New supplementary feeds for sterlet in industrial cultivation

Elena Ponomareva[1,3*], *Peter* Geraskin[2], *Angelika* Kovaleva[1], *Marina* Sorokina[1], *Uliana* Aleksandrova, and *Dmitry* Rudoy[3]

[1]Federal Research Centre The Southern Scientific Centre of the Russian Academy of Sciences (SSC RAS), Chekhov Street, 41, 344006 Rostov-on-Don, Russia
[2]Astrakhan State Technical University, Tatishchev St., 16, 414056 Astrakhan, Russia
[3]Don State Technical University, Gagarin sq. 1, 344003 Rostov-on-Don, Russia

Abstract. An increasingly significant deficit of fish meal necessary for the production of quality fish feed requires the use of new components characterised by high levels of protein and fat. This article presents the results of a feed supplements study with a protein component based on soldier fly and probiotics. The study of fish-biological parameters of fish revealed that the absolute and average daily gain in the variant with feed KV54 + soldier fly is higher by 40.9 %, the mass accumulation ratio by 33.3 %, compared with the control variant, with survival rate of 100 %. Feeding coefficient in the experiment variant KV54+soldier fly is lower compared to the control by 1.3 units, i.e. by 34 %. The physiological and biochemical parameters of fish blood were within the normal range for fish from a natural reservoir.

1 Introduction

Fishmeal is a major component of fish feed, but the constant high demand for fishmeal has led to a decrease in production and an increase in fishmeal prices. Insects and their larvae are being considered as an alternative to fishmeal as a source of protein and healthy fats. Fly larvae reared by feeding biological waste have been found to be excellent for the production of animal feed. The production of fly larvae has a lower negative impact on the environment (such as greenhouse gas emissions) than the production of other animal feedstuffs. The industrial production of H. illucens can cover the demand for animal protein, as this raw material is characterised by high nutritional properties. Therefore, the industrial importance of this species is gradually increasing in Russia and globally [1].

Of course, soldier fly larvae are still under study, but a number of benefits have already been identified. Animal feed with H. illucens is currently valued for its high protein and lipid content and is not only used for feeding carnivorous fish, but is also present in the diets of other animals [2]. The studies showed that the feed conversion ratio of H. illucens fly is higher than that of crickets and worms.

* Corresponding author: kafavb@mail.ru

The study of the feeding effect of H. illucens fly larvae on the health and productivity of cattle showed positive results. Melanin protein-energy supplement obtained from H. illucens fly larvae was used in the work. Melanin is an antioxidant and contributes to the long-term storage of feed. It has the ability to be partially digested in the body of animals, and also acts as an enterosorbent in the intestines of animals. According to the results of feeding, acceleration of growth and development of young animals was noted: the condition of intestinal microflora improved [1, 3].

The quality of H. illucens larvae can be improved by adding fish by-products to the diet, from which the larvae obtain omega-3 fatty acids [4]. Feeding a diet supplemented with such larvae produces growth results similar to feeding a traditional fishmeal-based diet [5].

The studies showed that addition of 10% of H. illucens larvae to the fish diet increases the growth rate and a number of other indicators, compared to the control variant [5, 6]. Estimation of nutritional quality and physico-chemical properties of fish fillets at complete replacement of fish meal in the diet with fly meal showed that this replacement did not affect the physico-chemical properties of fish fillets [7].

Nowadays, probiotics are as common as vitamins. They are used to rehabilitate the organism after antibiotic therapy, immunosuppression and to repair the gastrointestinal tract after diseases. There are studies on feeding probiotic feed to fish [8–11]. It is much easier to take care of their natural immunity than to diagnose and treat a disease [12].

As research by Dawood et al. has shown [13] probiotics can help aquaculture development by increasing fish growth and disease resistance, but as long as they are safe not only for aquatic animals, but also for the environment and humans [14].

When probiotics get into the body, they work in a number of ways. They are very active in destroying other bacteria, using both physical and chemical methods. By actively colonising the gastrointestinal tract, probiotic bacteria create a dense physical barrier on the surface of the epithelium, preventing other bacteria from taking hold.

Many of the probiotic bacteria produce organic acids, particularly lactic acid, which lowers the pH of the gastrointestinal tract below the optimal range for pathogens. Organic acids contribute to the creation of a protective barrier that inhibits the possibility of adhesion of pathogens on the inner surface of the intestine.

They produce hydrogen peroxide (H2O2), which breaks down disulfide bridges in bacterial proteins. Probiotics can produce proteolytic proteins that destroy bacterial walls, produce bacteriocins - natural antibiotics that act to kill Gram-positive bacteria.

An important quality of probiotics is immunostimulation. Due to the stimulation of the lymphatic system of fish increases the level of immune cells that synthesise immunoglobulins IgA, which protect the body from harmful factors of the external and internal environment, responsible for the neutralisation of pathogenic bacteria, viruses, toxins and enzymes produced by microorganisms.

The study by Shubhadeep Ghosh and his team [15] recorded more growth of fish in all experimental groups as compared to control group, increase in the number of B. subtilis bacteria in the gastrointestinal tract of fish. With the passage of time, the number of bacteria decreased but still retained more compared to other probiotic bacteria. It was explained that Bacilus subtilis forms endospores covered with a thick cortex (the thickest layer of the spore) and a three-layer wall, which makes it possible to survive even in conditions where the temperature is above 100 °C, under drought conditions, UV radiation, and in the absence of food. When studying the effect of probiotics on aquarium fish reproduction, higher fecundity, higher gonado-somatic index, higher survival rate of fry, fewer deformities and faster growth were observed [16].

In 2014, Telley et al. [17] studied the effect of *Bacillus subtilis* on immunity of Nile tilapia under stressful conditions at high planting densities of 50 fish per 800 litres in aquaria.

An interesting result was the positive effect of B. subtilis on fish immunity. Phagocytic index and lysozyme activity under stress increased in fish that received probiotics, in contrast to the control group. Phagocytes are innate immune system, they find and block the cells of pathogens. Lysozyme breaks down the cell walls of bacteria. Thus, the probiotic is able to increase its secretion by the host's immune system, as well as produce it independently.

Probiotics also provide positive benefits for fish by improving their nutritional quality through the synthesis of essential ingredients (vitamins, fatty acids and enzymes). Moreover, they break down food that without their participation could remain undigested. They have a favourable effect on reproduction, the quality of offspring and their survival rate [18–20].

According to the above, the goal of the study is to study the effect of introducing probiotic feed additives in complex with a protein component based on the soldier fly in mixed fodder for fish on fish-biological and physiological-biochemical parameters of young sterlet fish. (*Acipenser ruthenus*).

2 Material and research methods

The studies were conducted at the Kagalnik Coastal Research and Expedition Base (Rostov region, Russia) under the conditions of a recirculating water supply system. 1 m3 polypropylene tanks with circular water flow and central drainage were used (Figure 1). Juvenile sterlets (Acipenser ruthenus) (Figure 2) in the number of 62 individuals per tank were selected as the model object for experiments on the approval of new probiotic feed additives. The experiment lasted 29 days.

Fig. 1. Rearing of juvenile sterlets in RAS system tanks

https://doi.org/10.1051/bioconf/20248401045

Fig. 2. Sterlet juveniles (*Acipenser ruthenus*)

Standard mixed feed was used for the feeding of experimental fish (c/k - control), experimental variants 1 (c/k + probiotic KV54) and 2 (c/k + probiotic KV54 + protein component based on the larvae of the black soldier fly *Hermetia illucens*). The daily feeding rate for juvenile sterlets was 3% of body weight.

Fish oil with probiotics was added to the pelleted feeds to incorporate probiotic components into the diet. The drug was added directly to the fat mixture. The fat emulsion was injected into the unfatted pellet until the crude fat content of the pelleted feeds reached 14%. Prior to injection, the fat emulsion was heated to 40 °C, a probiotic was added and a fat antioxidant was introduced.

Standard methods were used to comprehensively assess the condition of fish following trials of new feed additives [21]. AZ Instrument 86031 thermo-oximeter was used to control hydrochemical parameters of water. Temperature measurements were made twice a day in the morning at 8.30 and in the evening at 19.30.

The following methods were used to assess the biological parameters of juvenile fish and to calculate feed costs [21–23] and equipment. Analysis of fish-biological characteristics of fish was conducted after stable adaptation of juveniles to rearing conditions.

To assess the physiological condition of the fish, blood parameters were examined: erythrocyte sedimentation rate (ESR), haemoglobin content. In addition, the levels of total protein, cholesterol, beta-lipoproteins, triglycerides in blood serum were measured using the methods we published earlier [24].

The main data obtained in the course of the study were subjected to statistical processing according to G.F. Lakin [25].

3 Results and discussion

The results of the feed additives approbation in comparison with the control variant, including the following data: survival rate, growth rate, mass accumulation coefficient, feed costs per unit of growth, were obtained in the course of the conducted work.

Water quality in the tanks was in accordance with the industry standard for fish farm water OST 15.372-87 and the water quality requirements for sturgeon. [26].

The concentration of the main hydrochemical compounds was within the norm. Acidity was at pH = 7-7.6, which is the norm even for such demanding species as trout and sturgeon.

The minimum water temperature was 19 °C and the maximum 22.5 °C. The average temperature was 20.9 °C.

One of the most important hydrochemical factors is dissolved oxygen. During the research, oxygen was measured daily. The average dissolved oxygen content was 7.4 mg/l, minimum - 6.5 mg/l, maximum - 8.4 mg/l. Dynamics of oxygen content is usually associated with increased consumption (up to 1-2 mgO2/l) during and after feeding, when metabolic reactions of nutrient assimilation take place. Water saturation was achieved by water movement in the system circuit. No additional aeration equipment was used.

At the beginning of the experiment, the average weight of juvenile sterlets was 46 g. During the rearing period, the weight of juvenile sterlets increased in all groups.

In terms of absolute and average daily gain, the highest values were observed in experiment 2 (by 40.9 % compared to the control), while the control variant and variant 1 had practically no differences (Table 1).

As a result of analysing the growth parameters obtained when growing young sterlet on different feeds, it can be noted that feeds containing the tested additives promoted intensive growth and high survival rate up to 100%.

Table 1. Fish-biological parameters of juvenile sterlet fish

Indicators	**Control**	**Experience variants**	
		1	**2**
Initial weight, g	46,6±1,53	45,76±1,74	46,61±1,39
Final weight, g	57,38±2,17	56,54±2,73	61,64±1,87
Absolute gain, g	10,79	10,69	15,02
Average daily weight gain, g/day.	0,37	0,37	0,52
Mass accumulation factor, units.	0,03	0,03	0,04
Survival rate, %	100	100	100

The growth rate of the juvenile fish was characterised by a steady increase in weight throughout the rearing period (Figure 3), which was supported by Dawood et al. in their work with probiotics [13], and Giannettoa et al. in their work with the soldier fly larvae [5].

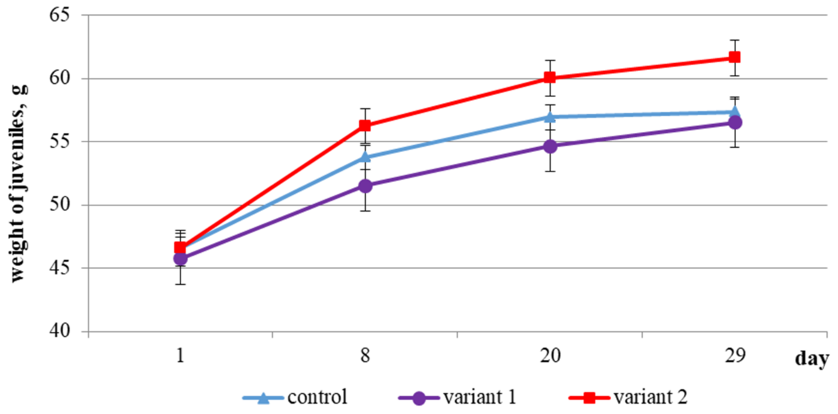

Fig. 3. Sterlet growth rate

The lowest feed costs per unit of sterlet juvenile growth were obtained in variant 2 (Figure 4), which is associated with better assimilation of the introduced feed. Compared to the control, this indicator was lower by 1.3 units, i.e. by 34%. The same effect was observed in a previous study on this topic. [18–20].

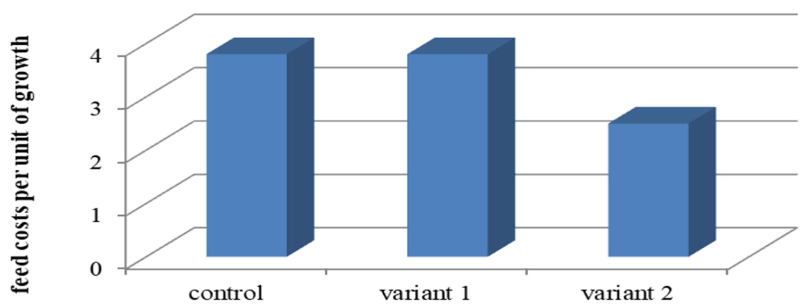

Fig. 4. Feed costs per unit of sterlet juvenile growth

Studies of physiological and biochemical parameters revealed that the erythrocyte sedimentation rate (ESR), according to the results of the experiments, was initially characterized as low - from 2.2 mm/h (variant 2) to 2.6 mm/h (variant 1), but within the physiological norm for fish reared under industrial conditions (1–6 mm/h) [27]. In the control, the average value for all experiments (2.5 mm/h) was recorded. By the end of the experiment period, there was an increase in ESR in all variants: in the control, the ESR level was 2.8 mm/h (12% higher than the baseline), in option 1 - 2.75 mm/h (5.8% higher) , in option 2 - 3.1 (by 40.9%). An increase in ESR may indicate the normalization of metabolism and the restoration of the body after stress.

During the experiment, the haemoglobin level in the blood of the fish was within the physiological norm for fish from the natural environment (50-70 g/L) [28, 29]. At the same time, a decrease in haemoglobin concentration was observed at the end of the experiment [30]. In addition, in variant 1, haemoglobin levels at the end of the experiment were significantly different from those at the beginning of cultivation ($P < 0.05$). In variant 2, at the end of the study, the haemoglobin content in the blood of juvenile fish in the experimental group was 9.7% higher than in the control group (differences not significant at $P > 0.05$) and

22.3% higher than in variant 1 (differences significant at P > 0.05). 3% (differences are significant at P < 0.05) [31, 32].

It can be assumed that the increased hemoglobin at the beginning of the experiment is associated with a low oxygen content in the water in the period before the experiment. The cause of high hemoglobin may also be stress, which in the experiment was leveled by the strengthening of natural immunity, since the action of probiotics is characterized by high immunomodulatory activity.

The protein concentration in the blood serum increased from the beginning to the end of the experiment. The addition of probiotics to the fish feed contributed to the increase in blood protein levels by 10–15 % due to the fact that bacteria contain protein in an amount that reaches 80% of the total dry mass of the cells. In addition, the presence of probiotics in the fish's digestive tract improves metabolic processes, the synthesis of enzymes, amino acids and vitamins during digestion, as confirmed by previous studies. [33].

The values of the concentration of beta-lipoproteins and cholesterol in the blood serum of sterlets in the variant 2 decreased by 8.9 % и 9.5 % at the end of the study, while an increase in the concentration of cholesterol was recorded in the control (by 4.7 %). At the same time, the concentration of beta-lipoproteins was slightly lower than in the experimental groups. The reason for the decrease in blood serum lipids was probably the more efficient assimilation of food in the experimental fish and its use for energy needs due to the synthesis of enzymes by probiotics, especially lipase. The changes we observed in the condition of the experimental fish are also confirmed by other literature. In particular, M.K. Koylytbayeva et al. argue that probiotic organisms, when concentrated in the intestine, among other actions, act as immunomodulators; neutralise toxins, allergens; reduce the level of cholesterol in the blood [33]. The level of beta-lipoproteins in the blood of fish from experimental variant 1 (1.24±0.049 g/l) differed significantly (P < 0.01) from the control (1.0±0.061 g/l). Other physiological and biochemical parameters were not significantly different.

Thus, by inhibiting the growth of pathogenic micro-organisms, probiotics contribute to the development of normal intestinal microflora, whose role in the life of the host organism is important and multifunctional: providing resistance to colonisation, performing digestive, synthetic, immunomodulatory and detoxifying functions.

According to the indicators of growth and mass accumulation of sterlet fry, the most intensive growth was observed in the variant of compound feed with probiotic KV54 and a protein component based on the larvae of the black soldier fly *Hermetia illucens* (variant 2). This indicator was 7% higher than in the control. A similar trend was observed for other fish-rearing and biological indicators. At the same time, the feed coefficient in the variant of the experiment KV54 + soldier fly is lower by 1.3 units, i.e. by 34% in comparison with the control variant, which indicates a better assimilation of feed.

The ESR indicators returned to normal as a result of the body's recovery from stress. The level of haemoglobin in the blood of the fish was within the physiological norm for fish from a natural environment. Its decrease during the experiment may be related to the strengthening of the natural immunity, since the action of probiotics is characterised by a high immunomodulatory activity. An increase in the concentration of protein in the blood by 10–15% may be the result of the addition of probiotics to fish feed, which improve metabolic processes, the synthesis of enzymes, amino acids and vitamins. The reason for the decrease in blood serum lipids was probably the more efficient assimilation of food in the experimental fish and its use for energy needs due to the synthesis of enzymes by the probiotics, in particular lipase.

4 Acknowledgements

This publication was prepared within the framework of the State Order of SSC RAS, Grant No. 122020100328-1, using the Unique Scientific Installation "Modular Unit-Complex" and «Bioresource Collection of Rare and Endangered Fish Species No. 73602» of the SSC RAS.

References

1. M.Y. Syromyatnikov, A.V. Lopatin, E.P. Danshina, V.N. Popov, *Recycling biological waste using the fly Hermetia illucens, environmental risks and biosafety for Russia*, IOP Conf. Series: Earth and Environmental Science, **640**, 062028 (2021) https://doi:10.1088/1755-1315/640/6/062028
2. T. Spranghersa, A. Noyez, K. Schildermans, P. De Clercq, Cold hardiness of the black soldier fly (Diptera: Stratiomyidae) Journal of Economic Entomology, **110**, 1501-1507 (2017) https://DOI:10.1093/jee/tox142
3. R. V. Nekrasov, A. A. Zelenchenkova, M. G. Chabaev, N. A. Ushakova, Melanin protein energy supplement from larvae in Hermetia illucens calf nutrition Agricultural biology, **53(2)**, 374-384 (2018)
4. D.C. Sheppard, G.L. Newton, S.A. Thompson, S. Savage, A value-added manure management-system using the black soldier fly Bioresource Technolgy, **50**, 275-279 (1994) https://doi.org/10.1016/0960-8524(94)90102-3
5. A. Giannettoa, S. Olivaa, C.F.S. Lanesb, F.A. Pedronb, and et. al., Hermetia illucens (Diptera: Stratiomydae) larvae and prepupae: Biomass production, fatty acid profile and expression of key genes involved in lipid metabolism. J. Biotechnology, **307**, 44-54 (2019) https://doi: 10.1016/j.jbiotec.2019.10.015
6. M.R. Chaklader, M.A.B. Siddik, R. Fotedar, J. Howieson, Insect larvae, Hermetia illucens in poultry by-product meal for barramundi, Lates calcarifer modulates histomorphology, immunity and resistance to Vibrio harveyi Scientific Reports, **9(1)**, 703 (2019) https://doi: 10.1038/s41598-019-53018-3
7. Y.S. Wang, M. Shleomi, Review of Black Soldier Fly (Hermetia illucens) as animal feed and human food Foods, **6**, 2-23 (2017) https://doi:10.3390/foods6100091
8. S.H. Hoseinifar, Y.-Z. Sun, A. Wang, Z. Zhou, Probiotics as Means of Diseases Control in Aquaculture, a Review of Current Knowledge and Future Perspectives. Front. Microbiol., **9**, 2429 (2018) https://doi.org/10.3389/fmicb.2018.02429.
9. S.H.T. Shefat, Probiotic Strains Used in Aquaculture. Int. Res. J. Microbiol, **7(2)**, 043-055 (2018) https://doi.org/10.14303/irjm.2018.023
10. B.A. Ige, Probiotics use in intensive fish farming African Journal of Microbiology Research, **7(22)**, 2701-2711 (2013) https://doi.org/10.5897/AJMRx12.021.
11. B.A. Ige, Probiotics use in intensive fish farming African Journal of Microbiology Research, **7(22)**, 2701-2711 (2013) https://doi.org/10.5897/AJMRx12.021.
12. https://tropicaledu.com/probiotics-in-fish-food/ (Last accessed 12.07.2023)
13. M.A. Dawood, S. Koshio, M. Ishikawa, M. El-Sabagh, M.A. Esteban, A.I. Zaineldin, Probiotics as an environment-friendly approach to enhance red sea bream, Pagrus Major growth, immune response and oxidative status, Fish & Shellfish Immunology, **57**, 170-178 (2016) https://doi: 10.1016/j.fsi.2016.08.038
14. E. Muñoz-Atienza, B. Gómez-Sala, C. Araújo, C. Campanero, R. Del Campo, P.E. Hernández, et al., Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture, BMC Microbiology, **13(1)**, 1-15 (2013) https://doi: 10.1186/1471-2180-13-15

15. S. Ghosh, A. Sinha, C. Sahu, Dietary probiotic supplementation in growth and health of live-bearing ornamental fishes. Aquaculture Nutrition, **14**, 289-299 (2008) https://doi.org/10.1111/j.1365-2095.2007.00529.x
16. S. Ghosh, A. Sinha, C. Sahu, Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquaculture Research, **38**, 518-526 (2007) https://doi.org/10.1111/j.1365-2109.2007.01696.x
17. G. Telli, Dietary administration of Bacillus subtilis on hematology and non-specific immunity of Nile tilapia Oreochromis niloticus raised at different stocking densities. Fish & Shellfish Immunology, **39**, 305-311 (2014) doi: 10.1016/j.fsi.2014.05.025
18. N. Mehdinejad, M.R. Imanpour, V. Jafari, Combined or individual effects of dietary probiotic, Pediococcus acidilactici and nucleotide on reproductive performance in goldfish (Carassius auratus). Probiotics and Antimicrobial Proteins, 1–8 (2018) http://doi.org/10.1007/s12602-017-9297-3
19. M.D. Ibrahem, Evolution of probiotics in aquatic world: Potential effects, the current status in Egypt and recent prospective, Journal of Advanced Research, **6**, 765-791 (2015) https://doi.org/10.1016/j.jare.2013.12.004
20. C. Sayes, Y. Leyton, C. Riquelme, Probiotic Bacteria as an Healthy Alternative for Fish Aquaculture, Antibiotic Use in Animals, 164 (2017) ISBN 978-953-51-3751-1 https://doi.org/10.5772/intechopen.71206
21. M.A. Shcherbina, E.A. Gamygin, Feeding fish in freshwater aquaculture, 360 (Moscow, VNIRO Publishing House, 2006)
22. V.V. Seliverstov, Guidelines for conducting a hematological examination, (Moscow, Min. agriculture and food, 1999)
23. I.F. Pravdin, Guide to the study of fish, mainly freshwater, 374 (Moscow, Food industry, 1966)
24. G.F. Metalov, E.N. Ponomareva, P.P. Geraskin, A.V. Kovaleva, V.A. Grigoryev, Vestnik ASTU. Ser.: Fishery, **4**, 128-135 (2017) doi: 10.24143/2073-5529-2017-4-128-135
25. M.N. Ponomareva, V.A. Sorokina, Grigoriev et al. Results of the development of methods for the formation of sterlet broodstock in conditions of closed water supply, Vestnik ASTU, Ser.: Rybnoe khozyaystvo, **1**, 86–90 (2010)
26. M.S. Chebanov, E.V. Galich, Yu.N. Chmyr, Guide to breeding and growing sturgeons, 148 (Moscow FGO "Rosinformagrotech", 2004)
27. E.V. Sementina, G.G. Serpunin, Fish-breeding-biological and hematological characteristics of the repair-brood stock of sterlet grown in closed water supply installations, Fisheries, **4**, 76-78 (2011)
28. H. Alamandari, S.V. Ponomarev, The use of probiotic drugs in feeding sturgeon fish: test results at a water temperature below optimal, Bulletin of the AGTU. Ser.: Fisheries, **3**, 133-139 (2013)
29. O.N. Gutsulyuk, The effect of probiotic additives on hematological and fish-breeding indicators of yearlings of the Russian-Lena sturgeon, Samara Scientific Bulletin, 4(13), 113-116 (2015)
30. M.N. Ponomareva, V.A. Sorokina, Grigoriev et al. Results of the development of methods for the formation of sterlet breeding herds in closed water supply conditions, Bulletin of AGTU, Ser.: Fisheries, 1, 86-90 (2010)

31. N.A. Ushakova, S.V. Ponomarev, V.G. Pravdin, L.Z. Kravtsova, S.A. Liman, D.S. Pavlov, Evaluation of the effectiveness of the synbiotic drug "Prostor" in the diet of juvenile sturgeon fish, Fundamental research, **6**, 1174-1177 (2013)
32. N.A. Ushakova, A.I. Bastrakov, A.A. Kozlova, S.V. Ponomarev, and et. al., Features of the effect of a complex probiotic with bacteria of the genus Bacillus and biomass of Hermetia illucens larvae on the young of the red tilapia Oreochromis mossambicus × O. niloticus and the Russian sturgeon Acipenser gueldenstaedti, Izvestiya RAS. Ser. biologicheskaya, **5**, 525-531 (2016)
33. M.K. Koilytbaeva, G.O. Ustenova, D.Zh. Batyrbaeva, Zh.S. Alibayeva, K.K. Mustafina, Prospects for the use of probiotics based on bacteria of the genus Bacillus, Bulletin of KazNMU, **4**, 181-184 (2018)