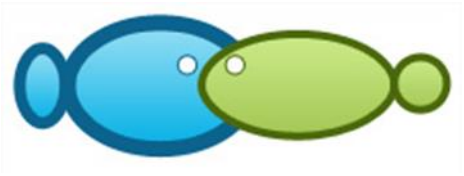

The effect of Sviteco PWC probiotic product on growth, physiological and biochemical status and non-specific resistance of sturgeon fish juveniles

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Abstract. Modern methods of industrial fish farming involve intensive technologies that do not always correspond to the physiological characteristics of the fish organism. This is especially relevant when cultivating sturgeon in recirculating aquaculture systems (RAS), where a number of technological factors affect the fish organism, which causes the development of oxidative stress and leads to a decrease in resistance and an increase in lethality. Therefore, increasing the viability and ensuring high growth intensity of sturgeons at all stages of their cultivation is one of the most urgent problems in fish farming. In recent years, in order to increase the immune potential, viability and efficiency of the growth processes of sturgeon juveniles, probiotic products, among which *Bacillus* strains occupy a prominent place, have been successfully used. In view of this, the aim of this work was to find out the effect of Sviteco PWC probiotic product, which contains *Bacillus subtilis*, *B. pumilus*, *B. licheniformis* and *B. amyloliquefaciens* complex, on growth, efficiency of feed consumption, physiological and biochemical status of the organism and the activity of natural factors of protection of hybrid sturgeon (♀ bester (♀ *Huso huso* X ♂ *Acipenser ruthenus*) and ♂ freshwater sterlet (*Acipenser ruthenus*)) juveniles. The study was conducted in two groups of fish (control and experimental, 30 individuals in each). For 28 days, the sturgeons of the control group consumed the commercial feed Star Alevin from Alltech Coppens, while the individuals of the experimental group received Sviteco PWC probiotic complex in the amount of 0.7 cm^3 x kg^{-1} feed together with this feed. After the experiment, which lasted for 28 days, the growth rate, feed conversion ratio, and survival rates were evaluated. To determine the biochemical profile of the fish organism, the following parameters were tested: liver - amylase activity, alkaline phosphatase and protein content; blood serum - the content of phosphorus, calcium, total protein and the ratio of its fractions, albumin, urea, triacylglycerols, cholesterol, as well as alkaline phosphatase activity. The activity of natural protective factors was assessed by lysozyme activity of blood serum, phagocytosis indicators and the content of circulating immune complexes. The conducted studies showed that the use of Sviteco PWC probiotic complex caused an increase in the average weight of the sturgeons of the experimental group compared to the control group by 9.57%, and also improved the adaptation potential of their organisms due to an increase in amylase activity in the liver by 6.75%; on the contrary, it led to a decrease in the intensity of dephosphorylation processes, as evidenced by a 27.02% decrease in alkaline phosphatase activity levels ($p < 0.05$) in the hepatopancreas of the fish. Determination of the biochemical parameters in the blood serum confirmed the ability of the study probiotic microorganisms to maintain the homeostasis of the organism, improving the functioning of its individual parts, in particular, the protein synthesis function of the liver of sturgeons while increasing the activity of metabolic processes. The activating effect of the probiotic product Sviteco PWC on the state of cellular and humoral factors of non-specific resistance of the fish organism was established. This is evidenced by the tendency to increase in lysozyme and phagocytic activity and decrease in the content of circulating immune complexes in the blood of fish.

Key Words: Acipenseridae, *Bacillus*, non-specific resistance, physiological and biochemical status, probiotic, sturgeon.

Introduction. The organism of sturgeons during the early ontogenesis undergoes significant step-by-step physiological, biochemical and morphological changes, which makes it particularly sensitive to environmental factors (Zoltowska 1999; Williot et al 2018). The intensification of the cultivation of these fish species in recirculating aquaculture systems (RAS) is accompanied by a significant number of stress factors, including high stocking density, increased concentration of biogenic compounds in water, artificial feeds and bacterial pressing (Kamaszewski et al 2014). Previous studies have not identified which genome transcription factors affect larval mortality (Lebeda et al 2020). As a result, due to mortality and morbidity, there is a decrease in the profitability of cultivation of these valuable fish species.

In order to improve the health and increase the viability and efficiency of the growth processes of fish juveniles, probiotic products are used (El-Saadony et al 2021; Rohani et al 2022; Frishtak et al 2022; Arun 2023). Probiotics are characterized by their antimicrobial activity against pathogenic and opportunistic bacteria, immunomodulatory and anti-inflammatory functions, as well as the ability to influence intestinal motility and digestive processes of the host's organism (Kuebutornye et al 2019; Zaloilo et al 2021). Their effect on the activation of growth processes and the metabolism of fish juveniles is due to their direct effect on the expression of insulin-like growth factor mRNA (IGF-1) (Arun 2023). The analysis of literature data showed that probiotic microorganisms from the *Bacillus* genus are quite often used, since they are natural members of the intestinal microbiota of fish and can transfer some of their abilities to the host organism (Kuebutornye et al 2019; Anokyewaa et al 2021). *Bacillus velezensis* (Kang et al 2022), *B. albus*, *B. safensis* (Kim et al 2021), *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. amyloloquefaciens*, and *B. megaterium* (Kuebutornye et al 2019) are used in aquaculture. They are used in the form of both individual strains and complexes, which include representatives of other genera (Puvanasundram et al 2021). The immunostimulating effect of SFSK4 probiotic complex containing strains of *B. licheniformis*, *B. amyloloquefaciens*, *B. subtilis* and *Pseudomonas* sp., which was used in the process of growing whiteleg shrimp (*Litopenaeus vannamei*) has been proven (Fernandes 2021).

Despite the long-term research of probiotics in aquaculture, in particular in sturgeon fish farming, the issue of the efficacy of selecting appropriate probiotic microorganisms to ensure the growth, survival and resistance of sturgeon juveniles in intensive aquaculture conditions remains relevant. The purpose of our study was to find out the physiological and biochemical mechanisms of protection in fingerlings sturgeon fish in the process of growing them in RAS under the influence of Sviteco PWC complex probiotic product containing *Bacillus subtilis*, *B. pumilus*, *B. licheniformis*, *B. amyloliquefaciens* and enzymes.

Material and Method

The fish and the ration. The object of the study was sturgeon juveniles, namely a hybrid of a bester ♀ (♀ *Huso huso* X ♂ *Acipenser ruthenus*) and a ♂ freshwater sterlet (A*cipenser ruthenus*). Experimental rearing of sturgeon juveniles in duplicate was carried out in RAS on the basis of the Lviv Research Station of the Institute of Fisheries of the National Academy of Agrarian Sciences of Ukraine in August 2022. A number of 30 specimens of fish with an average weight of 30.69±1.12 g was planted in each pool with an area of 1 m^2. The water temperature was maintained within 20.0±1.0°C. The content of ammonium nitrogen, nitrites and nitrates did not exceed permissible concentrations. The average saturation of water with oxygen was 70%. The duration of the experiment was four weeks – 28 days. Feeding was carried out with commercial feed Star Alevin from Alltech Coppens.

The complex probiotic product Sviteco PWC contains four strains of bacteria *Bacillus* genus in equal amounts: *B. subtilis*, *B. pumilus*, *B. licheniformis* and *B. amyloliquefaciens*, as well as 1% of enzymes. The average number of cells in the working solution was $1x10^9$ CFU mL^{-1}. The working solution was prepared by diluting 0.7 mL of the drug concentrate in 50 mL of water heated to a temperature of 35°C. The solution was applied in an even layer on the feed before feeding at the rate of 50 mL of the working preparation per 1 kg of feed, which was fed to the experimental group of fish, which was further designated as "probiotic". The fish of the control group consumed feed without the use of probiotics and were designated as "control".

Sampling and performance of fish growth. The studied feed was fed to the fish for 28 days. After that, the fish were anesthetized with clove (*Syzygium aromaticum*) oil at a concentration of 60 mg L^{-1}, their blood and liver were collected for testing. Sampling for studies was carried out in compliance with national and international standards for conducting experiments and experimental processing of fish for scientific purposes. During cultivation, every 7 days the following indicators were studied:

$$W = W_f - W_o$$

where: W = weight gain (g);
W_f = final weight (g);
W_0 = initial weight (g).

$$SGR = \frac{LnWf - LnWo}{t} \times 100$$

where: SGR – specific growth rate (% day^{-1}),
W_f = final weight (g);
W_0 = initial weight (g);
t = period of time (day).

$$FCR = \frac{F}{Wf - W0}$$

where: FCR = feed conversion ratio;
F = the weight of consumed feed (g);
W_f = weight of fish at the end of the feeding period (g);
W_0 = weight of fish at the beginning of the feeding period (g).

$$SR = \frac{Nf}{N0} \times 100$$

where: SR = survival rate (%);
N_f = final number of fish (specimens);
N_0 = initial number of fish (specimens).

Biochemical tests. Blood and liver samples served as material for biochemical tests. Liver samples were subject to freezing in liquid nitrogen. After defrosting, the tissue was homogenized in cold-blooded Ringer's solution and centrifuged at a speed of 10 g for 15 min. The activity of the studied enzymes was determined in the supernatant of the homogenate. The protein content in liver samples was determined by the Lowry method, which is based on the use of Folin`s reagent (Lowry et al 1951).

The activity of α-amylase (EC 3.2.1.1.) was determined by the Caravey method based on a decrease in the optical density of the substrate solution after its hydrolysis by an enzyme. For this a ready-made set of reagents from Reagent company were used (TU U 24.4-13433137-050:2006, 2016).

The activity of alkaline phosphatase (EC 3.1.3.1) was determined by the reaction with phenylphosphate using a ready-made set of reagents from Reagent company (TU U 24.4-13433137-047-2003, 2016).

Determination of biochemical indicators of sturgeon blood serum (cholesterol (C), triacylglycerol (TG), calcium, phosphorus, albumin, urea content, alkaline phosphatase activity (EC 3.1.3.1) was performed on the Humalyzer 2000 biochemical analyzer.

Electrophoretic tests of sturgeon blood serum proteins were carried out by electrophoresis on acetate cellulose films (Knuppel et al 1984).

Hematological and immunological analysis. After anesthetizing the fish, blood was collected from the tail vein. The phagocytic reaction in heparin-stabilized blood was assessed by phagocytic activity (PA), phagocytic index (PI) and phagocytic count (PC). Lysozyme activity (LABS) was tested in blood serum by the nephelometric method.

Determination of LABS and PA was carried out considering the specific features of experiments in hydrobionts, using the relevant daily cultures of laboratory strains and the temperature regime of cultivation. The content of circulating immune complexes (CIC) in blood serum was determined by precipitation with polyethylene glycol with a molecular weight of 6000. All biochemical and immunological studies were performed according to the procedures described in Vlizlo et al (2012).

Statistical analysis. The obtained results were analyzed using the Statistica Ver. 12.0 software. The probability of a difference in the results was determined using a One-way analysis of variance (ANOVA) using Tukey`s comparison test. The normality of data distribution was analyzed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. The Mann-Whitney test was used to assess the difference between individual non-parametric data. Significant difference was set at $p < 0.05$.

Results. The conducted studies showed that feeding the feed with the probiotic product Sviteco PWC had a stimulating effect on sturgeon growth (Table 1, $p < 0.05$). This is confirmed by the greater mass of fish in the experimental group compared to the control group throughout the experiment. At the same time, we did not find any significant differences between the average growth rates in the study groups, but there is a clear tendency to their increase in sturgeons of the experimental group.

At the end of the experiment, the body weight of the fish of the experimental group exceeded that of the control group by 9.57% ($p < 0.05$). Regarding the increase in SGR and decrease in FCR at the end of the experiment in sturgeons that consumed the probiotic product, we can only speak of trends, since the variance analysis did not reveal any significant differences between these indicators in the experimental and control groups.

During the study period, no mortality was detected in any of the test groups of fish, so the percentage of survival in all groups was 100%.

Table 1

Sturgeon growth rates and feed utilization efficiency under the influence of Sviteco PWC probiotic product (M±m, $p < 0.05$)

Experimental group	*Weight of fish at the beginning of the feeding period, g*	*Weight of fish at the end of the feeding period, g*	*Specific growth rate, % day^{-1}*	*Feed conversion ratio*
Probiotic	30.69±1.12^{a}	90.76±3.48^{a}	3.87±0.42^{a}	0.65±0.50^{a}
Control	30.52±1.24^{a}	82.83±1.56^{b}	3.55±0.14^{a}	0.70±0.50^{a}

The value is expressed as the arithmetic mean (M) ± the error of the arithmetic mean (m); different indices above the values are indicators of a significant difference between groups with a probability of $p < 0.05$.

After the completion of the 28-day experiment, the activity of two signaling enzymes was measured in the sturgeon's liver. The activity of the digestive enzyme α-amylase (hydrolase) allows to assess its hydrolytic potential. Alkaline phosphatase (transferase) is an intracellular enzyme, which participates in dephosphorylation, affects calcium metabolism in bone tissue and plays an important role in lipid and energy metabolism. The level of activity of both studied enzymes after the end of feeding with a probiotic complex product is presented in Table 2 ($p < 0.05$).

As can be seen from the data presented in Table 2, feeding the sturgeons of the experimental group with Sviteco PWC probiotic complex causes certain metabolic changes in the liver. Thus, an increase in α-amylase activity by 6.75% and a decrease in alkaline phosphatase by 27.02% were found ($p < 0.05$) in the fish of the experimental group. At the same time, the average value of the protein content in the liver of sturgeons of the experimental group compared to the control group tended to decrease.

Table 2

α-amylase and alkaline phosphatase activity and protein content in the liver of sturgeons after completion of the experimental feeding with Sviteco PWC probiotic complex

Indicators	*Groups of fish*	
	Probiotic	*Control*
α-amylase, mg x sec^{-1} x mg^{-1} protein	4.283 ± 0.512^{a}	4.012 ± 0.465^{b}
Alkaline phosphatase, μmol x sec^{-1} x mg^{-1} protein	0.848 ± 0.046^{a}	1.162 ± 0.078^{b}
Protein content, mg of protein x g^{-1} tissue	70.440 ± 3.614^{a}	79.317 ± 4.618^{a}

The value is expressed as the arithmetic mean (M) ± the error of the arithmetic mean (m); different indices above the values are indicators of a significant difference between groups with a probability of $p < 0.05$.

From the data presented in Table 3, we can see that feeding the sturgeons of the experimental group during the experiment with Sviteco PWC probiotic complex did not significantly affect the biochemical parameters of blood serum. At the same time, it should be noted that the content of triacylglycerols in the blood serum of the sturgeons from the experimental group was, respectively, 19.0% higher ($p < 0.05$), while the activity of alkaline phosphatase (AL) was 18.6% ($p < 0.05$) lower than in fish from the control group. The differences between the experimental and control groups of fish in terms of the content of albumin, urea, calcium and phosphorus in blood serum were improbable.

Table 3

The influence of Sviteco PWC probiotic complex on the biochemical parameters of sturgeons' blood serum

Indicators	*Groups of fish*	
	Probiotic	*Control*
Phosphorus, mmol L^{-1}	6.16 ± 0.26^{a}	5.60 ± 0.50^{a}
Calcium, mmol L^{-1}	2.20 ± 0.13^{a}	2.08 ± 0.06^{a}
Total protein, g L^{-1}	25.14 ± 1.11^{a}	26.92 ± 2.63^{a}
Albumin, g L^{-1}	30.70 ± 0.92^{a}	30.90 ± 1.41^{a}
Urea, mmol L^{-1}	2.20 ± 0.27^{a}	2.10 ± 0.20^{a}
Triglycerols, mmol L^{-1}	7.15 ± 0.32^{a}	9.56 ± 0.47^{b}
Cholesterol, mmol L^{-1}	1.55 ± 0.27^{a}	1.72 ± 0.27^{a}
Alkaline phosphatase, E L^{-1}	750.83 ± 8.55^{a}	622.72 ± 24.69^{b}

The value is expressed as the arithmetic mean (M) ± the error of the arithmetic mean (m); different indices above the values are indicators of a significant difference between groups with a probability of $p < 0.05$.

Protein exchange in an organism is an integrating link responsible for homeostasis, since the intracellular response to the action of exogenous factors is carried out with the participation of proteins. The content of the total protein and the ratio of its fractions in the blood serum characterize the degree of reactivity, allow to assess the state of protein metabolism and the level of their synthesis in the organism.

The fractional composition of blood serum proteins in different fish species is known to be a genetically determined heterogeneous system. Electrophoretic studies of sturgeon blood serum proteins showed that the use of the probiotic product caused certain changes in the ratio of protein fractions (Table 4).

Table 4

The ratio of individual protein fractions (%) in the blood serum of sturgeons used with Sviteco PWC probiotic complex

Experimental group	*Albumins*	*a_1-globulin*	*a_2-globulin*	*β-globulin*	*γ-globulin*
Probiotic	20.61 ± 1.84^{a}	23.38 ± 2.37^{a}	35.01 ± 3.66^{a}	15.12 ± 2.47^{a}	5.38 ± 1.10^{a}
Control	23.61 ± 0.74^{a}	24.51 ± 1.43^{a}	28.25 ± 2.68^{b}	14.5 ± 1.73^{a}	9.32 ± 0.37^{b}

The value is expressed as the arithmetic mean (M) ± the error of the arithmetic mean (m); different indices above the values are indicators of a significant difference between groups with a probability of $p < 0.05$.

The analysis of different blood fractions shows the absence of a probable difference between albumins, α_1-globulin and β-globulin fractions in individuals of the experimental group compared to the control group. Instead, trends were revealed that show the directionality of biochemical processes in the blood during the period of use of Sviteco PWC probiotic complex. It was found that the content of the α_2-globulin fraction of the blood of the sturgeons of the experimental group was 6.8% higher, and the content of the γ-globulin fraction was 3.9% lower than in the control group.

The state of natural defense mechanisms in the sturgeons of the experimental and control groups was assessed by indicators of cellular and humoral links of non-specific resistance (Table 5).

Table 5

Indicators of cellular and humoral links of non-specific resistance of the blood of sturgeons after feeding them with Sviteco PWC probiotic complex

Indicators	*Groups of fish*	
	Probiotic	*Control*
Lysozyme activity in blood serum (LABS), %	52.40±1.86[a]	54.86±1.56[a]
Circulating immune complexes (CIC), mmol dm^{-3}	36.40±3.15[a]	34.84±2.69[a]
Phagocytic activity (PA), %	44.80±0.86[a]	45.80±1.01[a]
Phagocytic index (FI), units	9.64±0.30[a]	9.58±0.42[a]
Phagocyte count (FC), units	4.32±0.19[a]	4.42±0.15[a]

The value is expressed as the arithmetic mean (M) ± the error of the arithmetic mean (m); different indices above the values are indicators of a significant difference between groups with a probability of $p < 0.05$.

As can be seen from the data presented in Table 5, feeding the studied probiotic complex to sturgeons did not cause significant changes in LABS, only trends to its increase were revealed. On the other hand, when studying another indicator of the humoral link of natural defense mechanisms, in particular, the content of circulating immune complexes, a tendency to their decrease under the influence of probiotics was recorded. At the same time, as with LABS, the differences in the content of CICs in relation to the fish of the control group turned out to be improbable. It is known that when the body's immune homeostasis is disturbed, CICs accumulate in blood vessels and can cause inflammatory processes.

The results of studies of phagocytosis indicators under the influence of the probiotic complex also indicate the absence of a significant effect on the cellular mechanisms of natural immunity. Similar changes were observed as in the study of the humoral link of the organism nonspecific resistance, as evidenced by the tendency to increase phagocytic and lysozyme activity in the blood of fish of the experimental group relative to the control group.

Discussion. The results of our studies are consistent with data from other autors on the positive effect of probiotics, which include the genus *Bacillus*, on the growth and non-specific resistance of yearlings of sturgeon and other fish species. Addition of probiotic strain *B. amyloliquefaciencs* and *Yarrowia lipolytica* to the main diet during 12 weeks of experimental feeding allowed to reliably increase the growth rate by 10.65% and body weight gain by 33.00% in sturgeon hybrid (*Acipenser schrenckii* X *Acipenser baeri*) fingerlings (Fei et al 2018). Also, under the conditions of feeding iridescent striped catfish (*Pangasianodon hypophthalmus*) fingerlings with feed containing a probiotic, which included a strain of *Bacillus subtilis* together with other microorganisms of *Enterococcus*, *Lactobacillus* genera and *Saccharomices* yeasts, a similar positive correlation was found between the product concentration in the feed and an increase in average weight of fish, their growth rate and decrease in FCR (Abdel-Latif et al 2023). The use of *B. licheniformis* as a probiotic for 60 days together with the addition of butyric acid had a positive effect on enhancing the body growth processes of trout yearlings. The authors associated this with the possibility of creating a favorable pH environment in the intestine of fish for the development of positive microflora and inhibition of pathogenic microorganisms (Taherpour

et al 2023). Also, the effect of *Bacillus* strains on improving the digestibility of protein substances, which increases the efficacy of hydrolytic and transport processes, is known.

In our studies, we found that the use of a complex of probiotic microorganisms based on four *Bacillus* strains contributed to the improvement of the histostructure of the intestinal surface of sturgeons, which led to an increase in the area of absorption and better digestion of nutrients. This was reflected in the intensification of growth processes, which manifested itself in a reliably higher body weight of the experimental group fish after 28 days (Table 1).

A number of biochemical processes occur in the liver during four weeks with using of a complex probiotic from four *Bacillus* strains, which support the metabolic homeostasis of the sturgeon's organism (Table 2). The increase in amylase activity in this organ indicates an increased potential for hydrolysis of sugars, in particular, in the process of breakdown of glycogen reserves. In a certain way, this feature increases the stress resistance in organism, ensuring its energy supply in the process of intensive growth. Alkaline phosphatase in the liver plays an important role in phosphate metabolism, bile formation, as well as in detoxification, contributing to the breakdown and excretion of toxic substances with bile. This enzyme ensures growth processes due to the biomineralization of cartilage and bone tissue, and its hepatic form indirectly plays an important role in this process (Orimo 2010). The activity of alkaline phosphatase in the liver of the experimental group fish is lower by 27% compared to the control group, which may indicate a decrease in the intensity of dephosphorylation processes. Understanding of the reasons for these processes requires more detailed further research.

The analysis of the biochemical parameters of the blood serum indicates an insignificant effect of the studied probiotic on the biochemical profile of the sturgeon's blood. Similar results were obtained when studying the effect of other probiotic products on the body of iridescent shark catfish juveniles (Abdel-Latif et al 2023). A more significant effect of probiotic products on biochemical indicators of blood serum was found in trout juveniles (Taherpour et al 2023). At the same time, a decrease in the activity of blood alkaline phosphatase was noted in the sturgeons of the experimental group compared to the control group, similarly to the measurements of this enzyme in the liver ($p < 0.05$). As already noted, this indicates a decrease in the intensity of dephosphorylation processes. It is known that this enzyme is located in cells in a bound state with plasma membranes. The entry of the enzyme from various tissues into the blood depends on the age and physiological state of the organism. Considering this, the decrease in the AP activity in the blood of the experimental group sturgeons compared to the control group can probably be explained by changes in the intensity of their growth (Table 1). The content of total protein in the blood serum of sturgeons from the experimental group tended to increase (Table 3). At the same time, it should be noted that, unlike its concentration in the liver, the content of total protein in the blood serum of the experimental group sturgeons was 7% higher than that of the control group individuals. Despite the fact that the differences were not probable, these data indicate a stimulating effect of the studied probiotic complex on the protein synthesis function of sturgeon liver. This can probably be associated with an increase in the activity of metabolic processes in sturgeon’s organism under the influence of the studied probiotic complex.

Analyzing individual fractions of blood proteins, it is worth noting that the fraction of α2-globulins, the amount of which is 6.8% higher in the experimental group, mainly includes proteins of the acute phase: α2-macroglobulin (involved in the development of infectious and inflammatory reactions), haptoglobin (forms a complex with hemoglobin released from erythrocytes during intravascular hemolysis), ceruloplasmin (specifically binds copper ions, and is also an oxidase of ascorbic acid, adrenaline, dioxyphenylalanine, capable of inactivating free radicals), as well as apolipoprotein B (participates in the lipid transport) (Christiansen et al 2015; Aversa-Marnai et al 2022). Blood serum γ-globulins are carriers of most immune bodies and reducing their number by 3.9% relative to the control group indicates a suppressive effect of the humoral link of fish immunity on this indicator. Also, it can serve as a compensatory factor for the trends we have established towards the increase of other indicators of the humoral and cellular link of non-specific resistance. It should be noted that a tendency towards an increase in the content of

albumins and a decrease in β-globulins was revealed in the blood serum of the sturgeons from the experimental group, compared to the control group. It is known that albumins play an important role in maintaining osmotic balance and serve as a source of amino acids for the organism. In addition, the transport of a number of both low- and high-molecular substances is associated with the content of albumins. Beta globulins contain transferrin, a protein that binds and transports iron ions (Subbotkin & Subbotkina 2016). Thus, the results of the conducted studies indicate that the use of the probiotic drug in sturgeons of the experimental group has a stimulating effect on the processes of protein biosynthesis.

Lysozyme is one of the most important humoral factors of the body's non-specific resistance (Biller et al 2021). Therefore, the assessment of lysozyme activity in sturgeon blood serum is an important informative indicator. This defense factor is part of such an integral indicator as the bactericidal activity of blood serum and, owing to its capability of directly influencing the cells of both the microorganism and the immune system, plays an important role in humoral protection. It is present in many biological fluids, is localized in the lysosomes of phagocytic cells – polymorphonuclear leukocytes and macrophages, from where it enters, in particular, blood serum. The antimicrobial properties of lysozyme are due to its ability to hydrolyze the β-1,4 bond between N-acetylglucosamine and N-acetylmuramic acid of the polysaccharide component of the cell wall of most Gram-positive and some Gram-negative bacteria. The main place in the organism protection against microorganisms is occupied by the mechanism of non-specific resistance of cellular protection – phagocytosis, which is formed faster in the process of ontogenesis than the humoral link of the immune response. Non-specific factors function independently or in combination, with the aim of active absorption of pathogenic particles with their subsequent cleavage with the participation of macrophages and neutrophilic granulocytes.

Analysis of the results of studies of natural defense mechanisms showed (Table 5) that feeding Sviteco PWC probiotic complex to the experimental group sturgeons during the 28 days of the experiment did not significantly affect the activity of individual indicators of cellular and humoral links of non-specific resistance. This is evidenced by the absence of probable changes in the content of CIC, lysozyme and phagocytic activity in the blood of sturgeons of the experimental group compared to the control group. At the same time, a tendency to increase of LABS and PA of blood granulocytes and decrease the content of CIC was revealed in the experimental group sturgeons. This may be due to the fact that lysozyme has not only antibacterial, but also immunoregulatory activity (Ragland & Criss 2017). The protein exhibits local immunoregulatory activity, which is manifested, in particular, by stimulating the adhesion of probiotic microorganisms to the surface of epitheliocytes, which, in turn, supports the functional activity of the intestinal microflora at the proper level. Lysozyme has particularly intense antiviral and antibacterial activity in the composition of granulocytes, monocytes and macrophages (Ferraboschi et al 2021).

Determining the CIC content in blood serum is important for assessing the metabolism in organism as a whole, III type allergic reactions, and the organism immune status. The number of CICs in the blood serum indicates the overall efficiency and balance of immune system reaction and allow detecting significant deviations in both cellular and humoral immunity before the appearance of clinical signs of pathology. An increase in the level of CICs in the blood serum indicates the development of the immunotoxicosis syndrome associated with a certain pathology. Antigen neutralization is one of the important physiological and biochemical functions of CICs. It has been established that the formation of CICs is an important response of the organism to the immune system activity (Patriche 2008). It has been shown that when the immune status of a fish organism is disturbed, CICs accumulate in blood vessels and can cause inflammatory processes. It has also been proven that the intensity of formation and biological activity of CICs largely depends on the nature and ratio of antibodies and antigens that are part of them (Vlizlo et al 2012). Any stressful influences that occur through metabolic disorders can lead to dissociation of the CICs with the release of potentially dangerous pathogenic factors (Broda et al 2014). One of the important characteristics of CICs circulating in the blood is the size of their molecules. It has been established that CICs formed with an excess of antigens have a relatively small molecular size and, for the most part, do not activate complement and do not cause inflammatory processes. CICs formed with an excess of antibodies are

able to activate complement, but their size is quite significant, they are quickly phagocytosed and have little pathogenicity. When the number of CIC molecules increases, they are deposited mainly in the cortical layer of the kidneys, causing complement activation and inflammatory processes (Ezeani et al 2021).

Conclusions. Based on the results of the analysis of the conducted experiment, it has been shown that the use of Sviteco PWC probiotic complex in the proposed quantity for 28 days improves the growth and health of hybrid sturgeon juveniles. This allowed to achieve an increase in the average weight of experimental group sturgeon by 9,57 % compared to the control. The use of a probiotic complex based on four *Bacillu*s strains contributes to the stress resistance of an organism due to the increased activity of sugar hydrolysis by 6.75% in the liver, but leads to a 27.02% decrease in dephosphorylation processes. Studies of biochemical indicators of blood serum confirmed the ability of probiotic microorganisms to maintain the homeostasis of an organism, improving the functioning of its individual parts, in particular, the protein synthesis function of the liver of sturgeons while increasing the intensity of metabolic processes. The studied concentration of Sviteco PWC probiotic complex for 28 days caused a tendency to increase the activity of cellular and humoral protective factors with a concomitant decrease in the relative content of γ-globulin fraction of sturgeon blood serum proteins.

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Conflict of interest. The authors declare that there is no conflict of interest.

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