Normalization of the symbiotic intestinal microflora of sturgeon’s juvenile with the introduction of a new generation of probiotic preparations into their diet

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Abstract. Modern aquaculture is characterized by new approaches to the fish maintenance in conditions of high stocking density, associated with the restoration of the natural ecology of the body and based on the use of biologically active products. One aspect of this approach is to normalize altered the microbial environment of the organism in water of a recirculating aquaculture system with probiotics. Probiotics have the ability to regulate the microbiocenosis of the digestive tract, making a significant contribution to the absorption of nutrients. The use of probiotics can solve a number of problems related to the physiological state of animals and fish, increasing the efficiency of digestion, stimulating growth and development. Probiotic preparations of the new generation include probiotics“ Ferm-KM “and” ProStor”, which are sorbed forms containing bacteria immobilized on solid sorbent particles. The article presents the results of the use of probiotics “Ferm-KM” and ”ProStor” in the composition of production compound feeds for sturgeon fish. It was found that the use of new generation probiotic preparations in the diet of sturgeon fish contributes an increasing in the number of beneficial microorganisms in the intestine (up to 8.5x10^7 CFU/g) of Russian sturgeon. The effectiveness of introducing a new generation of probiotics into the compound feed for sturgeon fish has been proven, which contributes to increasing the resistance of the macroorganism to pathogenic microorganisms, improving the functioning of the digestive system. Probiotic strains introduced with preparations interact with the community of intestinal bacteria, releasing metabolites that affect the digestive, immune and hormonal systems of the body.

1. Introduction
The microflora of fish is diverse and depends on many factors: the microbial background of water, the type of fish and the conditions of its habitat. The gastrointestinal tract of fish contains many pathogenic microorganisms that serve as a source of various infections. Bacteria get into it from the water along with mixed feeds. Among the intestinal microflora there are all the inhabitants of water, as well as anaerobes-spore-forming clostridia. Pathogenic clostridium and E. coli are common [1-4].

In different parts of the intestines of fish, different numbers of bacteria were found. The maximum number of them was found in the anterior and middle parts, which suggests the participation of bacteria in the breakdown of nutrients. The presence of a large number of bacteria in the posterior part of the intestine can be associated with their participation in the destruction of ballast substances and with the provision of secondary nutrients to fish. In the intestinal tract of fish, there is a population of
bacteria characterized by a high density – up to $10^8$ aerobic heterotrophic bacteria and approximately $10^5$ anaerobic bacteria per gram of content [5-7].

Individual physiological groups of microorganisms have the ability to destroy food substrates. Microorganisms, destroying proteins, carbohydrates and other compounds in the intestinal cavity, provide themselves and the macroorganism with the necessary substances [5]. It is established that the consumption of artificial feeds contributes to the violation of the composition of the intestinal microflora, as a result of which the number of microbes of certain species sharply decreases or disappears, which can lead to pathology or dysbiosis [8-10].

In this regard, the purpose of this work was to assess the effect of new-generation probiotic preparations "Ferm-KM" and ProStor" in production compound feeds on the intestinal microflora of sturgeon’s juveniles.

2. Materials and methods

Experimental work was carried out in the Innovation Center "Bioaquapark - Scientific and Technical Center of Aquaculture" of the Astrakhan State Technical University for 14 days. The fry of Russian sturgeon (Acipenser queldenstadtii Brandt et Ratzeburg, 1833) was used as the objects of research.

Two types of probiotic preparations were used for the research. The first sample – preparation "Ferm-KM" is a complex of bifidobacteria Bifidobacterium bifidum, Bifidobacterium longum, as well as lactobacillus strains Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus plantarum. The second sample - symbiotic additive "ProStor" - includes live bacillus cells and metabolites immobilized on a phytosorbent: strains of Bacillus subtilis, Bacillus licheniformis, a complex of lactic acid bacteria and products of their metabolism.

The selection of microbiological samples of fish intestines was carried out in the research laboratory of microbiological monitoring of the Department of "Applied Biology and Microbiology" of the FSBEI HE "ASTU" (Astrakhan). Sampling was carried out twice: 1 sample - before the use of probiotic preparations, 2 samples - at the end of the experiment. The generic and species identity of the obtained samples of microorganisms was determined using biochemical plates differentiating staphylococci and enterobacteria (developed by SPO “Diagnostic Systems”, Nizhny Novgorod). To determine the amount of lactic acid microorganisms in the intestines of fish, the method of deep seeding was used at the rate of 0.66 and 2.16 g of intestines on the medium cabbage agar. The inoculation was incubated for 5 days at a temperature of 30°C. Determination of the number of microorganisms was determined from calculations for 1 g.

3. Results

Series of experiments was conducted to analyze the intestinal microflora of Russian sturgeon fry before and after feeding with mixed feeds with the addition of new-generation probiotic preparations. The farmed fish were divided into 3 groups: a control group and two experimental ones. The control group consumed mixed feed without the addition of probiotic preparations, the first experimental group – mixed feed with symbiotic additive "ProStor", the second experimental group – mixed feed with probiotic preparation "Ferm-KM". For feeding, we used production compound feed for sturgeon fish of the OT-7 formulation. For autopsy, 5 specimens of Russian sturgeon fry were selected from each variant of the experiment and control, and a microbiological study of the intestinal microflora was carried out. After 14 days of feeding the fish with probiotic preparations, a second autopsy was performed.

Analysis of the intestines of Russian sturgeon fry before feeding revealed the presence of microorganisms of different taxa: g. Pseudomonas, fam. Enterobacteriaceae, coccoid forms, g. Bacillus, fungi. The dominant position among the identified microorganisms in all variants of the experiment was occupied by representatives of g. Citrobacter, their average number was 22x10^3 CFU/g. In smaller quantities, there are microorganisms of g. Morganella, which cause putrefactive processes in the intestines of fish. The amount of Escherichia coli varied from 1.4 to 2.5x10^3 CFU/g.
(figure 1). At the end of feeding, there is a tendency to increase the lactic acid bacteria *g. Bacillus* (figure 2).

**Figure 1.** The number of bacteria of *g. Bacillus* and *E. coli* in the intestines of Russian sturgeon fry before feeding, x $10^3$ CFU/g.

**Figure 2.** The number of *g. Bacillus* bacteria in the intestines of Russian sturgeon fry after feeding, x $10^7$ CFU/g.
Quantitative characteristics of the microbial background of the intestine of the Russian sturgeon fry had some variations not only in the control, but also in the experimental variants. In all the presented experiments, there was a tendency to increase the number of microorganisms from the beginning of the experiment to its end (Table 1). The total number of intestinal microorganisms in the control variant before feeding was $3.3 \times 10^5$ CFU/g, but at the end of the experiment, this indicator increased to $1.2 \times 10^7$ CFU/g.

| Indicator   | Control     | Experimental group 1 | Experimental group 2 |
|-------------|-------------|----------------------|----------------------|
| Before feeding | $3.3 \times 10^5$ | $2.4 \times 10^5$ | $3.1 \times 10^5$ |
| After feeding   | $1.2 \times 10^5$ | $6.0 \times 10^6$ | $8.5 \times 10^7$ |

In this article, the effectiveness of the use of new-generation probiotic preparations "Ferm-KM" and "ProStor" in the composition of production compound feeds for young sturgeon fish was scientifically substantiated. The positive effect of the introduction of preparations into the feed is to normalize the symbiotic intestinal microflora of juvenile sturgeon fish. As a result of the use of compound feeds with probiotics by local microflora, non-specific protection of the intestine from pathogenic viruses and bacteria with genetically determined invasive properties will be carried out by creating an antagonistic barrier (colonization resistance of the intestine). When in contact with the intestinal mucosa, it covers its surface with a thick layer, thereby protecting it from the penetration of pathogenic microorganisms.

4. Discussion
In the body of fish, significant deviations in the autoflora are noted, caused by such phenomena as violation of the conditions of maintenance and feeding, constant stress effects of various etiologies, contributing to negative shifts in the microflora. Against the background of normoflora deficiency, the normal relations between obligate intestinal microorganisms are violated, causing an imbalance between normal and conditionally pathogenic microorganisms and dysbacteriosis. Therefore, preventive measures include the use of means for the formation of normobiosis and colonization resistance, among which probiotics are of no small importance.

The most important aspects of the interaction of probiotic strains with the intestinal microflora and the animal body include: the formation of antibacterial substances, competition for nutrients and the place of adhesion, changes in microbial metabolism (decrease or increase in enzymatic activity), stimulation of the immune system.

Based on the conducted studies, it was found that the use of probiotic preparations "Ferm-KM" and "ProStor" in the composition of mixed feeds contributes to a significant reduction in the growth of enterobacteria, representatives of the genera *Pseudomonas* and *Staphylococcus*, and also increases the number of lactic acid organisms in the intestines of fish.

5. Conclusion
In this article, the effectiveness of the use of new-generation probiotic preparations "Ferm-KM" and "ProStor" in the composition of production compound feeds for young sturgeon fish was scientifically substantiated. The positive effect of the introduction of preparations into the feed is to normalize the symbiotic intestinal microflora of juvenile sturgeon fish. As a result of the use of compound feeds with probiotics by local microflora, non-specific protection of the intestine from pathogenic viruses and bacteria with genetically determined invasive properties will be carried out by creating an antagonistic barrier (colonization resistance of the intestine). When in contact with the intestinal mucosa, it covers its surface with a thick layer, thereby protecting it from the penetration of pathogenic microorganisms.
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