Part 1. Veterinary medicine

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THE IMPACT OF PLANT CRYOEXTRACT ON PRODUCTIVITY AND FACTORS OF INNATE IMMUNITY OF POND FISH AGAINST THE BACKGROUND OF STRESS IN THE EXPERIMENT

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Summary. The search for effective and environmentally friendly means to increase the productivity and natural resistance of animals, especially in aquaculture, remains an urgent socio-economic task. This article presents the results of the study of the effect of the cryobiological supplement 'Immunolife-Fish', based on medicinal plants, on the weight gain of juvenile common carp (*Cyprinus carpio*) and indicators of innate immunity of two-year-old common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*) under the influence of thermal stress factor in aquarium conditions. It was found that the weight gain of juvenile carp treated with the cryobiological supplement 'Immunolife-Fish' was higher than that of the control group at all stages of the study. The increase was most pronounced during the first 42 days of feeding. A significant increase in the number of leukocytes was found in the blood of fish (common carp and silver carp) exposed to a heat stressor compared to control values. In fish that received a supplement of the herbal preparation during stress, this indicator only tended to increase. The effect of the stressor in fish of both species is manifested by a significant increase in the leukocyte content, a decrease in the indicators characterizing the functional state of cellular immunity — phagocytic activity and number of phagocytes, as well as a tendency to decrease the phagocytic index of neutrophils in the blood. The use of the cryobiological supplement 'Immunolife-Fish' in fish exposed to stress not only prevented the decrease in the phagocytic activity of neutrophils but also contributed to a slight increase in their activity in comparison with the control. A significant decrease in the activity of lysozyme and the level of circulating immune complexes, as well as an increase in the content of seromucoids, was found in the blood serum of common and silver carp under the influence of the stress factor. The use of the drug against the background of stress leveled the negative changes in the above indicators characterizing the state of the humoral link of innate immunity. In addition, a mild immune stimulating effect of the cryobiological supplement on fish of both species, which were kept in optimal conditions, was noted. The research is aimed at the development and testing of organic, environmentally friendly anti-stress and immunostimulant agents in commercial fish farming

Keywords: common carp, silver carp, thermal stress, medicinal plants

Introduction. Today, global aquaculture provides half of the world's fish stocks and is one of the fastest-growing food production sectors. It is believed that the development of environmentally and socially sustainable aquaculture is based on three main principles: first, aquaculture should take into account the full range of ecosystem functions and services; second, aquaculture should contribute to the well-being of people representing all stakeholders; and third, aquaculture should be developed taking into account other sectors, policies and their goals (Vdovenko, 2013; Prysiazhniuk et al., 2019). Success in achieving the above objectives requires, on the one hand, proper management of aquaculture development at the institutional level and, on the other hand, social acceptance of the measures taken. Pond carp farming is a type of aquaculture that can meet all of the above criteria (Zhu, 2020). Other steps to improve the environmental performance of aquaculture include the development of 'multitrophic aquaculture' using nutrient-rich by-products; 'polyculture' (e.g., a combination of carps with different feeding niches in one pond) (DG MARE, 2021).

Carp is the main fish species growing well and raised in slow-flowing or stagnant water ponds. In particular, the Ukrainian common carp (*Cyprinus carpio*) grows better than other carp subspecies, is 17–20% more viable, and effectively utilizes the natural food base of ponds. Carp is an omnivorous fish: at a young age, it consumes

zooplankton, at an older age — benthos, it eats well artificially produced feed, grain waste, weed seeds, etc. Herbivorous fish, such as silver carp (*Hypophthalmichthys molitrix*), are often grown in polyculture with carp, as the period of its cultivation to marketable weight is the same as for carp (two to three years) (Pustova N., Pustova Z. and Balickiy, 2023).

In conditions of intensive fish farming, where the natural feed base has an insignificant share in fish feeding or is absent, the use of artificial feeds, especially with the use of various biogenic elements, the so-called functional feeds, plays an important role. They can be used to increase growth, and natural resistance and optimize the antioxidant status of fish and other aquaculture objects (Pacitti et al., 2015, 2016; Oleshko et al., 2021; Prysiazhniuk et al., 2023). The main factors of fish diseases and mortality against the background of reduced resistance are the effects of stress factors and pathogens of infectious and invasive diseases (Horchanok and Prysiazhniuk, 2020). At the same time, the constant use of various agents for disease control can lead to many problems: increased resistance of pathogens, pollution of water bodies, and loss of phyto- and zooplankton. Therefore, it is recommended not to use drugs or chemicals unless absolutely necessary. An alternative can be the use of biological control methods and environmentally friendly measures to prevent fish diseases and increase their natural resistance (Abdel-Ghany, El-Sisy and Salem, 2023).

As shown in numerous literature sources (Ekasari et al., 2014; Ibrahim et al., 2021; Novitskyi and Horchanok, 2022), increasing the resistance of fish to both pathogens and abiotic environmental factors is one of the modern and important problems of pond fish farming. The immune system of fish is self-regulated by direct interconnection of cells (macrophages, neutrophils, etc.), as well as with the participation of humoral defense factors.

The main components of the nonspecific (innate) immune system of fish are macrophages, granulocytes, monocytes, and humoral elements such as lysozyme (Saurabh and Sahoo, 2008). Bioactive compounds act as immunostimulants to enhance the immune response of fish (Salinas, Zhang and Sunyer, 2011; Mohammadi et al., 2020; Effendi et al., 2022), namely lysozyme, phagocytosis, complement system, reactive oxygen and nitrogen species, antiproteases, glutathione peroxidase, against bacterial, fungal, viral and parasitic diseases (Oleksiuk and Yanovych, 2010; Effendi et al., 2022; Janssens et al., 2000). The immune system of fish is a combination of cellular and humoral immunity factors, which are respectively realized by cells of the lymphoid-macrophage complex and humoral components (Kovalchuk, 2011; Mohammadi et al., 2020; Bavia, 2022). Cellular elements of the immune system are organized into tissue and organ structures (Rubio-Godoy, 2010; Salinas, Zhang and Sunyer, 2011). A significant part of immunocompetent cells is a component of blood and lymph. The immune system of fish differs from that of higher vertebrates in the absence of lymph nodes, bone marrow, and the pharyngeal bursa in birds; immunoglobulins in fish are represented only by IgM-like antibodies (Ibrahim et al., 2021).

The natural resistance of pond fish is influenced by many factors: housing conditions, adequate feeding, a hydrochemical regime in the reservoir, the content of toxic substances of various kinds in the water, etc. This is evidenced by a decrease in the resistance of fish to pathogens under the influence of negative factors. In pond fish farming, a variety of drugs and supplements are actively used to prevent the emergence and spread of pathogens, as well as immunostimulants that activate the system of non-specific resistance factors in fish (Ekasari et al., 2014). Phytotherapy is one of the modern ways to increase the natural resistance of animals.

The mechanisms and processes related to the use of medicinal plants in the world of fisheries are not well understood. Generally, people's attention is focused on mice, chickens, and humans (Mohammadi et al., 2020). Medicinal plants can be administered as parts or whole plants (seeds, leaves, roots, bark, or fruits) or complex extracts, as a feed supplement, through a water regimen, alone or as a combination of extract compounds, or even as a mixture with pro- and prebiotics (Effendi et al., 2022).

Plants and their extracts have been reported to have various effects, such as antistress, appetite and growth stimulation, and immunostimulation in various aquaculture targets, due to their various active components, such as alkaloids, terpenoids, tannins, saponins, polysaccharides, and flavonoids (Mohammadi et al., 2020). This makes the plants suitable for the treatment of multifactorial diseases, and their use can be an alternative to antibiotics with a low risk of developing resistance (Reverter et al., 2017).

One of the modern technologies for processing medicinal plants is cryoextraction (or cryoextension). The method is based on the obtaining of extracts from plant materials using low temperatures to isolate biologically active compounds. It is believed that the cryoextraction system preserves the active essence of plants, allows the use of plants that act in synergy, and their biological activity is 5–10 times higher than that of dried plant extracts. This method allows the preservation of the biological activity of substances and can be particularly effective for obtaining extracts from plants containing thermolabile compounds (Verkin, Zinov'ev and Povstyanyy, 1985). The low temperature stops the action of oxidizing enzymes and the oxidation process. The bonds of biologically active compounds with large protein molecules are broken, which increases their digestibility. This contributes to the 'cryoactivation' of

plant materials and increases the bioavailability of substances many times due to their transition to a state unrelated to proteins (Verkin, Dmitriev and Pavlyuk, 1986).

According to the literature, plant species with the greatest potential for use in aquaculture include garlic (*Allium sativum*), pomegranate (*Punica granatum*), Bermuda grass (*Cynodon dactylon*), ashwagandha (*Withania somnifera*), and ginger (*Zingiber officinalis*) (Reverter et al., 2017).

The study aimed to investigate the effect of the cryobiological supplement 'Immunolife-Fish' on the productivity of juvenile common carp (*Cyprinus carpio*) and innate immunity of two-year-old pond fish (common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*)) under the influence of heat stress in the experiment.

Materials and methods. To perform the tasks under aquarium conditions, two experiments were carried out using the cryobiological supplement 'Immunolife-Fish', produced by cryo-grinding technology. For this purpose, a composition of medicinal plants was used: golden root (*Rhodiola rosea*) roots and rhizomes, hawthorn (*Crataegus*) flowers, licorice (*Glycyrrhiza glabra*) roots, pine (*Pinus*) needles, and purple coneflower (*Echinacea purpurea*) roots in equal proportions. The drug was mixed with standard feed (feed concentrate Multigain™) in a final concentration of 1.5%. The experimental sample of the cryobiological additive was produced by the pharmaceutical company 'AIM' (Kharkiv, Ukraine).

The experiment to study the effect of cryobiological supplementation on fish productivity was carried out using two aquariums with a capacity of 100 liters, in which 20 individuals of juvenile common carp with an initial weight of 20.4 ± 0.25 g were kept. The water temperature was 24–25 °C, and the average oxygen concentration during the experiment was 5.2 mg/l. The fish of the experimental group were fed with the cryobiological additive 'Immunolife-Fish' together with the food, and the fish of the control group were fed only with the standard food. The feed was given 6 times a day in equal parts in the amount of 15% of the fish's weight. The duration of the experiment was 8 weeks.

Every 14 days, the fish were weighed on electronic scales to determine the dynamics of growth and development of the fish. Growth intensity was evaluated by absolute, relative, and average daily growth (Korol-Bezpala, 2020).

An experiment to study the effect of the cryobiological supplement 'Immunolife-Fish' on the state of innate immunity was conducted on two-year-old common carp and silver carp with an average weight of 450 ± 3.5 g and 570 ± 4.6 g, respectively, of which 4 groups (3 experimental and 1 control) were formed on the principle of analogs, 5 individuals per group. The fish were kept in aquariums with a capacity of 150 liters. Fish were fed in the morning and evening in equal parts in the amount of 15% of the fish weight per day. Fish of groups I and III of both species were exposed to a stress factor, which was modeled by increasing the water temperature in the aquarium to 26–27 °C, fish of groups II and III of both species received the cryobiological supplement 'Immunolife-Fish' with food.

The material for the study was blood taken from the heart of fish on 11th day of the experiment using a Pasteur pipette after anesthesia with essential oil of clove (*Eugenia caryophyllus*). In the heparin-stabilized blood, the number of leukocytes and the effectiveness of phagocytosis, namely phagocytic activity, phagocytic number, and phagocyte index, were determined (Vlizlo, 2016; Stegniy et al., 2013). In fish blood serum, lysozyme activity, seromucoid level, and circulating immune complexes level were studied according to generally accepted methods using standard reagent kits of PJSC 'Reagent'.

The studies were conducted in the Laboratory of Toxicological Monitoring, Clinical Biochemistry, Quality and Safety of Agricultural Products of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' of the National Academy of Agrarian Sciences of Ukraine (Kharkiv, Ukraine).

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 2010/63/EU (CEC, 2010), and under Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017). Under the current procedure, the research program was reviewed and approved by the Bioethics Committee of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'.

Statistical analysis. The results are presented as the mean ± standard deviation (x ± SD). Analysis of variance (ANOVA) was used to compare the mean difference between experimental and control groups. The p-value $p < 0.05$ was considered a significant value.

Results. It was found that the weight gain of juvenile common carp treated with the cryobiological supplement 'Immunolife-Fish' was higher than that of the control group at all times during the study, reaching a maximum of 17.7% on 42nd day of the experiment and finally 5.2% (Table 1).

According to the results of determining the calculated indicators of productivity of common carp in the dynamics of the experiment, it was found that the most pronounced effect of the drug on the absolute, average daily, and relative growth occurred on 29th–42nd days of

feeding, when the indicators of the experimental group maximally exceeded the control ones by 29.2%, 28.3%, and 10.6%, respectively (Table 2).

In the blood of common carp and silver carp of the first experimental groups exposed to the stress factor, there was a significant ($p < 0.05$) increase in the number of leukocytes by 22.2% and 19.6%, respectively, compared to the control values. In the fish of the third group, which received a plant supplement during the stress, this indicator only tended to increase, reaching 12.2% and 11.3%, respectively. A slight stimulating effect of the cryobiological supplement was also observed in the fish of the second group, which were kept in optimal conditions (Table 3).

Table 1 — Body weight (g) dynamics of common carp under the influence of cryobiological supplement 'Immunolife-Fish' ($n = 20$, $x \pm SD$)

Group	Day of the experiment				
	1	14	28	42	56
Control	20.3 ± 0.18	87.60 ± 0.23	173.6 ± 1.74	247.6 ± 1.96	329.0 ± 2.03
Experimental	20.5 ± 0.08	93.68 ± 0.18	195.5 ± 1.22	291.1 ± 1.54	389.2 ± 1.85

Table 2 — Calculated average group productivity indicators of common carp under the influence of cryobiological supplement 'Immunolife-Fish'

Group	Experimental period, day			
	1–14	15–28	29–42	43–56
Absolute weight gain, g				
Control	67.3	86.0	74.0	81.4
Experimental	73.2	101.8	95.6	98.1
Average daily weight gain, g				
Control	4.8	6.1	5.3	5.8
Experimental	5.2	7.3	6.8	6.4
Relative weight gain of fish, %				
Control	331.5	98.2	42.6	32.9
Experimental	357.0	108.7	48.9	34.6

Table 3 — The level of leukocytes ($\times 10^9$/dm^3) in the blood of pond fish under the influence of stress factor and application of cryobiological supplement 'Immunolife-Fish' ($n = 5$, $x \pm SD$)

Fish species	Group			
	Control	**I** (stress)	**II** (supplement)	**III** (stress + supplement)
Common carp	19.41 ± 0.48	23.72 ± 0.76*	20.38 ± 0.57	21.77 ± 0.93
Silver carp	22.32 ± 0.33	26.69 ± 0.51*	23.43 ± 0.71	24.84 ± 0.56

Note. * — $p < 0.05$ relative to control.

The study of the functional state of neutrophils in the blood of fish of groups I and II of both species revealed a decrease in their phagocytic activity by 29.4% ($p < 0.05$) and 22.8% ($p < 0.05$), phagocytic index by 23.8% and 20.9%, phagocytic number by 24.2% ($p < 0.05$) and 22.6% ($p < 0.05$), respectively. In fish of groups II and III, which received the cryobiological supplement 'Immunolife-Fish', an increase in phagocytic activity by 14.8% and 12.1%, 11.6% and 9.4%; phagocytic index by 14.1% and 11.3%, 10.8 and 10.2%; phagocytic number by 13.2% and 10.8%, 11.0%, and 9.6%, respectively, was observed compared to the control (Table 4).

The study of the indicators characterizing the state of the humoral link of the innate immunity of fish organisms revealed a decrease in the activity of lysozyme in the blood serum of the groups I of common carp and silver carp by 32.4% ($p < 0.05$) and 29.8% ($p < 0.05$), as well as a decrease in the level of circulating immune complexes by 26.8% and 27.4%, respectively. On the contrary, the level of seromucoid in fish of these groups increased by 43.2% ($p < 0.05$) and 39.6% ($p < 0.05$). In the blood serum of common carp and silver carp of the groups II, an increase in serum lysozyme activity by 16.3% and 17.8%, as well as an increase in circulating immune complexes' levels by 15.5 % and 14.6 %, respectively, was recorded. In the third experimental group of both fish species, serum lysozyme activity tended to increase (Table 5).

Table 4 — Indicators of the functional state of the cellular link of nonspecific resistance of pond fish under the influence of stress factor and the use of cryobiological supplement 'Immunolife-Fish' (n = 5, x ± SD)

Group	Phagocytic activity, %		Phagocytic index, units		Phagocytic number, units	
	Common carp	Silver carp	Common carp	Silver carp	Common carp	Silver carp
Control	26.34 ± 1.02	31.22 ± 0.94	3.18 ± 0.26	4.12 ± 0.28	5.36 ± 0.19	6.11 ± 0.18
I (stress)	18.59 ± 0.38*	24.10 ± 0.67*	2.42 ± 0.14	3.25 ± 0.18	4.06 ± 0.16*	4.73 ± 0.13*
II (supplement)	30.24 ± 1.38	34.99 ± 1.12	3.63 ± 0.11	4.58 ± 0.21	6.06 ± 0.21	6.77 ± 0.17
III (stress + supplement)	29.39 ± 0.84	34.15 ± 1.08	3.52 ± 0.16	4.54 ± 0.11	5.94 ± 0.14	6.69 ± 0.12

Note. * — $p < 0.05$ relative to control.

Table 5 — Indicators of the humoral link of the innate immunity of pond fish under the influence of stress factors and application of cryobiological supplement 'Immunolife-Fish'

Group	Serum lysozyme activity, %		Seromucoids, mg/cm^3		Circulating immune complexes, mg/cm^3	
	Common carp	Silver carp	Common carp	Silver carp	Common carp	Silver carp
Control	29.78 ± 1.68	31.36 ± 1.84	0.180 ± 0.010	0.170 ± 0.020	0.330 ± 0.018	0.360 ± 0.024
I (stress)	20.13 ± 1.13*	22.01 ± 1.63*	0.257 ± 0.017*	0.237 ± 0.013*	0.241 ± 0.011*	0.261 ± 0.018*
II (supplement)	34.63 ± 2.06	36.94 ± 2.16	0.185 ± 0.014	0.178 ± 0.011	0.381 ± 0.017	0.412 ± 0.013
III (stress + supplement)	31.27 ± 1.74	32.92 ± 1.48	0.188 ± 0.016	0.179 ± 0.015	0.316 ± 0.015	0.344 ± 0.012

Note. * — $p < 0.05$ relative to control.

Discussion. One of the areas of further intensification in aquaculture is to increase the natural resistance of fish to biotic and abiotic factors (Rudenko, Vishchur and Kovalenko, 2016; Hrytsyniak and Gurbyk, 2016; Solopova, 2020), particularly through the use of phyto-preparations that have appetite-stimulating, anti-stress, and immunostimulating effects. The aquaculture industry is increasingly favoring phytomedicine-based methods/compounds to develop resistance in farmed fish because they are inexpensive and safe for the environment (Semwal, Kumar A. and Kumar N., 2023).

Our studies have proven the positive effect of using 'Immunolife-Fish', produced by the technology of cryo-grinding a mixture of medicinal plants: golden root (*Rhodiola rosea*) roots and rhizomes, hawthorn (*Crataegus*) flowers, licorice (*Glycyrrhiza glabra*) roots, pine (*Pinus*) needles, and purple coneflower (*Echinacea purpurea*) roots on the growth of live weight of young common carp. Absolute growth rates, in a certain sense, reflect the growth rate of animals and are of great practical importance, as they allow us to compare actual results with planned ones and control the fulfillment of tasks. A

t the same time, juvenile fish grow unevenly, so that absolute weight gain does not accurately reflect the intensity of actual growth processes, namely the ratio between body weight gain and growth rates. Therefore, relative growth rates were determined. The analysis of the calculated data allows us to state the positive effect of the use of the cryobiological supplement 'Immunolife-Fish' on the productivity of juvenile common carp, which is most pronounced in the first 42 days of feeding.

According to Effendi et al. (2022), the greasy grouper (*Epinephelus tauvina*) fed a diet supplemented with a mixture of methanolic herbal extracts (*Cynodon dactylon*, *Piper longum*, *Phyllanthus niruri*, *Tridax procumbens*, and *Zingiber officinalis*) had 41% more weight than control fish. The increase in weight gain is attributed to improved digestibility and availability of nutrients with the introduction of plant extracts, which leads to increased feed conversion and increased protein synthesis in fish (Mohammadi et al., 2020). It has been proven that medicinal plants activate digestive enzymes, thereby increasing the growth of fish and animals. For example, an artemium-rich herbal diet improves the survival and growth rate of the Indian prawn, *Fenneropenaeus indicus* (Zhu, 2020). The herbal components of traditional Chinese medicine have a beneficial effect on carp growth, in particular, feed supplemented with herbs increases the efficiency of fish digestion (Reverter et al., 2017). The activation of metabolic processes can also contribute to the growth of fish weight (Deren, 2009).

As mentioned earlier, medicinal plants also have antibacterial, antifungal, and immunostimulating properties (Korylyak, 2015; Mohammadi et al., 2020). The analysis of our data obtained in the study of the effect of the cryobiological supplement 'Immunolife-Fish' on the organism of pond fish of the carp family allows us to assert that a compensatory increase in the number of leukocytes was found in the blood of common carp and

silver carp of the experimental groups I exposed to the stress factor. At the same time, in fish that received a supplement of the herbal preparation during stress, this indicator only tended to increase. In addition, a mild stimulating effect of the cryobiological supplement on fish of the second group, which was kept in optimal conditions, was recorded.

Leukocytes are the main protective elements and a key part of immunity, and their increase in fish can indicate both the development of inflammatory processes against the background of stress (groups I of common carp and silver carp) and an increase in the activity of non-specific immunity (groups II and III). This fact is supported by data on the activity of phagocytosis, which is the main protective function of leukocytes (Honcharov, 2019).

We found that the phagocytic activity of blood leukocytes of common carp and silver carp treated with the drug (groups II and III) was higher than that of fish in the control groups. At the same time, the phagocytic number, which indicates the average number of microorganisms per active phagocyte, and the phagocytosis index, which characterizes the number of microorganisms captured by an active phagocyte, were also increased in the blood of both fish species of groups II and III. These data indicate an increase in the activity of the cellular link of non-specific resistance in the blood of these fish. On the contrary, the data on phagocytosis activity in the body of common carp and silver carp of group I indicate a suppression of the functional capacity of the cellular link of non-specific resistance, which can negatively affect the condition of fish and lead to the development of pathological processes.

According to the literature, *Astragalus* and *Lonicera* extracts increased the production of intracellular superoxide anion by tilapia leukocytes (Ardó et al., 2000). *Lonicera* flower extract contains many different active components, one of them, chlorogenic acid, can activate macrophages through the calcineurin pathway (Effendi et al., 2022) and act as a macrophage activator *in vivo* (Mohammadi et al., 2020) and thereby activate the cellular link of fish innate immunity.

The application of herbal products also stimulates humoral factors of non-specific immunity (Mohammadi et al., 2020; Ibrahim et al., 2021). It should be noted that lysozyme activity is an integral factor of the body's natural resistance of the humoral type, which indicates the blood's ability to self-purify (Pukalo et al., 2008). The formation of circulating immune complexes is a natural defense mechanism of the body; it promotes the rapid elimination of endogenous and exogenous antigens through phagocytosis and the reticuloendothelial system. The study of the level of immune complexes in blood serum is important for the diagnosis of acute inflammatory processes and type 3 allergic reactions when the level of circulating immune complexes can increase, as well as for determining the effectiveness of treatment (Solopova and Vishchur, 2020).

The increase in serum lysozyme activity and circulating immune complexes in the organism of common carp and silver carp treated with the cryobiological supplement 'Immunolife-Fish' indicates the self-regulation of the immune system against the background of the use of a preparation rich in biologically active substances and a significant increase in the factors of nonspecific resistance of the experimental fish, especially against the background of the negative effect of the stress factor. Similar results were obtained by other researchers. For example, in the Nile tilapia (*Oreochromis niloticus*), which was fed with mistletoe (*Viscum album coloratum*) for 80 days, an increase in lysozyme and complement activity, phagocytic activity was recorded, which provided a 42% increase in survival when infected with the bacterial pathogen *Aeromonas hydrophila* (Park and Chai, 2012), and a similar effect was observed with the use of the Asteraceae plant *Eclipta alba* in the Mozambique tilapia (*Oreochromis mossambicus*) (Christybapita, Divyagnaneswari and Dinakaran Michael, 2007).

In addition, fish treated with herbal medicine, in particular, an alcoholic tincture of *Echinacea purpurea*, showed higher levels of red blood cells, lymphocytes, monocytes, and hemoglobin (Reverter et al., 2017; Deren, 2009). The study of the action of pure garlic components allicin and ajoene in aquaculture demonstrated their immunostimulatory ability and effectiveness against the pathogenic protozoa *Spironucleus vortens, Ichthyophthirius multifiliis* and the bacterium *Aeromonas hydrophila* (Millet et al., 2011; Nya et al., 2010; Tanekhy and Fall, 2015).

Ethanol extract from green tea added to the diet also improves lysozyme activity, lipid utilization, and recovery from stress, and reduces total cholesterol levels in black rockfish, *Sebastes schlegeli* (Hwang et al., 2013). *Astragalus* extract, the main active ingredients of which are polysaccharides, as an immunostimulant increased plasma lysozyme activity (Hanif et al., 2005; Kim and Austin, 2006).

Conclusions. It has been established that the cryobiological supplement 'Immunolife-Fish', produced using the technology of cryo-grinding of a composition of medicinal plants: golden root (*Rhodiola rosea*) roots and rhizomes, hawthorn (*Crataegus*) flowers, licorice (*Glycyrrhiza glabra*) roots, pine (*Pinus*) needles, and purple coneflower (*Echinacea purpurea*) roots in the amount of 1.5% of the main feed for 8 weeks increased the live weight gain of juvenile common carps by 5.2% in comparison with the control values.

Feeding the cryobiological supplement 'Immunolife-Fish' for 10 days against the background of heat stress to two-year-old common carp and silver carp leads to an

increase in the number of leukocytes by 22.2% and 19.6%, respectively, and activation of the phagocytic activity of leukocytes by 14.8% and 12.1%, respectively, compared to the control values.

The use of the supplement contributed to an increase in serum lysozyme activity by 16.3–17.8% and the level of circulating immune complexes by 14.6–15.5%. A mild stimulating effect of the cryobiological supplement was also observed in fish kept under optimal conditions.

Thus, the herbal food supplement 'Immunolife-Fish', produced using cryo-grinding technology, helps to increase the productivity and non-specific resistance of the organism and to adapt the fish body to the stress factor.

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