Evaluation of the effectiveness of feed additives in the diet of trout

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Abstract. The paper studied the effect of feed bioadditives Levabon Rumen E and Digestarom P.E.P. 1000 and a complex of organic acids on the dynamics of mass accumulation, indices of visceral organs, anatomical pathological changes, and hematological parameters of juvenile trout. The maximum positive effect on the morphophysiological parameters and health of fish was revealed for yeast in combination with a phytobiotic as a feed bioadditive. The immune response was found to increase in fish fed bioadditives. A complex of organic acids adversely affects the state of the visceral organs and tissues of fish. A combined use of feed additives in the diet of juvenile trout is recommended in the following concentrations: 2% Levabon Rumen E and 1.5% Digestarom P.E.P. 1000. This combination helps to reduce intensive development of pathological processes in adult fish and improve the physiological parameters of juvenile trout.

1 Introduction

The development of modern fish farming is closely related to artificial feed; its formulas are balanced in terms of macro- and micronutrient composition. At the same time, the needs of fish are individual for each species, and feed additives can optimize the nutritional composition of feed to provide healthy development of individuals and prevent a number of diseases.

The world market offers a wide variety of fish feed bioadditives actively used as vitamin-mineral premixes and protein-vitamin hydrolysates, pro- and prebiotics, and other substances. However, the current economic environment poses the most important challenges of import substitution and production of fish feed based on domestic raw materials. Therefore, it is highly relevant to study the effectiveness of the domestic feed additives in fish diets in order to elaborate practical recommendations for their use.

The aim of the study was to analyze the effectiveness of bioadditives from the Russian company Biomin in the feeding diet of juvenile rainbow trout.

2 Materials and methods

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In the experiment, we tested 4 formulas of granulated feed for salmon fish species produced at the Bisco LLC plant, Krasnodar Territory; three of the four formulas included biologically active additives from the Biomin company.

A formula of granulated feed for trout was used as a control diet, balanced for juveniles in terms of basic nutrients, proteins, fats and carbohydrates, and macro- and microelement composition (Table 1).

Table 1. The content of the main feed nutrients in the basic formula.

Nutrients	Content	Nutrients	Content
Crude protein, %	41	Lysine, %	2.8
Crude fat, %	18	Methionine + Cystine, %	1.55
Cellulose, %	3	Phosphorus	1.61
Ash, %	7.2		

Bioadditives Levabon Rumen E, Digestarom P.E.P. 1000 and a feed additive with a complex of organic acids, which include different biologically active substances, were used as additional ingredients (Table 2).

Table 2. Characteristics of feed additives.

Indicator	Levabon Rumen E (LVB)	Digestarom P.E.P. 1000 (PEP)	Feed additive with a complex of organic acids (COA)
Description of characteristics	Feed additive to improve digestion and increase productivity, a crude protein content of not less than 30%	Feed additive (phytobiotic) to improve the palatability of feed and increase fish appetite. It has antioxidant, anti-inflammatory and antibacterial properties, favorably affects the intestinal mucosa, improves absorption and intake of nutrients, and stimulates stress resistance.	Feed additive to reduce the level of pathogenic microflora in feed and increase fish performance. It reduces microbial contamination of feed, improves its sanitary and hygienic and taste qualities. It improves feed intake.
Ingredients	It contains inactivated yeast (94.9%); maltadextrin (not more than 4%); diatomaceous earth (no more than 1%); silica (no more than 0.1%)	It contains aromatic components (96%) of essential oils (anethole, carvacrol, limonene) on an inorganic carrier (4%) with an anti-caking agent	It contains a complex of organic acids (20% formic acid, 10% acetic acid and 5.5% propionic acid), flavor (cinnamaldehyde) and vermiculite.

The experiments were performed in 2021 at the Research Center for Aquaculture, Petrozavodsk State University. The test objects were morphogenetically homogeneous groups of juvenile trout (0+) of the Kamloops breed (Oncorhynchus mykiss val.) with an initial average weight of 80±5.26 g in aquatic environment. The experiment lasted for 60 days (Table 3).

Table 3. Setting up the experiment (10 individuals per group).

Fish group	Diet features	Formulation
Control	Control diet (CD)	Control Diet – basic formula (granulated trout feed with a granule diameter of 3 mm)
Experiment 1	CD+ 4% LVB	Diet No. 1 – basic formula + 4% feed additive Levabon Rumen E
Experiment 2	CD+ 2% LVB + 1.5% PEP	Diet No. 2 – basic formula + 2% feed additive Levabon Rumen E + 1.5% phytobiotic Digestarom P.E.P. 1000
Experiment 3	CD+COA	Diet No. 3 – basic formula + a complex of organic acids (propionic, acetic, formic)

Juvenile trout species were grown in 4 independent aquariums, 560 liters each, with a closed water supply system and a separate biofilter. The conditions maintained in the aquariums throughout the experiment were optimal: water temperature of 14.2 ºC, content of oxygen dissolved in water of 10.8–11.2 mg/l, pH of 6.0–6.5. The fish were fed 2 times a day at a rate of 1.5% feed of body weight.

Evaluation of the effectiveness of Biomin bioadditives for juvenile trout was performed with respect to fish-biological (weight gains) and morphophysiological (indices of visceral organs) indicators, and pathological changes in visceral organs in fish before and after the experiment. Additionally, hematological parameters and bactericidal activity of blood serum were analyzed. All studies were performed by conventional methods [1-4].

3 Results

Figure 1 shows dependencies of the dynamics in body weight gain in trout that received the test feed. The analysis employed linear functions, which are the easiest to interpret on the one hand, and exhibit sufficiently high correlation coefficients on the other hand.

The obtained linear dependences indicate that the dynamics of the increase in body weight of fish that received the test feed remained constant within the entire observation period. In this case, the slope tangents in the weight gain–time coordinates are constants that indicate the weight gain when trout species are grown under specific conditions and receive a certain diet.

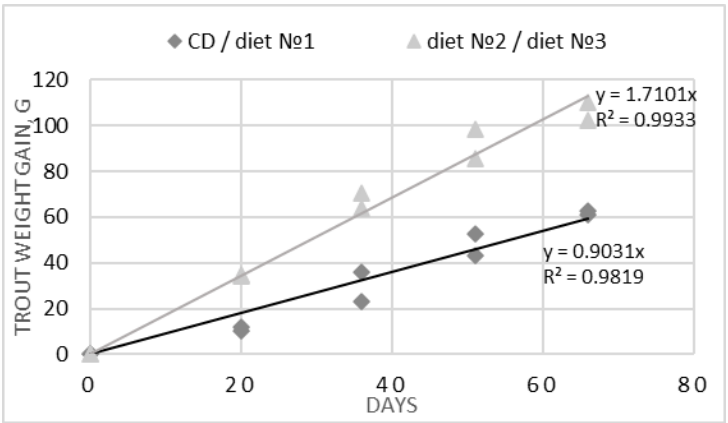

Fig. 1. Dynamics in changes in the live weight of rainbow trout during the experimental period.

It should be noted that the control feed and feed supplemented with 4% yeast (diet No. 1) exhibit similar functional dependence. This feature is typical of feed supplemented with 2% yeast + 1.5% phytobiotic (diet No. 2) and feed supplemented with a complex of organic acids (diet No. 3). Therefore, they are given in pairs in Figure 1.

As follows from the graphs, the weight gain constant for fish fed the control diet (CD), and for fish that received feed supplemented with 4% yeast (diet No. 1), is 0.9. The weight gain constant for diets No. 2 and No. 3 is 1.7, which is almost twice as much compared to the control (CD) and diet No. 1 (CD + 4% LVB). In addition, it should be noted that the control diet and the diet supplemented with 4% feed additive Levabon Rumen E yields not only a low weight gain constant, but also negatively affects the behavioral patterns of fish, which lacked appetite and activity.

Supplementation of 2% to 4% yeast and 1.5% phytobiotic to the control diet (CD) does not significantly affect the energy value of the feed, therefore the change in weight gain constants can be due to the effects of yeast and phytobiotic when applied in combination.

The increased constant in case of diets supplemented with phytobiotics can be attributed to the effect of essential oils in their composition that improve feed palatability and increase the fish appetite. In combination with yeast, which improves digestion, absorption of digested nutrients proceeds more efficiently.

Supplementation of a mixture of organic acids (diet No. 3) increased the body weight gain that can be attributed to the decreased level of pathogenic microflora in feed and palatability of feed improved by flavors.

Diet No. 2 (CD + 2% LVB + 1.5% PEP) affects not only the dynamics in biometric characteristics, but also the liver index, which has the lowest value (1.73%) among the fish from experimental groups. This indicates enhanced digestion and general metabolism in fish species that consume a complex feed additive (Fig. 2).

In the control diet and experimental diets No. 1 and No. 3, the liver index at the end of the experiment in all fish exceeded the established norms for juvenile trout (1.0–1.5%), which was accompanied by enlargement of the liver beyond its normal size due to elevated load on the organ. As a result, in adult trout it can cause fatty liver disease and health deterioration [5].

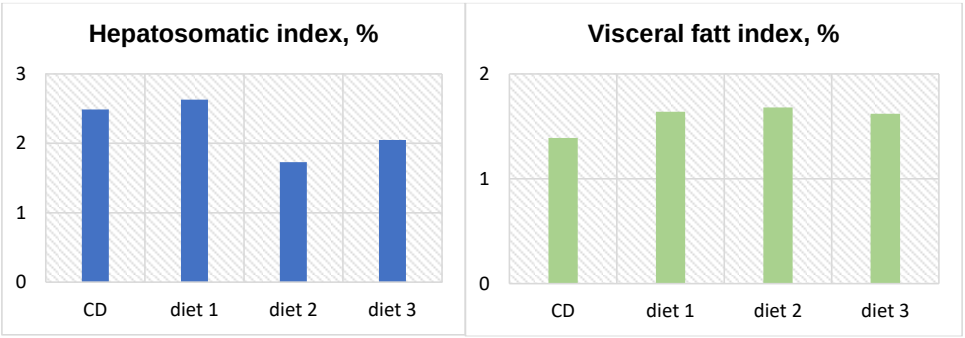

Fig. 2. Morphophysiological parameters of trout with regard to the diet: liver index in % of fish weight, visceral fat index in % of fish weight

The relative content of visceral fat in juvenile trout at the end of the experiment in all experimental groups was similar, but it was about 20% higher than that in the control (Fig. 3). This indicates the effect of the three feed additives on lipid metabolism. At the same time, after 60 days, the fat content did not exceed the reference values (above 3.5%) in the control and in the experimental groups, which does not exclude its pathological accumulation in fish in case of longer feeding.

No significant differences were found in indices of other visceral organs (spleen, heart, and kidneys). All of the indices remained within the reference levels.

Examination of the visceral organs after the experiment showed the greatest number of pathologies (5 points) in juvenile trout fed diet No. 4 supplemented with a complex of organic acids (Fig. 3). The trout looked healthy, yet almost all individuals were found to have one or more pathologies. The fish autopsy revealed hyperemia of muscle tissue, weak turgor, separation of bones from muscles, and pathophysiological changes in the liver and spleen.

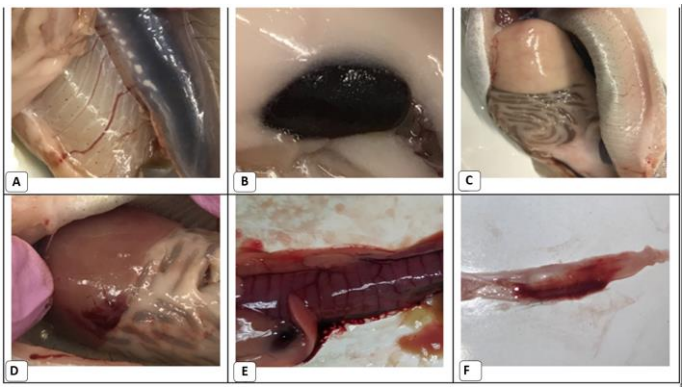

Fig. 3. Pathologies of the visceral organs in juvenile trout

(A – hyperemia of muscle tissue; B – changed morphology of the spleen surface; C – changed liver color intensity (pale); D – hemorrhages in the liver; E – inflammation of the intestine; F – inflammation of the swim bladder)

The incidence of pathologies of visceral organs with regard to the type of diet is presented in Table 4.

Table 4. The incidence of pathologies of visceral organs with regard to the diet.

No.	Indicator	Prior to the experiment	Control diet (CD)	CD+ 4% LVB	CD+ 2% LVB + 1,5% PEP	CD+COA
1	Weak muscle turgor, %	0	0	0	0	50
2	Hyperemia of muscle tissue, %	0	30	0	0	50
3	Hemorrhages in the liver, %	10	50	20	0	50
4	Change in the liver color intensity (pale), %	30	50	30	0	20
5	Change in the morphology of the spleen surface, %	0	0	10	10	20
6	Changes in the intestines, %	30	0	0	0	0
In total (maximum 6)		3:6	3:6	3:6	1:6	5:6

Liver damage found in 70% of the fish fed diet No. 3, which exhibited muscle pathologies, indicates detrimental effect of a complex of organic acids in specified concentrations.

A comparative analysis of three other feed samples showed that 4% Levabon Rumen additive supplemented to the basic formula improves the physiological state of muscles and reduces the number of pathological processes in the liver, thereby reducing the risk of its fatty degeneration in fish.

The smallest number of pathologies of visceral organs was found in juvenile trout fed the diet supplemented with 2% Levabon Rumen in combination with 1.5% Digestar P.E.P. 1000. Externally and at autopsy, juvenile trout looked healthy, well-fed, with dense muscle tissue

and well-developed visceral organs. The obtained results confirm a positive effect of both feed additives.

Changes in the morphology of the spleen surface were detected in a small number of fish.

Analysis of fish blood showed the values of the erythrocyte series in all the studied groups of trout within the reference levels, which indicates a good state of fish health (Table 5). Polymorphic erythrocytes were found in the blood of all the studied individuals due to the simultaneous presence of cells of various maturity degrees – erythroblasts, basophilic normoblasts and oxyphilic normoblasts, which is typical of juvenile fish [6. 7].

Table 5. Blood cell count for rainbow trout.

Blood cell count	Control (CD)	Diet No. 1	Diet No. 2	Diet No. 3	Reference values
Erythrocytes, %	89	87	87	88	78–98
Lymphocytes, %	5.7	6.5	6.6	6	1–12
Granulocytes, %	0.92	1.2	1.45	0.97	0–4
Monocytes, %	0.10	0.13	0.13	0.13	No values
Platelets, %	4.3	5.4	5.3	5.1	1–7

The white blood indicators of fish in the experimental groups were within the normal range for trout under the age of 1 year (Table 4). Despite this, an increased content of granulocytes (on average by 40%) and lymphocytes (on average by 12%) was observed in fish fed diets No. 1 (CD + 4LVB) and No. 2 (CD + 2LVB + 1.5PEP) and diet No. 3 (CD + COA) compared to the control (CD).

Granulocytes and lymphocytes are responsible for adaptive immunity; therefore, Levabon Rumen and Digestarom P.E.P. 1000 have a positive effect on immune response reactions.

The performed analysis of the bactericidal activity of blood serum in fish confirmed the results obtained in the description of the leukocyte formula. In diets No. 1 (OR + 4LVB) and No. 2 (OR + 2LVB + 1.5PEP) with Levabon Rumen and Digestar P.E.P. 1000, the intensity of humoral immunity factors in juvenile trout was much lower than that in the control (CD) and group No. 3 (CD + COA) (Table 5).

Table 6. Indicators of bacterial activity of fish blood serum.

Indicator	Control (CD)	Diet No. 1	Diet No. 2	Diet No. 3
Bactericidal activity of blood serum (%)	70.32	39.76	30.21	52.19

The obtained results show a beneficial effect of Levabon Rumen and Digestarom P.E.P. 1000 feed additives on the formation of a sufficiently high level of natural resistance of fish to a number of diseases associated with keeping and feeding conditions.

4 Conclusion

The study results show that the growth and development of juvenile trout, as well as the potentiation and prevention of pathologies, are affected not only by feed balanced in terms of macro- and micronutrient composition, but also by other feed ingredients, including essential oil components, inactivated yeast, and organic acids. Despite the fact that the olfactory organs and taste buds of fish are less developed than those of animals, flavorings enhance feed palatability and, consequently, feed intake.

Inactivated yeast that contains easily digested amino acids, short peptides and vitamins help to improve digestion and increase weight gain in fish. Glutathione, which is part of the yeast, plays a crucial role in the antioxidant defense. At the same time, a combined use of inactivated yeast and phytobiotics shows the maximum effect.

Of relevance is the role of individual components of feed additives in the activation of the immune response. A combined use of inactivated yeast in the composition of feed additive Levabon Rumen and essential oils enhance fish immunity, both at the cellular and humoral levels.

A complex of organic acids in the feed composition in a specified concentration triggers pathological processes in the organs and tissues of fish. For this reason, the expansion of range of additives and different quantitative ratios in feed require a more detailed study.

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