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Dynamics of the Juvenile Carps' Hematological Parameters under the Impact of Herbicides†

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Changes of the hematological parameters of the underyearling carps under the herbicide load have been studied. Availability of the urgent and long-term stages of the adaptation to the „roundup" herbicide has been shown. This fact is explained by the chemical nature of the herbicide – water-soluble N-(phosphonomethyl) glycine.

KEYWORDS: *herbicides, underyearling carps, hematological parameters.*

Introduction

Blood is a special type of the connective tissue, consisted of mobile cells, which need interaction with inductive hemopoietic microenvironment for division and differentiation [1]. Most vertebrates have multiple hemopoietic sites, which easily move in the course of ontogenesis and under the experimental impacts [1]. Liver, spleen, gonads, heart and intestine are considered as hemopoietic organs in fishes. Hematological parameters reflect state of the gas, nutritional and biologically active matters, metabolites' transport system; they can be changed in the fish organisms dependently on the ecological conditions and pollutants' impact. These last include many different synthetic compounds, including herbicides, which come into the open water bodies and cause unfavorable effect on the aquatic organisms. Their effect can appear as direct toxic impact, as well as not direct (decreasing of the dissolved oxygen content, change of the chemical composition of water). Response of fish to the impact of any kind of toxicant appears first of all as main blood parameters changes. Hematological analysis enables to elicit latent course of the toxicosis, giving warning of danger even when all other parameters indicate relative well-being. The aim of this work is to study impact of the herbicides "zenkor" and "roundup" on the hematological parameters dynamics in the juvenile carps (0+) within 7, 14, 21 days of stay under the toxic conditions.

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Material and Methods

Experiments were carried out in the 200-l aquariums with settled tap water. Fishes were placed on the basis of 1 fish per 20 liters. Herbicides' concentration (2 MPC – maximal permissible concentration) was produced by inoculation of the calculated amounts of the 70% "zenkor" powder and 36% "roundup" water solution. Stable conditions were controlled and maintained during all experiment: pH 7.4 ± 0.5, oxygen content 5.5 ± 0.3 mg/l, water temperature corresponded to the natural. Fish blood was sampled by the heart puncture. Initial blood sample without the first drop was used for the coagulability determination [9]. The rest of sample was stabilized by addition of heparin – 0.01% [10]. For the morphofunctional state of the organism assessment such hematological parameters were determined: erythrocytes number, blood sedimentation rate, hemoglobin concentration by Sahli method [7]. On the basis of the obtained data hemoglobin content in one erythrocyte (HCE) and globular value were calculated. Besides, blood viscosity was determined by the viscosimeter "BK-4" [2]. Calcium content in blood was determined by de Vaard method [8]. Results were statistically processed according to I.A. Oyvin [6].

Results and Discussion

Erythrocytes content is one of the main indices of fishes' vital functions. On the 7th day of the experiment their maximal amount was registered in the blood of the control fishes – 870 000 per 1 microliter (Fig. 1).

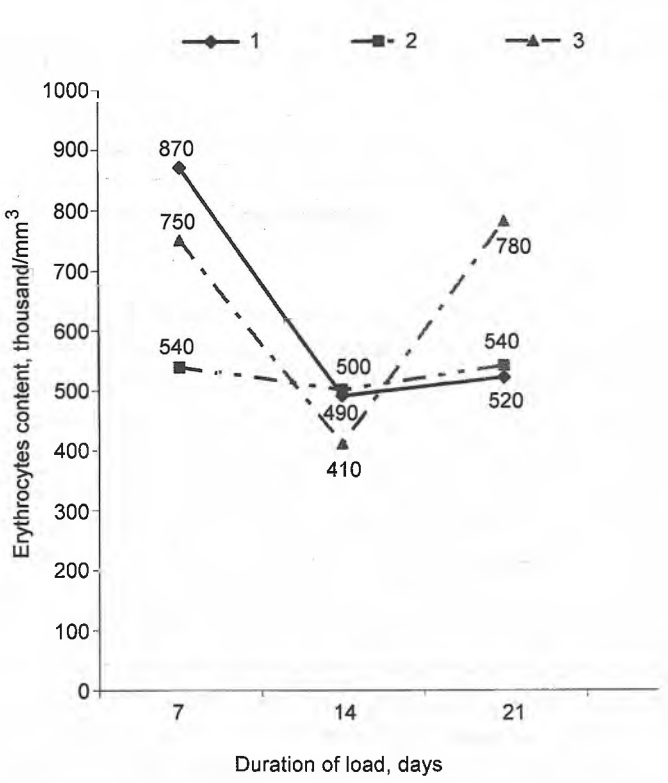

Fig. 1. Erythrocytes content dynamics in the carps' (0+) blood under the herbicide impact. Here and on the Fig. 2–7: 1– control; 2 – "roundup"; 3 – "zenkor".

Table

Ca^{2+} (mg%) content dynamics if the carps' (0+) blood serum under the herbicide load ($M \pm m$, $n = 6$)

Exposure	Ca^{2+} content, mg%		
	control	"zenkor"	"roundup"
7th day	17.77 ± 4.83	41.77 ± 11.22*	23.30 ± 6.40
14th day	25.43 ± 1.20	30.37 ± 6.17	63.10 ± 5.91*
21st day	42.10 ± 1.60	28.63 ± 5.01	25.90 ± 6.41*

* significant difference from control ($p < 0.05$–0.001).

Under the impact of the "roundup" herbicide erythrocytes number in the blood of underyearling carps decreased by 61%, and under the impact of "zenkor" – by 16%. Possible explanation of this fact can be herbicide's chemical structure. "Zenkor" (4-amino-6-*tret*-butyl-3-(methylthio)-1,2,4-triazin-5-(4H)-ene) by its chemical structure belongs to the group of the heterocyclic compounds (triazines), it is low soluble in water and highly soluble in the organic solvents. "Roundup" (N-(phosphonomethyl)glycine), on the contrary, is water-soluble, but not soluble in the most or-

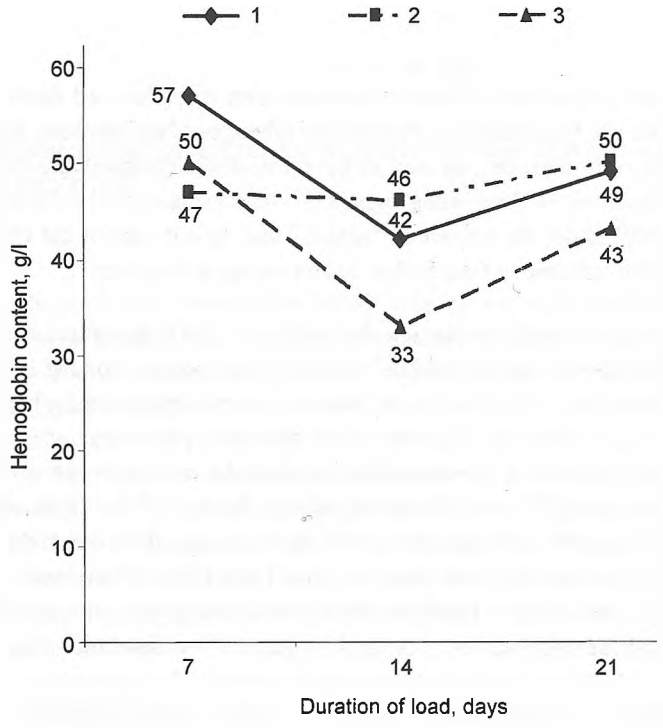

Fig. 2. Hemoglobin content dynamics in the carps' (0+) blood under the herbicide load.

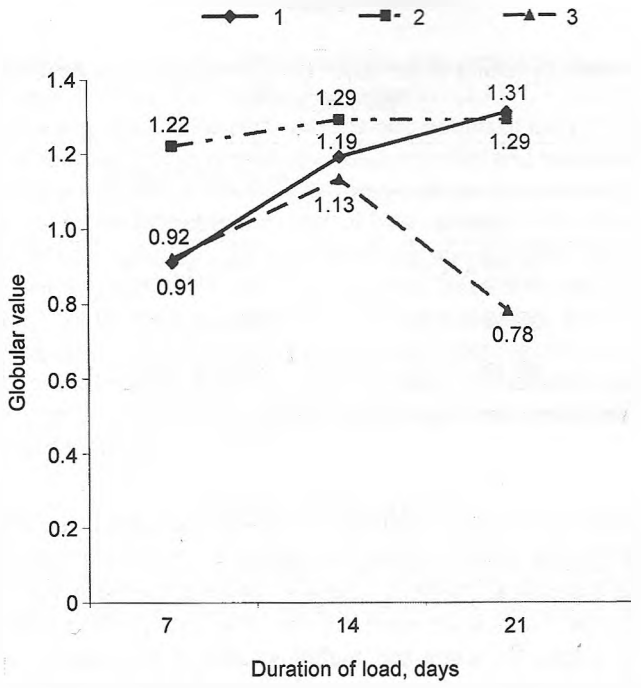

Fig. 3. Globular value dynamics in the carps' (0+) blood under the herbicide load.

ganic solvents. Their impact can influence apoptosis start (programmed death of the cells). It is known that apoptosis can be initiated by intracellular (changes of the hormonal background, growth of the calcium ions concentration), as well as by extracellular (temperature or pressure changes, some chemical compounds etc.) initiating signals [3]. Increasing of the calcium ions content in the blood serum by 240% under the impact of "zenkor" and by 43% under the impact of "roundup" demonstrates possible influence of the herbicides on the apoptosis start.

Sharp decreasing of the erythrocytes number in the blood of both control and experimental fish, especially those under the impact of "zenkor" (almost twice) occurred on the 14th day of the experiment. Possible explanation of this fact can be hemopoiesis disturbance in the blood-forming organs of the juvenile carps or further development of the apoptosis processes under the follows factors: hunger, toxicants' impact and, as a consequence, calcium ions content growth in the blood serum by 33% ("zenkor" effect) and 2.5 times ("roundup" effect). By the 21st day of the experiment erythrocytes number in both control and experimental fishes increased again, but in the control group to a less extent; calcium ions content in the blood serum of these fishes is maximal – 42.1 mg%. Hemoglobin is the main protein contained in the erythrocytes. Hemoglobin content dynamics in the juvenile carps' blood had the same tendency as erythrocytes content dynamics (Fig. 2).

The lowest indices were registered on the 14th day of the fishes' stay under the toxic conditions. On the 7th day maximal hemoglobin amount was in the control fishes' blood (57 g/l), under the effect of "zenkor" its content decreased by 14%, and under the effect of "roundup" – by 21%. How-

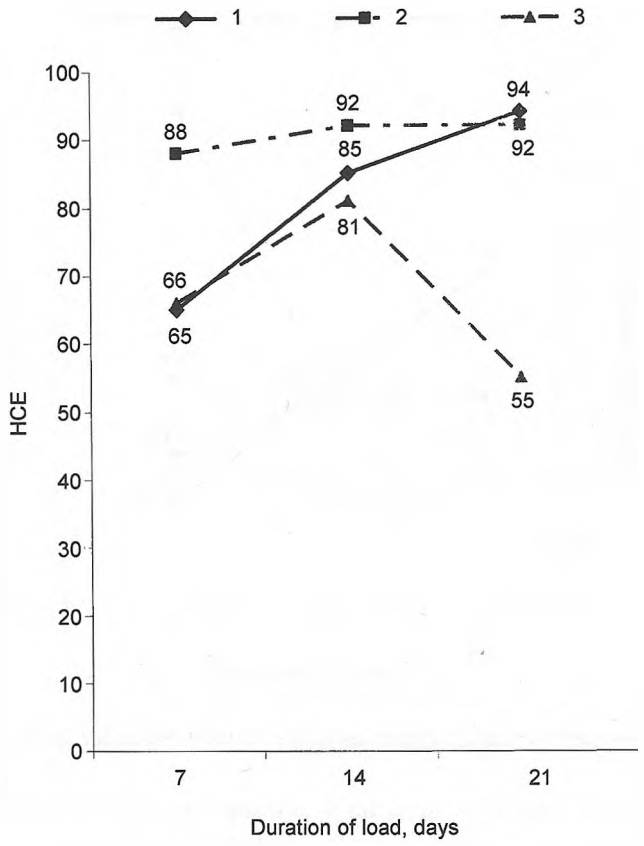

Fig. 4. HCE (hemoglobin content per one erythrocyte) dynamics in the carps' (0+) blood under the herbicide load.

ever by the 21st day hemoglobin content under the effect of this herbicide increased and amounted to 50 g/l. May be, this is an indication of the long-term adaptation forming. For the determination of the erythrocytes saturation by hemoglobin globular value and hemoglobin content per one erythrocyte (HCE) were calculated (Fig. 3, 4).

On the 7th day of the experiment globular value of the blood under the "roundup" effect was by 34% higher than in the control and under "zenkor" effect. By the 14th day globular value increased in all fish groups, and by the 21st day only under the effect of "zenkor" this parameter became lower than one and amounted to 0.78 (Figure 3). Dynamics of the hemoglobin content per one erythrocyte (HCE) in the underyearling carps was similar to that of the globular value (Figure 4). Just under "zenkor" impact on the 21st day of the fishes' stay in the toxic conditions erythrocytes saturation with hemoglobin was minimal. This caused incompetence of the organism and possibly is associated with the biochemical processes in the carp's blood under the certain type of herbicide. Blood sedimentation values (Fig. 5) did not exceed standard parameters during all the experiment, except on the 21st day under the impact of "zenkor" (5 times increase comparatively to control). This indi-

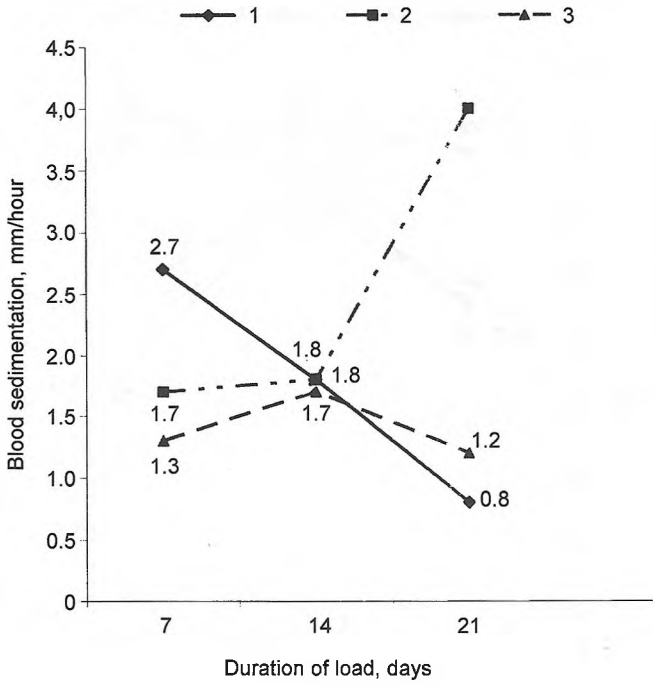

Fig. 5. Blood sedimentation dynamics in the carps' (0+) under the herbicide load.

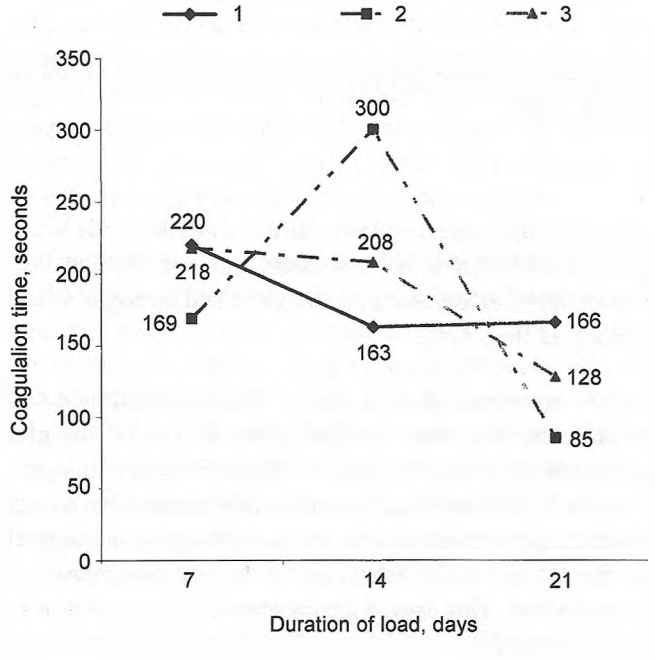

Fig. 6. Blood coagulability dynamics in the carps' (0+) under the herbicide load.

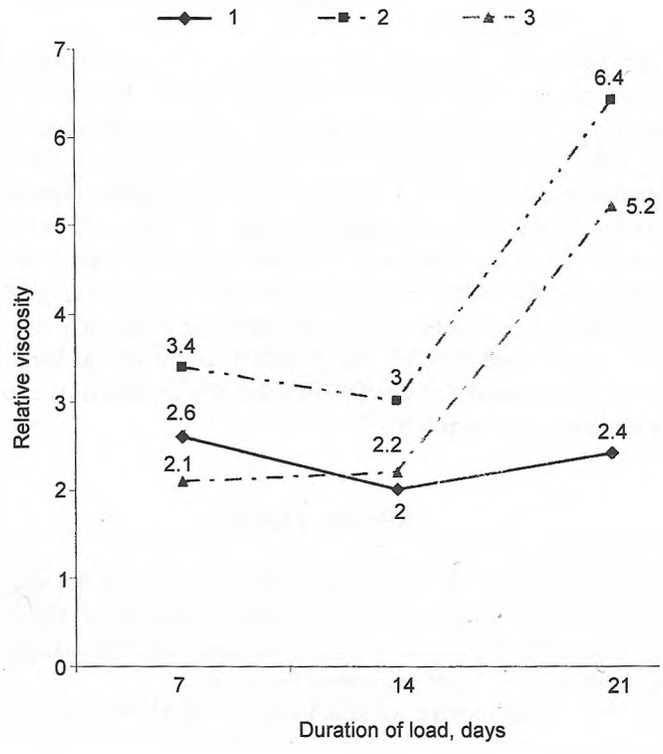

Fig. 7. Relative blood viscosity dynamics in the carps' (0+) under the herbicide load.

cates growth of the proteins-globulins content in the blood plasma, which has an influence on increase of the relative blood viscosity up to the value 6.4.

Dynamics of the relative blood viscosity is presented on the Figure 7, and dynamics of the blood coagulability – on the Figure 6. Variations of these parameters correlated. Thus, on the 7th day of the experiment maximal viscosity value – 3.4 – was registered under the effect of "roundup" (Fig. 7), and it corresponded to the lowest coagulability time – 169 seconds, that is to the maximal coagulability rate (Fig. 6). On the 21st day of the experiment maximal viscosity value – 6.4 ("roundup") – corresponded to the lowest coagulability time – 85 seconds, and the lowest viscosity value – 2.4, that is to the minimal coagulability rate. On the 14th day no such clear correlation was observed, possibly fishes organisms have not yet adapted to the toxic impact. Urgent adaptation stage with morphofunctional mobilization of the internal resources has not turned into the intermediate or long-term stages [9]. While comparing hematological parameters' dynamics in the juvenile (0+) and two-years-old carps it is worth to note similarity and distinctions [5]. Herbicide impact resulted in the changes of the hematological parameters: in the two-year-old carps the worst parameters were registered on the 7th day, and in the juveniles – on the 14th day. General level of these parameters in the adult specimens is higher.

Conclusion

So, analysis of the obtained results showed specificity of the hematological parameters variations in the juvenile carps under the impact of different herbicides. Thus, at the end of 7 days of the experiment changes of the blood indices under the effect of "roundup" (decreasing of the erythrocytes content by 61%, hemoglobin content by 21%, growth of the globular value by 34%, growth of the blood sedimentation by 35%) are more significant than those under the effect of "zenkor" (Figures 1–7). Between the 7th and 21st days under the effect of "roundup" some stabilization of the fishes' status occurred, which was conformed by slight variations of the blood parameters values (within 10% as compared to the control). Under the effect of "zenkor" divergence of the parameters values amounted to 50%. So it is possible to consider formation of the long-term adaptation only regarding "roundup". This is explained by the chemical nature of the herbicide: water-soluble glyphosate, derivative of the amino acid glycine. Possibly it is capable of the faster joining into the metabolism of the underyearling carps.

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