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BIOLOGICAL SCIENCES

STUDY OF FACTORS INFLUENCING THE FORMATION OF IMMUNITY OF SCALY, NAKED AND WILD CARP

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ABSTRACT

The article analyzes and summarizes the available in foreign and domestic literature data on the features of the immune system and immunological reactions of fish conditions of increased anthropogenic load. It is noted that often the cause

Immunological disorders in fish are immunotoxicity of heavy metals. Reasoned the need for further research on the immune parameters of fish as bioindicators pollution of the environment with heavy metals.

The results of our studies showed that feeding carp fish for a month fat-soluble vitamins and trace elements Selenium, Zinc and Iodine in the dietary supplement causes a tendency to decrease lymphocyte activity and immunoregulatory index in carp and carp.

The use of vitamins A, D3, E and trace elements Selenium, Zinc and Iodine in the supplement Enervik to the diet of carp did not significantly affect the number and functional activity of antigen-independent blood B-lymphocytes.

The immune system of fish is a set of cellular and humoral immune factors and consists of cells of the lymphoid-macrophage complex (lymphocytes, granulocytes, Kupffer cells, Langerhans, etc.) and humoral components (immunoglobulins, system of components, complement) lysine, hemolysins, hemagglutinins, etc.). Cellular elements of the immune system are organized in tissue and organ structures. These include: thymus, spleen, liver, lymphoid tissue of the head and trunk of the kidneys, accumulation of lymphoid cranial tissue box, intestine, pericardium. Much of the immunocompetent cells are a component part of the blood and lymph.

Keywords: immune system, analysis, regulation, cell, blood.

Topicality.

Over the last decade, the immune system of fish has become important as an object of study of immunotoxicity of important metals [2]. It has been found that fish are much more sensitive than higher vertebrates to important metals, which have a significant effect on immunological reactions that allows us to consider the immune system of fish as an important bioindicator of environmental pollution.

The purpose of this paper is to determine the effects of seasonality factors, as well as fat-soluble vitamins A, D3, E and trace elements Zinc, Selenium and Iodine on the hematological profile, the state of the immune and antioxidant systems in different breeds of carp fish.

The first step was to determine the effect of seasonality factors on the activity of the immune and antioxidant systems in carp fish. The studies were performed on three groups of two-year-old fish. Carp scaly and framed were grown side by side in one pond, and carp - separately, in the placed near. The material for the study was blood, which was taken from the hearts of fish in different: spring (May), summer (August) and autumn (October). Studies have shown that the content of intermediate and final products

FLOOR in the blood plasma of scaly and framed carp and carp in the autumn significantly higher ($p < 0.01–0.001$), and the content of lipid hydroperoxides in summer is lower ($p < 0.001$), than in the spring research period.

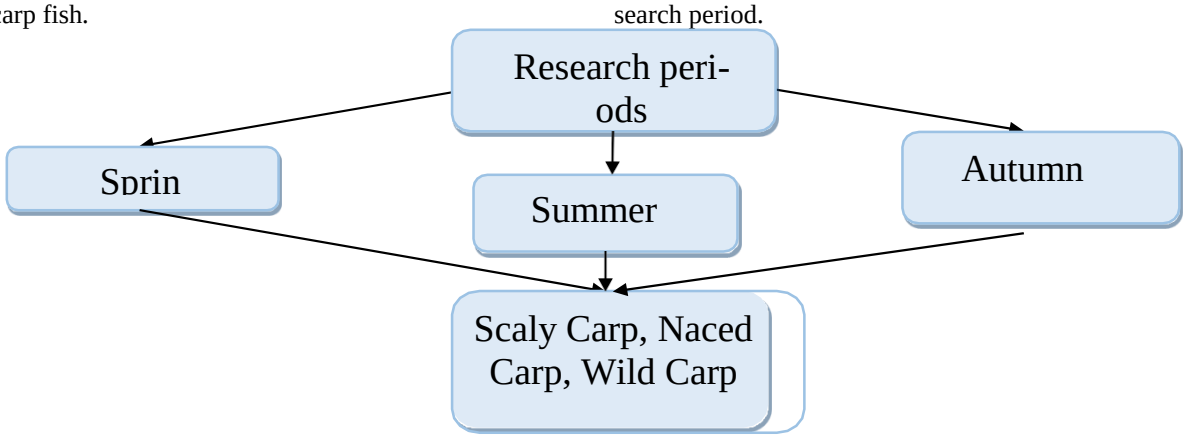

Fig 1. Experimental scheme

Hydrochemical parameters in the ponds were within normal limits (see Annex A). The average statistical water temperature in May was 17.6 ± 1.010C in August- 20,0 ± 0,50C, in October – 13,6 ± 0,280C.

Thus in blood at all breeds of fishes in autumn period of researches, in comparison with spring higher catalase activity is fixed (p <0,01–0,001), which indicates the dependence of the intensity of peroxide processes in the body of fish from seasonal factors and the activity of enzymes of the system antioxidant protection.

The second step was to determine the effect of the complex action fat-soluble vitamins A, D3, E and trace elements Zinc, Selenium and Iodine in the composition of the biologically active additive to the diet on the intensity of oxidative processes, activity of the antioxidant defense system, the state of cellular and humoral mechanisms of nonspecific resistance and adaptability in carp framed and carp at the end of the growing season.

The experiment was conducted in the autumn on two groups of fish (carp and carp), two years of age, which on the principle of analogues were divided into two control and two experimental groups of 10 individuals. The fish were kept in special trays under conditions permanent closed system of water circulation fish of control groups during. Granulated feed was fed for 30 days, experimental specimens - similar compound feed with the additive "Enervik".

Studies have shown that the use of biologically active additives to compound feed of fat-soluble vitamins A, D3, E and trace elements Zinc, Selenium and Iodine leads to an increase in the content of Zinc and Selenium in hepatopancreas and skeletal muscles in carp and carp. Magnification content of Zinc and Selenium in the studied tissues of carp fish caused increasing superoxide dismutase and glutathione peroxidase activity in hepatopancreas and kidneys of carp. These data indicate stimulating effect of fat-soluble vitamins and trace elements, and especially. Selenium and Zinc in the composition of biologically active additives, for activity antioxidant enzymes in the body of carp fish.

In the study of the cellular link of the specific immune response the stimulating effect of vitamin and mineral supplements on the total was stated the number of T-lymphocytes in the blood of carp.

In general, the results of studies have shown that the application carp fat-soluble vitamins and trace elements Selenium, Zinc and Iodine in the composition feed additives have a positive effect on the oxygen transport function of the blood, reduces the intensity of lipid peroxidation, increases activity antioxidant defense system, promotes the growth of immune potential and productivity in carp and carp. This effect was greater to a greater extent in the body of carp than in carp.

The functional relationship between the content of selenium and is proved and analyzed Zinc in the organs and tissues of carp frame and carp and the intensity of processes POL, antioxidant enzyme activity and immune potential studied fish under the conditions of use of vitamins A, D3, E and trace elements Zinc, Selenium and Iodine as part of feed additives.

The research results are of great theoretical importance biochemical and immunological processes that underlie seasonal changes and breed features of fish metabolism.

Ukrainian framed and Ukrainian scaly carp are bred in Ukraine. Both Ukrainian carp breeds were bred under the guidance of breeder Kuzyoma O.I. The structure of Ukrainian carp breeds now includes intrabreed and zonal (ecological) types (arrays), adapted to different physical and geographical zones of Ukraine, namely: Nyvkivsky (scaly form), small-scaly (frame form), Lubin (scaly and frame forms) intrabreed types and Antonino-Zozulenets (scaly and frame forms) and Nesvitsky (scaly and frame forms) zonal types (arrays).

Compared to the framed carp, the scaly carp is more elongated and has smaller head. Frame carp by the nature of nutrition belong to fattening type. They grow intensively in the conditions of garden cultivation on warm waters [10]. Several carp hybrids obtained by crossing it with carp. Genetic features of carp to a large extent affect its morphological, immunological and physiological and biochemical features [12].

The main life processes in carp are most intense relatively high water temperatures. Optimal temperature limits for growth and carp development range from 25 to 27 at C. Under conditions of increase or decrease water temperature, the intensity of nutrition and assimilation of food it decreases [6].

Lipid peroxidation is important for renewal biological membranes, rotation of their protein and lipid components, regulation physicochemical properties of cell membranes and subcellular structures, development normal physiological metabolism.

The AOS system and the intensity of LPS processes are among the most sensitive. The most intense course of peroxide processes occurs in the gills fish with changes in temperature, aeration, water hardness, in the spleen - with infection and immune reactions, in the liver - in intoxication and fast-reacting systems in the body of fish, which varies greatly depending on their species biological features, which are due mainly to their conditions existence, seasonal factors and unbalanced feeding and insufficient content in diet of trace elements that are part of antioxidant enzymes [3].

The main abiotic factor that determines the distribution of fish in fresh reservoirs, there is a temperature regime. The temperature affects the fish directly - changing the intensity of enzymatic processes occurring in the body, activity of food consumption, metabolism, the course of development of the gonads and more. It is proved that during the winter changes in the chemical composition of the body, in general there are changes in the lipid, carbohydrate and protein metabolism. Species features of the enzymatic link were formed under the influence of factors external environment and largely depend on the type of food in fish. Free radical processes due to reactive oxygen species lie in based on lipid peroxidation [8]. Significant impact on the intensity of peroxide processes and the activity of the antioxidant system in the body of fish has the content of dissolved oxygen, hypoxia and hyperoxia.

It should be noted that during the winter in the tissues of pond fish the content of endogenous fat-soluble antioxidants - tocopherol and retinol, which reduces the activity of the non-enzymatic part of the system AOZ.

In addition, in the energy processes in the tissues of fish, in particular in carp, winter, intensively used amino acids that are released in processes of proteolysis of reserve tissue proteins. There is a direct link between antioxidant and immune activity systems in fish intensity of peroxide processes and the state of the antioxidant defense system in the body of carp fish to a large extent depends on the influence of seasonal factors, temperature and oxygen regimes [1, 9].

The living conditions of fish affect the morphological composition and quantity indicators of red and white blood. Based on this, the blood picture changes to epending on temperature, hydrochemical regime, composition and quantity of natural feed, stocking density, age and general physiological condition of fish.

Seasonal fluctuations in hemoglobin concentration are potentially associated with change water temperature and changes in oxygen concentration in water. The fish adapts to change water temperature by increasing the total concentration of hemoglobin or by other mechanisms, such as changes in red cells nucleoside

In the conditions of winter starvation in the body of fish temperature and resource-deficient stress develops. The answer is increase during the winter period by 1.5 times the content of erythrocytes and size hematocrit. The total hemoglobin content is reduced by 2 times, which correlates with data on the direct dependence of hemoglobin content in carp on water temperature.

Nonspecific immunity plays a key role in the acquired immune response and homeostasis through a system of receptor proteins. These receptor proteins are identified molecular models that are characteristic of pathogenic microorganisms, including polysaccharides, lipopolysaccharide (LPS), peptidoglycan bacterial DNK, viral RNK and other molecules that are not normally found on the surface multicellular organisms [2].

One of the most pressing problems of modern pond fish farming of Ukraine has a decrease in fish resistance due to the negative effects of a number factors: poor feeding, anthropogenic impact of environmental factors, violation of technologies for their cultivation.

Analysis of recent research and publications.

The first work on the study of immunobiological reactivity of some vertebrates were conducted by one of the founders of modern immunology II Mechnikov (1883,1913, 1947, 1950). Phagocytic and inflammatory phenomena were noted in fish, regenerative processes were described [14]. Immunological reactivity of the organism is determined by its ability recognize and neutralize genetically alien.

The cellular link of nonspecific resistance of an organism of fishes, as well as mammals, is characterized by phagocytic activity of blood. Phagocytosis is considered to be the main process of nonspecific resistance. Phagocytosis - is the process of active absorption by cells of the body of pathogenic living or dead microorganisms, as well as other foreign particles with their subsequent digestion by intracellular enzymes. The main cells involved in the process phagocytosis, there are neutrophilic granulocytes [5].

Investigation of nonspecific mechanisms of response of the immune system of fish provide protection against infections and preservation of individual integrity of an organism, is of both theoretical and practical interest in determining the role of the immune system in the implementation of adaptation processes to toxic and biotic factors, in assessing the causes reducing the intensity of natural immunity in the environment and the development of problems biotesting, determination of norm and pathology, etc. [7]. The most important task of ecological and immunological research is to clarify the general patterns of rearrangements of the immunological reactivity of fish under the influence of environmental factors, the ability to predict and manage these changes. This problem cannot be solved without careful study of the main basis of the body's immune response – immunocompetent cells, which include primarily lymphocytes, as well as macrophages / monocytes and granulocytes [12].

The immune system of fish, which protects the internal environment from invasion foreign antigens, is a very sensitive indicator of the state of both the organism and habitat. In this regard, the study of various components of the immune system fish are quite important for assessing the immunotoxicity of various compounds. From the literature it is known that hematological parameters are the most important parameters for the assessment of immune fish status [4].

Thus, the immune system as a system of protection of the body from foreign exposure is extremely sensitive to the toxic effects of chemicals that are present in very low concentrations in the environment.

Methods for determining biochemical parameters

Determination of soluble protein content. The concentration of total protein in the serum was determined spectrophotometrically by the Lowry method [12]. The essence of the method is the formation of colored products as a result of the reaction of aromatic amino acids with Folin-Chekalteu reagent in combination with the biuret reaction.

Determination of the content of lipid hydroperoxides in plasma and homogenate. The content of lipid hydroperoxides (LPL) in blood and homogenates was determined by the method described by VV Myronchik [9,17]. The principle is based on the precipitation of proteins, which is carried out by trichloroacetic acid (THO) with the addition of ammonium thiocyanate. To 0,2 ml of blood plasma or tissue homogenate was added 2,8 ml of ethanol and 0,05 ml of 50% THO solution. Covered with corks and shaken for 5 minutes. Then centrifuged for 10 min at 3000 rev/min.

The resulting supernatant was taken 1,5 ml and added 1,2 ml of ethanol, shaken, then added 0.02 ml of HCl, 0.03 ml of 1% Moro salt solution, shaken and after 30 s was added 0,2 ml of 20% ammonium thiocyanate solution. Optical density measurements were performed for 10 min after the addition of ammonium thiocyanate on a spectrophotometer at a wavelength of 480 nm.

$$\Delta D_{480}(GPL)=D_{480}(D)-D_{480}(K)$$

Determination of hemoglobin concentration. The concentration of hemoglobin was performed by cyanomethemoglobin method. according to the methodical recommendations of Selvestrov V.V, (1999). 5 ml of Drabkin's transforming solution was added to the test tube and 20 μl of whole blood was added. The tube was mixed thoroughly and left in the refrigerator for 20 minutes.

The optical density of the resulting solution was determined on a spectrophotometer at 540 nm against the transforming solution. The calculation of the hemoglobin concentration was performed according to the formula:

x = d540 x 367,1 g / l,

where: d 540 - FEC readings;

367.1 - conversion factor that takes into account the dilution of blood.

CONCLUSIONS

The intensity of fish growth and their resistance to disease are genetically determined and largely depend on the action of seasonal factors that significantly affect the metabolism in their body [9]. During the annual cycle of fish farming can withstand significant changes in ambient temperature and oxygen content in the water [13, 10]. This, in turn, affects the state of metabolism in their body. In particular, the intensity of lipid peroxidation processes and the activity of the antioxidant defense system in fish changes throughout the year.

The most pressing problems in the cultivation of carp is the need to increase their adaptation to environmental factors and a comprehensive study of biological characteristics.

Table 1

Seasonal dynamics of the content of LPO products in the blood plasma of the studied fish (M±m, n=5)

Indicators	Research periods	Breeds of fish		
		Scaly curp	Naked curp	Wild curp
TBK, mкmol/l	Spring (kontr.)	2,28±0,05	1,60±0,13***	2,47±0,06*
	Summer	2,42±0,14	2,19±0,07^{oo}	2,42±0,05
	Autumn	3,53±0,13^{ooo}	2,29±0,04***^{ooo}	3,72±0,14^{ooo}
GPL, un.E/ml	Spring (kontr.)	1,24±0,02	1,26±0,04	1,24±0,02
	Summer	0,62±0,01^{ooo}	0,63±0,01^{ooo}	0,87±0,02***^{ooo}
	Autumn	1,74±0,11^{oo}	1,32±0,05**	1,86±0,06^{ooo}

Note *— p<0,05; **— p<0,01; ***— p<0,001

The content of intermediate and final LPO products in the blood of scaly and framed carp and carp in the autumn period (October) is much higher than in the spring (May) period of research (p <0.01–0.001). It should be noted that the content of lipid hydroperoxides formed in the intermediate stage lipid peroxidation, in the blood plasma of the studied species of fish in the summer (August) period is less than in the spring (p <0,001) and, especially, the autumn periods.

The obtained results indicate a significant increase in peroxide processes in the body of fish in the autumn, which is apparently due to temperature factors and a significant decrease (p <0,01-0,001) in glutathione peroxidase and superoxide dismutase activity in the blood of the studied fish. The high content of HPL and TBA-active products in the blood plasma of fish in the autumn can also be explained by the increase in polyunsaturated fatty acids, which initiate lipid peroxidation in cell membrane lipids under conditions of lower ambient temperature [1, 20].

Studies have shown that the hematological profile of the studied carp fish during the annual cycle of cultivation depends largely on seasonal factors. As can be seen from the data number of erythrocytes, hemoglobin content, hematocrit and the average concentration of hemoglobin in the erythrocyte in the blood of scaly carp, carp and carp in the summer and especially in autumn is much higher (p <0,05 – 0,001) than in the spring.

The results of research indicate a significant decrease in oxygen transport function of blood in fish in winter and early spring, compared with summer and autumn.

In scientific work it was breed and seasonal features of immune and antioxidant system in carp fish and the effect of fat-soluble vitamins A, D3, E and trace elements Zinc, Selenium and Iodine on the activity of these systems.

Table 2

Hematological parameters and total protein content in the serum of carp fish (M±m; n=5)

Indicators	Research periods	Breeds of fish		
		Scaly curp (Kontr)	Naked curp	Wild curp
protein,g/l	Spring (Kontr)	21,35±0,29	19,98±0,27**	21,70±0,21
	Summer	32,66±0,31^{ooo}	32,3±0,43^{ooo}	34,1±0,61^{ooo}
	Autumn	23,35±2,75	22,67±1,57	28,97±1,30^{ooo}
hemoglobin,g/l	Spring (Kontr)	61,3±2,3	62,7±2,6	66,7±3,1
	Summer	79,9±1,6^{ooo}	77,1±1,34^{ooo}	80,8±1,9^{oo}
	Autumn	91,04±1,5^{ooo}	90,8±1,9^{ooo}	96,7±2,4^{ooo}
hematocrit, %	Spring (Kontr)	21,4±1,28	21,2±0, 9	24±1,22
	Summer	21,0±0,5	20,4±0,5	22,4±0,5
	Autumn	27,9±0,4^{ooo}	29,7±1,3^{ooo}	24,9±1,6
erythrocytes,t/l	Spring (Kontr)	2,0±0,3	2,2±0,2	2,26±0,2
	Summer	2,0 ±0,3	2,26±0,15	2,32,5±0,2^{o}
	Autumn	2,04±0,1	2,4±0,3	2,7±0,2*

This is also indicated by higher blood indices (color index, average concentration of hemoglobin in the erythrocyte and the amount of hemoglobin in one erythrocyte) in the studied fish in the summer ($p < 0,05$-$0,001$) compared to spring.

It is known, that the sensitivity of fish to changes in water temperature is also associated with the properties of hemoglobin: with increasing water temperature, the body's need for oxygen increases, but the ability of hemoglobin to bind it decreases.

Use for carp and carp as part of feed additives fat-soluble vitamins and trace elements Selenium, Zinc and Iodine promotes increase body weight by 13,2 and 6,4%, respectively, and body length by 10 and 6,9%, respectively control.

In order to increase the immune potential and antioxidant protection and growth of carp fish at the end of the growing season during the month is recommended to apply a vitamin and mineral supplement according to the developed methodical recommendations.

Today, as a result of expansion human activity in almost all natural areas and irrational human attitudes to the surrounding nature, many immunological parameters of fish began to be used as biomarkers for monitoring the immunotoxicity of chemical pollutants existence and to predict the toxicological risk associated with contamination aquatic environments [5, 8].

The most widely used immune parameters are concentration lysozyme, antibodies and leukocytes in the blood of fish, as well as tests of functional activity complement, macrophages and lymphocytes. Research of indicators of immune system of fishes can be used in the development of effective methods of immunological monitoring condition of fish and solving problems to assess the quality of the environment, as well as to serve basis for solving practical problems such as efficient industrial breeding fish, ecological modeling and reliable prediction of changes in the ecological situation biogeocenoses.

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