The use of Akwa-Biot-Norm biogenic feed additive in the cultivation of Lena sturgeon (*Acipenser baeri, Brandt*)

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Abstract. Growing fish in industrial conditions is accompanied by the pressure of various environmental factors on it. Any changes in the environment affect the physiological state and general resistance of the fish, resulting in reduced growth rates, resistance to adverse factors of different nature, and increased mortality. In such conditions, the issue of finding the means and methods of enhancing the protective functions of objects of breeding and cultivation is particularly relevant. Currently, the drugs that have pronounced biological activity are used to improve the functional state of the fish, as well as to increase the value of the artificial feed. In the conditions of the fish farm of LLC YUTAS of the Chuvash Republic, extensive production studies were conducted to assess the effectiveness of the use of nutrient feed additive Akwa-Biot-Norm to the Lena sturgeon, developed by scientists of the Chuvash State Agricultural Academy. Research results indicate that the use of nutrient feed additives in the technology of growing Lena sturgeon (*Acipenser baeri, Brandt*) has a positive effect on some fish-breeding indicators, such as growth rates and survival rates for fish, etc. Thus, the use of biogenic feed additives contributed to a significant increase in the overall weight gain of fish by 5.06% and safety by 2.6% for the period of the experiment (240 days). The results of hematological and immunological studies show a general improvement in the physiological state of the growing objects. The use of biogenic feed supplements had a positive effect on the number of erythrocytes and leukocytes, hemoglobin content, the ratio of lymphocytes and neutrophils and the concentration of total protein in blood serum. In fish, there was an increase in the level of hemoglobin in the blood, as well as an increase in the total protein content in the blood serum by 10.9% to the control indicator, which is a favorable prerequisite for optimizing metabolic processes and guaranteeing high nonspecific resistance. The application of nutrient feed additives has improved the resistance of fish to diseases associated with stress, such as saprolegnia. The conducted studies confirm the efficiency of application of nutrient feed additives Akwa-Biot-Norm for growth stimulation and disease prevention of fish due to the activation of nonspecific resistance of the organism in terms of the pressing environmental and technological stress factors.

1. Introduction

It is possible to meet the population’s demand for marketable fish products, including sturgeon, in modern conditions of a decrease in commercial fish production, due to aquaculture, which is a reliable source of increasing fish food production and serves as a guarantor of Russian food security. Currently, industrial fish farming, based on intensive methods of cultivation in cages and swimming pools with direct and closed water supply, is gaining more and more development. At the same time, the technology
of sturgeon cultivation with a high density of planting in warm-water farms is used, which allows growing fish all year round and get maximum indicators on fish productivity and its quality [1-6].

However, in conditions of artificial reproduction of fish under high biomass loads per unit volume, artificial feeding of farmed objects, water pollution with organic substances, various technical operations, etc. are constant stress factors. Their continuous influence contributes to reducing the overall resistance of the organism of fish, resulting in reduced growth rates, resistance to the action of adverse factors of different nature and, as a result, preservation decreases [4, 7, 8, 9].

In such conditions, the issue of finding the means and methods of enhancing the protective functions of objects of breeding and cultivation is particularly relevant. Currently, the introduction of antibacterial, probiotic and other biologically active drugs into the rations for fish is practiced [10, 11].

The determination of the physiological and immunological state of the fish organism provides valuable information that allows evaluating the effectiveness of the use of biogenic preparations for enhancing nonspecific resistance [12-14].

The purpose of this work is to study the physiological and immunological state of the body of fish against the background of the application of a biogenic feed additive based on a polysaccharide complex of yeast cells.

2. Material and methods

Research work was carried out in a specialized full-system fish farm LLC YUTAS, Cheboksary, Russian Federation, in which sturgeon fish are grown. In the farm for feeding fish using extruded feed Efiko Sigma, manufactured by BioMar, Denmark. Scientists of the Chuvash State Agricultural Academy have developed a technology for introducing the developed biogenic feed additive into the feed to improve the biological usefulness of animal feed, ensuring that its initial biological activity is preserved, and eliminating the loss of the drug as a result of washing out with water.

The objects of study were individuals of the Siberian sturgeon of the Lena population (Acipenser baerii Brant, 1869). Two groups of fish were formed for the experiment, 500 copies each, experimental and control, according to the principle of groups of analogs, taking into account the clinical and physiological state and body weight. The average live weight of fish in the control group was 245.1 ± 3.59 g, in the experimental group 243.6 ± 3.48 g. The biogenic feed additive Akwa-Biot-Norm, developed by scientists of the Chuvash State Agricultural Academy, Russian Federation, was additionally introduced into the diet of fish from the experimental group, in two courses lasting 5 days with a break of 2 days. At the beginning of the experiment and every decade of the experiment, control weights of the fish were carried out with a threefold random sample of 10 specimens to determine the growth dynamics. In addition, before the experiment and upon completion, blood samples were taken from 10 samples from each group for hematological, biochemical and immunological studies. Morbidity and safety of fish were recorded in the groups.

The biogenic feed additive Akwa-Biot-Norm is a suspension of 50 mass parts of a 1% gelatin solution, 1 mass part of the concentrate of the purified Saccharomyces cerevisiae polysaccharide complex and 3 mass parts of levomizol.

Research work was performed using the following methods:

1) **hematological** - determined the number of erythrocytes, erythrocyte sedimentation rate, hemoglobin concentration, leukocyte count, additional leukogram differentiation was performed microscopically, making blood smears and staining using Romanovsky method, Giemsa modification, blood for analysis was taken in vivo from the tail artery, immediately after extraction fish out of water;

2) **biochemical** - the level of total protein in the blood serum was studied using an IRF-454B-2M refractometer (A.M. Akhmedov), the protein spectrum using a turbidimetric method (S.A. Karpyuk);

3) **immunobiological** - phagocytic activity of leukocytes was determined using a daily agar culture of Staph. aureus, strain 0-55 (V.S. Gostev), lysozyme activity of blood plasma using daily agar culture of M. lysodeiticus, strain ML-43-29-1 (V.G. Dorofeychuk), bactericidal activity of serum
using daily agar E.coli cultures (O.V. Smirnova et al.);

4) zootechnical - determined the live weight and average daily gain of the Lena sturgeon by periodic weighing, exterior measurements;

The digital material of the experiments was processed by the method of variation statistics on the reliability of the difference between the compared indicators (P < 0.05-0.01) using the Microsoft Office Excel 2007 software package.

Diagnostic studies and data processing were performed under the Department of Parasitology, epizootiology and veterinary-sanitary examination of FBOU HE the Chuvash State Agricultural Academy, BU CR "Chuvash Republican Veterinary Laboratory" of the state veterinary service of the CR.

3. Research results

The results of monitoring the safety and increase in live weight of the Lena sturgeon are shown in Table 1.

Table 1. Growth and safety of Lena sturgeon.

| Indicator             | Group            | Group            |
|-----------------------|------------------|------------------|
|                       | control n=500    | experimental n=500 |
| Average fish weight, g| At the beginning of the experiment | 245.1±3.59 | 243.6±3.48 |
|                       | Upon completion of the experiment | 930.8±5.98 | 964.2±6.39** |
| Live weight gain, g   | 685.7            | 720.6            |
| Safety, %             | 94.4             | 97.0             |
| Ichthyomass, kg       | At the beginning of the experiment | 122.55 | 121.80 |
|                       | Upon completion of the experiment | 439.33 | 467.68 |

** P<0.01

From the data obtained it can be seen that upon the completion of the experiment, the sturgeon of the control group had an average weight of 930.8 g, experienced - 964.2 g, which is 33.4 g more, or - by 3.65%. The total weight gain in the control group was 685.7 g, the experimental group - 720.6 g, which is more by 34.9 g. High preservation of fish had a positive effect on the ichthyomass of the experimental group, which amounted to 467.68 kg, against 439.33 kg of the control group, which is 28.35 kg more. These results indicate a positive effect of feed supplementation on fish growth and survival.

At the beginning of the experiment, the number of erythrocytes in the blood of fish in the control group was 0.70 x 10^{12} / l, which is within the physiological norm. Upon the completion of the experiment, this indicator did not change significantly and had a value of 0.79 x 10^{12} / l. In the blood of the fish of the experimental group, this indicator significantly increased by 26.4% and amounted to 0.86 x 10^{12} / l.

The hemoglobin concentration in the blood of the fish of the experimental group at the beginning of the experiment had a value of 33.3 ± 1.07 g / l, the control - 32.73 ± 1.21 g / l (Table 2). Upon the completion of the experiment, this indicator increased, the hemoglobin content in the blood of fish in the control group was 75.7 ± 2.23 g / l, and in the experimental one - 83.2 ± 1.91 g / l, which is 9.9% statistically significantly more benchmark.

An analysis of the dynamics of hemoglobin concentration indicates that, against the background of the use of biogenic feed additive Akwa-Biot-Norm in the technology of feeding Lena sturgeon, its increase is observed. When comparing this indicator with the number of erythrocytes, it becomes obvious that an increase in hemoglobin concentration occurs both due to an increase in the number of erythrocytes and due to an increase in their functional activity, which indicates activation of hemopoiesis, an increase in the intensity of redox processes and metabolism in general.
When determining the dynamics of the content of leukocytes in the blood of fish against the background of the use of a biogenic feed additive, leukocytosis was established, which was within the physiological norm in the blood of the fish of the experimental group. At the beginning of the experiment, the number of leukocytes in the blood of the Lena sturgeon of the experimental group was 24.9 ± 0.69 x 10⁹ / l, and the control group - 24.3 ± 0.53 x 10⁹ / l. Upon the completion of the experiment, this indicator did not have significant differences from the initial and control indicators in the blood of fish of the experimental group.

The percentage of eosinophils in the blood of the Lena sturgeon of the experimental group at the end of the experiment increased to 74.65%. Upon completion of the experiment, this indicator did not have significant differences from the initial and control indicators in the blood of fish of the experimental group, and that of the experimental group was 74.6%. Upon the completion of the experiment, the number of leukocytes in the control group, amounted to 24.3 ± 0.53 x 10⁹ / l, was statistically unreliable.

At all periods of observation, the relative amount of monocytes in the blood of fish did not have statistically significant differences between the groups and was within the physiological norm.

Analysis of the leukocyte picture shows that when using the biogenic feed additive Akwa-Biot-Norm in fish, an increase in the absolute number of leukocytes and a decrease in the relative number of eosinophils is observed. There is an increase in lymphocytes in the blood of fish of the experimental group. The decrease in the relative amount of eosinophils can be explained by an increase in the proportion of lymphocytes. The tests indicate leukocytosis within the physiological norm, with pronounced lymphocytosis, having an absolute majority in the leukocyte picture. These data confirm the high degree of activity of the cellular component of the immune system.

At the beginning of the experiment in the blood serum of Lensky sturgeon of the experimental group, the amount of total protein was 19.41 g/l. At the end of the experiment, this figure increased and amounted to 24.9 ± 0.69 x 10⁹ / l or 10.9% more than in the control, but statistically unreliable.

An increase in the protein concentration in the blood serum of the Lena sturgeon of the experimental group correlates with an increase in ichthyomass, which indicates the activation of synthetic processes in the body of fish and, as a consequence, an increase in their growth.

### Table 2. Morphological and biochemical parameters of the blood of Lensky sturgeon.

| Indicator       | At the beginning of the experiment | Upon completion of the experiment |
|-----------------|-----------------------------------|----------------------------------|
|                 | Control  | experimental | Control  | experimental |
| Red blood cells, 10¹² / l | 0.70±0.01 | 0.68±0.04  | 0.79±0.02 | 0.86±0.01*   |
| Hemoglobin, g/ l    | 32.73±1.21 | 33.3±1.07  | 75.7±2.23 | 83.2±1.91*   |
| Leukocytes, ×10⁹ / l | 24.3±0.53 | 24.9±0.69  | 34.10±2.10 | 33.8±2.01    |
| Neutrophils, %      | 13.2±0.39 | 13.3±0.99  | 16.6±1.17 | 16.9±1.54    |
| Eosinophils, %      | 9.8±0.27  | 9.9±0.31   | 5.75±0.34 | 5.04±0.55    |
| Basophils, %        | 0.3±0.01  | 0.3±0.03   | 0.4±0.02  | 0.4±0.01     |
| Lymphocytes, %      | 74.7±1.53 | 74.6±1.23  | 74.65±1.65 | 75.36±1.58   |
| Monocytes, %        | 1.91±0.04 | 1.83±0.03  | 2.6±0.67  | 2.3±0.55     |
| Total protein, g/ l | 19.08±0.57 | 19.41±0.78 | 25.4±1.63 | 28.18±1.77   |

* P<0.05
The results of the study of the immunological properties of the blood of Lensky sturgeon against the background of the use of biogenic feed additives based on the polysaccharide complex of yeast cells are presented in Table 3.

**Table 3. Immunological properties of the blood of Lena sturgeon.**

| Indicator                  | At the beginning of the experiment | Upon completion of the experiment |
|----------------------------|-----------------------------------|----------------------------------|
|                            | Control                           | experimental                     |
|                            | 13.7±0.56                         | 13.8±0.84                        |
| Bactericidal activity of blood serum, % | 14.3±1.02                         | 16.1±0.98*                       |
| Phagocytic activity of neutrophils, % | 18.3±0.18                         | 18.48±0.16                       |
|                            | 19.3±0.27                         | 20.92±0.47*                      |
| Lysozyme, µg / l           | 7.18±0.16                         | 7.2±0.18                         |
|                            | 9.4±0.29                          | 10.12±0.37                       |

* P<0.05

The bactericidal activity of the blood serum of the control and experimental fish at the beginning of the experiment did not differ significantly and was 13.7 ± 0.56% and 13.8 ± 0.84%, respectively. Upon the completion of the experiment, this indicator in the fish of the control group increased by 4.38% of the initial value but had no statistically significant differences. The bactericidal activity of the blood serum of the experimental fish at the end of the experiment was significantly higher than in the control and was 16.1 ± 0.98%. This indicator was higher than the original by 16.67%, and the control by 12.58%.

From the table, it follows that the phagocytic activity of blood neutrophils in the control group increased, but the value of this indicator is not statistically significant. The results of the experiment indicate that the phagocytic activity of the blood neutrophils in the fish of the experimental group increased. At the beginning of the experiment, this indicator was 18.48 ± 0.16%, and at the end of 20.92 ± 0.47%, which was significantly higher than the control by 8.39%.

Consequently, the biogenic feed additive Akwa-Biot-Norm activated cellular factors of nonspecific protection of the body of fish, with a more pronounced effect of the latter.

The concentration of the proteolytic enzyme at the beginning of the experiment in the blood serum of the control and experimental fish groups was 7.18±0.16 µg/l and 7.2±0.18 µg / l, respectively, within the physiological norm. Upon the completion of the experiment, lysozyme concentration in the control and experimental groups were also within physiological norms. In the blood of fish of the experimental group, the indicated indicator of nonspecific resistance increased by 40.55% and amounted to 10.7 ± 0.12 µg / l, but not statistically significant.

The efficiency of freshwater aquaculture depends on many factors, such as the sanitary and hygienic regime of the aquatic environment, the quality of animal feed, compliance with technical processes, etc. These requirements are not always met in a production environment. Fish are constantly exposed to various stress factors associated with the use of low-quality feed, violation of the temperature, oxygen regime, etc. Favorable conditions are created for the development of an infectious process caused by the association of microorganisms, while not necessarily pathogenic, and often even a complex of saprophytic bacteria that contaminate the fish's body as a result of a decrease in its resistance.

One of these diseases is saprolegniosis. During the work, we noted fish diseases with the following clinical signs: on the skin and fins, the presence of white thin filaments perpendicularly extending from the surface of the fish body. After a few days, a white bloom of white color consisting of interlaced hyphae appears on these places. Microscopic examination of scrapings from the skin showed clearly visible hyphae of the fungus. Based on these signs, a preliminary diagnosis was made - saprolegniosis. Selected pathological material for identification of the fungus in the laboratory.

The percentage of lesion of the Lena sturgeon in the control and experimental groups was established with careful examination of the fish. The results of the examination showed that the level of fish...
affection by saprolegnosis in the control group was 33.68%, and in the experimental group - 15.46%. Fishes of both groups are subjected to treatment-and-prophylactic treatment.

In the laboratory, in the study of pathological material, after obtaining a clean culture and growing on the environment of Chapek, the causative agent of saprolegniosis is established. Other infectious and invasive diseases, as well as non-infectious etiology, were not revealed. The safety of the fish in the experimental group for the entire experimental period was 97.0% against 94.4% in the control group.

The results of the experiment showed that the use of the nutrient feed additive Akwa-Biot-Norm to the Lena sturgeon increases their resistance to diseases associated with stress, such as saprolegniosis.

Thus, the use of the Akwa-Biot-Norm biogenic feed additive to the Lena sturgeon with two courses of 5 days duration with a break of 2 days has a positive effect on their body, activating nonspecific resistance factors and causing protection of fish from the negative effects of various environmental factors.

4. Conclusion
Against the background of the application of the biogenic feed additive Akwa-Biot-Norm based on the Saccharomyces cerevisiae polysaccharide complex of yeast cells in Lena sturgeon, an increase in live weight of fish is observed by 5.09%.

A significant increase in hemoglobin concentration by 9.9% was found due to an increase in the number of red blood cells and an increase in their functional activity.

A significant increase in the absolute number of leukocytes by 35.7% was noted, with a decrease in the relative amount of eosinophils. The relative number of lymphocytes, although not statistically significant, increased by 1.02%. Thus, leukocytosis within physiological norms, with pronounced lymphocytosis, with an absolute majority in the leukocyte formula and a slight decrease in the proportion of eosinophils, while maintaining their high numbers indicates a high degree of development of the cellular link of the immune system.

The amount of total protein in the blood serum of the Lensky sturgeon of the experimental group relative to the control indicator was higher by 10.9 %, which indirectly indicates the activation of synthetic processes in the body of sturgeons and, as a consequence, an increase in the intensity of fish growth.

The study of the immunological properties of the blood of Lensky sturgeon against the background of the use of biogenic feed additive Akwa-Biot-Norm revealed a significant increase in the value of such indicators of nonspecific resistance of the fish organism as bactericidal activity of blood serum by 16.6%, phagocytic activity of neutrophils by 13.2%, as well as an increase in the concentration of the proteolytic enzyme lysozyme by 40.5%. These indicators are higher than the corresponding indicators of the control group. These changes of the immunological properties of blood Lensky sturgeon indicate activation of cellular and humoral components of nonspecific resistance of the organism of the fish, and confirm effectiveness of application of nutrient feed additives Akwa-Biot-Norm based on polysaccharide complex of yeast cells Saccharomyces cerevisiae to enhance nonspecific resistance of the organism of sturgeon fish in conditions of environmental and technological pressures.

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