Chapter 2
The Effect of Modern Probiotic Preparations on Enterosorbents on the Piscicultural and Microbiological Parameters of Sturgeon Fish

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Abstract Currently, in order to prevent and treat a large number of pathological conditions caused by a violation of endoecology, enterosorbent-based preparations that help cleanse the body of metabolic products, heavy metals, radionuclides and various toxins have been widely used. However, the presence of only enterosorbents in the preparation can have a negative impact on the body, since these substances eliminate not only toxins, but also metabolites necessary for the full functioning of the body, and also reduce the number of beneficial microorganisms. To solve this problem, it is necessary to use complex preparations, in particular probiotics on enterosorbents, which have increased efficiency of sorption and desorption of eubiotic bacteria. Microorganisms located in the pores, as well as microbial cells located close to the outer surface of the sorbent, differ in their desorption properties. The porous carrier, being an enterosorbent, relieves the effects of toxicosis, which contributes to the survival of the bacteria of the drug and the intestinal microflora, and also reduces the load on the detoxification organs of fish. The presence of bifidobacteria and lactobacilli in the preparation contributes to an increase in the rate of biomass accumulation in the conditions of the technological process by preserving enzymatic activity and increasing the efficiency of the use of nutrient media. It was found that the introduction of a probiotic drug immobilized on an enterosorbent,

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"Ecoflor" in a dosage of 4 g/kg of compound feed contributes to an increase in fish-breeding biological and microbiological indicators of farmed fish. There will be an improvement in the physiological state of fish and an increase in the protective properties of the immune system. "Ecoflor" has pronounced sorption properties, the ability to fix and remove bacteria, toxins and allergens from the intestine.

Keywords Russian sturgeon · Probiotic preparation · Enterosorbent · Fish-breeding and biological indicators · Microbiological indicators

2.1 Introduction

Currently, it is urgent to search for new approaches to the maintenance and cultivation of sturgeon species of fish, allowing to obtain the maximum yield of products, spending the minimum amount of resources. One of such directions is the normalization of the bacterial flora of hydrobionts grown in closed water supply installations, based on the use of various kinds of probiotic preparations.

It is proved that the density of the microbial population of the contents of the gastrointestinal tract of sturgeon fish depends on the density of the microbial population in the reservoir. Even during periods of wintering and starvation, the intestines of fish are not freed from bacterial population, however, the number of bacteria decreases by 10 times or more in comparison with their number with intensive nutrition.

The survival and viability of all fish species directly depends on their diet. The cultivation of sturgeon species of fish is based on a properly formulated feeding technology, and this is what ensures the maximum survival rates and growth rate, while spending the minimum amount of feed.

To date, it is necessary to create recipes for highly effective compound feeds for fish, including sturgeon species, and for this you need to carefully study the nutrients of natural fish food and the characteristics of all body systems. Also, to create a complete feed, it is necessary to select all the components correctly, while studying not only their production properties, but also to fully balance the feed in terms of nutrient content, based on the needs of the cultivated species at a certain stage of development.

2.2 Materials and Methods

A promising direction in feed production is the production of compound feeds with the use of probiotic preparations on enterosorbents necessary for the prevention and treatment of bacterial and viral etiology of fish diseases, normalization of intestinal microflora by removing pathogenic and conditionally pathogenic microorganisms, toxins and metabolic products from the body (Zhandalgarova et al. 2017). Such preparations can mitigate the stresses caused by the change of compound feeds and

technological effects on the body of fish, serve to increase the resistance of the body and the tension of immunity, allow to increase the digestibility of compound feeds (Zhandalgarova 2014).

This trend in the development of feed production makes it necessary to conduct scientific research, the purpose of which is to evaluate the effectiveness of the use of probiotic preparation on enterosorbent in production compound feeds for sturgeon fish.

To achieve the goal, the following tasks were set:

(1) To study the piscicultural indicators of two-year-old Russian sturgeon when grown on mixed feed with the addition of enterosorbent;
(2) Analyze the microflora of the gills and intestines of the Russian sturgeon when feeding with compound feed with the addition of the "Ecoflor" preparation.

Experimental studies were conducted at the Innovation Center "Bioaquapark—Scientific and Technical Center of Aquaculture" of the Federal State Educational Institution of Higher Education "ASTU". The objects of research were two-year-old Russian sturgeon (Acipenser gueldenstaedtii Brandt et Ratzeburg, 1833).

For the research, the probiotic preparation "Ecoflor" was used, which is a consortium of natural strains of lacto- and bifidobacteria: B. bifidum, B. longum and L. acidophilus, L. casei, L. plantarum, immobilized on the carbon-mineral sorbent SUMC-1. The normative content of bifidobacteria in the preparation is 108 CFU / g, lactobacilli—107 CFU / g.

Throughout the experimental work, fish were kept in fiberglass tanks with rounded corners with a volume of 1 m^3. At the same time, the stocking density was 15 individuals per tank. The content of dissolved oxygen in water during the entire growing period ranged from 7–9 mg/ l, pH—6.5–7.0, and the water temperature was maintained at 18–22° C.

Studies on the effectiveness of the use of a probiotic preparation as part of production compound feeds were carried out in laboratory conditions for 30 days. To do this, the fish were divided into 4 groups. The control group was fed with compound feed of the OT-7 formulation. Feeding of the first experimental group was carried out with compound feed OT-7 with the addition of the "Ecoflor" preparation at a dosage of 2 g per 1 kg of feed, the second experimental group—with the addition of the preparation at a dosage of 4 g per 1 kg of feed, the third experimental group—with the addition of 6 g of the preparation per 1 kg of feed.

Throughout the experiment, the daily feeding rate was calculated based on data on the body weight of fish and the water temperature in the tanks, in accordance with the generally accepted cultivation technology (Ponomarev et al. 2002).

The size and weight parameters of the studied individuals of the Russian sturgeon were determined by the method of Pravdin I.F. (1966).

In order to assess the symbiotic microflora of gills and intestines of farmed fish, their microbiological analysis was carried out. Before the selection of fragments of gills and intestines, the fish body was treated with alcohol. The autopsy was performed with sterile instruments. The test samples were placed in pre-prepared

sterile penicillin vials. The microflora was studied according to the method of I.V. Burlachenko and L.I. Bychkova (2005). Sampling was carried out three times: 1 sample—before feeding with experimental compound feed with the addition of the "Ecoflor" preparation, 2 sample—in the middle of the experiment (during feeding with experimental compound feed), 3 sample—after feeding. After sampling of the fish under study, seeding was carried out on various media: cabbage agar, Bleakfiltd, Li, nutrient agar (PA) using the method of deep seeding. To analyze the microbiological seeding of the gills and intestines of fish, the method of deep seeding on various media was used. The crops were incubated for 5 days at a temperature of 30° C. The number of microorganisms was determined based on 1 g.

2.3 Results

The effectiveness of feed with the addition of the "Ecoflor" in the diet of two-year-old Russian sturgeon was evaluated on the basis of its feeding for 30 days. The grown individuals were divided into 4 groups: 3 experimental and 1 control. The control group was fed OT-7 compound feed throughout the experiment, 1 experimental group was fed OT-7 compound feed with the addition of the probiotic "Ecoflor" with a dosage of 2 g/kg of feed, 2 experimental group—4 g/kg, 3 experimental group—6 g/kg (Table 2.1).

For the entire experimental period of cultivation, the best results in linear-weight indicators are characteristic of the 2 experimental group, in which the dosage of the drug was 4 g/kg. The absolute increase in the second experimental group was 70,1 g, which is 34 g more than the increase in the control variant.

It should be noted that feeding two-year-old Russian sturgeon with OT-7 feed in the dosage of the "Ecoflor" 4 g/kg allowed to reduce feed costs to 1,2 units, as well as increase the growth rate.

Table 2.1 Piscicultural indicators of growing two-year-old Russian sturgeon

Indicator	Group			
	Control	Test 1	Test 2	Test 3
Initial weight, g	575,3 ± 5,6	564,2 ± 8,2	567,1 ± 8,7	570,5 ± 5,5
Final weight, g	611,4 ± 0,3	632,4 ± 6,9	637,8 ± 9,2	629,5 ± 2,5
Absolute growth, g	36,1	68,2	70,1	59
Average daily growth, g	1,20	2,27	2,34	1,97
Average daily growth rate, %	0,20	0,38	0,39	0,33
Mass accumulation coefficient, units	0,017	0,032	0,033	0,038
Feed ratio	1,5	1,3	1,2	1,3
Survival rate, %	100	100	100	100
Duration of the experiment, day	30	30	30	30

Table 2.2 Data on the quantitative analysis of microorganisms on the gills of experimental individuals of Russian sturgeon before feeding with compound feed with the addition of the "Ecoflor"

Name of the medium	Control group, CFU/g	Test group, CFU/g
Cabbage agar	$2{,}81*10^4$	$2{,}75*10^5$
Bleakfeltd	$4{,}36*10^5$	$3{,}98*10^5$
Li	$3{,}54*10^4$	$2{,}62*10^5$
PA	$1{,}1*10^5$	$1{,}5*10^5$

Throughout the experiment, the piscicultural indicators of two-year-old Russian sturgeon raised in the second experimental group were significantly better than in the control variant. At the same time, if we compare the results in the three experimental groups, then it was in the second that there was a rapid improvement in linear-weight characteristics.

During the experimental cultivation, a qualitative and quantitative analysis of bacterial microflora on the gills of the Russian sturgeon was carried out before and after feeding with a probiotic preparation immobilized on the enterosorbent "Ecoflor". The method of deep seeding on various media was used: cabbage agar, Bleakfeltd, Li and PA (Table 2.2). The individuals of Russian sturgeon were divided into two groups: control and experimental. Feeding of the experimental group was carried out with compound feed with the addition of 4 g/kg of the probiotic drug "Ecoflor", since this dosage is the most optimal.

During the study of isolated cultures of microorganisms before the start of experimental studies, the following colonies were identified: colonies of beige-milky color, slimy, rounded; colonies of yellow color, rounded, glossy; colonies of milky color, creeping along the substrate; colonies of brown color, irregular shape; colonies of pale pink color, round, convex glossy. Among the above colonies, gram-positive bacillus (group 19; Lactobacillus), gram-positive cocci (group 17; p. *Pedioccocus, Leuconoctoc*) and gram-positive spore-forming bacillus (group 18; p. *Bacillus*) (Bergey 1997).

After the end of feeding the experimental group with compound feed with the addition of a probiotic preparation immobilized on enterosorbent, an additional analysis of the quantitative and qualitative microbiological state of the gills of experimental individuals of Russian sturgeon was carried out. Samples were also taken from the control and experimental groups. The method of deep seeding on various media was used (Table 2.3).

During the study of the isolated cultures of microorganisms after the completion of feeding with compound feed with the addition of the "Ecoflor", the following colonies were identified: colonies that eat away the substrate; colonies of orange color, rounded, wrinkled, glossy; colonies of milk color, irregular shape, matte; colonies of beige-milk color, slimy, rounded; colonies of brown color, oval, convex; colonies of pale pink color, round, convex glossy. As a result of the analysis of

Table 2.3 Data on the quantitative analysis of microorganisms on the gills of experimental individuals of Russian sturgeon after the end of feeding with the addition of the "Ecoflor"

Name of the medium	Control group, CFU/g	Test group, CFU/g
Cabbage agar	$1{,}57*10^4$	$2{,}75*10^5$
Bleakfeltd	$1{,}57*10^5$	$1{,}63*10^5$
Li	$1{,}42*10^4$	$1{,}62*10^5$
PA	$1{,}42*10^6$	$1{,}5*10^6$

microbiological seeding, lactic acid microorganisms were identified: gram-positive bacillus and cocci belonging to the genera *Lactobacillus, Pedioccocus, Leuconoctoc*.

When feeding two-year-olds of Russian sturgeon with compound feed with the addition of the "Ecoflor" at a dosage of 4 g/kg, an increase in the concentration of microorganisms on the gills was observed.

At the second stage of experimental work, a qualitative and quantitative analysis of the bacterial microflora in the intestines of the Russian sturgeon was carried out before and after feeding with a probiotic immobilized on the enterosorbent "Ecoflor". The method of deep seeding on various media was used: cabbage agar, Bleakfeltd, Li and PA (Table 2.4).

When analyzing the microbiological seeding of the intestines of experimental individuals of Russian sturgeon, several varieties of bacterial colonies were found: milky colonies, creeping along the substrate; beige-milky colonies, slimy, rounded; yellow colonies, rounded, glossy; brown colonies, irregular shape; pale pink colonies, round, convex glossy. Among them, gram-positive bacillus (group 19; Lactobacillus); gram-positive cocci (group 17; p. *Pedioccocus, Leuconoctoc*) and gram-positive spore-forming bacillus (group 18; p. *Bacillus*) (Bergi 1997).

After the experimental group was fed with compound feed with the addition of the probiotic "Ecoflor" immobilized on enterosorbent, an additional analysis of the quantitative and qualitative microbiological state of the intestines of experimental individuals of Russian sturgeon was carried out. Samples were also taken from the control and experimental groups. The method of deep seeding on various media was used (Table 2.5).

During the study of the cultural properties of microorganisms identified on crops, the following bacterial colonies were found: colonies of a corroding substrate; colonies of milk color, irregular shape, matte; colonies of beige-milk color, slimy,

Table 2.4 Data on the quantitative analysis of microorganisms in the intestines of experimental individuals of Russian sturgeon before feeding with compound feed with the addition of the "Ecoflor"

Name of the medium	Control group, CFU/g	Test group, CFU/g
Cabbage agar	$2{,}55*10^4$	$2{,}9*10^5$
Bleakfeltd	$3{,}02*10^5$	$2{,}85*10^5$
Li	$3{,}12*10^4$	$4{,}63*10^5$
PA	$2{,}38*10^5$	$3{,}2*10^7$

Table 2.5 Data on the quantitative analysis of microorganisms in the intestines of experimental individuals of Russian sturgeon after the end of feeding with compound feed with the addition of the "Ecoflor"

Name of the medium	Control group, CFU/g	Test group, CFU/g
Cabbage agar	$3{,}9*10^5$	$1{,}38*10^6$
Bleakfeltd	$1{,}85*10^5$	$3{,}45*10^5$
Li	$1{,}63*10^6$	$3{,}4*10^6$
PA	$1{,}2*10^7$	$5{,}8*10^7$

rounded; colonies of orange color, rounded, glossy; colonies of brown color, oval, convex; colonies of orange color, wrinkled; colonies of milk color, irregular shape, matte; colonies of brown color, oval, convex, rough; pale pink colonies, round, convex glossy; colonies are pale pink, round, convex glossy. In the course of the study of the data obtained as a result of microbiological analysis of the intestines of experimental individuals of Russian sturgeon, lactic acid microorganisms were identified: gram-positive bacillus and cocci belonging to the genera: *Lactobacillus, Pedioccocus, Leuconoctoc* (Bergi 1997).

It should be noted that feeding two-year-olds of Russian sturgeon with compound feed with the addition of the "Ecoflor" had a beneficial effect on the intestinal biocenosis. An increase in the quantitative composition of the bacterial biota of the intestine was observed both during feeding with experimental compound feed and after feeding.

2.4 Discussion

When an immobilized probiotic preparation is introduced into the production feed at a dosage of 4 g/kg, an improvement in piscicultural indicators is observed in the reared individuals of Russian sturgeon (the absolute increase was 70,1 g, the average daily growth rate was 0,39%, the mass accumulation coefficient was 0,33 units), a reduction in feed costs to 1,2 units.

As a result of the analysis of microbiological seeding of gills, lactic acid microorganisms were identified: gram-positive bacillus and cocci belonging to the genera *Lactobacillus, Pedioccocus, Leuconoctoc*. When feeding two-year-olds of Russian sturgeon with compound feed with the addition of the "Ecoflor" at a dosage of 4 g/kg, an increase in the concentration of microorganisms on the gills was observed when feeding with experimental compound feed, immediately after the end of feeding, a decrease in this indicator was observed.

During the study of the data obtained as a result of microbiological analysis of the intestines of experimental individuals of Russian sturgeon, lactic acid microorganisms were identified: gram-positive bacilluus and cocci belonging to the genera:

Lactobacillus, Pedioccocus, Leuconoctoc. It should be noted that feeding two-year-olds of Russian sturgeon with compound feed with the addition of the "Ecoflor" had a beneficial effect on the intestinal biocenosis. An increase in the quantitative composition of the bacterial biota of the intestine was observed both during feeding with experimental compound feed and after feeding.

If an infectious pathology occurs as a result of a violation of the balance between normal and pathogenic intestinal microflora, antibacterial therapy is used. This method involves the use of various kinds of antibiotics. If antibacterial therapy does not bring proper results, but on the contrary increases the load on the animal's body, it is necessary to immediately introduce probiotics into the diet, but the use of probiotics as the main medicine is excluded.

The addition of the drug "Ecoflor" allows you to influence a wide range of problems that are directly related to the physiological state of animals. It is also possible to stimulate the growth and development of the body, while increasing the efficiency of digestion. From which it follows that this preparation is an effective element in the production technology of fish farming products that are safe for humans.

2.5 Conclusion

As a result of the conducted research, it was found that it is advisable to introduce a probiotic on the enterosorbent "Ecoflor" into the production compound feeds for sturgeon fish. The rate of probiotic input is 4 g/kg of compound feed. This dosage contributes to an increase in piscicultural and hematological parameters of fish, as well as to the improvement of the microbiological status of farmed fish.

The use of Russian sturgeon with productive feeds in combination with immobilized probiotic preparations can significantly increase profits and minimize feed costs.

Production compound feed with the addition of an immobilized form of probiotic preparation for sturgeon fish species helps to eliminate the existing negatives in fish farming, as it allows to increase the volume of products obtained, improve the quality of compound feeds. Therefore, it is one of the ways to increase the efficiency of cultivation and rational use of compound feeds.

References

Burlachenko KV, Bychkova LK (2005) Method of clinical assessment of the condition of sturgeon fish during their cultivation in installations with a closed water supply cycle. Fisheries 6:70–72 (in Russian)

Hoult J, Krig N, Snitt P (eds) (1997) The determinant of bacteria Bergi. m The determinant of bacteria Berg. Mir., Moscow, Russia (in Russian)

Ponomarev SV, Gamygin EA, Nikonorov SI, Ponomareva EN, Grozesku YuN, Bakhareva AA (2002) Technologies of cultivation and feeding of aquaculture objects in the South of Russia. Nova Plus, Astrakhan, Russia (in Russian)

Pravdin PF (1966) Guide to the study of fish. Food industry, Moscow, Russia (in Russian)

Zhandalgarova AD (2014) The use of probiotic drugs as sorbents in the toxic effect of surfactants on sturgeon. In: Matishov GG (eds) Proceedings from: topical issues of fisheries and aquaculture of the basins of the southern seas of Russia. Publishing House of the SSC RAS, Rostov-On-Don, Russia (in Russian)

Zhandalgarova AD, Bakhareva AA, Grozesku YuN, Pravdin AI (2017) The influence of a new generation synbiotic feed additive "PROSTOR" on the fish-breeding, biological and hematological parameters of the young Russian sturgeon. Chief Zootechnik 7:30–35 (in Russian)