# The effectiveness of the use of astaxanthin in production feeds for promising aquaculture objects

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Abstract. 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. 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 article discusses the experience of using the natural antioxidant astaxanthin in feeding tilapia. Astaxanthin is a powerful antioxidant that has a huge impact on the functioning of all systems and overall health. But this practice has not affected mass thermophilic aquaculture species like tilapia, their 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 - 20.0 and 40.0 mg/ kg to the composition of production feeds allowed to increase productivity by 22.0 and 39.2%, respectively, as well as to have a positive effect on the physiological state of fish.

#### 1 Introduction

In fish farming, the determining factors that ensure high production rates are optimal habitat conditions and properly organized feeding. The latter often plays a decisive role. In feeding fish, the quality of feed that fully meets the needs of the raised aquatic organisms is of great importance [1-3]. Many specialists and scientists continue to work on the problems of improving the quality of compound feeds by introducing new components into the formulation that allow increasing the productivity, balance and usefulness of feed. 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

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natural origin, in particular to increase the efficiency of industrial cultivation of such mass thermophilic aquaculture species as tilapia [4-10]. 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. They play a different role in the metabolism of fish and are also pronounced antioxidants that protect the body from the action of free radicals that have a damaging effect on the membranes of fish cells [11-12].

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, 6000 times stronger than vitamin C. In addition, it is 10 times more effective than zeaxanthin, lutein, canthaxanthin and various forms of beta-carotene [13-15].

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.

The aim of the research was to study the effect of the antioxidant astaxanthin on the efficiency of cultivation and the physiological state of juvenile tilapia.

#### 2 Material 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 the yearlings of the Mozambique tilapia (*Oreochromis mossambicus*).

The study examined the effectiveness of the use of the natural antioxidant astaxanthin, trade name "Astaped" (manufactured in India). The study was carried out on three experimental groups. The first group (control) received a food product balanced in all nutrition elements, according to physiological needs. The second group (Test 1) received the diet of the 1st group with the addition of the natural antioxidant astaxanthin (AX) in the amount of 20 mg/kg. The third group (Test 2) received the diet of the 1st group with the addition of the natural antioxidant astaxanthin in the amount of 40 mg/ kg (Table 1). Astaxanthin is a lipophilic compound, it dissolves well in oils. Before being introduced into experimental feeds, astaxanthin was previously dissolved in liquid fish oil. Experimental feed was produced in laboratory conditions using feed components of domestic production by wet pressing. Cultivation was carried out at the same planting density and constant temperature regime in accordance with the biological characteristics of the species. Fish feeding was carried out manually 3 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. The scheme of the study is presented in table 1.

The daily feeding rate was set according to the feeding tables, depending on the average weight of fish and water temperature. 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. The survival rate of fish was taken into account by the piece method. The concentration of hemoglobin in the blood was determined photometrically, the rate of erythrocyte sedimentation (ESR) was determined on the device of R.P. Panchenkov. For biochemical blood analysis, blood samples were taken into test tubes without heparin, left to coagulate to obtain serum. The concentration of total protein, cholesterol and the level of beta-lipoproteins were determined according to accepted methods.

Parameter	Control	Test 1	Test 2	
Dist	.D.ff.	«Biff» + 20 mg/kg	«БИФФ» + 40	
Diet	«DIII»	of AX	mg/kg of AX	
Granule size, mm	2,0 2,0 2,0			
Fish-breeding tanks	Aquarium (400 l)			
Stocking density	100 pcs./m <sup>3</sup>			
pcs./m <sup>3</sup>				
Feeding method	Manually, by eatability			
Temperature regime	26.5+0.5	26,2±1,1	26,4±0,4	
of tanks, °C	20,5±0,5			
pH, units.	7,5	7,5	7,5	
Research period	30 days			

Table 1. Scheme of the main parameters of the experiment.

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 t-criterion.

#### **3 Research results**

The study used fish weighing from 150.0 g to 210.0 g, while the coefficient of variation did not exceed 3.0%. Evaluation of the effectiveness of the use of the natural antioxidant astaxanthin in production compound feeds showed that the best growth rates were characteristic of the group of fish that consumed feed with the addition of astaxanthin in the amount of 40 mg/kg – option No. 2 (Figure 1).

The dynamics of the growth rate corresponded to the change in absolute values. In the course of the study, it was revealed that significantly ( $p \le 0.05$ ) lower values of the increase were characteristic of the control group of fish.



□ The begininf of experiment ■ The end of experiment



During the experiment, it was found that a high intensity of growth was observed in all groups.

Over the entire period of the experiment, the absolute and average daily increase in the fish of the experimental groups was 22.0-39.2% higher than that of the fish of the control group. The best result of the absolute increase was established for option No. 2, this indicator was 1.4 times higher than the control (p<0.001) (Figure 2).





The average daily growth rate in the control group was lower by 22.2–39.1%, in contrast to variants 1 and 2, respectively (Table 2).

For all variants of the study, growing conditions were maintained at an optimal level, and feeding was carried out according to established recommendations, obviously energy resources for the sample from the control group obtained from compound feed to a lesser extent degrees were spent on plastic exchange. This is confirmed by lower data on fishbreeding and biological indicators.

Fable	2.	Dynamics	of the growth o	f Mozambique tilar	pia (Oreochrom	<i>is Mossambicus</i> ) (n	=25).
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Parameter	Control	Test 1	Test 2
Average daily growth, g	2,07	2,53*	2,88*
Average daily growth rate, %	13,0	13,8*	14,0**
Mass accumulation coefficient, units.	0,0012	0,0014	0,0015
Duration of cultivation, day.	30	30	30
Survival rate, %	100,0	100,0	100,0

Note: at \* p<0.05, \*\* p<0.001 – the differences are significant

An adequate indicator of the quality and balance of the feed consumed are hematological and biochemical blood parameters (Table 3).

Parameter	Control	Test 1	Test 2
Hemoglobin, g/l	$\frac{72,0\pm3,8}{79,1\pm2,9}$	$\frac{70,6\pm4,4}{80,0\pm2,1}$	$\frac{74,00\pm3,5}{82,7\pm4,0}$
ESR, mm/h	$\frac{4,0\pm0,1}{2,9\pm0,3}$	$\frac{5,1\pm0,3}{2,4\pm0,3}$	$\frac{4,5\pm0,2}{2,2\pm0,6}$
Total serum protein, g/l	$\frac{24,0\pm1,7}{26,2\pm0,9}$	$\frac{25,1\pm1,4}{32,1\pm1,3^*}$	$\frac{22,4\pm1,5}{35,5\pm0,1^*}$
Cholesterol, mmol/l	$\frac{3,3\pm0,2}{3,5\pm0,3^{**}}$	$\frac{3,7\pm0,1}{2,5\pm0,3}$	$\frac{3,5\pm0,2}{2,2\pm0,3}$

**Table 3.** Hematological and physiological-biochemical parameters of tilapia blood (*Oreochromis* Mossambicus) (the numerator is the beginning of the experiment, the denominator is the end of the experiment) (n=25).

Note: at \* p<0.05, \*\* p<0.001 – the differences are significant

Analysis of red blood indicators did not reveal significant changes. There were no significant differences in the level of hemoglobin in the fish of the control and two experimental groups (p>0.05).

However, it was noted that hemoglobin in the blood of fish of all variants of the study was at a high level, and during the study period there was an increase of 10.8% and 14.3% in fish raised on a diet with the addition of astaxanthin, which indicates a positive effect of feed components on the metabolism of the studied fish. In the control variant, an increase in hemoglobin occurred by 9.7% and corresponded to the level of this indicator in the blood of fish of the experimental groups.

The ESR analysis revealed a downward trend, but the indicator remained within the normative values, and the data obtained is consistent with the data of other researchers. In addition, a slight decrease in ESR is evidence of the constancy of the protein composition of blood plasma when testing new diets.

One of the elements of the biochemical assessment of the physiological state of the studied object is the characteristic of the metabolic function of blood, and in particular, the dynamics of proteins and lipids transported by blood.

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. In the conditions of the study, a slight increase (9.2%) in total whey protein was noted in the fish of the control variant. The greatest changes occurred in the blood of fish that consumed the tested feed. The level of total whey protein increased (p<0.05) by 27.8% and 58.5% in variants 1 and 2, respectively. Such dynamics correspond to the data on the growth rate. Considering that the diet of the three experimental groups of fish contained the optimal amount of amino acids that meets the needs of the current period of life, the indicators of the protein composition of the blood serum can be conditionally considered normal.

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. The cholesterol level in the blood of fish above 3.7 g / 1 is considered pathological and indicates the effect of stressful environmental factors.

In the conditions of the study, there was an unambiguous tendency to decrease cholesterol in the blood of fish that consumed feed with the addition of astaxanthin in the feed formulation. At the same time, the greatest changes occurred in option No. 2 (59.0%), while in option No. 1 the indicator decreased by 18.6%. In the control variant, the cholesterol level was significantly higher (p<0.001) and did not even change slightly in comparison with the initial data

Thus, the indicators of protein and lipid metabolism in all three variants of the experiment were within the normative values, and the dynamics of changes is consistent with fish-breeding and biological data.

# 4 Conclusion

The conducted studies indicate the effectiveness of the use of astaxanthin in feeding promising aquaculture objects, in particular tilapia. The positive effect of the natural antioxidant astaxanthin on the growth and development of cultured juveniles has been established. In general, the obtained results of hematological and biochemical parameters are consistent with the data of other authors [14].

The fish during the experiment with astaxanthin did not show any anxiety after feeding. At the end of the experiment, tilapia meat was compared visually, by the shade of the muscles. Astaxanthin did not show a pigmenting role in the coloring of tissues on tilapia.

Thus, according to biological growth indicators, we can talk about the effectiveness of adding astaxanthin to tilapia feed in an amount of 40 mg/ kg, since this sample is characterized by a high growth rate and a higher level of total whey proteins.

The results obtained complement the existing ideas about the fields of application of antioxidants, and also prove the prospects of using astaxanthin as an antioxidant feed additive.

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## References

- M. W. Dethlefsen, N. H. Hjermitsleva, S. Frosch, M. E. Nielsen, Animal Feed Science and Technology 221, 157-166 (2016) doi.org/10.1016/j.anifeedsci.2016.08.007
- N. P. C. Tu, N. N. Ha, N. T. T. Linh, et al., Aquacultur 553, 738048 (2022) doi.org/10.1016/j.aquaculture.2022.738048
- 3. S. Ahmadkelayeh, S. K. Cheema, K. Hawboldt, Journal of Cleaner Production **371**, 133609 (2022) doi.org/10.1016/j.jclepro.2022.133609
- 4. F. Fernando, C. L. Candebat, J. M. Strugnell, N. A. L. Nankervis, Aquaculture Reports **26**, 101266 (2022) doi.org/10.1016/j.aqrep.2022.101266
- 5. R. Haque, P. B. Sawant, P. Sardar, Aquaculture **15**, 738828 (2022) doi.org/10.1016/j.aquaculture.2022.738828
- 6. S. Gao, M. Yang, K. Xu, X. Jiang, et al., Aquaculture 555, 738203 (2022) doi.org/10.1016/j.aquaculture.2022.738203
- H. H. Fang, J. Niu, Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 260, 109407 (2022) doi.org/10.1016/j.cbpc.2022.109407
- K. C. Lim, F. M. Yusoff, et al., Fish & Shellfish Immunology 114, 90-101 (2021) doi.org/10.1016/j.fsi.2021.03.025

- J. Jiang, W. Nuez-Ortin, A. Angell, et al., Algal Research 42, 101596 (2019) doi.org/10.1016/j.algal.2019.101596
- K. Hamre, G. Micallef, M. Hillestad, J. Johansen, et al., Aquaculture 551, 737950 (2022) doi.org/10.1016/j.aquaculture.2022.737950
- 11. L. Liu, J. Lia, X. Cai, et al., Aquaculture Reports **24**, 101124 (2022) doi.org/10.1016/j.aqrep.2022.101124
- 12. X. Zhu, R. Hao, J. Zhang, et al., Fish & Shellfish Immunology **122**, 38-47 (2022) doi.org/10.1016/j.fsi.2022.01.037
- 13. X. Zheng, Q. Huang, Food Chemistry **395**, 133584 (2022) doi.org/10.1016/j.foodchem.2022.133584
- 14. Z. Tuan Harith, S. Mohd Sukri, N. F. S. Remlee et al., Aquaculture and Fisheries 24 (2022) doi.org/10.1016/j.aaf.2022.06.001
- 15. Z. Fei, J. Liao, F. Fan, M. Wan, W. Bai, M. He, Y. Li, Biochemical Engineering Journal **183**, 108456 (2022) doi.org/10.1016/j.bej.2022.108456