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The results of tilapia juveniles growing on production compound feeds with the antioxidant astaxanthin addition

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Abstract. One of the main stages of metabolism in the body is the digestibility and digestibility of feed nutrients, the effectiveness of which to a certain extent depends on the use of biologically active substances in diets that have antioxidant properties and have a stimulating effect on vital body functions. An important criterion for choosing feed additives is their environmental safety. An ideal antioxidant should be easily absorbed by the body and prevent the formation of free radicals at physiologically significant levels. Therefore, the use of natural antioxidants, namely carotenoids, is of particular interest. Carotenoids play a different role in the metabolism of fish and are also pronounced antioxidants that protect the body from the action of free radicals. The experience of using the natural antioxidant astaxanthin in feeding tilapia juveniles is considered. Astaxanthin is a powerful antioxidant that has a huge impact on the functioning of all systems and overall health. But the practice of its use has not affected mass thermophilic aquaculture species, such as tilapia, whose muscle tissues and caviar are not stained under the action of astaxanthin, and its effect on the physiological status of fish, as well as antioxidant properties have not been studied. It was found that the addition of astaxanthin to the composition of production feeds at a dosage of 20.0; 30.0; 40.0 mg/kg allowed to increase productivity by 38.5; 30.1; 17.03%, respectively, and also had a positive effect on the physiological state of the fish. As a result, after feeding, the indicators of total protein in the experimental variants 1-3 were higher than the control group by 48.3; 41.6 and 31.6%, respectively. As a result of the analysis of the data obtained after the completion of experimental work, a significant increase in the level of hemoglobin concentration in experimental variants 1-3 was established by 1.2-1.3 times, respectively, compared with the control variant.

Keywords: feeding, astaxanthin, juveniles, tilapia, growth, physiological and biochemical parameters

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Научная статья

Результаты выращивания молоди тиляпии на продукционных комбикормах с добавлением антиоксиданта астаксантина

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Аннотация. Одними из главных этапов обмена веществ в организме являются переваримость и усвояемость питательных веществ корма, эффективность которых в определенной степени зависит от использования в рационах биологически активных веществ, обладающих антиоксидантными свойствами и оказывающих стимулирующее воздействие на жизненно важные функции организма. Важным критерием выбора кормовых добавок является их безопасность в экологическом отношении. Идеальный антиоксидант должен легко усваиваться организмом и предотвращать образование свободных радикалов на физиологически значимых уровнях. Поэтому особый интерес представляет использование природных антиоксидантов, а именно каротиноидов.

Каротиноиды играют различную роль в метаболизме рыб и также являются выраженными антиоксидантами, которые защищают организм от действия свободных радикалов. Рассматривается опыт применения природного антиоксиданта астаксантина в кормлении молоди тиляпии. Астаксантин – мощнейший антиоксидант, который оказывает огромное влияние на работу всех систем и общее состояние здоровья. Но практика его применения не коснулась массовых теплолюбивых видов аквакультуры, таких как тиляпия, мышечные ткани и икра которых не окрашиваются под действием астаксантина, а его влияние на физиологический статус рыб, а также антиоксидантные свойства так и не изучены. Установлено, что добавление в состав продукционных кормов астаксантина в дозировке 20,0; 30,0; 40,0 мг/кг позволило увеличить продуктивность на 38,5; 30,1; 17,03 % соответственно и оказало положительное влияние на физиологическое состояние рыб. В результате после кормления показатели общего белка в опытных вариантах 1–3 были выше контрольной группы на 48,3; 41,6 и 31,6 % соответственно. В результате анализа данных, полученных после завершения экспериментальных работ, установлено достоверное повышение уровня концентрации гемоглобина в опытных вариантах 1–3 в 1,2–1,3 раза соответственно по сравнению с контрольным вариантом.

Ключевые слова: кормление, астаксантин, молодь, тиляпия, прирост, физиолого-биохимические показатели

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Introduction

A characteristic feature of industrial aquaculture is the cultivation of fish under controlled conditions using dry granular compound feeds with a lack or complete absence of live feed organisms in the diet. An urgent task is to improve the composition of compound feeds and feeding technologies, especially at the early stages of development. To increase the resistance of fish to the action of peroxides of poor-quality feed, unfavorable environmental factors, it becomes necessary to search for and use new feed components of natural origin, in particular to increase the efficiency of industrial cultivation of such mass thermophilic aquaculture species as tilapia. When developing the composition of recipes for complete dry combined feeds in industrial aquaculture, in addition to their balance in terms of basic nutrients, it is necessary to pay attention to the presence of a number of irreplaceable biologically active feed components in them. Among them, along with vitamins and minerals, are carotenoids – natural pigments contained in the natural food of fish [1]. The role of carotenoids for the course of normal physiological processes is indisputable. A large number of carotenoid pigments were found in the tissues and organs of hydrobionts [2]. Being natural substances, carotenoids are synthesized by plants and some microorganisms. This causes the need for their entry into the body of animals with food. The function of carotenoids in the body is not limited only to conversion into vitamin A. Their other properties, such as photoprotective and antioxidant, have also been proven. In addition, they are capable of the saving action of vitamins and enzymes [1, 3]. Scientists have found out the immunostimulating role of carotenoids. Carotenoids increase the cytostatic activity of killer cells, slow down tumor growth and accelerate wound healing. Their importance in increasing the body's resistance to exposure to toxic substances in hypoxia is also noted [4, 5]. Carotenoids, even with long-term use in human and animal food and sufficiently high doses, do not show toxicity [6].

One of the most powerful carotenoids is astaxanthin, it is 10 times more effective than zeaxanthin, lutein, canthaxanthin and various forms of beta-carotene. Astaxanthin shows higher activity than other antioxidants, because due to its chemical structure it binds the inner and outer cell membranes. Astaxanthin is 550 times stronger than vitamin E, 6,000 times stronger than vitamin C.

In these scientific studies, it is planned to investigate natural astaxanthin, it has antioxidant, provitamin and antimutagenic activity, is used in the food industry, agriculture and medicine [1].

The aim of the research was to study the effect of the antioxidant astaxanthin on the functional state of juvenile tilapia.

Materials and methods of research

Experimental work was carried out on the basis of the Innovation Center Bioaquapark – STC of Aquaculture of the Astrakhan State Technical University. The objects of the study were fingerlings of the Mozambique tilapia (*Oreochromis mossambicus*).

The study investigated the effectiveness of the use of the natural antioxidant astaxanthin, trade name *Astaped* (manufactured in India) (Fig. 1).

Fig. 1. Antioxidant astaxanthin

The bioavailability (the ability to be absorbed by the body) of astaxanthin is not too high, but the absorption of astaxanthin improves when combined with edible oils, such as fish oil. Astaxanthin is a lipophilic compound, it dissolves well in oils. Before being introduced into experimental feeds, astaxanthin was previously dissolved in liquid fish oil.

The study was carried out according to the following scheme: test I - 20.0 mg/kg of feed; test II - 30.0 mg/kg of feed; test III - 40.0 mg/kg of feed. Feed without the addition of astaxanthin was used as a control (Table 1).

Table 1

Scheme of the main parameters of the experience

Indicator	Groups			
	Control	Test I	Test II	Test III
	Basic diet (BD)	BD + astaxanthin 20 mg/kg	BD + astaxanthin 30 mg/kg	BD + astaxanthin 40 mg/kg
Granule size, mm	2.0			
Fish-breeding tanks	Aquarium (400 l)			
Stocking density	40 pcs./m^3			
Feeding method	Manually, by eatability			
Temperature regime of aquariums, °C	26.44 ± 0.46	26.51 ± 0.18	26.39 ± 0.16	26.60 ± 0.34
pH, units	7.5			
Research period	40 days			
Survival rate, %	100			

The control and experimental feeds were made in laboratory conditions using feed components of domestic production by wet pressing, the conditions for the manufacture of all feed variants were the same. Fish feeding was carried out manually 2 times in the daytime. The daily feeding rate was determined according to the feed tables depending on the average weight of fish and water temperature [7].

Cultivation was carried out at the same planting density and constant temperature regime in accordance with the biological characteristics of the species.

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. Weighing and measuring of fish was carried out according to the recommendations adopted in fish farming using laboratory scales *Mass-K VK-3000*, for a comparative assessment of the fatness of tilapia fingerlings, the Fulton coefficient (FC) was used [8] (Fig. 2).

a

b

Fig. 2. Control measurement *Oreochromis mossambicus*: *a* – weighing fish; *b* – measuring fish

The survival rate of fish was taken into account by the piece method. The physiological state of the studied objects was assessed by biochemical parameters of protein, lipid and carbohydrate metabolism (blood composition), according to the developed methods [9–12]. Blood was taken in vivo from the tail vein of fish into Eppendorf tubes [11]. The following indicators were determined: the concentration of hemoglobin photometrically using a set of reagents from *Agat-Med* [11], the rate of erythrocyte sedimentation (ESR) on the device of R. P. Panchenkov [10]. A Unico 2100 spectrophotometer was used to measure the optical density of the samples obtained.

Blood smears were prepared using a dye fixative from *Olvex-diagnosticum* (Russia) using the May-Grunwald method [13]. Identification of leukocytes by stages of their cytogenesis, evaluated by differential counting of cell types. 200 leukocytes were identified on each blood smear of juvenile tilapia, taking into account their cytogenesis according to the classification of N. T. Ivanova [13].

The study of the smallest details on histological blood samples was performed with an *Olympus* electron microscope (Japan).

The results of the research were processed using generally accepted methods of biological statistics and the Microsoft Excel program. The level of differences was assessed using the Student's reliability criterion [14].

Research results

Table 2 shows the results of growing tilapia juveniles on tested experimental feeds (*n* – number of measuring fish in each group).

Table 2

Fish-breeding and biological indicators of *Oreochromis mossambicus* cultivation on experimental compound feeds (*n* = 40)

Indicator	Control	Test I	Test II	Test III
Weight, g:				
initial	37.4 ± 11.4	35.9 ± 10.7	36.81 ± 13.2	37,0 ± 15,1
final	100.3 ± 14.5	122.3 ± 22.3**	117.9 ± 12.7	110,3 ± 17,3
Length, cm:				
initial	13.51 ± 1.4	13.03 ± 1.5	13 24 ± 1.6	13.45 ± 1.1
final	17.03 ± 1.2	17.95 ± 2.2*	17.77 ± 1.6	17.61 ± 2.6
Fulton fatness coefficient:				
initial;	1.55 ± 0.3	1.57 ± 0.1	1.54 ± 0.4	1.53 ± 0.6
final	2.03 ± 0.4	2.12 ± 1.1	2.10 ± 0.5	2.02 ± 0.9
Absolute growth, g	62.63	86.31**	81.18*	73.34*
Average daily growth, g	1.56	2.16**	2.03*	1.83
Average daily growth rate, %	3.0	3.7*	3.5	3.3
Weight accumulation coefficient	0.09	0.12*	0.11	0.11

* $P \le 0.05$; ** $p \le 0.001$ – the differences are significant.

Evaluation of the effectiveness of the use of astaxanthin in production compound feeds showed that the best growth rates were characteristic of a group of fish that consumed feed with the addition of astaxanthin 20 mg per 1 kg of feed (Test I).

The absolute increase in live weight in the fish of the first group was 86.31 g versus 81.18 and 73.34 g in the second and third groups, respectively ($p < 0.05$). The average daily increase in variants 1, 2, 3 was: 2.16, 2.03 and 1.83 g, which is 38.5, 30.1 and 17.03%, respectively, higher than the control group. The average daily growth rate in Tests I, II, III was 3.7, 3.5 and 3.3%, respectively, which is significantly higher than the control group in Test I and II by 1.2 times ($p < 0.05$) and in Test III this difference was 1.1 times. The mass accumulation coefficient in fish in the first Test was 0.12 units, in the second and third Tests - 0.11 units, which is 0.03 and 0.02 units, respectively, higher than the control. The survival rate in the experimental tests and control was 100%.

The Fulton fatness coefficient, reflecting the relationship between the average body weight and the length of the fish, did not differ significantly and varied in the range of 1.53-1.57 units at the beginning of the experiment, by the end of the study these indicators ranged from 2.02-2.12 units.

The data obtained during the study indicate that the use of astaxanthin at concentrations of 20.0, 30.0 and 40.0 mg per 1 kg of feed for tilapia juveniles has a positive effect on growth rates. However, the most effective concentration of astaxanthin in the diet of younger age groups of tilapias is 20.0 mg/kg of feed, this is confirmed by the results obtained during the experiments, expressed in higher growth rates. The best growth rates were characteristic of Test I, in this sample the highest absolute growth, average daily growth, average daily growth rate and mass accumulation coefficient were observed.

Tilapia is one of the most resistant to viral, bacterial and invasive diseases compared to other cultivated fish. Knowledge about blood components and their functions is important for understanding the normal and pathological state of the body. To assess the effect of astaxanthin in the compound feed for juvenile tilapia, the dynamics of physiological blood parameters were studied.

One of the elements of the biochemical assessment of the physiological state of cultured fish is the characteristic of the metabolic function of blood [15]. In order to identify changes in metabolic processes, the dynamics of hematological and biochemical parameters of blood was studied.

The results of the analysis of the physiological state of juvenile tilapia are presented in Table 3.

Table 3

Hematological parameters of *Oreochromis mossambicus* blood* (*n* = 40)

Indicator	Control	Test I	Test II	Test III
Hemoglobin, g/l	48.8 ± 1.5 / 55.8 ± 3.8	46.2 ± 2.1 / 70.4 ± 5.4**	50.9 ± 2.3 / 66.3 ± 3.4**	48.9 ± 1.9 / 67.6 ± 4.7**
ESR, mm/h	2.8 ± 0.6 / 2.7 ± 0.3	3.2 ± 0.8 / 2.3 ± 0.7	3.5 ± 0.3 / 2.6 ± 0.4	3.4 ± 0.2 / 2.5 ± 0.8

* The numerator is the beginning of the experiment; the denominator is the end of the experiment; ** $p < 0{,}05$ – the differences are significant.

The most important element of the realization of respiratory function is the level of hemoglobin. A decrease in hemoglobin concentration is a symptom of functional tension in the body's oxygen supply system [16]. Analyzing the data obtained after the completion of experimental work, a significant increase in the level of hemoglobin concentration in Tests I, II, III was found by 14.6, 10.5 and 11.8 g/l, respectively, compared with the control variant. The high hemoglobin content in the experimental groups (within the reference values) may be associated with a more intensive metabolism in the body of fish. Since the antioxidant astaxanthin was used in the feed, which increases the rheological parameters of the blood, which suggests a positive effect on the microcirculation of blood in the body of fish. It is difficult to underestimate this property of astaxanthin [1]. The blood circulation depends on how well the organs are supplied with nutrients and oxygen.

According to the ESR indicator, no differences were found in the study tests ($p > 0.05$). However, it was found that in three experimental groups there was a tendency to decrease the indicator by the end of the experimental work. Thus, in the fish of the first experimental group, the erythrocyte sedimentation rate decreased from 3.2 to 2.3 mm/h, while in the control variant this indicator remained almost at the same level and amounted to 2.7 mm/h. It should be noted that in all variants of cultivation, the ESR did not exceed the normative indicators, which may indicate the absence of inflammatory processes and physiological changes in the body of farmed fish [17].

The analysis of the leukocyte blood formula also confirmed the good physiological condition of the experimental groups of fish (Table 4).

Table 4

Leukocyte formula of *Oreochromis mossambicus* blood (*n* = 40)

Indicator	Lymphocytes	Monocytes	Eosinophils	Neutrophils	Basophils
Control	78.85 ± 2.15	2.45 ± 0.53	3.16 ± 0.72	14.17 ± 0.74*	1.37 ± 0.14
Test I	77.82 ± 1.04	2.97 ± 0.11	3.83 ± 0.94	13.40 ± 0.13	1.98 ± 0.29
Test II	78.01 ± 1.33	2.88 ± 0.35	3.89 ± 0.80	13.64 ± 0.22	1.58 ± 0.09
Test III	77.53 ± 1.19	2.59 ± 0.44	3.99 ± 0.81	13.95 ± 0.14	1.94 ± 0.10

* $P < 0{,}05$ – the differences are significant.

The differential number of leukocytes was characterized by the predominance of lymphocytes in all variants of the study. Five types of leukocytes were identified in the circulating blood: lymphocytes, neutrophils, monocytes, eosinophils and basophils.

White blood cells are important cells involved in the immune response. When the physiological balance is disturbed, the body produces more white blood cells, which indicates lymphocytes as immunocompetent cells. The maximum number of lymphocytes was observed in smears of the control group and amounted to 78.85 ± 2.15%, but the number of lymphocytes in Mozambique tilapia fingerlings did not differ significantly and varied between 77.53-78.85%. In comparison with the experimental groups and the control, the difference between the content of lymphocytes ranged from 0.84 to 1.32% and was not statistically significant. In the leukocyte profile of fish blood in the control, it was noted that a significant part of leukocytes are neutrophils (14.17 ± 0.74%). As a result, the number of neutrophils in the control group was 1.1 times higher compared to the experimental variants, which probably indicates the activation of granulopoiesis in the fish body in response to a pathological state of the body in which neutrophils secrete substances with bactericidal and antitoxic properties into the blood. The number of monocytes in the control and three experimental groups was at the same level and did not exceed 2.97% of the total volume of calculated cells. A low percentage of monocytes in the leukocyte formula indicates a good physiological condition of the farmed fish [18, 19].

The dynamics of biochemical indicators serves as a marker of the state of the fish organism in artificial and natural reservoirs, characterizes the quality and quantity of nutrition, planting density, adaptive abilities of fish, the intensity of anthropogenic factors. Low levels of total protein, glucose, beta-lipoproteins and cholesterol in the blood serum may reflect both an intensification of their use for the needs of the body and a weakening of the functional activity of the liver [15, 16]. The results of the biochemical study are presented in Table 5.

Table 5

Physiological and biochemical parameters of *Oreochromis mossambicus* blood* (*n* = 40)

Indicator	Control	Test I	Test II	Test III
Total serum protein, g/l	22.3 ± 1.2 24.0 ± 10.4	23.0 ± 1.7 35.6 ± 14.0**	24.6 ± 0.6 34.0 ± 12.7**	22.8 ± 0.4 31.6 ± 11.7**
Glucose, g/l	3.2 ± 1.2 3.5 ± 0.8	2.6 ± 1.7 4.0 ± 0.6	2.7 ± 0.2 3.8 ± 0.5	2.8 ± 0.2 3.2 ± 0.7
Cholesterol, mmol/l	3.0 ± 0.2 3.5 ± 0.2	3.5 ± 0.3 2.9 ± 0.2	3.4 ± 0.2 3.0 ± 0.1	2.8 ± 0.6 3.1 ± 0.3
β-lipoproteins, g/l	2.3 ± 0.3 2.9 ± 0.3	2.5 ± 0.2 4.0 ± 0.6**	2.3 ± 0.4 3.5 ± 0.2	2.7 ± 0.6 3.0 ± 0.4

* The numerator is the beginning of the experiment; the denominator is the end of the experiment; ** $p < 0.05$ – the differences are significant.

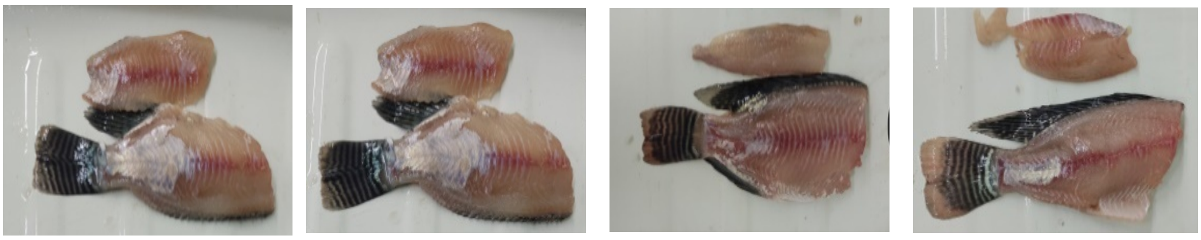
Most of the most subtle biological functions are performed by proteins or with their participation. The most important function of whey proteins is the transport of substances that provide the cells of the animal's body with building material and energy. The level of total protein can vary significantly and depends on the state of the internal environment, nutrition conditions, diet and the level of energy metabolism [16]. The concentration of total protein in the blood serum significantly changed ($p \leq 0.05$) during the study period, before the experiment in Tests I, II and III, the indicators were low, after feeding with the addition of astaxanthin to the experimental feed, the indicators changed significantly, while in the control, the indicators before and after the study remained at the same level. As a result, after feeding, the indicators of total protein in the experimental tests (I, II and III) were higher by 48.3, 41.6 and 31.6%, respectively, of the control group.

At the end of the experiment, the glucose dynamics in the studied groups was within the physiological norm (from 3.0-4.0 g/l), which is the result of the normal operation of the enzymatic system that catalyzes the transformation of glucose.

Fats and fat-like substances belong to lipids. Among them, cholesterol is of considerable interest. For the growth of the body and cell division, the concentration of cholesterol in the blood plays an important role, which comes from food or is produced by its own cells and synthesized in the liver. According to the level of cholesterol stimulating the body's immune system, the changes ranged from 3.0 to 3.5 mmol/l, the data were within the normative values [15, 16].

Beta-lipoproteins are the most cholesterol-rich particles (they contain up to 45% cholesterol). This is actually low-density cholesterol (LD-C) in combination with protein and other fats and fat-like substances [16]. In our experiment, this indicator was as follows: in the first experimental group – 4.0 ± 0.6 g/l, in the second experimental group – 3.5 ± 0.2 g/l and in the third group – 3.0 ± 0.4 g/l. In the control, this indicator was the lowest – 2.9 ± 0.3 g/l. Thus, the concentration of beta-lipoproteins was within the physiological norm in all variants of the studies. However, the values of beta-lipoproteins in Test I were slightly higher than the control, the discrepancies were statistically significant ($p < 0.05$).

The fish during the experiment with astaxanthin did not show any anxiety after feeding. Astaxanthin did not show a pigmenting role in the coloring of tissues on tilapia juveniles (Fig. 3).



a *b* *c* *d*

Fig. 3. Results of astaxanthin effect on tissue pigmentation:
a – control; *b* – Test I; *c* – Test II; *d* – Test III

However, astaxanthin showed an effect on the externa pigmentation of fish (Fig. 4).

a *b* *c* *d*

Fig. 4. Results of astaxanthin exposure to external integuments *Oreochromis mossambicus*:
a – control; *b* – Test I; *c* – Test II; *d* – Test III

The figure shows the results of staining after 40 days of feeding with astaxanthin with the dosage: Test I – 20.0 mg/kg of feed; Test II – 30.0 mg/kg of feed; Test III – 40.0 mg/kg of feed. The control feed was without the addition of astaxanthin.

Thus, the results obtained indicate the effective utilization of consumed feed and activation of plastic metabolism, which is confirmed by the data of physiological-biochemical and fish-breeding-biological analysis, as well as the absence of any disturbance in the transformation of substances in the body. The data obtained during the study indicate that the use of astaxanthin at a concentration of 20.0 mg per 1 kg of feed gives the best growth rate, and the effectiveness of this dosage is also confirmed by the results of the physiological state of fish obtained during the experiments.

In general, the obtained results of hematological and biochemical parameters are consistent with the data of other authors [20, 21].

Conclusion

The conducted studies indicate the effectiveness of the use of astaxanthin in feeding industrial aquaculture facilities, in particular tilapia. The positive effect of the natural antioxidant astaxanthin on the growth and development of cultured juveniles has been established.

It was found that the addition of the antioxidant astaxanthin to the feed contributes to a sharp increase in productivity, fish are distinguished by better fish–breeding and biological indicators, contributing to an increase in fish weight gain. As a result, the inclusion of the antioxidant astaxanthin in the feed has contributed to a more efficient use of nutrients in the diet.

Thus, according to the biological indicators of growth and physiological state, we can talk about the effectiveness of adding astaxanthin to the feed for tilapia juveniles in an amount of 20 mg/kg, since this sample is characterized by a high growth rate and higher indicators of protein, carbohydrate and lipid metabolism.

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