Corrective effect of probiotics on the work of the fish body in industrial aquaculture

Elena Romanova[1*], *Vasily* Romanov[1], *Vaselina* Lyubomirova[1], *Ludmila* Shadyeva[1], *Tatyana* Shlenkina[1], *Elena* Turaeva[1], and *Alexander* Vasiliev[1]

[1]Ulyanovsk State Agrarian University named after P.A. Stolypin, Ulyanovsk 432017, Russia

Abstract. The world community associates the solution of the problem of food security with the development of aquaculture. The search for means of intensifying the production of a complete and easily digestible food protein in aquaculture is an important and urgent problem. Recently, probiotics, which have long been in demand in medicine, have been used in aquaculture to improve health, accelerate growth and improve the quality of fish in aquaculture. However, the correct choice of a probiotic for aquaculture is not an easy task, since in the modern world a wide range of probiotic preparations is produced in various forms, the dosage of which for aquaculture needs has not been developed, as well as the dosage and duration of their use have not been developed. Meanwhile, the use of probiotics in aquaculture can be expected to increase the production of marketable fish of improved quality. Probiotics for aquaculture should be resistant to gastric juice, able to colonize the intestines of fish, interact with the normobiota of the intestine and suppress pathogens. These properties are possessed by the probiotic "Sporotermin". It consists of freeze-dried spores of the bacteria Bacillus subtilis and Bacillus licheniformis. The content of viable spores in the preparation is not less than 3x10/9 CFU/g. The research results showed that the use of the probiotic "Sportermin" in aquaculture has a healing effect on fish, normalizes the structure of intestinal and liver tissues, affects the leukogram, activates the immune response system, reduces the level of oxidative stress, activating antioxidant defense enzymes and reducing the level of malondialdehyde. The research was carried out on the instructions of the Ministry of Agriculture of the Russian Federation.

1 Introduction

The problem of food security is invariably one of the most urgent; The global scale of the food problem was clearly identified in the 20th century [1]. The result of mankind's awareness of the importance of this problem was the creation of the Food and Agriculture Organization (FAO) [2] and the report of experts to the Rome Club, developed in 1972, which outlined the "Limits to Growth", when the intensification of agriculture almost reached its limit [3].

* Corresponding author: vvr-emr@yandex.ru

Nowadays, aquaculture is considered as the main segment of agricultural production, on which the solution to the problem of food security depends [4]. Feed costs for the production of food protein in aquaculture are 5 times less than for the production of the same amount of beef [5].

The development of aquaculture is necessary to achieve the goals of sustainable development of mankind. The outstripping pace of aquaculture development is global and outpaces population growth. The sustainable development of civilization in terms of the production of animal protein depends on the sustainable development of aquaculture, which is the future. The potential for the development of aquaculture has not been fully disclosed to date [6, 7].

Aquaculture has become a public policy priority in most developed countries. Today, more than 50% of the fish consumed by the population is grown in aquaculture. The leaders in this area are China, Vietnam, Norway

Fish and fish products in the conditions of modern civilization are the most demanded food product, not only because it is a historically established pattern, but also because of their unique nutritional value. Fish and fish products in the modern world are classified as staple foods [9].

The world's largest giants in the production of feed for aquaculture are developing high-tech technologies for designing high-quality innovative feeds of a new generation that increase the growth rate and feed conversion, correct the functioning of the fish body, reduce the risk of developing diseases, and improve the quality and nutritional value of fish [10-12].

FAO has established global principles of aquaculture for the safety of fish products and the environment, which include: the health of cultivated objects, food safety, environmental protection, socio-economic aspects [13]. To implement these principles, you need to pay attention to probiotics.

Probiotics in aquaculture are used to improve the microbiocenosis of the fish habitat, to protect against pathogenic microbiota, to normalize the intestinal microbiocenosis of fish, as an alternative to antibiotics and as a source of biologically active substances [14-16].

With artificial breeding, a high level of water pollution by metabolic products of fish is noted, i.e. organic. This creates favorable conditions for the development of opportunistic and pathogenic microbiota, which is dangerous for cultivated fish.

In the digestive system of fish, the microbiota freely drifts along with the contents of the digestive tract, is present as a biofilm on the surface of the epithelium, or is concentrated in the crypts of the intestinal mucosa. Each type of intestinal microbiota has its own functional specialization [17, 18].

It must be understood that the microbiota of the digestive tract is directly or indirectly involved in all metabolic processes of the host organism. Its main function is to protect the host organism from pathogenic and conditionally pathogenic organisms. This happens by stimulating the barrier function of the intestinal epithelium against pathogens and is due to the fact that the immune system of fish coevolved in interaction with the symbiotic microbiota. As a result, protective reactions to pathogens in the body of fish are produced by a complex: the immune system-microbiota [19].

The stability of the microbiota system - the host organism ensures the normal course of metabolic processes and the constancy of the internal environment of the fish organism. Today it is known that the microbiota is an epigenetic factor that changes the gene expression of the host organism.

We have already noted that probiotics are antagonists of pathogens. By using probiotics in aquaculture, you can avoid the need for antibiotics and reduce the level of microorganisms in the aquatic environment.

Probiotics are also effective in restoring the microbial ecology of the whole fish organism [20]. It is known that probiotic microbiota penetrates from the gastrointestinal tract into various organs and systems of the host organism. This explains the systemic effect of probiotics [21]. Having completed the life cycle, the microbiota becomes a source of protein for the host organism.

The aim of the work is to investigate the mechanisms by which the microbiota of probiotics corrects the work of the fish organism.

2 Materials and methods

The studied object was a catfish – Clarias gariepinus. From five-month-old individuals, two groups of 50 individuals weighing 900-1000g were formed. The fish were kept in polypropylene containers, at 24-26єC and 90% oxygen saturation. The water was purified by quartz sand filters. The daily water change in the pools was 25%.

The control group was grown on specialized feed brand "Som" by LimKorm (Russia), the experimental group, in addition to feed brand "Som" by LimKorm, additionally received the probiotic "Sporotermin" in the amount of 2 g/kg of feed. The probiotic was dissolved in water, then irrigated with a solution of the probiotic and dried in a thermostat at 30єC. "Sporotermin" refers to the latest generation of probiotics, created from spore forms of cultures of microorganisms Bacillus subtilis and Bacillus licheniformis with a filler - lactose. The duration of the experiment was 3 months.

Upon completion of the experiment, the histological structure of the intestines and liver of catfish was studied. The material for histological studies was obtained in triplicate. The samples were fixed with formalin, dehydrated, embedded in paraffin, cut, stained with Mayer's hematoxylin-eosin, and placed in mounting medium [22].

The preparations obtained were analyzed using a ZEISS Axio Imager 2 direct microscope at the Research Center of Ulyanovsk State Pedagogical University.

Blood for research was taken from the tail artery of the catfish. The smears were stained according to Pappenheim.

In smears, the percentage ratio of morphologically different functional groups of leukocytes was calculated. Leukocyte cells were identified by their morphological features according to the classification of N.T. Ivanova (1983) using a Keyence VHX 1000E microscope with a Z500 objective [23].

The study of antioxidant defense enzymes was carried out using a UV-1800 spectrophotometer (Shimadzu). The activity of superoxide dismutase and catalase was given in nmol substrate/min per mg of protein.

The enzymatic activity of catalase was evaluated according to the method of Aeby, (1984) [24]. The enzymatic activity of superoxide dismutase (SOD) in the blood was assessed by the method of Giannopolitis, Ries, (1977), [25].

The determination of malondialdehyde in catfish muscles was carried out on a Hitachi AAA 835 analyzer in the laboratory of Ulyanovsk State University.

3 Research results

The efficiency of the process of growing commercial fish in aquaculture depends on the quantity and quality of feed consumed and their digestibility. An important role in this is played by the state of the digestive system of fish. Therefore, at the 1st stage, we examined the state of the catfish digestive system. For this, histological studies of the intestinal wall were performed. The results of the studies showed that the rearing of fish in industrial aquaculture is characterized by pathological changes in the intestinal wall (fig. 1). Changes in the muscular layer of the intestinal wall, serous membrane were recorded. Thinning and destruction of the villi, a decrease in their density, contacts, length and branching were revealed. There are areas of thinning and edema of the mucosa proper, which are interspersed with areas of edema of the muscular layer and serous membrane (fig. 1).

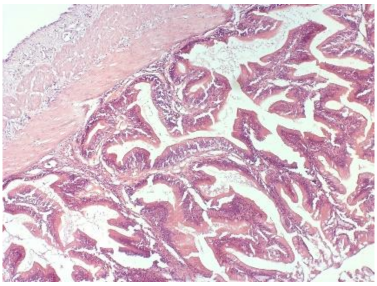

Fig. 1. Intestinal wall (hematoxylin+eosin, 10x40.). Destruction, shortening and weak villi branching. Muscle layer edema, swollen serosa.

At the next stage of our work, we studied the state of the liver in catfish grown in industrial aquaculture. The liver was chosen for the study not by chance, but because it is sensitive to changes in metabolism and can be considered as an indicator of the state of the body.

The liver is an external secretion gland that performs many important functions in the body of fish. It neutralizes foreign substances entering the body, excessive amounts of its own hormones, mediators, toxic metabolites such as ammonia, ethanol, acetone, ketone acids, etc. The liver regulates carbohydrate metabolism, replenishes the energy needs of the body, synthesizing glucose from non-carbohydrate components, stores glycogen. The liver accumulates fat-soluble and water-soluble vitamins and is their storage, it is involved in the metabolism of vitamins and folic acid. The liver is a regulator of lipid metabolism, synthesizes cholesterol, various lipids, hormones, bile acids, bilirubin, produces and secretes bile; is a blood depot. This is not a complete list of liver functions.

Our histological studies of the liver of fish grown in industrial aquaculture have revealed pathological changes in the liver parenchyma in a number of cases. At fig. 2 reveals fatty vacuolization in combination with signs of parenchymal necrosis.

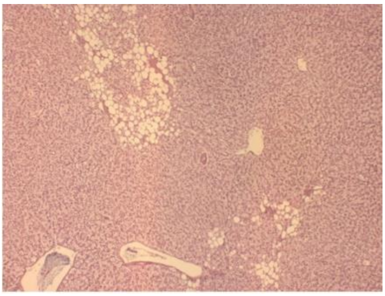

Fig. 2. Liver parenchyma of fish reared without probiotics (hematoxylin-eosin 10x10).

When analyzing a series of histological preparations, fatty degeneration, necrosis of hepatocytes, hemorrhage, loss of polarity of the hepatocyte nucleus were found; erythrostasis was observed in the vessels, indicating blockage of the vessels.

We did not conduct deeper histological studies, since we were faced with the task of identifying the presence or absence of intestinal or liver pathology in fish grown in industrial aquaculture, and not the specifics of this pathology.

The results showed that growing fish in an artificially created environment is accompanied by pathological changes in cells, intestinal and liver tissues, which certainly require adjustment. Fish with such a pathology poorly consumes and digests feed, grows slowly. To correct the revealed pathology, the probiotic "Sporotermin" was used, containing spore forms of bacteria B. subtilis and B. licheniformis.

Analysis of histological preparations of the intestines of a catfish (Clarias gariepinus), which received a probiotic for three months, showed that the microbiota of the probiotic had a positive effect on the histological structure of its intestinal wall (fig. 3).

On the preparations of the intestinal wall of fish treated with the probiotic, loose connective tissue lamina propria mucosae, penetrated by blood capillaries, is visible. Outside is a single-row stratified integumentary epithelium. It consists of enterocytes, glandular sac-like cells, basal cells, villi and crypts. The mucous membrane is separated from the Tela submucosa by a muscular layer. Tela submucosa consists of amorphous connective tissue, which is surrounded by blood and lymphatic vessels. This structure of the intestinal wall corresponds to the norm (fig. 3).

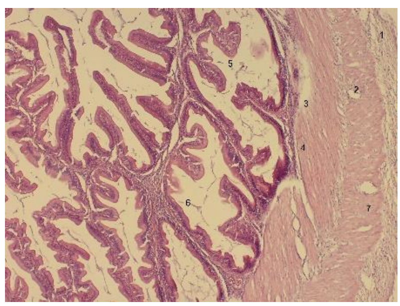

Fig. 3. Fragment of the intestinal wall of the catfish treated with probiotic (hematoxylin-eosin; 10x10); 1 – serous membrane, 2 – blood vessel, 3 – submucosa, 4 – inner muscle layer, 5 – mucous membrane, 6 – intestinal lumen, 7 – outer longitudinal muscle layer. Without pathology.

Analysis of the research results allowed us to conclude that the introduction of the probiotic "Sporotermin" into fish feed had a corrective effect on the cellular and tissue structures of the intestinal wall and improved it.

In fish treated with the probiotic, the histological structure of the liver was normal (fig. 4).

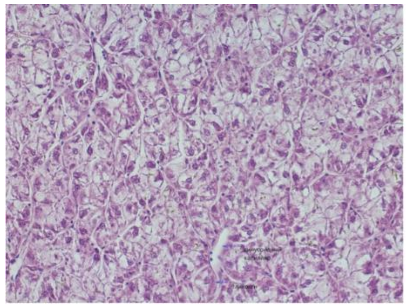

Fig. 4. The structure of liver parenchyma of catfish treated with probiotic (hematoxylin-eosin; 10 x40). Without pathology.

https://doi.org/10.1051/e3sconf/202236303066

The results of the studies showed that the probiotic "Sporotermin" had a pronounced healing effect and normalized the structure of cells and liver tissues of catfish grown in industrial aquaculture.

When breeding fish in artificial conditions, it is necessary to monitor the health of the fish. The blood system is the most informative in this regard. In determining the indicator indicators of health, an important role is assigned to the leukogram. All groups of leukocytes are immunocompetent cells and protect the body from foreign invasion. At the same time, each group of leukocytes performs its evolutionarily developed functions.

Practical experience shows that under conditions of artificial breeding at high stocking densities, fish develop chronic stress, which reduces the stability of their immune system. The structure of the leukogram makes it possible to obtain information about the state of health of fish even at the initial stages of the pathological process. We set the task to evaluate the effect of the microbiota of the probiotic "Sportermin" on the structure of the leukogram, i.e. on the white blood cells of fish responsible for immunity.

In catfish, the leukogram is of the lymphocytic type. In our studies, the proportion of lymphocytes in fish grown without probiotics reached 76.8%. Lymphocytes detect and destroy everything foreign in the body. These cells are responsible for immunocompetent reactions, their regulation and immune imprinting. The results of the studies showed that the proportion of lymphocytes was higher in fish treated with the probiotic, reaching 81.3% (fig. 5).

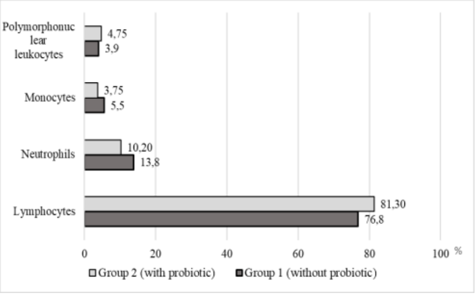

Fig. 5. Leukocyte formula of catfish blood cells

Consider monocytes. It carries out phagocytosis of dead cells and their fragments, as well as microorganisms in the body. In fish treated with the probiotic, monocytes were 3.75%. When growing fish without any additives, there were more monocytes. It accounted for 5.5%.

Our previously published studies have shown that the use of probiotics in aquaculture can reduce the level of bacterial water pollution in pools by 4 orders of magnitude [26]. It cannot be ruled out that this phenomenon was the trigger that led to the redistribution of leukocytes in the leukogram.

Consider neutrophils. Their production in the body is controlled by cytokines. These cells are part of the innate immune system and have the highest ability to penetrate tissues (compared to other leukocytes) and phagocytize decay products, pathogenic microbiota and fungi. At the fish. those not treated with the probiotic had 13.8% neutrophils, while the fish raised with the probiotic had 10.2%. It can be assumed that the introduction of the probiotic reduced the body's need for this group of leukocytes.

Polymorphonuclear cells. In fish treated with the probiotic, we observed a trend towards an increase in the proportion of all forms of polymorphonuclear cells (fig. 5).

At the next stage of our work, we studied the antioxidant defense system of fish grown in industrial aquaculture and the effect of probiotic microbiota on it.

We also conducted a comparative study of the activity of antioxidant defense enzymes and the content of the end product of lipid peroxidation, malondialdehyde, in fish grown in industrial aquaculture without probiotics and in the presence of probiotics.

It is known that the conditions for growing fish in industrial aquaculture cannot be called optimal due to the high stocking density and organic pollution of the habitat by metabolic products. In fish, chronic stress is formed, which reduces its productivity and quality. It triggers the mechanism of lipid peroxidation with the formation of free radicals that damage biomolecules and membrane structures of cells. These damages lead to numerous morphological and functional pathological changes, disruption of homeostasis and irreversible processes in the body. It is necessary to look for solutions to this problem. Therefore, we used the probiotic "Sporotermin". We believed that the enzymes produced by the microbiota of the Sporothermin probiotic can increase the efficiency of the antioxidant system and reduce the level of malondialdehyde (MDA). This metabolite has attracted attention because it is regarded as the end product of lipid peroxidation. To test our hypothesis, we evaluated the content of MDA.

The results of the studies showed that in catfish grown in an artificially created environment, the enzymatic activity of catalase in the blood serum was at the level of 1.04±0.15 nmol substrate/min. Catalase found higher activity in erythrocytes – 1.96±0.45 nmol substrate/min (fig. 6).

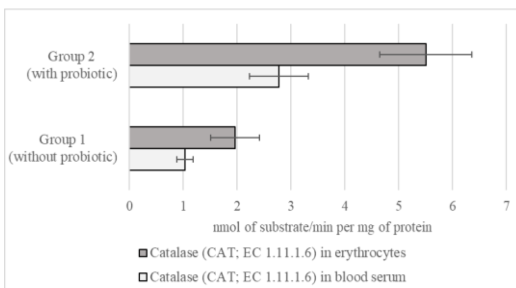

Fig. 6. Catalase activity in catfish.

In fish reared under conditions of artificial breeding with the addition of a probiotic, we found a pronounced increase in catalase activity (fig. 6). Enzyme activity increased 2.68 times in serum and 2.81 times in erythrocytes. Catalase in erythrocytes showed activity – 5.5±0.85 nmol substrate/min. A similar picture was observed in the study of SOD activity. The results are presented in fig. 7.

In fish that did not receive the probiotic, SOD activity was significantly lower in blood serum (189.02±7.1 nmol substrate/min) and in erythrocytes (268.92±13.3 nmol substrate/min) than with "Sporotermin", when the enzymatic activity in serum increased to 221.4 nmol substrate/min, and in erythrocytes to 343.44±18.7 nmol substrate/min (Fig. 7).

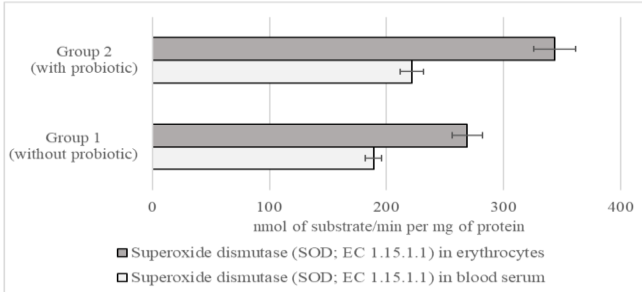

Fig. 7. Superoxide dismutase activity in blood serum and erythrocytes of catfish.

When studying the level of MDA in fish muscles, it was shown that the use of a probiotic based on the spore forms of B. subtilis and B. licheniformis in fish feeding reduced the level of this metabolite. It was found that fish grown in industrial aquaculture without a probiotic, the content of malondialdehyde (MDA) was 7201.01 mmol/100 g of tissue. In fish grown with the probiotic, the level of MDA is 3194.12 mmol/100 g of tissue. resistance to oxidative stress, healing the body of fish.

4 Discussion

The obtained results showed that the microbiota of the probiotic protects against toxic damage and normalizes the histological structure of the tissues of the intestines and liver of fish. Probiotic microbiota, optimizes the structure of intestinal microbiocenosis, inhibiting the growth of pathogenic and conditionally pathogenic microbiota. Together with the liver, the obligate microbiota and the probiotic microbiota form a powerful complex detoxification system for the entire fish organism [27].

The microbiota of the probiotic and the microbiota of the intestinal microbiocenosis normalized by it, acting in total, reduces the load on the liver due to the detoxification of products dangerous to the body. The unity of the goals of the liver and intestinal microbiota unites them, forming a complex hepatoenteric complex, the functioning of which is important for maintaining homeostasis [28].

High-tech fish breeding systems, as shown by our research, cause pathological changes in the body of fish that need to be corrected. These changes were characteristic of both the fish intestine and the liver. This leads to serious endoecological problems at the level of the organism, complicating the process of growing marketable fish and reducing its quality. Under the action of bacteria B. subtilis and B. licheniformis, the probiotic "Sportermin", which produce a complex of biologically active substances, the pathology of the tissues of the intestinal wall and liver parenchyma is corrected and the general improvement of the fish organism occurs.

In the study of the white blood of catfish, it was found that the leukogram of fish grown without a probiotic differs from the leukogram of fish grown with a probiotic. Under the action of a probiotic, the structure of the leukogram changes. It was established that against the background of the probiotic, the proportion of lymphocytes increased, the level of neutrophils decreased, and the proportion of polymorphonuclear formed elements increased. There was a redistribution of functionally unequal forms of leukocyte cells under the influence of the probiotic microbiota, which to a certain extent assumed the function of protection against pathogens.

The microorganisms Bacillus subtilis and Bacillus licheniformis are not human pathogens. They have GRAS status –generally regarded as safe. They constantly enter the gastrointestinal tract and lungs, reaching a level of 107 CFU/g there. They dominate in the intestinal normobiota and are comparable in quantitative content with Lactobacillus. Microorganisms Bacillus subtilis are able to acidify the environment and produce antibiotics [29]. The uniqueness of Bacillus subtilis and Bacillus licheniformis bacteria lies in the fact that up to 5% of their genome contains a program for the synthesis of biologically active substances that suppress the microbiota that provokes intestinal infections. These bacteria are also considered as the most promising as the basis of a new generation of drugs – metabiotics.

In the study of the activity of antioxidant defense enzymes and the content of MDA, it was found that the microbiota of the probiotic "Sporotermin" is able to increase the activity of catalase and superoxide dismutase enzymes and reduce the level of MDA.

5 Conclusion

The fish intestine has digestive, immune and endocrine functions. An important role in this is played by the obligate and symbiotic microbiota that colonizes the digestive system of fish and is responsible for the microecology of the whole organism.

Microbiocenosis of the digestive system of fish ensures the constancy of the internal environment of the body in the process of metabolism under the influence of external or internal stimuli.

In the process of vital activity, the microbiota produces a large number of biologically active substances – metabiotics and many other low molecular weight compounds.

The microbiota of probiotics exhibits enzymatic activity, synthesizes biologically active substances, such as vitamins, amino acids, lipids, organic acids. Under the influence of the microbiota, incoming nutrients are absorbed faster and more efficiently by the body, increasing the growth rate of fish [6].

Intestinal microbiota spreads throughout the organs and tissues of fish, making adjustments to metabolic processes. Probiotics have the same effect. They improve the digestibility of feed, the absorption of calcium, iron. The probiotic microbiota produces detoxifiers; to protect against pathogenic microbiota, symbiotics synthesize specific substances, including alcohol-containing compounds [6, 16].

Summing up the results of the studies, it should be noted that the microbiota of the probiotic "Sporotermin" has a corrective effect on the structure of organs and metabolism in the body of fish.

It was found that under the influence of Bacillus subtilis and Bacillus licheniformis, the histological structure of the intestinal wall and liver parenchyma of fish was normalized. Against the background of a probiotic, it was possible to get away from toxic damage to liver cells. The nuclei of hepatocytes again acquired polarity, the signs of fatty degeneration and necrosis disappeared. The topography of the liver parenchyma returned to normal, and hemorrhages in the vascular region disappeared. Against the background of the probiotic, there were changes in the structure of the fish leukogram associated with the redistribution of different forms of leukocytes. There was an increase in the proportion of lymphocytes due to a partial decrease in the proportion of neutrophils and monocytes. But all the revealed changes did not go beyond the physiological norm for this fish species.

When studying the antioxidant system, it was found that probiotic microorganisms, producing biologically active substances, are able to increase the activity of antioxidant defense enzymes and reduce the level of toxic metabolites, in particular MDA.

As a result, based on the results obtained, it can be concluded that the systemic effect of the action of the microbiota of the probiotic "Sportermin" on the fish organism is manifested.

References

1. K. Pawlak, M. Kołodziejczak, Sustainability **12(13),** 5488 (2020).
2. M. Larionova, E. Safonkina, Int. J. of Agr. Manag. 7(4), 96-121 (2018).
3. E. Weizsacker, A.B. Lovins, L.H. Lovins, Factor Four: Doubling Wealth, Halving Resource Use - A Report to the Club of Rome, 1-322 (2013).
4. T.E. Marinchenko, IOP Conf. Ser.: Earth and Env. Sci. Int. Baikal Investment and Construction Forum "Spatial Restructuring of territories", 012052 (2021).
5. Official website www.aqua-spark.nl.
6. E.M. Romanova, V.V. Romanov, V.N. Lyubomirova, L.A. Shadyeva, T.M. Shlenkina, Bio Web of Conf. Int. Scient.-Pract. Conf. "Agr. and Food Security: Techn., Innov., Markets, Human Resources" (FIES 2020), 00132 (2020).
7. J.K. Pinnegar, G.H. Engelhard, K.G. Hamon, et al., Frontiers in Marine Sci. **7**, 568219 (2020).
8. Z.-M. Hu, Q.-S. Zhang, T.-F. Shan, et al., Rev. in Aquacul. **13(117),** 1-28, (2021) DOI:10.1111/raq.12524.
9. Z. Usydus, J. Szlinder-Richert, M. Adamczyk, U. Szatkowska, Food Chemistry **126(1),** 78-84 (2011).
10. M. Hussain, H. Ul Hassan, M.A.M. Siddique, et al., Egyptian J. of Aquatic Res. **47(3),** 329-334 (2021).
11. M. Garcia-Vaquero, M. Hayes, Food Rev. Int. **32(1),** 15-45 (2016).
12. M. Mulumpwa, J. of Insects as Food and Feed **4(4),** 301-312 (2018).
13. Official website www.fao.org.
14. M. Jobling, Aquacul. Int. **24(3),** 767-786 (2016).
15. M.I. Hossain, M. Sadekuzzaman, S.-D. Ha, Food Res. Int. **100**, 63-73 (2017).
16. T.M. Shlenkina, E.M. Romanova, V.N. Lyubomirova, V.V. Romanov, L.A. Shadyeva, Bio Web of Conf. Int. Scient.-Practical Conf. "Agr. and Food Security: Technology, Innovation, Markets, Human Resources" (FIES 2020), 00133 (2020).
17. G.I. Izvekova, E.I. Izvekov, A.O. Plotnikov, Biology Bulletin **34(6),** 610-618 (2007).
18. G.H. Hansen, J.A. Olafsen, Microbial Ecology **38**(1), 0001-0026 (1999).
19. V.N. Chernov, B.M. Belik, A.I. Poliak, L.I. Vasil'eva, L.E. Bragina, J. of Microbiology Epidemiology Immunobiology **4,** 70-74 (1999).
20. R.S. Mukhammadiev, A.S. Mukhammadieva, E.V. Skvortsov, L.R. Valiullin, A.P. Glinushkin, IOP Conf. Ser.: Earth and Env. Science. Ser. "All-Russian Conf. with Int. Part. Econ. and Phytos. Rat. for the Introd. of Feed Plants", 012008 (2021).
21. M. Van de Wouw, H. Schellekens, T.G. Dinan, J.F. Cryan, J. Nutr. **147,** 727–745 (2017) doi: 10.3945/ jn.116.240481.
22. D.E. Korzhevsky, Fundamentals of histological technique (2010)
23. N.T. Ivanova, Atlas of fish blood cells (M.: Light and food industry, 1983)
24. H. Aeby, Methods Enzymol. **105**, 121-126 (1984).
25. C.N. Giannopolitis, S.K. Ries, Plant Physiol. **59**, 309-314 (1972).
26. M.E. Mukhitova, E.M. Romanova, V.V. Romanov, Bulletin of the Ulyanovsk state agricultural academy **2(46),** 126-131 (2019).

27. R.G. Kerry, P. Pradhan, D. Samal, et al., Microbial Biotechnology. Application in Food and Pharmacology. Singapore: Springer Nature Singapore Pte. Ltd., 141–152 (2018) doi: 10.1007/978-981-10- 7140-9_7.
28. A.A. Shevandova, I.I. Fomochkina, A.V. Kubyshkin, L.E. Sorokina, A.I. Gordienko, A.A. Gorbunov, T.P. Makalish, K.A. Yurchenko, Medical News of North Caucasus **16(2),** 222-226 (2021).
29. E. Romanova, E. Spirina, V. Romanov, V. Lyubomirova, L. Shadyeva, E3S Web of Conf. "13th Int. Scient. and Practical Conf. on State and Prospects for the Development of Agribusiness, Interagromash 2020", 02013 (2020).