ТОВАРНАЯ АКВАКУЛЬТУРА И ИСКУССТВЕННОЕ ВОСПРОИЗВОДСТВО ГИДРОБИОНТОВ

COMMODITY AQUACULTURE AND ARTIFICIAL REPRODUCTION OF HYDROBIONTS

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Evaluating effectiveness of biologically active additives with antioxidant effect in combination with vitamin E in fish food composition

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Abstract. Success of the industrial methods of fish farming depends primarily on the quality and balance of the combined feeds used. The results of a study of the effectiveness of the use of a flavonoid antioxidant, dihydroquercetin, and an assessment of the synergistic effect of this antioxidant in combination with the immunostimulant arabinogalactan and vitamin E are presented. To evaluate the effectiveness of dihydroquercetin in fish diets, two series of experiments were carried out. The research results showed that the best growth rates are owed to the addition of a complex of biologically active substances – dihydroquercetin in combination with vitamin E. The growth increased by 37.0-46.0%, and the fatness coefficient according to Fulton was 0.07 units, then as in the fish of the control group, the indicator did not exceed 0.04 units. When using an antioxidant in combination with vitamin E, the average weight of fish in the control group increased by 18.0%, while in the experimental variants this figure increased by 30.0-31.0%. Fatness coefficient according to Fulton in fish of the control group was 0.04 units, and when the diet was enriched with complexes of antioxidant additives, it was 0.07 units. The indicators of energy metabolism also indicate a better accumulation of plastic substances in the fish of the experimental groups: ESR 1.92 ± 0.30 mm/h and 1.83 ± 0.27 mm/h, the level of total protein is significantly ($p \leq 0.01$) lower values of the control group (test 1 with dihydroquercetin – 25.70 ± 2.9 and test 2 with dihydroquercetin and arabinogalactan – 23.38 ± 0.90 g/l), and the cholesterol level is lower by 11.5-24.1%. Thus, it can be inferred that the complex of antioxidants provided more favorable trophic and biochemical conditions necessary, in particular, for the normal growth and development of fish. The results obtained in the course of the research can serve as a basis for improving the technologies for the production of mixed feed when raising fish on an intensive basis.

Keywords: dihydroquercetin, arabinogalactan, antioxidant, vitamin E, immunostimulant, tilapia, aquaculture, feeding, growth

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Научная статья

Оценка эффективности применения биологически активных добавок с антиоксидантным действием в сочетании с витамином Е в составе продукционных кормов для рыб

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Аннотация. Успех индустриальной формы рыбоводства в первую очередь зависит от качества и сбалансированности применяемых комбинированных кормов. Представлены результаты исследования эффективности применения антиоксиданта флавоноидной природы – дигидрокверцетина – и оценка синергетического действия этого антиоксиданта в сочетании с иммуностимулятором арабиногалактаном и витамином Е. Для оценки эффективности дигидрокверцетина в рационах рыб проводили две серии экспериментов. В результате исследований установлено, что лучшие показатели по приростам наблюдались при добавлении комплекса биологически активных веществ – дигидрокверцетина в сочетании с витамином Е. Прирост увеличился на 37,0–46,0 %, а коэффициент упитанности по Фультону составил 0,07 ед., тогда как у рыб контрольной группы показатель не превышал 0,04 ед. При использовании антиоксиданта в сочетании с витамином Е у рыб контрольной группы средняя масса увеличилась на 18,0 %, тогда как в опытных вариантах этот показатель увеличился на 30,0–31,0 %. Коэффициент упитанности по Фультону у рыб контрольной группы составил 0,04 ед., а при обогащении рациона комплексами антиоксидантных добавок – 0,07 ед. Показатели энергетического обмена также свидетельствуют о лучшем накоплении пластических веществ у рыб экспериментальных групп: СОЭ 1,92 ± 0,30 мм/ч и 1,83 ± 0,27 мм/ч, уровень общего белка достоверно ($p \leq 0,01$) ниже значений контрольной группы (вариант 1 с дигидроквертецином – 25,70 ± 2,9, вариант 2 с дигидроквертецином и арабиногалактаном – 23,38 ± 0,90 г/л), а уровень холестерина ниже на 11,5–24,1 %. Таким образом, можно говорить о том, что комплекс антиоксидантов обеспечил более благоприятные трофические и биохимические условия, необходимые, в частности, для нормального роста и развития рыб. Результаты, полученные в ходе исследований, могут служить основой для совершенствования технологий производства комбикормов при выращивании рыб на интенсивной основе.

Ключевые слова: дигидрокверцетин, арабиногалактан, антиоксидант, витамин Е, иммуностимулятор, тиляпия, аквакультура, кормление, прирост

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Introduction

Analysis of the work of aquaculture enterprises shows that the technological features of industrial fish farming (high stocking densities, regular sorting of fish, oxygen level drops, etc.) are stressful for fish and can provoke free radical oxidation processes and disrupt metabolism, which leads to a delay in the growth and development of cultivated objects and affects the fish adaptive mechanisms [1-6].

In this regard, further improvement of modern technologies of industrial fish farming, in particular, in recirculation aquaculture systems, involves a detailed study of metabolism and, especially, effect of antioxidant protection in conditions of artificially created ecosystems. This will allow identifying the most vulnerable stages, monitoring, and if necessary, correcting the physiological state of the fish.

At the same time, an important role is assigned to organizing rational feeding balanced with the needs of the cultivated species. Deficiency or imbalance of vitamins, macro- or microelements in the diet of fish leads to characteristic disturbances in metabolic processes, contributing to a decrease in the efficiency of cultivation. In addition, non-compliance with certain stages of feeding technology (production of feed from low-quality raw materials, improper storage) can cause producing peroxides dangerous for the fish body.

Loss of appetite, decreased growth rate, decreased activity and high mortality are signs of weakening of the body's antioxidant defense. In addition, muscular dystrophy, fatty degeneration of the liver, accumulation of fluid in the abdominal cavity, hemolysis of erythrocytes, decrease in hematocrit, etc. are noted [7, 8].

One of the ways to improve the biotechnics of fish breeding is using the biologically active substances (BAS) that have a stimulating effect on the vital functions of the body.

An important argument when choosing BAS is their antioxidant and adaptogenic effect. Thus, the diet of fish should not only be balanced, according to the biological needs of cultivated species, but also additionally enriched with antioxidant complexes.

Developing the new drugs with antioxidant properties makes it possible to improve the technology of fish feeding and increase the pro-oxidant – antioxidant balance.

Due to the fact that an important criterion for choosing feed additives is environmental safety, natural bioantioxifiers of flavonoid nature, in particular, dihydroquercetin, are of interest. Dihydroquercetin, a natural flavonoid isolated from the larch wood has a wide range of properties, for example, it participates in the synthesis of vitamin P, helps to reduce the permeability and fragility of capillaries [9]. Being an effective antioxidant, dihydroquercetin interrupts the processes of lipid peroxidation in cell membranes, penetrates into the cytoplasm of the cell and protects the cell from the damaging effects of free radicals.

Dihydroquercetin also 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 [10-14].

The aim of the study was to examine the metabolic processes in the fish body when using dihydroquercetin in diets and to evaluate the synergistic effect of this antioxidant in combination with the immunostimulator arabinogalactan (AG) and vitamin E.

Materials and methods of research

The study was conducted in the innovation center "Bioaquapark – scientific and technical center of aquaculture" under Astrakhan State Technical University. To evaluate the effectiveness of dihydroquercetin (DHQ) in fish diets, two series of experiments were conducted. The work was carried out according to the scheme presented in Table 1.

Table 1

Scheme of the experiment

Indicator	Control	Test 1	Test 2
Feeding	Basic diet (BD)	BD + 50.0 mg DHQ	BD + 25.0 mg DHQ + 25.0 mg AG
		BD + 50.0 mg DHQ + 50.0 mg vitamin E	BD + 25.0 mg DHQ + 50.0 mg vitamin E
The object of the study	Tilapia hybrid *Oreochromis mossambicus* × *Oreochromis niloticus*		
Stocking density, pcs/m^3	100		
Granule size, mm	3.5	3.5	3.5
Temperature regime, °C	26.5 during a day		
pH	7.5	7.5	7.5
Feeding method	Manually, by eatability		
Research period	28 days		

The drug Flavitol (CJSC SPF "FLAVIT") was used as a source of DHQ, which contains highly purified DHQ (94-96%) with a preserved native form. The drug dissolved in distilled water was introduced into the compound feed in the process of its producing. Experimental batches of dry compound feeds were made under laboratory conditions by wet pressing followed by drying.

The daily feeding rate was determined according to the feed tables depending on the average weight of fish and water 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, weight accumulation coefficient). Measurements and calculations were carried out in accordance with the recommendations adopted in fish farming [15-17].

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 and without an anticoagulant to obtain blood serum (by centrifugation at 3 000 rpm) and to study biochemical parameters.

Hemoglobin was determined by the hemoglobin cyanide method [18]. The erythrocyte sedimentation rate (ESR) was determined by the Panchenkov method [19].

Determination of biochemical parameters of blood serum (total serum protein, cholesterol) was carried out according to certified methods and reagent kits of Olvexdiagnosticum-Yug, LLC (Russia) and Agat-Med, LLC (Russia) [20-22]. Blood smears were prepared using a May-Grunwald dye fixative from Olvex-Diagnosticum [23, 24].

The research results were processed using generally accepted methods of biological statistics [25] and the Microsoft Excel program. Statistical analysis was carried out using the Student's t-criterion, differences were considered significant at $p \leq 0.05$.

Research results

Analysis of fish-breeding and biological indicators (Table 2) showed that the growth rate of fish whose diet was enriched with DHQ exceeded these indicators in fish of control groups.

Table 2

Dynamics of growth indicators of tilapia hybrid when using DHQ and AG

Indicator	Control	Test 1	Test 2
Weight, g: initial; final	 99.43 ± 10.51 131.73 ± 10.01	 80.27 ± 6.12 119.53 ± 7.33	 79.88 ± 7.48 124.00 ± 8.91
Length, cm: initial; final	 18.27 ± 0.52 19.54 ± 0.46	 17.68 ± 0.42 19.23 ± 0.39	 17.39 ± 0.44 19.03 ± 0.42
Fulton fatness coefficient: initial; final	 1.54 ± 0.04* 1.7 ± 0.03	 1.41 ± 0.03* 1.76 ± 0.14	 1.45 ± 0.04 1.77 ± 0.03
Absolute growth, g	32.28	39.26	44.12
Average daily growth, g	1.15	1.40	1.58
Average daily growth rate, %	1.01	1.43	1.58
Weight accumulation coefficient	0.05	0.07	0.07
Duration of cultivation, days	28	28	28

* $P \le 0.05$.

During the experiment, it was found that high growth rate was observed in all groups. The average daily increments fluctuated in the range of 1.1-1.5 g.

The best indicators for increments 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 44.12 g in the second group, which is 17.7 and 27.2% higher than in fish of the control group (Table 3).

Table 3

Dynamics of growth indicators of tilapia hybrid when combined in the diet of DHQ and vitamin E

Indicator	Control	Test 1	Test 2
Weight, g: initial; final	 142.00 ± 12.17 173.68 ± 10.88	 121.65 ± 27.20 172.45 ± 38.56	 130.50 ± 7.87 189.16 ± 8.36
Length, cm: initial; final	 20.29 ± 0.54 21.13 ± 0.60	 20.18 ± 4.51 21.35 ± 4.77	 20.22 ± 0.36 21.36 ± 0.22
Fulton fatness coefficient: initial; final	 1.63 ± 0.03* 1.91 ± 0.10	 1.46 ± 0.03* 1.77 ± 0.05**	 1.55 ± 0.03*** 1.92 ± 0.04**
Absolute growth, g	31.68	50.80	58.66
Average daily growth, g	1.13	1.81	2.10
Average daily growth rate, %	0.72	1.25	1.33
Weight accumulation coefficient	0.04	0.07	0.07
Duration of cultivation, days	28	28	28

* $P \le 0.001$; ** $p \le 0.01$; *** $p \le 0.05$.

When using an antioxidant in combination with vitamin E in fish of the control group, the average weight increased by 18.0%, whereas in experimental variants this indicator increased by 30.0-31.0%. The Fulton fatness coefficient in fish of the control group was 0.04 units, and when enriching the diet with complexes of antioxidant additives 0.07 units.

During the growing period, the absolute body weight gain of fish in the control group was 37.6-45.9% lower than that of fish in the experimental groups. A similar trend is observed for other piscicultural indicators. The best piscicultural 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%. 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 growth indicators (increased weight gain and length. fatness of fish) and survival indicate a positive effect of adding a complex of antioxidants to the diet of hybrid tilapia. The functional state of the organism in the proposed growing conditions was assessed by physiological and biochemical parameters of blood, which act as specific indicators of physiological or pathological changes. The results of studies of blood biochemical parameters are presented in Tables 4 and 5.

Table 4

Biochemical blood parameters of tilapia hybrid when used in the diets of DHQ and AG

Indicator	Control	Test 1	Test 2
Hemoglobin, g/l	48.38 ± 5.84	80.91 ± 11.01*	54.57 ± 2.65**
ESR, mm/h	1.92 ± 0.33	1.90 ± 0.24	1.83 ± 0.28
Total serum protein, g/l	21.33 ± 0.88	23.67 ± 1.86	33.00 ± 2.0**
Cholesterol, mmol/l	3.23 ± 0.35	3.62 ± 0.52	3.75 ± 0.57
Glucose, g/l	5.79 ± 0.42	6.01 ± 0.16	5.21 ± 0.18**
Total lipids, g/l	2.95 ± 0.18	3.27 ± 0.15	3.34 ± 0.20

*$P \le 0.01$; **$p \le 0.001$.

Table 5

Biochemical blood parameters of tilapia hybrid using DHQ and vitamin E in diets

Iidicator	Test 1	Test 2	Control
Hemoglobin, g/l	60.07 ± 3.89	54.32 ± 2.87	59.62 ± 2.68
ESR, mm/h	1.92 ± 0.30	1.83 ± 0.27	1.50 ± 0.19
Total serum protein, g/l	25.70 ± 2.93	23.38 ± 0.90*	30.88 ± 2.34*
Cholesterol, mmol/l	3.22 ± 0.24*	3.75 ± 0.22	4.24 ± 0.32*

*$P \le 0.05$.

The obtained results of hematological and biochemical parameters are consistent with the data of other authors [26-32]. The erythrocyte sedimentation rate in all experimental variants remained within the normative values, which is also consistent with the literature data [33, 34] and indicates a constant protein composition of blood plasma.

The concentration of hemoglobin varied from 40.0 to 80.0 g/l. When the BAS complex was added to the diet, the hemoglobin level was 30.0-40.0% higher compared to other experimental variants and indicates a positive effect of the feed additive on the metabolism of the studied fish.

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

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.

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 enrichment of the diet of DHQ in combination with vitamin E also influenced some biochemical parameters of the blood of fish, a significant difference ($p \le 0.01$) of which indicate a positive effect of biologically active substances on the physiological state of the body of fish.

In the fish of the control group the ESR was slightly lower than in the fish of the experimental groups. The revealed difference may indicate a change in the protein composition of blood plasma due to a more intensive diet of fish, and may also be the result of adaptation of tilapia to other habitat conditions, in this case, to a different diet [35, 36]. Hemoglobin concentration in the fish of the control and experimental groups differed slightly ($p \ge 0.01$).

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 ($p \le 0.01$) 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.

According to the level of cholesterol stimulating the body's immune system, the changes ranged from 2.8 to 5.5 mmol/l ($p \ge 0.01$). However, this indicator in the fish of the control group was 11.5-24.1% higher than in the fish of the experimental groups. The increased level of cholesterol in the blood contributes to a change in blood viscosity, which leads to a violation of active metabolism in the body, so it can be assumed that the dynamics of lipid metabolism in fish of the experimental groups also contributed to the normal process of accumulation of energy resources.

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 fish normal growth and development.

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 object under study long before the appearance of clinical signs of emerging pathologies. Table 6 shows the leukocyte formula of the blood of the fish under study.

Table 6

Leukocyte blood formula (%) of tilapia hybrid using DHQ and AG in the diets

Indicator	Control	Test 1	Test 2
Lymphocytes	86.94 ± 2.62	88.92 ± 1.87	89.48 ± 1.59
Monocytes	2.95 ± 0.71	2.94 ± 0.65	2.24 ± 0.47
Neutrophils	9.08 ± 1.57	7.45 ± 1.27	7.53 ± 1.21
Basophils	1.03 ± 0.14	0.69 ± 0.06**	0.75 ± 0.17

** $P \leq 0.01$.

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%).

Thus, the obtained hematological and biochemical indicators indicate a positive effect of BAS on the health of fish, and the results of size and weight characteristics confirm the high activity of metabolic processes.

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 results obtained 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 DHQ in combination with vitamin E as part of the production feed for hybrid tilapia during its commercial cultivation.

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