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Study of the effectiveness of the use of bioflavonoids in the composition of production feeds on the sturgeon physiological state

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Abstract. The appearance of new preparations with antioxidant properties allows us to improve the technology of fish feeding and increase the pro-oxidant-antioxidant balance. Due to the fact that an important criterion for choosing a feed additive is environmental safety, natural bioantiooxidants of flavonoid nature, in particular dihydroquercetin, isolated from larch wood, are of interest. It is known that dihydroquercetin has capillaroprotective, lymphokinetic and other properties, but it has a low solubility in water (about 1 g/l), which probably limits its pharmacological activity. In a recent study, it was found that the use of dihydroquercetin (DHA) in a composition with plant polysaccharides increases its pharmacological effect, which is associated with an increase in solubility. One of these commonly used polysaccharides is arabinogalactan, also isolated from larch. The scientific and practical significance of the work consists in testing the effectiveness of bioflavonoids in the composition of production feeds for sturgeon fish as sources of antioxidants, immunostimulants and prebiotics.

1. Introduction

Bioflavonoids, also known as flavonoids, are substances with low molecular weight that are found in vascular plants. They are found in all parts of plants, but especially in photosynthetic cells [1] and have a wide spectrum of action as antioxidants, enzymatic inhibitors, precursors of toxic substances, protection against ultraviolet radiation, and also participate in energy conversion [1-2]. Other functions include giving color, taste and texture to food [3].

More than 4,000 bioflavonoid compositions were found in plants [4]. However, most of all flavonoids are known for their antioxidant properties, despite other numerous useful qualities [5]. These include: 1) anti-inflammatory effect through their ability to influence the biosynthesis of eicosanoids and their effect on multiple pathways of the inflammatory process; 2) antithrombotic effect by preventing the aggregation of blood platelets; 3) antihypertensive and antiarrhythmic effect by enhancing the relaxation of smooth muscles of the cardiovascular system; 4) antitumor effect; 5) hepatoprotective effect; 6) antiviral effect [3-4]. Flavonoids also show their antioxidant effect through the sparing effect of other antioxidants, and can also affect the digestive tract, protecting molecules during digestion from oxidative damage, as well as protecting the intestinal epithelium [6].

The appearance of new preparations with antioxidant properties allows us to improve the technology of feeding fish and increase the pro-oxidant-antioxidant balance. Due to the fact that an important criterion for choosing a feed additive is environmental safety, natural bioantioxidants of

flavonoid nature, in particular dihydroquercetin, isolated from larch wood, are of interest. It is known that dihydroquercetin has capillaroprotective, lymphokinetic and other properties, but it has a low solubility in water (about 1 g/l), which probably limits its pharmacological activity. In a recent study, it was found that the use of DHA in a composition with plant polysaccharides increases its pharmacological effect, which is associated with an increase in solubility. One of these commonly used polysaccharides is arabinogalactan, also isolated from larch [7].

The scientific and practical significance of the work consists in testing the effectiveness of bioflavonoids in the composition of production feeds for sturgeon fish as sources of antioxidants, immunostimulants and prebiotics.

The aim of the work is to test the effectiveness of the bioflavonoid dihydroquercetin and the immunostimulator arabinogalactant in the composition of mixed feeds.

2. Materials and methods

The experiments were carried out on the basis of the Innovation Centre "Bioaquapark – Scientific and Technical Centre of Aquaculture" of the FSBE HE ASTU. The objects of research were two-year-old Russian sturgeon (*Acipenser gueldenstaedtii*, Brandt).

Square fiberglass tanks with rounded corners measuring 1x1m and a depth of 0.8 m with constant flow and aeration were used for fish breeding. Additional water heating was used with the help of thermoregulators. Thus, the temperature in the tanks with Russian sturgeon was maintained at 22-24 ^{0}C. The content of dissolved oxygen in water was determined using a thermal oximeter daily – no oxygen drops below 6 mg/l were recorded during the experiment. The main diet for the experimental fish was mixed feed of the "Coppens" company (Netherlands), medium-energy production feed with a protein content of 46%, fat-15%, fiber-1.9%, total energy-21.1 MJ/kg. At the same time, feeding recommendations were applied for optimal growth – 1.12% of the biomass per day [8].

The study was carried out on three experimental groups. The first group (control) received a production feed balanced for all elements of nutrition, according to physiological needs. The second group (test 1) received a diet of the 1st group with the addition of the antioxidant dihydroquercetin in the amount of 50 mg/kg. The third group (test 2) received a diet of the 2nd group with the addition of the antioxidant dihydroquercetin (25 mg/kg) in combination with the immunostimulant arabinogalactan (50 mg/kg) (table 1). Experimental feed was produced in laboratory conditions using feed components of domestic production by wet pressing. The daily feeding rate was determined depending on the fish body weight and water temperature, in accordance with the generally accepted cultivation technology [8].

Table 1. Scheme of experiments.

Group	Characteristics of feeding	Numbers, pcs.
1	Balanced feed (control feed " Coppens Supreme-15»)	22
2	Balanced feed with dihydroquercetin (50 mg per 1 kg of Coppens Supreme-15 feed) (test 1)	22
3	Balanced feed with dihydroquercetin and arabinogalactan (25 mg of dihydroquercetin and 50 mg per 1 kg of Coppens Supreme-15 feed)	22

Throughout the entire study period, the hydrochemical conditions were constantly monitored using Tetra express methods (table 2).

In general, these hydrochemical indicators meet the standards established for fish farms.

The condition and development of fish was determined by a set of indicators, analyzing the rate of increase in body size and muscle mass building.

The pharmacological effect of the preparations and the physiological state of the studied juveniles were evaluated by biochemical parameters of protein, lipid and carbohydrate metabolism, according to the developed methods [9-11].

Table 2. Hydrochemical parameters under experimental conditions.

Indicator	Standard	Control	Test 1	Test 2
Hydrogen index (pH), units	6-8	7.28±0.08	7.62±0.14*	7.52±0.17
Ammonium nitrogen, mg / l	2-4	0.87±0.12	0.94±0.21	0.84±0.14
Nitrites (NO_3), mg / l	up to 0.1-0.2	0.063±0.03	0.059±0.07	0.047±0.04
Nitrates (NO_2), mg / l	up to 60	24.71±1.54	27.64±1.37	25.71±1.47
Phosphates, mg / l	0.2-0.5	0.114±0.15	0.128±0.16	0.117±0.12

Note: * $p \leq 0.01$

Blood was collected in vivo from the caudal vein in Eppendorf tubes. For hematological analysis (hemoglobin concentration, erythrocyte sedimentation rate, leukocyte formula), heparin was used as an anticoagulant.

The concentration of hemoglobin in the blood was determined photometrically using a set of reagents from Agat-Med [9], ESR was determined by the Panchenkov method. Blood smears were prepared with the use of a latch-dye according to May-Grunwald of "Olvex-Diagnostikum" Company.

The content of serum protein was determined using reagent kits of the company "Agat-med", the level of cholesterol in the blood was determined by the enzymatic method [10-11]. The concentration of glucose in the blood serum was determined by enzymatic colorimetric method without deproteinization (Trinder reaction). To measure the optical density of the obtained samples, a Unico 2100 spectrophotometer was used.

The results of the experiments were analyzed by methods of biological statistics using computer programs. The level of differences was assessed using the Student's criterion.

3. Results

Evaluation of the effectiveness of the use of dihydroquercetin and arabinogalactan in production feed showed that the best growth rates were characteristic of the group of fish that consumed feed with the addition of two components dihydroquercetin (25 mg) and arabinogalactan (50 mg) (table 3).

In this group (Test 2), the highest absolute growth, average daily growth, average daily growth rate and mass accumulation coefficient were observed. The average daily growth rate in this variant was 0.78 %, which is significantly higher than the control group by 0.42 % ($p \geq 0.05$). The data obtained indicate, in accordance with figures 1 and 2, that the best growth rates were characteristic of the group of fish that consumed feed with the addition of two components-dihydroquercetin and arabinogalactan.

In test 1, fish that consumed feed with the addition of BAS (dihydroquercetin 50 mg / kg), the average daily growth rate was higher than the control by 0.21 %. When biologically active substances were added, the live weight gain in the fish of the first group was 66.0 g compared to 92.0 g in the second group, which is 36.4% and 54.3% higher than in the fish of the control group. The coefficient of mass accumulation in fish in the first variant was 0,044 units and in the second 0.061 units, which is 0.02 and 0.03 units, respectively, higher than the control.

The average daily increase in variants 1 and 2 was: 2,2 g. and 3,1 g, which is 1,6 and 2,2 times higher, respectively, than the control group. The survival rate in the experimental variants and control was 100%.

It was found that the best growth rates were typical for test 2 - fish that consumed feed with the addition of two components of the bioflavonoid dihydroquercetin (25 mg) + the immunostimulator arabinogalactan (50 mg). This is quite understandable, according to the literature, a new generation antioxidant-dihydroquercetin-destroys radicals of peroxide compounds of feed fats and increases the

efficiency of using the diet. Fish feeds are characterized by a high fat content in their composition. The addition of the immunostimulator arabinogalactan to the feed contributes to a sharp increase in productivity by 20% and a high survival rate of fish compared to foreign feeds by 15.9%, fish have a better fatness coefficient. Studies have shown the effectiveness of the combined use of dihydroquercetin and arabinogalactan in mixed feeds for fish, which contributes to an increase in fish weight gain. Thus, the inclusion of a new generation of antioxidant dihydroquercetin and the immune stimulator arabinogalactan in mixed feeds contributed to the reliable destruction of fat peroxide compounds and more efficient use of nutrients in the diet.

Table 3. Fish-breeding and biological indicators of growing two-year-old Russian sturgeon on experimental compound feeds.

Indicators	Control feed «Coppens Supreme-15»	Test 1 (50 mg per 1 kg of Coppens Supreme-15 feed)	Test 2 (25 mg of dihydroquercetin and 50 mg per 1 kg of Coppens Supreme-15 feed)
Absolute growth, g	42.0	66.0	92.0*
Average daily growth, g	1.4	2.2	3.1*
Average daily growth rate, %	0.36	0.57	0.78*
Mass accumulation coefficient, units.	0.028	0.044	0.061*
Survival rate, %	100	100	100
Duration of the experiment, day	30	30	30

Note: * differences are significant at p≥0,001

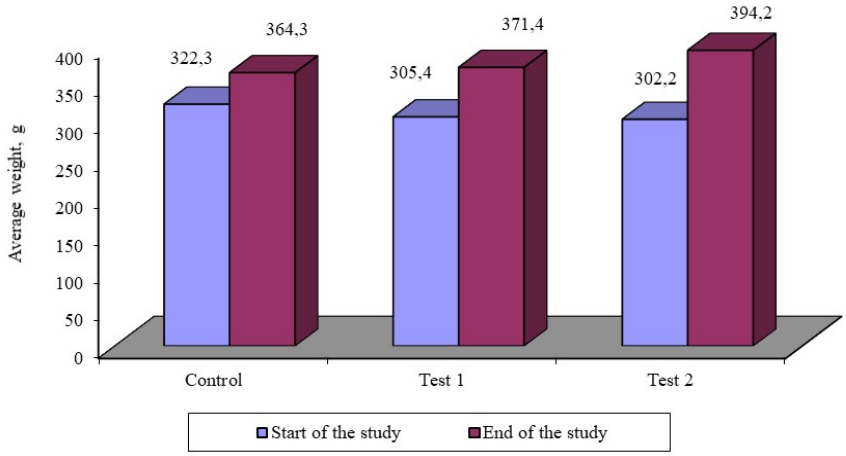

Figure 1. Weight accumulation of Russian sturgeon during the enrichment of the BAS diet.

Objectively assess the state of the body can be based on the physiological and biochemical parameters of blood, which act as specific indicators of physiological or pathological changes in the body. Blood, as the most labile tissue, quickly reacts to the influence of the external environment and the quality of food consumed, so the study of blood parameters gives objective assessments of the physiological state of fish. The study of hematological parameters of fish is of great importance for substantiating the adaptive capabilities of the body and assessing the conditions of growing and

feeding. Hematological indicators objectively reflect the physiological state of fish. Fish blood is an average of 4 % of body weight, has an oily to the touch consistency of bright red color, a specific smell of fish oil, pH 7,2-7,5. Studies in the field of fish feeding have shown that even short-term full-fledged feeding causes significant changes in fish blood parameters [12-16]. Fish-breeding indicators, as a rule, correlate with the physiological state of fish, which is confirmed by hematological indicators (table 4).

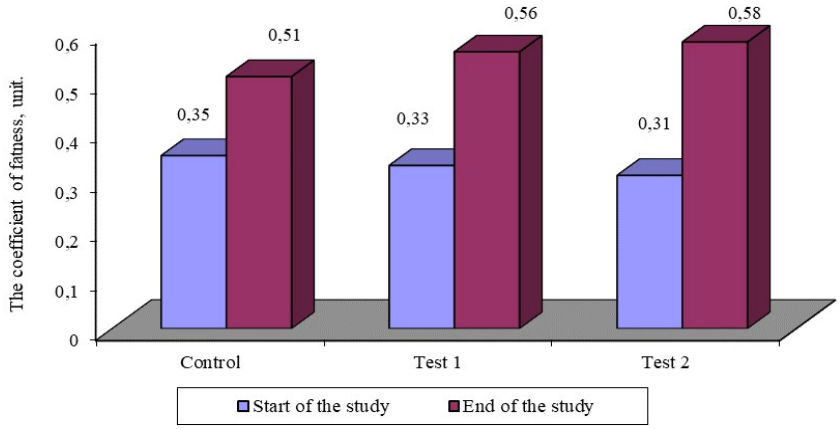

Figure 2. Fatness coefficient (according to Fulton) of two-year-olds of Russian sturgeon when enriching the diet of BAS.

The obtained results of hematological and biochemical parameters are consistent with the data of other authors [17]. The rate of erythrocyte and cholesterol sedimentation in all variants of the experiment remained within the standard values, which is also consistent with the literature data [18] and indicates a constant protein composition of blood plasma.

As can be seen from the above results in accordance with figure 3, there is a positive trend in the use of two drugs dihydroquercin and arabinogalactan, which affects the increase in hemoglobin levels, maintaining cholesterol and ESR levels at the level of standard values.

The hemoglobin concentration varied from 50,0 to 80,0 g/l. When the BAS complex was added to the diet in test 2, the hemoglobin level was 75,91±11,01 g / l, which is 25,0-35,0% higher in comparison with other experimental groups and indicates a positive effect of the feed additive on the metabolism of the studied fish.

A similar dynamic is observed in the change in glucose level ($p \leq 0.01$), and its maintenance in the range of 5,0-6,0 g / l is the result of the normal operation of the enzymatic system that catalyzes the transformation of glucose.

Table 4. Physiological and biochemical blood parameters of two-year-olds of Russian sturgeon

Indicator	Control feed «Coppens Supreme-15»	Test 1 (50 mg per 1 kg of Coppens Supreme-15 feed)	Test 2 (25 mg of dihydroquercetin and 50 mg per 1 kg of Coppens Supreme-15 feed)
Cholesterol, mmol / l	3.13±0.57	3.27±0.35	3.42±0.52
Glucose, mmol / l	4.87±0.18	6.01±0.16	6.21±0.42*
ESR, mm / h	1.83±0.28	1.92±0.33	1.90±0.24

Note: * $p \leq 0.01$

In comparison with the control, BAS contributed to the activation of plastic metabolism, which is confirmed by a lower level of total whey protein and a high growth rate.

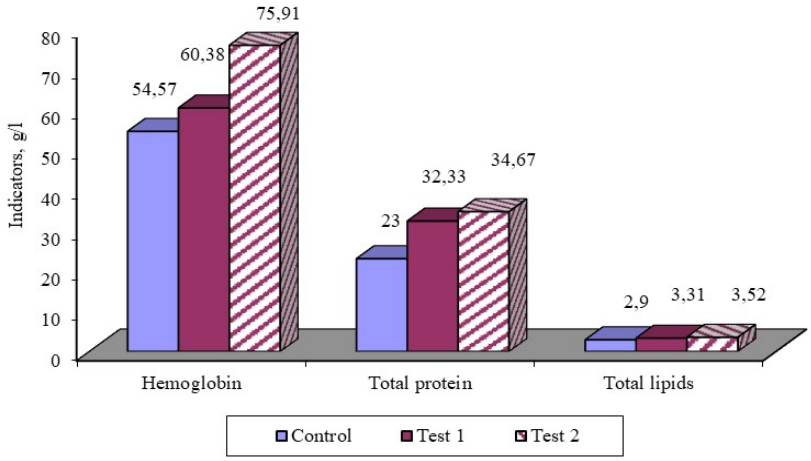

Figure 3. Physiological and biochemical blood parameters of two-year-olds of Russian sturgeon.

Under experimental conditions, the level of total serum lipids changed slightly. Its important component is cholesterol, which stimulates the body's immune system and plays a role in protecting against stress. The dynamics of lipid metabolism contributed to the normal process of accumulation of energy resources.

The high content of hemoglobin in the experimental groups (within the reference values) may be associated with a more intensive metabolism in the fish body. Since the feed used bioflavonoid dihydroquercetin, which increases blood circulation in the body of fish. It is difficult to underestimate this property of flavonoids. How well the organs are supplied with nutrients and oxygen depends on the blood circulation. Cholesterol in the blood of fish, as well as other animals, is one of the key factors of the state of lipid metabolism in the body, this indicator in variants 1 and 2 was similar to the control, minor discrepancies were found ($p>0.05$).

A fairly informative indicator in assessing the overall physiological state of the body is the leukocyte blood formula, which reflects not only the physiological state of fish, but also some aspects of cellular immunity. Changes in the leukogram can detect metabolic disorders and deterioration of the condition of the studied object long before the appearance of clinical signs of emerging pathologies. The leukocyte formula of the blood of the studied fish is presented in accordance with figure 4.

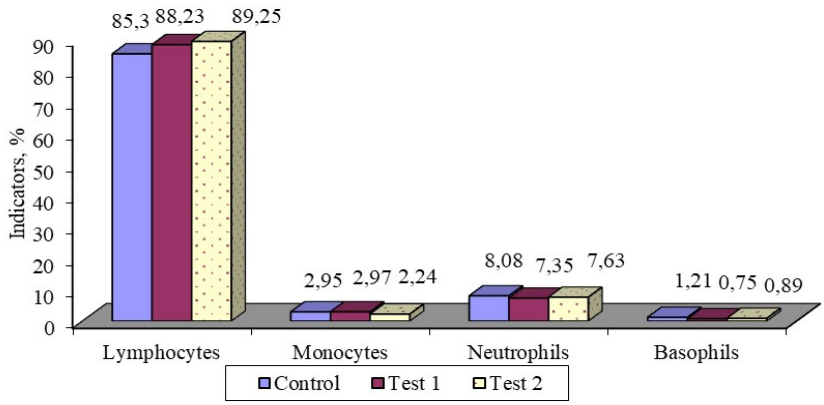

Figure 4. Ratio of blood elements (%) in two-year-olds of Russian sturgeon.

Thus, as a result of the obtained data, the feed with the addition of two components dihydroquercin +arabinogalactan had the most positive effect on the physiological status of fish. These components

indicate a positive effect on the metabolism of the studied fish.

4. Discussion

In the pilot study, on the basis of Innovation Centre "Bioaquapark – Scientific and Technical Centre of Aquaculture" of the FSBE HE ASTU, experiments were conducted on feeding two-years olds of Russian sturgeon feed, which was used bioflavonoids.

Bioflavonoids are a group of polyphenolic compounds of plant origin. They are found in the leaves, roots, flowers, fruits, and wood of many plants. The world knows more than 4000 varieties of flavonoids. These compounds take an active part in plant metabolism and are widely distributed among higher plants. For pharmacological action and properties, bioflavonoids are classified as vitamins of group P. All bioflavonoids have a powerful antioxidant effect and help the body fight free radicals and toxins. Many scientific studies confirm the beneficial properties of bioflavonoids [1-7].

As a result, the introduction of dihydroquertecin and arabinogalactan into the composition of mixed feeds has a positive effect on the complex of fish-breeding and biological indicators when growing fish in industrial conditions: absolute and relative increments, average daily growth rate, mass accumulation coefficient, as well as physiological and biochemical parameters: hemoglobin, cholesterol, total protein, total lipids, glucose, ESR.

The highest growth rates were demonstrated by the group of fish that consumed feed with two components dihydroquertecin (25 mg) + arabinogalactan (50 mg) compared to the control sample. In the second place in terms of the effectiveness of feeding results, the severity of fish – breeding and biological indicators was noted in the sample that consumed food only with the addition of dihydroquercetin (50 mg per 1 kg of feed).

5. Conclusion

The conducted studies indicate the effectiveness of the use of bioflavonoids in feeding promising aquaculture objects, in particular sturgeon fish species. The positive effect of the tested BAS on the growth and development of cultured groups of fish was established. The results obtained complement the existing ideas about the fields of application of antioxidants, and also prove the prospects of using plant-based products as antioxidant feed additives.

Thus, as a result of the obtained data, the feed with the addition of two components dihydroquercin +arabinogalactan had the most positive effect on the physiological status of fish. These components indicate a positive effect on the metabolism of the studied fish.

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