Feed additive for fishdiet with antioxidant and immunostimulating effect

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Abstract. The increase in the production of aquaculture products confirms the relevance of the development of feed production in order to provide the industry with high-quality compound feeds and feed additives that meet modern requirements and biological needs of cultivated fish. Therefore, the development of aquaculture requires special attention to the feeding process and the use of full-fledged and environmentally safe feeds for all types of aquacultures. Antioxidants of natural origin are increasingly used in feed production. In the course of the work, the positive properties of antioxidant complexes containing the bioflavonoid dihydroquercetin in combination with vitamin E were studied when enriching the diet of a tilapia hybrid (Oreochromis mossambicus × Oreochromis niloticus). As a result of the research, it was found that the inclusion of dihydroquercetin in the composition of production feeds significantly improved productivity indicators and the main criteria for the formation of muscle mass in fish. The use of bioflavonoid dihydroquercetin allowed to increase productivity by 30.0%. The positive effect of the feed additive on morphological and biochemical parameters of blood was noted, which indicates an increase in natural resistance. The study of feed quality indicators confirmed the inhibition of oxidation processes in feed with the addition of dihydroquercetin. During the period of storage of feed in the experimental batch of feed, the level of peroxide number decreased by 3 times, and the acid number decreased by 28.4%. Taking into account the positive properties of bioflavonoid, it is recommended to further study the effectiveness of this biologically active additive in order to introduce it more widely into the practice of feed production.

1 Introduction

The development of aquaculture is one of the tasks of the fisheries industry, which makes it possible to provide the consumer market with valuable products. An increasingly important place is occupied by the technology of obtaining marketable products in conditions of closed water supply, which allows achieving complete independence of the production

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process from natural and climatic conditions, modeling conditions close to natural, and producing environmentally friendly fish products in all climatic zones of the world.

Modern technologies of industrial fish farming make it possible to obtain high-quality products. However, stressful conditions of the artificial ecosystem (high planting density, violation of optimal hydrochemical conditions) they can negatively affect nutritional activity, growth rate, lead to the development of alimentary diseases and depletion of antioxidant potential.

The growth of fish directly depends on the composition and quality of the feed [1]. Therefore, one of the ways to improve aquaculture technologies is the use of biologically active substances (BAS), in the form of feed additives, vitamins and vitamin-mineral complexes, which have a stimulating effect on the fish body - increasing the digestibility of feed, eliminating anti-nutritional factors, strengthening the body's resistance and, in general, correcting the physiological state of cultivated fish and increasing the profitability of a fish-breeding enterprise [2, 3].

In recent years, much attention has been paid to the use of natural food additives in aquaculture. Studies on the effect of certain antioxidants in the enrichment of fish diets are conducted by both foreign and domestic scientists [4-10]. At the same time, there is a positive effect on productivity, metabolic processes of fish and the immune status of fish.

An important criterion for creating a rational feeding technology using antioxidant complexes is environmental safety. Currently, flavonoid compounds, including dihydroquercetin, are of particular interest, capable of normalizing water phosphate and lipid metabolism and having a positive physiological effect on the body.

Dihydroquercetin is an effective antioxidant agent that interrupts the processes of lipid peroxidation in cell membranes, is able to penetrate into the cytoplasm of the cell and protect the cell from the damaging effects of free radicals, effectively corrects violations in various parts of the antioxidant system of the body. Provides complex antioxidant protection of the body, active prevention of the development of oxidative stress, degenerative-dystrophic processes in tissues.

Dihydroquercetin has a synergistic effect on ascorbic acid and the membrane antioxidant vitamin E, promoting the regeneration of the active form of the latter and preventing the formation of tocopherylquinone.

However, an analysis of the current state of research on this problem shows that there is insufficient scientifically substantiated information in the literature on technological approaches to the use of such feed components, there is no information on physiologically and economically feasible schemes for the use of these biologically active substances in the cultivation of aquaculture facilities.

The article presents the results of research on the use of the bioflavonoid dihydroquercetin (DQ) in the composition of food for thermophilic fish species, using the example of a tilapia hybrid, provides data from comparative tests of DQ in combination with vitamin E, as well as evaluates the process of oxidation of feed with the addition of an antioxidant.

2 Material and methods of research

The study was conducted in the conditions of the innovation center of the Astrakhan State Technical University "Bioaquapark – scientific and Technical center of aquaculture" (Astrakhan).

The object of the study was a juvenile hybrid tilapia (Oreochromis mossambicus × Oreochromis Niloticus). The study was carried out on 4 experimental groups (n=25). The first group (control - OR) received a food product balanced in all nutrition elements, according to physiological needs. The second group (option 1 - OR 50) received a group 1

diet with the addition of the antioxidant dihydroquercetin (50.0 mg/kg). The third group (option 2 - OR 50/50) received the diet of the 2nd group with the addition of vitamin E (50.0 mg / kg). The fourth group (option 3 - OP 25/50) received a diet with the addition of dihydroquercetin (25.0 mg/ kg) in combination with vitamin E (50.0 mg/kg).

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 body weight of the fish and the water temperature, in accordance with the generally accepted cultivation technology. Feeding was carried out manually, according to the feedability.

Throughout the entire study period, thermal and hydrochemical regimes were monitored using a MARK-302 thermooximeter and a HANNA pH meter. The studied hydrochemical parameters were within the limits permissible for growing tilapia. During the day, the temperature was maintained at the level of 26.0-27.0 ° C, the pH did not exceed 7.0-8.0 units.

As a source of dihydroquercetin, the drug Flavitol (CJSC NPF FLAVIT) was used, which contains highly purified dihydroquercetin (94%-96%) with a preserved native form. The drug was added into the compound feed during its manufacture, previously dissolved in distilled water. Experimental batches of dry compound feeds were produced in laboratory conditions by wet pressing followed by drying.

The quality of feed with the addition of dihydroquercetin was assessed by the level of peroxide and acid number. The analysis of the control and experimental batches of feed was carried out during the storage of feed in a warehouse under conditions of natural change of daily temperatures, and after the completion of the experiments (July-August) – in the laboratory at room temperature.

The dynamics of the growth rate was assessed based on the analysis of changes in linear and weight indicators and their dependencies among themselves (absolute and average daily growth, average daily growth rate, Fulton fatness coefficient, mass accumulation coefficient). Measurements and calculations were carried out in accordance with the recommendations adopted in fish farming [11, 12].

The assessment of the functional state of cultured fish was carried out on the basis of a comprehensive physiological and biochemical study of blood, taking into account species and age characteristics. Blood was taken in vivo from the caudal vein into Eppendorf tubes with the addition of an anticoagulant (heparin) for hematological analysis. For biochemical blood analysis, blood samples were taken into test tubes without heparin, left to coagulate to obtain serum.

Hemoglobin was determined by the hemiglobin cyanide method. The erythrocyte sedimentation rate (ESR) was determined by the Panchenkov method. Blood smears were prepared using a dye fixative according to May-Grunwald of the Olvex-Diagnosticum company. The concentration of total protein, cholesterol, and total serum lipids and glucose was determined according to accepted methods [13].

The results of the research were processed using generally accepted methods of biological statistics and the Microsoft Excel program. Statistical analysis was carried out using the Student's t-criterion, differences were considered significant at $p \leq 0.05$.

3 Research results

Long-term storage of feed at high temperatures often leads to the oxidation of fat and the formation of toxic peroxides, which shortens its shelf life, negatively affects the physiological state of cultivated fish and reduces their productivity.

The degree of oxidation of fatty acids during the storage of feed is determined by the indicators of peroxide (% iodine) and acid (mg KOH) numbers. The permissible norms of

the peroxide number of fat in mixed feeds for fish should be no more than 0.3% iodine, and the acid number should not exceed 50.0 mg KOH in production feeds and 30.0 mg KOH in starter feeds.

The dynamics of feed quality indicators during storage for 4 months is shown in Figure 1.

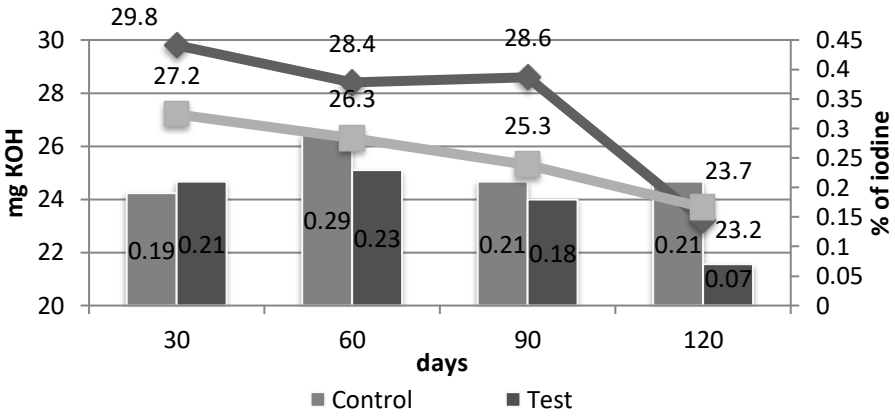

Fig. 1. Dynamics of feed quality indicators of control and experimental (OR 50) batches.

Studying the properties of dihydroquercetin in feed, it was found that during 4 months of storage, in an experimental batch of feed, the level of peroxide number decreased by 3 times, and the acid number decreased by 28.4%. In the control variant, the values were at the same level, and the acid number decreased by no more than 15.0%.

From the data obtained, it can be seen that the values of feed quality indicators are within the normal range, which indicates an inactive process of formation of peroxides during the storage of compound feed, especially in the experimental batch of feed.

To assess the possibility of using feeds containing dihydroquercetin, experiments were conducted on the cultivation of hybrid tilapia juveniles using production feeds with antioxidant additives. The analysis of fish-breeding and biological indicators showed that the growth rate of fish whose diet was enriched with DQ exceeded these indicators in fish of control groups (Figure 2).

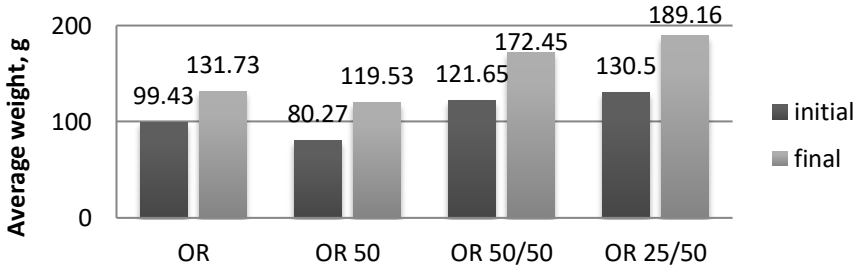

Fig. 2. Change in the weight of the tilapia hybrid when the diet is enriched with antioxidants.

During the experiment, it was found that high growth intensity was observed in all groups. The average daily gains fluctuated in the range of 1.1-2.1 g.

The best growth rates were observed with the addition of a complex of biologically active substances — the live weight gain in fish of the first group was 39.26 g versus 50.80 g and 58.66 g with the addition of vitamin E, which is 36.46% and 44.9% higher than in

fish of the control group. The lowest Fulton fatness coefficient was observed in the fish of the control group and amounted to 1.7 ± 0.03 units. Whereas when adding actioxidants to the diet, the indicator was 1.77 ± 0.05 units. The highest value of this indicator is observed in fish of group 4 (OR 25/50) - 1.92 ± 0.04 units ($p \leq 0.01$).

A similar trend is observed for other fish-breeding and biological indicators. The best fish-breeding and biological indicators according to the results of cultivation were shown by an experimental group of fish that consumed the main diet with the addition of 25.0 mg of Flavitol and 50.0 mg of vitamin E. Survival in all variants of the study was 100%.

Table 1. Dynamics of growth indicators of tilapia hybrid when enriching the diet with antioxidants.

Indicator	OR	OR 50	OR 50/50	OR 50/25
Absolute growth	32,28	39,26	50,80	58,66
Average daily growth	1,15	1,40	1,81	2,10
Average daily growth rate	1,01	1,43	1,25	1,33
Mass accumulation coefficient, units.	0,05	0,07	0,07	0,07

The feed coefficient characterizing the feed conversion in the control variant was 1.4 units, which is 14.3% higher than in the experimental variants. The best assimilation of the feed was with the addition of a complex of antioxidants, and the feed coefficient was 1.2 units.

Thus, the results of biological indicators of growth and survival indicate a positive effect of adding a complex of antioxidants to the diet of hybrid tilapia.

One of the elements of the biochemical assessment of the physiological state of cultured fish is the characteristic of the metabolic function of blood.

Hematological analysis revealed a wide variation in hemoglobin concentration in all groups of fish. However, the average value was at the same level. The concentration of hemoglobin varied from 40.0 to 80.0 g/l. However, a slightly higher indicator (by 9.2%) was observed in fish fed with an antioxidant complex, which indicates a positive effect of the feed additive on the metabolism of the studied fish.

The erythrocyte sedimentation rate in all variants of the experiment remained within the normative values, which is also consistent with the literature data [14-16] and indicates a constant protein composition of blood plasma.

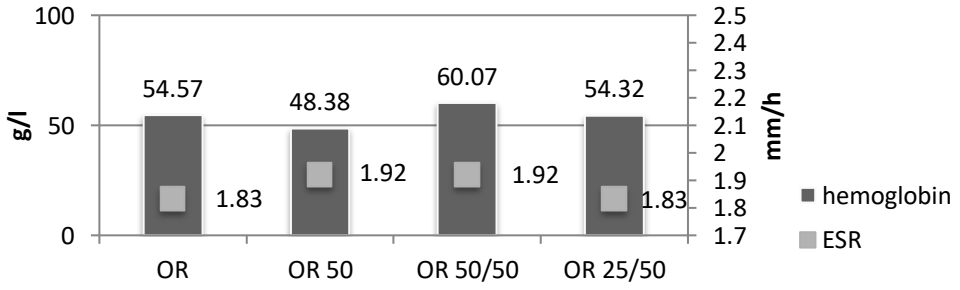

Fig. 3. Hematological parameters of juvenile tilapia hybrid under experimental conditions.

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. Table 2 shows the leukocyte formula of the blood of the fish under study.

Table 2. Leukocyte blood formula (%) of tilapia hybrid when used DQ in diets

Indicators	OR	OR 50	OR 50/50	OR 25/50
Lymphocytes	86,94±2,62	88,92±1,87	89,48±1,59	87,54±1,47
Monocytes	2,95±0,71	2,94±0,65	2,24±0,47	2,18±0,95
Neutrophils	9,08±1,57	7,45±1,27	7,53±1,21	9,16±0,93
Basophils	1,03±0,14	0,69±0,06**	0,75±0,17	1,12±0,29

Note: **p≤0,01

The number of lymphocytes, monocytes, neutrophils, basophils remained at the same level in all three experimental groups. The leading group in the studied smears were lymphocytes, which made up the majority of the total number of leukocytes (from 86.94 to 89.48%).

The results of a biochemical study of blood parameters are presented in Table 3.

Table 3. Biochemical blood parameters of tilapia hybrid under experimental conditions

Indicators	OR	OR 50	OR 50/50	OR 25/50
Total whey protein, g/l	33,00±2,0***	21,33±0,88	25,70±2,93	23,38±0,90*
Cholesterol, mmol/l	3,75±0,57	3,23±0,35	3,22±0,24*	3,75±0,22
Glucose, g/l	5,21±0,18***	5,79±0,42	5,38±0,27	5,64±0,37
Total serum lipids, g/l	3,34±0,20	2,95±0,18	3,24±0,14	2,87±0,24

Note: ** p≤0,01; *** p≤0,001

Depending on the conditions of nutrition, diet and the level of energy metabolism, the amount of total whey protein changes, the excess or shortage of which indicates a decrease in the viability of fish. In all variants of the study, the indicator was within the normative values for this type of fish and varied from 19.08 to 36.40 g / l. The lower protein level in fish of the experimental groups ($p \leq 0.01$) is explained by the better growth rate of fish, since it affects the structure of the body, which is confirmed by the data of fish-breeding and biological analysis.

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. Under experimental conditions, the cholesterol level was within the reference values and did not experience significant changes during the growing period. thus, the dynamics of lipid metabolism contributed to the normal process of accumulation of energy resources.

A similar dynamic can be traced in the change in glucose level ($p \leq 0.001$), and its maintenance within 5.0-6.0 mmol / l is the result of the normal operation of the enzymatic system that catalyzes the transformation of glucose.

The obtained results of hematological and biochemical parameters are consistent with the data of other authors [17-21].

Thus, the obtained hematological and biochemical indicators indicate a positive effect of BAS on the health of fish, and the results of the size and weight characteristics confirm the high activity of metabolic processes. Considering that the fish of all groups were kept in the same conditions, the processing of the material was carried out uniformly, and the difference was only the diet, we can say that the complex of antioxidants provided more favorable trophic and biochemical conditions necessary, in particular, for the normal growth and development of fish.

4 Conclusion

The conducted studies indicate the effectiveness of the use of bioflavonoids in feeding promising aquaculture objects, in particular tilapia and its hybrids. The positive effect of the tested BAS on the growth and development of cultured juveniles has been established.

The obtained results complement the existing ideas about the fields of application of antioxidants, and also prove the prospects of using herbal remedies as antioxidant feed additives.

The data obtained allow us to recommend the complex use of DQ in combination with vitamin E as part of the production feed for hybrid tilapia during its commercial cultivation.

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