74.4±5.2[1]	63.2±6.6
de Ritis ratio (ALT/AST)	1.06–1.12	1.11±0.08	1.17±0.06	1.18±0.1
ALP U/L	195.5–252.1	240.4±16.8	264.8±9.8	220.4±10.4*
GGT U/L	18.4–22.4	24.0±0.8	30.2±1.1	20.0±2.2

Table 3. Concentration of macronutrients in the blood serum of red deer (M±m, n=10)

Parameter	Reference values	Animal group		
		D1	D2	D3
Ca, mmol/L	1.89–1.99	2.12±0.18	2.05±0.34	1.90±0.40
P (inorganic), mmol/L	1.12–1.36	1.44±0.1	1.36±0.1	1.32±0.3
Mg, mmol/L	0.46–0.50	0.54±0.06	0.52±0.04	0.54±0.06
Na, mmol/L	136.7–142.2	141.7±4.8	140.4±10.2	136.6±8.8
K, mmol/L	5.5–6.3	6.4±0.64	6.4±0.4	6.1±0.8

Table 4. Biochemical parameters of blood serum of red deer of the English population and Latvian crossbreeds (M±m, n=10)

Parameter	Reference values	Animal group	
		D4	D5
Protein total, g/L	63.6–66.3	64.4±2.8	66.6±6.6
Albumin, g/L	35.7–37.5	36.1±4.2	34.2±3.8
Globulins, g/L	27.2–29.5	26.3±2.2	32.4±4.0
α-globulin, g/L	10.1–10.9	10.2±0.8	12.9±0.6
β-globulin, g/L	4.8–5.7	4.4±0.4	5.6±0.4
γ-globulin, g/L	11.8–13.5	11.7±0.5	19.9±0.6
A/G ratio	1.31–1.27	1.37±0.08	1.06±0.08
Glucose, mmol/L	8.5–9.7	8.8±0.6	9.2±0.2
Urea, mmol/L	5.1–6.0	5.6±0.2	5.8±0.4
Creatinine, μmol/L	147.6–159.4	154.4±8.2	162.4±6.0

Table 5. Serum enzyme activity of red deer from different populations (M±m, n=5)

Parameter	Reference values	Animal group	
		D4	D5
ALT U/L	51.7–56.7	64.1±3.6	60.2±4.4
AST U/L	55.0–63.3	68.2±6.8	69.4±7.0
de Ritis ratio (ALT/AST)	1.06–1.12	1.06	1.15
ALP U/L	195.5–252.1	240.4±14.6	264.2±10.8
GGT U/L	18.4–22.4	22.8±1.4	20.2±0.8

Table 6. Electrolyte composition of blood serum of red deer of different origin (M±m, n=5)

Parameter	Reference values	Animal group	
		D4	D5
Ca, mmol/L	1.89–1.99	1.90±0.44	2.08±0.32
P (inorganic), mmol/L	1.12–1.36	1.39±0.3	1.78±0.4*
Mg, mmol/L	0.46–0.50	0.44±0.08	0.54±0.06
Na, mmol/L	136.7–142.2	136.6±18.2	142.0±8.8
K, mmol/L	5.5–6.3	5.8±0.40	5.4±0.34

As a result of the experiment, it was stated that deviations in such indicators such as the content of total protein, its fractional composition, concentration glucose, urea, creatinine were insignificant and generally within the average values, recommended for red deer as reference. The activity of serum enzymes in the blood of deer of two subpopulations are presented in table 5. It was found that the activity of AST and ALP was higher in animals of group D5, and ALT and GGT in deer of group D4, although all obtained results were within the reference range.

The electrolyte composition of the blood serum of deer from the English population and the Latvian crossbreed was slightly different (table 6). It was found that the concentration of calcium ions, Phosphorus, Magnesium and Sodium ions in animals of group D5 (Latvian crossbreed) was comparable to with those of deer of group D5 was 9.4, 28.0, 22.7, and 4.0% higher than in the D4 group.

Tables 7, 8 and 9 show the results of biochemical studies of blood serum of young, 3–4 month old fawns, depending on sex. It was found that the content of protein total protein content in females was 6.4% ($P<0.05$) higher

Table 7. Biochemical parameters of blood serum of young red deer (M±m, n=5)

Parameter	Reference values	Animal group	
		D6	D7
Protein total, g/L	56–70	54.4±4.4	57.9±6.8*
Albumin, g/L	24–33	34.2±2.6	33.6±5.0
Globulins, g/L	—	20.2±1.8	24.3±3.2
α-globulin, g/L	—	10.4±0.6	8.2±0.8
β-globulin, g/L	—	3.2±0.2	6.0±0.4
γ-globulin, g/L	—	6.6±0.6	10.1±0.6
A/G ratio	—	1.69±0.04	1.38±0.06
Glucose, mmol/L	4.7–8.0	5.4±0.6	5.2±0.2
Urea, mmol/L	3.6–7.5	4.2±0.4	4.0±0.4
Creatinine, μmol/L	63–93	112.4±7.2	88.6±6.0*

Note. In this and the following table, reference values are given for fawns without the use of tranquilizers.

Table 8. Serum enzyme activity of young red deer depending on sex (M±m, n=5)

Parameter	Reference values	Animal group	
		D6	D7
ALT U/L	7.0–54.0	46.6±3.0	44.2±4.2
AST U/L	30.0–91.0	40.4±5.2	34.4±5.0**
de Ritis ratio (ALT/AST)	4.29–1.69	0.87±0.02	0.78±0.02
ALP U/L	129–475	312.4±26.0	300.6±14.8
GGT U/L	7–44	40.1±1.4	36.4±3.8

Table 9. Macronutrient composition of blood serum of young males and females of red deer (M±m, n=5)

Parameter	Reference values	Animal group	
		D6	D7
Ca, mmol/L	1.89–1.99	2.86±0.26	2.16±0.44*
P (inorganic), mmol/L	1.12–1.36	1.82±0.4	1.66±0.2*
Mg, mmol/L	0.46–0.50	0.62±0.05	0.56±0.08
Na, mmol/L	136.7–142.2	174.4±12.2	180.2±20.2
K, mmol/L	5.5–6.3	7.8±0.5	7.0±0.6

than in males. At the same time, according to the analysis of fractional composition of protein in the blood of females revealed a higher level of globulins, in particular α-globulins. The determined ratio of albumin to globulins (A/G) was also lower in them. Of the other studied indicators, the value of creatinine concentration in blood serum of young females was significantly lower, by 21.2% (P<0.05).

The activity of the studied enzymes in the blood of young deer was within the reference values. However, it was noted that the activity of AST in the blood of males was by 17.4% (P<0.01) higher than in females.

The analysis of the macronutrient composition of the blood serum revealed that the concentration of calcium ions in males was 32.4% (P<0.05) higher than in females, and Phosphorus, on the contrary, prevailed in males. The ratio of Ca to P in males was at the level of 1.57, and in females — 1.30.

So, summarizing, we conclude that a higher level of proteinin the blood serum of adult deer (table 1), compared to the same indicator in young individuals of this species (table 7) is obviously the result of catabolic changes in their body. We cannot exclude possible anabolic

abnormalities in metabolism in adult animals, against the background of estrogen action during the rut (blood sampling was carried out at the end of September).

We found that in the blood of young deer, compared to adults, the following levels were higher levels of Ca, P, Na, and K ions compared to adults. Authors report similar results in their studies [3]. It is believed that young deer during this period of postnatal development is characterized by high activity of osteoblasts and osteoclasts in tissue remodeling, which leads to an increased need for macro- and microelements.

Comparing the activity of the studied enzymes in adult and young animals it was found that in fawns the activity of GGT and ALP was higher and the activity of AST was lower.

1. The biochemical parameters of deer blood undergo significant fluctuations against the background of technological stress. When taking blood from deer immobilized by the use of sedative *Xyla*, the results obtained are within the reference values determined for this species of wildlife.

2. The results of biochemical studies of blood in red deer did not have significant deviations at both the population and individual levels.

3. According to the results of biochemical studies of blood of non-dewormed deer the probable development of a chronic inflammatory process in animals of this group was established, which confirms the importance of preventive deworming.

4. The biochemical profile of blood of young deer has certain peculiarities and is determined by morphological changes and functional development of their organism in this period of postnatal development.

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Біохімічний профіль крові оленів (*Cervus elaphus*), вирощених в умовах Полісся Волині

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З'ясовано, що біохімічні показники крові благородних оленів (*Cervus elaphus*), вирощених в умовах Полісся Волині, загалом відповідають загальним закономірностям, характерним для цієї популяційної підродини, хоч і мають певні коливання. Ми виявили, що вміст загального протеїну, його фракційний склад, концентрація глюкози, сечовини і креатиніну в сироватці крові оленів англійської популяції та латвійського кросбріду була в межах референтних величин, характерних для цього виду диких тварин. При цьому встановлено, що активність АлАТ і ГГТ була вищою у тварин англійського походження, а АсАТ і ЛФ — в оленів латвійського кросбріду. В останніх був вищим і вміст у крові іонів Ca, P, Mg та Na — відповідно, на 9,4; 28; 22,7 і 4%. Підтверджено дані інших дослідників щодо залежності біохімічних показників крові в оленів від способу знерухомлення. Встановлено, що на тлі стресу, спричиненого фізичним способом відлову тварин для забору крові, в сироватці крові оленів зростає рівень загального протеїну та його альбумінова фракція. Концентрація сечовини була вищою на 22,5%, глюкози — на 18,4% а креатиніну — на 6,5%, ніж у групі тварин за медикаментозної седації. За технологічного стресу в оленів у крові зростає активність АлАТ і АсАТ — на 11 і 5,5%, та ЛФ і ГГТ — на 9,1 і 20,0% відповідно. За дослідження біохімічних показників крові у молодих тварин (3–4 міс.) з'ясовано, що вміст протеїну загального в самочок на 6,4% вищий, ніж у самців. Водночас активність АсАТ у сироватці крові самців була на 17,4% вищою, ніж у молодих самок. Концентрація іонів Ca у самців перевищувала аналогічний показник у самочок на 32,4%, а P — навпаки, була нижчою в самців. Співвідношення Ca до P в крові самців було на рівні 1,57, а в самок — 1,30. Встановлено, що вміст загального протеїну у тварин з віком зростав і був найвищим в дорослих оленів. Концентрація досліджуваних макроелементів (Ca, P, Na, K) мала вищий рівень у крові молодих тварин.

Ключові слова: олені, стрес, кров, біохімічні показники

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