Conference Paper

Clinical and Biochemical Blood Parameters of White-Tailed Deer Under Stress Conditions

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Abstract

White-tailed deer have been introduced into Russia, andthey are exposed to stress factors. The aim of this article was to study the clinical and biochemical blood parameters of white-tailed deer under stress. The experiment was conducted on 24 adult animals. Under stress the content of the red blood cells and hemoglobin increased, and the content of the leukocytes decreased with an increase in the share of segmented neutrophils and a decrease in the share of lymphocytes. In the blood serum, the content of glucose, total bilirubin, creatinine, urea, cholesterol, and enzymes KFK, LDH and GGT increased, and the content of calcium, phosphorus, magnesium and iron decreased.

Keywords: odocoileus virginianus, adaptation, introducers, stress factor, immunosuppression.

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1. Introduction

Reindeer husbandry is a promising agricultural sector. White-tailed deer (Odocoileus virginianus) are of interest as hunting trophies and a source of dietary meat for functional nutrition [1, 2].

For Russia, white-tailed deer are introducers, as their homeland is North and South America [3]. Since 2013, white-tailed deer have been a hunting species in Leningrad region; currently, there are hunting species in other regions of the European part of the Russian Federation (Voronezh, TverskayaSmolenskaya, Nizhny Novgorod, etc.). The introduction of white-tailed deer has increased due to the fact that the number of wild boars has decreased in the European part of the Russian Federation; it is necessary to replace them with other animals that are a hunting resource. White-tailed

deer have a low stress resistance, adapt to new conditions for a long time and poorly react to rearrangement and other stressors, including transportation, strangers or dogs, veterinary procedures, etc. [4–7].

When importing white-tailed deer into the Russian Federation, they are subject to transport stress [3].

Stress affects the body, causing multiple disorders, including deaths. Stress consequences are a decrease in the reproductive ability, a slowdown in growth rates due to starvation, a predisposition to infectious diseases, especially pneumonia due to the suppressed immune system [8, 9].

Since white-tailed deer are introducers, Russian scientific literature does not provide information on their adaptation to new conditions, as well as data on changes in the clinical and biochemical blood parameters under stress.

The aim of the article is to study clinical and biochemical blood parameters of white-tailed deer under stress.

2. Methods and Equipment

The study was conducted on 24 adult white-tailed deer, aged 2 years transported from Poland to Tver region. Transportation took place in winter; as a result of stressors, 16 animals died.

Blood sampling was performed after quarantine procedures. Samples were taken from the jugular vein into plastic tubes for a hematological analysis with the addition of K3EDTA as an anticoagulant. For biochemical studies, 3 ml tubes with gel were used as a coagulant activator. A blood test was performed within 4 hours after taking blood.

A general clinical blood test was performed using an HTI MicroCC-20Plus hematology analyzer (HTI USA) and Clinical Diagnostic Solutions reagents (Russia).

The number of leukocytes, red blood cells, platelets was counted by a unified method in the Goryaev's counting chamber. The leukocyte formula was derived by a unified method. Microscopy of smears of peripheral blood was performed using a phase-contrast light microscope Leica DM 1000 (Austria). Blood smears were stained according to the Romanovsky-Giemsa's method.

Blood for serum production was centrifuged using Armed 80-2 (Russia) at a speed of 3000 rpm for 10 minutes.

A biochemical analysis of blood serum was carried out using an HTI Biohaem SA biochemical analyzer (USA). The following studies were performed with Diacon-DS

reagents: total protein – biuret, albumin – colorimetric, alkaline phosphatase (ALP) – standardized optimized kinetic, α-amylase and LDH – standardized optimized kinetic enzyme, the concentration of total and direct bilirubin was studied by the method of IndrassicGrof, and the concentration of glucose – by enzymatic colorimetric methods.

To determine activity of alanine aminotransferase (AlAT), aspartate aminotransferase (AsAT) in blood serum using the Reitman-Frenkel method, Olveks Diagnostic reagents were used.

The concentration of calcium was studied by the unified colorimetric method, and the concentration of inorganic phosphorus and magnesium – by the unified spectrophoto-metric method.

The data obtained by M. L. Smith (2011), D. D. Waid (1984), S. R. Klinger, (1986) [6, 10, 11] were taken as reference values for the general clinical and biochemical blood parameters of white-tailed deer.

The results were processed using statistical analysis methods in Statistica.

3. Results

The functions of the hematopoietic organs are sensitive to any changes in the body, in particular to stressful effects.

The results of studies of general clinical indicators of blood of white-tailed deer are presented in Table 1.

To identify the effects of transportation stress and its impact on hematopoiesis, the following indicators were studied: the number of red blood cells, the level of hemoglobin and hematocrit and the integrated assessment of red blood cell indices (the color index, MCV, MCH, MCHC).

The average number of red blood cells was 14.31 ± 1.14 x 1012/μl, which exceeds the upper limit. The hemoglobin content was higher – 16.504 ± 1.32 g/l.

The medium erythrocyte volume (MCV) and the mean erythrocyte hemoglobin content (MCH) corresponded to the average reference values. At the same time, the mediun concentration of hemoglobin in the erythrocyte (MCHC) was at the upper limit of the norm and amounted to 38.4 ± 2.69 %, which indicates a high saturation of red blood cells with hemoglobin during stress as a compensatory reaction of the body.

The color indicator of blood was below normal and amounted to 0.39 ± 0.01, which may indicate an iron deficiency in blood serum.

The platelet count was at the lower limit of the reference values.

To analyze the links of non-specific immune reactions, a leukogram was built. The number of leukocytes averaged 3.7 x103/ml, which is lower than the reference values. Catecholamines released during stress can suppress activity of white blood cells, weakening the immune system [3].

Young, stab neutrophils, monocytes and basophils were not detected. Lymphopenia was observed with a significant increase in segmented neutrophils to 70.8 % and a decrease in lymphocytes to 24.9 $\pm$ 0.99 %.

The number of eosinophils was reduced to 2.0 $\pm$ 0.15 %, which is typical for acute stress.

Thus, the results can be interpreted as the stress of the hematopoietic and immune units towards immunosuppression. Against the background of stress-induced immunod-eficiency, activation of latent infections, aggravation of viral infections, and suppression of the specific immune response during vaccination can be observed [3].

TABLE 1: General clinical blood indicators for white-tailed deer

Indicators	Measurement unit	white-tailed deer (n = 24) M$\pm$m	Reference values
Red blood cells (RBC)	x 10^{12}/µl	14.31$\pm$1.14	10.0-13.0
Average volume of red blood cells (MCV)	µm^3	34.41$\pm$1.7	20.0-40.0
Hematocrit (HCT)	%	43.27$\pm$3.89	20.0-50.0
Hemoglobin (HB)	g/l	16.504$\pm$1.32	12.4-15.0
Color indicator	–	0.39$\pm$0.01	0.9-1.1
The medium content of hemoglobin in the red blood cell (MCH)	pkg	12.75$\pm$0.9	8.0-13.0
The medium concentration of hemoglobin in the red blood cell (MCHC)	%	38.4$\pm$2.69	14.0-38.0
White blood cells (WBC)	x 10^3/ml	3.7$\pm$0.26	4.0-8.0
Platelets (PLT)	x 10^3/µl	273.33$\pm$5.73	270.0-500.0
Leukocyte formula:			
Eosinophils (EOS)	%	2.0$\pm$0.15	3.0-8.0
Monocytes (MON)	%	0.5$\pm$0.05	0.0-6.0
Basophils (BAS)	%	0	0 – 0.93
Lymphocytes (LYM)	%	24.9$\pm$0.99	40.0-75.0
Neutrophils:			
Young	%	0	0
Bacillary	%	0	0
Segmental	%	70.8$\pm$4.25	10.0-50.0

Microscopy of blood smears revealed anisocytosis of different levels of severity and species poikilocytosis (drepanocytes). In 41.67 % of the animals, insignificant poikilocytosis (Fig. 1) was observed, in 25 % of the animals moderate poikilocytosis (Fig. 2) and anisocytosis were observed. In 33.33 %, poikilocytosis and anisocytosis were pronounced (Fig. 3).
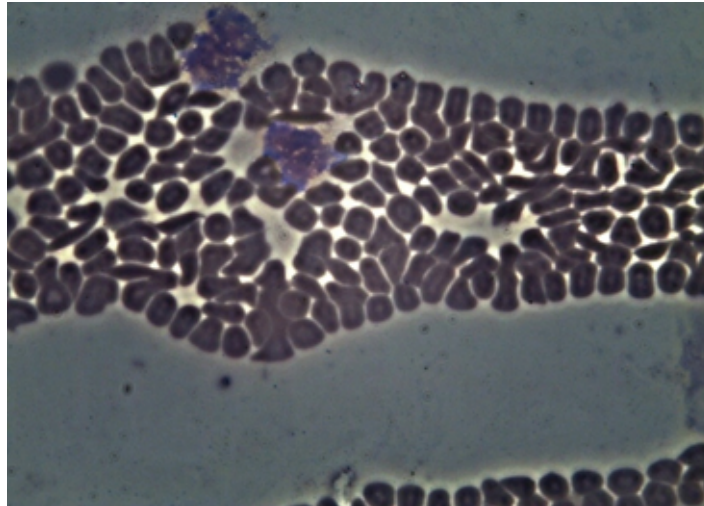

Figure 1: Blood smear of white-tailed deer (x900), Romanovsky-Giemsa stain. Minor poikilocytosis
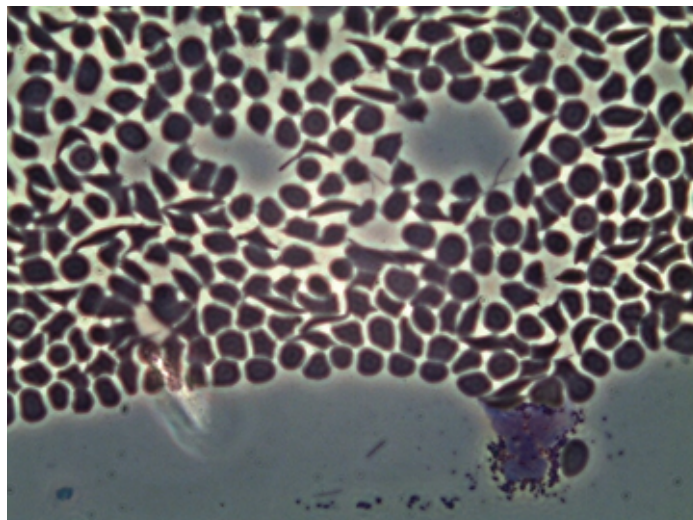

Figure 2: Blood smear of white-tailed deer (x900), Romanovsky-Giemsa stain. Moderate poikilocytosis

To assess the functional state of organs and systems of white-tailed deer after transportation stress, we studied biochemical parameters of blood serum; the research results are presented in Table 2.

The total protein content was within the reference values and averaged 65.0 ± 12.0 g/l.
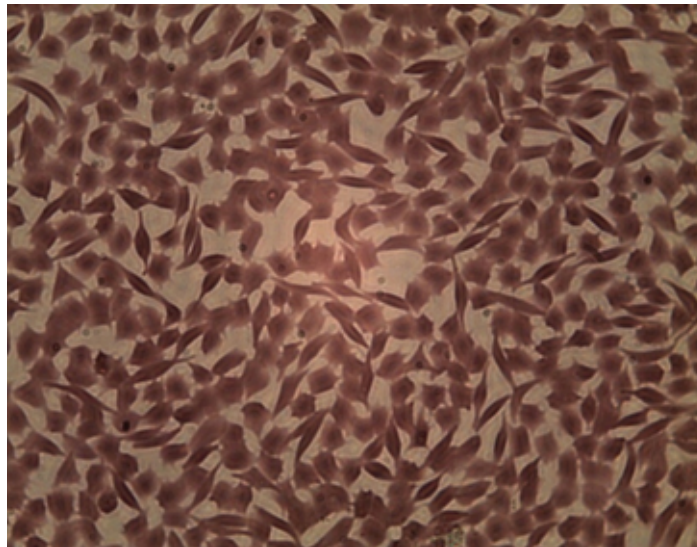
Figure 3: Blood smear of white-tailed deer (x900), Romanovsky-Giemsa stain. Drepanocytes, anisocytosis.

Albumin fractions of protein in serum were 36.0 ± 3.88 g/l, globulin fractions were 35.0 ± 3.08 g/l. Blood albumin is an endogenous reserve of amino acids. It binds hydrophobic compounds which is important to take into account for the post-stress syndrome.

Thus, all indicators characterizing protein metabolism were within the normal range.

Serum glucose was higher than normal and amounted to 7.6 ± 0.67 mmol/L, which can reflect both the state of acute stress caused, and the development of the post-stress syndrome caused by transportation.

The content of total bilirubin amounted to 12.9 ± 0.69 μmol/L. This is probably due to an increased functional load of the liver and endogenous intoxication.

Levels of aspartate aminotransferase (AST), alanyl aminotransferase (ALT), alkaline phosphatase (ALP) were within normal limits. The Ritis coefficient was 6.3 ± 0.49. In scientific literature, there are no data on the Rhysis coefficient of white-tailed deer; when evaluating the results, we relied on the reference range for cattle which is 1.33–2.5. An increase in the Ritis coefficient can indicate the destruction of cardiomyocytes caused by stress and species specificity.

The level of GGT (gamma-glutamyltransferase) was 80.0 ± 3.09 E/L, which exceeds the upper limits of the reference value. This significant increase may be due to destructive disorders of the gastrointestinal tract, pathological processes in the kidneys and the heart muscle.

The level of urea was 11.5 ± 0.35 mmol/L, which slightly exceeds the reference values. The creatinine content exceeded the upper limits of reference values and amounted

TABLE 2: General clinical blood indicators for white-tailed deer

Indicators	Measurement units	white-tailed deer (n = 24) M±m	Reference values
Total protein	g/l	65.0±12.0	60–80
Albumen	g/l	36.0±3.88	30–40
Globulin	g/l	35.0±3.08	32–50
Albumen/Globulin	–	1.0±4.0	0.2–1.5
Glucose	mmol/l	7.6±0.67	2.2–4.5
Bilirubin (TB)	µmol/l	12.9±0.69	0–9
Bilirubin (PR)	µmol/l	3.0±0.22	0–5
Kreatinine	µmol/l	190,0 ± 10,65	76–180
Urea	mmol/l	11.5±0.35	4–11
Calcium	mmol/l	2.8±0.27	6.4–13.5
Phosphorous	mmol/l	1.5±0.14	1.9–26.4
Magnesium	mmol/l	0.9±0.06	1.4–4.6
AST	E/L	125.0±11.3	30–130
ALT	E/L	20.0.±1.89	15–45
AP	E/L	73.0±6.46	50–240
Amylase	E/L	143.0±12.58	140–270
Chlorides	mmol/l	90.0±7.9	100–120
Cholesterol	mmol/l	3.1±0.08	1–2.5
Triglycerides	mmol/l	0.5±0.035	0.22–1.15
KFK	E/L	468.0±4.76	10–200
LDH	E/L	1420.0±36.54	80–470
GGT	E/L	80.0±3.09	20–44
Iron	µmol/l	23.0±1.84	27–40
Ritis coefficient	–	6.3±0.49	–

to 190.0 ± 10.65 mmol/L. Thus, an increase in GGT, urea and creatinine indicate an impaired renal function induced by a stress response.

The content of calcium and phosphorus was reduced and amounted to 2.8 ± 0.27 mmol/L and 1.5 ± 0.14 mmol/L, respectively. The magnesium content corresponded to 0.9 ± 0.06 mmol/L, which is lower than normal.

The results indicate a violation of mineral metabolism.

Maintaining the level of magnesium in the blood plasma refers to strictly controlled physiological processes that reflect the electrolyte balance of the body. Electrolyte imbalance is dangerous, as electrolytes control the heart rate, conducting nerve impulses. Calcium and magnesium are antagonists; the level of calcium is affected by its magnesium content [9].

The level of iron was reduced to 23.0 ± 1.84 µmol/L, which indicates an iron deficiency, which confirms the results of clinical and hematological studies.

The concentration of chloride is slightly lower than the reference values – 90.0 ± 7.9 mmol/L.

A significant increase in the level of LDH (lactate dehydrogenase) to 1420.0 ± 36.54 E/L was revealed. Blood LDH activity is a sensitive indicator of hepatocellular lesions, non-chronic renal pathologies, liver hypoxia, and erythrocyte hemolysis.

The concentration of CPK (creatine phosphokinase) exceeded the reference values. It was 468.0 ± 4.76 E/L. CPK reflects the energy metabolism in the body, since the white-tailed deer were mature; an increase in the level of CPK indicates violations of the cardiovascular system.

The level of cholesterol was 3.1 ± 0.08 mmol/L, which is typical for stress.

Thus, biochemical studies of serum of white-tailed deer showed an increase in glucose, total bilirubin, creatinine, urea, cholesterol and enzymes KFK, LDH, GGT, and the levels of calcium, phosphorus, magnesium and iron were significantly lower than standard values, which indicates the development of multi-organ disorders, including cardiovascular, excretory, digestive systems, mineral and lipid exchanges.

Under stress, the immunity weakens, and the number of leukocytes and lymphopenia decreases. At the same time, susceptibility to diseases increases, fatness decreases, and quality of meat deteriorates. Stressful effects affect the reproduction of animals, which is an important aspect for farms [3]. White-tailed deer are very stress-sensitive animals. The main factors causing stress are as follows: regrouping, transportation, changing a food regime, climate conditions, ambient temperatures [2, 8, 12]. When transporting animals from Poland, where the average winter temperature was –4 °C, to Tver region, where the temperature reached –30 °C, the animals developed a post-stress syndrome, the body's resistance decreased, and thermoregulation was disturbed.

During transportation, stressful effects can cause deaths of animals and serious financial losses [4–7].

Under stress conditions, deer breathing quickens, general agitation, aggression, frequent vocalizations are observed. If these symptoms last for a long time, animals are in chronic stress, lose weight and hair. Therefore, it is necessary to minimize stress factors. In order to eliminate stress factors, it is important to determine the source of the problem and eliminate it.

Under proper conditions, the body compensates for changes caused by stress factors [5].

4. Conclusion

The results of the study of the Russian reindeer husbandry show positive trends. White-tailed deer meat has a high palatability and nutritional qualities and is in demand. White-tailed deer are prolific animals [2].

The results confirmed that under stress, white-tailed deer have multiple organ disorders that can cause their deaths. Therefore, the prevention of stressful effects is more effective To do this, and cost-effective than the elimination of consequences. It is recommended to monitor clinical and biochemical blood parameters, eliminate adverse keeping conditions, which can prevent diseases and deaths, improve quality of meat products.

When importing white-tailed deer, the biological characteristics, especially pronounced stress tolerance, should be taken into account. Transportation stressors are changes in the feeding order, in the climate conditions, ambient temperatures.

The further studies are aimed to conduct monitoring of general clinical and biochemical blood parameters of white-tailed deer in a larger sample of animals of different sex and age groups to improve reliability of the results.

Acknowledgement

Conflict of Interest

The authors have no conflicts of interest.

References

[1] Blood, D.A. (2000).White-tailed deer in British Columbia, British Columbia Ministry of Environment. Lands and Parks, p. 6. http://www.env.gov.bc.ca/wld/documents/whttail.pdf

[2] Guiascón,Ó. and Paz,S.E. (2018).Hunting and Use of White-Tailed Deer in Mayan Communities, *Tropical and Subtropical Agroecosystems* , vol. 21, issue 2,pp. 283–294.

[3] Marco,I., *et al.* (2002).Transport Stress in Roe Deer (Capreolus Capreolus): Effect of a Short-Acting,*Animal Welfare,* vol. 11, issue 4, pp.405-417.

[4] Kennedy-Slaney, L., *et al.*(2018). Northward Bound: The Distribution of White-Tailed Deer in Ontario under a Changing Climate.*Wildlife Research*,vol. 45,pp. 220–228.

[5] Lashley,M.A. and Harper,C.A.(2012).The Effects of Extreme Drought on Native Forage Nutritional Quality and White-Tailed Deer Diet Selection. *Southeastern Naturalist*,vol. 11, issue 4,pp. 699–710.

[6] Smith,M.L.(2011).Blood Chemistry of Free-Ranging and Captive White-Tailed Deer (*Odocoileus virginianus*) in Texas.(Master's thesis,Texas A&M University,2011).

[7] Webb,S.L.(2012).Biology and Management of White-Tailed Deer. *Journal of Wildlife Management* , vol. 76, issue 1,pp. 220–221.

[8] Wesson,J.A., *et al.*(2011).Influence of Chemical Immobilization and Physical Restraint on Packed Cell Volume, Total Protein, Glucose, and Blood Urea Nitrogen in Blood of White-Tailed Deer. *Canadian Journal of Zoology*, vol. 57, issue 4,pp. 756–767.

[9] Boesch,J.M,*et al.*(2011). Biochemical Variables in Free-ranging White-tailed Deer (*Odocoileus virginianus*) after Chemical Immobilization in Clover Traps or Via Ground-Darting, *American Association of Zoo Veterinarians, Journal of Zoo and Wildlife Medicine* , vol. 42, issue 1,pp. 18–28.

[10] Waid,D.D. and Warren, R.J.(1984). Seasonal Variations in Physiological Indices of Adult Female White-Tailed Deer in Texas. *Journal of Wildlife Diseases*, vol. 20, issue 3,pp. 212–219.

[11] Klinger, S.R., *et al.*(1986). Blood characteristics of white-tailed deer from northeastern Kansas. *Journal of Wildlife Diseases*, vol. 22, issue 3,pp. 385–388.

[12] Chapple,R.S., *et al.*(1991).Haematology and Serum Biochemistry of Captive Unsedated Chital Deer (*Axis axis*) in Australia. *Journal of Wildlife Diseases,* vol. 27, issue 3,pp. 396–406.